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Alpha-2 Adrenergic Challenge with Guanfacine One Month after Mild Traumatic Brain Injury: Altered Working Memory and BOLD Response

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Abstract

Alterations in working memory (WM) are common after traumatic brain injury (TBI). Frontal catecholaminergic systems, including the alpha-2 adrenergic system, modulate WM function and may be affected in TBI. We hypothesized that administration of an alpha-2 adrenergic agonist might improve WM after mild TBI (MTBI). Thirteen individuals with MTBI one month after injury and 14 healthy controls (HC) were challenged with guanfacine and placebo prior to administration of a verbal WM functional MRI task. Guanfacine was associated with improved WM performance in the MTBI but not the HC group. On guanfacine the MTBI group showed increased activation within a WM task-specific region of interest. Findings are consistent with the hypothesis that alterations in WM after MTBI may be improved with the alpha-2 agonist guanfacine.

Keywords

traumatic brain injury; alpha-2 adrenergic agonist; working memory; functional MRI; guanfacine

Introduction

Initial and persistent cognitive deficits are the most common complaints after traumatic brain injury (TBI) (Whyte et al. 2000) and are the major hindrance to return to baseline function (Cicerone et al. 2000). There are several predictable areas of cognitive impairment

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after TBI (Binder et al. 1997; Levin 1998; Levin et al. 1987; McMillan 1997), including working memory (WM), which is the ability to hold information in mind and to manipulate that material in light of incoming information. We previously used functional MRI (fMRI) to demonstrate that one month after mild TBI (MTBI) performance on a moderate difficulty WM task was associated with significantly greater cerebral activation (“compensatory activation”) and increased memory complaints despite WM task performance similar to that seen in controls (McAllister et al. 1999). We subsequently showed that MTBI patients were unable to further increase brain activation with a higher WM processing load (McAllister et al. 2001). We interpreted these results as evidence for abnormalities in the activation and allocation of WM processing resources after MTBI. In essence, patients were “working harder” to achieve the same results with a moderately difficult task, and thus had fewer additional processing resources to recruit during the more difficult task condition. Other groups have subsequently shown similar alterations in cerebral activation following MTBI (Chen et al. 2004; Perlstein et al. 2004).

Elucidation of the neural mechanisms underlying these observations might inform pharmacological strategies for treatment of WM complaints associated with TBI. A significant body of work suggests that catecholaminergic mechanisms play important roles in WM function, especially in the prefrontal cortex (PFC; see (Arnsten et al. 1998)), and there is evidence that TBI alters catecholamine tone and regulation after TBI (see McAllister et al 2004; Kobori et al. 2010 for reviews). Such abnormalities may play a role in alterations in activation and allocation of WM processing resources after MTBI. Although difficult to test this hypothesis directly, it is possible to use pharmacological challenges to probe whether individuals with MTBI differ in their response to catecholaminergic agents. Altered behavioral (cognitive) and neurophysiological (task-related cerebral activation) responses to a catecholamine agonist would provide indirect support for this hypothesis.

The catecholamine systems are complex, however, and different catecholamine agonists can have different effects on cognitive function. For example, in monkeys and rats localized prefrontal depletion of catecholamines (dopamine and norepinephrine) caused by infusion of 6-hydroxydopamine as well as global catecholamine depletion with agents such as reserpine lead to impaired performance on spatial WM tasks similar to that seen with ablation of neural tissue in the PFC (Brozoski et al. 1979; Cai et al. 1993). Normal aging in these animals results in lower levels of both dopamine (DA) and norepinephrine (NE) and is associated with declining performance on WM tasks (Arnsten 1998; Bartus et al. 1978; Luine et al. 1990), as is the use of alpha-2A (A2A) antagonists (Steere and Arnsten 1997; Tanila et al. 1996).

