ANESTHESIOLOGY

Midazolam and Ketamine Produce Distinct Neural Changes in Memory, Pain, and Fear Networks during Pain

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ANESTHESIOLOGY 2021; XXX:00–00

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Light sedation with general anesthetics is known to decrease recollection of conscious painful experiences
- Pharmacologically distinct anesthetic drugs differentially affect pain perception and memory encoding at varying levels of sedation
- The neural correlates underlying these drug-specific differential effects are incompletely understood

What This Article Tells Us That Is New

- In this randomized within-subject crossover study of healthy volunteers using an experimental memory paradigm using periodic pain under light sedation, recollection memory was reduced by midazolam compared with saline and ketamine
- The paradigm-related brain activity differed between the two drugs: Whereas midazolam mostly increased, ketamine predominantly decreased functional connectivity from brain regions involved in memory encoding, pain processing, and threat response
- These observations highlight how pharmacologically distinct general anesthetics may engage distinct neural dynamics to modulate cognitive experience under threat of pain

ABSTRACT

Background: Despite the well-known clinical effects of midazolam and ketamine, including sedation and memory impairment, the neural mechanisms of these distinct drugs in humans are incompletely understood. The authors hypothesized that both drugs would decrease recollection memory, task-related brain activity, and long-range connectivity between components of the brain systems for memory encoding, pain processing, and fear learning.

Methods: In this randomized within-subject crossover study of 26 healthy adults, the authors used behavioral measures and functional magnetic resonance imaging to study these two anesthetics, at sedative doses, in an experimental memory paradigm using periodic pain. The primary outcome, recollection memory performance, was quantified with d' (a difference of z scores between successful recognition *versus* false identifications). Secondary outcomes were familiarity memory performance, serial task response times, task-related brain responses, and underlying brain connectivity from 17 preselected anatomical seed regions. All measures were determined under saline and steady-state concentrations of the drugs.

Results: Recollection memory was reduced under midazolam (median [95% CI], d' = 0.73 [0.43 to 1.02]) compared with saline (d' = 1.78 [1.61 to 1.96]) and ketamine (d' = 1.55 [1.12 to 1.97]; $P < 0.0001$). Task-related brain activity was detected under saline in areas involved in memory, pain, and fear, particularly the hippocampus, insula, and amygdala. Compared with saline, midazolam increased functional connectivity to 20 brain areas and decreased to 8, from seed regions in the precuneus, posterior cingulate, and left insula. Compared with saline, ketamine decreased connectivity to 17 brain areas and increased to 2, from 8 seed regions including the hippocampus, parahippocampus, amygdala, and anterior and primary somatosensory cortex.

Conclusions: Painful stimulation during light sedation with midazolam, but not ketamine, can be accompanied by increased coherence in brain connectivity, even though details are less likely to be recollected as explicit memories.

(ANESTHESIOLOGY 2021; XXX:00–00)

Memory formation and pain perception are among the cognitive functions fundamental to the human experience of consciousness. Anesthetic drugs are routinely used to modulate these and other elements of consciousness during what would otherwise be intolerably painful experiences. However, the neural correlates of anesthetic action in humans are incompletely understood. Comparative studies have demonstrated how pharmacologically distinct

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). Preliminary reporting of these results has occurred in public presentations: Society for Neuroscience in Anesthesiology and Critical Care Annual Meeting, San Francisco, California, October 12, 2018; Society for Neuroscience in Anesthesiology and Critical Care, Neuroscience Symposium on Perioperative Neuroscience, Virtual Meeting, May 16, 2020; and National Institutes of Health, Pain Consortium, Symposium on Advances in Pain Research, Technologies for Improved Understanding and Management of Pain, Virtual Meeting, June 3, 2020.

Submitted for publication October 6, 2020. Accepted for publication March 8, 2021. From the Department of Anesthesiology and Perioperative Medicine, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania (K.M.V., J.W.I., C.T.S., A.T.C., C.M.N., A.M.); Department of Bioengineering, Swanson School of Engineering (K.M.V., H.T.K., A.M., H.J.A.), Department of Psychiatry (H.T.K., H.J.A.), Department of Bioinformatics (A.M.), Department of Psychology (J.A.F.), Department of Neuroscience (J.A.F.), and Clinical and Translational Science Institute (J.W.I.), University of Pittsburgh, Pittsburgh, Pennsylvania; Center for the Neural Basis of Cognition, Pittsburgh, Pennsylvania (K.M.V., V.P., L.M.R., J.A.F.); Department of Anesthesiology, Surgical Service Line, Veterans Affairs Pittsburgh Healthcare System, Pittsburgh, Pennsylvania (J.W.I.); and Department of Psychology, Carnegie Mellon University, Pittsburgh, Pennsylvania (V.P., L.M.R.).

 Anesthesiology 2021; XXX:00–00. DOI: 10.1097/ALN.0000000000003774

<zdoi;. DOI: 10.1097/ALN.0000000000003774>

anesthetic agents differentially affect pain perception[1–3] and memory encoding at varying levels of sedation.[4–6] However, these coarse behavioral measures are limited in revealing how different drugs affect underlying brain activity. Functional neuroimaging provides a unique tool to better understand the complex milieu of anesthetic action during the experience of pain.

We performed a within-subject comparative neuroimaging study of two commonly used anesthetic agents, midazolam and ketamine. These distinct drugs are well-known to have different effects on discrete aspects of cognition. Whereas midazolam provides anxiolysis and reversible anterograde amnesia, ketamine produces analgesia and a dissociated mental state. Both drugs are used at low doses to provide sedation during experiences that could otherwise be characterized as unpleasant, painful, or anxiety-provoking. The brain regions involved in memory formation, pain processing, and fear learning are engaged by these experiences, and their inhibition may be important in preventing psychologic sequelae. We attempted to experimentally model such an experience, using repeated, unpredictable painful stimulation and a task that allowed later quantification of successful memory encoding.

Functional neuroimaging can quantify and localize distinct features of brain activity that underlie drug-induced differences in mental state. The blood oxygen level–dependent effect has been used for decades as a reliable surrogate for localized neuronal activity induced by task performance.[7] Additionally, low-frequency (less than 0.1 Hz) fluctuations of the signal reflect meaningful periodic changes in neuronal activity that can be observed at rest or in the context of task performance.[8] Functional connectivity is a measure of the coherence in these fluctuations over time, reflecting neuronal communication.[9] In this study, we analyzed both task-related activity and functional connectivity to determine the neurosignature of midazolam and ketamine during memory encoding and acute pain.

As the primary outcome in this study, we expected reductions in explicit memory with both drugs but that recollection would be more impacted than familiarity, with both assessed during next-day recognition. We hypothesized that midazolam would cause a greater reduction in recollection compared with ketamine. Neuroimaging measures of brain activity and connectivity were evaluated as secondary outcomes. We predicted that, compared with saline, fewer task-related changes would be detected under both anesthetic agents in the brain regions associated with memory, pain, and fear processing. It was less clear how the drugs would impact functional connectivity between brain regions in the setting of experimental pain, but we anticipated reductions in long-range functional connectivity for both agents.

