Cannabis Use and Memory Brain Function in Adolescent Boys: A Cross-Sectional Multicenter Functional Magnetic Resonance Imaging Study

Gerry Jager, Ph.D., Robert I. Block, Ph.D., Maartje Luijten, M.Sc., Nick F. Ramsey, Ph.D.

Objective: Early-onset cannabis use has been associated with later use/abuse, mental health problems (psychosis, depression), and abnormal development of cognition and brain function. During adolescence, ongoing neurodevelopmental maturation and experience shape the neural circuitry underlying complex cognitive functions such as memory and executive control. Prefrontal and temporal regions are critically involved in these functions. Maturational processes leave these brain areas prone to the potentially harmful effects of cannabis use. **Method:** We performed a two-site (United States and the Netherlands; pooled data) functional magnetic resonance imaging (MRI) study with a cross-sectional design, investigating the effects of adolescent cannabis use on working memory (WM) and associative memory (AM) brain function in 21 abstinent but frequent cannabis–using boys (13–19) years of age and compared them with 24 nonusing peers. Brain activity during WM was assessed before and after rule-based learning (automatization). AM was assessed using a pictorial hippocampal-dependent memory task. **Results:** Cannabis users performed normally on both memory tasks. During WM assessment, cannabis users showed excessive activity in prefrontal regions when a task was novel, whereas automatization of the task reduced activity to the same level in users and controls. No effect of cannabis use on AM-related brain function was found. **Conclusions:** In adolescent cannabis users, the WM system was overactive during a novel task, suggesting functional compensation. Inefficient WM recruitment was not related to a failure in automatization but became evident when processing continuously changing information. The results seem to confirm the vulnerability of still developing frontal lobe functioning for early-onset cannabis use. J. Am. Acad. Child Adolesc. Psychiatry, 2010;49(6):561–572. **Key Words:** cannabis, adolescence, early-onset, fMRI, memory

Early initiation of cannabis use increases the risk of later use/abuse of other drugs and drug dependence, and is associated with mental health problems such as psychosis and depression. The strength of this association appears to be dependent on the age when cannabis use begins.[1] A major concern that has only recently gained attention is the effect of early-onset cannabis use on adolescent brain function and neurodevelopment.

The still-developing adolescent brain differs anatomically and neurochemically from the adult brain[2,3] and is likely more susceptible to drug-induced adaptive neuronal plasticity.

Animal studies on the neural consequences of chronic cannabis exposure during the peri-adolescent period report changes in brain structure (predominantly limbic brain regions) and altered emotional and cognitive performance in later life.[4] However, these effects were mostly observed at relatively high doses of synthetic cannabinoids (Win 55,212-2; CP 55,940) and therefore may not be comparable to the human situation.

Studies in cannabis-using human adolescents

are still limited, but a number of functional magnetic resonance imaging (fMRI) studies have linked adolescent cannabis use to increased parietal activation along with diminished prefrontal activation during spatial working memory, and to increased parietal and prefrontal activation during inhibition, indicating reorganization of neural networks and recruitment of additional neural resources,[5,6] as previously reviewed.[7-9] Throughout early and late adolescence, maturational processes occur at different rates in various brain regions. Maturation is slightly delayed in the temporal and prefrontal cortex, regions critically involved in memory and learning and cognitive control.[2,10] The aim of the present study was to investigate the effects of adolescent regular cannabis use on memory-related brain function, with a focus on temporal and prefrontal regions. As there is evidence for gender differences in the rate and timing of neurodevelopment[11] and neurodevelopmental responses to cannabinoid exposure,[12] only boys were included in this study. We performed Blood-Oxygen-Level-Dependent (BOLD) fMRI using two tasks that reliably engage prefrontal and/or temporal brain regions, i.e., a verbal working memory (WM) task and a pictorial associative memory (AM) task, and compared regularly cannabis-using boys with age-matched nonusing peers. Previous fMRI studies from our laboratory in adult cannabis users and nonusing controls applied the same task paradigms.[13,14] These studies yielded no effects on task performance. However, with regard to brain activity, adult cannabis users displayed subtle alterations in superior parietal brain activity patterns during WM[13] and overall hypoactivity in a network of prefrontal and parahippocampal areas during AM.[14] In the present study, therefore, we would not expect a performance deficit, as the adults (having used for several years, i.e., longer than the current adolescent users) did not exhibit a deficit. For brain activity, we anticipated two possible outcomes. For one, effects of cannabis on brain activity could mimic those in the adults, i.e., altered parietal brain activity during WM and reduced activity during AM, suggesting age-independent effects of cannabis use. Alternatively, brain activity findings could differ from those found in adult users. In this case, we hypothesized hyperactivation to compensate for cannabis-induced dysfunction, most prominently in parahippocampal and prefrontal brain areas.

TABLE 1 Study Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
Male sex	Medical or neurological problems
Age 12–19 years	Regular use of illegal drugs other than cannabis (>10 episodes lifetime), except alcohol or nicotine
Right-handedness	Axis I psychiatric diagnosis, except for conduct disorder, which is a common diagnosis in cannabis-using boys
	IQ scores <80
	Use of psychotropic medication
	Contraindications for MRI (claustrophobia, metal objects, full dental braces)

Note: MRI = magnetic resonance imaging.

METHOD

This study was a joint venture of the University Medical Center Utrecht (the Netherlands) and the University of Iowa (United States). Data were pooled to increase statistical power.

Participants

In total, 47 boys, aged 13 through 19 years, were included in the study; 23 regular cannabis users (11 Dutch, 12 US) with at least 200 lifetime episodes of cannabis use, the others nonusing controls (12 Dutch, 12 US). Eligibility was ascertained through a screening procedure involving questionnaires on drug use, medical history, and mental health. Table 1 reports inclusion and exclusion criteria. Written consent was obtained from adolescents and their parent/legal guardian in accordance with the local IRB and conformed to the Helsinki Declaration of 2004. Adolescents were informed in advance that a parent/legal guardian had to provide signed informed consent and would be informed by the researchers about the group the adolescent was in (e.g., user/nonuser group) without revealing any details on history and/or pattern of cannabis use or other substances. Hence, parents/guardians knew in which group their son was, but we did not share information with them on the details of cannabis use, to promote the integrity of the drug use information we obtained from the youngsters. Subjects were reimbursed for participation with gift certificates.

Procedure

Subjects participated in two separate sessions separated by 1 week. The first involved screening for inclusion and exclusion criteria using self-report questionnaires on drug use history and a semistructured psychiatric inter-

view (NIMH Diagnostic Interview Schedule for Children; C-DISC–version IV). To estimate cannabis use parameters, a semi-structured self-report questionnaire was used, containing questions on the number of times subjects used cannabis during the last 3 months, the last year, and (if relevant) previous years, as well as the number of joints/blunts smoked per occasion. Lifetime number of cannabis use episodes was estimated through extrapolation. In addition, we estimated the number of joints smoked the previous year (number of episodes $\times$ average number of joints used per occasion) and number of joints lifetime, where we took into account periods of reported more or less frequent use.

