Neuroimaging analysis of an anesthetic gas that blocks human emotional memory

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It is hypothesized that emotional arousal modulates long-term memory consolidation through the amygdala. Gaseous anesthetic agents are among the most potent drugs that cause temporary amnesia, yet the effects of inhalational anesthesia on human emotional memory processing remain unknown. To study this, two experiments were performed with the commonly used inhalational anesthetic sevoflurane. In experiment 1, volunteers responded to a series of emotional and neutral slides while under various subanesthetic doses of sevoflurane or placebo (no anesthesia). One week later, a mnemonic boost for emotionally arousing stimuli was evident in the placebo, 0.1%, and 0.2% sevoflurane groups, as measured with a recognition test. However, the mnemonic boost was absent in subjects who received 0.25% sevoflurane. Subsequently, in experiment 2, glucose PET assessed brain-state-related activity of subjects exposed to 0.25% sevoflurane. Structural equation modeling of the PET data revealed that 0.25% sevoflurane suppressed amygdala to hippocampal effective connectivity. The behavioral results show that 0.25% sevoflurane blocks emotional memory, and connectivity results demonstrate that this dose of sevoflurane suppresses the effective influence of the amygdala. Collectively, the findings support the hypothesis that the amygdala mediates memory modulation by demonstrating that suppressed amygdala effectiveness equates with a loss of emotional memory.

amygdala | anesthesia | brain imaging | cerebral metabolism | sevoflurane

The memory modulation hypothesis proposes that emotional stimuli have a mnemonic advantage over neutral stimuli in long-term memory (1, 2). The influence of emotional arousal on memory is thought to be mediated through a neurobiological mechanism involving the amygdala and its interactions with other brain regions (3). Gaseous anesthetic agents are powerful amnesic agents, causing temporary amnesia at doses that are a fraction of those required to produce unconsciousness (4). The effects of inhalational anesthesia on human emotional memory processing have yet to be investigated. These effects are clinically relevant, because the amnesic component of general anesthesia can fail and allow patients to have recall of intraoperative events (5). It is important to know whether a particular anesthetic agent might prevent or exacerbate the sequelae associated with episodes of intraoperative awareness.

Findings from animal studies suggest that the commonly used inhalational anesthetic sevoflurane should disrupt human emotional memory processing. Evidence suggests that the basolateral amygdala (BLA) is the locus for interaction between arousal and memory modulation (3). Numerous studies show that drug manipulations of BLA activity can either enhance or impair memory performance, especially for long-term memory of emotionally arousing stimuli (3). Animal research on amygdala functioning has been consistent with human research findings, including the study of amnesic agents, such as benzodiazepines. The amnesic effect produced by benzodiazepines depend on the BLA (6). Lesions of the BLA block the amnesic effect of benzodiazepines in rats (7). Interestingly, in humans, benzodiazepines impair long-term memory for emotionally arousing stimuli (8). Recently, lesions of the BLA were found to block the amnesic effect of sevoflurane (9). Integrating these animal findings with the results from the human benzodiazepine study suggests that sevoflurane, at some dose, should block human emotional memory.

In experiment 1, we first determined whether exposure to a low dose of sevoflurane would block the mnemonic advantage associated with emotional arousal. Volunteers ($n = 28$; 19 male) were exposed to either placebo (i.e., 100% oxygen) or one of three constant doses of sevoflurane (i.e., 0.1%, 0.2%, or 0.25%) during viewing of pictures, per a design used in earlier studies (10, 11). Subjects rated each image for its emotional arousal intensity on a 1–4 scale. One week later, with no anesthesia exposure, memory was tested. Memory results were analyzed with respect to picture emotionality (i.e., emotional vs. neutral ratings).

From experiment 1, an emotional memory-blocking dose of sevoflurane was determined. Brain-activity changes associated with this dose of sevoflurane were assessed with PET. The cerebral metabolic rate of glucose utilization (rCMRglu) was measured in 11 male subjects with ^{18}fluoro-deoxyglucose (FDG) on a high-resolution PET camera. The regionally specific effects and the brain area interactions of sevoflurane were analyzed with statistical parametric mapping (SPM) and structural equation modeling (SEqM), also called path analysis. Subjects were scanned on two occasions during a resting state, once while they were under the influence of 0.25% sevoflurane and once while breathing only oxygen. We hypothesized that sevoflurane might demonstrate a localized regional effect on amygdala activity and would disrupt its effective connectivity influences (12).

Results

Sevoflurane Shifts Emotional Ratings. Items were grouped into two categories based on emotional reactivity scores of either "neutral" (items rated 1 or 2) or "emotional" (items rated 3 or 4). As shown in Fig. 1, subjects exposed to sevoflurane rated significantly more slides as neutral than emotional when compared with placebo ($P < 0.05$, for each sevoflurane group).

