ANESTHESIOLOGY®

Effects of Sedative Doses of Propofol, Dexmedetomidine, and Fentanyl on Memory and Pain in Healthy Young Adults: A Randomized, Controlled, Single-blind Crossover Study Using Functional Magnetic Resonance Imaging at 7 Tesla

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ABSTRACT

Background: Anesthetic agents are well known for their effects on memory and pain; however, previous studies quantifying anesthetic modulation of memory have not included experimental noxious stimulation. This study used functional magnetic resonance imaging to determine how low doses of propofol, dexmedetomidine, and fentanyl affect the brain systems for memory encoding and pain perception.

Methods: This was a single-blind, 1:1:1, randomized, placebo-controlled crossover study of 92 healthy volunteers ages 18 to 40 yr. Effect-site concentrations were targeted for propofol (1.0 mcg/ml), dexmedetomidine (0.15 ng/ml), or fentanyl (0.9 ng/ml). Participants listened to a series of 80 words creating a mental picture. Thirty were accompanied by a 2-s painful shock. Blood oxygen–weighted images were obtained at 7 T using a custom head coil. The primary outcome was next-day memory performance, measured by d′, a normalized measure of correct identifications *versus* false positives. Mixed models were fit to test outcome differences between drug groups. Only statistically significant ($P < 0.05$) changes are reported, after adjustment for multiple comparisons.

Results: Recollection, reported as mean d′ (95% CI), was 1.16 (95% CI, 0.97 to 1.34) under no drug. This was reduced under propofol (0.51; 95% CI, 0.182 to 0.842; $P = 0.006$) but not under dexmedetomidine (1.04; 95% CI, 0.73 to 1.35; $P = 0.99$) or fentanyl (0.98; 95% CI, 0.68 to 1.28; $P = 0.99$). Propofol decreased memory-encoding activation of the hippocampus and amygdala. Propofol reduced pain-related activation in the insula, anterior cingulate, hippocampus, and amygdala. Dexmedetomidine showed decreased memory-related activation in the hippocampus but did not change pain ratings or show activation differences in pain-processing areas. Fentanyl showed decreased memory activation in the hippocampus and amygdala. During painful stimulation, fentanyl decreased activation in the primary somatosensory cortex and insula and increased activation in the anterior cingulate, hippocampus, and amygdala.

Conclusions: These findings add important details to the complex framework of how these distinct anesthetics affect different aspects of cognition through diverse neuroanatomic targets in the brain.

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Different classes of anesthetic drugs—such as fentanyl, propofol, and dexmedetomidine—produce quite different brain states, as estimated using functional magnetic resonance imaging.
- From previous clinical and brain imaging results, it is believed that propofol is the most potent and fentanyl the least potent drug in impairing memory encoding.
- The functional brain imaging of how these classes of anesthetic drugs alter the interaction between memory and pain is unknown.

What This Article Tells Us That Is New

- Subjects receiving painful stimulation under mild sedation with propofol showed less pain intensity and less activation in brain regions associated with attention and memory encoding.
- At similar levels of sedation, dexmedetomidine resulted in no amnesia and no changes in pain perception or increased activity in pain-related brain regions.
- Those subjects who received fentanyl demonstrated no amnesia but decreased activation of memory encoding systems. On painful stimulation, there was less activation in sensory attention brain regions but increased activity in memory systems.

This article is featured in "This Month in ANESTHESIOLOGY," page A1. This article is accompanied by an editorial on p. 253. 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). This article has a video abstract. Part of the work presented in this article has been presented at the International Anesthesia Research Society Annual Meetings, virtual, March 17 to 20, 2022, and in Denver, Colorado, April 13 to 16, 2023; the Association of University Anesthesiologists Annual Meeting, virtual, March 17 to 18, 2022, and in Denver, Colorado, April 13 to 14, 2023; the International Society of Anesthetic Pharmacology Annual Meeting in Philadelphia, Pennsylvania, October 18, 2024; and the American Society of Anesthesiologists Annual Meeting in Philadelphia, Pennsylvania, October 18 to 22, 2024.

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Abbreviations: CS, conditioned stimulus; **fMRI**, functional magnetic resonance imaging; **MRI**, magnetic resonance imaging

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Clinical anesthesia has long used drugs with widely distinct pharmacology to radically alter consciousness, with primary goals including the provision of amnesia and analgesia during otherwise painful experiences. Responsiveness to stimulation is the most common endpoint of titration, but we have previously shown that functional magnetic resonance imaging (fMRI) can reveal how distinct agents may induce dramatically different brain states in equally responsive subjects.[1] The objective of the current study was to determine, in healthy subjects under controlled experimental conditions, how low doses of three drugs (propofol, dexmedetomidine, and fentanyl) would affect the brain systems for two important, clinically relevant aspects of cognition: memory encoding and pain perception

Recognizing the obvious differences in clinical effects and receptor pharmacology of these agents, we expected diverging effects on memory and pain, including an experimental interaction between drug modulation of these two cognitive processes affecting multiple distinct structures within the brain. This localization is difficult to predict based on molecular pharmacology alone. As an example, the hippocampus, critical for long-term memory encoding, has γ-aminobutyric acid type A,[2–4] noradrenergic (α_2),[5] and opioid[6,7] receptors. Whether the hippocampus is effectively inhibited by any of the three agents in this study can be elucidated with real-time neuroimaging. These drugs also have been previously characterized to have differing effects on memory, showing impairment by propofol,[8,9] dose-dependent decreases under dexmedetomidine,[10,11] and smaller reductions with higher doses of fentanyl.[12,13]

Notably, previous work quantifying anesthetic effects on memory has not included controlled experimental noxious stimulation. As painful stimulation activates areas throughout the brain (including the insula, anterior cingulate, and thalamus),[14,15] and stimulates the attention network,[16] pain may affect memory.[17–19] Further adding to a nuanced picture, analgesia during ongoing noxious stimulation can change activation throughout the brain.[20,21] Functional imaging studies with propofol[9] and dexmedetomidine[22] have shown inhibition of the hippocampus along with persistent activity in the amygdala while simply viewing emotion-inducing images, but how acute pain affects these systems under these (and other) anesthetic agents is largely unknown. We anticipated that findings from this study, using periodic painful experimental stimulation, would reveal important comparative neural correlates of how three distinct agents act on brain memory systems during the clinically relevant paradigm of sedation while experiencing acute pain.

