Published in final edited form as:

Cerebellum. 2012 March ; 11(1): 300–310. doi:10.1007/s12311-011-0311-0.

An fMRI Investigation of Cerebellar Function During Verbal Working Memory in Methadone Maintenance Patients

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Abstract

Working memory is impaired in opioid-dependent individuals, yet the neural underpinnings of working memory in this population are largely unknown. Previous studies in healthy adults have demonstrated that working memory is supported by a network of brain regions that includes a cerebro-cerebellar circuit. The cerebellum, in particular, may be important for inner speech mechanisms that assist verbal working memory. This study used functional magnetic resonance imaging (fMRI) to examine brain activity associated with working memory in 5 opioid-dependent, methadone-maintained patients and 5 matched, healthy controls. An item recognition task was administered in two conditions: 1) a low working memory load “match” condition in which participants determined whether target letters presented at the beginning of the trial matched a probe item, and 2) a high working memory load “manipulation” condition in which participants counted two alphabetical letters forward of each of the targets and determined whether either of these new items matched a probe item. Response times and accuracy scores were not significantly different between the groups. FMRI analyses indicated that, in association with higher working memory load (“manipulation” condition), the patient group exhibited hyperactivity in the superior and inferior cerebellum and amygdala relative to that of controls. At a more liberal statistical threshold, patients exhibited hypoactivity in the left prefrontal and medial frontal/pre-SMA regions. These results indicate that verbal working memory in opioid-dependent individuals involves a disrupted cerebro-cerebellar circuit, and shed light on the neuroanatomical basis of working memory impairments in this population.

Keywords

addiction; fMRI; cognition; working memory; cerebellum; methadone

1. Introduction

Working memory involves the maintenance and manipulation of information held in mind over a brief duration lasting several seconds [1]. Verbal working memory specifically pertains to online information that is *verbalizable*; that is, information that can be expressed in speech, such as letters, words, numbers, colors, or nameable objects. Information does not

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All authors have declared that they have no conflicts of interest.

need to be spoken aloud during verbal working memory. This online process likely involves elicitation of language and speech-related brain regions [2-4] because internal speech mechanisms (e.g., an inner voice) are thought to support working memory, and may more generally represent, maintain, and organize task-relevant information and conscious thoughts [5, 6]. Neuroimaging studies of verbal working memory in healthy adults have consistently described a neural circuit that includes inferior frontal and dorsolateral prefrontal brain regions, premotor and supplementary motor areas (SMA), the inferior parietal lobe (IPL), and the cerebellum [7-14]. Moreover, functional magnetic resonance imaging (fMRI) studies have demonstrated that brain activation patterns during verbal working memory share functional overlap with overt and covert speech, particularly within the cerebellum [10, 15, 16]. Thus, verbal working memory appears to engage inner speech mechanisms that are part of a language-related cerebro-cerebellar circuit.

Within the cerebro-cerebellar circuit, there appears to be a topographic functional organization of verbal working memory [5]. Specifically, superior aspects of the cerebellum (Lobule VI/Crus I) activate in response to encoding new verbal content [8, 9], along with motor- and language-related frontal regions (inferior frontal cortex, premotor and SMA), presumably to initiate an internal motor trace of the verbal content [5, 11, 17]. The inferior cerebellum (Lobule VIII), however, appears to be associated with storage functions that are independent of motoric aspects of the working memory process [10]. The inferior cerebellum functions with cortical brain regions, such as the prefrontal cortex, anterior cingulate (ACC) and IPL.

Activity within the cerebro-cerebellar circuit increases with greater working memory demands [12]. For example, when verbal content must be manipulated versus simply maintained, cerebro-cerebellar activity increases in intensity and duration. This ramping of activity may represent intensification of inner speech as the task at hand becomes more difficult. Supporting this view, Marvel and Desmond (submitted) found that, under high verbal working memory demands, superior cerebellar activity in healthy young adults was greatest in those who committed the most errors on the task, even when analyses were conducted on correct trials only. This finding seemed to suggest that those who found the task most effortful (i.e., made the most errors) used inefficient working memory strategies (even on correct trials), which involved a heavy reliance on inner speech and corresponded with hyperactivity of the superior cerebellum.