The role of the adrenergic system in WM varies as a function of receptor type, brain region, dose, and task difficulty. For example, in rodents and primates, blockade of the A2A system with infusion of A2A antagonists produces spatial WM impairment (Steere and Arnsten 1997; Tanila et al. 1996), whereas alpha-1 and beta adrenergic antagonists do not (Arnsten and Li, 2005). WM performance deficits brought about by normal aging, chemical depletion, or infusion of A2A antagonists can be reversed by A2A agonists (see Arnsten et al. 1988 for review; Arnsten and Goldman-Rakic 1985; Carlson et al. 1992; Franowicz and Arnsten 1998; Rama et al. 1996). However, A1A *agonists* can impair WM function, suggesting that different adrenergic receptors have opposing effects on cognitive function (Arnsten 1998; Birnbaum et al. 1999). In humans the A2A agonist guanfacine seems particularly effective at enhancing WM performance with a relatively low side effect profile (Arnsten et al. 1998; Arnsten 1998; Rama et al. 1996; Sirvio et al. 1991; Steere and Arnsten 1997). Jakala et al. (Jakala et al. 1999; Jakala et al. 1999) administered three different doses of clonidine (also an A2A agonist) or two doses of guanfacine to healthy controls using a double-blind, placebo-controlled design. Both low and high dose clonidine were associated

with poorer performance on WM, and showed little effect on the other tasks. At the higher dose (29mcg/kg), guanfacine was associated with significant improvement on WM, the Tower of London task, and a verbal paired associates learning task. The researchers interpreted these results as consistent with guanfacine-enhanced frontal functioning in both spatial WM and planning. Taken together, these studies suggest that A2A effects may depend on individual characteristics of the agonist used and the dose, as well as the nature of the task tested. There are no studies of the ability of A2A agonists to enhance WM in individuals with TBI; however, there is evidence from other clinical populations that augmentation of the A2A system can improve aspects of WM and other cognitive functions (Arnsten et al. 1996; Hunt et al. 1995); see also Arnsten (1998) and McAllister and Arnsten (2008) for thorough discussion.

The mechanism by which A2A receptor stimulation results in improved WM function is not clear. A2A receptors are located both pre-synaptically on noradrenergic neuronal terminals, and post-synaptically on dendritic spines, as well as sites removed from traditional synapses. Presynaptically, A2A stimulation results in inhibition of NE release; post-synaptically A2A stimulation appears to result in increased signal to noise ratio, apparently the opposite of what is seen with alpha-1 receptor stimulation (Arnsten 1998). Adrenergic neuronal cell bodies arise in the brain stem within the locus coeruleus (LC) and project throughout most of the CNS. Thus LC activity is capable of altering a wide array of CNS functions. However, there are particularly strong reciprocal connections between PFC and LC, suggesting that adrenergic tone in one area can affect adrenergic tone in the other (Arnsten 1998). It is relevant that guanfacine is ten times weaker than clonidine in assays of pre-synaptic function, and this may in part explain why clonidine is more sedating than guanfacine (clonidine also likely has more potent effects on A2B receptors in the thalamus, which have powerful effects on arousal). However, the majority of A2A receptors in the brain are actually localized post-synaptic to NE neurons, and these are of particular relevance to WM.

The above literature suggests that the adrenergic system plays an important but complex role in the modulation of WM that is receptor, brain region, dose, and task specific. Enhancement of A2A system functioning can improve WM through stimulation of post-synaptic receptors in the PFC, which is a critical component of WM circuitry. However, depending on the A2A agonist used, there can be opposing effects. For example, agents that stimulate pre-synaptic A2A receptors in LC (e.g., clonidine) can result in reduced adrenergic tone in PFC and reduced arousal, both of which could impair WM function. Alternatively, excessive stimulation or conditions of stress (including perhaps arousal and stress associated with performance of difficult cognitive tasks), will impair WM and other cognitive functions (Arnsten and Li 2005). Guanfacine, because of its lower propensity for pre-synaptic A2A receptors (and hence less inhibition of LC firing) is ideally positioned to be of selective benefit to WM.

In this study we tested the hypothesis that problems in activation and allocation of WM resources in PFC after MTBI are related to dysregulation of prefrontal A2A post-synaptic receptors. We therefore hypothesized that stimulation of these receptors with a moderate dose of guanfacine would selectively improve WM performance and normalize task associated cerebral activation in WM circuitry relative to noninjured controls.

Methods

This was a prospective, placebo-controlled, double-blind, crossover study of consecutive patients with MTBI referred to Dartmouth-Hitchcock Medical Center (DHMC), a Level 1 Trauma Center.