We defined our primary outcome as the effect on long-term explicit memory performance and hypothesized that this would be reduced by propofol and dexmedetomidine but not fentanyl. Because of the inherent complexity in making predictions for thousands of brain imaging data points, we considered fMRI results as secondary outcomes and conceptually framed our approach as data-driven rather than hypothesis-driven. Nonetheless, we expected decreases in activation during our memory encoding task, particularly in the hippocampus, parahippocampus, and surrounding medial temporal lobe structures (which, for brevity we have simply labeled as "hippocampus" in the remainder of the article) that would follow the pattern propofol then dexmedetomidine then fentanyl. The predicted pattern for decreases in activation with painful stimulation was expected to follow the progression fentanyl then dexmedetomidine then propofol, localized to key regions in pain processing such as the insula, anterior cingulate, and somatosensory cortex. Regardless of specific findings, we anticipated that the pattern of brain system modulation observed would guide future research, with an aim toward better understanding the neuroscience of clinical anesthesia practice.

Materials and Methods

Basic Study Design and Oversight

This was a 1:1:1 randomized, placebo-controlled, single-blind neuroimaging drug study comparing propofol,

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dexmedetomidine, and fentanyl. Each participant was scheduled to receive one of the anesthetic agents and also assigned a separate no-drug control session, with these two sessions performed in randomized order. Informed consent was obtained in advance of any study activity, and participants were informed that they were free to withdraw at any time. The protocol was preregistered as a clinical trial at ClinicalTrials.gov (NCT04062123), with first record entered August 19, 2019, and Keith M. Vogt listed as principal investigator. The study was approved by the University of Pittsburgh (Pittsburgh, Pennsylvania) Biomedical Institutional Review Board (STUDY19030183). All applicable ethical standards for the responsible conduct of research were followed.

Participants Screening and Preparation

Healthy volunteers between the ages of 18 and 40 yr were recruited from the community and compensated up to $500 for completing all study visits and procedures. As described in Supplemental Digital Content 1 (https://links.lww.com/ALN/D954), 207 potential subjects were screened, and 116 of these were enrolled in the study. Ultimately, data were successfully collected from 92 participants. All participants denied chronic medical problems, including chronic pain, hearing impairment, and memory impairment. They denied previous adverse reaction to agents used in the study and had no recent or routine use of antidepressants, antipsychotics, antihistamines, anxiolytics, stimulants, sleep aids, or analgesic medications. Potential participants were excluded for excessive ethanol or regular marijuana use and for any illicit drug use, except remote isolated history. Potential participants also completed a brief in-person memory screening test before enrollment, and performance at greater than 0.5 SDs above chance guessing was an inclusion criterion. A urine drug screen for opioids, marijuana, and other illicit drugs was obtained at the first study visit to confirm no measurable levels of any of these agents were present before participation. Participants also underwent a preanesthetic evaluation by an anesthesiologist, urine pregnancy testing for females, and magnetic resonance imaging (MRI) compatibility screening to rule out other contraindications to safe sedation inside a high-field magnet. Participant weight was confirmed with a scale. Subjects were instructed on all experimental tasks and given an opportunity to practice them in advance. All participants adhered to the American Society of Anesthesiologists (Schaumburg, Illinois) guidelines for preoperative fasting[23] and additionally abstained from tobacco and caffeine for at least 8 h before the MRI sessions. No restrictions were placed on intake before the next-day memory testing sessions.

Overall Study Flow

Aside from the screening visit and separate consent discussion with the principal investigator, this was designed as a four-visit study. Two scan visits were planned, each of these followed by a memory testing session held in person sometime the next day. Scan visits were scheduled at least 2 days apart. Enrollment and dropouts were considered as random processes. Given the relatively small scale of the study, replacement assignments were made to target balanced trial arms. There were roughly equal numbers of both sexes in each group and roughly equal sequences of the drug *versus* no-drug session occurring first. Subject numbers in each allocation group are shown in Supplemental Digital Content 1 (https://links.lww.com/ALN/D954).

Painful Stimulation

A battery-powered electric nerve stimulator (EzStim II; Life Tech, USA) was used to deliver noxious stimulation with a 100-Hz waveform. Outside the scanner, the nerve stimulator was connected *via* two small electrodes secured to the lateral aspect of the left index finger. The current was slowly titrated up to a subjective rating of 7 out of 10 pain intensity using a numerical rating scale with anchors at 0 as no pain and 10 being the worst imaginable. Several test shocks were delivered to confirm the rating had stabilized. This was repeated with two additional test shocks once in the scanner, and the current level adjusted upward as needed to begin the experiment at 7 out of 10 pain intensity rating. Ratings of pain unpleasantness were also obtained and recorded, but no specific value was targeted. Subsequent pain ratings were obtained after each in-scanner experimental segment. No further adjustment of nerve stimulator intensity occurred during the experiment.

Drug Administration

Intravenous access was obtained in each participant's hand or arm with a 22-gauge catheter. Once the participant was positioned in the MRI scanner and connected to standard American Society of Anesthesiologists (Schaumburg, Illinois) monitors, a crystalloid carrier infusion was run at 100 ml/h until the end of the experiment. If the participant was assigned to receive a drug, the bolus was given, without announcement, shortly after acquisition of a localizer scan. For no-drug scans, a 5-ml bolus of saline was given at this time to assist in maintaining subject blinding to drug condition. Drug bolus and infusion rates were determined by presimulation using the open-source online software stanpumpR (interface at https://stanpumpr.io/; code available at https://github.com/StevenLShafer/stanpumpR). Steady state effect site concentrations were targeted according to established pharmacokinetic models accounting for age, sex, height, and weight. The target concentration for propofol[24] was 1.0 mcg/ml, for dexmedetomidine[25] was 0.15 ng/ml, and for fentanyl[26] was 0.9 ng/ml. To grossly quantify drug effects, the Observer's Assessment of Awareness/Sedation score[27] was recorded at each break in scanning. As we could not easily visualize the participant's face, we relied on a composite of only the responsiveness and speech domains in assigning the score 0 to 5. On this scale, 5 indicates

normal brisk verbal response, and 0 indicates no response to noxious stimulation. Any subjective complaints or observed side effects were also obtained from participants at each break in scanning.

Stimulus Presentation and Recording

Experimental tasks were implemented with E-Prime version 3.0 (Psychology Software Tools, USA), using a laptop computer running Microsoft (USA) Windows 10. A USB-emulated serial port connection enabled E-Prime to control a relay board that provided precise computer control of the nerve stimulator, and timing was coordinated with other experimental events. Audio files used for the prerecorded words were played through MRI-compatible audio ear plugs. An MRI-compatible right hand response glove was used, and button presses were captured by the E-Prime software. E-Prime was configured such that a 5-V square wave signal was generated to precisely mark audio output and shock timing; these were captured along with an MRI scanner trigger signal to mark acquisition of each imaging volume. These marker signals were recorded using a BIOPAC MP 160 (BIOPAC Systems, Inc., USA) data acquisition system with AcqKnowledge 5.0 (BIOPAC Systems, Inc.) running on a separate laptop computer.

