

# Event-related Functional Magnetic Resonance Imaging of a Low Dose of Dexmedetomidine that Impairs Long-term Memory

Hiroki R. Hayama, Ph.D.,\* Kristin M. Drumheller, M.A.,† Mark Mastromonaco,‡  
Christopher Reist, M.D., M.B.A.,§ Lawrence F. Cahill, Ph.D.,|| Michael. T. Alkire, M.D.#

## ABSTRACT

**Background:** Work suggests the amnesia from dexmedetomidine (an  $\alpha_2$ -adrenergic agonist) is caused by a failure of information to be encoded into long-term memory and that dexmedetomidine might differentially affect memory for emotionally arousing material. We investigated these issues in humans using event-related neuroimaging to reveal alterations in brain activity and subsequent memory effects associated with drug exposure.

**Methods:** Forty-eight healthy volunteers received a computer-controlled infusion of either placebo or low-dose dexmedetomidine (target = 0.15 ng/ml plasma) during neuroimaging while they viewed and rated 80 emotionally arousing (e.g., graphic war wound) and 80 nonarousing neutral (e.g., cup) pictures for emotional arousal content. Long-term picture memory was tested 4 days later without neuroimaging. Imaging data were analyzed for drug effects, emotional

## What We Already Know about This Topic

- Dexmedetomidine impairs long-term memory by weakening the strength of the initial memory trace, but it is not known where in the brain dexmedetomidine causes failure of information to be encoded into memory
- Functional neuroimaging and memory testing were used to study the effects of dexmedetomidine in healthy human volunteers

## What This Article Tells Us That Is New

- Dexmedetomidine had region-specific effects on brain activity and sensory input processing such that reduced long-term memory correlated with reduced hippocampal activity at the time of learning

processing differences, and memory-related changes with statistical parametric mapping-8.

**Results:** Dexmedetomidine impaired overall (mean  $\pm$  SEM) picture memory (placebo:  $0.58 \pm 0.03$  vs. dexmedetomidine:  $0.45 \pm 0.03$ ,  $P = 0.001$ ), but did not differentially modulate memory as a function of item arousal. Arousing pictures were better remembered for both groups. Dexmedetomidine had regionally heterogeneous effects on brain activity, primarily decreasing it in the cortex and increasing it in thalamic and posterior hippocampal regions. Nevertheless, a single subsequent memory effect for item memory common to both groups was identified only in the left hippocampus/amygdala. Much of this effect was found to be larger for the placebo than dexmedetomidine group.

**Conclusion:** Dexmedetomidine impaired long-term picture memory, but did not disproportionately block memory for emotionally arousing items. The memory impairment on dexmedetomidine corresponds with a weakened hippocampal subsequent memory effect.

**T**HE induction of a temporary reversible state of amnesia (defined as memory loss for information learned in the presence of a drug) is considered a fundamental component of anesthesia.<sup>1</sup> Amnesic doses of anesthetics are generally

\* Postdoctoral Researcher, ‡ Undergraduate Researcher, Department of Anesthesiology and Perioperative Care, University of California, Irvine, Irvine, California. † Research Coordinator, VA Long Beach Healthcare System, Long Beach, California. § Associate Professor in Residence, VA Long Beach Healthcare System, and Department of Psychiatry and Human Behavior, University of California, Irvine. || Professor, Department of Neurobiology and Behavior, University of California, Irvine. # Associate Professor in Residence, Department of Anesthesiology and Perioperative Care, University of California, Irvine, and VA Long Beach Healthcare System.

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Address correspondence to Dr. Alkire: Department of Anesthesiology and Perioperative Care, University of California, Irvine, 101 City Drive South, Bldg 53, Route 81-A, Orange, California 92868. malkire@uci.edu. Information on purchasing reprints may be found at [www.anesthesiology.org](http://www.anesthesiology.org) or on the masthead page at the beginning of this issue. ANESTHESIOLOGY's articles are made freely accessible to all readers, for personal use only, 6 months from the cover date of the issue.

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a fraction of those needed to produce unresponsiveness.<sup>2,3</sup> Dexmedetomidine is an  $\alpha_2$ -adrenergic agonist often used for procedural sedation.<sup>4–6</sup> Its sedative effect is thought to involve its noradrenergic influences in the brainstem locus coeruleus,<sup>7</sup> along with some contribution coming from suppressed hippocampal serotonergic signaling.<sup>8</sup> Both of these cellular mechanisms could influence how dexmedetomidine affects the processing of new memories. Ebert *et al.* found suppressed recall and recognition memory using sedative doses of dexmedetomidine.<sup>9</sup> Pryor *et al.* used an evoked potential paradigm to investigate the memory impairment associated with low doses of dexmedetomidine at 0.20 and 0.40 ng/ml plasma targets, which caused an approximate 40% and 60% reduction in long-term memory of visual stimuli across a 6-h study-test interval.<sup>10</sup> They concluded dexmedetomidine impairs long-term memory by weakening the strength of an initial memory trace.

Yet, where in the brain might dexmedetomidine be causing a failure of information to be encoded into memory? A number of candidate regions could be hypothesized, ranging from sensory processing networks (*i.e.*, the information never gets to higher cognitive areas),<sup>11</sup> to the hippocampus itself (*i.e.*, a medial temporal lobe structure critical for forming new long-lasting conscious memories),<sup>12</sup> or even the amygdala, a small almond-shaped formation located anterior to the hippocampus in each hemisphere.<sup>13</sup> The amygdala appears important for aversive learning and mediating the amnesic effect associated with a number of anesthetics, including propofol,<sup>14,15</sup> sevoflurane,<sup>16,17</sup> and the benzodiazepines.<sup>18</sup> As norepinephrine neurotransmission in the amygdala is critical to memory formation, especially for emotionally arousing material,<sup>19,20</sup> it is plausible that dexmedetomidine could differentially affect memory for emotionally arousing *versus* nonemotionally arousing (*i.e.*, neutral) material, in a manner similar to how  $\beta$ -blockers prevent the enhancement of memory for emotionally arousing material.<sup>21</sup>

To investigate the neural correlates of low-dose dexmedetomidine action, we employed event-related functional magnetic resonance imaging (fMRI) and focused our analyses on three primary issues. First, we determined whether dexmedetomidine had generalized effects on functional brain activity and sensory input processing. Second, we determined whether dexmedetomidine differentially affects responses to emotionally arousing material. Third and finally, we examined where in the brain the failure of memory formation might occur with dexmedetomidine using the “subsequent memory procedure.”<sup>22–24</sup> In this procedure, subjects are presented with a series of items during a study session. In any particular study, encoding these items into memory can either be intentional (*i.e.*, learn and remember these) or incidental (*i.e.*, classify these items). Then, based on a subsequent memory test, the brain activity evoked by each item that occurred at the time of viewing can be sorted into those items that are later remembered *versus* those items that are later forgotten. The

difference is referred to as the “subsequent memory effect” and indicates the presence of neural activity that supported successful formation of a long-term memory.

## Materials and Methods

In the present experiment, subjects viewed a series of pictures while inside a magnetic resonance imaging machine and were either administered a placebo saline infusion or a very low-dose infusion of dexmedetomidine at 0.15 ng/ml plasma target delivered through a STANPUMP software-controlled Harvard 22 infusion pump (Harvard Apparatus, Holliston, MA). Based on the Pryor *et al.* study,<sup>10</sup> we anticipated this to be about the lowest dose of dexmedetomidine that could be used and might still cause a measurable impairment of long-term memory. This low dose was used to minimize the potential confounding influence of the drug on the blood oxygenation level-dependent (BOLD) signal itself and to minimize sedation-related effects on the cognitive processes underlying long-term memory encoding. An incidental encoding paradigm was used to help mitigate directed attention effects on memory processing. To ensure that each item was seen and given the opportunity to be encoded, subjects were instructed to signal whether they found the content of each picture to be emotionally arousing (*e.g.*, a graphic war wound) or not emotionally arousing (*e.g.*, a blue coffee cup) with a button press response. It is assumed that a response to a picture indicates sufficient cognitive resources were available to provide the opportunity for the successful encoding of the contents of the picture. A significant increase in response latency or large numbers of missing responses would indicate that even at this low dose, dexmedetomidine was having a strong sedative effect. Long-term memory of the incidentally encoded images was tested 4 days later without any neuroimaging. This study-test delay timeframe was selected after pilot testing showed it was long enough to allow some level of forgetting to occur in the placebo condition while at the same time allowing some memory retention in the dexmedetomidine condition.