Materials and Methods

Study Design and Oversight

This was a randomized, single-blind, within-subject crossover neuroimaging trial comparing the effects of two anesthetics, midazolam and ketamine. A study flowchart is shown in figure 1. Written informed consent was obtained in person, at the first study visit, after a full discussion of risks and benefits. The study was approved by the University of Pittsburgh Institutional Review Board (PRO 14050609) and conformed to all relevant standards for the ethical and responsible conduct of research. The trial was prospectively registered with clinicaltrials.gov (NCT02515890).

Participants

Healthy volunteer participants between the ages of 18 and 39 yr were recruited from the community and compensated up to $200 for participation. Demographic information from all 26 participants is tabulated in table 1. All participants acknowledged being free from significant memory or hearing impairment, chronic pain, other chronic medical problems, and recent or regular use of antidepressants, antipsychotics, antihistamines, anxiolytics, stimulants, sleep aids, and analgesics. A preanesthetic evaluation by a study anesthesiologist, urine pregnancy test for females, and magnetic resonance imaging compatibility screening confirmed no other contraindications to safe sedation inside a high-field magnet. In addition to following American Society of Anesthesiologists (Schaumburg, Illinois) guidelines for fasting,[10] participants abstained from tobacco and caffeine for 8 h before the magnetic resonance imaging sessions.

Painful Stimulation and Monitoring

An electric nerve stimulator (EzStim II; Life Tech, USA) was employed using a 100-Hz tetanic stimulation waveform. The stimulator was connected to electrodes on the left index finger, and current was slowly titrated to a subjective rating of 7 of 10 pain. The numerical rating scale for pain had anchors at 0 being no pain and 10 being the worst imaginable. Two 1-s test shocks were delivered once in the scanner, and a pain rating was obtained, as listed in table 2. The stimulator was readjusted at that time, if necessary, but not further manipulated during the experiment. Pain scores and any subjective complaints were obtained from participants and recorded at the end of the saline segment and after each of the three blocks in the drug segment (timing is diagrammed in fig. 2).

Infusions

Intravenous access was obtained in each participant's right hand with a 22-gauge catheter. Once positioned in the magnetic resonance imaging scanner and connected to standard American Society of Anesthesiologists monitors,

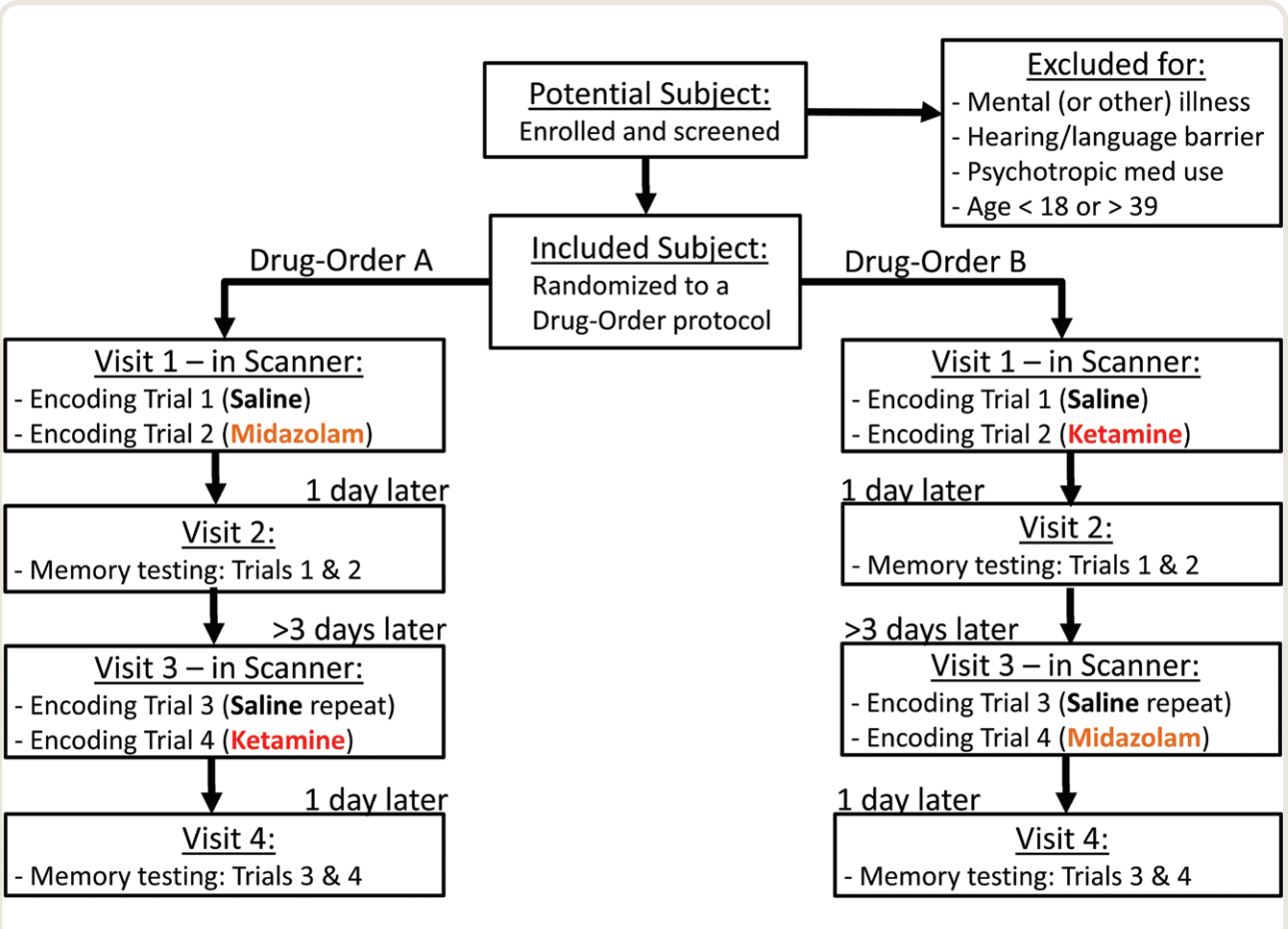

Fig. 1. Study flowchart showing participant flow through the four experimental visits.

a saline carrier infusion was run at 75 ml/h until the end of the experiment. After completion of tasks during the saline control condition, drug administration was started. Pre-experimental computer simulation was used to determine the optimal bolus and infusion doses to efficiently achieve and maintain steady-state drug concentrations, using the open-source software STANPUMP (Stanford University, USA [http://opentci.org/code/stanpump; accessed April 3, 2021]). Brain effect site concentrations of 10 ng/ml for midazolam and 200 ng/ml for ketamine were targeted, using pharmacokinetic models[11,12] that accounted for age, sex, height, and weight. The total drug dose administered to each participant is listed in table 1.