Given that superior cerebellar activity increases with verbal working memory demands, one might expect similar hyperactivity in populations with known working memory impairments, such as in addiction [18]. However, only a handful of studies have applied neuroimaging to the study of verbal working memory in addiction. Desmond and colleagues [19] used fMRI to study verbal working memory in alcohol-dependent individuals. Participants were asked to subvocally rehearse a set of letters during a brief delay. Relative to controls, the alcohol-dependent group exhibited hyperactivity in the right superior cerebellum, left inferior frontal gyrus (BA 44), and left prefrontal cortex (BA 9), even though behavioral performance was equated between the two groups. The authors interpreted the hyperactive cerebro-cerebellar circuit as evidence of compensation that enabled the alcohol dependent participants to maintain normal performance on the task. Separate fMRI studies of individuals addicted to cocaine [20] and to opioids [21] examined response conflict, which may be considered, indirectly, a verbal working memory task given that verbalizable stimuli need to be held in mind and continuously judged throughout the task. Both studies found hyperactivity in prefrontal and cerebellar brain regions, again consistent with compensatory behavior.¹ Taken together, all three studies indicate consistent involvement of the frontal cortex and cerebellum in addicted populations. Interestingly, a recent fMRI study of cue-induced craving in heroin-addicted individuals [22] found

remarkably similar results. Following abstinence from heroin and water, addicted individuals were shown drug- and water-related cues. Relative to the water-related cues, the heroin-related cues elicited greater activity in the bilateral inferior frontal gyrus (BA 9/44/45) and bilateral superior cerebellum. Although verbal working memory was not examined directly, it is notable that cue-induced craving elicited a verbal working memory-like circuit in the heroin users. This suggests that working memory, and perhaps inner speech, plays a role in drug craving.

The present study used fMRI to directly examine the neural correlates of verbal working memory in opioid-dependent, methadone-maintained patients versus healthy controls. The primary aim was to clarify the role of the cerebellum in addiction by measuring brain activity while participants performed a verbal working memory task that, in healthy, young adults, was known to reliably elicit cerebro-cerebellar activity in association with working memory demands (Marvel & Desmond, submitted). We hypothesized that activity in the cerebro-cerebellar circuit would be abnormal in the patient group. Special attention was paid to activity in the superior cerebellum (Lobules VI/Crus I) and inferior cerebellum (Lobule VIII) because of the putative contributions of these regions to working memory. For example, given that the superior cerebellum may be involved in the motor-related aspects of inner speech, hyperactivity in this region could reflect intense engagement of inner speech while one struggles to perform the task. By contrast, hyperactivity in the inferior cerebellum could indicate general difficulties with information storage independent from motor-related inner speech functions. Similarly, we explored activations in the cerebrum, with special interest in regions previously shown to be involved in working memory and addiction: the inferior frontal and dorsolateral prefrontal brain regions (BA 44/45 and 9), the premotor (BA 6) and supplementary motor areas, the anterior cingulate (BA 32), and the inferior parietal lobe (BA 40).

2. Methods

2.1. Study Participants

Five opioid-dependent, methadone-maintained patients (2 males) and five healthy controls (2 males) without histories of drug dependence were tested. Patients were recruited from the Johns Hopkins Behavioral Pharmacology Research Unit (BPRU). Controls were recruited from the community. All participants were native English speakers, right-handed, with no known neurological conditions or history of head trauma. All participants were given a Structured Clinical Interview for DSM-IV Axis I (SCID) [23], with consensus ratings conducted by authors CLM and MLF. Information obtained from the SCID was used to 1) establish a history of heroin and/or other drug dependence in the patient group (and lack thereof in the control group), 2) determine the presence of alcohol dependence (exclusionary for both groups), and 3) determine a history of other Axis I disorders (exclusionary for both groups, with the exception of addiction to caffeine or nicotine). Consequently, participants were excluded if there was a prior history of depression. All participants were cigarette smokers, and abstained from smoking 2-4 hours prior to their MRI scan. Patients were in methadone treatment for at least 10 months (mean duration = 389 days, range = 307-2003 days; mean daily dose = 88 mg, range = 35-120 mg) and on a stable dose (within 5% of current dose) for at least 2 months. Three patients had a history of cocaine dependence, but all patients had abstained from illicit drug use for 204-892 days prior to testing. Duration of drug abstinence in the patient group was confirmed by: 1) regular (at least bi-monthly) urinalysis taken as part of their treatment within the BPRU, 2) counselor-provided