To estimate IQ, subjects performed four subtests (similarities, block design, vocabulary and matrix reasoning) of the Wechsler Intelligence Scale for Children–4th edition (WISC-IV). The second session, 1 week after the first, included neuropsychological testing (results to be reported elsewhere) and fMRI scanning. Participants abstained from cannabis, alcohol, and other substances for at least 24 hours before the first session and remained abstinent until completion of the second session. Smoking was allowed until 2 hours before the scanning session (to avoid nicotine withdrawal). On both sessions, urine samples were collected for drug screening (enzyme-multiplied immunoassay for cannabis, alcohol, amphetamines, ecstasy, opiates, cocaine, and benzodiazepines). Exclusion followed on positive testing on any psychoactive substance on the second test day, except for cannabis, which can linger in the body for several weeks because of its lipophilic properties and thus can induce a positive test result even after 1 week of abstinence.[15] Instead, cannabis-using subjects were excluded only when their urine toxicology test failed to show a decrease in quantified levels (μg/L) of cannabinoid metabolites (THCCOOH) between the first and second urine sample taken. Based on the laboratory results, two subjects had to be excluded from analyses. Two other subjects had a positive urine test on cannabinoids during the second session, but THCOOH-levels were decreased compared with the first measurement 1 week earlier, which was consistent with self-reported abstinence. All other subjects had negative urine tests on the day of testing.

Assessment of WM and AM
Two fMRI tasks were administered: a verbal WM task (Figure 1) based on Sternberg's item-recognition paradigm (denoted STERN), and a pictorial AM task (denoted PMT; Figure 2). Both tasks have previously been described in detail.[13,14] STERN assesses the WM system before and after practice (automatization). Subjects were instructed to memorize a set of five letters (memory set) and subsequently to respond to single letters (probes) by pressing a button if the probe was in the memory set (target). A novel (NT) and a practiced task (PT) were administered. In PT, a fixed memory set was used repeatedly, on which subjects were trained before scanning to induce automatization. In the NT, the composi-

FIGURE 1 Temporal sequence of events for the working memory task. Note: Each epoch starts with presentation of the memory set (a set of five consonants, for example, "FGMPT"), and is followed by 10 trials showing a single consonant. Subjects have to press a button as fast as possible if the letter belongs to the memory set.
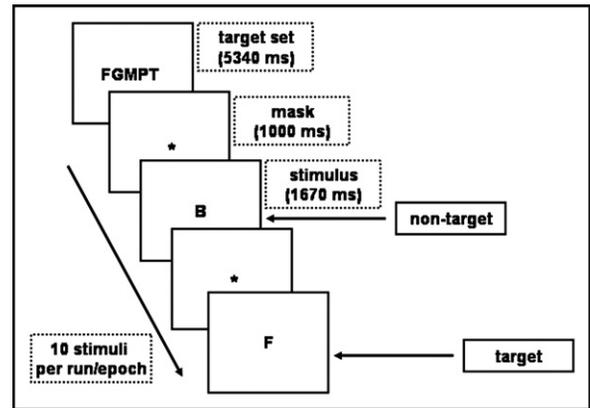

tion of the memory set was changed after every epoch. An additional reaction time control task (CT) was included during which subjects made a button press when the symbol "< >" appeared. In the scanner, each task (CT, PT, and NT) was presented in six epochs (duration, 29 seconds) of 10 stimuli each, as well as six rest periods of equal epoch duration.

PMT assesses (para)hippocampal-dependent AM and involves three tasks. First, an associative learning task (AL) is performed that requires subjects to establish a meaningful connection between two pictures and to memorize the combination. Next, single pictures have to be classified (SC), which serves as a control task; i.e., compared with AL it requires the same amount of perceptual processing and a motor response, but it lacks the associative learning component. Finally, the retrieval task (RE) asks subjects to recognize specific combinations previously presented during AL, and provides a performance measure. Each task was presented in three epochs (duration, 65 seconds) of eight stimuli each, as well as three rest periods of equal duration.

fMRI acquisition
Imaging was performed using a clinical 3 T MRI scanner (Philips Achieva [the Netherlands] and Siemens Magneton Trio [US]), both with an eight-channel head coil. Pilot data on various T1 and EPI scan sequences from three subjects (i.e., researchers G.J. and N.F.R. and a research assistant, J.Z.) were tested for homogeneity of signal-to-noise (SNR) and temporal signal-to-noise (tSNR) ratios across sites and vendors. The scan parameters yielding highest similarity between SNR and tSNR maps across scanners were used for both tasks: TE/TR 35/2000 ms, flip angle 70°, field of view (FOV) 256 $\times$ 256 mm, acquisition matrix 64 $\times$ 64, slice thickness 3.6 (plus a

FIGURE 2 Temporal sequence of events for the associative memory task. Note: Each epoch starts with an instruction slide (5 seconds) followed by a fixation cross (2.5 seconds). This is followed by eight trials of 7.5 seconds each (picture pair 5 seconds, fixation cross 2.5 seconds). Subjects respond by pressing one of two buttons, according to the instruction in each task condition.
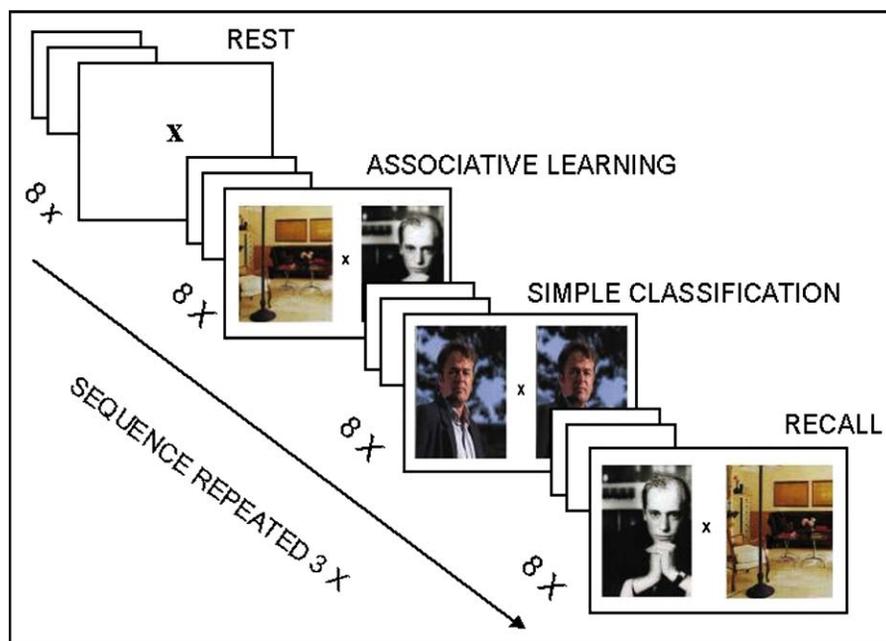
0.4-mm gap), voxel size 4.0-mm isotropic, 26 slices, scan orientation transaxial for STERN and parallel to the long axis of the hippocampus for PMT. Details on the issue of multisite studies and scanner compatibility are presented in Supplement 1 and Figure S1 (available online). For STERN, a single run of 312 scans was acquired over a period of 10.4 minutes. For PMT, a single run of 324 scans lasted 10.8 minutes. In addition, a volumetric T1-weighted MR anatomical scan was acquired for spatial localization (TR 25 ms, flip angle 30°, FOV read 256 mm, voxel size 1.0 mm isotropic, 176 slices, scan duration 7.8 minutes).

Statistical Analysis

Sample Characteristics and Drug Use. Cannabis parameters were as follows: age of onset, cumulative use lifetime (estimated number of joints), current or recent use (number of joints last year), frequency and duration of use, and abstinence (weeks since last use). Additional drug use data included last year use of alcohol (average number of drinks/week), tobacco (average number of cigarettes/week), and lifetime use of other illegal substances (number of occasions lifetime). Because distribution of the drug variables was skewed, scores were log-transformed, except for age of onset of cannabis, which was normally distributed. Other variables included age, estimated IQ, country (US versus NL), and a diagnosis of conduct disorder (yes/no). Group differ-

ences were tested using *t* tests and nonparametric Kolmogorow-Smirnov Z tests.