Participants

Participants were diagnosed with MTBI by a Neuropsychiatrist (TM) using the American Congress of Rehabilitation Medicine criteria for mild brain injury (Kay et al. 1993). Exclusion criteria included a history of other neurologic disorders, significant systemic medical illness, or current DSM-IV Axis I diagnosis of psychiatric illness as assessed by the Structured Clinical Interview for DSM-IV (First et al. 1997). Healthy controls (HC) were recruited through community advertisements and were screened for neurologic, medical, or any past or current psychiatric illness. After complete description of the study to the participants, written informed consent was obtained. The study protocol and the informed consent document were approved by the Dartmouth College Committee for the Protection of Human Subjects.

Procedures

Participants were studied on two occasions approximately one week apart. The sequence of guanfacine and placebo administration was randomized and balanced across groups. The order of neuropsychological and fMRI task administration was also balanced, using alternate forms when available.

Study Protocol—Approximately two and a half hours after oral drug (2.0 mg guanfacine) or placebo ingestion, participants were positioned in the MRI scanner using laser alignment beams and a non-magnetic deformable foam head holder to stabilize head position. Stimuli for the cognitive tasks were programmed in Presentation (Neurobehavioral Systems, Inc., Albany, CA) and presented visually through an MRI compatible goggle system (Resonance Technology, Van Nuys, CA).

fMRI Scan Procedure—All scans were acquired using the same GE Horizon 1.5T LX scanner. A gradient echo, echo-planar sequence was used to provide whole brain coverage: TR=2500 ms, TE=40 ms, FOV=24 cm, NEX=1, 29 interleaved 5 mm thick sagittal slices with no skip, yielding a 64×64 matrix with 3.75 mm² in-plane resolution. Initial volumes prior to spin saturation were discarded.

WM Task—As in our previous studies (McAllister et al. 2006; McAllister et al. 1999; McAllister et al. 2001), a visually presented verbal “N-back” task was used to test WM. During scanning participants viewed a string of consonant letters (except L, W, and Y) presented at a rate of one every three seconds, in a four-condition, blocked design. Conditions were 0-, 1-, 2-, and 3-back. For each consonant seen, participants used a button press device (Photon Control Inc., Burnaby, BC) to signify whether the current letter was a match (i.e., was the same as the designated target or the letter presented 1, 2, or 3 back in the sequence, depending on the condition instructions) or was a non-match. Each task condition was presented in 27-second epochs preceded by three seconds of instruction (e.g., “the match is D” or “the match is one back”). The four experimental conditions were each presented three times in pseudo-random order for a total of 12 task blocks. Participants rehearsed a practice version of the task on a laptop prior to scanning to ensure comprehension of task demands.

Clinical Assessment—After the scan session, participants completed a neuropsychological test battery that assessed level of estimated general intellectual functioning (WRAT-3, Reading subtest (Wilkinson 1993); WAIS-III, Block Design subtest (The Psychological Corporation 1997)), verbal memory (California Verbal Learning Test (Delis et al. 1987) or California Verbal Learning Test-II (Delis et al. 2000)), psychomotor speed (WAIS-III, Digit Symbol-Coding subtest (The Psychological Corporation 1997)) and WM, executive, and attentional functioning (Trail Making Test, Parts A and B (Reitan and

Wolfson 1993) or Delis-Kaplan Executive Function System (D-KEFS), Trail Making Test Conditions 2 and 4 (Delis et al. 2001)); D-KEFS Color-Word Interference Test (Delis et al. 2001); Controlled Oral Word Association Test (Lezak 1995; Spreen and Strauss 1998); Paced Auditory Serial Addition Test (PASAT) (Gronwall 1977); Gordon Continuous Performance Test (Gordon et al. 1986)).

Statistical Analysis

Cognitive Measures—WM in-scanner performance data and neuropsychological variables for both groups were compared using a repeated measures ANCOVA taking into account drug condition (guanfacine vs. placebo), diagnosis (MTBI vs. HC), age, education, and order of drug administration (drug/placebo vs. placebo/drug). For neuropsychological test data, raw or standard scores were used as indicated, with the exception of Trail Making. Because the version of the test changed during the protocol, raw scores for Trails A and B and D-KEFS conditions 2 and 4 were z-transformed using the HC group means in the placebo condition, with z-scores utilized for group comparisons. All models were fit using PROC MIXED in SAS V9, specifying a random subject effect for the repeated measures covariance structure.