Memory Encoding Task

The experiment was implemented with E-Prime version 2.0 (Psychology Software Tools, USA). Shock delivery synchronization to follow experimental word items was accomplished with E-Prime control of custom-built hardware. A schematic timeline is shown in figure 2. Participants made category judgments about each word's meaning (Alive or not?) and responded by pressing a button with their right index (yes) or middle finger (no).[13] Ninety words were used in each segment, 30 of which were immediately followed by a 1-s electric shock. No more than two pain-paired words nor five non-pain words occurred consecutively. Word order was randomized between repetition blocks, although pain pairing was kept consistent. To increase statistical power for detecting task events in the imaging data, 0- to 6-s periods of jitter were included between items.[14]

Response Times

During the encoding segments, the participant response window began at the start of the word being played and closed after 6 s. Response time outliers were removed using RStudio (USA; version 1.0.153 [https://www.rstudio.com/; accessed April 3, 2021]) running R version 3.2.5. The median absolute deviation was calculated,[15] and response time values greater than 3.5 times the median absolute deviation from the grand median (across subjects) were defined as outliers. Outliers represented less than 1% of all data.

Table 1. Subject Demographic Data and Total Drug Doses Received

Subject	Age (yr)	Sex	Height (cm)	Mass (kg)	Drug Dose (mg)	
					Midazolam	Ketamine
1	23.2	Male	175	75.0	—	36.8
2	21.9	Male	183	88.6	1.89	41.1
3	32.3	Male	173	69.0	1.86	37.9
4	21.8	Female	157	56.8	1.13	26.2
5	32.4	Female	168	75.9	1.60	32.9
6	23.9	Male	180	85.9	—	44.9
7	28.4	Male	185	75.5	1.47	32.6
8	19.5	Female	173	63.6	0.79	—
9	22.4	Female	168	73.6	1.59	33.3
10	22.3	Male	185	80.5	1.04	36.0
11	23.5	Female	170	68.2	1.40	32.9
12	22.6	Female	165	70.5	1.43	—
13	37.0	Male	157	65.9	1.28	30.8
14	23.3	Male	178	81.8	1.73	38.0
15	27.8	Female	154	50.9	1.03	26.3
16	28.3	Female	168	92.3	1.94	25.9
17	22.5	Male	175	65.0	2.08	38.1
18	33.3	Male	180	88.6	2.08	42.7
19	21.8	Male	178	81.8	—	48.7
20	23.9	Male	183	75.0	1.62	—
21	19.5	Female	170	54.5	—	27.1
22	22.3	Male	173	63.6	1.49	—
23	20.0	Female	155	56.8	1.18	25.3
24	24.8	Male	175	72.7	1.57	36.8
25	33.2	Male	168	70.5	1.61	37.1
26	25.1	Female	157	53.2	1.12	28.1
Average	25.3	(15 Male)	171	71.4	1.50	34.5

Dash indicates the subject did not participate in the experimental session.

Memory Testing

Explicit memory testing occurred the next day, 20 to 32 h after the scanning session. Recognition testing used the Remember-Know-New scheme[16] (subject instructions were published previously[13]). Recollection (of specific details) was indicated by a Remember response, whereas a Know response indicated familiarity (recognized, but with no specific details recollected). Participants were instructed to mark the word as New if they did not recognize it. All words heard the previous day were played intermixed with an equal number of foils, in randomized order. As in previous similar studies,[17] memory performance was summarized using the signal detection metric, d',[18] which is calculated from the difference in z scores between cumulative Gaussian distributions: z(hits) – z(false alarms); hits are correctly recognized previously heard items, and false alarms are foils incorrectly identified with Remember or Know responses. This more rigorous metric accounts for subjects' false alarm rate, in a way that correct responses alone would not. As an example, a 98% hit rate with 98% false alarms would yield a d' of zero, reflecting the inability to discriminate between previously heard and unheard words. Thus, measures of both recollection and familiarity were determined separately and treated as distinct components of long-term explicit memory.

Magnetic Resonance Imaging

Imaging was performed on a Siemens (USA) Prisma 3 T scanner using a 32-channel head coil. Functional images were obtained using a gradient-echo echo-planar imaging sequence, with the following parameters: echo time = 30 ms, repetition time = 1 s, flip angle = 45°, bandwidth 2,004 Hz/pixel, and anterior-posterior phase encoding. Sixty slices at 2.3 mm isotropic spatial resolution gave whole brain coverage (including the cerebellum), and these were obtained in interleaved fashion using multiband acceleration (factor 5). For subsequent correction of magnetic field in homogeneities, a gradient echo field map was acquired after each functional imaging run in the same coordinate space. For later registration, a T1-weighted anatomical image was obtained with 1 mm isotropic spatial resolution.

Statistical Analysis

The primary outcome, recollection memory performance, was evaluated by comparing d' scores (where d' represents a difference of z scores between successful recognition *versus* false identification). Statistical analysis of response time data was carried out in SPSS Statistics 26 (IBM, USA). The common logarithm ($\log_{10}$) transformed the data to a normal distribution, and a linear mixed model with autoregressive covariance structure was used. Occurrence number, drug condition, and pain association were included as repeated fixed factors, including interaction terms. Data for both recollection and familiarity performance failed tests for normality, so statistical comparisons for d' were performed with independent-sample Kruskal–Wallis tests in SPSS. Recollection and familiarity data were analyzed separately and not compared with one another. All statistical analyses were two-tailed; reported P values are Bonferroni-adjusted for the number of comparisons, with $P < 0.05$ used as the threshold for significance. A power analysis for the primary outcome, based on extrapolations from pilot memory performance data, indicated that 16 participants would be needed to detect a 50% decrement in recollection performance with 80% power. No statistical power calculation was possible for the imaging analyses, but overall sample size was based on general estimates[19,20] suggesting that 24 subjects are adequate for task-based functional magnetic resonance imaging studies.

In addition to the eight subjects who did not return for their second set of experimental sessions, unrecoverable scanner errors corrupted the imaging data from two subject sessions, which were not able to be included in the analysis. Functional images were preprocessed using Statistical Parameter Mapping software (University College London, United Kingdom; SPM12 v7219 [http://www.fil.ion.ucl.ac.uk/spm/; accessed April 3, 2021]) using a Windows 10 computer running MATLAB

Table 2. Nerve Stimulator Intensities and Pain Scores for Each Subject, by Session

	Midazolam Session						Ketamine Session					
		Pain Scores						Pain Scores				
Subject	Intensity (mA)	After Test Shocks	After Block 3 Saline	After Block 1 +Drug	After Block 2 +Drug	After Block 3 +Drug	Intensity (mA)	After Test Shocks	After Block 3 Saline	After Block 1 +Drug	After Block 2 +Drug	After Block 3 +Drug
1	—	—	—	—	—	—	15	7	7	7	7	7
2	16	5	6.5	7	7	7	19	7	7	5.5	5.5	5.5
3	13	5	5	7	7	7	11	7	5.5	2.5	0	1.5
4	13	6	7	6	5.5	5.5	11	6.5	6	4	3.5	3.5
5	13	7	8	7.5	7.5	7.5	13	7	7	5.5	6	7.5
6	—	—	—	—	—	—	9	6.5	5.5	5.5	5.5	5.5
7	22	5.5	7	7	7	7	23	7	7	6	6	6.5
8	10	6	6	5	4	3	—	—	—	—	—	—
9	13	7	7	7	7	7	11	6	7	7	7	7
10	25	6	7	x	7	7	21	5.5	6	6	6	5.5
11	14	7	7	6.5	7	7	10	7	7	5	5.5	6
12	14	6	7	7	6	7	—	—	—	—	—	—
13	25	7	7	7	7	7	20	7	7	3	1	0
14	14	7	7	7	6	6	42	7	7	5	5	5
15	11	7.5	7.5	7.5	7.5	7.5	11	7	7	2	2	2
16	11	7	7	5.5	6	4.5	17	5.5	6	5.5	4.5	4
17	23	7	7	7	7	7	24	7	7	x	5	4.5
18	23	7	7	7	6	6	18	7	7	3	x	3
19	—	—	—	—	—	—	19	7	7	7	7	7
20	24	7	7	6	7	5	—	—	—	—	—	—
21	—	—	—	—	—	—	18	7	7	7	7	7
22	12	7	7	7	7	7	—	—	—	—	—	—
23	21	7	7	7	7	7	16	7	6	4	4	3.5
24	18	7	5	5	4	4	16	7	5.5	4	4	3
25	22	7	7	7	7	7	22	7	7	6	7	7
26	11	7	7	7	7	7	18	6	7	7	7	7
Average	16.7	6.6	6.8	6.7	6.5	6.4	17.5	6.7	6.6	5.1	5.0	4.9