Physiologic Recordings

Electrodermal activity was recorded during scanning with a specializer amplifier (EDA100C-MRI; BIOPAC Systems, Inc.) for the BIOPAC system. Electrodermal activity electrodes were applied to the left thenar and hypothenar eminences. Despite using MRI-compatible versions of the electrocardiograph (ECG100C-MRI; BIOPAC Systems, Inc.) and two-channel electroencephalograph (EEG100C-MRI; BIOPAC Systems, Inc.) lead systems, we were unable to record any meaningful signals from the 7-T environment with these sensors due to electrical interference.

Memory and Conditioning Task

The primary task included 80 auditory word items. Words were preselected randomly in advance and drawn from a bank of more than 700 possible words. All were common nouns short enough to be spoken in a 0.75-s recording (more details on word list generation were previously explained[28]). Each word was played four times during a 6-s window. In a mental imagery memory encoding task, subjects were tasked with creating a mental picture or story surrounding the word and instructed to add more details as the word repeated. Words were recorded in two recognizably distinct voices (masculine and feminine) and employed in a partial (75%) reinforcement classical conditioning paradigm.[29] Voices were the conditioned stimuli (CS), with breakdown shown in the blue box in figure 1. Forty of the words were played in one voice and never paired with shocks. Thus, this voice was designated CS-. The feminine voice is shown as an *example* in figure 1, but assignment of voice gender was randomized across CS conditions. Thirty of the words were played in the other voice and paired with nerve stimulation (the unconditioned stimulus). Again, masculine voice is shown paired with CS+ in figure 1, but assignment was randomized across subjects. Shocks were 2 s long and delivered in the second half of the auditory window, after the word had already played twice. These 30 words were designated "CS+/unconditioned stimulus" and intended to form a conditioning association to voice. Additionally, 10 words were played in the same voice, but without associated shock. These unreinforced stimuli were designated CS+ (alone). This paradigm was split into two 8-min blocks with a scanning break to allow for subject assessment. To increase statistical power for detecting task events in the imaging data, a jitter of 0 to 1 s was included between items,[30] which were otherwise spaced 7 s apart.

Pain Task

Nerve stimulation alone was used in the Pain task fMRI scan. A block design was employed with five 10-s periods of stimulation, as shown in the gray box in figure 1. Periods between stimulations averaged 20 s, but the duration of the rest period varied by 1 to 4 s to reduce predictability of shocks.

Brief Cognitive Task Battery

Three tasks were included in a 3.5-min behavioral battery (green box in fig. 1) administered three times throughout the experiment under steady state drug conditions in an attempt to quantify anesthetic effects on different aspects of cognition (inspired by previous work[31]). Functional imaging data were not collected concurrently with these tasks. First was a psychomotor vigilance task in which subjects were asked to respond by pressing a button with their right index finger as quickly as possible upon hearing a tone. Subjects heard an unpredictable series of 10 tones with 2 to 5 s between them. This was followed by a dual-task serial object recognition paradigm (detail shown in the bottom right of fig. 1), modeled after previous work,[8] which employed a series of colored geometric images shown visually. Subjects indicated with different right-hand buttons whether they recognized each image. Images were presented for 4 s (regardless of button press) with a 5-s interstimulus interval. The design included nine three-back pairs, interspersed with 12 additional items (shown once, but not used in the three-back task) for which long-term memory was assessed. Thus, there were 30 total items presented. The interval between initial presentation and recognition test in the long-term memory task was 66 to 71 s, far exceeding the capacity of working memory. The proportions of correct recognitions and false-positive identifications were calculated separately for the three-back and visual memory tasks. For both, as in previous similar studies,[1,9] memory performance was summarized using the signal detection