fMRI Analyses—Spatial realignment using a six parameter model was performed on all raw scan data to remove any minor (subvoxel) motion-related signal change. All volumes for each subject were normalized into standardized Montreal Neurological Institute atlas space using SPM5 (Wellcome Department of Cognitive Neurology, University College, London). During spatial normalization all scans were resampled to 2 mm³ isotropic voxels. Spatial smoothing to a full width half maximum of 8 mm was performed prior to statistical analysis. fMRI analyses included statistical parametric mapping, on a voxel-by-voxel basis, using a general linear model approach (Friston et al. 1995) as implemented in SPM5. Smoothed normalized scans for all subjects were entered into the model, and contrast images comparing pairs of the WM processing load conditions (1-back > 0-back, 2-back > 0-back, 3-back > 0-back) were created for each subject. These contrast images were then used for the second level multi-subject/between-group random effects analyses. The random effects procedure performs a mixed model analysis to account for both random effects (scan) and fixed effects (task condition) (Holmes and Friston 1998).

Random effects analyses were conducted using ANCOVA to construct contrast maps of voxels in which brain activation differed between group and drug conditions (full factorial model in SPM5). Comparisons were conducted within an omnibus group- (two independent levels: MTBI, HC) by-drug (two non-independent levels: guanfacine, placebo) ANCOVA, covarying for age, education, and order of drug/placebo administration. The design matrix therefore included both conditions for both groups, accounting for the repeated measures nature of the drug factor (i.e., the matrix included four columns, one for each group on placebo and guanfacine). The critical significance threshold (P_{crit}) was set to 0.01. Given the constraints of the sample size, only clusters of activated voxels with a whole-brain search cluster-level $P_{uncorrected} < 0.05$ are discussed. For illustrative purposes, figures and tables also include results at a P_{crit} of 0.05 where noted. Within the omnibus SPM5 design matrix between-group comparisons were conducted using weighted contrast vectors. For example, pair-wise comparisons of brain activation on guanfacine (MTBI vs. HC) were conducted by entering values of 1 and -1 in the appropriate columns in the matrix. In this manner determination of regions where HC showed greater activation than MTBI on guanfacine would be identified by entering 1 in the HC guanfacine column and -1 in the MTBI guanfacine column.

Results

Demographics

Thirteen subjects with MTBI and 14 HC completed the protocol (see Table 1). There were no significant group differences in WRAT-3 Reading scores, an estimate of baseline verbal intellectual ability. The groups did differ with respect to years of education, largely because several of the MTBI subjects were students at the time of study participation. Parental education did not differ between groups. MTBI participants were studied a mean of 37.2 days after injury (± 17.6), and had an average Glasgow Coma Scale score of 13.9 (± 2.1). All participants were injured either by falls or in motor vehicle accidents, and none of the MTBI participants had focal injuries visible on computerized axial tomography (CT) scans done at the time of injury. The protocol was well tolerated by both groups.

Cognitive Performance

There was a main effect of drug order in our initial modeling of cognitive outcomes (better performance on the second assessment), probably because the protocol called for repeated cognitive assessments over a short interval and both test performance and imaging results may be vulnerable to the influence of practice and exposure effects. Even when alternate forms of a task are given, there can be improvement in performance related to improved strategy/efficiency in task approach. Thus we included order (drug or placebo first) as a covariate in subsequent analyses. Although the two groups did not differ significantly with respect to age, the literature supports a relationship between age and performance across a variety of cognitive measures, including the age range spanned by our participants. In addition, there is evidence that age affects task-related blood oxygen level dependent (BOLD) response. Thus subsequent analyses reported used age, education and order of drug administration as covariates.

N-Back Performance—The MTBI and HC groups showed similar performance on the N-back task. Both groups showed the expected WM load effect on performance, regardless of drug condition (see Table 2), with percentage of correct responses (adjusted for guessing) decreasing with increasing WM load requirements. The HC group generally did worse on guanfacine relative to placebo, although this was not statistically significant. The MTBI group showed a statistically significant improvement on the moderate WM load (2-back) condition on guanfacine relative to placebo ($p=0.018$), and there was a significant drug-by-diagnosis interaction ($p=0.007$) for the 2-back as well as a trend for the mean back ($p=0.07$), with the MTBI group demonstrating improved performance on guanfacine relative to placebo and the HC group showing a decline in performance on the drug.