¹Even though the authors of each study interpreted the hyperactivity as compensatory in their respective patient groups, it should be noted that the cocaine-dependent group [20] showed behavioral deficits, whereas the opioid-dependent group [21] performed at normal levels.

information, and 3) patient-provided information. In controls, drug abstinence was based on information provided by the participant. In addition, both groups were drug and pregnancy tested by urinalysis on the day of the initial eligibility screening and again on the day of the MRI (conducted within two weeks of the initial eligibility screening). The drug screen (Aim Screen MultiDrug 9 by Germaine Laboratories) tested for the presence of the following substances in the urine: cocaine, amphetamine, methamphetamine, marijuana, methadone, opiates, phencyclidine, barbiturates, and benzodiazepines. A detailed history of lifetime drug and alcohol exposure was obtained in both groups using the Lifetime Drug Use Questionnaire [24]. The two groups were equated [reported as mean (standard deviation) and p-value] for age in years [patients: 46.8 (5.1); controls: 45.4 (5.1); $p = .69$], nicotine in pack-years [patients: 12.8 (7.4); controls: 14.7 (9.9); $p = .74$], and lifetime alcohol consumption in Liters [patients: 573 (899); controls: 459 (509); $p = .81$]. Patients had a significantly lower education level (in years) than did controls [patients: 11.8 (1.8); controls: 14.8 (1.8); $p < .05$].

This research was approved by the Johns Hopkins Institutional Review Board and in accordance with the Helsinki Declaration of 1975. All participants gave their written informed consent and were paid for their participation.

2.2. Task Procedures

All participants performed a computerized working memory test using a delayed item recognition paradigm while undergoing fMRI. The task consisted of a low working memory load “match” condition and a high working memory load “manipulation” condition (see Figure 1). In both conditions, one or two uppercase consonants, or targets, were presented for one second (encoding phase). This was followed by a blank screen for four or six seconds (delay phase). During the delay phase, participants were asked to silently rehearse the targets in the match condition, or to count two alphabetical letters forward of each of the targets (thereby creating new targets) in the manipulation condition. Finally, a single lowercase letter, or probe, was presented for one second (retrieval phase). Participants were given six seconds to indicate with a button press (yes = right index finger; no = right middle finger) whether the probe matched an original target (match condition) or a new target (manipulation condition). Thus, the two test conditions differed only by the instructions for the delay phase, in which letters were either silently rehearsed or manipulated.

Match and manipulation conditions were performed during separate blocks of 64 trials each, the order of which was counterbalanced across participants. Participants were instructed to respond as quickly and accurately as possible while thinking silently “in your head.” Response time (RT) and accuracy were recorded for each trial. In order to familiarize participants with the rules of the task, participants practiced 10 trials of each condition prior to entering the MRI environment.

Trials were jittered with an inter-trial interval (ITI) that lasted six to nine seconds. Failure to respond did not inhibit the start of the next trial. Consonant target letters were randomly generated prior to testing to create a “match” list and a “manipulation” list. Target letters were unique within a trial and randomly placed across six possible spatial positions, as in prior studies [9, 10, 13]. Pound (“#”) placeholders were displayed in the unused positions within a trial. Sixteen trials (25%) did not include any probe at all so that brain activity measured during the delay phase could be visualized more clearly, and in the absence of activity related to the subsequent retrieval phase. In the no-probe trials, participants viewed a blank screen throughout the retrieval phase, and no response was expected. For the remaining 48 trials, probes corresponded to a target on half of all trials. In such trials, the location of the target letter was counterbalanced across spatial positions. For the manipulation condition, the probe did not directly match the originally presented target

letters within a trial. There were an equal number of 1- and 2-target trials, and 4- and 6-second delay intervals. The number of targets (1 or 2), length of delay (4 or 6 sec), expected response (yes or no), and duration of ITI (6 to 9 sec) were pseudorandomized so that the presentation of identical parameters was limited to three consecutive trials.