Sevoflurane Effects on Memory. ***Free recall.*** Sevoflurane dose-dependently reduced free recall. The mean (±SEM) in the placebo group was 6.8 ± 1.3 slides. The 0.2% dose significantly reduced recall by 49%, to 3.5 ± 0.8 slides ($P < 0.05$). The 0.25% dose also reduced recall by 53%, to 3.2 ± 1.1 slides ($P < 0.05$). Fig. 2 shows that the expected mnemonic boost associated with arousal occurs in the placebo group and the 0.1% sevoflurane group ($P < 0.05$, paired t test, for both). However, a mnemonic

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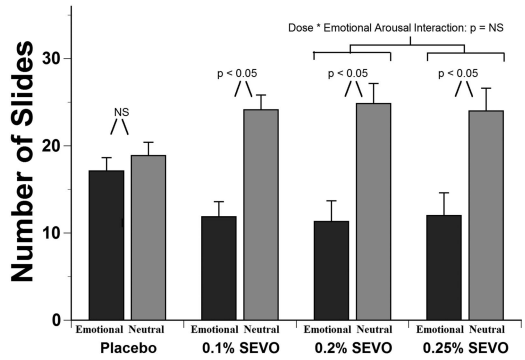

Fig. 1. The number of pictures rated as either neutral or emotional for their arousal reaction is shown (mean ± SEM). Placebo subjects rated the same number of items neutral as emotional. Subjects that received sevoflurane (SEVO) rated significantly more items as neutral vs. emotional. There was no significant effect of increasing dose from 0.2% to 0.25% on how subjects rated the pictures, as shown by the dose × emotionality interaction. NS, not significant.

boost was not evident with the 0.2% and 0.25% doses. An apparent trend for the 0.1% sevoflurane group vs. the placebo group to have a mnemonic boost in free recall of the emotional items over the neutral items is suggested in Fig. 2 by the larger emotional bar. However, this effect did not approach statistical significance for a dose × emotionality interaction comparing placebo with 0.1% sevoflurane ($P = 0.30$).

Recognition memory. Table 1 shows the dose-dependent changes in recognition memory performance. The 0.25% dose significantly reduced the total hit rate proportion (i.e., confident plus nonconfident hit rate). The increase in false alarm rates was primarily driven by an increase in false alarms to nonconfident items.

Recognition memory performance was determined as the discrimination index − Pr, the proportion of studied items correctly identified as old, corrected by the false alarm rate to the foils [Pr = p(Hit) − p(False Alarm)] (13). Sevoflurane dose-dependently reduced total Pr. This effect reached significance for the 0.2% dose compared with placebo ($P < 0.05$), where the drug suppressed total Pr 21%. Total Pr dropped from 73 ± 9% on placebo to 58 ± 14% on 0.2% sevoflurane. The effect was greater in the 0.25% group, where total Pr fell by 43% compared with placebo ($P < 0.001$), dropping to a value of 42 ± 5%.

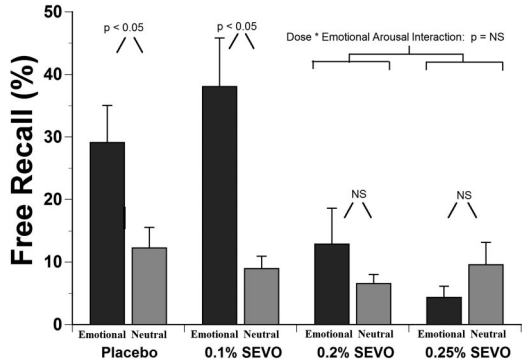

Fig. 2. The dose-dependent changes in recall memory performance are shown (mean ± SEM). The expected mnemonic boost for emotionally arousing items is evident in the placebo and the 0.1% sevoflurane groups. The mnemonic boost is not seen in the 0.2% or 0.25% groups. There was no significant effect of increasing dose from 0.2% to 0.25% on recall performance related to emotionality of the items, as shown by the dose × emotionality interaction. NS, not significant.

Pr recollection (confident hits). Sevoflurane caused a dose-dependent reduction in Pr − recollection memory (defined as confident hits − confident false alarms). Mean probability of recollecting the slides was reduced by 11%, 12%, and 30% for the 0.1%, 0.2%, and 0.25% sevoflurane doses, respectively. This effect was significant only at the 0.25% dose vs. placebo ($P < 0.05$).

Pr familiarity (nonconfident hits). Sevoflurane dose-dependently reduced Pr for familiarity memory (defined as nonconfident hits − nonconfident false alarms). The effect was significant only for the 0.25% dose showing a 73% reduction of Pr-familiarity memory ($P < 0.05$, vs. placebo).

Sevoflurane Blocks Emotional Recollection Memory. Fig. 3 shows the effect of sevoflurane on the Pr-confident recollection memory performance as related to slide emotionality. The placebo, 0.1%, and 0.2% groups significantly recollected more emotional slides than neutral slides ($P < 0.05$, for each group). However, in the 0.25% group, there was no significant difference in recollection between the emotional and neutral slides. This change in performance was dose-related, because a dose-by-emotionality ANOVA–interaction term comparing the 0.2% and the 0.25% groups on Pr-recollection memory was significant ($P < 0.05$).