## Subjects

Forty-eight subjects gave written informed consent to participate and were randomly assigned to receive either a placebo infusion ( $n = 24$ , mean  $\pm$  SD age  $21 \pm 3.7$ , 11 females) or a very low dose dexmedetomidine infusion ( $n = 24$ , age  $21 \pm 2.8$ , nine females). All subjects reported themselves to be in good general health, right-handed, to have no history of neurologic disease or other contraindications for magnetic resonance imaging, and to have attained fluency in English by age 5. Subjects underwent a brief physical exam and medical history review to verify eligibility. Subjects were recruited from the University of California, Irvine community and remunerated for their participation, in accordance with the human subjects procedures approved by the Institutional Review Board of University of

California, Irvine. Data from six subjects were excluded from all analysis because of issues with imaging data or task performance. One placebo subject had image clipping during data acquisition and a high false alarm rate (0.67), indicating random guessing on the memory test. Five dexmedetomidine subjects were excluded. Two subjects were too sedated and failed to respond to one third of the items during encoding. Three subjects were excluded on the basis of head movement in excess of 3 mm in the x- or y-plane or rotational movement in excess of 3 degrees.

### Stimulus Materials

Extensive literature suggests emotionally arousing items have a mnemonic advantage over nonemotionally arousing items.<sup>25</sup> This is often referred to as emotional memory, and the modulation of emotional memory is thought to occur in a manner that is proportional to the magnitude of the emotional arousal induced at the time of encoding, with more arousing items being better remembered.<sup>23,26</sup> The valence of an item, (*i.e.*, the degree to which an item is considered to be pleasant or unpleasant) also has an effect on memory, but much less so than arousal.<sup>27</sup> To maximize event-related fMRI signals, a binary emotionally arousing *versus* neutral stimulus set was constructed. The critical stimuli consisted of a pool of 240 pictures that were selected to be at the extremes of the emotionally arousing scale. Approximately 60% of the contexts were taken from the International Affective Picture System,<sup>28</sup> which consists of a series of pictures standardized for arousal ratings (*i.e.*, to what extent does one experience an emotionally arousing reaction to the contents of the picture) and valence (*i.e.*, do the contents of the picture induce a positive, happy pleasant feeling or a negative, unpleasant aversive feeling). This picture set is limited in size so the remaining pictures were obtained from a variety of Internet sources and were selected to parallel the content of the Lang series. All pictures were rated for arousal and valence by 10 age-matched subjects (four females) who did not participate in the fMRI experiment, to validate each picture's *a priori* categorization for subsequent use in the scanner as either an emotionally arousing or neutral target in a representative sample of the local subject population. In this sample, the ratings were acquired with two 5-point Likert-type scales that assessed arousal (1 = very calming, 3 = neutral, 5 = very arousing) and valence (1 = very negative, 3 = neutral, 5 = very positive). The pictures selected as experimental materials consisted of 120 highly arousing negatively valenced pictures (mean arousal: 4.14, SD: 0.33; mean valence: 1.50, SD: 0.23) and 120 neutral pictures (mean arousal: 2.81, SD: 0.28; mean valence: 3.16, SD: 0.21). No positively valenced pictures were included, as these tend to show greater interindividual variability in arousal-response reactions. For each participant in the fMRI scanning sessions, 80 highly emotionally arousing pictures and 80 neutral pictures were randomly assigned to the encoding sessions, and the remaining 80 pictures (40

emotionally arousing and 40 neutral) served as "new" items on the memory test that was conducted 4 days later.

### Subject Preparation

All subjects were American Society of Anesthesiologists physical classification I. Subjects arrived at the magnetic resonance imaging suite having consumed neither food nor liquid for at least 8 h before the imaging session. Intravenous access was obtained and normal saline was used as the carrier fluid to keep the intravenous catheter open. Subjects received approximately 250 ml of normal saline during the course of the experiment. Normal saline also served as the placebo liquid. Subjects were placed onto the scanner bed and then were connected to an In Vivo 3150 magnetic resonance compatible patient monitor (In Vivo Research Inc., Orlando, FL.). The subjects had their electrocardiogram, pulse oximetry, blood pressure, and temperature monitored throughout the imaging session and into recovery. In addition, while in the scanner the subjects wore an oxygen facemask which delivered 35% O<sub>2</sub> and was used to monitor the subject's expired carbon dioxide. Once all equipment was attached and working properly with the subjects properly positioned on the magnetic resonance imaging table, the anesthetic infusion was started. The blood target level for dexmedetomidine was 0.15 ng/ml plasma, using height-adjusted parameters. The subjects always underwent the structural neuroimaging scan first to allow time for the drug to reach pseudosteady state equilibrium. Given the low target dose, the target was reached within a few minutes. Nevertheless, experimental scanning did not start until the subjects had been on the infusion for about 20 min. As the target dose administered was near the lower limit for blood level quantification of the drug, no blood samples were taken. Following the scanning all subjects were able to easily walk under their own power from the scanner to a nearby recovery area. All subjects tolerated the procedure well and were discharged to home in the normal expected time frame.

### Experimental Tasks and Procedures

**Memory-encoding Procedure.** Brief instructions and a short practice were administered inside the scanner. The subjects were first shown a series of 12 practice pictures, when the scanner was off, that emulated the timing and content of the forthcoming study slides. The subjects were instructed to rate the pictures into one of two categories depending on their personal emotional arousal reaction to the content of each picture. They rated each item as either emotionally arousing or neutral with an index or middle finger button-press response (response hand was counterbalanced across the subjects). Subjects were not asked to rate the pictures as fast as possible, but they were told to complete their rating as accurately as possible before the next picture appeared. Subjects then rested as an alignment scan and a structural magnetic resonance imaging brain scan were performed during the next 10–15 min.

The encoding study phase of the experiment proper consisted of the presentation of two blocks of pictures each lasting 10 min, during which BOLD signals were measured. The two encoding blocks were separated by a brief rest period (approximately 1 min). Each individual study trial (*i.e.*, event) began with the presentation of a red fixation character in the center of the display frame for 500 ms. The character was replaced by a picture to be encoded that was presented for 1,500 ms. Immediately after picture offset, a centrally presented black fixation character was presented for 2,500 ms, completing the trial. Subjects rated each picture as emotionally arousing or not, and reaction times to make this rating were recorded. The stimulus onset asynchrony (*i.e.*, the timing of when the next picture appeared) of the study trials was stochastically distributed (*i.e.*, not a fixed determined interval) with a minimum of 4,500 ms modulated by the addition of 80 randomly intermixed null trials (*i.e.*, the black fixation character).<sup>29</sup> Trials were presented in pseudorandom order, with no more than three trials of one item type (arousing, neutral, or null) occurring consecutively. Each study block consisted of 124 trials, comprising 40 emotionally arousing pictures, 40 neutral nonarousing pictures, 40 null events, and four buffer items (two items at the beginning and end), to give a total of 160 critical study items across the two blocks.

**fMRI Data Acquisition.** High resolution T1-weighted anatomical images (240 × 240 matrix, 1 mm<sup>3</sup> voxels, 160 slices, sagittal acquisition, three-dimensional magnetization prepared rapid acquisition gradient echo recalled sequence) and BOLD, T2\*-weighted echoplanar images (80 × 79 matrix, 3 × 3 mm in-plane resolution, axial acquisition, flip angle 70°, echo time 30 ms) were acquired using a 3-Tesla Philips Achieva magnetic resonance scanner (Philips Medical System, Andover, MA). An eight-channel parallel imaging headcoil with a sensitivity-encoding reduction factor of 1.5 was used in data acquisition. During each study session, 290 functional volumes were acquired, and two 10-min encoding sessions were imaged. Each echoplanar image volume comprised 30 of the 3-mm-thick axial slices oriented parallel to the anterior commissure–posterior commissure plane and separated by 1-mm gaps. The images were positioned to give whole brain coverage. Volumes within sessions were acquired continuously in an ascending sequential order. Data were acquired with a repetition time of 2 s/volume. The use of a 4.5 s stimulus onset asynchrony allowed for an effective 2 Hz sampling of the hemodynamic response.