Stimuli were delivered using E-Prime 1.1 software [25] on a Hewlett Packard xw4300 workstation running Windows XP Pro. The computer-generated visual display was rear-projected onto a screen situated behind the participant's head. The display was then reflected onto a mirror directly within the participant's line of view just outside the head coil. Responses were collected using two fiber optic button boxes (MRA, Inc., Washington, PA) that were held in the subject's right hand.

2.3. MRI Data Acquisition

One structural MRI scan using a T1-weighted MPRAGE and two fMRI scans using a T2*-weighted gradient echo EPI pulse were administered on a 3.0T Philips Intera scanner. The structural MRI protocol consisted of a T1-weighted MPRAGE (TR = 7 ms; TE = 3.3 ms; flip angle = 8°; inplane resolution = 0.75 mm; slice thickness = 1 mm, no gap; 170 sagittal slices; FOV = 240 mm; 320 × 320 matrix; 1 NEX, SENSE factor = 2). The total MPRAGE scan duration was less than 6 minutes. FMRI data were collected using a T2*-weighted gradient echo EPI pulse sequence (TR=1000 ms; TE = 30 ms; flip angle = 61°; inplane resolution = 3.75 mm; slice thickness = 6 mm, skip 1 mm; 20 oblique-axial slices; FOV = 240 mm; 64 × 64 matrix; 1 NEX; SENSE factor = 2). The total scan duration was less than 16 minutes for each of the two fMRI scans. Statistical Parametric Mapping (SPM2) (Wellcome Department of Cognitive Neurology) software was used for routine preprocessing steps: timing, motion correction, co-registration, spatial normalization to the Montreal Neurological Institute (MNI) stereotaxic space, and spatial smoothing (FWHM = 5 mm). Individual parameter estimates of fit between the reference waveform and the fMRI time series were subjected to a high-pass filtering of 234 seconds. T2*-weighted images were acquired in the oblique-axial plane rotated 25° clockwise with respect to the AC-PC line in order to provide whole-brain imaging of the cerebellum and neocortex. The start of the fMRI scan was triggered by E-prime software at the beginning of each block.

2.4. Functional Data Analysis

Reference waveforms were created for the delay phase of the task, combining 1- and 2-target trials, by convolving a canonical hemodynamic response function with the square wave representation of the delay period. Individual parameter estimations were derived using the least squares estimates of fit between the reference waveform and the fMRI time series at each voxel on correct trials only. A random effects analysis for each group was then performed to map an average blood oxygen level dependent (BOLD) response to the delay phase of the task. This analysis was performed by computing one contrast volume (manipulation minus match condition) per participant and using these volumes to calculate one-sample t-test values at every voxel. Finally, a 2-sample t-test was performed on these values, voxel by voxel, to test whether groups differed in their BOLD signal contrast between manipulation and match conditions during the delay phase. MNI coordinates were transformed into the coordinate system of the Talairach and Tournoux stereotaxic atlas [26], using the MNI to Talairach transformation described by Lancaster et al. [27], and referenced with the Talairach atlas. However, MNI coordinates are reported in the text and depicted in the figures. For the cerebellum, MNI coordinates were referenced with the cerebellar atlas of Schmahmann et al. [28].

Due to the relatively low degrees of freedom, additional non-parametric tests were run to confirm the findings of the parametric tests. This was done in two ways. First, Statistical

nonParametric Mapping (SnPM), an SPM2 toolbox (known as “SnPM3 on SPM2”) (Wellcome Department of Cognitive Neurology), was used to perform multiple voxel-level nonparametric permutation/randomization tests [29]. Second, Mann-Whitney U Tests were conducted, using PASW Statistics 18, on the contrast values of manipulation minus match conditions that were created from regions of interest (ROI) determined by the original parametric t-tests. These functionally defined ROIs were circumscribed from the activation cluster using locally developed software [30]. A gray matter mask, derived from the segmentation in SPM2, was applied to each ROI such that only voxels greater than or equal to a gray matter probability threshold of 0.5 were considered in computing the average contrast value within the ROI. This approach was used to adjust for possible gray matter volume differences between the groups [31]. Thus, for each participant, the average activation within an ROI was determined by calculating the mean parameter estimate value per gray matter voxel. The resultant contrast values per participant were then entered into Mann-Whitney U Tests to test for significant group differences for each ROI.