Dash indicates the subject did not participate in experimental session; x indicates missing data.

2018b (Mathworks, USA). Initial steps included motion correction and unwarping (using the acquired field inhomogeneity maps) of the functional imaging data and tissue segmentation of the anatomical image. Excessive motion precluded use for 10 of the 168 independent functional datasets acquired. Before further analysis, the CompCor algorithm[21] was used to reduce physiologic noise related to respiration and cardiac pulsatility. Thus, the principal components (first five Eigenseries) derived from the average white matter and cerebrospinal fluid signal timecourses were included as covariates of no interest, along with the motion parameters. Spatial smoothing was performed with a 5-mm Gaussian kernel. Event-related task activation was calculated, using an event window that started with onset of the word audio being played and ended once the participant had made their response. Four subject-level contrasts were included: all experimental items, items subsequently recollected (correct Remember responses), items subsequently recognized as familiar (correct Know responses), and all pain-paired items. Interactions between item types (correct Remember responses for pain-paired items) were not analyzed, because the number of available observations would be small for some subgroupings. Group average task activation maps were generated by averaging data within a drug condition. Voxels were initially thresholded for significance at $P < 0.001$, and then a cluster-determining threshold was applied that adjusted the family-wise error rate to $P < 0.05$.

Connectivity analyses were performed using Conn Toolbox[22] version 18b (Massachusetts Institute of Technology, USA [https://www.nitrc.org/projects/conn/; accessed April 3, 2021]). As part of data preprocessing, Artifact Removal Tool (Conn Toolbox)–based outlier detection was used. Data were band-pass filtered (0.008 to 0.09 Hz); linear detrending was applied. A seed-to-voxel analysis was used, with seed regions defined in the Harvard-Oxford atlas available in Conn Toolbox. Seventeen seed regions were chosen, for hypothesized roles in memory, pain, or fear networks. These were (bilateral): anterior and posterior cingulate, amygdala, anterior and posterior parahippocampus, hippocampus, insula, precuneus,

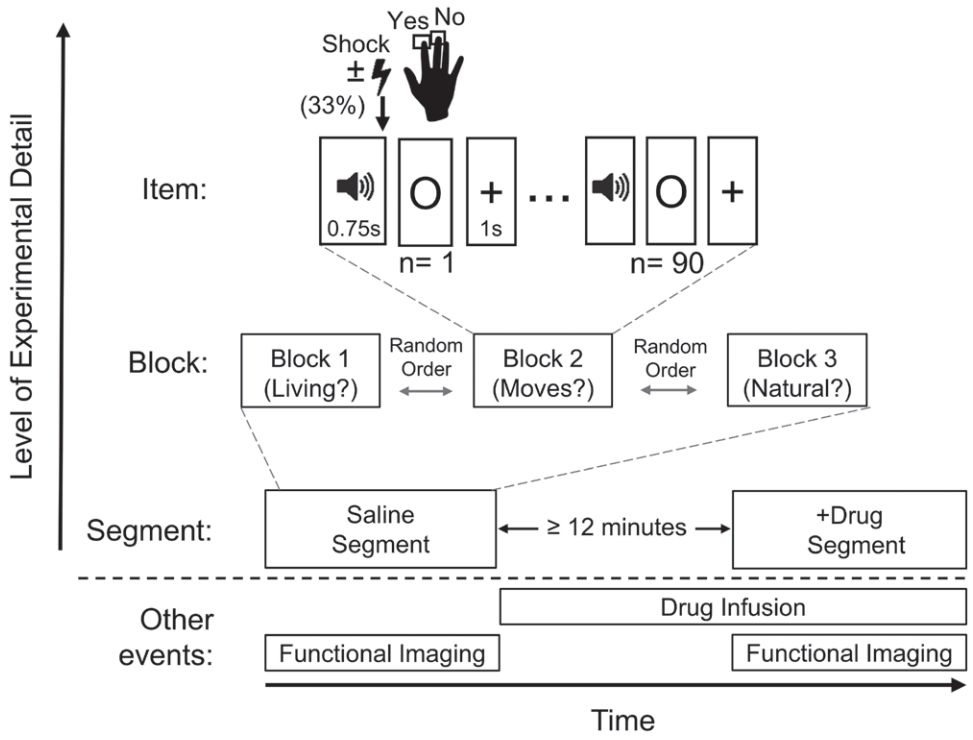

Fig. 2. Graphical representation of detailed timeline for experimental procedures performed in the scanner (during visits 1 and 3).

primary somatosensory cortex, and thalamus. In addition to CompCor and motion parameters, timing of all experimental events was included as a covariate of no interest, essentially removing task responses from the signal time-course. This allows background connectivity to be assessed with minimal contamination from task events.[23,24] Group-level connectivity contrasts for saline greater than midazolam and saline greater than ketamine were calculated and thresholded within Conn Toolbox, correcting the overall significance for a cluster false-discovery rate of $P < 0.05$.[25] Complete lists of all clusters showing statistically significant connectivity change with drug are available (see tables, Supplemental Digital Content 1 [http://links.lww.com/ALN/C585] for saline greater than midazolam, and Supplemental Digital Content 2 [http://links.lww.com/ALN/C586] for saline greater than ketamine). Connectivity changes are summarized in tables 3 and 4, where identified target regions are organized by network in which a putative role may be assigned (such as the insula for pain processing[26]). All statistically significant connectivity changes are represented in the summary tables, but functionally related target areas (such as the left ventrolateral and the right and left dorsolateral prefrontal regions) are collapsed into a single row. Based on manual inspection of the data, functional neuroscience labels were assigned in the summary tables, in place of anatomical atlas labels, such as ventrolateral prefrontal cortex, rather than superior frontal gyrus.

Results

Behavioral Measures

Pain scores (listed in table 2) from the encoding trial segments performed during drug infusion were significantly reduced under ketamine (median decrease of 1.6 on a 0 to 10 numerical rating scale; $P < 0.001$) but not midazolam ($P = 0.194$). Data noted as missing in table 2 also reflect that eight subjects did not return for their second drug session. Subject reports attributed to dissociation or dysphoria were recorded in 6 of 22 ketamine sessions (and no midazolam sessions). Subject reports of sedation were recorded in 4 of 22 ketamine and 8 of 22 midazolam sessions.

