

Marquette University

e-Publications@Marquette

College of Nursing Faculty Research and
Publications

Nursing, College of

4-15-2006

Peripheral Blood Pressure Changes Induced by Dobutamine Do Not Alter BOLD Signals in The Human Brain

Heng Liu

Medical College of Wisconsin

Charles Rainey

Medical College of Wisconsin

Kathryn K. Lauer

Medical College of Wisconsin

Linda B. Piacentine

Marquette University, linda.piacentine@marquette.edu

Alan S. Bloom

Medical College of Wisconsin

See next page for additional authors

Follow this and additional works at: https://epublications.marquette.edu/nursing_fac



Part of the [Nursing Commons](#)

Recommended Citation

Liu, Heng; Rainey, Charles; Lauer, Kathryn K.; Piacentine, Linda B.; Bloom, Alan S.; Risinger, Robert C.; Ward, B. Douglas; Stein, Elliot; and Li, Shi-Jiang, "Peripheral Blood Pressure Changes Induced by Dobutamine Do Not Alter BOLD Signals in The Human Brain" (2006). *College of Nursing Faculty Research and Publications*. 681.

https://epublications.marquette.edu/nursing_fac/681

Authors

Heng Liu, Charles Rainey, Kathryn K. Lauer, Linda B. Piacentine, Alan S. Bloom, Robert C. Risinger, B. Douglas Ward, Elliot Stein, and Shi-Jiang Li

Marquette University

e-Publications@Marquette

Nursing Faculty Research and Publications/College of Nursing

This paper is NOT THE PUBLISHED VERSION; but the author's final, peer-reviewed manuscript. The published version may be accessed by following the link in the citation below.

NeuroImage, Vol. 30, No. 3 (April 15, 2006): 745-752. [DOI](#). This article is © Elsevier and permission has been granted for this version to appear in [e-Publications@Marquette](#). Elsevier does not grant permission for this article to be further copied/distributed or hosted elsewhere without the express permission from Elsevier.

Peripheral Blood Pressure Changes Induced by Dobutamine Do Not Alter BOLD Signals in The Human Brain

Heng Liu

Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI

Charles Rainey

Department of Psychiatry, Medical College of Wisconsin, Milwaukee, WI

Kathryn K. Lauer

Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, WI

Linda Piacentine

Department of Psychiatry, Medical College of Wisconsin, Milwaukee, WI

Alan Bloom

Department of Pharmacology, Medical College of Wisconsin, Milwaukee, WI

Robert Risinger

Department of Psychiatry, Medical College of Wisconsin, Milwaukee, WI

B. Douglas Ward

Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI

Elliot Stein

Neuroimaging Research Branch, Intramural Research Program, The National Institute on Drug Abuse, Baltimore, MD

Shi-Jiang Li

Department of Biophysics, Medical College of Wisconsin, Milwaukee, WI

Abstract

In extending the use of functional MRI to neuropharmacology, a primary area of concern is that peripheral blood pressure changes induced by pharmacological agents could independently produce a change in the blood oxygenation level-dependent (BOLD) signal, resulting in difficulties distinguishing or interpreting drug-induced neural activations. In the present study, we utilized intravenous dobutamine, a beta-adrenergic receptor agonist, to increase the mean arterial blood pressure (MABP), while examining the effects of MABP changes on the BOLD signal in cocaine-dependent participants. Dobutamine infusion significantly increased the MABP from 93 ± 8 mm Hg to 106 ± 12 mm Hg ($P < 0.0005$), but did not produce a significant global BOLD signal. Yet, a few voxels in the anterior cingulate showed BOLD signal changes that paralleled the changes in blood pressure (BP). Our observations support the conclusion that following the infusion of psychoactive agents, brain BOLD signals accurately reflect neuronal activity, even in the face of relatively large peripheral cardiovascular effects that transiently increase systemic BP.

Keywords

fMRI, Dobutamine, Cocaine, Brain, BOLD, Blood pressure

Introduction

Functional MRI (fMRI) is increasingly being employed to study brain functions in patient populations following various pharmacological manipulations. For example, blood oxygenation level-dependent (BOLD) contrast techniques have been recently applied to detect changes in regional brain activity following the acute administration of drugs such as nicotine (Stein et al., 1998), heroin (Xu et al., 2000, Xi et al., 2002), and psychostimulants (Breiter et al., 1997, Li et al., 2000, Luo et al., 2003, Schwarz et al., 2004, Risinger et al., 2005) in humans and animals. The term pharmacological MRI (phMRI) (Chen et al., 1997) has been used to denote the use of pharmacological probes during fMRI either to study the effects of drug action in the brain or the actions of a drug on specific cognitive, affective, or sensory processes (Stein, 2001, Salmeron and Stein, 2002, Leslie and James, 2000). However, before such manipulations can be routinely employed, the influence of such potential peripheral effects as changes in arterial blood pressure (BP) and heart rate (HR) on the BOLD signal must be elucidated.

As first modeled by Ogawa et al. (1993), the BOLD signal reflects changes in a number of hemodynamic parameters, including: (1) region cerebral blood flow (rCBF), (2) regional cerebral blood volume (rCBV), and (3) the local cerebral metabolic rate of oxygen (CMRO₂). As such, if pharmacological agents change systemic BP, they could cause global or local CBF changes independent of, or in addition to, change in

neuronal activity. Therefore, it is conceivable that such peripheral effects could confound the interpretation of drug-specific effects on neural activity.

This issue has not yet received much attention, with the exception of a few studies addressing the issue, yielding inconsistent results. Hypotension has been induced following blood volume depletion (Zaharchuk et al., 1999, Kalisch et al., 2001), while hypertension has been studied following administration of cocaine methiodide (Luo et al., 2003) and norepinephrine (Tuor et al., 2002) in rats and cold application or the Valsalva maneuver in humans (Harper et al., 2000). Luo et al. (2003) and Zaharchuk et al. (1999) concluded that BP changes have little effect on the BOLD signal as long as BP remains within the range of compensatory cerebral autoregulation. On the other hand, Kalisch et al. (2001) and Tuor et al. (2002) suggested that changes in BP correlate with regional BOLD changes. Some of these inconsistencies may be attributable to the models used to produce BP changes, which are purely of peripheral origins.