Task Performance. Outcome measures included reaction times (STERN only) and accuracy. General linear model (GLM) repeated measures analysis was applied, with task condition (CT, PT, and NT for STERN; AL, SC, and RE for PMT) and outcome measure (reaction time and accuracy) as within-subject factor, and group (user versus control) as between-subject factor. To adjust for normal developmental effects, i.e., younger boys performing relatively worse than older ones, age was included as a covariate.

fMRI. Imaging data were analyzed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm). Pre-processing included realignment (motion correction) and unwarping, coregistration, normalization, and smoothing with an 8-mm (FWHM) Gaussian kernel. First, statistical activity maps were generated for each subject for all task conditions (CT, PT, and NT for STERN; AL, SC, and RE for PMT) by analyzing time series data with multiple regression analyses using a vector representing the design of a task and including cosine basis functions to remove low frequency drifts in the signal (details on preprocessing and first-level statistical analysis in Supplement 1, available online). Next, for STERN, individual contrast maps were created for NT and PT, corrected for individual offset activity levels (i.e., subtracting activity levels during the control task [CT], representing baseline activity unrelated to WM processing, from activity levels during

TABLE 2 Regions of Interest during Working Memory and Associative Memory Tasks

Task	Region	Brodmann area	No. of voxels	X	Y	Z	T-max
STERN	ACC	6/24	77	−4	12	48	9.55
	l-PCC/DLPFC	9/46	48	−40	0	36	6.21
	l-IFG	47	35	−36	24	0	6.13
	l-SPC	39	6	−28	−60	36	4.73
PMT	r-PHG/MOG	36/37/19	310	36	−52	−20	13.49
	l-PHG	36/37	139	−36	−48	−20	12.80
	r-DLPFC	9/46	43	48	28	20	8.74
	ACC	6/24	20	4	20	44	7.68
	l-DLPFC	46	17	−44	20	20	7.18
	l-MOG	19	17	−24	−92	4	7.60

Note: Montréal Neurological Institute (MNI) coordinates for the regions of interest involved in working memory (Sternberg item-recognition task [STERN]) and associative memory (pictorial memory task [PMT]). The coordinates X, Y, and Z represent location of the voxels with the highest t value in the group map. ACC = anterior cingulate cortex; IFG = inferior frontal gyrus; l = left; MOG = middle occipital gyrus; PCC/DLPFC = precentral/dorsolateral prefrontal cortex; PHG = parahippocampal gyrus; r = right; SPC = superior parietal cortex.

NT and PT, respectively). Similarly, for PMT, we created individual activity maps for AL and RE, corrected for individual offset activity levels during the control condition, contrasting both AL and RE with SC. Contrast maps were then used in a second-level whole-brain analysis to test for effects of cannabis use on brain activity. Age and a dichotomized variable for country (0 for the Netherlands; 1 for the United States) were added as covariates, to take into account potential systematic effects of age and/or differences in MRI scanners across sites. In addition to whole-brain analysis, region-of-interest (ROI) analyses were performed. To check whether users and nonusers activated similar networks of brain regions during WM (as expected, as there is no *a priori* reason to assume that cannabis users show significant functional reorganization of the brain), we defined ROIs for both groups separately (group contrast maps; NT-CT for STERN and AL-SC for PMT, p < .05, FWE corrected). Visual inspection of the group activation maps indicated no differential activation patterns between users and controls. Therefore, ROIs used for further analyses (Table 2) were derived from group contrast maps (NT-CT for STERN and AL-SC for PMT) of all subjects combined (p < .05, FWE corrected). For STERN (Figure 3), this yielded activated regions in the left superior parietal cortex (l-SPC), left inferior frontal gyrus (l-IFG), left precentral and dorsolateral prefrontal cortex (l-PCC/DLPFC), and anterior cingulate cortex (ACC). For PMT, activated regions included bilateral regions in the parahippocampal gyrus, middle occipital gyrus, and prefrontal areas (Figure 4). ROIs for both tasks involved regions known from previous studies from our laboratory, using the same task paradigms,[13,14,16] and hence, met *a priori* expectations. ROIs were marked and activity values for all subjects were obtained per ROI by averaging β-values across all contained voxels for NT and PT, and for AL and RE (corrected for individual offset activity levels), using the Marsbar toolbox in SPM5.[17] Finally, these

variables were entered into GLM repeated-measures analyses with task conditions (NT and PT for STERN, AL and RE for PMT) and ROIs as within-subject factors, and group as between-subject factor. Similar to the whole-brain analyses, country and age were entered as covariates in all ROI analyses.

Potential Confounders. Nine of 12 US cannabis users met criteria for a diagnosis of conduct disorder, which might act as a confounding factor. However, as the co-occurrence of conduct disorder was restricted to the US users, it was strongly correlated with the factor country (which was included as a covariate in all analyses, together with age), and the effects of conduct disorder could not be disentangled from those of country. Cannabis use in both US and Dutch users correlated significantly with lower IQ, alcohol use, and tobacco use. Any significant group difference found on output parameters (task performance, brain activity) resulting from the main analyses (see previous paragraph) was therefore considered rather an effect of a "cannabis-using lifestyle" than an effect of cannabis use alone. Yet, to explore the relative strength of the effect of cannabis alone, output parameters were recomputed after controlling for confounding effects of IQ, use of alcohol and tobacco by means of multiple regressions, i.e., saving the standardized residuals. In a subsequent analysis, we then re-entered these standardized residuals into group analyses (analysis of variance [ANOVA], GLM repeated measures). This constitutes a conservative approach whereby interactions between effects of cannabis and other effects are prevented from leaking into the "cannabis" effect.

RESULTS

Two users were excluded based on positive urine drug test results, and STERN fMRI data were lost for one control because of technical malfunction.

FIGURE 3 Regions of interest for the working memory task. Note: (A) Left inferior frontal gyrus. (B) Left precentral/ dorsolateral prefrontal cortex. (C) Left superior parietal cortex. (D) Anterior cingulate cortex. Regions of interest are based on the contrast between novel and control task (p < .001). Numbers above slices indicate z coordinates of the Montreal Neurological Institute system. Slices are in neurological orientation (i.e., left side is left hemisphere).

Results are reported for 45 participants (21 users, 24 controls) for sample characteristics, drug use, and PMT data, and for 44 participants (21 users, 23 controls) for STERN.