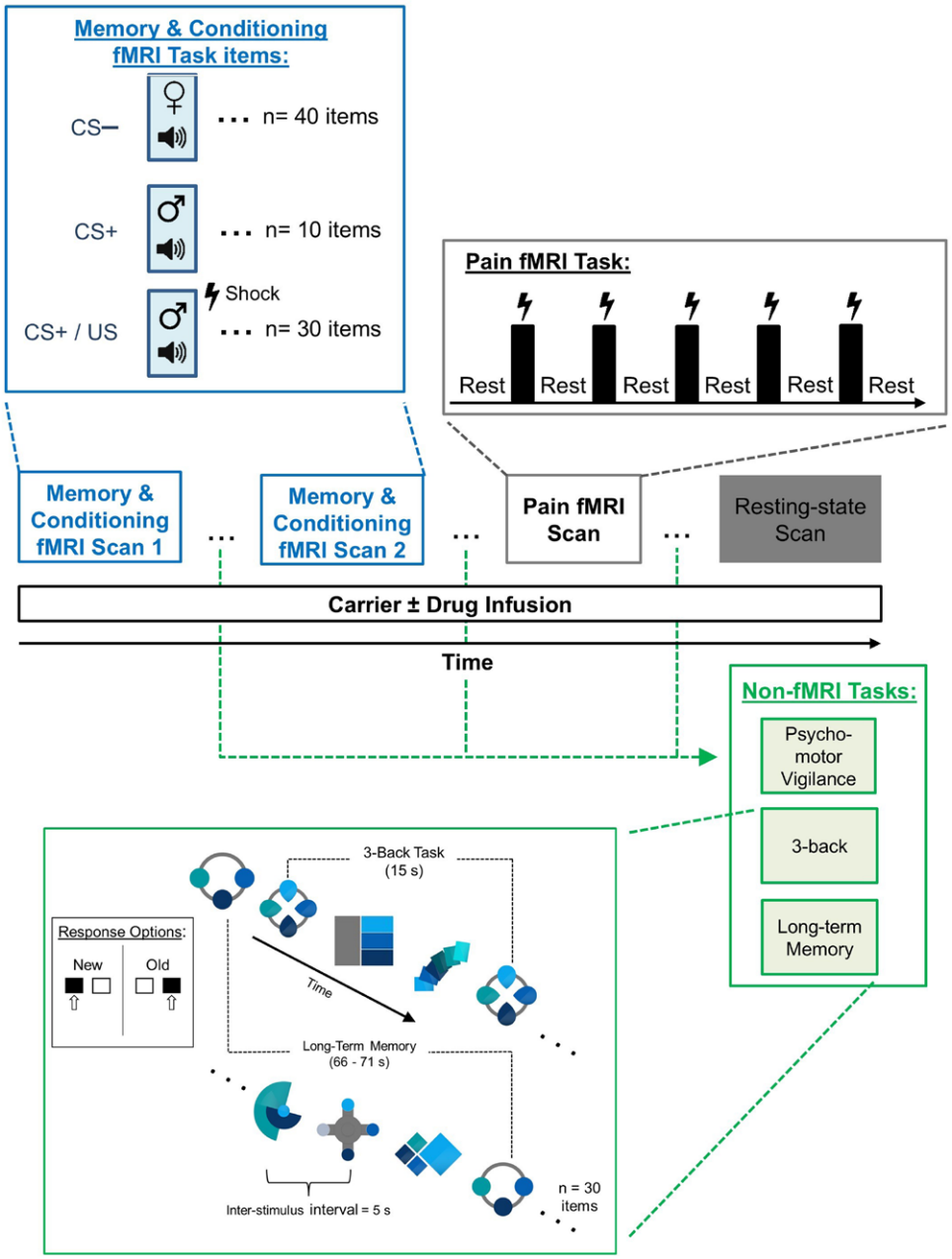

Fig. 1. Scan session experimental design overview. The 80 total items in the Memory and Conditioning task were split between the first two 8-min functional magnetic resonance imaging (fMRI) scans, with item details shown in the *blue box*. Note that assignment of the gender of spoken voice was randomized between conditions. The pain task fMRI block followed, with five 10-s shocks, as shown in the *gray box*. A task-free resting-state scan (not analyzed) ended the session. Immediately after each of the fMRI task scans, a brief battery of three cognitive tasks (*green boxes*) was performed during structural scanning. Additional details of the combined object recognition paradigm with three-back and memory tasks are shown in the *bottom left box*. CS+, conditioned stimulus (associated); CS- conditioned stimulus (unassociated); US, unconditioned stimulus.

metric, d′.[32] This is calculated as the difference in Z-scores between cumulative Gaussian distributions (calculated with NORMSINV() in Microsoft Excel), using the formula Z(hits) minus Z(false alarms), where hits are correct identifications of a previously seen picture and false alarms are incorrect indication of recognition of a picture not previously seen. To avoid any confusion between repetitions of the image recognition task, images in each occurrence of this task battery used a distinct color family (*e.g.*, blue-green, then yellow-orange, and then red-pink).

Memory Testing

Subjects returned the day after the scan visit for a separate memory testing session in a dedicated room with a laptop computer and headphones. Auditory recognition testing used the Remember-Know-New paradigm[33] with standardized instructions given to and reviewed with the subject (exact wording published previously[28]). Participants could indicate recollection (including some specific details recalled) with a Remember response. Familiarity, with no specific details recalled, could be indicated by a Know response. The last option was to identify a word as New if they did not recognize it from the previous day. Words were played once, and there was no time limit on response. In addition to all words heard the previous day, an equal number of foils (not previously used words) were included in randomized order and drawn from the same bank of possible words. Thus, the next-day memory test reflected the ability to discriminate between previously heard and unheard words. These were analyzed in a signal detection framework, as described for the object recognition task. Measures of recollection and familiarity were analyzed separately for the behavioral data to examine effects on these distinct components of explicit memory. All words that were recognized by participants with either a Remember or Know response were followed by a second question. In this case, the same word they just heard and identified was presented visually on the screen, and they indicated whether they thought the word was previously paired with a painful shock the day before by selecting Yes, No, or Unsure responses. This was an attempt to collect data on source memory for pain pairing.

Magnetic Resonance Imaging

A Siemens 7-T Magnetom scanner was used in conjunction with a custom-built Tic-Tac-Toe radiofrequency head coil design[34] that improves excitation homogeneity and performance consistency between different subjects.[35] For later registration, a T1-weighted anatomic image was obtained with 0.75-mm isotropic spatial resolution. This was obtained first, including during the wait period for the drug to reach steady state. Functional images were obtained using blood oxygen level–dependent contrast, with an accelerated echo-planar imaging sequence.[36,37] Parameters were as follows: echo time, 20 ms; repetition time, 1 s; flip angle, 65 degrees; bandwidth, 1,624 Hz/pixel; and posterior–anterior phase encoding direction. In-plane field of view was 220 mm. Sixty slices at 2 mm isotropic spatial resolution gave whole brain coverage. Slices were obtained in interleaved fashion using partial Fourier (7/8) phase encoding, partial k-space sampling (Generalized Autocalibrating Partially Parallel Acquisitions factor 3), and multiband acceleration (factor 3). Iterative higher-order shimming was performed at the beginning of each scan session to reduce inhomogeneity. A pair of spin echo field maps was acquired after each functional imaging run in the same coordinate space for offline inhomogeneity correction. As noted in the experimental overview in figure 1, a task-free resting-state scan was obtained at the end of each experimental run, but analysis of these data is not presented in this report.

Statistical Analysis

Analysis of numerical behavioral data, including memory performance data, was carried out with R software (version 4.3.1, https://www.r-project.org/, accessed June 30, 2023). Memory and three-back task performance were evaluated by comparing d′ scores. Next-day memory performance was compared between drug conditions as the primary outcome. Secondary outcomes were also compared, including ratings of pain intensity and unpleasantness, in-scanner three-back and long-term memory performance, and sedations scores. Data were first tested for normality with the Kolmogorov–Smirnov test before application of two-tailed parametric statistical methods for analysis. Separate mixed models were fit for each outcome, with drug group as a fixed effect and participant as a random effect. Interaction terms between time and drug group were fit and tested and included in models if statistically significant. $P < 0.05$ was used as the threshold for statistical significance, and values are reported both raw and P_a, indicating Bonferroni adjustment for the number of comparisons performed in the behavioral data analysis.

fMRI Data Analysis

fMRI datasets were preprocessed using FSL 6 (https://fsl.fmrib.ox.ac.uk/, accessed June 3, 2025) on several machines running MacOS (Apple Inc., USA). Subsequent acronyms are subroutines within the FSL software package. Correction for magnetic field distortion, using the acquired field maps, was performed on all functional data using TOPUP. Brain extraction was performed with BET and tissue segmentation with FAST. Based on the principal components of signal within the white matter and CSF in the functional time-series, the CompCor algorithm[38] was used to reduce physiologic noise related to respiration and cardiac pulsatility. Motion parameters were included as a covariate of no interest in the first-level model, with censoring of any time point with root mean squared frame displacement motion greater than 0.9 mm. Spatial smoothing was performed with a 5-mm Gaussian kernel.