In this study, we modulated peripheral BP in human subjects by infusing dobutamine and examined the effects of BP changes on the BOLD signal. Dobutamine was selected because it increases peripheral BP in the absence of drug-induced neural activity. Dobutamine is used clinically to increase cardiac output and increase blood pressure during shock and heart failure. Also, it is used to increase the work of the heart in cardiac stress testing. In vitro, dobutamine is a moderately selective β_1 -adrenergic receptor agonist. Clinically, dobutamine may activate β_1 , β_2 , and α_1 adrenergic receptors (Ruffolo, 1987). Its cardiovascular effects include a prominent inotropic rather than chronotropic effect on the heart, resulting in increased BP (Wahl et al., 2004, Hardman and Limbird, 2001). This inotropic effect usually has a rapid onset (within 1 to 2 min) and a short half-life of about 2 min. Adrenergic β_1 receptors predominate in the heart, kidney, and brain (Minneman et al., 1979, Engel et al., 1985). High levels of β_1 receptors have been observed in the rat cingulate cortex, hippocampus, mediodorsal, and ventral nuclei of the thalamus, etc. (Rainbow et al., 1984). However, it has been reported that peripherally infused dobutamine does not interact with brain β_1 receptors (Conway et al., 1987), nor does it have a direct vasomotor effect on rodent brain microvessels (Kawamura and Yasui, 1998). Dobutamine, therefore, is believed to have no direct effect on brain activity. Thus, any drug-induced changes in BOLD signal would be interpreted as peripheral in origin as a result of change in BP.

Materials and methods

Human subjects selection and risk minimization procedure

Approximately 50 cocaine users were screened and 13 were recruited. Among the 13 subjects, six were eliminated due to a left ganglion cyst, high blood pressure, history of hand injuries, and subjects' decision to abandon the study. Seven right-handed cocaine-dependent individuals finally participated in this study (demographic data shown in Table 1). This study was approved by the Institutional Review Board of the Medical College of Wisconsin. After receiving a complete description of the study, written informed consent was obtained. Current cocaine users were included to compare the effects of a pure peripheral agent on BOLD signal changes to the effects of cocaine, which we have previously used in fMRI studies (see e.g. Risinger et al., 2005). In addition to cocaine's CNS effects, it is known to cause significant increases in BP which, as stated above, could independently influence BOLD signal. Subjects underwent an evaluation that included a medical history, physical, and mental status examination.

Laboratory studies (including blood and urine batteries and a 12-lead EKG) excluded potential subjects with medical conditions. A careful vascular history was also elicited. Subjects were excluded if they were positive for HIV, hepatitis, or had a history of drug dependence other than nicotine, cocaine, marijuana, or caffeine use. Participating subjects could be occasional marijuana users relieving symptoms of acute cocaine use, but cocaine was clearly their drug of choice. Urine toxicology (Triage®) screens were completed at each visit to identify use of other illicit substances. All subjects had experience in at least one prior fMRI study, which may have included the administration of cocaine. Other study exclusion criteria included pregnancy, hypertension, presence of cardiac dysrhythmia, a history of, or current diagnosis of cardiovascular, gastrointestinal, renal, or hepatic impairment. They also had to be negative for all DSM-IV criteria for Axis I psychiatric conditions.

Table 1. Demographic data of all subjects, in addition to the subjects whose data were included in the fMRI data analyses during scans 1, 2, and 3

	All seven subjects	Scan 1 (6 subjects)	Scan 2 (7 subjects)	Scan 3 (6 subjects)
Age (years)	37.9 ± 6.5	39.7 ± 4.8	37.9 ± 6.5	36.8 ± 6.5
Gender	2F/5M	2F/4M	2F/5M	1F/5M
Education (years)	11.6 ± 1.5	12.0 ± 1.1	11.6 ± 1.5	11.5 ± 1.6
Cocaine use (years)	10.8 ± 4.1	10.6 ± 4.3	10.8 ± 4.1	11.8 ± 3.5
Times of use/week	5.8 ± 1.2	5.8 ± 1.3	5.8 ± 1.2	5.6 ± 1.2
\$ spent/week	307 ± 313	325 ± 339	307 ± 313	317 ± 342

Since an arterial line was inserted during the study to record real-time arterial BP, ultrasound evaluations of the radial and ulnar arteries were performed before any experimental procedures in order to evaluate the patency of the palmar arch to minimize the possibility of ischemic damage. Likewise, an evaluation was performed, 1 day and 1 week after cannulation, to ensure the absence of significant occlusion or damage caused by the arterial catheter placement.

On the day of an experiment, an intravenous catheter was inserted into a forearm vein to deliver saline and dobutamine and to draw blood for drug level measurement during the experiment. Prior to fMRI scans, a drug toleration procedure was employed to ensure the safety of dobutamine infusion for each subject, as well as to develop dosing guidelines and infusion profiles for subsequent scanning. This allowed the subject to become familiar with the experience of having the drug injected while monitoring vital signs for any idiosyncratic response to the dobutamine or the monitoring procedures in the safety of a Clinical Research Center rather than in an MRI scanner room. During the drug tolerance procedure, both saline and dobutamine injections were completed sequentially. Blood was withdrawn after each injection for arterial blood gases and drug level measurements. All subjects tolerated this assessment procedure well and no idiosyncratic response to dobutamine was found. All vital signs remained within the delimited safety limits. The subjects proceeded to the fMRI session after their BP returned to baseline levels. After scanning, BP was monitored until the effects of dobutamine subsided. Subjects were admitted overnight and a wrist ultrasound was performed the next day to verify circulatory status. All subjects underwent a brief physical exam prior to discharge. Subjects returned 1 week after the scan session to have wrist circulation checked with a repeat

ultrasound examination again. Throughout the drug tolerance procedure and the subsequent fMRI experiments, the following physiological parameters were monitored and recorded electronically: (1) heart rate, (2) SpO₂, (3) respiration rate, (4) end tidal CO₂, and (5) blood pressure from both indwelling arterial line and BP cuff. Also, an EKG was continuously monitored. All physiological data, except cuff BP, which was sampled every 2–3 min, were acquired at 1 Hz.