**Memory Test Procedure (Not Scanned).** Subjects returned 4 days poststudy encoding session. There was no scanning or drug administration during the memory-testing session. An incidental memory-testing strategy was used such that the subjects were only informed of the memory testing on the return visit. The memory test consisted of two blocks of 80 study items (40 emotionally arousing and 40 neutral) and 40 randomly interspersed unstudied (20 new emotionally

arousing and 20 new neutral) items. No more than 3 items of one type were presented consecutively. The widely used “remember/know” procedure<sup>30,31</sup> was used for memory testing because some evidence suggests that memory modulation for emotionally arousing content will be primarily evident for items that are associated with episodic details (remember), as opposed to those items that are simply familiar (know).<sup>26,32</sup> Subjects were instructed to make one of three responses to each test picture: remember, know, or new. The “remember” response (indicative of recollection) was described as indicating recall of some contextual detail about the picture’s study episode. Examples of such related details were given, such as memory for a specific thought associated with the picture, memory for a mental image generated in association with the picture, or memory for some distinctive aspect of the study item. Subjects were instructed that if there was a strong sense of prior occurrence of a picture in the absence of recollection (indicating familiarity), they should signal that they “know” an item is old. If there was no sense of prior occurrence of a picture, or if there was uncertainty as to whether a picture was presented during study, subjects were instructed to respond “new.” To encourage compliance with the instructions, subjects were instructed to describe the specific detail or details they retrieved in association with each “remember” response during the practice test. A self-paced timing was used to maximize the potential for memory responses. Each test trial began with a red fixation presented in the center of a gray frame for 500 ms, followed by a centrally presented test picture for 1,500 ms. After the picture presentation, a black fixation cross was centrally presented and then the computer prompted the subject for memory response and emotional arousal rating. Each test block lasted approximately 10 min.

### fMRI Data Analysis

Imaging data were analyzed with Statistical Parametric Mapping version 8 (Wellcome Department of Imaging Neuroscience, London, United Kingdom) that was run under Matlab R2009b (The Mathworks Inc., Natick, MA). The functional volumes underwent standard spatial realignment and slice timing correction procedures. Images were aligned to the first volume of the first study block and, subsequently, to the across-block mean. The data in each volume were temporally shifted to the onset of the middle slice and the resulting volumes were reoriented, coregistered with the anatomical volume, spatially normalized to a standard echo planar image template (based on the Montreal Neurologic Institute reference brain), resampled into 3-mm isotropic voxels using nonlinear basis functions,<sup>33</sup> and smoothed to an 8-mm full-width half maximum Gaussian kernel. Functional time series were concatenated across the two sessions. Each subject’s anatomical volume was segmented into gray and white matter,<sup>34</sup> and then processed with the diffeomorphic anatomical registration through exponentiated lie algebra statistical parametric mapping toolbox to create



an across-subjects ( $n = 42$ ) template.<sup>35</sup> Parameters were determined by an affine transformation of the template to Montreal Neurologic Institute space, along with the diffeomorphic anatomical registration-based transformation parameters, and were then applied to each subject's anatomical (resampled to 1 mm) before averaging to create an across-subjects mean image.

Statistical analyses on the study phase data were performed using a General Linear Model in a two stages mixed effects design. In the first stage, neural activity elicited by the study pictures was modeled by  $\delta$  functions (impulse events) that occurred with the onset of each picture. Next, to model the BOLD response, this function was then convolved with a canonical hemodynamic response function,<sup>36</sup> and its temporal and dispersion derivatives (which model variances in latency and duration, respectively). A substantial number of subjects failed to respond "remember" to 10 or more neutrally rated items. This meant that the BOLD signal could not be reliably modeled for this category of response and the power to detect image differences between remembered neutral events and other responses would be low. Thus to enhance the memory-related imaging signals associated with the neutral events, the "remember" and "know" responses were collapsed into a single response category of "old," which signified memory of an item.<sup>37,38</sup> The "remember" and "know" responses were also collapsed into a single category for the items rated emotionally arousing. The principal analyses were thus confined to four events of interest: emotionally arousing and neutral-rated pictures that were later correctly identified as being old (emotionally arousing remembered and neutral remembered, respectively) and studied emotionally arousing and neutral-rated pictures that were later forgotten (emotionally arousing forgotten and neutral forgotten, respectively). A fifth category of trials was comprised of events of no interest, namely buffer trials and trials associated with erroneous study responses (*i.e.*, where subjects failed to respond or made multiple responses to a single event). For each study block, the model also included as covariates the across-scan mean and six regressors representing motion-related variance (three for rigid-body translations and three for rotations). For each voxel, the image time-series was highpass-filtered to 1/128 Hz and an autoregressive1 model was used to estimate and correct for nonsphericity of the error covariance.<sup>39</sup> The general linear model was then used to obtain parameter estimates representing the activity elicited by the four conditions of interest (hits, "remembered," and misses, "forgotten," for the subjectively rated emotionally arousing and neutral pictures, respectively) for each participant and these were carried forward to a second-level group-wise random effects analysis. In this random effects analysis, individual participants' parameter estimates for the four conditions of interest were entered into a repeated-measures three-way full factorial design, with factors of group (placebo, dexmedetomidine) subjective arousal

rating (emotionally arousing, neutral) and response category (remembered, forgot).

For unidirectional contrasts, a voxel-wise statistical threshold of  $P < 0.001$  uncorrected, combined with a cluster extent threshold of 20 contiguous voxels, was employed to account for multiple comparisons. This significance threshold is often used for event-related studies of memory.<sup>40–42</sup> Only independent contrasts were masked. Regions of overlap between the outcomes of two contrasts were identified by inclusive masking and the conjoint significance was computed using Fisher exact test of estimating the conjoint significance of independent tests.<sup>43</sup> In the present study, the contrasts to be masked were thresholded at  $P < 0.001$ , whereas the inclusive mask was thresholded at  $P < 0.05$ , resulting in a conjoint probability of  $P < 0.0005$  while maintaining a 20-voxel extent threshold. Exclusive masking was used to eliminate voxels that were not shared between two contrasts. For this, the contrasts to be masked were thresholded at  $P < 0.001$  and the statistical parametric map constituting the exclusive mask was thresholded at  $P < 0.1$  (F contrasts were used, thus the resulting threshold is  $P < 0.05$  one-sided.) It is important to note that the use of a more liberal exclusive mask results in a more conservative masking procedure (more voxels are eliminated by exclusion). The resultant threshold of an exclusively masked statistical parametric map is  $P < 0.001$  with an extent threshold of 20 voxels. The effects of interest are displayed onto sections of the included subjects' mean normalized structural image, or are rendered onto a standard inflated fiducial brain in statistical parametric mapping  $-5$  space.<sup>44</sup>

### Behavioral Data Analysis

The behavioral data were analyzed using an ANOVA procedure implemented in IBM SPSS version 19 (International Business Machines Corporation, Armonk, NY). Reaction times and picture ratings were analyzed as a  $2 \times 2$  ANOVA. A  $P$  value of 0.05 was considered significant. Memory data were analyzed for scanning as hits *versus* misses (raw score of items marked as remembered *vs.* items forgotten). Absolute hit rate analysis, however, is not the same thing as memory performance. Memory performance also needs to take into account the rate at which subjects are biased for guessing on the memory test. This biased guessing is called the rate of false alarms and it reflects the likelihood of how often a subject will report that a picture they have never before seen was part of the study items. As the study looked for subsequent memory effects in the neuroimaging data that were obtained at the time of encoding, there is no way to further classify any specific event as a possible false alarm and, therefore, the neuroimaging data must be analyzed based on the hit *versus* miss categorization. Yet, the actual memory performance levels will be lower than those for just hit rates, once the hit rates are adjusted for the rate of false alarms. For calculating memory performance the discrimination index was used. The real probability that an item is remembered is equal to the proportion of remembered responses minus the proportion

**Table 1.** Behavioral Responses to Study Items

	Placebo			Dexmedetomidine		
	Arousing	Neutral	Total	Arousing	Neutral	Total
Number rated (#)	70.8 (5.6)	88.9 (5.5)	159.7 (0.2)	68.6 (5.1)	82.2 (5.7)	150.7 (3.2)
Missed or multiple responses (#)	N/A	N/A	0.3 (0.2)	N/A	N/A	9.3 (3.2)
Proportion of rated (%)	0.44 (0.03)	0.56 (0.03)	1.00	0.46 (0.03)	0.54 (0.03)	1.00
Reaction time (ms)	1,257 (87)	1,173 (91)	1,211 (89)	1,228 (95)	1,229 (117)	1,229 (107)

SE is in parenthesis.

N/A = not applicable.

of false alarms signaled as remembered. “Remember” and “know” responses are mutually exclusive (only one response is allowed). Therefore, if the “know” proportions are not corrected for the proportion of “remembered” responses, they will underestimate the probability that an item is familiar. To compensate for the underestimation, the independent “remember/know” method was used to calculate “know” hits and “know” false alarms (see Yonelinas<sup>45</sup> for review). Thus, “know” hits = (proportion of items responded to as “know”/[1 – proportion of items responded to as “remembered”]). “Know” false alarm rates were calculated in an analogous manner, and probability familiar is calculated by subtracting the “know” false alarm rate from “know” hits.