Event-related task activation was calculated based on timing of events. For the memory and conditioning task data, three subject-level contrasts were included: items subsequently recognized correctly (with either a correct Remember or Know response at next-day testing), items forgotten (a false-negative response at next-day testing), and the timing of all electric nerve stimulations (regardless of memory performance for the corresponding word). In the pain task data, only shock timing was used as a first-level contrast. Average task activation and drug condition difference maps were generated by grouping data from each drug condition in a group-level mixed effects model. Two hardware and one software upgrades to the scanner during

Table 1. Subject Characteristic Information Summarized by Groups According to Available Data by Drug Administered in Scan Session

	Age, yr		Mass, kg		Height, cm		Body Mass Index, kg/m²	
	Mean ± SD	Min/Max	Mean ± SD	Min/Max	Mean ± SD	Min/Max	Mean ± SD	Min/Max
No drug	24.9 ± 5.6	18.4/40.6	69.3 ± 12.4	46.7/101.2	170.4 ± 8.9	152.4/190.5	23.8 ± 3.2	18.5/32.9
Propofol	25.0 ± 6.2	19.0/40.6	70.3 ± 14.5	46.7/100.0	169.4 ± 11.1	154.9/190.5	24.3 ± 3.7	19.0/33.2
Dexmedetomidine	25.6 ± 6.5	18.4/40.2	69.3 ± 11.4	49.1/95.5	168.5 ± 7.9	152.4/182.9	24.3 ± 2.9	19.2/30.2
Fentanyl	23.9 ± 5.0	18.4/40.1	70.5 ± 10.4	50.0/90.1	170.7 ± 8.5	157.5/188.0	24.2 ± 3.3	18.5/30.8

Max, maximum; Min, minimum.

the course of the study were accounted for at the group level as effects of no interest. Individual voxels were initially thresholded with a cluster-determining threshold of $Z > 2$. Then an additional threshold was applied that adjusted the overall analysis-level cluster false discovery rate to $P < 0.05$. All results displayed in figures and listed in tables reflect statistically significant changes in brain activation, including correction for multiple comparisons. No statistical power calculation was possible for the imaging analyses, but overall sample size was based on general estimates[39,40] suggesting that 24 subjects are adequate for task-based fMRI studies.

In an exploratory analysis, we also analyzed sex-based differences in activation under drug and no-drug conditions. For this analysis, we focused on the successful recognition and shock contrasts in the Memory and Conditioning task data. Data were grouped based on self-reported sex assigned at birth, and male and female participant data were contrasted for differences.

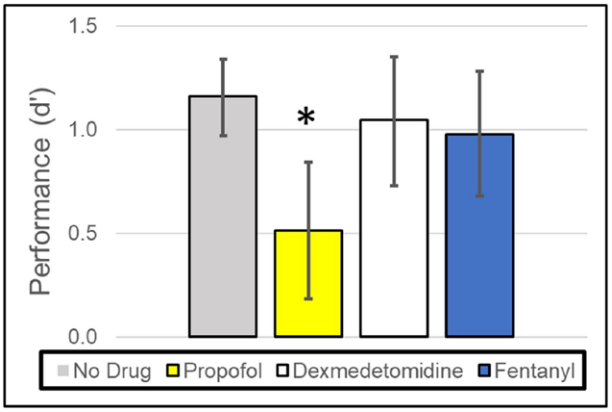

Fig. 2. Next-day auditory recollection memory performance for words heard in the scanner, using signal detection metric d′ (see text for details) across drug conditions. *Bars* represent 95% CI. *$P < 0.05$ compared to no-drug condition.

Results

Subject and Group Demographics

Characteristic information from all participants is tabulated by drug group in table 1. Several subjects were lost to follow-up at various points in the experimental flow, and this is displayed in Supplemental Digital Content 1 (https://links.lww.com/ALN/D954). Not all participants completed both of their assigned no-drug and drug scan sessions. Summary statistics for the amounts of drug administered by bolus and infusion as well as the times spent receiving the drug infusions are listed in Supplemental Digital Content 2 (https://links.lww.com/ALN/D955). Data on the intervals between scan sessions and the average between scan session and next-day memory testing are included in Supplemental Digital Content 3 (https://links.lww.com/ALN/D956).

Next-day Memory Testing Results

Results for next-day memory testing (the primary outcome) were initially visualized separately by pain-pairing, but no differences were evident (Supplemental Digital Content 4, https://links.lww.com/ALN/D957). As pain did not directly affect item-level memory, subsequent analyses were collapsed across pain-pairing to analyze all word items. Hit and false alarm rates for the next-day auditory memory testing are tabulated according to experimental condition in Supplemental Digital Content 5 (https://links.lww.com/ALN/D958). Calculated d′ values for Know responses (reflecting familiarity without recollection) were near zero (indicating chance guessing performance) for all drug groups. Next-day memory performance for recollection (d′ for correct Remember responses) is shown in figure 2. Data are additionally shown in more granular violin plots in Supplemental Digital Content 6 (https://links.lww.com/ALN/D959). Performance with no drug (1.16; 95% CI, 0.97 to 1.34) was reduced under propofol (0.51; 95% CI, 0.182 to 0.842; $P = 0.006$; $P_a = 0.018$), but not dexmedetomidine (1.04; 95% CI, 0.73 to 1.35; $P = P_a = 1$) or fentanyl (0.98; 95% CI, 0.68 to 1.28; $P = P_a = 1$). Despite the predominance of a recollection response, results for the source memory question for pain-pairing were worse than chance guessing in all drug conditions (data not shown). This indicates that participants mostly recollected details