fMRI experiments

Two consecutive fMRI sessions were conducted on a GE Signa 1.5 T scanner separated by a 30–60 min rest interval between sessions. The first session consisted of two 25-min scans. During each scan, a single infusion of either saline (scan 1) or dobutamine (scan 2) (Fig. 1A) was administered 5 min into the respective scan. The saline infusion always delivered a total of 10 ml at a rate of 0.7 ml/s. Because of variations in individual BP responses to the dobutamine, the rate of drug infusion varied from 7–10 mg/kg/min during the infusion period, as determined by the subject's response during the drug tolerance procedure. In the second session, a 30-min scan with a double dobutamine infusion paradigm (scan 3) was conducted. As shown in Fig. 1B, the first infusion of dobutamine started 5 min into the scan and lasted for 3 min. The second infusion started at 17 min into the scan and also lasted for 3 min. The dobutamine infusion rate was fixed for each subject for the single and both of the double infusion trials. Two SPGR image sets were acquired before scan 1 and scan 3 and used for subsequent image registration. In order to assess potential behavioral effects from the alterations in BP, during scans 1, 2, and 3, subjects were prompted to evaluate their feelings of “liking drug,” “high,” “queasy,” “light headed,” and “racing” in a random order once every minute with a joystick-type wheel device. They turned a wheel to move a tab on a visual analog scale displayed via computer, then pressed a button to record the final tab position. Whole-brain axial images were collected using a hybrid pulse sequence. The superior portion of the brain was acquired with a standard EPI sequence, while the inferior portion of brain was acquired with an EPI pulse sequence with z-shimmed background gradient compensation to overcome susceptibility artifacts (Li et al., 2002). A total of 19 continuous 5-mm slices were acquired with FOV of 24 cm, matrix size of 64 × 64, TE of 30 ms, and an equivalent TR of 6 s. Only the four most inferior slices required the z-shim method, which covered the brain region where susceptibility artifacts are most significant, including the orbitofrontal cortex.

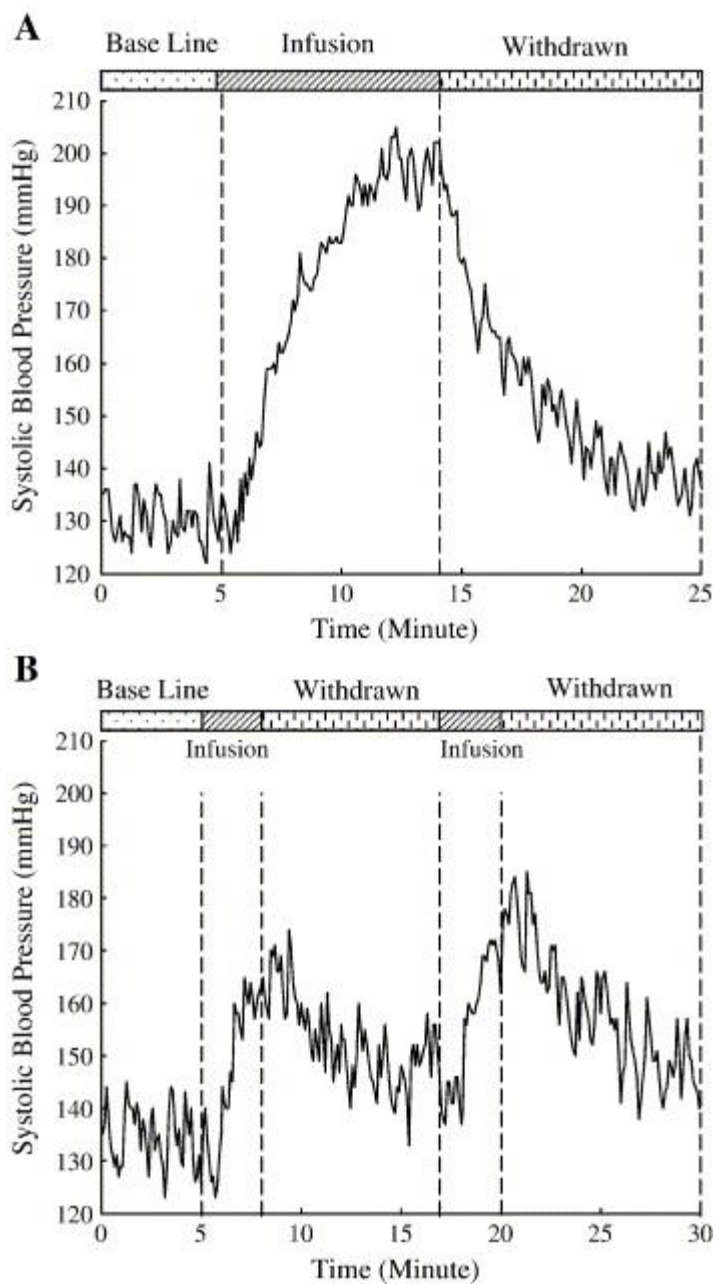


Fig. 1. Representative arterial systolic blood pressure (SBP) profile from a single subject during a single- (A) and double- (B) dobutamine infusion experiment. The shadowed bars indicate infusion periods.

Data analysis

Analyses of Functional NeuroImages (AFNI) software (Cox, 1996) were employed to perform all fMRI analyses. One subject did not participate in scan 3 and one saline scan (scan 1) data set was eliminated due to excessive body motion. The rejection threshold was set at displacement of 2.5 mm and rotation of 2.5°, as estimated by the AFNI 3dvolreg program. Subject demographics for the scan sessions are shown in Table 1. To assess whether BP changes influenced the BOLD signal, two data analysis methods were employed: (1) a cross-correlation (cc) calculation to evaluate the relationship between the fMRI time course and the downsampled systolic BP profile (only systolic BP was used, as it has been reported that dobutamine infusion does not alter diastolic BP (Wahl et al., 2004), which was

confirmed in this study); (2) a nonlinear regression with a differential exponential model (Ward et al., 1998) to calculate the percent change in the area under the fitted curve (AUC%). The cc and %AUC maps were converted to standard Talairach coordinates (Talairach and Tournoux, 1988). Analyses across subjects with individual significance cc thresholds and F statistic values corresponding to a Bonferroni corrected $P < 0.05$ were performed to obtain group maps. One-tailed t tests on the cc data (i.e., correlation between BP and BOLD) from the single- and double-dobutamine infusions vs. the null hypothesis of zero correlation, and a two-tailed, t test on the %AUC from the single dobutamine infusion vs. saline control, were performed, respectively. A minimum cluster activation volume of 350 μl was required to achieve an omnibus corrected P of 0.05.