Sample Characteristics and Drug Use
Table 3 summarizes sample characteristics and drug use for users (US and Dutch) and nonusers (US and Dutch). Users were, on average, abstinent for 5.1 weeks (±4.2). Groups did not differ in age, but users had significantly lower IQ-scores than controls (p < .01), and reported significantly higher previous-year consumption

of alcohol (mean alcoholic drinks per week 13.3 [± 13.6] for users compared to 3.4 [± 5.8] for controls) and tobacco (mean number of cigarettes smoked per week 63.8 [±53.4] for users compared with 6.1 [±14.9] for controls). In addition, demographic and cannabis use parameters were compared for the user and nonuser groups separately, comparing US subjects with Dutch subjects, to identify potentially site related biases. Dutch users were older than US users (mean age 17.9 years [++ 0.9] and 16.7 [++ 0.8] respectively, p < .05), but no significant differences were found on IQ scores or cannabis use param-

FIGURE 4 Regions of interest (ROI) for the associative memory task. Note: (A and B) Left and right (para)hippocampal gyrus (PHG). (C) Left middle occipital gyrus. (D and E) Right and left dorsolateral prefrontal cortex. (F) Anterior cingulate cortex. ROIs are based on contrast-associative learning versus simple classification (p < .001). Numbers above slices indicate z coordinates of the Montreal Neurological Institute (MNI) system. Slices are in neurological orientation (i.e., left side is left hemisphere).
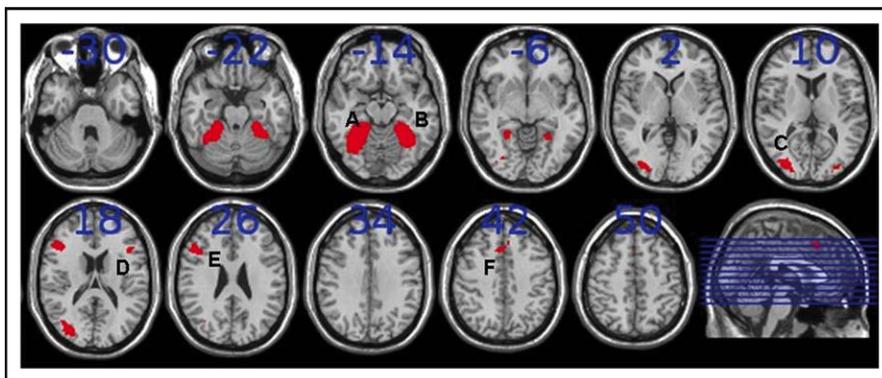
TABLE 3 Demographic Characteristics and Drug Use (N = 45)

Characteristic	Users (N = 21)	Controls (N = 24)	p^a
Age	17.2 (1.0, 15–19)	16.8 (1.3, 13–19)	NS
IQ	101 (10.7, 82–116)	111 (11.6, 94–138)	<.01
Cannabis use			
Lifetime (no. of joints)	4,006 (7,555, 224–32,850)	1.8 (4.0, 0–15)	<.001
Last year (no. of joints)	741 (772, 208–3528)	1.0 (2.9, 0–12)	<.001
Age of onset (years)	13.2 (2.3, 8–16)	15.0 (1.6, 12–17)	NS
Abstinence (weeks since last use)	5.1 (4.2, 1–16)		
Other substances (use last year)			
Alcohol (drinks/week last year)	13.3 (13.6, 0–46)	3.4 (5.8, 0–24)	<.01
Tobacco (cigarettes/week last year)	63.8 (53.4, 0–144)	6.1 (14.9, 0–53)	<.001
Ecstasy (episodes lifetime)	0.3 (0.9, 0–4)	0.1 (0.4, 0–2)	NS
Amphetamines (episodes lifetime)	1.3 (2.4, 0–7)	–	
Cocaine (episodes lifetime)	0.4 (0.9, 0–4)	–	
Psilocybin (magic mushrooms) (episodes lifetime)	0.4 (0.6, 0–2)	0.1 (0.4, 0–2)	NS
LSD (episodes lifetime)	0.1 (0.2, 0–1)	–	
Laughing gas (episodes lifetime)	0.7 (2.1, 0–9)	0.1 (0.4, 0–2)	NS
Benzodiazepines (episodes lifetime)	2.86 (8.8, 0–40)	0.2 (0.8, 0–4)	NS
C-DISC			
Conduct disorder (yes/no)	n = 9 (yes), n = 12 (no)	n = 24 (no)	<.001

Note: Data are mean with standard deviations and ranges in parentheses. C-DISC = Children's Diagnostic Interview Schedule; LSD = Lysergic acid diethylamide; NS = not significant.

^{a}Significance of differences calculated using independent-samples t tests (age, Intelligence Quotient [IQ], age of onset) and nonparametric Kolmogorov-Smirnow Z tests, two-tailed.

eters. Nine US users had a C-DISC diagnosis of conduct disorder, whereas none of the Dutch users or the controls (both US and Dutch) had such a diagnosis.

Task Performance
Figure 5 shows performance data for the study participants.
WM (STERN). Users did not differ from controls on reaction times and accuracy during the STERN task. On average, reaction times and accuracy were similar to adult performance levels as observed in our previous study,[13] indicating normal behavioral WM capacity and efficiency. A main effect of age was observed (F(1,40) = 11.91, p = .001), indicating that older boys performed faster and more accurately than younger boys, but this was independent of cannabis use.
AM (PMT). Users performed equally accurate as controls, and accuracy levels were comparable to those observed in adults,[14] indicating that AM performance was unaffected by cannabis use. No effects of age were found.

fMRI data
Scanner compatibility (details in Supplement 1, available online) was quantified by a direct com-

parison of temporal signal-to-noise maps (tSNR) maps for both STERN and PMT imaging data between the two sites, using nonparametric statistics in SPM5 (SnPM5-toolbox; see also www.sph.umich.edu/~nichols/SnPM/). The results revealed some areas with differential tSNR between sites, predominantly in the orbito- and ventromedial prefrontal regions. However, there was no overlap between regions showing scanner-related differences in tSNR and regions of interest (ROI; see below) for the WM or AM task.
WM (STERN). A second-level whole-brain analysis in SPM5 (p < .05, FWE corrected) revealed no significant differences between users and controls in working-memory–related brain activity before or after practice (NT-CT and PT-CT contrast respectively). A subsequent ROI- GLM repeated-measures analysis (Table 2 and Figure 3 for ROIs), with group as between-subjects factor, task (NT, PT) and ROI (l-IFG, l-SPC, l-PCC/DLPFC, ACC) as within-subjects factors, and age and country as covariates, yielded a significant task × group interaction (F(1,40) = 12.85, p = .001). Separate GLM analyses for NT and PT showed that the WM system tended to be overactive before automatization (NT) in users (F(1,40) = 2.77, p = .10; Figure 6). They showed