## Results

### Behavioral Performance

**Study Task.** Mean picture rating raw scores, proportions, and reaction times are listed in table 1. A  $2 \times 2$  ANOVA (factors of group, placebo *vs.* dexmedetomidine, and picture rating, emotionally arousing *vs.* neutral) was used to assess the influence of dexmedetomidine on item-emotionality ratings. This revealed both a main effect of picture rating ( $F[1,40] = 4.23$ ,  $P < 0.05$ ), and a main factor of group ( $F[1,40] = 9.382$ ,  $P < 0.005$ ). This shows that both dexmedetomidine and placebo subjects rated significantly more pictures as neutral rather than emotionally arousing and that subjects on dexmedetomidine responded to significantly fewer pictures than those on placebo. Of the 160 pictures seen during scanning, the dexmedetomidine subjects failed on average to respond to  $9 \pm 3$  (mean  $\pm$  SEM) items. In contrast, the placebo subjects failed to respond to only  $0.3 \pm 0.2$  items. These values demonstrate that the task itself (judging the emotional content of a picture while lying in a scanner) was not too difficult for these normal subjects to perform, because 19 subjects out of the 23 responded to every picture. In contrast, some sedative effect of the drug was clearly evident, because 15 out of the 19 subjects on dexmedetomidine missed responding to at least one or more of the pictures. In addition, the ANOVA did not reveal a significant rating by drug group interaction. This shows that dexmedetomidine administration did not significantly affect the proportion of items that were rated as emotionally arousing *versus* neutral.

Mean reaction times for judgments on emotionally arousing and neutral pictures were analyzed with a  $2 \times 2$  ANOVA (factors of group, placebo *vs.* dexmedetomidine, and picture rating, emotionally arousing *vs.* neutral) to assess if the dexmedetomidine differentially affected response times to emotionally arousing or neutral items. This analysis failed to reveal any significant effects. Response times were statistically equivalent across all four emotional arousal response categories and there was no significant interaction on response times for dexmedetomidine. Thus, during the encoding phase of the study, a behavioral effect of low-dose dexmedetomidine was not evident on the emotional arousal content processing of the pictures or on the response timing associated with making contextual judgments about the pictures.

**Retrieval Task.** Hit rates for pictures rated emotionally arousing and neutral are listed in table 2. A  $2 \times 2$  ANOVA (factors of group, placebo *vs.* dexmedetomidine, and picture rating, emotionally arousing *vs.* neutral) was used to assess if dexmedetomidine differentially affected memory hit rates for emotionally arousing *versus* neutral items. This revealed both a main effect of group ( $F[1,40] = 6.623$ ,  $P < 0.05$ ) and a main effect of hit type ( $F[1,40] = 50.332$ ,  $P < 0.001$ ), but no interaction between group and hit type was observed. The main effects demonstrate that of the pictures rated at encoding, the dexmedetomidine subjects had significantly fewer hits (a 14% reduction) and that across both groups, memory for emotionally arousing items was greater than that for neutral items (a 17% mnemonic advantage for emotional items). The lack of an interaction indicates that dexmedetomidine exposure during learning was not associated with a differential modulation of memory for emotionally arousing items. This suggests that whatever effects dexmedetomidine may have had on long-term memory encoding, occurring around the time of learning, these effects were not specifically modulated by the emotional context of the pictures.

False alarm rates (how often a never-before-seen item is falsely identified as being seen before) are also listed in table 2. A  $2 \times 2$  ANOVA (factors of group, placebo *vs.* dexmedetomidine, and picture rating, emotionally arousing *vs.* neutral) revealed a single main effect of false alarm type ( $F[1,40] = 5.08$ ,  $P < 0.05$ ). Irrespective of group, subjects were significantly

**Table 2.** Memory Performance Tables

	Placebo			Dexmedetomidine		
	Arousing	Neutral	Total	Arousing	Neutral	Total
Overall hits	0.78 (0.03)	0.67 (0.03)	0.71 (0.03)	0.69 (0.03)	0.55 (0.03)	0.61 (0.03)
False alarms	0.15 (0.03)	0.13 (0.02)	0.13 (0.02)	0.20 (0.04)	0.13 (0.02)	0.16 (0.03)
Hits corrected for FA	0.63 (0.04)	0.54 (0.03)	0.58 (0.03)	0.49 (0.04)	0.42 (0.03)	0.45 (0.03)
Remember hits	0.36 (0.04)	0.21 (0.04)	0.28 (0.04)	0.29 (0.04)	0.17 (0.04)	0.22 (0.04)
Remember FA rate	0.03 (0.02)	0.02 (0.01)	0.02 (0.01)	0.03 (0.01)	0.03 (0.02)	0.03 (0.01)
Probability remember	0.33 (0.04)	0.19 (0.04)	0.25 (0.03)	0.26 (0.04)	0.15 (0.03)	0.19 (0.03)
Know hits	0.65 (0.04)	0.59 (0.04)	0.60 (0.04)	0.53 (0.05)	0.44 (0.04)	0.48 (0.04)
Know FA rate	0.11 (0.02)	0.12 (0.02)	0.11 (0.02)	0.17 (0.04)	0.11 (0.02)	0.14 (0.02)
Probability know	0.53 (0.04)	0.48 (0.03)	0.49 (0.03)	0.35 (0.05)	0.34 (0.04)	0.34 (0.04)

SE is in parenthesis.

FA = false alarms.

more likely to call a new picture old if the content was perceived to be emotionally arousing, rather than neutral.

### Memory Performance

Hit rate performance adjusted for the rate of false alarms revealed that long-term memory performance actually decreased 22%, as it fell from 58% item memory on placebo to 45% on dexmedetomidine. Further examination of memory performance (corrected for false alarms) specific to the “remembered” or “know” responses (table 2) was performed using a  $2 \times 2 \times 2$  ANOVA with factors of group (placebo *vs.* dexmedetomidine), arousal rating (emotionally arousing *vs.* neutral), and memory type (remember *vs.* know). This revealed four significant effects: (1) a significant main effect of group ( $F[1,40] = 11.82$ ,  $P < 0.005$ ), (2) a significant main effect of arousal rating ( $F[1,40] = 25.76$ ,  $P < 0.001$ ), (3) a significant main effect of memory type ( $F[1,40] = 24.16$ ,  $P < 0.001$ ), and (4) a significant arousal rating by memory type interaction ( $F[1,40] = 9.09$ ,  $P < 0.001$ ). The main effect of group indicates that dexmedetomidine decreased memory performance for both “remembered” and “know” items, whereas the main effect of arousal suggests that emotionally arousing items were better remembered than neutral items; the main effect of memory type also demonstrates that more items were associated with “know” responses rather than “remembered” responses. The interaction demonstrates that emotionally arousing items were better remembered for those items signaled as “remembered” *versus* items signaled with the “know” response. In other words, the mnemonic boost associated with emotional arousal was evident in the “remembered” items, but not so much in the “know” items. Importantly, no factors interacted with drug. Thus, even for the items reported to elicit contextual details (those items marked “remembered”), dexmedetomidine did not differentially modulate memory for emotionally arousing items.

The memory impairment caused by the drug could have differentially affected recollection *versus* familiarity memory processes. As we collapsed data across trials later afforded a “remember” *versus* “know” response, we have only limited

ability to investigate this. However, the proportion of identified studied items afforded a “remember” response *versus* a “know” response for placebo and dexmedetomidine subjects was not significantly different between the groups (34% *vs.* 36% for “remembered” and 66% *vs.* 64% for “know” for the placebo and dexmedetomidine groups, respectively). This suggests only minimal differences in the imaging data might be attributable to a drug specific effect on one type of memory.

### fMRI Results

Analysis of the fMRI data were directed at three things. First, we identified regions where brain activity differed as a function of drug administered. Second, we looked at the subjective effects of emotional arousal (pictures rated emotionally arousing more than pictures rated neutral) to see if this mental process differed as a function of drug administration and to verify that the emotionally arousing items caused the anticipated regional brain responses. Third and most importantly, we searched for differences as well as commonalities in subsequent memory effects (pictures later remembered more than pictures later forgotten).