Sevoflurane Suppresses Cerebral Metabolism. The global and regional cerebral metabolic effects of 0.25% sevoflurane are shown in Fig. 4. Supporting information (SI) Table 2 shows that physiologic parameters did not change significantly during 0.25% sevoflurane. Mean (±SD) global rCMRglu significantly decreased 17 ± 13% from baseline with sevoflurane (Fig. 4*A*, $P < 0.05$). The region-of-interest (ROI) analysis (Fig. 4*B*) revealed glucose metabolism was suppressed in a nonuniform manner throughout the brain. The regional percent change of metabolic activity is shown in Fig. 4*C*. In Fig. 4*D*, the subtraction analysis showed a relative decrease of activity in the thalamus (the central cluster; $x = 5$, $y = -14$, $z = 12$; z-score = 3.07), occipital cortex ($x = 4$, $y = -72$, $z = 1$; z-score = 2.99) and cerebellum ($x = -4$, $y = -81$, $z = -26$; z-score = 3.67). A relative suppression or activation of the amygdala was not found. Fig. 4*E* further localizes the thalamic suppression effect to an area containing the intralaminar thalamic nuclei. These nuclei interact with the amygdala and are known to play a role in mediating arousal and controlling the level of consciousness during anesthesia (14).

Amygdala to Hippocampal Effective Connectivity Changes. Fig. 5 shows the SEqM path diagrams for the two scan conditions: baseline (i.e., placebo or no anesthesia; Fig. 5*A*) and 0.25% sevoflurane (Fig. 5*B*). In the baseline state, the effective connectivity of the amygdala onto the hippocampus is shown as a large positive influence in both hemispheres (Fig. 5*A*, solid red arrows from amygdala to hippocampus). A positive path weight with a value of 1 implies that if all regions within a network model were held constant, then a one-unit increase of activity in an upstream brain region would increase the activity in a downstream brain region by one unit. A negative path weight implies the inverse, such that if activity increases by one unit in an upstream brain region, then activity in a downstream brain region will decrease by one unit (15). The path weights thus show the effective influence one brain region has on another within the modeled network. During 0.25% sevoflurane, a number of path weights change and take on a negative influence, primarily in the right hemisphere (Fig. 5*B*, dashed red arrows). Most importantly, the amygdala to hippocampal pathway in the right, but not left, hemisphere takes on a negative influence (Fig. 5*B*). A large negative influence from the thalamus onto the amygdala also becomes evident in both hemispheres. Fig. 5*C* shows the subtraction analysis of path weights between both conditions. Only two significant changes in path weights between conditions

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Table 1. Mean (SD) sevoflurane-induced dose-dependent changes in memory performance rates

	Hit rate			False alarms			Pr				
Dose	Total	Confident	Non-con	Total	Confident	Non-con	Total	Confident	Non-con	Miss	CR
Placebo	0.78 (0.10)	0.59 (0.17)	0.19 (0.10)	0.05 (0.05)	0.02 (0.03)	0.03 (0.03)	0.73 (0.09)	0.57 (0.16)	0.16 (0.09)	0.22 (0.10)	0.95 (0.05)
0.10%	0.80 (0.12)	0.55 (0.23)	0.25 (0.16)	0.13 (0.13)	0.04 (0.05)	0.09 (0.09)	0.67 (0.16)	0.51 (0.21)	0.17 (0.17)	0.20 (0.12)	0.87 (0.13)
0.20%	0.68 (0.25)	0.54 (0.23)	0.14 (0.09)	0.10 (0.07)	0.03 (0.03)	0.06 (0.06)	0.58 (0.14)*	0.50 (0.18)	0.08 (0.11)	0.32 (0.17)	0.90 (0.07)
0.25%	0.59 (0.16)*	0.44 (0.13)	0.16 (0.09)	0.17 (0.05)*	0.04 (0.03)	0.13 (0.07)*	0.42 (0.05)*	0.40 (0.07)*	0.03 (0.07)*	0.41 (0.05)*	0.83 (0.05)*

Pr, discrimination index; CR, correct rejections; non-con, nonconfident responses. *, $P < 0.05$ vs. placebo.

were found and are highlighted in blue ($P < 0.05$ for significant effective connectivity changes). The influence of the amygdala onto the hippocampus in the right hemisphere was significantly different between conditions. Additionally, the right nucleus basalis area onto the right hippocampus also showed a significant network change between conditions.

Discussion

There are six major findings from this work. (*i*) The mnemonic boost associated with free recall of emotional memory did not occur at sevoflurane doses of 0.2% or 0.25%. (*ii*) The confident recollection of emotional memory demonstrated a sharp dose-related cutoff, such that the mnemonic boost observed at 0.2% sevoflurane was not present at 0.25%. (*iii*) Each dose of sevoflurane changed how the subjects rated the emotionality of the slides, such that significantly more slides were rated neutral rather than emotional. (*iv*) By using the PET data, SEqM revealed that 0.25% sevoflurane suppressed the effective connectivity between the right amygdala with the right hippocampus. (*v*) SEqM also showed that sevoflurane suppressed the effective connectivity between the right nucleus basalis and the right hippocampus. (*vi*) By using the PET data, SPM subtraction analysis revealed that 0.25% sevoflurane did not significantly change regional amygdala activity, although it did suppress regional thalamic activity.