FIGURE 5 Behavioral data. Note: (A) Working memory task: mean reaction time ± standard error of mean (SEM) of correct responses on targets for both groups and mean percentage of errors as percent of all trials (±SEM) during the control task (CT), after (PT), and before practice (NT). (B) Associative memory task: accuracy during simple (percentage correct responses) classification (SC) and recognition (RE) (± SEM) for both groups. CAN = cannabis users; DN = drug-naive controls.
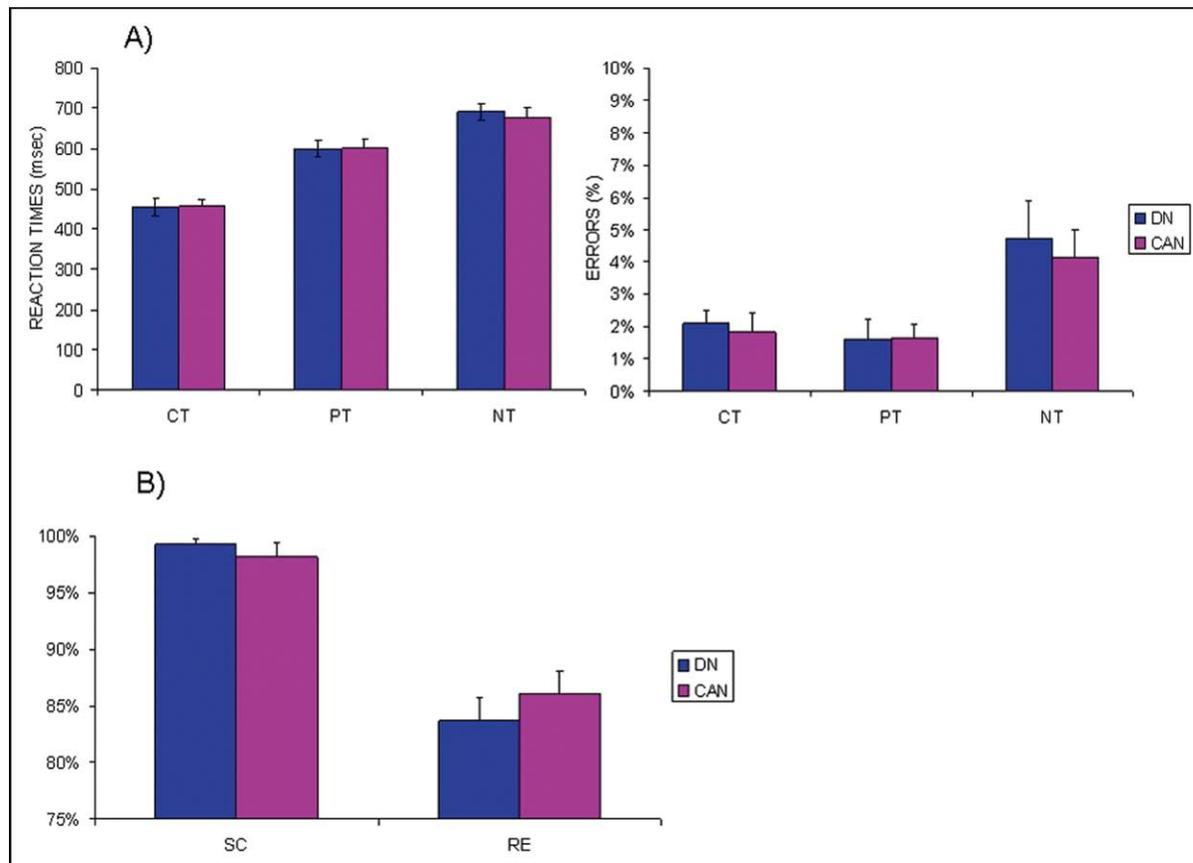
significantly larger differences in activity before and after practice, e.g., NT-PT contrast values in the l-IFG (F(1,40) = 12.04, p = .001), the l-PCC/ DLPFC (F(1,40) = 22.37, p < .001), and ACC (F(1,40) = 5.42, p = .03). As learning (PT) reduced activity in the WM system to the same level in both groups in most areas, this indicates overall excessive effort in users to achieve normal performance when a task is novel (Figure 7).

AM (PMT). A second-level whole-brain analysis in SPM5 (p < .05, FWE corrected) yielded no significant group differences in associative learning or recognition-related brain activity (AL-SC and RE-SC contrasts, respectively). Neither did a subsequent ROI analysis (all p values >.20; Table 3 and Figure 4 for details on ROIs).

Potential Confounders. To explore the relative strength of the effect of cannabis alone on ROI between-group differences in WM brain activity,

average β values across all contained voxels per ROI were recomputed by running multiple regression analyses, including IQ, alcohol, and tobacco use as regressors, and saving the standardized residuals. Standardized residuals, which were now devoid of effects of IQ, alcohol, and tobacco, were entered into GLM repeated-measures analyses. The task × group interaction remained significant (F(1,40) = 6.81, p = .01). *Post hoc* ANCOVA with age and country as covariates revealed that even after adjustment for effects of IQ or alcohol and tobacco use, the group differences in NT-PT contrast remained significant in l-IFG and l-PCC/ DLPFC (F(1,40) = 5.99, p = .02 and F(1,40) = 12.05, p = .001, respectively) but became marginally significant in ACC (F(1,40) = 2.56, p = .10).

In users (N = 21), we assessed whether cannabis use parameters (number of joints, age of onset) predicted ROI-specific difference in activity before

FIGURE 6 Activity levels per region of interest for working memory task (in arbitrary units [AU]) after practice (PT) and before (NT) for both groups. Note: Activity levels were marginally higher in users compared with controls during NT ($F(1,40) = 2.77$, p = .10), but no significant group effects were found during PT. ACC = anterior cingulate cortex; l-IFG = left inferior frontal gyrus; l-PCC/DLPFC = left precentral/dorsolateral prefrontal cortex; l-SPC = left superior parietal cortex.
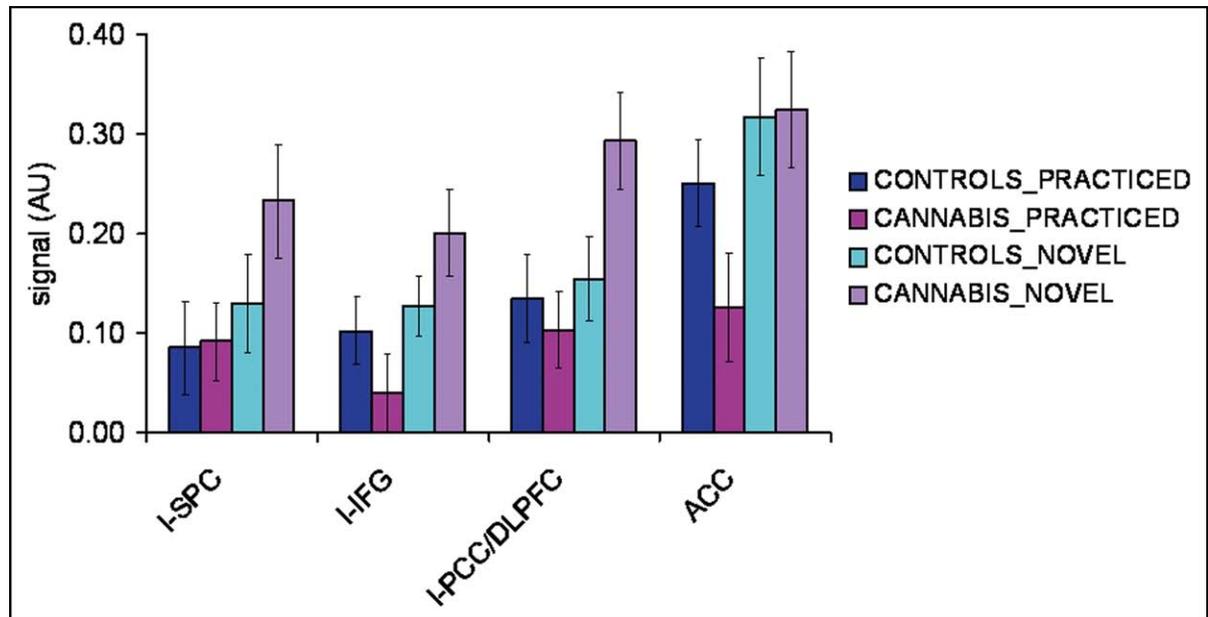
and after automatization (NT-PT contrast). In the l-IFG, number of joints last year was significantly correlated to the difference in activity before and after automatization (Spearman's rho = 0.52, p < .05), whereas for number of joints lifetime there was a trend (rho = 0.42, p = .07), tentatively indicating that part of the effects found was selectively associated with cannabis use.