**Drug Effects on Regional BOLD Activity.** Effects attributable to the drug condition regardless of ultimate behavioral performance were assessed by combining all four events of interest (emotionally arousing hit, emotionally arousing miss, neutral hit, neutral miss) across each group separately and then by comparing activity that was greater for one group than the other and *vice versa*. As is detailed in table 3 and illustrated in figure 1, regions of the brain that demonstrated greater activity for placebo than dexmedetomidine were primarily cortical in nature, consisting of bilateral primary and supplementary motor cortex, bilateral visual cortex, and anterior prefrontal cortex bilaterally (blue areas of fig. 1). By contrast, bilateral regions of the thalamus, including the pulvinar, hypothalamus, and portions of the posterior hippocampal area, appeared more active in the dexmedetomidine group than in the placebo group (table 3 and fig. 1, red areas). These results are illustrated at both a typical event-related related threshold of  $P < 0.001$ , with a voxel extent threshold of 20 voxels

**Table 3.** Brain Regions Associated with the Main Effect of the Drug

Hemisphere	Region	Brodmann Area	Coordinates (x, y, z)	Z-score (No. Voxels)
Placebo more than dexmedetomidine				
L	Cuneus	18	(-18, -91, 28)	7.12 (2,205)
R	Precentral gyrus	6	(48, -1, 34)	6.53 (3,755)
R	Inferior parietal	40	(48, -34, 46)	6.24*
L	Precentral gyrus	6	(-51, -4, 40)	5.97*
L	Fusiform/cerebellum		(-21, -55, -11)	6.22 (324)
L	Superior parietal	7	(-27, -49, 46)	5.29 (337)
L	Inferior occipital/cerebellum		(-12, -88, -14)	4.94 (60)
LR	Medial prefrontal	6	(-3, -16, 52)	4.55 (69)
R	Anterior prefrontal	9	(30, 50, 22)	4.41 (73)
R	Precentral gyrus	6	(63, 5, 4)	4.29 (51)
L	Anterior prefrontal	10	(-33, 50, 19)	4.27 (70)
R	Cerebellum		(30, -43, -35)	4.01 (67)
R	Middle frontal gyrus	9	(36, 41, 34)	3.92 (27)
Dexmedetomidine more than placebo				
LR	Thalamus/pulvinar		(9, -34, 4)	6.01 (708)
R	Hippocampus/parahippocampal		(36, -25, -14)	4.84 (106)
LR	Caudate		(0, 2, 16)	4.59 (132)
L	Medial frontal gyrus	10	(-3, 65, 4)	3.78 (47)

\* These regions are subpeaks of the larger precentral gyrus cluster, thus the numbers of voxels were not available for these regions.

L = left; LR = both left and right; R = right.

(fig. 1A) and a liberal statistical threshold ( $P < 0.05$ , uncorrected) as an exploratory analysis to allow the reader to see all areas that might be affected by the drug (fig. 1B).

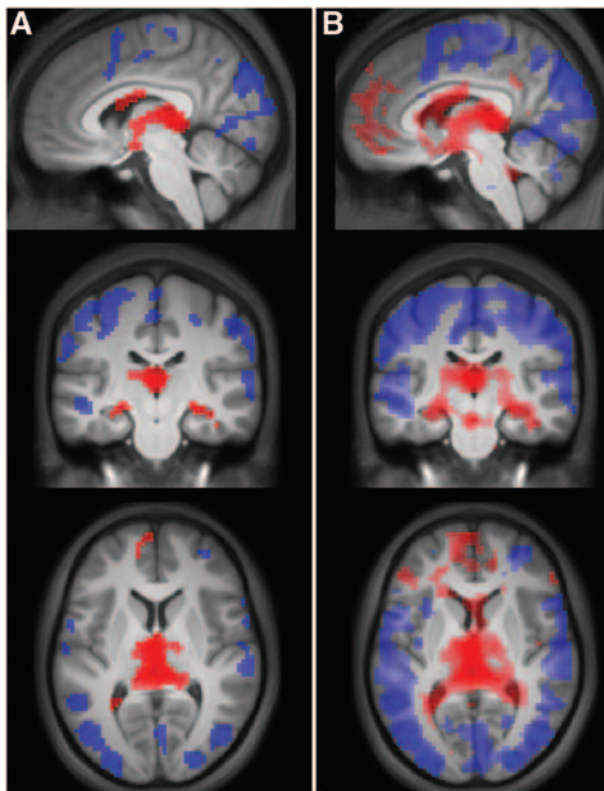
As the regional suppressive effect of the drug is primarily cortical in nature, this result is better visualized on an inflated cortical surface. Figure 2 shows the cortical brain regions where there is a main effect of the drug across all events, along with time activity graphs from two representative voxels. The blue shaded areas (fig. 2A) show where the BOLD signal is significantly weaker for the dexmedetomidine condition compared with placebo. BOLD signal time-activity course changes are shown for representative voxels in the occipital lobe (fig. 2B, marked with a green dot) and the parietal lobe (fig. 2B, marked with a yellow dot). A significant effect of the drug compared with placebo is found in the occipital lobe (fig. 2B, green dot), as the peak of the red line that represents the mean summated activity across all events for those on dexmedetomidine is significantly lower than the peak of the blue line, which represents the summated activity across all events for those on placebo (error bars are  $\pm$  SEM). The plotted BOLD response in the parietal cortex (fig. 2B, yellow dot) shows no main effect of the drug, despite the fact that this is thought to be an important region in some subsequent memory studies.<sup>46</sup> To further examine these cortical effects as potentially relating to subsequent memory effects, the time-activity course graphs are also plotted with the individual events parsed into remembered and forgotten items (fig. 2C). Note that if a change in cortical processing contributed to a memory encoding failure, then parsing the data into these remembered and forgotten signals should show

a significant difference across memory performance, which it does not. In other words, a subsequent memory effect is not occurring in those sensory motor regions that show a primary effect of the drug (fig. 2A, blue areas). This makes it unlikely that the behavioral change in memory performance is being driven by the drug-induced reduction of BOLD activity that is occurring in these sensory-processing areas.

**Subjective Emotional Arousal Effects.** Subjective emotional arousal effects were collapsed across the factor of response type (*e.g.*, hits and misses) for each group. Common subjective emotional arousal effects were identified by exclusively masking the main effect of emotional arousal (collapsed across dexmedetomidine and placebo; thresholded at  $P < 0.001$ ) by the two-sided emotional arousal  $\times$  drug interaction ( $P < 0.1$ ; note that the more liberal the threshold of an exclusive mask, the more conservative is the masking procedure), thus removing all voxels where emotional arousal effects significantly differed (at  $P < 0.05$ , one-sided) as a factor of drug. As detailed in table 4 and shown in figure 3, common emotional arousal effects were identified in the amygdala, the anterior cingulate cortex, and the medial prefrontal cortex bilaterally, as well as in the left thalamus. These findings show that the emotionally arousing *versus* neutral stimuli used were sufficient for activating the brain regions associated with processing of emotional arousal.<sup>40,47</sup>

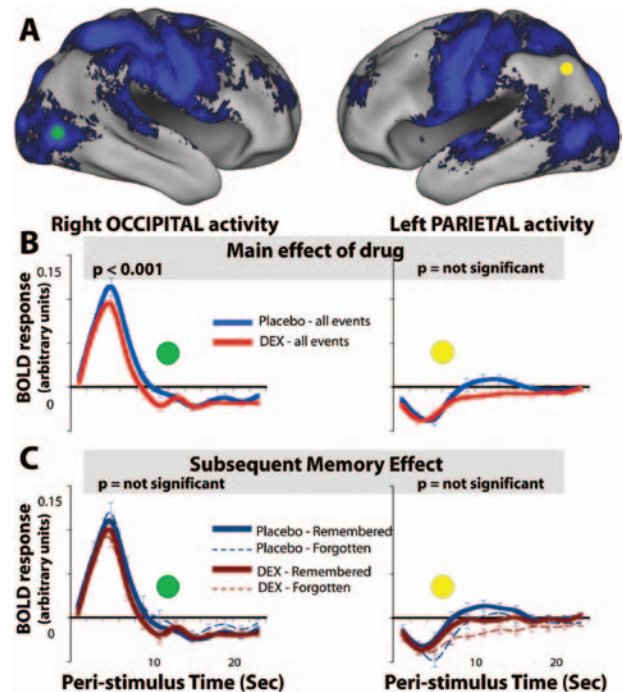
Subjective emotional arousal effects greater for one group compared with the other were identified by inclusively masking the main effect of subjective emotional arousal ( $P < 0.001$ ) with the one-sided drug  $\times$  emotional arousal interaction (*e.g.*, emotional arousal effects greater in placebo than dexmedetomidine,