Experiment 1 revealed a compelling demonstration for blockade of human emotional memory using an anesthetic manipulation. In the free-recall data, subjects at 0.1% sevoflurane recalled the emotional slides far better than the neutral slides. However, subjects at 0.2% and 0.25% sevoflurane did not show this mnemonic boost, because they recalled an equal number of emotional and neutral slides. Thus, the mnemonic boost of emotional arousal associated with long-term free recall appears to be blocked if learning occurs during exposure to 0.2% or 0.25% sevoflurane. It is intriguing that the 0.1% group appears to have a slight mnemonic advantage for emotional over neutral items in comparison with the performance in the placebo group (see Fig. 2). However, this effect was not statistically significant. Nonetheless, this dose of sevoflurane was found to have a memory-enhancing effect in rats (16). Together, these observations suggest further work might clarify the parameters needed to document a memory enhancing effect of sevoflurane in humans.

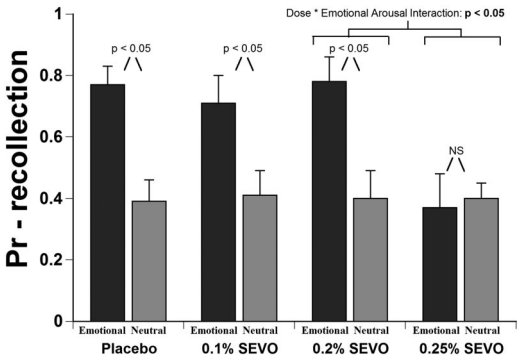

Fig. 3. The Pr for recollection memory is shown (mean ± SEM). A mnemonic boost for emotional pictures is noted in the placebo, 0.1%, and 0.2% sevoflurane groups. The mnemonic advantage of emotional material fails to occur in subjects exposed to 0.25% sevoflurane. There was a significant effect of increasing dose from 0.2% to 0.25% on recollection related to arousal intensity of the items, as shown by the dose × emotionality interaction. NS, not significant.

A blockade of emotional memory was also clearly evident in the recollection data. A robust mnemonic boost effect was seen in the placebo, 0.1%, and 0.2% groups. However, in the 0.25% group, the emotional and neutral items were recollected equally (see Fig. 3). An ANOVA comparing emotional vs. neutral recollection at 0.2% vs. 0.25% revealed a significant emotionality interaction (dose × emotional arousal intensity). At 0.2%, the mnemonic boost is present, yet at 0.25%, the effect is gone; thus, the emotional memory-blocking effect of sevoflurane on recollection memory is sharply dose-dependent. The emotional memory-blocking dose effect differs slightly between recall and recollection memory performance (e.g., 0.2% vs. 0.25%). This is likely due to the inherent differences in task difficulty between recall and recognition memory.

Sevoflurane shifted the emotional ratings of the pictures (Fig. 1), where significantly more items were rated as neutral than emotional. However, the block of emotional recollection memory found at 0.25% sevoflurane cannot be attributed to this shift, because an identical shift occurred in the 0.2% group, and this group still retained its mnemonic boost. Overall Pr-recognition memory was impaired by 43% with exposure to 0.25% sevoflurane. This human amnesic dose for contextual information is similar to the rat amnesic dose for avoidance learning, where the 24-hr ED_{50}-amnesic dose of sevoflurane was found to be 0.24% (4).

Results from experiment 1 showed that 0.25% sevoflurane blocks emotional recollection. Therefore, experiment 2 investigated brain activity associated with this dose of sevoflurane. Glucose PET was used for experiment 2, rather than functional MRI, because PET directly identifies both the global and regional changes in brain activity induced by the anesthetic. A subtraction analysis on the PET data did not show any site-specific effect of sevoflurane on amygdala activity. However, SEqM results showed that 0.25% sevoflurane reduced the effective influence of the right amygdala onto the right hippocampus. Additionally, the effective influence of the right nucleus basalis area onto the right hippocampus was reduced. This suggests sevoflurane's behavioral effects involved interactions with multiple brain regions, which ultimately altered the effective network connectivity interactions of the amygdala with its target areas of influence, such as the hippocampus (3). The subtraction analysis and the SEqM results suggest that sevoflurane does not exert its effects on emotional memory simply through a site-specific amygdala effect. A site-specific effect could have occurred if the amygdala contained a high density of sevoflurane-sensitive or insensitive receptors. For example, the amygdala has a high regional density of GABA receptors with