To help establish the false positive level for the two dobutamine scans, we applied a bootstrap statistical method. Artificial data sets were generated from the actual data sets in a random manner and the correlation of such artificial fMRI signals and actual SBP profiles was tested for statistical errors. Therefore, these artificial data sets can be characterized as randomized noise rather than true signal and no significant correlation was expected between the “noise” and SBP profile. A bootstrap method (Zoubir and Iskander, 2004) was implemented to rearrange time courses and produce 10 artificial data sets for each scan, leading to a total of 20 artificial data sets. For all voxels in the data set, a block length of 10 points was used to divide the fMRI time courses consisting of 250(300) time points sequentially to 25(30) blocks for scan 2(3). A rearrangement sequence, which was applied to all voxels, was then randomly generated to pick blocks 25(30) times to create one new artificial data set. In other words, a particular block in the original time course could be used more than once or not used at all, such as the example in Fig. 4A, where the bootstrap sequence was [22, 8, 14, 0, 4, 16, 20, 0, 18, 19, 20, 19, 10, 8, 5, 24, 0, 9, 14, 19, 0, 13, 19, 21, 1] for a single dobutamine paradigm. The cross-correlation between artificial data sets and the original SBP profiles was then calculated.

During infusion scans, the visual analog scale subjective ratings provided by the subjects usually consisted of sudden rating variations as time progressed. One of the causes for this variability could have been the discomfort created by the arterial line and the lightheaded feeling due to the dobutamine infusion, which may prevent subjects from effectively performing the rating task. Also, we took into consideration the limited number of subjects who participated in this study. We determined that a mean rating curve was not the best or most reliable way to understand the behavioral data. Instead, the absolute change of the mean VAS behavioral ratings during dobutamine infusion vs. baseline was calculated for each of the five behavioral constructs on a per subject basis. The baseline rating was obtained by averaging ratings during the baseline period, i.e., min 0–5 for the single infusion paradigm (scan 2) and min 0–5 and 13–17 for the double infusion paradigm (scan 3). Similarly, ratings during dobutamine infusion were acquired during min 5–25 for the single infusion paradigm scans and min 5–13 and 17–30 for the double infusion paradigm scan. The Wilcoxon nonparametric signal-rank test was then applied. P values were estimated according to the signed rank of absolute rating change and the W -estimate was calculated to represent treatment effect as shown in Table 2.

Table 2. Wilcoxon statistics of percentage changes of mean VAS behavioral ratings during elevated SBP vs. during baseline SBP in single- ($n = 7$) and double- ($n = 6$) dobutamine infusions scans (*italic font denotes significant ratings*)

Visual analog scale behavior ratings						
Scan rating		Like drug	Lightheaded	High	Queasy	Racing
Single infusion	W-estimate	-2.6	13.8	2.2	8.9	14.4
	<i>P</i> value	2e-9	< 1e-12	7e-12	< 1e-12	< 1e-12
Double infusion	W-estimate	-0.4	2.0	-1.2	-3.7	3.3
	<i>P</i> value	0.3	0.1	0.2	3e-4	0.02
Single vs. double infusion	W-estimate	-3.3	8.2	2.7	5.1	11.7
	<i>P</i> value	0.2	<1e-12	3e-4	1e-8	<1e-12

The “W-estimate” is an estimate of the difference in location of the two populations, which is used in calculating the nonparametric Wilcoxon signed-rank test as described by Noether (1991).

Results

Seven subjects participated in the single-dobutamine infusion paradigm and six of the seven subjects participated in the double dobutamine infusion paradigm. With the exception of systolic blood pressure, analysis of physiological data (HR, ECG, SpO₂, respiration rate, end tidal and inhaled CO₂, diastolic BP) showed no significant change during dobutamine infusion.

In the single-dobutamine paradigm, the mean arterial blood pressure (MABP) ($1/3$ SBP + $2/3$ DBP) significantly increased from 93 ± 8 mm Hg (during min 0–5) to 106 ± 12 mm Hg (during min 5–25) ($P < 0.0005$). In the double dobutamine infusion paradigm, the MABP also significantly increased from 104 ± 7 mm Hg (during min 0–5 and 13–17) to 109 ± 6 mm Hg (during min 5–13 and 17–30) ($P < 0.0004$). A set of representative systolic BP (SBP) responses from one subject following single- and double-dobutamine infusions is shown in Fig. 1. In the single-infusion paradigm (Fig. 1A), the SBP was elevated to about 150% of its baseline value after 7 min of dobutamine infusion, sustained for 2 min and gradually dropped back to baseline after the infusion was stopped. Fig. 1B shows the SBP changes seen as a result of the double infusion paradigm. With the same infusion rate, SBP was elevated to 125% of the baseline during both 3-min dobutamine infusion periods. The rates of SBP elevation were consistent between the single-infusion and the double-infusion paradigms and verify dobutamine's characteristic rapid onset and a short half-life effect on acute BP.

Increases in SBP after either a single- or double-dobutamine infusion had no effect on the global BOLD signal. Only limited significant BOLD regional changes were seen and these were restricted to the anterior cingulate region (BA32). In the single-dobutamine injection trials, very similar changes were seen based on the %AUC (Fig. 2A) and the cc analysis methods (Fig. 2B). However, these regional “activations” were absent in the double-infusion paradigm (Fig. 2C). Further analysis revealed that the averaged SBP profile induced by the single dobutamine infusion was strongly correlated with the mean time course of the activated voxels across all subjects ($R = 0.69$, $P < 1 \times 10^{-36}$) (Fig. 3). The mean time course was obtained by averaging the mean time courses of activated voxels of each subject. In addition, the z-shim gradient compensation EPI pulse sequence was used to minimize susceptibility-

induced signal loss in the inferior portions of the brain. Therefore, the detection of ‘activation’ was not confounded by susceptibility.