Response times for the categorization decision task performed in the scanner were not statistically different between drugs ($P = 0.065$), suggesting equal psychomotor impairment (fig. 3). Items with no recorded response were infrequent (449/23,220), at fewer than 2% of all trials (see table, Supplemental Digital Content 3 [http://links.lww.com/ALN/C587], for individual response rates), demonstrating that participants maintained voluntary motor responsiveness to verbal cues.

Individual hit rates tabulated according to experimental conditions are available for review (see table, Supplemental Digital Content 4 [http://links.lww.com/ALN/C588], for

Table 3. Saline *versus* Midazolam Functional Connectivity Contrast, Results Summary

Seed Region		Target Identified		
Network (Role)	**Specific Area**	**Network (Role)**	**Specific Area**	**Connectivity Change**
Default mode	Precuneus	Cognitive processing	Ventrolateral prefrontal cortex	Decreased
Default mode	Precuneus	Memory encoding	Temporal pole, middle temporal gyrus	Increased
Default mode	Precuneus	Somatosensory	Primary somatosensory cortex	Increased
Default mode	Posterior cingulate	Fear/pain response	Anterior cingulate	Decreased
Default mode	Posterior cingulate	Cognitive processing	Ventrolateral prefrontal cortex	Decreased
Default mode	Posterior cingulate	Memory encoding	Hippocampus, middle temporal gyrus	Increased
Default mode	Posterior cingulate	Somatosensory	Thalamus, primary somatosensory cortex	Increased
Default mode	Posterior cingulate	Visual processing	Occipital lobe	Increased
Pain processing	Insula	Associative processing	Lateral parietal lobe	Increased
Pain processing	Insula	Cognitive processing	Dorsolateral and ventromedial prefrontal cortices	Increased
Pain processing	Insula	Fear/pain response	Anterior cingulate	Increased
Pain processing	Insula	Memory encoding	Middle temporal gyrus	Increased
Pain processing	Insula	Motor	Motor cortex	Increased
Pain processing	Insula	Motor	Caudate nucleus	Decreased

Increases in the Connectivity Change column indicate a greater connectivity value seen under midazolam compared with saline. Anatomic location labels were systematically determined using mutual information from three atlases (described in the Materials and Methods section). Network (role) labels were added by the investigators, based on likely roles in the experimental framework.

Table 4. Saline *versus* Ketamine Functional Connectivity Contrast, Results Summary

Seed Region		Target Identified		
Network (Role)	**Specific Area**	**Network (Role)**	**Specific Area**	**Connectivity Change**
Fear/pain response	Anterior cingulate	Cognitive processing	Orbitofrontal and ventromedial prefrontal cortices	Increased
Fear/pain response	Anterior cingulate	Motor	Supplementary motor area	Decreased
Fear response	Amygdala	Motor	Supplementary motor area, putamen	Decreased
Fear response	Amygdala	Somatosensory	Primary somatosensory cortex	Decreased
Memory encoding	Hippocampus	Associative processing	Medial parietal cortex	Decreased
Memory encoding	Hippocampus	Cognitive processing	Ventrolateral prefrontal cortex	Decreased
Memory encoding	Hippocampus	Memory encoding	Inferior temporal gyrus	Decreased
Memory encoding	Hippocampus	Motor	Motor cortex, supplementary motor area	Decreased
Memory encoding	Hippocampus	Somatosensory	Primary somatosensory cortex	Decreased
Memory encoding	Parahippocampus	Motor	Motor cortex	Decreased
Memory encoding	Parahippocampus	Somatosensory	Primary somatosensory cortex	Decreased
Somatosensory	Primary somatosensory cortex	Associative processing	Lateral parietal cortex	Increased
Somatosensory	Primary somatosensory cortex	Memory encoding	Hippocampus	Decreased
Somatosensory	Primary somatosensory cortex	Fear/pain response	Anterior cingulate	Decreased

Decreases in the Connectivity Change column indicate a lower connectivity value seen under ketamine compared with saline. Anatomic location labels were systematically determined using mutual information from three atlases (described in the Materials and Methods section). Network (role) labels were added by the investigators, based on likely roles in the experimental framework.

saline; Supplemental Digital Content 5 [http://links.lww.com/ALN/C589], for midazolam; Supplemental Digital Content 6 [http://links.lww.com/ALN/C590], for ketamine). False alarm responses were also tabulated (see table, Supplemental Digital Content 7 [http://links.lww.com/ALN/C591]). Figure 4 displays the results for recollection and familiarity from next-day recognition testing, using the summary statistic d'. Consistent with previous results[13] demonstrating that painful stimulations may affect memory for non-pain items within the same experimental block, no differences between pain-paired *versus* non-pain items were detected ($P = 0.813$; fig. 4A). Collapsing across pain-pairing (fig. 4B), median recollection under saline was $d' = 1.78$ (95% CI, 1.61 to 1.96). Recollection performance under midazolam ($d' = 0.73$ [0.43 to 1.02]) was significantly reduced compared with saline ($P < 0.0001$) and ketamine ($d' = 1.55$ [1.12 to 1.97]; $P < 0.001$), representing a meaningful performance difference of more than 0.75 SD units. Comparisons of recollection between saline ($d' = 1.78$ [1.61 to1.96]) and ketamine ($d' = 1.55$ [1.12 to 1.97]) were not considered different ($P = 0.081$). Aggregate performance for familiarity, indicated by correct Know responses, did not

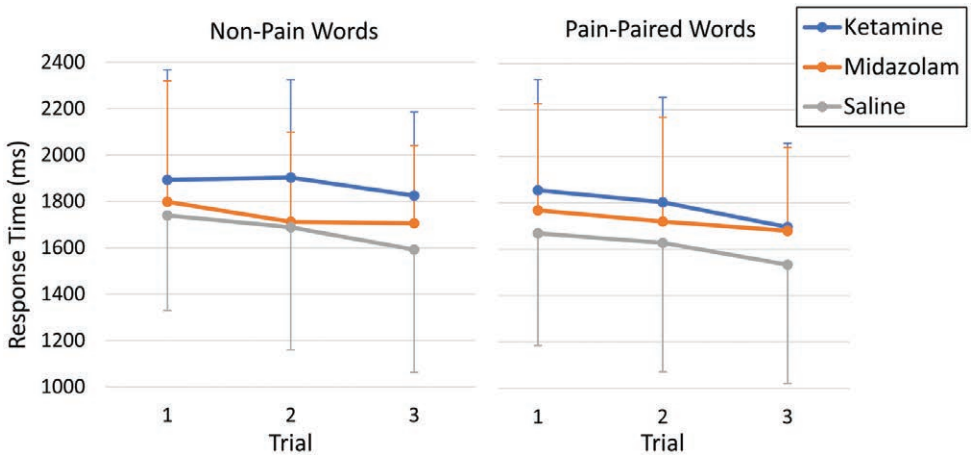

Fig. 3. Response times for each experimental block during encoding segments of experiment. *Error bars* reflect SD.

significantly differ between saline (d' = 0.36 [0.18 to 0.53]), midazolam (d' = 0.31 [0.03 to 0.59]), and ketamine (d' = 0.31 [0.02 to 0.61]), with overall P = 0.540. This predominance of impact on recollection has been previously shown for midazolam, which interferes with binding of words to their experimental context.[27]

Functional Magnetic Resonance Imaging Task-related Activation

A whole-brain annotated group average functional magnetic resonance imaging activation map is shown in figure 5, analyzed for event-related signal changes for items correctly recognized as familiar, based on the subsequent memory analysis. This represents a characteristic example of task-related activation, as similar maps were seen for correctly recollected items (see figure, Supplemental Digital Content 8 [http://links.lww.com/ALN/C592]) and items specifically paired with painful stimulation (see figure, Supplemental Digital Content 9 [http://links.lww.com/ALN/C593]). Note that all these maps represent average activation within the saline or drug conditions for one type of experimental item.