Out of Scanner Cognitive Tasks—Overall, the MTBI group and the HC group did not differ on out of scanner cognitive test performance, with the exception of the fastest condition of the PASAT (PASAT D), where HC demonstrated better performance than the MTBI group. There was a variable effect of guanfacine on performance in a small number of out of scanner cognitive tests, with a positive main effect of drug on the D-KEFS Color-Word Interference Test Word Reading Trial ($p=0.007$), but a negative main effect on CPT simple reaction time number correct ($p=0.036$); and CPT vigilance reaction time ($p=0.009$); as well as Trails 2/A ($p=0.02$).

fMRI

Both diagnostic groups showed the expected WM activation pattern on both drugs (Figure 1), including bilateral frontal, parietal, and cerebellar circuitry. This pattern of activation is consistent with previous results with the N-back task (McAllister et al. 1999; McAllister et al. 2001). As noted above, the two groups showed a significant diagnosis-by-drug

interaction in task performance at the moderate (2-back) WM load. All WM conditions were examined, but similar to the findings for task performance, cerebral activation differences were most marked for the 2-back condition and are displayed in Figures 1 and 2, and Table 3. Visual inspection of these maps indicates a prominent increase in right frontal activation in the MTBI group on guanfacine relative to placebo (Figure 1C compared to 1D). Within-group statistical comparisons are shown in Figure 2. Brain regions with significant activation in both groups on both drugs are displayed in red, while drug contrasts of interest are overlaid in blue (e.g., regions where HC showed greater activation on guanfacine than placebo are shown in blue in Figure 2A). Figure 2 again demonstrates increased right frontal activation on guanfacine relative to placebo in the MTBI group. In contrast, the HC group shows increased activation outside typical WM circuitry on guanfacine relative to placebo. This pattern is similar to that of the MTBI group on placebo, where greater activation is also noted outside WM circuitry. This pattern was also evident on the 3-back condition (not shown).

Discussion

These results indicate that individuals with MTBI have altered cognitive and cerebral activation patterns in response to the A2A receptor agonist guanfacine relative to controls, consistent with the main study hypothesis. Of particular interest is that the observed effect was largely specific to WM task performance. On guanfacine relative to placebo, the MTBI group showed increased activation in WM circuitry, particularly in the right prefrontal area, in and around the middle frontal gyrus. This change in activation was associated with improved performance on the 2-back WM task. The HC group showed no increase in activation in WM circuitry on guanfacine relative to placebo, but did show increased activation in regions not typically involved in WM and in particular N-back performance. This pattern was associated with generally poorer task performance. Although guanfacine is generally less sedating than other alpha-2 agonists such as clonidine, it may be that the decrements observed on some timed tests (CPT measures and Trails 2/A) may be related to decreased arousal associated with the drug, perhaps through pre-synaptic effects on noradrenergic neurons in the LC.

The observation that increased N-back performance was associated with increased activation in PFC, particularly in regions involved with WM processing, is of interest. The A2A system is complex and A2A agonist effects are regionally specific. At the level of central adrenergic tone, stimulation of pre-synaptic A2A receptors on noradrenergic neurons in the LC can reduce activity of these neurons. However, the majority of A2A receptors are found post-synaptic to noradrenergic neurons (Arnsten and Li 2005), particularly on the dendritic spines of pyramidal neurons in the superficial layers of the PFC, where they are positioned to play an important role in the cortical-cortical networks that subserve WM (Arnsten and Li 2005; McAllister and Arnsten 2008). Here the A2A receptors are co-localized with hyperpolarisation-activated cyclic nucleotide-gated (HCN) cation channels (Barth et al. 2008; Ramos et al. 2006). Stimulation of the A2A receptors (with subsequent reduction in production of cyclic AMP) “closes” the HCN channels, making them less leaky and increasing pyramidal cell excitability (Ramos et al. 2006). This in turn enhances local connectivity and is associated with reduced distractibility and enhanced WM (Wang et al. 2007). Additional work has suggested that TBI is associated with a hypercatecholaminergic state, at least in part related to increased tyrosine hydroxylase expression and activity with subsequent increased catecholamine synthesis (Kobori and Dash 2006). Thus it has been suggested that A2A agonists such as guanfacine might enhance WM pre-synaptically by reducing overall catecholamine release at the level of the LC, while also working post-synaptically through enhancing cortical to cortical networks in the PFC (McAllister and Arnsten 2008). In an animal model, guanfacine was found to increase regional cerebral

blood flow in monkeys performing a WM task (Avery et al. 2000). More recently Clerkin et al. (2009) found that guanfacine (1.0 mg) significantly reduced reaction time in a warning signal paradigm, and this was associated with increased activation in the dorsolateral PFC as well as related frontal subcortical circuitry. Our results suggest enhanced WM associated with guanfacine in the MTBI group as well as increased PFC activation. Although we did not find improved performance on timed tests, this could be related to the higher guanfacine dose used in our study.