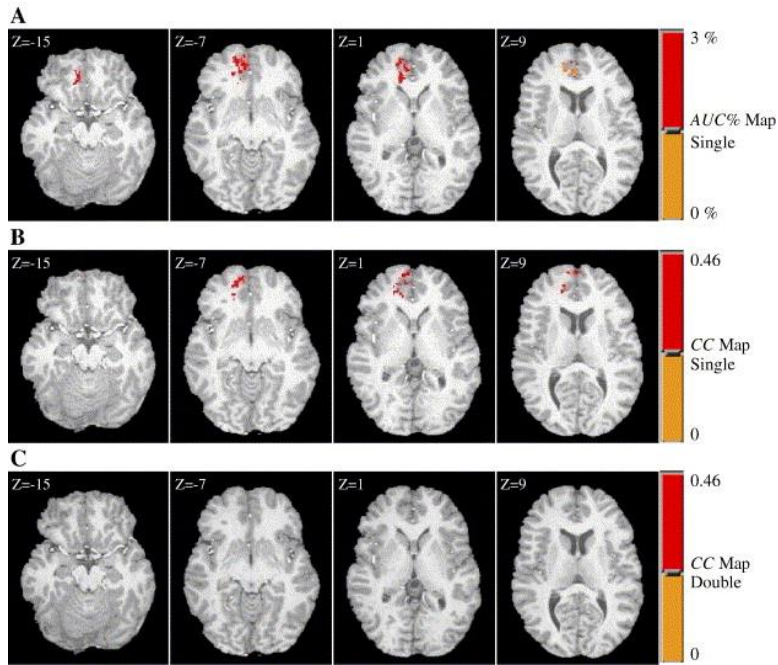


Fig. 2. (A) %AUC results from the single-dobutamine vs. saline infusion, $df = 11$, (B) cross-correlation (cc) results from the single-dobutamine infusion SBP profile and voxel BOLD time courses, $df = 6$, (C) cc map from the double-dobutamine infusion SBP profile and the voxel BOLD time courses, $df = 5$. Functional activation is overlaid on a standardized brain anatomy. (All figures are shown using neurological viewing convention: the left side of the image is the left side of the anatomy).

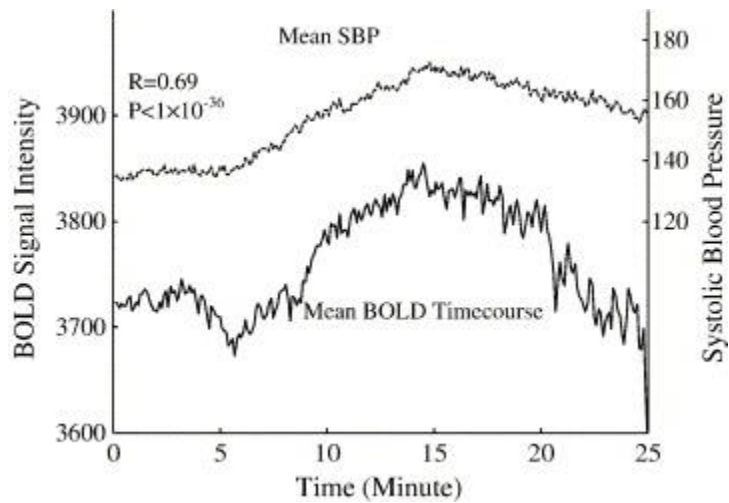


Fig. 3. Mean arterial systolic blood pressure curve (top, right side y-axis) obtained from all subjects and mean BOLD voxel time course averaged over the activated anterior cingulate region across all subjects (bottom, left y-axis) in a single-infusion paradigm (scan 2).

Table 2 summarizes the real-time VAS ratings during the single- and double-dobutamine infusions as a direct comparison between the two scans. All five ratings – “liking drug,” “lightheaded,” “high,” “queasy,” and “racing” – were significantly different during the single-dobutamine infusion vs.

baseline, while only “queasy” was significantly different during the double infusion. Further, a direct comparison between single- and double-infusion epochs shows that “lightheaded,” “high,” “queasy,” and “racing” were significantly decreased during the double infusion scan.

Discussion

This study demonstrated that in the face of robust increases in systemic BP induced by infusion of dobutamine, a peripherally acting agent, no significant correlation was seen with either global or regional brain BOLD signal changes, with the possible exception of a small, restricted area in the anterior cingulate region. The relative independence of the BOLD signal with peripheral BP is likely due to the well-known autoregulation of CBF under a wide range (60–160 mm Hg) of mean arterial blood pressure. Pharmacological agents such as dobutamine can increase MABP by changing cardiac function, as well as by modifying the systemic vasculature, particularly arterial resistance. Changes in peripheral circulation engage multiple homeostatic compensatory mechanisms in order to maintain a normal MABP. For example, an increase in MABP will induce systemic vasodilation and a decrease in MABP will induce systemic vasoconstriction. In contrast, the cerebral vasculature primarily controls blood flow rather than pressure. Thus, the cerebrovascular response to an increase in MABP is the opposite of that in the periphery. These concepts can be summarized in the following way: MABP depends on cardiac output (CO) and total peripheral resistance (TPR): $MABP = CO \times TPR$. Total CBF depends on MABP and cerebrovascular resistance (CVR): $CBF = MABP / CVR$. Many pharmacological agents affect MABP, CO, and TPR and some, which cross the blood–brain barrier, may also affect CVR and CBF (Ishiyama et al., 1998, Ganjoo et al., 1998). The BOLD signal has been shown to be dependent upon CBF, CBV, and blood oxygenation (Ogawa et al., 1993, Buxton et al., 1998, Hoge et al., 1999). Therefore, the effects of a change in MABP induced by a pharmacological agent on the BOLD signal will be largely dependent on whether CBF, CBV, and $CMRO_2$ have changed. These principles are required to interpret the current and historic data. For example, in reporting an apparent correlation between BP change and BOLD signal, Tuor et al. (2002) and Kalisch et al. (2001), not only changed BP but also changed CBF or CBV, calling into question the causative relationship between BOLD and BP.

In contrast, similar to our findings, Luo et al. (2003) administered cocaine methiodide, a quaternary derivative of cocaine that shares the same peripheral vascular actions of cocaine but does not cross the blood–brain barrier, to increase BP while measuring BOLD change in rats. In the face of large changes in BP, only scattered, weak, and transient changes in BOLD signal were observed that were not dose-dependent. Similarly, Zaharchuk et al. (1999) showed that changing MABP did not alter CBF due to presumed autoregulatory mechanisms, resulting in little effect on brain BOLD signal.

Although the BP increase following dobutamine infusion did not induce a significant global BOLD signal change, it did correlate with small regional changes in the anterior cingulate. This correlated activity may be attributed to one or more possibilities. The first possibility is that the observed anterior cingulate activation from the single infusion scan may have resulted from a false positive error. In fact, when we reran the *cc t* test with individual significance threshold increased to $cc > \pm 0.37$, the single infusion then gave a null activation map, exactly as that from double infusion data. As described below, the bootstrap analysis helps explain why the correlation threshold for the single infusion should be ± 0.37 in order to retain a false positive rate of 0.05. Quantitatively, based on the bootstrap simulation, the application of a Bonferroni correction ($\alpha = 0.05$) is equivalent to a correlation threshold of 0.29 for

single and 0.27 for the double-dobutamine infusion paradigms. The difference between correlation thresholds is a result of the different data lengths for each paradigm. As shown in Fig. 4B, approximately 12% of the voxels for the single infusion data had a correlation coefficient greater than 0.29 or less than -0.29 . In contrast, only approximately 2% of voxels had a correlation coefficient exceeding the threshold of 0.27 for double-infusion data (Fig. 4C). If a false positive rate of 0.05 is to be retained, the correlation threshold should be ± 0.37 for single infusion and ± 0.21 for double infusion according to the bootstrap simulation. By applying this correlation threshold of ± 0.37 to the single-infusion data, no activation was seen in the anterior cingulate region. These findings indicate that a Bonferroni correction procedure, which assumes independent white Gaussian noise, remains too liberal to correct the rate of false positives in the single-dobutamine infusion data, perhaps due to the inherent temporal autocorrelation of fMRI time series. The same bootstrap simulation indicates that the Bonferroni correction procedure is sufficient for the double infusion data analysis. Clearly, since the rate of false positive error is higher in the case of single infusion than that of double infusion, the identified anterior cingulate activation in the single-infusion scan may be due to a false positive error.