Statistically significant task-related brain activity under saline was seen in specific areas within different functional networks. These include primary memory encoding areas: the hippocampus, parahippocampus, and temporal gyrus (predominantly right-sided). Activation also occurred in the left amygdala, dorsal (or mid-) anterior cingulate, and bilateral insula. Activity in somatosensory network structures included the left primary and bilateral secondary somatosensory cortices, and the bilateral thalamus and brain stem. Motor network activation was seen in the cerebellum, supplementary motor area, and left primary motor cortex. Task-related activity was also detected in the left ventrolateral and right dorsolateral prefrontal cortices, in the lateral and medial parietal association areas, and in the posterior cingulate cortex and precuneus.

Figure 5 (and figures in Supplemental Digital Content 8 [http://links.lww.com/ALN/C592] and 9 [http://links.lww.com/ALN/C593]) also shows task activation for experimental items experienced under both drugs. It is important to note that the visual differences between columns in these figures are not definitive; when analyzed as a saline *versus* drug contrast, no clusters survive thresholding (analyses not shown).

Functional Connectivity Changes with Drug Administration

Of the 17 seed regions investigated, only the precuneus, posterior cingulate, and left insula showed significant changes in background connectivity under midazolam. Table 3 summarizes the 28 independent changes in connectivity seen under midazolam (for a complete list of clusters and coordinates of local maxima, see the table in Supplemental Digital Content 1 [http://links.lww.com/ALN/C585]). The direction of change was predominantly increased in these data (20 of 28 statistically significant changes detected), which were acquired during task performance and thus the periodic experience of pain. Increases in background connectivity included distant targets identified across frontal, temporal, occipital, and parietal lobes. Although they were the minority of changes, decreases in long-range connectivity were also seen with midazolam. Characteristic examples include decreases from the posterior cingulate to bilateral targets in the anterior cingulate and ventrolateral prefrontal cortex.

With ketamine administration in this experimental paradigm, statistically significant changes in background connectivity were detected from 8 seed regions to 19 independent target clusters throughout the brain, summarized

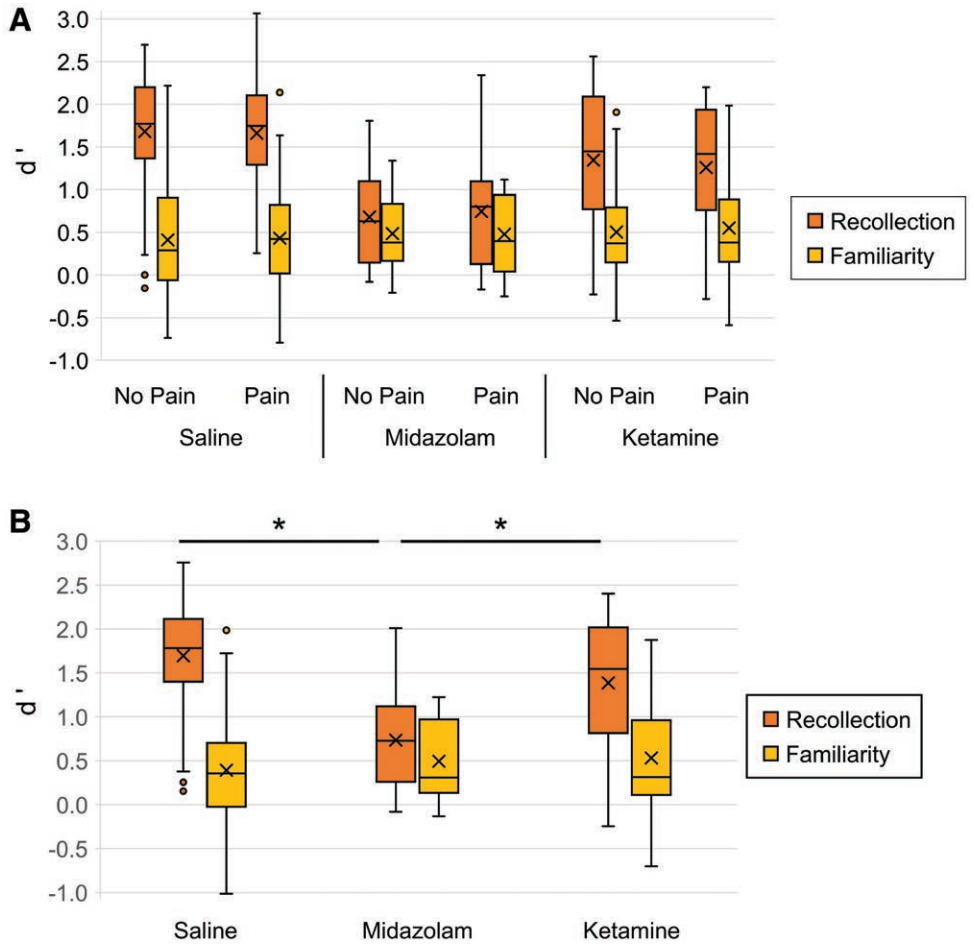

Fig. 4. Overall memory performance shown as *box and whisker plots* of *d'* for word items experienced under different experimental conditions, with drug segment along the *horizontal axis*. The mean value is marked with an X. Whisker length is 1.5× the interquartile range from the first and third quartile values (limits of the box). (*A*) Performance for items separated by pain pairing. Because there was no significant effect of pain on memory performance, *B* shows performance collapsed across pain pairing, with significant differences indicated with an *asterisk* (*).

in table 4 (complete list of clusters and coordinates of local maxima in table, Supplemental Digital Content 2 [http://links.lww.com/ALN/C586]). Two changes with ketamine were increases in connectivity: from the anterior cingulate to left medial frontal lobe and from the left posterior cingulate to left lateral parietal lobe. The remaining 17 changes under ketamine were decreases in functional connectivity, which occurred both between and within the networks for memory, pain, and fear. Notable between-systems decreases in connectivity were from the right amygdala to the right primary somatosensory cortex and from the right hippocampus to the right primary somatosensory cortex. Additionally, ketamine disrupted connectivity between the seed regions investigated and prefrontal and parietal areas (both targets identified as having decreased connectivity from the hippocampus). Finally, decreased connectivity was detected from the posterior cingulate to targets identified in the hippocampus and anterior cingulate.