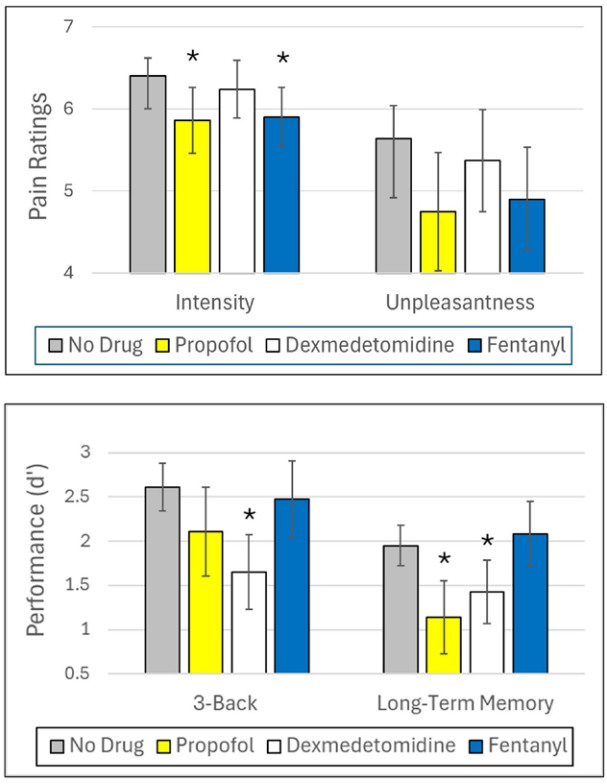

Fig. 3. Behavioral data from during scan sessions, averaged across subjects, by drug condition. (*Top*) Pain scores. (*Bottom*) Performance on the two image recognition tasks in the brief in-scanner cognitive battery. *Bars* represent 95% CI. *$P < 0.05$ compared to no-drug condition.

of their self-generated mental picture or story, rather than accurately recalling whether or not a word item was paired with a painful shock.

Behavioral Measures from Scan Session

Details of mixed modeling analyses of numerical data are presented in Supplemental Digital Content 7 (https://links.lww.com/ALN/D960). Average sedation scores, reported as mean (95% CI) with no drug at 4.98 (95% CI, 4.92 to 5.04), were slightly higher than under propofol (4.75; 95% CI, 4.64 to 4.85; $P < 0.001$; $P_a = 0.001$), dexmedetomidine (4.87; 95% CI, 4.77 to 4.96; $P = 0.036$; $P_a = 0.107$), and fentanyl (4.81; 95% CI, 4.71 to 4.90; $P = 0.002$; $P_a = 0.006$). Realizing all these round to the scale maximum of 5, these differences indicate that minimal sedation[41] predominated. Only two participants became unresponsive to repeated verbal stimulation (Observer's Assessment of Awareness/Sedation, 2); both these events occurred while receiving propofol.

Pain ratings by drug condition are shown in figure 3 (top). Averaging across the three observations during the scan session, pain ratings with no drug were 6.4 (95% CI, 6.18 to 6.63) for intensity and 5.64 (95% CI, 5.24 to 6.04) for unpleasantness. Intensity ratings were lower under propofol (5.86; 95% CI, 5.46 to 6.27; $P = 0.016$; $P_a = 0.047$) and fentanyl (5.9; 95% CI, 5.54 to 6.25; $P = 0.012$; $P_a = 0.037$) but not dexmedetomidine (6.24; 95% CI, 5.89 to 6.59; $P = 0.390$; $P_a = 1$). Unpleasantness ratings were marginally lower under propofol (4.75; 95% CI, 4.03 to 5.47; $P = 0.024$; $P_a = 0.071$) and fentanyl (4.9; 95% CI, 4.27 to 5.53; $P = 0.038$; $P_a = 0.113$) but not dexmedetomidine (6.24; 95% CI, 5.89 to 6.59; $P = 0.421$; $P_a = 1$).

Response times for the psychomotor vigilance task were obviously not different between groups (data not shown). Variance within each drug data group was approximately 10 times the calculated differences in mean response time between groups. Thus, these data were not further analyzed.

Results from the in-scanner visual object recognition task are shown in figure 3 (bottom). Performance on the visual three-back task, listed as mean d′ (95% CI) with no drug, was 2.61 (95% CI, 2.34 to 2.88). This was lower under dexmedetomidine (1.65; 95% CI, 1.23 to 2.07; $P_a < 0.001$). There was no difference detected under propofol (2.11; 95% CI, 1.61 to 2.60; $P = 0.061$; $P_a = 0.183$) or fentanyl (2.48; 95% CI, 2.05 to 2.91; $P = 0.581$; $P_a = 1$). Performance on the long-term visual object recognition memory task with no drug was d′ = 1.95 (95% CI, 1.72 to 2.18). Memory was worse under propofol (1.14; 95% CI, 0.73 to 1.55; $P_a = 0.001$) and dexmedetomidine (1.43; 95% CI, 1.07 to 1.78; $P = 0.006$; $P_a = 0.017$) but not fentanyl (2.08; 95% CI, 1.71 to 2.44; $P = 0.533$; $P_a = 1$).

fMRI Task-related Activation

Brain activation for selected slices from both tasks in the Memory and Conditioning task scans are shown (without any overlaying labels or arrows) in Supplemental Digital Content 8 (https://links.lww.com/ALN/D961). Figure 4 shows brain activation for items successfully encoded into long-term memory, as reflected by successful recognition at the next-day testing session. The top rows of figure 4 show the average pattern under the drug conditions, with warm colors representing task-positive changes (increases in blood-oxygen contrast) and cool colors indicating task-negative correlations to fMRI signal changes. For purposes of interpretation, we operationally assume both represent stimulus-induced changes in neural activity. In the bottom three rows of figure 4, maps of statistically significant differences (no drug *vs.* drug contrast) are shown. In the three difference maps, warm colors represent Z-scores for activation that were increased under the drug condition. Cool colors in the difference maps represent decreased activation during memory encoding under the drug condition. Areas of interest for this task are labeled with colored arrows for the hippocampus in purple (realizing, as mentioned in the introduction, this includes the parahippocampal cortex) and amygdala in yellow, shown in the inferior slices of the brain. We note that our task engaged the primary auditory cortex and memory areas, as expected. Brain areas with strongest clusters of activation are listed in Supplemental