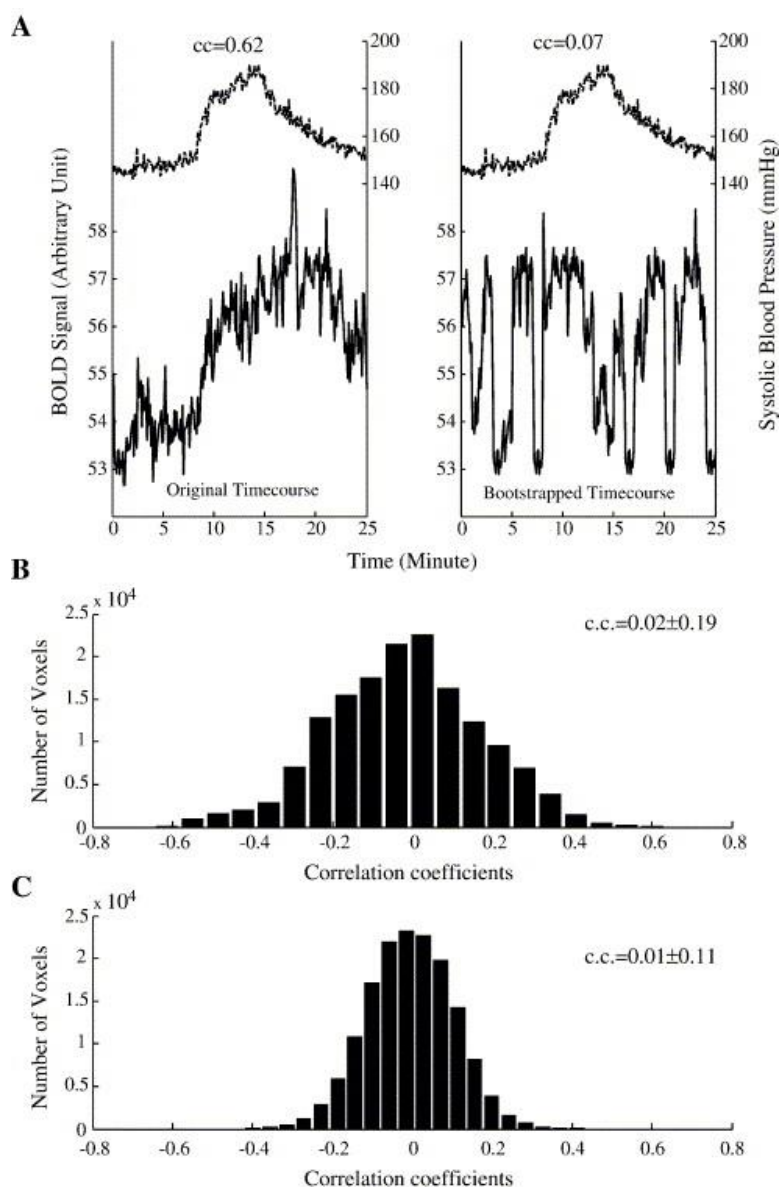


Fig. 4. Analysis of potential false positive activations related to the single and double dobutamine infusion paradigms. (A) A representative plot of the bootstrap rearrangement data on a single-infusion time course. The top curves are the SBP profiles induced by the single infusion (scaled and shifted for clarity). The bottom left panel of panel A is the original voxel time course and the right is the bootstrapped version. The cross-correlation coefficient dropped from 0.62 to 0.07 after bootstrap. Panels B and C show the correlation histograms of 10 artificial data sets of “false signal” for the single and double infusions, corresponding to 155,056 and 154,446 voxels, respectively.

The second possibility is that the observed anterior cingulate activity may be related to functional control of BP. It long has been implicated that the anterior cingulate is involved in control over autonomic responses (Kremer, 1947, Kaada et al., 1949). Both phasic increases and decreases in BP can be elicited with direct electrical stimulation of the cingulate in the rat (Kaada et al., 1949), dog (Kremer, 1947), and human (Pool and Ransohoff, 1949). In addition, pyramidal cells of the anterior cingulate cortex project directly to brain regions involved with homeostatic and autonomic control, including the hypothalamus (Ongur et al., 1998), pontine gray matter (Vilensky and van Hoesen, 1981, Porrino and Goldman-Rakic, 1982), and periaqueductal gray (An et al., 1998). It is thus conceivable that the observed anterior cingulate BOLD signal change may have resulted from a localized brain response to changes in systemic BP.

Since these subjects were active cocaine users, the third possibility is that the observed anterior cingulate activation following the single-dobutamine infusion may have been as a result of secondary emotional feelings to perceived changes in BP acting as drug-related internal cues. It is possible that the dobutamine-induced hypertensive effect may have mimicked the physiological and/or affective experience of cocaine use and evoked increased activity in the anterior cingulate. It could be argued, however, that if this conditioned cue hypothesis is true, such activations should have occurred in both the single- and double-infusion paradigms. The disparate results could be explained by rapid extinction or habituation of the conditioned response whereby after the single infusion and before the second infusion of the double infusion paradigm, the subjects may have learned (consciously or unconsciously) that no cocaine was or would be actually administered. The real-time behavior ratings support this possibility. As shown in Table 2, subjective ratings of “liking drug,” “lightheaded,” “high,” and “racing” were significantly altered during the single-infusion period but not during double-infusion period.