The pattern of greater activation outside typical WM circuitry in the MTBI group on placebo and the HC group on guanfacine accompanied by poorer task performance also merits comment. As shown in Figure 2A and 2D, activation in precentral and superior and middle temporal gyri was apparent in these contrasts. These regions are typically deactivated during WM processing, with prior research in healthy subjects and other clinical populations relating poorer WM task performance to a failure to deactivate such regions (in addition to failure to activate typical WM circuitry) (Anticevic et al. 2010; Crossley et al. 2009; Nejad et al. 2011; Tomasi et al. 2006). The current finding of relatively poorer task performance accompanied by greater activation (or failure to deactivate) outside of expected WM circuitry may offer further evidence that successful WM performance relies on region-specific alterations in brain activation, which can be differentially disrupted by pharmacological challenge in clinical populations relative to healthy controls.

Several limitations should be considered in the interpretation of these findings. We did not include MTBI participants with significant medical and psychiatric disorders; therefore these results may not generalize to all individuals with MTBI. This MTBI group had very mild injuries and the results may not apply to those with moderate and severe injuries. It is also important to point out that the regions of increased activity evident on guanfacine do not necessarily indicate the exact locations of the adrenergic effect. Although BOLD fMRI activation in these areas indicates local increased cerebral blood flow and metabolic activity, guanfacine administration could indirectly modulate this activity through striatal or other interconnected subcortical or cortical sites. This is a relatively small sample size and thus should be considered a preliminary finding. However, the fact that we are able to detect a specific effect on the hypothesized cognitive domain (WM) and that this effect is associated with increased activation in WM circuitry, even in this sample size, is of interest and suggests that psychopharmacological probes in association with fMRI may be a promising way to test hypotheses about the neural mechanisms of cognitive complaints and deficits after MTBI.

These results are consistent with the hypothesis that MTBI is associated with subtle dysregulation of frontal alpha-2 adrenergic systems in the first 4–6 weeks after injury, and that augmentation strategies with alpha-2 agonists might improve WM function. However, the majority of A2A receptors in the brain are actually localized post-synaptic to NE neurons, particularly in the dendritic spines of prefrontal neurons critical to cortical-cortical networks involved in WM (Arnsten and Li 2005; Ramos et al. 2006). Further studies are needed to clarify the effects that different dosing strategies, severity of injury, and the injury to treatment interval may all have on outcome.

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Figure 1. Group Activation Patterns during 2-Back in Response to Pharmacological Challenge. Activation maps (SPM5) for each group (MTBI and HC) under each drug condition (placebo or guanfacine). Note increased right frontal activation in the MTBI group on guanfacine when compared to placebo (1C and 1D).

Figure 2.

Within-Group Differences in Brain Activation during 2-Back in Response to Pharmacological Challenge

The combined SPM5 activation map for the 2-back > 0-back contrast for both groups (MTBI and HC) in both drug conditions (placebo and guanfacine) is displayed in red. Note the bilateral frontal, parietal, and cerebellar network typically activated during the N-back task. Group contrasts of interest (discussed further in text) are overlaid in blue, using the MRICroGL software package.

Table 1

Sample Characteristics

	HC [Mean±SD or N(%)] N=14	MTBI [Mean±SD or N(%)] N=13	p-value
Age (years)	32.0 ± 8.5	27.2 ± 11.7	0.23
Education (years)	16.5 ± 2.1	13.6 ± 2.8	0.005
WRAT-3 Reading SS	106 ± 4.7	107 ± 9.3	0.69
Mother's Education (years)	14.2 ± 2.5	14.1 ± 2.8	0.90
Father's Education (years)	14.6 ± 2.9	14.7 ± 3.0	0.97
Male Gender	6 (42.9%)	7 (53.8%)	0.57
Placebo first	8 (57.1%)	8 (61.5%)	0.82