3. Results

3.1. Behavioral Results

Mean accuracy and RT were calculated for the following trial types: 1-target match, 2-target match, 1-target manipulation, and 2-target manipulation, as shown in Figure 2. For accuracy, a 2 (condition: match vs. manipulation) $\times 2$ (target load: 1 vs. 2 letters) $\times 2$ (group: controls vs. patients) mixed-design analysis of variance (ANOVA) yielded a main effect of condition, $F(1,8) = 12.24$, $p < .01$, target load, $F(1,8) = 12.26$, $p < .01$, and an interaction of condition $\times$ target load, $F(1,8) = 14.87$, $p < .01$, but no main effect of group, $F(1,8) = 3.84$, $p = .09$. These effects appear to have been driven by low accuracy in the 2-target manipulation condition. A $2 \times 2 \times 2$ mixed-design ANOVA was also conducted on RT for correct responses. Results indicated a main effect of condition, $F(1,8) = 9.12$, $p < .02$, and target load, $F(1,8) = 16.14$, $p < .01$, but no main effect of group $F(1,8) = 1.53$, $p = .25$ and no condition $\times$ target load interaction, $F(1,8) = 3.32$, $p = .11$.

3.2. Imaging Results

In the parametric analyses, group differences in activation were significant at the $p < .001$ level. A cluster threshold of $p < .05$ (i.e., at least 7 voxels) was used to filter activations, except for those observed within *a priori* regions of interest, such as in the superior or inferior cerebellum. Non-parametric analyses were conducted on regions that met these threshold criteria, as described in section 2.4. Activations that did not survive the secondary non-parametric analyses were not considered reliable, and therefore, excluded from these results.

BOLD signal differences between patients and controls were observed in three regions (Figure 3): (1) the right superior cerebellum, (2) left superior cerebellum, and (3) right amygdala. An additional activation smaller than 7 voxels was observed in the right inferior cerebellum, which was part of the *a priori* hypothesis of this experiment. Because groups showed a significant difference in education, an additional analysis using education as a covariate was performed using SPM2. Results verified that the BOLD signal differences in these regions remained significant between groups.

To confirm that group differences were not due to outliers, ROIs were formed from each of these four activations, and data were examined within each ROI for each participant, as described in section 2.4. As shown in Figure 3, manipulation condition signal increases were observed for every patient in cerebellar Lobules VI (bilaterally), the amygdala, and right cerebellar Lobule VIII. By contrast, controls showed minimal signal change, or decreases, in

these same regions. Thus, the ROI analyses demonstrated consistent activation patterns for individual members within each group, indicating that findings were not driven by outliers. The possibility that education differences accounted for group differences was explored further in each ROI in a $2 \text{ (group)} \times 10$ (BOLD contrast: values for subjects 1-10) univariate analysis of covariance (ANCOVA) entering education level as a covariate. Group differences within each ROI remained highly significant, suggesting that BOLD differences between the groups were more likely due to addiction than to educational differences (ROIs right cerebellar lobule VI, left cerebellar lobule VI, and right amygdala $p < .001$; ROI right cerebellar lobule VIII, $p < .01$).

Given that other studies have observed group differences in frontal brain regions, the threshold was reduced to $p < .05$ in order to expose sub-threshold activations outside of the cerebellum and amygdala. Activations were recognized if they met the following criteria: 1) were significant at the $p < .05$ level, 2) reached a cluster size threshold of 120 voxels (cluster size significance $p < .05$), and 3) were located within an *a priori* ROI [inferior frontal region (BA 44/45), dorsolateral prefrontal cortex (BA 9), premotor cortex (BA 6), supplementary motor area, anterior cingulate (BA 32), and inferior parietal lobe (BA 40)]. Two regions met these criteria; both were hypoactivations in the patient group: the left middle frontal gyrus (BA 9) (-51, 24, 31; 153 voxels, including a sub-maxima in BA 46) and the right medial frontal gyrus (BA 6/pre-SMA) (15, 2, 59; 281 voxels).