Ideally, a counter-balanced order of single- and double-infusion scans could be employed to verify this argument. However, due to our initial subject safety considerations, the double infusion scan was designed to always run second. Clearly, an appropriate order-balanced protocol should be conducted in the future. This issue could be further investigated by applying the same experimental procedure to normal control subjects rather than cocaine abusers. In the case of normal control subjects, no cocaine conditional cue would be expected; thus a similar null activation map for the single infusion would confirm this conclusion.

In conclusion, while potential secondary effects of BP changes on cognitive and affective states need to be examined, and additional mechanistic issues of cerebral autoregulatory processes need to be further addressed by pulse sequences that are sensitive to CBF and or CBV, our data indicate that relatively large, acute changes in peripheral BP as a consequence of dobutamine infusion do not directly induce a BOLD signal change in the human brain. These observations suggest that, within the

range of cerebral autoregulatory capacity, peripheral BP changes alone will not confound the interpretation of BOLD signal changes induced following administration of CNS acting drugs and support the utility of extending fMRI techniques to map and quantify drug-induced neuronal activity. Such applications should extend new insights into the systems level sites and mechanisms of action of drugs in the brain and potentially provide a new tool for drug discovery and assessments.

Acknowledgments

This study was supported in part by NIH grants DA10214 and the MCW General Clinical Research Center (RR00058). We appreciate the inspiring statistical discussion with Mr. Douglas Ward and the editorial assistance from Ms. Carrie O'Connor.

References

- An et al., 1998. X. An, R. Bandler, D. Ongur, J.L. Price. Prefrontal cortical projections to longitudinal columns in the midbrain periaqueductal gray in Macaque monkeys. *J. Comp. Neurol.*, 401 (1998), pp. 455-479
- Breiter et al., 1997. H.C. Breiter, R.L. Gollub, R.M. Weisskoff, D.N. Kennedy, N. Malris, J.D. Berke, J.M. Goodman, H.L. Kantor, D.R. Gastfriend, J.P. Rorden, R.T. Mathew, B.R. Rosen, S.E. Hyman. Acute effects of cocaine on human brain activity and emotion. *Neuron*, 19 (1997), pp. 591-611
- Buxton et al., 1998. R.B. Buxton, E.C. Wong, L.R. Frank. Dynamics of blood flow and oxygenation changes during brain activation: the balloon model. *Magn. Reson. Med.*, 39 (1998), pp. 855-864
- Chen et al., 1997. Y.C.I. Chen, W.R. Galpern, A.L. Brownell, R.T. Mattews, M. Bogdanov, O. Isacson, J.R. Keltner, M.F. Beal, B.R. Rosen, B.G. Jenkins. Detection of dopaminergic neurotransmitter activity using pharmacologic MRI: correlation with PET, microdialysis, and behavioral data. *Magn. Reson. Med.*, 38 (1997), pp. 389-398
- Conway et al., 1987. P.G. Conway, S. Tejani-Butt, D.J. Brunswick. Interaction of beta adrenergic agonists and antagonists with brain beta adrenergic receptors in vivo. *J. Pharmacol. Exp. Ther.*, 241 (1987), pp. 755-762
- Cox, 1996. R.W. Cox. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput. Biomed. Res.*, 29 (1996), pp. 162-173
- Engel et al., 1985. G. Engel, R. Maurer, K. Perrot, B.P. Richardson. Beta-adrenoceptor subtypes in sections of rat and guinea pig kidney. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 328 (1985), pp. 354-357
- Ganjoo et al., 1998. P. Ganjoo, N.E. Farber, A. Hudetz, J.J. Smith, E. Samsó, J.P. Kampine, W.T. Scheiling. In vivo effects of dexmedetomidine on Laser-Doppler flow and pial arteriolar diameter. *Anesthesiology*, 88 (1998), pp. 429-439
- Hardman and Limbird, 2001. J.G. Hardman, L.E. Limbird. Goodman and Gilman's *The Pharmacological Basis of Therapeutics*. (10th ed.), McGraw-Hill, New York (2001)
- Harper et al., 2000. R.M. Harper, R. Bandler, D. Spriggs, J.R. Alger. Lateralized and widespread brain activation during transient blood pressure elevation revealed by magnetic resonance imaging. *J. Comp. Neurol.*, 417 (2000), pp. 195-204
- Hoge et al., 1999. R.D. Hoge, J.B. Atkinson, G.R. Crelier, S. Marrett, G.B. Pike. Investigation of BOLD signal dependence on cerebral blood flow and oxygen consumption: the deoxyhemoglobin dilution model. *Magn. Reson. Med.*, 42 (1999), pp. 649-663