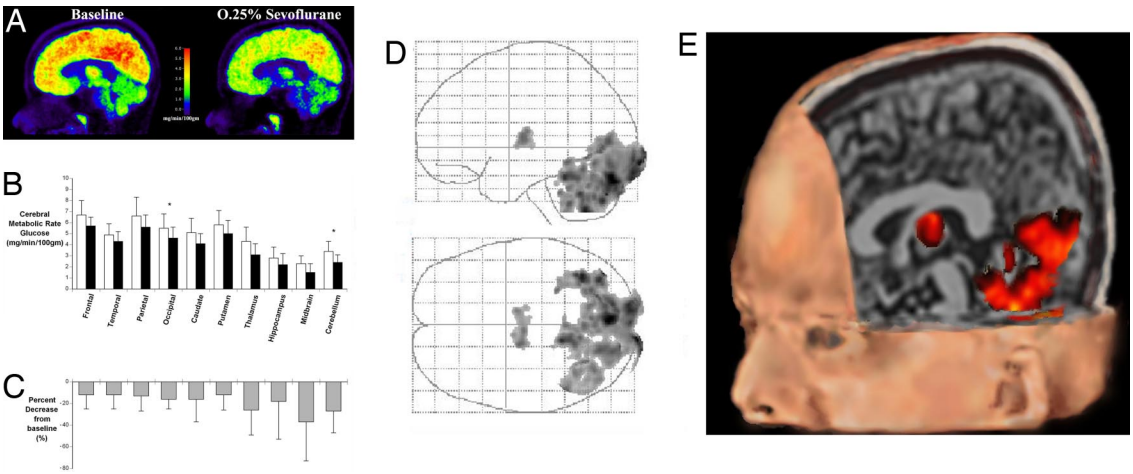

Fig. 4. The cerebral metabolic effects of 0.25% sevoflurane are shown. (*A*) Representative high-resolution PET scans. (*B*) Absolute (mean ± SD) regional metabolic changes (white bars, placebo, no anesthesia; dark bars, 0.25% sevoflurane; marked with * for $P < 0.05$, Bonferroni corrected, paired *t* test). (*C*) Relative percent decreases of regional metabolism. (*D*) Regional SPM results of sevoflurane induced metabolic suppression (*Upper*, sagittal; *Lower*, axial). *E* shows the regional thalamic finding (brain center) on a colorized MRI. The SPM effects are significant at $P < 0.001$, uncorrected; displayed at $P < 0.005$, with a 500-voxel extent.

the epsilon subunit, and these receptors are uniquely insensitive to the i.v. anesthetic, propofol (17).

A striking aspect of the SEqM results was that the change in amygdala connectivity was specifically in the right hemisphere. Our PET imaging sample consisted of only male subjects. Evidence from several laboratories has identified a sex-related hemispheric lateralization of amygdala function with respect to emotional memory. The amygdala in the right hemisphere is disproportionately involved with emotional memory for men (10, 11). Conversely in women, it is the amygdala in the left hemisphere that is more involved with emotional memory (10, 11). The right-hemisphere amygdala connectivity change in our male subjects suggests the possibility that sevoflurane might selectively disrupt amygdala functioning in the left hemisphere of women.

Neuroimaging studies support the memory modulation hypothesis of amygdala functioning by establishing that enhanced emotional memory is directly correlated with enhanced amygdala activity (18–20). Additional studies show that the mnemonic boost associated with emotional arousal enhances amygdala functional and effective connectivity (12, 21). Here we demonstrate the other side of the memory modulation concept by using anesthesia to suppress, rather than enhance, the functioning of the system. We show that a drug capable of suppressing the effective influence of the amygdala onto the hippocampus is also capable of blocking the mnemonic boost associated with emotional arousal.

The vast majority of drug and hormone modulations of memory depend critically on amygdala function, and animal studies of general anesthetic-induced amnesia follow this consistent trend in the literature (1, 7, 9, 22). This suggests studying the effects of other anesthetics on human emotional memory processing is warranted. Understanding how anesthesia affects arousal-related memory might also provide further insight for helping to prevent cases of intraoperative recall. Finally, the present findings suggest the study of sevoflurane's event-related memory arousal effects should be considered.

In conclusion, this study reports the discovery of an agent and method for blocking human emotional memory. An anesthetic gas blocked the mnemonic boost usually associated with emotional arousal, an effect not attributable to the drug's influence on emotional reactions. Brain imaging analysis of the emotional