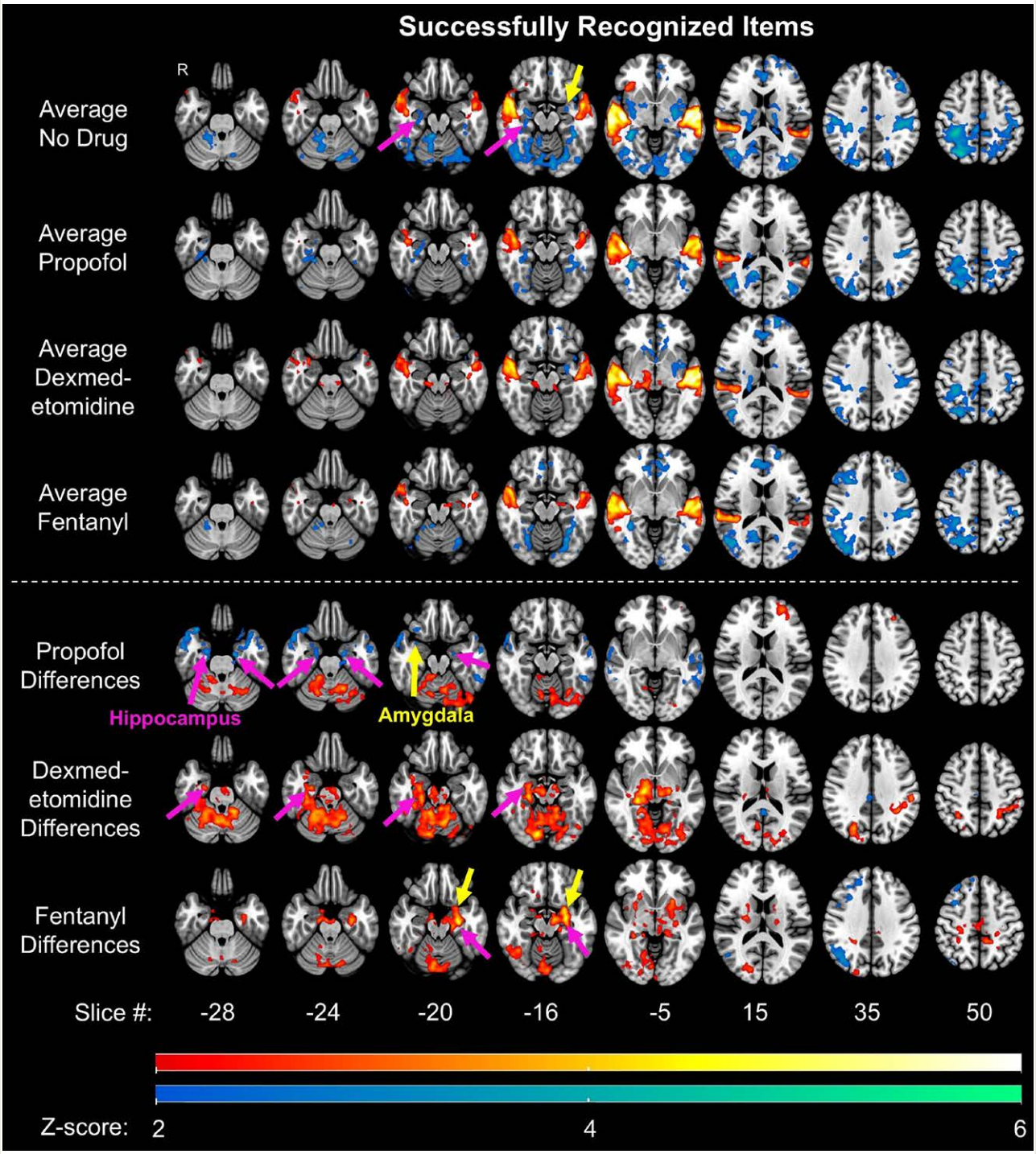

Fig. 4. All-subject average functional magnetic resonance imaging (fMRI) task activation in the Memory and Conditioning scan for words successfully recognized at next-day testing for selected axial slices, with Monteal Neurologic Institute slice numbers shown. The *color bar* indicates Z-score, according to the scale shown. The *top rows* show brain areas with statistically significant positive (*warm colors*) or negative (*cool colors*) correlation to the task under the drug conditions. The *bottom three rows* display areas of statistically significant differences under each drug condition (the no-drug *vs.* drug contrast). *Warm colors* indicate drug greater than no drug (increase under drug) and *cool colors* indicate no drug greater than drug (decrease under drug) differences. The amygdala is indicated with *yellow arrows*; the hippocampus (and/or parahippocampus) is indicated with *purple arrows*.

Digital Content 9 (https://links.lww.com/ALN/D962). Note that three different atlases are listed, often with incomplete concordance, to best represent how the results map to known brain areas.

Notable drug-based differences for items successfully recognized include a reduction in activity under propofol in the bilateral hippocampus, as well as a portion of the right amygdala. Successfully recognized items under dexmedetomidine were associated with decreased activity in the right hippocampus and bilateral midbrain and thalamus. Fentanyl showed decreased activity in the left hippocampus and amygdala in association with successful memory encoding. Brain areas with largest drug-based differences in activation within each cluster are listed in Supplemental Digital Content 9 (https://links.lww.com/ALN/D962).

Task activation from shocks received during the Memory and Conditioning scans for selected brain slices is shown in figure 5. Importantly, shocks were delivered while still hearing word items and presumably engaging in the mental imagery task. As shown in the first row, the series of 2-s shocks was associated with activation in areas commonly associated with acute pain, including the right (contralateral) primary somatosensory cortex, anterior cingulate, and bilateral insula. Additionally, increased activation was seen in the right amygdala and hippocampus/parahippocampus with decreased activation in a portion of the left hippocampus/parahippocampus. Brain areas with strongest activation and largest drug-based differences within identified clusters for the shock task are listed in Supplemental Digital Content 10 (https://links.lww.com/ALN/D963). Propofol was associated with decreased activation in the bilateral insula, right anterior cingulate, right hippocampus, and bilateral amygdala during shocks. Differences in shock-related activation under dexmedetomidine were limited to the cerebellum and posterior cortical areas outside the regions of interest for pain processing. Fentanyl was associated with decreased activity in the right (contralateral) primary somatosensory cortex, portions of the (predominantly right) insula, and a small portion of the left amygdala and left hippocampus. Fentanyl showed increased activation in portions of the hippocampus bilaterally.

Differences between male and female participants in brain activation during the Memory and Conditioning scan are shown in Supplemental Digital Content 11 (https://links.lww.com/ALN/D964). Calculating sex-based differences inherently reduced the size of the group data being compared (approximately in half). Thus, these findings should be viewed as exploratory. Activation during successful memory encoding under the no-drug condition was greater in men than women in the bilateral mid-hippocampus, bilateral insula, and left amygdala. Propofol exhibited greater activation in male participants, compared to females, in a small portion of the bilateral anterior cingulate cortex. Dexmedetomidine exhibited greater activation in male participants, compared to females, in the primary somatosensory cortex. Activation during shock under the no-drug condition was greater in women than men in the medial parietal cortex (right greater than left). Dexmedetomidine exhibited greater activation in female participants, compared to males, in the left ventrolateral prefrontal cortex.