- Ishiyama et al., 1998. T. Ishiyama, S. Dohi, H. Iida. The vascular effects of topical and intravenous α_2 -adrenoceptor agonist clonidine on canine pial microcirculation. *Anesth. Analg.*, 86 (1998), pp. 766-772
- Kaada et al., 1949. B.R. Kaada, K.H. Pribram, J.A. Epstein. Respiratory and vascular responses in monkeys from temporal pole, insula, orbital surface and cingulate gyrus. *J. Neurophysiol.*, 12 (1949), pp. 347-356
- Kalisch et al., 2001. R. Kalisch, G.-K. Elber, C. Gossel, M. Czisch, D.P. Auer. Blood pressure changes induced by arterial blood withdrawal influence BOLD signal in anesthetized rats at 7 tesla: implications for pharmacologic MRI. *NeuroImage*, 14 (2001), pp. 891-898
- Kawamura and Yasui, 1998. S. Kawamura, N. Yasui. Effects of dobutamine on brain surface microvessels in rats. *Neurol. Med. -Chir. (Tokyo)*, 38 (1998), pp. 137-141
- Kremer, 1947. W.F. Kremer. Autonomic and somatic reactions induced by stimulation of the cingulate gyrus in dogs. *J. Neurophysiol.*, 10 (1947), pp. 371-379
- Leslie and James, 2000. R.A. Leslie, M.F. James. Pharmacological magnetic resonance imaging: a new application for functional MRI. *Tips*, 21 (2000), pp. 314-318
- Li et al., 2000. S.-J. Li, B.B. Biswal, Y. Wang, Z. Li, R. Risinger, C. Rainey, J.-K. Cho, B.J. Salmeron, E.A. Stein. Acute cocaine administration alters functional connectivity in human primary visual cortex detected by fMRI. *Magn. Reson. Med.*, 43 (2000), pp. 45-51
- Li et al., 2002. Z. Li, G. Wu, X. Zhao, F. Luo, S.-J. Li. Multiecho segmented EPI with z-shimmed background gradient compensation (MESBAC) pulse sequence for fMRI. *Magn. Reson. Med.*, 48 (2002), pp. 312-321
- Luo et al., 2003. F. Luo, G. Wu, Z. Li, S.-J. Li. Characterization of effects of mean arterial blood pressure induced by cocaine and cocaine methiodide on BOLD signals in rat brain. *Magn. Reson. Med.*, 49 (2003), pp. 264-270
- Minneman et al., 1979. K.P. Minneman, L.R. Hegstrand, P.B. Molinoff. Simultaneous determination of beta1 and beta2-adrenergic receptors in tissues containing both receptor subtypes. *Mol. Pharmacol.*, 16 (1979), pp. 34-46
- Noether, 1991. G.E. Noether. *Introduction to Statistics: The Nonparametric Way*. Springer-Verlag, New York (1991)
- Ogawa et al., 1993. S. Ogawa, R.S. Menon, D.W. Tank, S.G. Kim, H. Merkle, J.M. Ellermann, K. Ugurbil. Functional brain mapping by blood oxygenation level-dependent contrast magnetic resonance imaging. A comparison of signal characteristics with a biophysical model. *Biophys. J.*, 64 (1993), pp. 803-812
- Ongur et al., 1998. D. Ongur, X. An, J.L. Price. Prefrontal cortical projections to the hypothalamus in Macaque monkeys. *J. Comp. Neurol.*, 401 (1998), pp. 480-505
- Pool and Ransohoff, 1949. J.L. Pool, J. Ransohoff. Autonomic effects on stimulating rostral portion of cingulate gyri in man. *J. Neurophysiol.*, 12 (1949), pp. 385-392
- Porrino and Goldman-Rakic, 1982. L.J. Porrino, P.S. Goldman-Rakic. Brainstem innervation of prefrontal and anterior cingulate cortex in the Rhesus monkey revealed by retrograde transport of HRP. *J. Comp. Neurol.*, 205 (1982), pp. 63-76
- Rainbow et al., 1984. T.C. Rainbow, B. Parsons, B.B. Wolfe. Quantitative autoradiography of beta1- and beta2-adrenergic receptors in rat brain. *Proc. Natl. Acad. Sci.*, 81 (1984), pp. 1585-1589

- Risinger et al., 2005. R.C. Risinger, B.J. Salmeron, T.J. Ross, S.L. Amen, M. Sanfilippo, R. Hoffmann, A.S. Bloom, H. Garavan, E.A. Stein. Neural correlates of high and craving during cocaine self-administration using BOLD fMRI. *NeuroImage*, 26 (2005), pp. 1097-1108
- Ruffolo, 1987. R.R. Ruffolo Jr. Review: the pharmacology of dobutamine. *Am. J. Med. Sci.*, 294 (1987), pp. 244-248
- Salmeron and Stein, 2002. B.J. Salmeron, E.A. Stein. Pharmacological applications of magnetic resonance imaging. *Psychopharmacol. Bull.*, 36 (2002), pp. 102-129
- Schwarz et al., 2004. A.J. Schwarz, A. Zocchi, T. Reese, A. Gozzi, M. Garzotti, G. Varnier, O. Curcuruto, I. Sartori, E. Girlanda, B. Biscaro, V. Crestan, S. Bertani, C. Heidbreder, A. Bifone. Concurrent pharmacological MRI and in situ microdialysis of cocaine reveal a complex relationship between the central hemodynamic response and local dopamine concentration. *NeuroImage*, 23 (2004), pp. 296-304
- Stein, 2001. E.A. Stein. fMRI: a new tool for the in vivo localization of drug actions in the brain. *J. Anal. Toxicol.*, 25 (2001), pp. 419-424
- Stein et al., 1998. E.A. Stein, J. Pankiewicz, H.H. Harsch, J.K. Cho, F.A. Fuller, R.G. Hoffmann, M. Hawkins, S.M. Rao, P.A. Bandettini, A.S. Bloom. Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. *Am. J. Psychiatry*, 155 (1998), pp. 1009-1015
- Talairach and Tournoux, 1988. J. Talairach, P. Tournoux. *Coplanar Stereotaxic Atlas of The Human Brain*. Thieme Medical Publishers, New York (1988)
- Tuor et al., 2002. U.I. Tuor, E. McKenzie, B. Tomanek. Functional magnetic resonance imaging of tonic pain and vasopressor effects in rats. *Magn. Reson. Imaging*, 20 (10) (2002), pp. 707-712
- Vilensky and van Hoesen, 1981. J.A. Vilensky, G.W. van Hoesen. Corticopontine projections from the cingulate cortex in the Rhesus monkey. *Brain Res.*, 205 (1981), pp. 391-395
- Wahl et al., 2004. A. Wahl, I. Paetsch, A.I. Gollesch, S. Roethemeyer, D. Foell, R. Gebker, H. Langreck, C. Klein, E. Fleck, E. Nagel. Safety and feasibility of high-dose dobutamine-atropine stress cardiovascular magnetic resonance for diagnosis of myocardial ischaemia: experience in 1000 consecutive cases. *Eur. Heart J.*, 25 (14) (2004), pp. 1230-1236
- Ward et al., 1998. B.D. Ward, H. Garavan, T.J. Ross, A.S. Bloom, R.W. Cox, E.A. Stein. Non-linear regression for fMRI time series analysis. *NeuroImage*, 7 (1998), p. 5767
- Xi et al., 2002. Z.-X. Xi, G. Wu, E.A. Stein, S.-J. Li. GABAergic mechanisms of heroin-induced brain activation assessed with functional MRI. *Magn. Reson. Med.*, 48 (2002), pp. 838-843
- Xu et al., 2000. H. Xu, S.-J. Li, J. Bodurka, Z.L. Zhao, Z.X. Xi, E.A. Stein. Heroin-induced neuronal activation in rat brain assessed by functional MRI. *NeuroReport*, 11 (2000), pp. 1085-1092
- Zaharchuk et al., 1999. G. Zaharchuk, J.B. Mandeville, A.A. Bogdanov Jr., R. Weissleder, B.R. Rosen, J.A. Marota. Cerebrovascular dynamics of autoregulation and hypoperfusion—an MRI study of CBF and changes in total and microvascular cerebral blood volume during hemorrhagic hypotension. *Stroke*, 30 (1999), pp. 2197-2205
- Zoubir and Iskander, 2004. A.M. Zoubir, D.R. Iskander. *Bootstrap Techniques for Signal Processing*. Cambridge Univ. Press, Cambridge, UK (2004)