4. Discussion

This study demonstrated abnormal cerebro-cerebellar activity in opioid-dependent, methadone-maintained patients in association with high working memory demands (manipulation condition). As hypothesized, cerebellar hyperactivity was observed in the patient group in the superior lobes bilaterally and in the right inferior lobe. Figure 4a illustrates that peak cerebellar hyperactivations in the current study (orange markers) were consistent with hyperactivations found in neuroimaging studies of alcoholism (red) [19] and cocaine dependence (green) [20]. In those studies, peak activation locations reported by the authors were converted into MNI coordinates and then mapped onto the superior cerebellum bilaterally (see Figure 4 caption for methodological details). Cerebellar hyperactivations by the patients in the present study also mapped directly onto cerebellar activations observed during high working memory demands in healthy, young adults (yellow) while performing this same task (Marvel & Desmond, submitted). Taken together, this pattern of activation corroborates the notion that 1) cerebellar activity increases under high working memory demands, and 2) a patient population with known working memory impairments will engage the cerebellum even more than controls do when attempting to achieve similar performance levels.

The observation of working memory-related hyperactivity in the inferior cerebellum in an addicted population is new. It has been proposed that the superior and inferior cerebellum make distinct contributions to working memory with the superior cerebellum supporting inner speech processes by creating motor memory traces [2], and the inferior cerebellum assisting with the phonological storage of information in a motor independent manner [11, 32]. Thus, functional abnormalities in both the superior and inferior cerebellum may reflect difficulties in working memory function on multiple levels. Future work, however, is warranted to tease apart whether working memory deficits lead to cerebellar hyperactivity or, alternatively, etiological disturbances in cerebellar function contribute to working memory deficits.

Patients additionally exhibited hyperactivity in the amygdala. The event-related fMRI analysis indicated that this hyperactivity was disproportionately associated with the high

working memory load manipulation condition. Visual inspection of amygdala activity on an individual participant basis (shown in Figure 3) clearly shows consistently increased activity in each of the patients, compared to consistently decreased activity in each of the controls. Thus, in the amygdala, the two groups responded differently to high working memory demands, but the source of this difference is not entirely clear. The amygdala is known to be part of the arousal and stress systems of addiction [33]. The stimuli in this paradigm were intended to be emotionally neutral, but it is possible that, on a trial by trial basis, the high working memory demands of the manipulation condition elicited anxiety and stress in the patients because it was harder for them to achieve a similar level of performance.

At a sub-threshold level of $p < .05$, hypoactivity in the left prefrontal cortex (BA 9) and in the medial frontal gyrus (BA 6/pre-SMA) was detected in the patient group. The locations of these hypoactivations were highly consistent with what has been reported in three previous studies of addiction, in which users (addicted to either alcohol, cocaine, or opiates) showed abnormal activation in these regions relative to controls during working memory-related tasks [19-21]. Figure 4b shows the location of activations in each of the three comparative studies, in the current study, and in a recent study conducted in healthy, young adults (Marvel & Desmond, submitted). Collectively, these reports suggest that two frontal regions—the left prefrontal cortex (BA 9) and the medial frontal/pre-SMA region (BA 6)—are involved in verbal working memory functions under high load demands, and are impacted by addiction. However, given that group differences in these regions were detectable only at a more liberal threshold, these differences were more subtle than those found within the cerebellum and amygdala.

Behavioral results between the groups did not differ, suggesting that the patient group was able to achieve normal levels of working memory performance. This contrasts findings from other studies that have found working memory deficits in opioid-dependent, methadone-maintained patients [34]. Visual inspection of the data suggests that with more participants, a RT difference could emerge between the groups. Regardless, the results at hand indicate that the fMRI data were more sensitive at detecting group differences than were the behavioral data, a dissociation that has been reported in several fMRI studies of addiction (e.g., [19, 21, 35-37]).