Discussion

We present a comparative neuroimaging study of two distinct anesthetic agents. Participants encoded words heard while periodically experiencing painful shock, which we expected to activate somatosensory and pain processing areas, as well as fear learning centers. The task-related activity under saline showed functional magnetic resonance imaging activation across many brain areas involved in these cognitive processes directly related to task response. Specifically, activations in the hippocampus, parahippocampus, and temporal gyrus, in combination with the lateral prefrontal cortex and posterior parietal cortex, represent the core memory network.[28,29] Activations in the amygdala and anterior

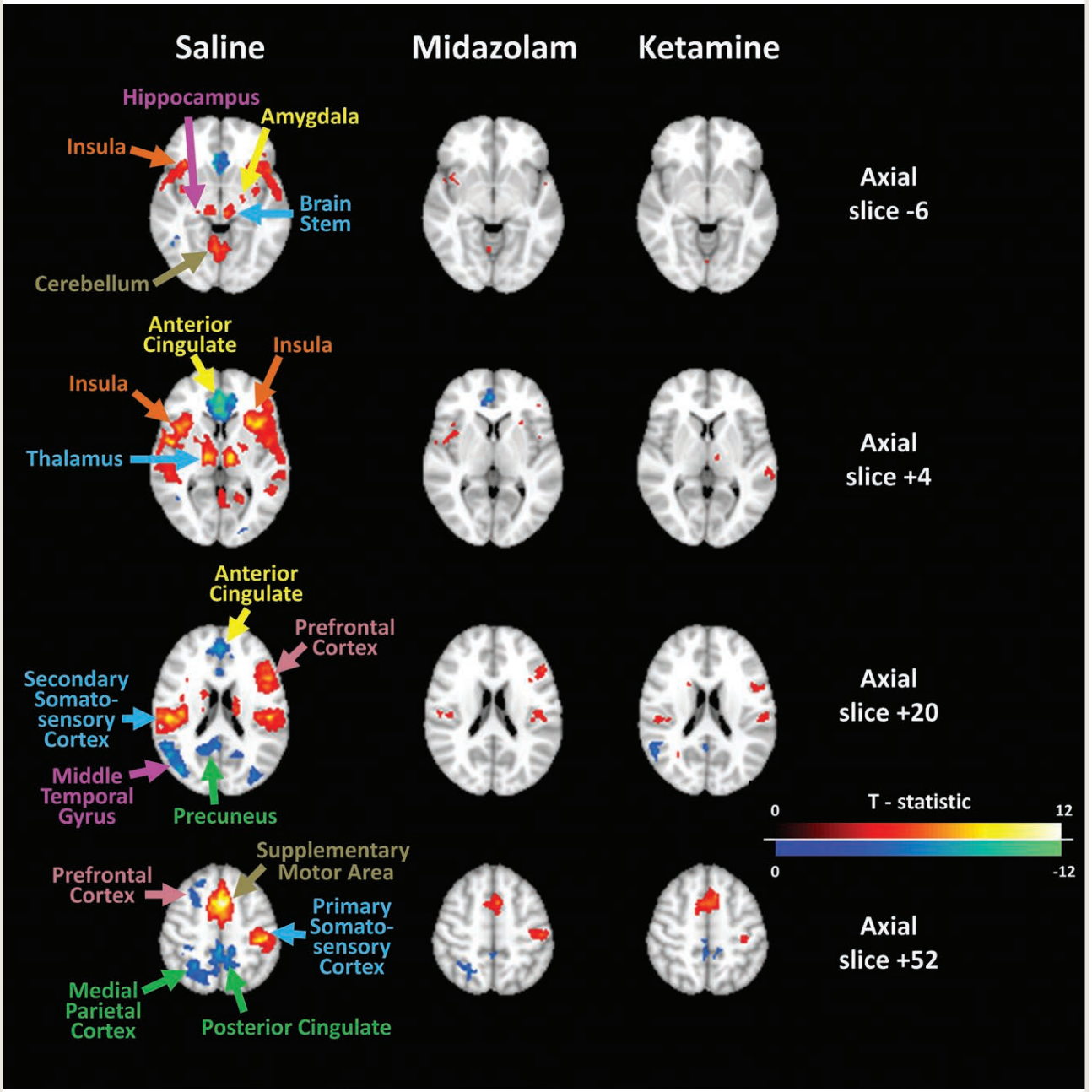

Fig. 5. Group average functional magnetic resonance imaging activation for experimental items subsequently recognized as familiar under saline and drug conditions. Clusters in all columns are thresholded for significance, controlling for a family-wise error rate of $P < 0.05$. The Montreal Neurologic Institute (Montreal, Canada) standard space brain was used as the underlay, with axial slice locations listed. Images are oriented with radiologic convention (right side of the brain shown on the left of the figure). Labels for anatomic regions of interest are color-coded based on predominant functional organization: memory encoding in *purple* (hippocampus and middle temporal gyrus), fear response in *yellow* (amygdala and anterior cingulate cortex), pain processing in *orange* (insula), somatosensory processing in *blue* (brain stem, thalamus, and primary and secondary somatosensory cortices), parietal areas (including default-mode network structures) in *green* (precuneus, posterior cingulate, and medial parietal cortex), prefrontal cortex in *pink*, and motor areas in *olive* (cerebellum and supplementary motor area).

cingulate demonstrate engagement of core elements in fear response.[30–33] These function in a larger network involved in classical conditioning that include the hippocampus,[34] parahippocampus,[35] and connections to the ventromedial prefrontal cortex.[34] The somatosensory network, which most prominently features the thalamus and primary and

secondary somatosensory cortices, is commonly activated in pain processing.[36] Activity seen in the insula represents a structure with specificity for pain processing,[36] although the insula is also active in fear conditioning.[30,33] Additional activation in the posterior cingulate and precuneus (core components in the default-mode network[37–39]) as well as cognitive processing areas in the prefrontal and parietal cortices likely reflect task-related attention shifts and higher-level cortical integration of the task experiences.

The recurring and unpredictable pattern of experiencing pain likely induced a state of threat during the entire experiment. This is supported by insula and somatosensory cortex activation in the saline average maps for the correctly recognized items (see fig. 5 for familiarity and figure, Supplemental Digital Content 8 [http://links.lww.com/ALN/C592], for recollection) even though only approximately one third of these items were paired with a painful shock. In fact, the map for successfully recognized items closely matches the average map for all pain-paired items (see figure, Supplemental Digital Content 9 [http://links.lww.com/ALN/C593]). A relatively larger magnitude of signal change after pain-paired items relative to non-pain items could explain some of this similarity in the calculated maps. However, another possibility is that the pain experience affects brain activation after nonpainful experimental trials as well. Behavioral evidence from a similarly designed experiment supports this notion, as memory for non-pain items were influenced by the presence of pain-paired items in the same experimental context.[13]

Signal changes in the hippocampus, parahippocampus, and amygdala did not surpass the threshold for significance in the average task activation maps for either drug. This suggests (but is not direct evidence of) less task-related neuronal activity under both drugs. This nondefinitive result was not supported by a significant saline *versus* drug contrast but may inform the design of future studies. Human neuroimaging evidence for the effect of midazolam on medial temporal lobe memory structures is unreported,[40] but previous qualitative results show reduced hippocampal activity under propofol in a similar paradigm.[17] Previous task-based functional studies under ketamine have shown reduced activation related to memory[41] and pain[42] tasks.