**Fig. 1.** Brain regions where event-related functional magnetic resonance imaging signals are modulated by dexmedetomidine during the picture-viewing and emotion-rating task. Across the whole brain the areas where the blood oxygen level-dependent signal is attenuated by dexmedetomidine are illustrated in shades of *blue*, and areas where the signal is enhanced by dexmedetomidine are shown in *red*. (A) Images are shown at a standard event-related functional magnetic resonance imaging (fMRI) significance threshold of  $P < 0.001$ , with a 20-voxel extent threshold. At this threshold, dexmedetomidine appears to enhance the signals present in the center parts of the brain, including the thalamus and hippocampal areas. At the same time, dexmedetomidine appears to suppress activity across large portions of the cortex. (B) Images are shown at a liberal significance threshold of  $P < 0.05$ , uncorrected, for viewing purposes only. The activity is displayed on the normalized across-subjects mean anatomical image.

and *vice versa*; thresholded at  $P < 0.05$ ), resulting in a conjoint threshold of  $P < 0.0005$  with a 20-voxel threshold extent. Placebo group subjective emotional arousal effects that were larger than those for dexmedetomidine were identified only in the right inferior frontal gyrus and the left inferior temporal gyrus (table 4). These effects overlapped with and are confounded by the main effect of the drug. Thus, interpreting these areas as specific foci for emotional-processing differences caused by dexmedetomidine is difficult because a behavioral change in emotional processing was not found and these interaction effects are localized to a subset of regions occurring within the confines of the main effect of the drug. In the case of dexmedetomidine, larger subjective emotional arousal effects were seen in a different portion of the right inferior frontal gyrus



**Fig. 2.** (A) Results from the main effect of drug analysis are displayed on an inflated rendering of cortical brain surface showing right and left hemispheres, respectively. *Blue* areas show where the blood oxygen level-dependent signal response is suppressed on dexmedetomidine relative to placebo at a significance level of  $P < 0.001$ , with a voxel extent threshold of 20. The uncolored areas (*light gray* regions) do not show a significant effect of the drug. The peristimulus time-activity course in the measured signal is shown for two representative areas; one shows a main effect of the drug (the *green dot* area, within the *blue* color in the occipital lobe) and one shows no significant effect (the *yellow dot* area, within the *gray* area in the parietal lobe). (B) Time courses of the main drug effect for all events are plotted for activity from the representative areas shown as the *green* ( $x = 27, y = -91, z = 10$ ) and *yellow* ( $x = -48, y = -73, z = 28$ ) dots in panel A. (C) Time courses from the same areas shown in figure 2B, but now the events are parsed into those items that were remembered *versus* those that were forgotten. No significant subsequent memory effects are found in these cortical regions, regardless of whether a significant main effect of the drug was found (mean activity  $\pm$  SEM). BOLD = blood oxygen level-dependent; DEX = dexmedetomidine.

overlapping with the temporal tip, along with a small cluster localized in the brainstem near the red nucleus. Again, a behavioral correlate for this difference is lacking and the significance of this finding is hard to interpret. Overall, these findings suggest that the engagement of emotional arousal network activity was relatively unchanged by this low-dose infusion of dexmedetomidine.

### Subsequent Memory Effects

Because dexmedetomidine did not differentially modulate behavioral performance of memory associated with emotionally arousing content, and as there were only minimal drug related

**Table 4.** Brain Regions Associated with Subjective Arousal Effects

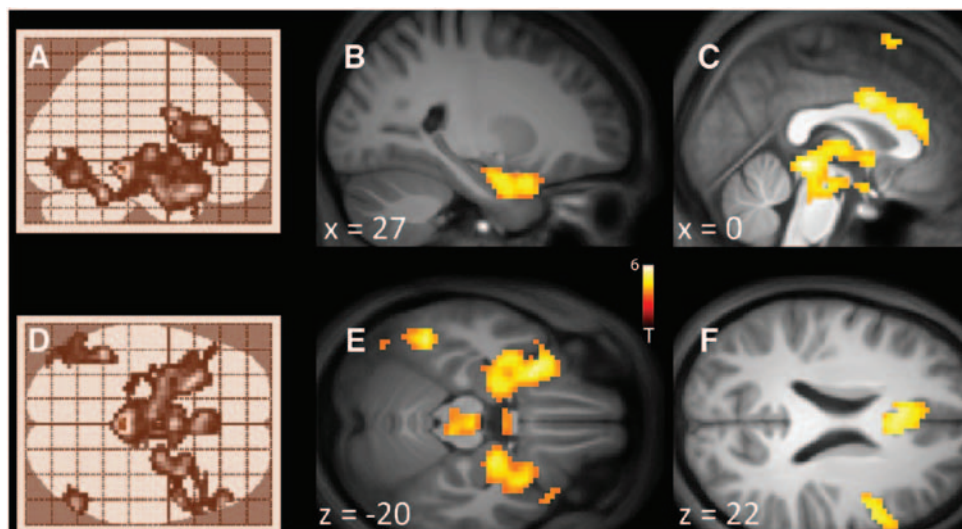
Hemisphere	Region	Brodmann Area	Coordinates (x, y, z)	Z-score (No. Voxels)
Common				
LR	Thalamus		(3, -31, -5)	5.73 (1,966)
R	Amygdala		(24, -7, -20)	4.98*
L	Amygdala		(-21, -4, -20)	4.73*
L	Middle temporal gyrus		(-48, -46, -23)	5.01 (399)
LR	Cingulate gyrus	24	(0, 2, 31)	5 (275)
R	Inferior frontal gyrus	9	(51, 8, 25)	4.77 (207)
R	Fusiform gyrus	37	(54, -64, -11)	4.77 (115)
LR	Superior frontal gyrus	6	(3, 17, 64)	3.82 (28)
Placebo more than dexmedetomidine				
R	Inferior frontal gyrus		(51, 8, 28)	4.76 (73)
L	Inferior temporal gyrus		(-48, -64, -2)	4.3 (22)
Dexmedetomidine more than placebo				
R	Inferior frontal gyrus		(36, 20, -32)	4.32 (35)
LR	Red nuclues		(-3, -19, -17)	4.06 (21)

\* Local peaks in the amygdala are listed for the thalamus cluster, thus the numbers of voxels were not available for these regions.

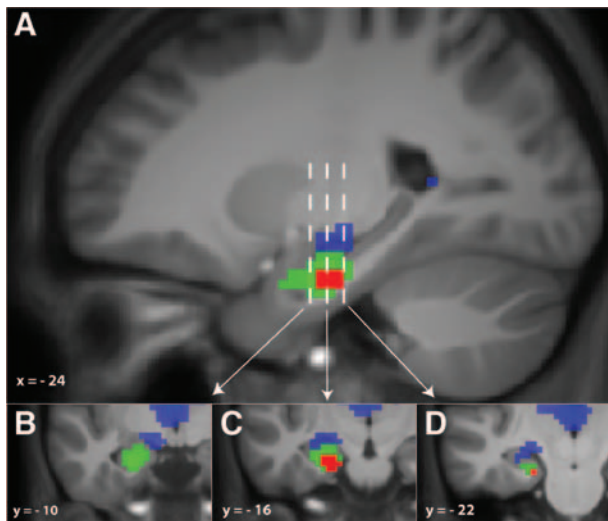
L = left; LR = both left and right; R = right.

effects because of emotional arousal in the neuroimaging data, subsequent memory effects were examined by collapsing over the factor of subjective emotional arousal rating (*e.g.*, emotionally arousing and neutral hits were collapsed). To identify subsequent memory effects common to the two groups, the main effect of subsequent memory collapsed over

placebo and dexmedetomidine (thresholded at  $P < 0.001$ ) was exclusively masked by the subsequent memory  $\times$  drug interaction ( $P < 0.1$ ), thus removing all voxels where effects significantly differed (at  $P < 0.05$ , one-sided) according to drug administered (note that a more liberal exclusive mask results in a more stringent masking procedure). As illustrated



**Fig. 3.** Results of the contrast examining where brain activity responses are significantly greater for emotionally arousing pictures compared with neutral nonarousing pictures. (A) The maximum intensity projection results are shown in a sagittal view that is displayed at a threshold of  $P < 0.001$ , with a 20-voxel extent. This displays the three-dimensional results occurring across the whole brain as seen when looking through the brain from a side view. The outline of the brain is shown in light color. The significant regional effects are shown as the dark areas within this brain outline. (B) When the results shown in figure 3A are projected onto a single parasagittal cross-section of the group mean anatomical image, it is evident that emotionally arousing pictures (compared with neutral pictures) significantly activate the amygdala. (C) When the results shown in figure 3A are projected onto a midsagittal cross-section, then activation of the anterior cingulate region just above the corpus collosum can be seen. Also evident in this slice is activation of thalamic and brainstem areas. (D) The same results as in figure 3A are now shown in the axial plane, as if looking through the brain from the top down. (E) When the results shown in figure 3D are projected onto the group mean anatomical image sliced in the axial plane, the amygdala activation can clearly be seen as bilateral. Other brainstem areas of activation can also be seen. (F) The anterior cingulate activation is shown in the axial plane.