FIGURE 7 Contrast values per region of interest for the working memory task (in arbitrary units [AU]), i.e., difference in activity before and after practice. Note: ACC = anterior cingulate cortex; l-IFG = left inferior frontal gyrus; l-PCC/DLPFC = left precentral/dorsolateral prefrontal cortex; l-SPC = left superior parietal cortex.
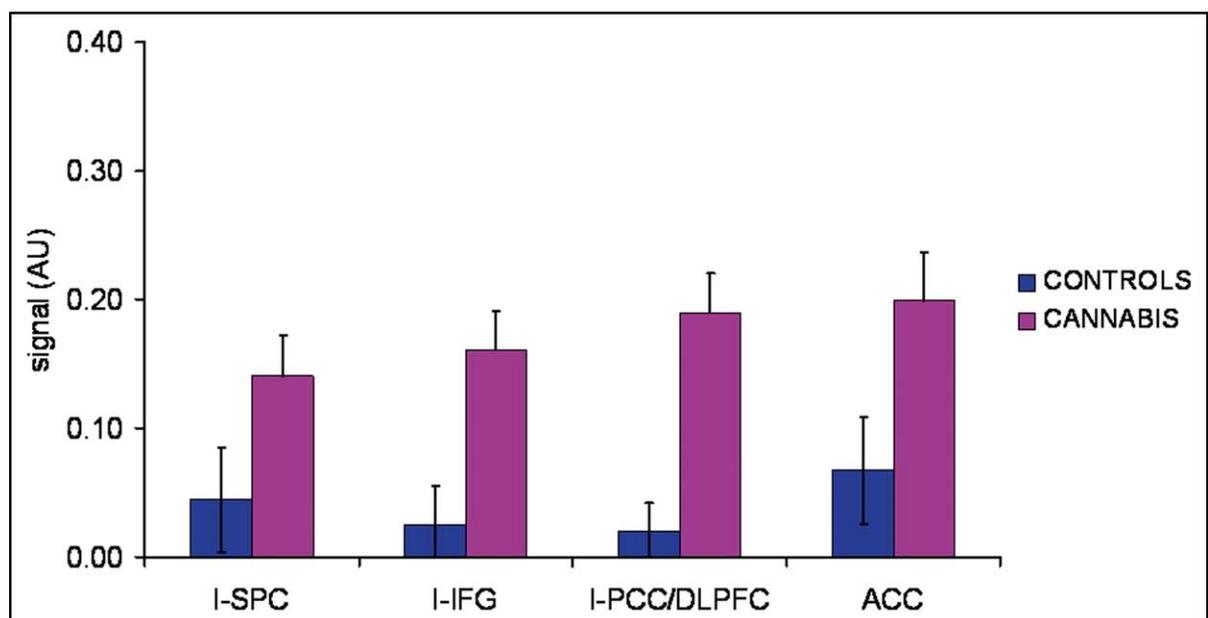
DISCUSSION

This study examined the effects of regular cannabis use on adolescent WM and AM brain function. No evidence was found for effects of cannabis use on AM, at either the behavioral or at the neurophysiological level, but the WM system was overactive in users during a novel task, whereas automatization reduced overall activity to the same level in users and controls.

Our findings extend those of previous studies in adolescents with cannabis and alcohol use disorders reporting increased dorsolateral prefrontal activation during a spatial WM task.[18] Increased activity levels in hippocampal and parietal regions have also been observed in abstinent cannabis using teens during verbal WM,[19] all with normal performance levels.

We have previously observed subtle alterations during WM in the superior parietal cortex in adult cannabis users, but not in prefrontal regions.[13] The present results, therefore, support our hypothesis of age-specific effects of adolescent cannabis use on still-developing brain function and seem to confirm the vulnerability of developing frontal lobe functioning.

Animal studies on the neural consequences of cannabis exposure during adolescence suggest greater, more persistent memory deficits and hippocampal abnormalities in adolescent than in adult animals.[20] Contrary to what we expected, our results yielded no proof of impaired AM performance or temporal lobe dysfunction. Our findings are, however, in line with neuropsychological data in cannabis-using teenagers indicating significant impact of cannabis on spatial WM and verbal learning but not on associative learning.[21] In adult cannabis users, we have found hypoactivity compared with that in controls in (para)hippocampal regions and the right dorsolateral prefrontal cortex during AM.[14] A recent study by Nestor et al.,[22] however, reports parahippocampal hyperactivity and frontocortical hypoactivity in adult users during an associative face–name learning task. The current study fails to replicate either of these results. One possible explanation for the discrepancies between effects of frequent cannabis use on AM brain function between adolescents and adults may be different abstinence periods, as they vary between short intervals (mean 15 hours; range 2–45),[22] at least 1 week, but on average not much longer,[14] and on average 5 weeks (range 1 week to 4 months) in the present study. It has been argued repeatedly that the nonacute effects of cannabis use on brain functioning might vary according to the duration of abstinence[23] and that this may even be different in adolescents.[8]

This study has several limitations. Groups differed on a number of key variables, with users displaying lower estimated IQ scores and greater alcohol use and tobacco-smoking histories. Although the main findings remained unchanged after controlling for these factors, and although cannabis use parameters were linked to the excessive activation in the left inferior frontal gyrus during the novel task, use of alcohol and tobacco pose the possibility of synergistic effects. Together, these factors may be considered to constitute a "cannabis-using lifestyle" that may be predictive for detrimental effects on development of cognition and brain function. The high prevalence of conduct disorder in the US users (nine of 12 subjects) may be related to site differences in recruitment strategies. In the Netherlands, subjects were recruited using a variety of strategies, including advertisements on the Internet, and with help of schools. In the United States, however, recruitment was restricted to local substance abuse councils and adolescent health and resource centers offering education and treatment programs to minors who got involved with the legal system because of possession of cannabis or other drugs. Boys ending up in these programs may display more externalizing behavior problems and more often meet criteria for conduct disorder. Banich et al.[24] compared brain activation patterns during a color–word Stroop interference task between adolescents with severe substance and conduct problems and controls. Similar to our results, these investigators found that patients needed to engage prefrontal brain regions to a greater extent than did controls during the interference condition to obtain the same level of performance. However, the question remains whether these differences in brain activation are a predisposing factor in patients with severe, comorbid conduct and substance problems, or whether the differences result from the prior ingestion of illicit substances. The design of our study did not permit conclusions on the nature of the potential confounding effect of conduct disorder, as presence of conduct disorder was country-specific and could not be disentangled from other site-related differences. Still, as any effects related to country differences were regressed out, we believe that we are justified in arguing that the confounding influence of conduct disorder on the main findings is limited.

Besides the advantage of increased power, the

multicenter design also poses a challenge in terms of scanner compatibility. We assessed scanner compatibility in the preparation stage of the study, opted for scan sequences that showed the greatest similarities in scans across sites, and, after study completion, used several statistical methods to further minimize and/or quantify systematic effects due to site-related differences in scanner equipment. Nonetheless, we cannot completely rule out the influence of scanner differences on between-subjects variability, predominantly in the orbito- and ventromedial regions. These areas are involved in inhibitory processes and decision making, and altered brain function in these areas has been reported in relation to chronic cannabis use/abuse and/or use of other drugs.[25,26] We acknowledge limited sensitivity to detect cannabis-related effects in orbito- and ventromedial regions in the present study. However, the experimental paradigms applied in the present study are unlikely to elicit activations in those areas. Hence, we feel confident that the results of our ROI analyses have not been compromised by the multicenter approach.