It is possible that group differences in neural activity were related to acute methadone exposure in the patient group. One might expect that, in the patient group, if opioid receptors were largely occupied by methadone, this would leave a significant number of opioid receptors unavailable for normal physiological function. A study by Kling et al. examined opioid specific binding in methadone-maintained former heroin users using positron emission tomography (PET) and the mu and kappa opioid receptor-directed binding radioligand [^{18}F]cyclofoxy [38]. Indeed, several brain regions showed significantly lower levels of radioligand binding in the patient group, meaning that many opioid receptors were occupied and unavailable. In decreasing order of overall specific binding levels, those regions were: thalamus, caudate, anterior cingulate, middle temporal cortex, and middle frontal cortex. Of these, only the middle frontal cortex was implicated (sub-threshold) in the current study. No differences in binding levels were observed between groups in the Kling et al. study in the amygdala or cerebellum, which were the two primary regions that showed between-group differences in the current study. Thus, it is possible that the observed hypoactivity in the prefrontal cortex of the patient group in the present study was related to high opioid receptor occupancy, thereby leaving a reduced number of receptors available for normal function. However, given that hyperactivity in the amygdala and cerebellum in the patient group did not follow this receptor-occupancy model, the observed group differences cannot be fully accounted for by this interpretation. Instead, it is more likely that the

observed effects are not solely related to methadone, but rather to years of chronic opioid use, or alternatively, to an inherent vulnerability that underlies general addiction pathology.

A limitation of this study is the small sample size. The criteria for inclusion into this study were highly selective in order to diminish the influence of potentially confounding variables that sometimes accompany research in drug-dependent populations. For example, none of the patients were co-morbid for depression or anxiety disorders. Though medicated, patients had been on a stable dose of methadone for at least 2 months, and had been in a methadone program for at least 10 months. None of the patients were co-morbid for alcohol dependence, which is important given the effects of alcohol on the cerebellum [39, 40], and groups were equated on lifetime alcohol exposure. Importantly, none of the patients had used any illicit substances in at least 7 months, meaning that the acute effects of intoxication and short-term abstinence had subsided. The highly selective process of screening patients for this study led to a lower number of study participants than in comparable studies described here (Yucel et al., $n = 24$; Hester and Garavan, $n = 15$; Desmond et al., $n = 10$). Notably, in two of those studies (Yucel et al.; Hester & Garavan), participants were active users. Due to the low N , neuroimaging results were rigorously tested in order to confirm that group differences were not due to outliers by conducting non-parametric analyses and inspecting individual ROI data (shown in Figure 3). The consistency of results across multiple analytic approaches indicated that these findings, particularly in the cerebellum and amygdala, were robust (at the $p < .001$ level) and reliable. Nevertheless, findings in a small sample size cannot be deemed conclusive, albeit compelling, and follow up studies will be needed to further explore cerebellar involvement of working memory problems in addiction.

Previous work in our laboratory has shown that when healthy adults silently manipulated letter stimuli while performing the same task that was used in this study, cerebellar activity in the superior cerebellum increased and accuracy decreased (Marvel & Desmond, submitted). This pattern of activity suggests that when working memory demands become exceedingly difficult, people continue to rely on inner speech mechanisms, perhaps intensely so, while struggling to perform the task. Thus, in the present study, the patients' hyperactivity in the superior cerebellum likely reflected a disproportionate degree of working memory difficulty that led to (or resulted from) increased inner speech activity. Interestingly, this same region of the cerebellum, along with the prefrontal cortex, has shown hyperactivity during drug cue-induced craving in studies of heroin, cocaine, and alcohol addiction [22, 41, 42]. Verbal working memory and drug craving, therefore, appear to be intertwined and to share common neural systems, of which the cerebellum is an integral part. Moreover, this pattern appears to be non-specific to dependence on any particular drug. Though highly speculative, a pattern of abnormal brain physiology, involving both the superior cerebellum and prefrontal cortex, could simply underlie a more general pathology in which working memory and aspects of inner speech may be involved (e.g., craving, ruminations, obsessions, preoccupations, and delusions) [5]. In addition to addiction, prefrontal and cerebellar abnormalities have been reported in a variety of psychiatric disorders involving working memory impairments, such as depression, anxiety, obsessive-compulsive disorder, and schizophrenia [43-50]. However, substantial research is needed to clearly identify the neural correlates of working memory, the limits of adaptive function within this neural system, and the indicators of brain functional impairment that are common and distinct within various psychiatric populations.