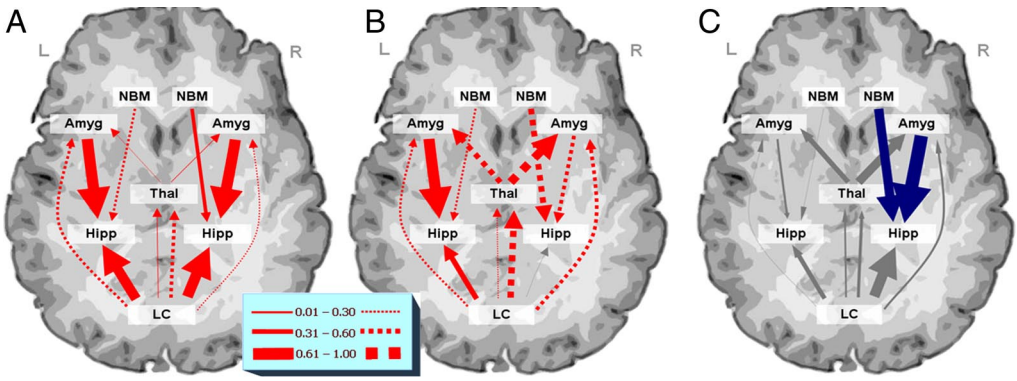

Fig. 5. Path diagrams for baseline (placebo, no anesthesia, *A*) and 0.25% sevoflurane (*B*) are shown. Positive influences of one region onto another are shown as solid lines and negative influences are shown as dotted lines. Line width represents the magnitude of the effective influence, larger widths indicating a larger influence, according to the scale shown. The numerical difference in path weights between conditions is shown in *C*. Paths that significantly contribute to the network model more in the placebo state vs. the anesthesia state are highlighted; all other paths are shown grayed out. A large change in path weights is noted for the effective influence of the amygdala and the nucleus basalis of Meynert (NBM) on the hippocampus during sevoflurane. Thal, thalamus; Amyg, amygdala; LC, locus coeruleous; Hipp, hippocampus.

memory-blocking dose revealed brain changes consistent with suppression of the neural circuitry hypothesized to mediate emotional memory (3, 12). These findings support the memory modulation hypothesis of amygdala functioning and demonstrate anesthesia might have great utility for helping to clarify the neurobiology of human emotional memory.

Materials and Methods

Research activities were conducted with institutional review board approval and subject-written informed consent.

Subjects. ***Experiment 1.*** Twenty-eight volunteers [19 male; age range 18–28 years, mean = 21.1 (SD = 2.9)] were recruited from the local college campus and compensated for participation.
Experiment 2. Eleven male volunteers [age range 18–22 years, mean = 20.4 (SD = 1.4)] were recruited for brain imaging. Subjects were right-handed, healthy, and with no family history of adverse anesthetic reaction.

Anesthesia procedures. ***Experiment 1.*** Subjects sat facing a computer monitor and keyboard. Standard anesthetic monitoring was used. A 20-gauge i.v. catheter was inserted for potential administration of antinausea medication (none was needed). Subjects were randomized to either 0.0% ($n = 9$), 0.1% ($n = 8$), 0.2% ($n = 6$), or 0.25% ($n = 5$) sevoflurane doses. Sevoflurane was delivered through a calibrated 19.1 vaporizer in 100% oxygen via a standard semicircle breathing circuit attached to a Dräger anesthesia machine. Subjects breathed through a face mask. Anesthetic level was monitored with a Datex-Ohmeda Ultima Capnomac. Once subjects reached the targeted dose, they stayed at that dose for at least 20 min before viewing the slides.
Experiment 2. A similar anesthetic procedure was followed, except subjects were placed supine on a gurney, and two i.v. catheters were placed, one for arterialized venous blood sampling and one for delivery of the ^{18}fluorodeoxyglucose (FDG) radiotracer. Subjects were on 0.25% sevoflurane for at least 20 min before assessment of rCMRglu. The subjects underwent glucose PET scanning in a dark quiet room with eyes closed. They performed no specific cognitive task, other than extending one to three fingers when asked for scoring on the modified observer's assessment of alertness/sedation scale (OAA/S). The scan order was randomized and counterbalanced. Scans were performed at least 1 week apart.

Visual Stimulus Materials. Visual stimuli were selected from the international affective picture system (IAPS) series. The 36 target stimuli selected were a subset of the 96 slides previously used by Canli *et al.* (11) and also used by Cahill *et al.* (10). The normative valence ratings for this set of pictures ranged from highly negative (1.17) to neutral (5.44), and the normative arousal ratings ranged from tranquil (1.97) to highly arousing (7.63). Stimuli were selected to generate a roughly equal distribution of ratings for each emotional arousal category from 1 to 4. Pictures were presented in a randomized order for 6.0 sec each. To ensure each picture was viewed, a small white ''x'' appeared on one of the four corners of each scene after 6.0 sec, and subjects made a key-press response. Subjects then entered their emotional arousal reaction ratings by key press on the number pad from 1 to 4. Then the next slide was shown. The interstimulus interval was 4 sec.

Recall and recognition memory testing occurred 1 week later. Recall testing was performed, as described (23). Briefly, subjects wrote down as many pictures as they could remember, with enough detail to allow a blinded rater to identify each picture. For recognition memory testing, all 36 target pictures were shown intermixed with a set of 35 foils, matched for valence and arousal ratings. Subjects identified those pictures previously seen and provided confidence ratings. Items remembered with 100% confidence were classified as recollected confident hits. Items remembered with $<$100% confidence were classified as familiar nonconfident hits. One subject failed to return for memory testing and was excluded.

PET Scanning. Global and relative CMRglu was measured by using FDG (5.5 mCi). Arterialized venous blood sampling was used (24). One subject was excluded due to failed blood sampling. Static FDG images were obtained on a CPS Innovations high-resolution research tomography. Attenuation correction was provided by a single-photon emission Cs-137 source. The axial and transaxial field of view is 25.3 and 17.5 cm, with 207 image planes obtained at 1.2-mm plane spacing.