A third limitation is the cross-sectional design, and hence, the possibility that the observed differences in WM related brain function predated the onset of regular cannabis use. There is ample literature on genetic, neurobehavioral, and personality profiles increasing the liability to substance use and abuse, as reviewed elsewhere.[27,28] Such pre-existing factors may both increase the tendency of adolescents to get involved in risky behaviors such as drug use/abuse, and may affect neurocognitive development. Finally, the inclusion of cannabis-using boys, only, excludes the possibility to explore gender-specific differences in the impact of adolescent cannabis use on cognition and brain function, for which there is tentative evidence from animal studies.[13]

Future neuroimaging studies should attempt to elucidate the structural and neurochemical correlates that underlie the observed alterations in brain activation in cannabis users during a cognitive challenge, because the clinical significance of these alterations is far from clear.[23] Findings of increased brain activation combined with normal performance are commonly interpreted as functional compensation to maintain normal task performance. Yet, a warning against simplistic or mechanistic interpretation of increased versus decreased brain activation in the absence of differences in task performance seems appropriate, especially with regard to fMRI studies in adolescents. During adolescence, both brain maturation and learning and experience shape the neural circuitry that underlies cognitive brain function, resulting in highly dynamic changes in brain function over time.[2] Hence, alterations in brain activity patterns as observed in the present study may signify persistent dysfunction but, alternatively, may also reflect a shift or delay in normal neurodevelopmental changes in cognitive brain function. In this context, it is interesting to note that our current findings of excessive activation in several prefrontal areas during the most demanding task condition show similarities with a study on automatization and WM capacity in schizophrenia by Van Raalten et al.[16] This study reports that patients with schizophrenia, who often display deficits in executive functioning, displayed similar levels of brain activity after automatization compared with controls; however higher levels of brain activity during the novel, more demanding task indicated inefficient WM function and a failure to properly engage the WM brain system when task demands increase.[16] The present results resemble these findings. It has been suggested, based on overlap in cognitive dysfunction associated with long-term cannabis use and schizophrenia, that with regard to the neurobiology underlying the dysregulation of higher-order cognitive processes, the endocannabinoid system may be implicated.[29] The endocannabinoid system could be involved, either directly or through its interactions with other neurotransmitter systems, notably dopamine, in the development of similar cognitive deficits associated with both cannabis abuse and schizophrenia.[30] This notion is consistent with the increasing number of studies detecting cognitive dysfunction in adolescent cannabis users[7-9] as well as a greater incidence of juvenile psychotic symptoms and other mental health problems.[31] During critical periods of neurodevelopment, in particular, adolescence, cannabis use may have more impact, especially in otherwise genetically predisposed individuals, and may precipitate the onset of psychosis.[32] This is not to say that cannabis use itself causes psychosis in young people, but that a complex association between cannabis use and schizophrenia may be due to dysfunction of the endocannabinoid system,[29] which may be reflected in similarities in abnormal neurophysiology underlying cognitive brain functions.

In conclusion, teenage cannabis use may reduce the ability to process information requiring frequent updating. The present study indicates that predominantly prefrontal brain regions are prone to adverse consequences of cannabis use during

this stage of life. Whether the effects of adolescent cannabis use on working memory–related brain activation persist over longer periods of abstinence, as well as their clinical relevance in terms cognitive dysfunction, remains to be determined. &

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SUPPLEMENT 1

Magnetic resonance imaging (MRI) scanner compatibility. This study was a joint venture of the University Medical Center Utrecht (the Netherlands) and the University of Iowa (United States). Imaging data were collected using two clinical 3.0-Tesla MRI scanners, both with an eight-channel head coil but from different vendors (Philips Achieva in Utrech and Siemens Magneton Trio in Iowa. As data were pooled to increase statistical power, the issue of scanner compatibility had to be dealt with, focusing both on scanner hardware and software. The following approach to assess scanner compatibility and to overcome problems resulting from scanner differences was adopted.

First, in the preparation stage of the study, pilot data were collected of three subjects (i.e., researchers G.J. and N.F.R.) and a research assistant (J.Z.) on both scanners, acquiring several try-out three-dimensional (3-D) anatomical scans and functional time series (using different TEs, TRs and flip angles). Data were tested for homogeneity of signal-to-noise (SNR) and temporal signal-to-noise (tSNR) ratios across sites and ven-

dors. Three pulse sequences were compared: 3D PRESTO, fast 2D-EPI, and slow 2D-EPI. The first two scans involved SENSE/GRAPPA (both scanners had the same eight-channel head coil) and yielded different tSNR maps. Moreover, we were not able to make the pulse sequences exactly the same for both scanners. The slow 2D-EPI scan, which uses the eight channels but does not apply any SENSE or GRAPPA, turned out to be the best in terms of the SNR and tSNR (data not reported) and was selected as the scan with which to proceed. All scan parameters were the same (TE/TR 35/2000 ms, flip angle 70°, FOV 256 × 256 mm, acquisition matrix 64 × 64, slice thickness 3.6 (plus a 0.4-mm gap), voxel size 4.0 mm isotropic, 26 slices, scan orientation transaxial for STERN and parallel to the long axis of the hippocampus for PMT). The only difference across scanners was an opposite step-direction of the phase-encoding gradient. This caused a difference in the orbitofrontal region, where macro-susceptibility causes some signal loss and signal becomes shifted away from the brain midline (due to rapid signal decay and a concomitant T2*