After regression of task-related signal changes, midazolam and ketamine had different effects on underlying brain functional connectivity during the experience of pain. Changes in background connectivity cut across functional networks, as targets were identified across all brain lobes, suggesting effects on higher-level integration of information. These cross-network changes in functional connectivity are exemplified well by the changes seen from the insula seed region under midazolam. The insula, with known specificity for pain processing,[26] showed increases in connectivity to the anterior cingulate (involved in pain processing and fear learning), the middle temporal gyrus (involved in higher-order memory processing), and prefrontal cortex (involved in higher-level cognitive processing).

Compared with saline, midazolam significantly altered connectivity from three seed regions identified in our analysis, and these were within the pain (insula) and default-mode (precuneus and posterior cingulate) networks. The direction of connectivity change was mixed with midazolam, but we found several decreases in long-range connectivity (such as from the posterior cingulate seed to prefrontal and anterior cingulate targets). This aligns with previous work (though a small study using older scanner technology and higher doses of midazolam) that showed reduced connectivity from the posterior cingulate to other areas.[43] In contrast, our findings of predominantly increased connectivity with midazolam diverge from some previous resting-state studies. A recent study, which showed reduced connectivity across many predefined brain networks, used a dose (2 mg) of midazolam comparable with our study. However, this was done in a smaller number of much older subjects, ranging from 55 to 73 yr.[44] Our midazolam connectivity findings align best with a previous study[45] that used a similar dose administered as a bolus 5 min before a 7-min-long resting-state scan. Although liberally thresholded (without a correction for multiple comparisons), increases in connectivity with midazolam were seen in sensorimotor networks bilaterally and in some portions of networks identified as "language" (anterior insula and temporal gyrus), "frontoparietal" (dorsolateral prefrontal cortex and posterior cingulate), and "salience" (dorsal anterior cingulate and left anterior insula).[45] In the same study, decreased connectivity under midazolam was found for other networks investigated.[45] With our finding of predominantly increased connectivity during a pain task and midazolam, it is important to note that low doses of midazolam can be disinhibitory.[46] Further, increases in connectivity between functionally distinct brain regions have been demonstrated with sedative doses of other anesthetics that similarly act as γ-aminobutyric acid receptor agonists.[47–49]

Under ketamine, connectivity changes between brain regions within our networks of interest were predominantly decreased, compared with saline. Between-systems decreases in connectivity under ketamine were notable from fear learning (right amygdala) and memory encoding (right hippocampus) areas to the right primary somatosensory cortex. Noting that the painful stimulation was left-sided, this finding suggests a disruption of the link between these two learning systems and brain region primarily processing the painful stimulus. Additionally, ketamine disrupted connectivity between the seed regions investigated and cortical areas involved in higher-level processing, such as prefrontal and parietal association areas; both were targets identified as having decreased connectivity from the hippocampus. Finally, decreased connectivity was detected from the posterior cingulate within the default-mode network (involved in attention) to targets identified in the hippocampus (memory) and anterior cingulate (fear learning and pain processing).

Previous ketamine connectivity findings[50] showed decreases in connectivity between a large somatosensory seed network (including the insula) and target regions in the amygdala, anterior cingulate, and hippocampus, consistent with the pattern we detected. Within-network connectivity decreases with ketamine[51] can be seen, as can relative increases in default-mode network connectivity at higher doses.[52] It should be noted that variability across studies in choosing how to group anatomically disparate seed and target regions of interest for connectivity analyses makes comparison with previous work not straightforward. It is our hope that granular reporting of connectivity changes for individual anatomically described regions of interest helps to resolve this issue in the future.

This study has important limitations. The use of a single drug dose limits the generalizability of our findings. Although there would be great value in collecting behavioral and imaging data in a similar paradigm across a range of drug dose responses, this was beyond the scope of the current project. Doses were chosen to target roughly equal sedation at a level that maintained some explicit memory ability, to allow analysis of neural activity of successfully recognized items. However, subjects did have considerable variability in drug effects. Poignant examples of interindividual pharmacodynamic differences include analgesic response to ketamine (table 2) and amnestic response from midazolam (compare tables in Supplemental Digital Content 4 [http://links.lww.com/ALN/C588] and 5 [http://links.lww.com/ALN/C589]). These differences in individual drug responsiveness capture population variability but decrease the power in our saline *versus* drug imaging comparisons. Also, the presence of fewer accurately recognized items under both drugs influenced the power of the activation maps for recollection (see figure, Supplemental Digital Content 8 [http://links.lww.com/ALN/C592]). This concern is ameliorated, however, by the similar numbers of saline and drug experimental items analyzed in the familiarity (fig. 5) and pain-paired (see figure, Supplemental Digital Content 9 [http://links.lww.com/ALN/C593]) maps. Another consideration is that activation was averaged over the three repetitions of the word. It is possible that different strengths of signal change occurred with each repetition of the word, and our analysis framework looks for changes in the average neural activity over these repetitions. Differing levels of neural activity with each repetition may modestly decrease the effect size observed for the activation results. Finally, the possibility of implicit memory was not addressed by our paradigm. We have previously shown, in an unsedated non–magnetic resonance imaging cohort, that our pain stimulation paradigm causes robust skin responses with shock delivery,[53] but this recording was not possible in the scanner. Further work in this area, using a more typical conditioning paradigm, could clarify whether learned sympathetic responses are modulated by these anesthetics.

In summary, in a clinically relevant paradigm of memory formation with periodic pain, low doses of midazolam reduced recollection memory. Midazolam and ketamine were associated with diverging functional connectivity changes, which we postulate reflects their differing activity in the modulation of the cognitive experience under threat of pain. This snapshot of the complex neural dynamics from the combined effects of pain and anesthesia underscores the need to better understand residual brain activity during sedation, particularly in the setting of distinct agents that differentially affect specific cognitive functions.

Acknowledgments

The authors are grateful for the critical comments and helpful suggestions from the handling editor and anonymous reviewers, who helped to sharpen this work into the current form, which is greatly improved from its initial version.

Research Support

This work was supported by the Department of Anesthesiology, University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania. Significant research funding and salary support was provided by a Mentored Research Training Grant (to Dr. Vogt) from the Foundation for Anesthesia Education and Research (Schaumburg, Illinois; MRTG-CT 2-2017). Salary support for Dr. Vogt during early development work directly related to this project was supported by an institutional training grant (T32GM075770), and support during data analysis and manuscript preparation came from a career development award (K23GM132755), both from the U.S. National Institutes of Health (Bethesda, Maryland), specifically the National Institute of General Medical Sciences (Bethesda, Maryland). Additional financial support for Dr. Vogt came from the National Institutes of Health Clinical Loan Repayment program (L30GM120759). Subject recruitment was assisted by the University of Pittsburgh Clinical Translational Science Institute Research Participant Registry, a project supported by the National Institutes of Health (UL1TR000005).

Competing Interests

The authors declare no competing interests.

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