ROI Analyses. CMRglu was determined from ROIs by using Volume Imaging in Neurological Research, Co-Registration and ROIs included (VINCI, Ver. 2.7) software from the Max Planck Institute for Neurological Research, Cologne, Germany. ROIs were constructed by intersecting the regional WFU_PickAtlas templates with each subject's normalized brain MRI and coregistered PET. The outline of the ROIs used is provided as SI Fig. 6.

SPM-2 Analysis. A voxel-based categorical comparison was performed by using SPM (SPM-2) software from the Wellcome Department of Imaging Neuroscience, University College London. The PET images were realigned, MRI-registered, and spatially normalized by using SPM-2 (www.fil.ion.ucl.ac.uk/spm) running in Matlab 6.5 (MathWorks). All normalized PET images were spatially smoothed with a 4-mm FWHM isotropic Gaussian kernel. By using a voxel-wise approach, a statistical parametric map of the t statistic (SPM{T}) was constructed within the framework of the General Linear Model and Gaussian Field Theory.

For each subject, high-resolution T1-weighted volumetric SPGR MRI scans were also acquired by using a 1.5-T clinical Philips Eclipse scanner (Philips Medical Systems). The background MRI used in Fig. 5 was reconstructed in 3D by using Chris Rorden's MRIcro, Freeware. The subject's skin was pseudocolorized in Adobe Photoshop CS2 (Ver. 9.0, Adobe Systems).

Structural Equation Modeling. There are two general ways in which effective connectivity can be assessed: (*i*) through implementation of regression models (25), and (*ii*) through implementation of structural equation models (15). Due to the small number of scans, we used SEqM. This procedure consists of four basic steps: (*i*) identify the network of interest, (*ii*) construct an anatomical model of the network, (*iii*) calculate the interregional correlations, and (*iv*) calculate the path coefficients and compare the resulting SEqMs (15).

Network Identification. A model network of amygdala–hippocampal interactions was constructed based primarily on theoretical grounds combining results from animal studies (26, 27). Network complexity was limited by sample size. The model contained five nodes and six paths per hemisphere. The effective influence of the amygdala (node 1: $x = \pm16$, $y = -06$, $z = -18$ mm) onto the hippocampus (node 2: $x = \pm28$, $y = -30$, $z = -10$ mm) was the primary theoretical focus of the model (12, 28). Noradrenergic influences from the locus coeruleus (node 3: $x = \pm04$, $y = -32$, $z = -26$ mm) were modeled for theoretical reasons (29). Acetylcholine related influences from the area of the nucleus basalis (node 4: $x = \pm12$, $y = 06$, $z = -12$ mm) were integrated into the model also for theoretical reasons (30). Thalamic (node 5: $x = \pm03$, $y = -16$, $z = -00$ mm) influences were added to the model, because this region was identified as a main effect of anesthesia (e.g., see *Results*). Metabolic measures from each node were extracted from the underlying subject samples by using the partial least-squares program ''PLSgui'' (15).

Anatomical Model. The anatomical model was determined from animal tracing studies of the modeled network connections (31). This generalized anatomical model only includes the putative influences of major efferent pathway connections.

Interregional Correlations. These were computed within condition and across all 11 subjects by using Matlab 6.5 (Mathworks). The data used were the representative mean centered relative CMRglu voxel values from within each node of interest.

Path Coefficients and SEqM Comparisons. The path coefficients or effective connections among the nodes of the network were determined by using the maximum-likelihood estimation function implemented in Lisrel 8.3 (Scientific Software). The method minimizes the difference between the observed covariances and those implied by the structural equation model. Comparison of path coefficients across conditions was determined by using a stacked model approach (15). This procedure determines the χ^2 goodness-of-fit statistic for both a null model, in which the path coefficients are constrained to be equal between conditions, and the alternative model, in which the coefficients are allowed to differ. The significance of the difference between the models is expressed as the difference in the χ^2 statistic with degrees of freedom equal to the difference in the degrees of freedom for the null model and alternative model. A P value of <0.05 was considered significant.

Other Statistical Analyses. The main drug effects on memory were assessed with ANOVA and posthoc t-tests. A probability level of $P < 0.05$ was considered significant, after Bonferroni/Dunn correction for multiple comparisons where appropriate. Data are mean and SEM, unless otherwise noted.