FIGURE S1A Graphic presentation of the results of a nonparametric two-sample t test for the working memory task (STERN) contrasting signal-to-noise (SNR) images from the US scanner (n = 23) and Dutch scanner (n = 21), family-wise error (FWE)–corrected p value = .05 (corresponding threshold value of T = 5.11). Note: In green, the areas that show significantly higher SNR in US scans compared with Dutch scans. In blue, the areas of the opposite contrast, i.e., significant higher SNR in Dutch scans compared with US scans. In red (superimposed), the regions of interest (ROI) for STERN, used in the ROI analysis. Images are in radiological orientation, i.e., left = right.
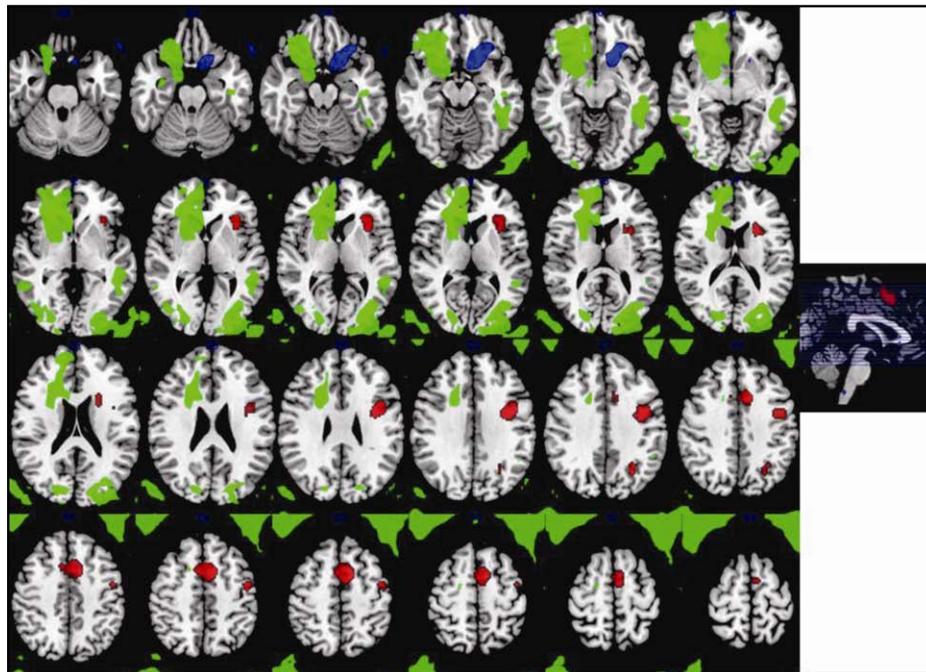
FIGURE S1B Graphic presentation of the results of a nonparametric two-sample t test for the associative memory task (PMT) contrasting signal-to-noise (SNR) images from the US scanner (n = 24) and Dutch scanner (n = 21), family-wise error (FWE)–corrected p value = .05 (corresponding threshold value of T = 5.18). Note: In green, the areas that show significantly higher SNR in US scans compared with Dutch scans. In blue, the areas of the opposite contrast, i.e., significant higher SNR in Dutch scans compared with US scans. In red (superimposed), the regions of interest (ROI) for PMT, used in the ROI analysis. Images are in radiological orientation, i.e., left = right.
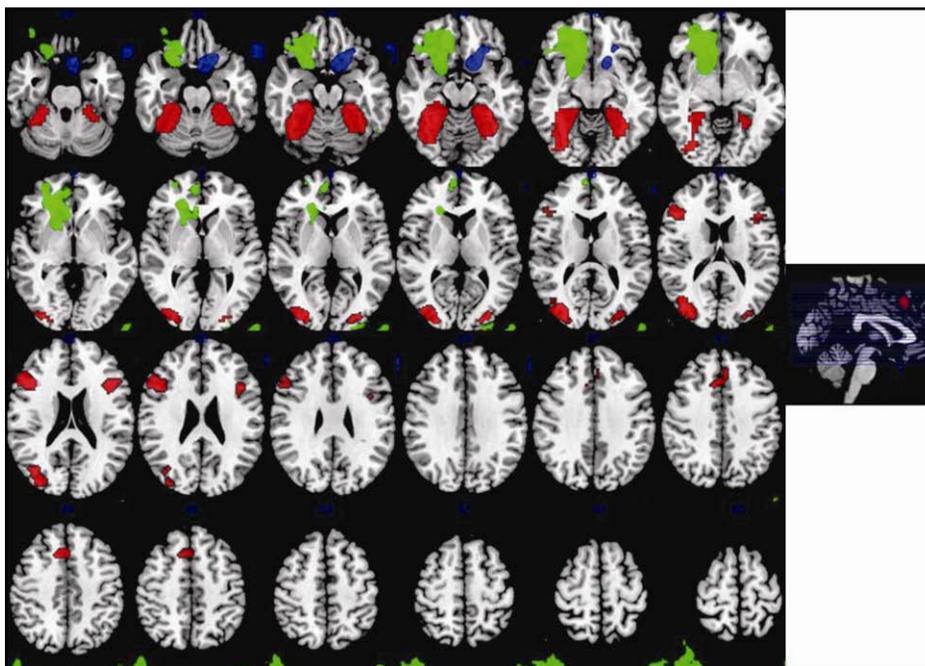
weighting across K-space), resulting in reduced tSNR locally and thus reduces sensitivity for BOLD-signal change. The step direction could not be made the same for both scanners, so in the lowest slices SNR (and, as a consequence, to some degree tSNR) is lower in the right hemisphere for the Philips scanner and in the left hemisphere for the Siemens.

Second, after completion of the study the tSNR maps were quantitatively compared between the two sites to ascertain the expected similarity, by directly comparing tSNR maps for both STERN (working memory) and PMT (associative memory) image data. As tSNR cannot be assumed to be normally distributed (both thermal and physiological noise contribute, which have different distributions), nonparametric statistics were applied, using the SnPM5b toolbox in SPM5 developed by Andrew Holmes and Tom Nichols (see also www.sph.umich.edu/~nichols/SnPM/). For each subject's time series, tSNR-images were created based on the preprocessed functional images (i.e., the unwarped, coregistered, normalized, and smoothed time series) for each task separately. tSNR-images were entered into a nonparametric

two-sample t test in SnPM, using an approximate test of 1,000 permutations (default procedure to reduce computation time when the exact test exceeds 5,000 permutations), a family-wise error (FWE)–corrected p value of .05, and contrasting tSNR-maps obtained on the US scanner (Siemens) with tSNR-maps obtained on the Dutch scanner (Philips) for both STERN and PMT. The results are shown in Figure S1, and revealed several areas that showed significant site-related differences in tSNR (as was expected based on the pilot data because of different step directions) and, hence, in activation effect size. Differences are most prominent in the orbito- and ventromedial regions, where the difference in step direction between scanners likely added to susceptibility in these areas to lower signal-to-noise ratios caused by artifacts resulting from local field distortions in the proximity of the eye sockets and nasal cavities.

Finally, for data analysis, two procedures were adopted to minimize systematic effects of scanner differences on the results. First, preprocessing included a smoothing step of 8 mm FWHM (i.e., two times the voxel size). This is not uncom-

mon in fMRI analyses because of the assumptions of Gaussian Random Field Theory needed for some algorithms, but is important in the context of scanner differences in that smoothness equalization (the procedure of smoothing image data from different scanners with scanner-related variability in "raw" smoothness to a constant FWHM) markedly reduces any possible activation effect size differences between scanners.[1] Second, country (US/Netherlands) was entered as a covariate in the group-wise comparisons on brain activity, as any site-related differences in tSNR would result in a systematic difference in the magnitude of activation (i.e., the contrast-to-noise ratio).

Preprocessing of fMRI data

Imaging data were analyzed using SPM5 (http://www.fil.ion.ucl.ac.uk/spm). Preprocessing included realignment (motion correction) and unwarping, coregistration, normalization, and smoothing with an 8-mm (FWHM) Gaussian kernel. For realignment (motion correction), all images were aligned to the first functional image using a rigid body transformation procedure (default option SPM). EPI images are sensitive to distortions due to magnetic field inhomogeneities, caused by magnetic susceptibility differences in neighboring tissues within the head, resulting in geometrical distortion and signal loss. SPM5 offers a default procedure (unwarping), using algorithms to calculate the geometric distortion with a field mapping sequence, and then compensates for these artifacts by geometrically unwarping the EPI images and by applying cost-function masking in registrations to ignore areas of signal loss. Coregistration involved moving all source images (the anatomical scan and the unwarped realigned EPI images) to a reference image (mean unwarped EPI image) using interpolation methods and affine transformation. An indirect normalization approach was used, coregistering EPI images from each individual subject with his high-resolution T1 anatomical scan (default settings in SPM5), and normalizing this anatomical scan to stereotaxic space (i.e., the MNI305-template). Parameters from this step were used to transfer the EPI images to stereotaxic space. No resampling was done (i.e., voxel size used was the size of acquisition (i.e., 4.0 isotropic). First-level fMRI time series model specifications included a vector representing the block designs of the tasks, and modeling the four task conditions (i.e., onset and durations (in scans) for CT, PT, NT, and instruction frames for STERN, and AL, SC, RE, and instruction frames for PMT) along with a basic set of cosine functions that high-pass filtered the data (cutoff value of 249.6 seconds for STERN and 324 seconds for PMT). Cutoff values for high pass filtering were calculated using in-house–developed software to determine the optimal high-pass filter taking into account the R-square between the factors included in the model (i.e., minimizing multicollinearity).

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