5. Summary

The results of this study indicated that, in opioid-dependent individuals, the superior and inferior cerebellum and amygdala were hyperactive in association with high verbal working memory demands. The location of the cerebellar hyperactivations, namely in the bilateral

superior lobes, and in the right inferior lobe, suggest the presence of inner speech and phonological storage deficits that help shed light on the nature of the working memory impairments in this population.

Acknowledgments

The authors would like to thank Jonathan Cooper for his assistance with scoring the data. MRI scans were obtained at the Kirby Center of the Kennedy Krieger Institute in Baltimore, MD. Parts of this research were presented at the Society for Neuroscience Annual Meeting 2009, and Organization for Human Brain Mapping Annual Meeting 2011. Funding for this study was provided by NIH grants K01 DA030442 (Marvel), K24 DA023186 (Strain), R01 MH060234 (Desmond), and the Nellie Ball Research Trust (Marvel).

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Delayed Item Recognition Task

a) Match Condition

b) Manipulation Condition

Figure 1.

A verbal working memory task was given under two conditions. a) In the match condition, participants encoded 1 or 2 targets (encoding phase). Note that in the example, 2 targets are shown, but on half the trials, only 1 target was shown. Then, participants silently rehearsed these targets across a delay (delay phase). Finally, at the presentation of a letter probe, subjects indicated whether the probe matched either of the targets (retrieval phase). b) In the manipulation condition, the encoding phase was identical to that in the match condition. However, in the delay phase, instead of simply rehearsing the targets, subjects counted two alphabetical letters forward of each. Then they rehearsed these newly identified targets. In the retrieval phase, subjects indicated whether the probe matched either of the newly identified targets (rather than the original targets). In both figures, the box wave indicates when stimuli were visible on the screen.

Figure 2.

Behavioral results. a) Mean accuracy for all trials as a function of condition (match vs. manipulation), target load (1 vs. 2 targets) and group (controls vs. patients). Significant accuracy effects were found for condition and target load, as well as for an interaction of condition $\times$ target load (i.e., low accuracy for the 2-item target load in the manipulation condition). b) Mean response times for correct responses as a function of condition, target load, and group. Significant response time effects were found for condition (slower on the manipulation condition) and target load (slower on 2-item trials). Error bars of 1 standard error are plotted for each bar in the graphs. Manip = Manipulation condition.

regions corresponding to the activations of interest are listed along with the proposed functional contributions of each region to the task. L = left, R = right.

Figure 4.

Consistency of group activation differences across studies of addiction and working memory. a) Location of increased cerebellar activations associated with high verbal working memory load as reported in four separate neuroimaging studies: 1) orange = opioid-dependent patients in the current study, 2) red = individuals with alcohol dependence [19], 3) green = individuals with cocaine dependence [20], and 4) yellow = healthy, young adults performing the same task that was administered in the current study (Marvel & Desmond, submitted). Peak activations in the comparative studies were obtained directly from the publications and converted to MNI coordinates using local software [27, 51]. Foci were plotted manually by CLM using Paint.NET software on coronal section $y = -58$, which was the mean y -axis location across the four studies, and overlaid onto the atlas section developed by Schmahmann et al. [28], shown in neurological orientation. Superior cerebellar activations were consistent across the studies. In addition, increased right inferior cerebellar activity was observed in the patients of the current study (orange) and in healthy, young adults (yellow). b) Location of group activation differences in the left prefrontal lobe (BA 9) reported in the comparative studies listed in part (a), plus a fifth study, shown in blue, that represents a study of opioid-dependence [21]. Activation locations were converted from Talairach to MNI coordinates as described in 4a. Foci were plotted using Tor Wager's

Meta Analysis Toolbox <http://www.columbia.edu/cu/psychology/tor/meta-analysis.html>. c) Location of group activation differences in the medial frontal gyrus (BA 6/pre-SMA), shown in the right hemisphere, with axes as in 4b. The orange markers in 4b and 4c represent hypoactivations by the patients in the current study, at the sub-threshold level of $p < .05$.