WRAT-3 = Wide Range Achievement Test, 3rd edition; SS = Standard Score

Table 2

N-Back Performance

	HC (N= 14)			MTBI (N= 13)			HC vs. MTBI p-values		Overall p-values		
	Placebo Mean(SE)	Guanfacine Mean(SE)	P-value	Placebo Mean(SE)	Guanfacine Mean(SE)	P-value	Placebo	Drug	Diagnosis	Drug	Interaction
N-Back Processing Load											
Corrected 1-Back	86.7(4.1)	90.0(4.4)	0.53	84.7(4.7)	89.2(4.1)	0.43	0.97	0.92	0.97	0.32	0.88
Corrected 2-Back	79.9(6.0)	70.7(6.6)	0.13	76.9(6.0)	88.8(3.1)	0.018	0.28	0.18	0.88	0.46	0.007
Corrected 3-Back	62.3(6.4)	55.9(6.4)	0.36	73.4(5.5)	68.5(7.1)	0.48	0.30	0.24	0.20	0.26	0.89
Mean-Back	76.3(4.0)	72.2(3.8)	0.19	78.3(4.2)	82.2(4.1)	0.19	0.99	0.22	0.51	0.97	0.07

Table 3
Regions of Significant Activation Differences for 2-back > 0-back Contrast as a Function of Diagnosis and Drug Condition

MNI coordinates (x y z)	$P_{\text{crit}} < .01$			$P_{\text{crit}} < .05$			T	Region description (for cluster peak)
	Cluster extent (k)	Cluster-level $P_{\text{corrected}}$	Cluster-level $P_{\text{uncorrected}}$	Cluster extent (k)	Cluster-level $P_{\text{corrected}}$	Cluster-level $P_{\text{uncorrected}}$		
HC Placebo > Guanfacine								
-44 24 -20	134	.965	.039	1440	.143	.001	3.77	Left superior temporal gyrus
34 -66 46	177	.822	.020	612	.952	.021	3.54	Right superior parietal lobule
12 -50 62				1244	.254	.002	3.50	Right precuneus
14 12 0				632	.939	.019	3.29	Right caudate
-24 -64 -8				609	.954	.021	3.26	Left fusiform gyrus
HC Guanfacine > Placebo								
52 -14 60	150	.926	.030	475	.996	.038	3.60	Right postcentral gyrus
-26 -44 70	160	.892	.026				3.43	Left postcentral gyrus
-40 -28 50				1135	.345	.003	3.64	Left postcentral gyrus (same cluster as previous line)
42 -44 -14				421	.999	.049	4.05	Right fusiform gyrus
-42 -52 6				1788	.052	.000	3.77	Left superior temporal gyrus
46 18 -32				419	.999	.049	3.27	Right superior temporal gyrus
28 -16 50				946	.562	.006	3.08	Right precentral gyrus
MTBI Placebo > Guanfacine								
52 -58 4	287	.324	.005	810	.747	.009	4.46	Right superior temporal gyrus
56 10 -6	172	.844	.022	931	.582	.006	4.22	Right superior temporal gyrus
-8 -78 0	424	.076	.001	4731	.000	.000	3.82	Left lingual gyrus
8 -64 6	185	.785	.018				3.74	Right cuneus (same cluster as previous line)

MNI coordinates (x y z)	$P_{crit} < .01$			$P_{crit} < .05$			Region description (for cluster peak)
	Cluster extent (k)	Cluster-level $P_{corrected}$	Cluster-level $P_{uncorrected}$	Cluster extent (k)	Cluster-level $P_{corrected}$	Cluster-level $P_{uncorrected}$	
-32 -80 -14	179	.813	.020				Left inferior occipital gyrus (same cluster as previous line)
-42 -50 -34	131	.970	.041				Left cerebellum (same cluster as previous line)
18 -58 -14	130	.972	.042				Right cerebellum (same cluster as previous line)
22 -76 44				514	.991	.032	Right precuneus
4 40 -16				442	.998	.044	Right medial frontal gyrus
<i>MTBI Guanfacine > Placebo</i>							
-48 -50 36				471	.997	.039	Left inferior parietal lobule
42 -6 30				1372	.175	.001	Right precentral gyrus
26 -36 58				496	.994	.034	Right postcentral gyrus
-4 20 16				860	.679	.008	Left anterior cingulate gyrus
-8 -34 68				1362	.180	.001	Left paracentral lobule
54 32 30				439	.999	.045	Right middle frontal gyrus