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1. McGaugh JL (2000) Memory–A century of consolidation. *Science* 287:248–251.
2. LaBar KS, Cabeza R (2006) Cognitive neuroscience of emotional memory. *Nat Rev Neurosci* 7:54–64.
3. McGaugh JL (2004) The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annu Rev Neurosci* 27:1–28.
4. Alkire MT, Gorski LA (2004) Relative amnesic potency of five inhalational anesthetics follows the Meyer-Overton rule. *Anesthesiology* 101:417–429.
5. Sebel PS, *et al.* (2004) The incidence of awareness during anesthesia: a multicenter United States study. *Anesth Analg* 99:833–839.
6. Dickinson-Anson H, McGaugh JL (1993) Midazolam administered into the amygdala impairs retention of an inhibitory avoidance task. *Behav Neural Biol* 60:84–87.
7. Tomaz C, Dickinson-Anson H, McGaugh JL (1992) Basolateral amygdala lesions block diazepam-induced anterograde amnesia in an inhibitory avoidance task. *Proc Natl Acad Sci USA* 89:3615–3619.
8. Buchanan TW, Karafin MS, Adolphs R (2003) Selective effects of triazolam on memory for emotional, relative to neutral, stimuli: differential effects on gist versus detail. *Behav Neurosci* 117:517–525.
9. Alkire MT, Nathan SV (2005) Does the amygdala mediate anesthetic-induced amnesia? Basolateral amygdala lesions block sevoflurane-induced amnesia. *Anesthesiology* 102:754–760.
10. Cahill L, Uncapher M, Kilpatrick L, Alkire MT, Turner J (2004) Sex-related hemispheric lateralization of amygdala function in emotionally influenced memory: an FMRI investigation. *Learn Mem* 11:261–266.
11. Canli T, Desmond JE, Zhao Z, Gabrieli JD (2002) Sex differences in the neural basis of emotional memories. *Proc Natl Acad Sci USA* 99:10789–10794.
12. Kilpatrick L, Cahill L (2003) Amygdala modulation of parahippocampal and frontal regions during emotionally influenced memory storage. *NeuroImage* 20:2091–2099.
13. Snodgrass JG, Corwin J (1988) Pragmatics of measuring recognition memory: applications to dementia and amnesia. *J Exp Psychol Gen* 117:34–50.
14. Alkire MT, McReynolds JR, Hahn EL, Trivedi AN (2007) Thalamic microinjection of nicotine reverses sevoflurane-induced loss of righting reflex in the rat. *Anesthesiology* 107:264–272.
15. McIntosh AR, Lobaugh NJ (2004) Partial least squares analysis of neuroimaging data: applications and advances. *NeuroImage* 23 Suppl 1:S250–S63.
16. Alkire MT, Nathan SV, McReynolds JR (2005) Memory enhancing effect of low-dose sevoflurane does not occur in basolateral amygdala lesioned rats. *Anesthesiology* 103:1–7.
17. Davies PA, Hanna MC, Hales TG, Kirkness EF (1997) Insensitivity to anaesthetic agents conferred by a class of GABA(A) receptor subunit. *Nature* 385:820–823.
18. Cahill L, *et al.* (1996) Amygdala activity at encoding correlated with long-term, free recall of emotional information. *Proc Natl Acad Sci USA* 93:8016–8021.
19. Canli T, Zhao Z, Brewer J, Gabrieli JD, Cahill L (2000) Rapid Communication: Event-related activation in the human amygdala associates with later memory for individual emotional experience. *J Neurosci* 20:1–5.
20. Hamann SB, Ely TD, Grafton ST, Kilts CD (1999) Amygdala activity related to enhanced memory for pleasant and aversive stimuli. *Nat Neurosci* 2:289–293.
21. Dolcos F, LaBar KS, Cabeza R (2004) Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. *Neuron* 42:855–863.
22. Alkire MT, Vazdarjanova A, Dickinson-Anson H, White NS, Cahill L (2001) Lesions of the basolateral amygdala complex block propofol-induced amnesia for inhibitory avoidance learning in rats. *Anesthesiology* 95:708–715.
23. Cahill L, Alkire MT (2003) Epinephrine enhancement of human memory consolidation: interaction with arousal at encoding. *Neurobiol Learn Mem* 79:194–198.
24. Huang SC, *et al.* (1980) Noninvasive determination of local cerebral metabolic rate of glucose in man. *Am J Physiol* 238:E69–E82.
25. Friston KJ (1995) Functional and effective connectivity in neuroimaging: a synthesis. *Hum Brain Mapp* 2:56–78.
26. Izquierdo I, *et al.* (2006) Different molecular cascades in different sites of the brain control memory consolidation. *Trends Neurosci* 29:496–505.
27. McGaugh JL (2002) Memory consolidation and the amygdala: a systems perspective. *Trends Neurosci* 25:456–461.
28. Kilpatrick LA, Zald DH, Pardo JV, Cahill LF (2006) Sex-related differences in amygdala functional connectivity during resting conditions. *NeuroImage* 30:452–461.
29. Jones BE (2003) Arousal systems. *Front Biosci* 8:s438–51.
30. Power AE, Thal LJ, McGaugh JL (2002) Lesions of the nucleus basalis magnocellularis induced by 192 IgG-saporin block memory enhancement with posttraining norepinephrine in the basolateral amygdala. *Proc Natl Acad Sci USA* 99:2315–2319.
31. McGaugh JL, McIntyre CK, Power AE (2002) Amygdala modulation of memory consolidation: interaction with other brain systems. *Neurobiol Learn Mem* 78:539–552.