**Fig. 4.** The only significant subsequent memory effect that was identified is located in the left hippocampus. (A) Sagittal view of the brain depicting the three different types of hippocampal effects found in comparing placebo with dexmedetomidine. The *green* areas represent those regions where blood oxygen level-dependent activity was greater for items that were subsequently remembered *versus* items that were subsequently forgotten common to both the placebo subjects and the subjects on dexmedetomidine. The *red* areas illustrate where there was a significant interaction, such that the placebo subjects had a larger subsequent memory effect than the dexmedetomidine subjects. Thus, this analysis identifies the *red* area as one neural correlate associated with the behavioral decrease in long-term memory performance that occurs on dexmedetomidine. The *blue* region is shown for comparison to reveal how nearby brain activity was greater for dexmedetomidine than placebo, even though this particular region did not show any subsequent memory effect. Three different close-up coronal views of the hippocampus are illustrated in the insets; (B) where the y coordinate is  $-10$ , (C) at the y coordinate of  $-16$ , and (D) at the y coordinate of  $-22$ . Images are displayed on sections of the normalized across-subjects mean anatomical image and thresholded at a minimum of  $P < 0.001$ , with a 20-voxel extent threshold.

in figure 4 (green areas), only a single common subsequent memory effect was identified at this significance threshold in left hippocampus ( $-27, -13, -23, z = 4.39, 82$  voxels). Subsequent memory effects that were greater for one group than the other were identified by inclusively masking the main effect of subsequent memory (thresholded at  $P < 0.001$ ) with the drug  $\times$  subsequent memory interaction (e.g., subsequent memory effects that were greater for placebo than dexmedetomidine, and *vice versa*; thresholded at  $P < 0.05$ ) resulting in a conjoint threshold of  $P < 0.0005$  with a 20-voxel extent threshold. In the case of the placebo group, a single subsequent memory effect centered on the left hippocampus ( $-24, -13, -26, z = 4.69, 21$  voxels, inclusively masked effective threshold of  $P < 0.0005$ ) survived the mask (figs. 4A, C, and D, red areas). No subsequent memory effects were found to be larger for the dexmedetomidine

than placebo group. However, the overall signal was greater for dexmedetomidine in the blue areas of figure 4.

Representative BOLD signal time-activity course changes for the common subsequent memory effect and the dexmedetomidine  $<$  placebo subsequent memory effect, along with a portion of nearby hippocampal activity that showed an increased response to dexmedetomidine, are shown in figure 5. Figures 5A, C, and E show the main effect of drug in regions corresponding to the green, red, and blue portions of figure 4. Figures 5B, D, and F show the subsequent memory effects occurring in these exact same voxels when the data are parsed into items that are remembered *versus* those that are forgotten. Figure 5B illustrates that in the posterior hippocampus there was no significant subsequent memory effect for either condition (corresponding with the blue areas of fig. 4). Figure 5D illustrates that there is a significant subsequent memory effect for both the placebo and dexmedetomidine groups (corresponding with the green areas of fig. 4). Importantly, the difference in subsequent memory performance across drug conditions is shown in figure 5F (corresponding with the red areas of fig. 4). In this portion of the hippocampus, the remembered items on dexmedetomidine did not elicit as strong of a BOLD signal as the remembered placebo items, and some slight change in the size of the signal for the forgotten items may be contributing to the nature of the drug-related interaction.

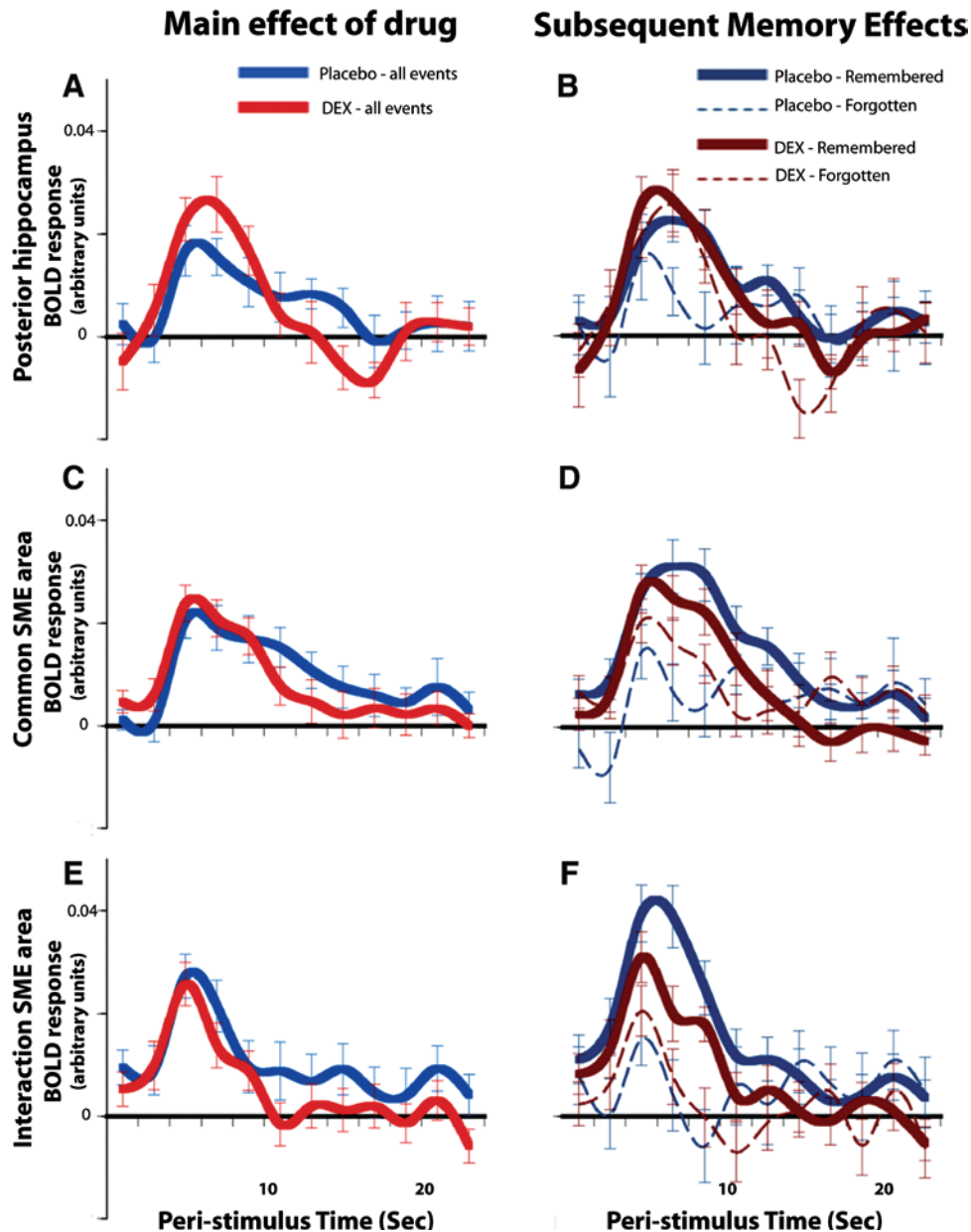
## Discussion

This human neuroimaging study investigated behavioral neural correlates associated with low-dose dexmedetomidine infusion. Dexmedetomidine did not differentially affect emotional reactivity to a series of emotionally arousing *versus* neutral pictures. It did impair long-term memory performance, but it did not show a specific ability to differentially affect memory for emotionally arousing *versus* neutral material. Dexmedetomidine caused regionally heterogeneous effects on brain activity, along with minimal changes in activity of emotional-processing networks, and a single subsequent memory interaction effect localized to the left hippocampal/amygdala area. The difference in the size of the subsequent memory effect on dexmedetomidine is consistent with the hypothesis offered by Pryor *et al.* that suggested the amnesic effect of dexmedetomidine is associated with a weaker memory trace being formed at the time of learning.<sup>10</sup>

## Behavioral Findings

The proportions of studied items rated to be emotionally arousing *versus* neutral and the ratio of test items afforded “remembered” *versus* “know” responses did not differ between the groups. Despite this performance similarity, on a test of long-term memory for the items seen in the scanner, the dexmedetomidine subjects forgot essentially one-third more items than controls. Forgetting was equally distributed across the emotionality and memory category dimensions. The





**Fig. 5.** Representative time courses to all events (*i.e.*, the blue areas of fig. 4) from a posterior region of the hippocampus that were greater for the dexmedetomidine than the placebo condition are shown (A). For this same voxel (B), parsing the data into remembered and forgotten events revealed no significant subsequent memory effects. A representative blood oxygen level-dependent response from the common subsequent memory effect area (*i.e.*, the green areas of fig. 4) shows that the dexmedetomidine and placebo responses were essentially identical when collapsed across all event types (C). However, parsing the data into remembered and forgotten events (D) revealed that a significant subsequent memory effect was present for both placebo and dexmedetomidine. A representative signal from the interaction subsequent memory effect area (*i.e.*, the red areas of fig. 4) shows that the dexmedetomidine and placebo responses did not significantly differ across all events (E). However, parsing the data into remembered and forgotten events (F) reveals a much larger subsequent memory effect for placebo than dexmedetomidine. BOLD = blood oxygen level-dependent; DEX = dexmedetomidine; SME = subsequent memory effect.

noradrenergic actions of dexmedetomidine were anticipated to modulate memory for emotionally arousing material in a manner comparable with that seen with  $\beta$ -blockers.<sup>48</sup>  $\beta$ -blockers prevent the mnemonic boost associated with emotional arousal,<sup>21</sup> likely because of reduced noradrenergic signaling occurring within the amygdala around the time of

learning.<sup>49</sup> Because  $\beta$ -blockers modulate emotional memory, they have been tried as therapy for posttraumatic stress disorder.<sup>50</sup> Here dexmedetomidine at the dose used caused a significant impairment of memory performance without affecting responsiveness to emotionally arousing material or specifically modulating emotional memory. Taken together,



this suggests dexmedetomidine would not be a therapeutic option for stress disorders.

At study, the dexmedetomidine subjects missed responses to about 6% of the items, whereas controls missed less than 1%. This difference suggests a mild sedative effect was occurring and that the dexmedetomidine subjects may have had fewer attentional resources available during encoding. However, there was no difference in the average reaction times to rate emotionally arousing and neutral pictures. This shows that when the subjects were attending to the task (for the vast majority of the items), the drug did not appear to slow emotional processing time. A similar lack of difference in reaction times was also reported by Pryor *et al.*, on a different task using even higher doses of dexmedetomidine.<sup>10</sup> This combination of findings establishes that the low dose used was sufficient to cause a memory-impairing effect, but it was not large enough to inhibit the perception of emotionally arousing content or slow psychomotor control. Subjects often described the subjective feeling of the drug as having had nothing at all or feeling similar to having had one or two beers.

### fMRI Findings

The event-related fMRI design allowed three different types of analyses. First, brain regions showing a main effect of the drug were identified. Second, we determined whether processing of emotionally arousing material was affected by drug. Lastly, we identified subsequent memory effects common to, as well as specific to, the experimental groups. Crucially, if the long-term memory-impairing effect of dexmedetomidine is caused by a weaker memory trace being formed at the time of initial encoding, then we predicted subsequent memory effects might be attenuated in the hippocampus, or other areas commonly associated with subsequent memory effects.<sup>51</sup>

Even with the low dose used, the drug caused heterogeneous effects on the BOLD signal throughout the brain. BOLD response reductions were largely cortical in nature and occurred primarily in the visual-processing stream and sensory-motor cortical areas. Although our paradigm was not specifically designed to quantify the magnitude of a drug-induced sensory-processing deficit, it did reveal which brain areas could contribute to such a deficit (figs. 1 and 2A). Given these changes in sensory areas, it remains plausible that weaker sensory processing contributed to a weaker initial memory trace during drug exposure. However, our subsequent memory behavioral paradigm analysis did not reveal these cortical areas as the neural correlates of the impaired long-term memory performance (fig. 2C).

BOLD response increases because of drug were identified in midbrain regions such as the hypothalamus, thalamus, and parts of the hippocampus/parahippocampal area. As higher sedative doses of dexmedetomidine will significantly reduce global cerebral blood flow,<sup>52</sup> and the imaging analysis assumes global normalization, these regional signal increases may represent areas least suppressed by drug, rather

than actual increased activity. Still, we speculate that these regional effects are related to the underlying regional mechanisms of drug action. Hypothalamic activation may fit with a “sleep” model of dexmedetomidine-induced sedation,<sup>53,54</sup> and widespread cortical suppression fits with an overall generalized pattern of metabolic suppression caused by anesthesia.<sup>55</sup> However, these regional effects must be interpreted cautiously as they represent an evoked response to a specific task. Things may differ in the unstimulated state. Indeed, the regional cortical changes seemed to avoid the areas of lateral frontal and lateral parietal cortex (fig. 2B) that are more commonly associated with the direct effects of various anesthetics,<sup>56,57</sup> and are thought important for attention processing and perhaps even consciousness.<sup>46,57</sup> Why dexmedetomidine would have less of an effect in these frontal/parietal regions during this task is not known, but we speculate extra attentional effort may have been needed to overcome sedative effects and keep response times similar between groups.

Regions involved in the processing of emotionally arousing content (emotionally arousing more than neutral stimuli) were largely unchanged by dexmedetomidine. Activity in the amygdala and the anterior cingulate cortex, two regions that play a role in processing of emotional arousal,<sup>58</sup> were activated by the arousing stimuli in a manner that was statistically equivalent across groups. This suggests the lack of a drug effect on memory modulation for emotionally arousing content was not because of an upstream effect of the drug only blocking reactivity to emotional arousal. Still, a few brain regions did show a significant difference in drug-related processing of emotionally arousing material (table 4). However, interpreting these effects as specific drug-related changes in arousal processing is difficult because these few specific regions overlapped with those broader areas that also showed a main effect of the drug. There were also very minimal differences in brain regions recruited by subjects administered placebo *versus* dexmedetomidine, none of which were in the vicinity of the amygdala. This lack of a direct interaction with the amygdala contrasts with that found for sevoflurane in humans with positron emission tomography neuroimaging,<sup>32</sup> and with some data from animal experiments<sup>15</sup> that suggest an amygdala focus might exist for some anesthetic memory interactions.

Subsequent memory effects were found in regions of the left hippocampus and amygdala for subjects in both groups. Yet, a differential subsequent memory effect was found in an adjacent region of the hippocampus that was greater for placebo subjects than the dexmedetomidine subjects. A component of this interaction may be related to how forgotten items were differentially processed on the drug, and this suggests that the change in hippocampal function is complex and difficult to interpret as a simple suppression of a memory trace on dexmedetomidine. The ultimate pathway by which the hippocampal activity changed likely involved numerous factors, such as decreased attention, easier distractibility, apathy toward the task, sedation effects, or global

metabolic suppression. Yet, whatever the factors were, they all culminated in a reduced BOLD response in a portion of the hippocampus that was not by itself a main target of the drug. Indeed, nearby posterior portions of the hippocampus showed an increase (rather than a decrease) of the BOLD signal on dexmedetomidine. Importantly, these posterior hippocampal regions were not differentially modulated by subsequent memory behavior (fig. 5). As memory performance was impaired for dexmedetomidine subjects, and as the medial temporal lobe is widely recognized as a key structure in memory storage and consolidation operations,<sup>38</sup> it is very likely that the differential subsequent memory activity in subjects who received dexmedetomidine reflects the inhibited ability of these subjects to encode the information into long-term memory.

In summary, the combination of findings reveal that the dose of dexmedetomidine used in the present study does not modulate processes associated with emotional arousal and that the failure of memory encoding during a low-dose dexmedetomidine infusion is associated with a reduction of subsequent memory effects in the left hippocampal region. This supports the general idea that insights into anesthetic-induced amnesia, which targets conscious memories, may be obtained by measuring hippocampal activity. The underlying mechanism for a hippocampal-dependent memory-suppression effect is not elucidated by this type of study. Nonetheless, these results strongly support further experimentation that is localized to the hippocampus. Indeed, a recent animal study that measured the electroencephalogram directly in the hippocampus investigated changes in hippocampal  $\theta$  rhythm as a mechanism of anesthetic-induced amnesia.<sup>3</sup> The mechanistic hypothesis offered in that animal study is consistent with the findings of the current study in humans.

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