Brain activation from the block-design Pain task scan is shown for selected slices in figure 6. As in previous figures, the top rows display average activation (warm colors) and deactivation (cool colors). Pain-related activation was seen throughout the bilateral insula, right primary and bilateral secondary somatosensory cortices, portions of the bilateral basal ganglia, and right amygdala. Deactivations during the Pain task were seen bilaterally in the precuneus, posterior cingulate, mid-cingulate, anterior cingulate, and hippocampus and broadly throughout the frontal, occipital, and lateral parietal lobes. Deactivations were also seen in the left primary somatosensory cortex, which was ipsilateral to the site of stimulation, and in the right cerebellar hemisphere. Brain areas with strongest activation for the Pain task scan are listed in Supplemental Digital Content 12 (https://links.lww.com/ALN/D965).

Drug *versus* no-drug differences in Pain task activation are shown for selected slices in the bottom three rows of figure 6. Propofol was associated with decreased activation to acute pain stimulation in the left insula, basal ganglia, and thalamus and increased activation in the right frontal and left parietal lobes, including a small portion of the precuneus. There were no statistically significant pain-related activation differences under dexmedetomidine. Fentanyl was associated with decreases in pain-related activation in the right somatosensory cortex and insula and with bilateral increases in pain-related activation in the hippocampus, amygdala, thalamus, basal ganglia, precuneus, posterior cingulate, mid-cingulate, and anterior cingulate, and broad portions of the frontal and parietal lobes. Brain areas with the largest drug-based differences for the Pain task scan are also listed in Supplemental Digital Content 12 (https://links.lww.com/ALN/D965).

Discussion

This study used low doses of three agents with distinct pharmacology that are central to anesthesia practice. We quantified cognitive-behavioral changes in pain perception and memory performance and related these tasks to differences in brain activation measured with fMRI. In tying these data together, we demonstrate some of the key neuroanatomical substrates that underpin both overlapping and distinct clinical features of these drugs when used in a clinically relevant paradigm including the experience of acute pain.

Subjective ratings of pain intensity were reduced under both propofol and fentanyl. For fentanyl, this change is not unexpected.[42,43] Propofol has some inconsistent previous findings for pain modulation[44] but reduces pain ratings in similarly framed experimental studies.[45,46] We found no difference in pain scores under dexmedetomidine. This is consistent with previous work demonstrating that

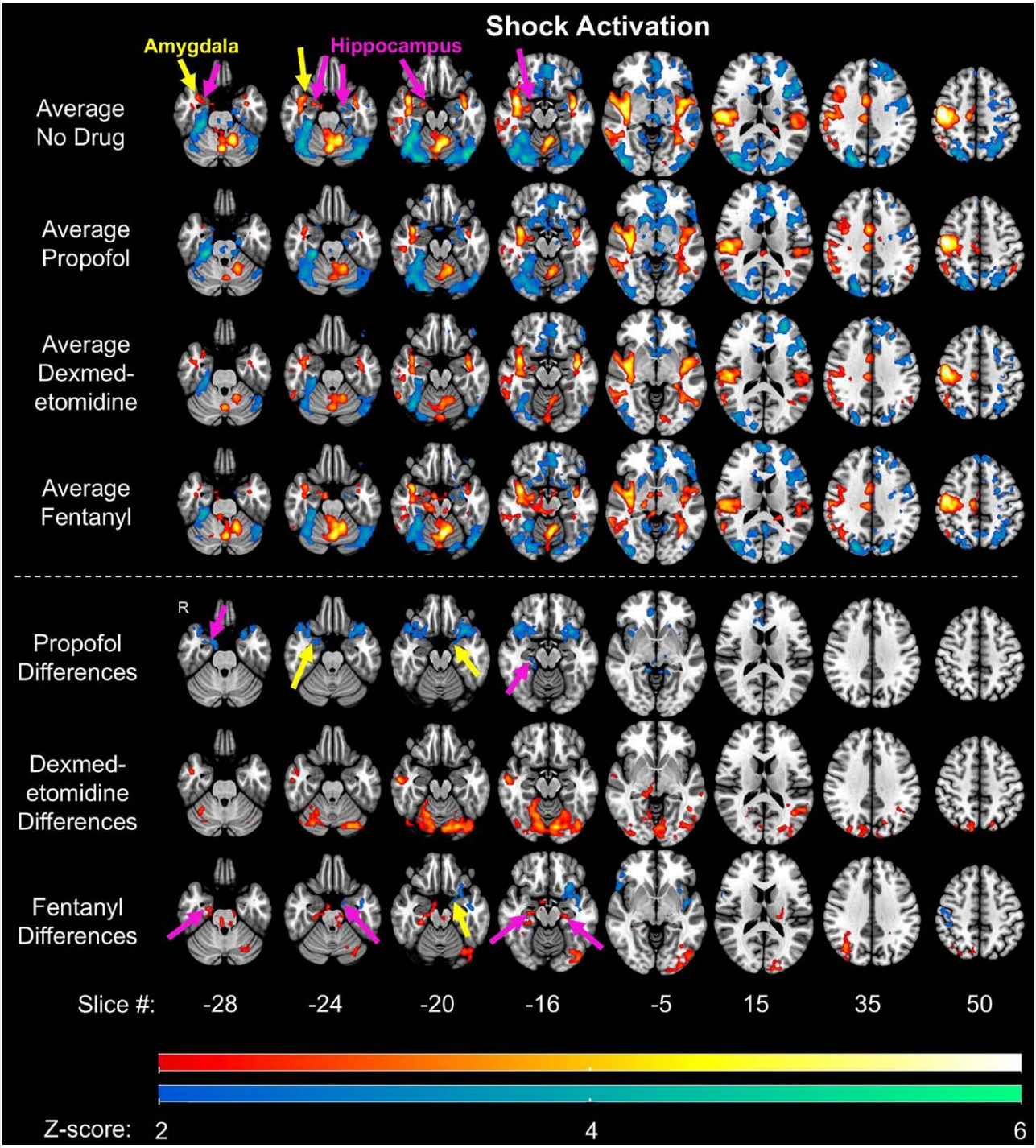

Fig. 5. All-subject average functional magnetic resonance imaging (fMRI) activation in the Memory and Conditioning scan, showing responses to painful electric nerve shock for selected axial slices, with Monteal Neurologic Institute slice numbers shown. The *color bar* indicates Z-score, according to the scale shown. The *top rows* show brain areas with statistically significant positive (*warm colors*) or negative (*cool colors*) correlation to shocks under the drug conditions. The *bottom three rows* display areas of statistically significant differences under each drug condition (the no-drug *vs.* drug contrast). *Warm colors* indicate drug greater than no drug and *cool colors* indicate no drug greater than drug differences. The amygdala is indicated with *yellow arrows*; the hippocampus (and/or parahippocampus) is indicated with *purple arrows*.

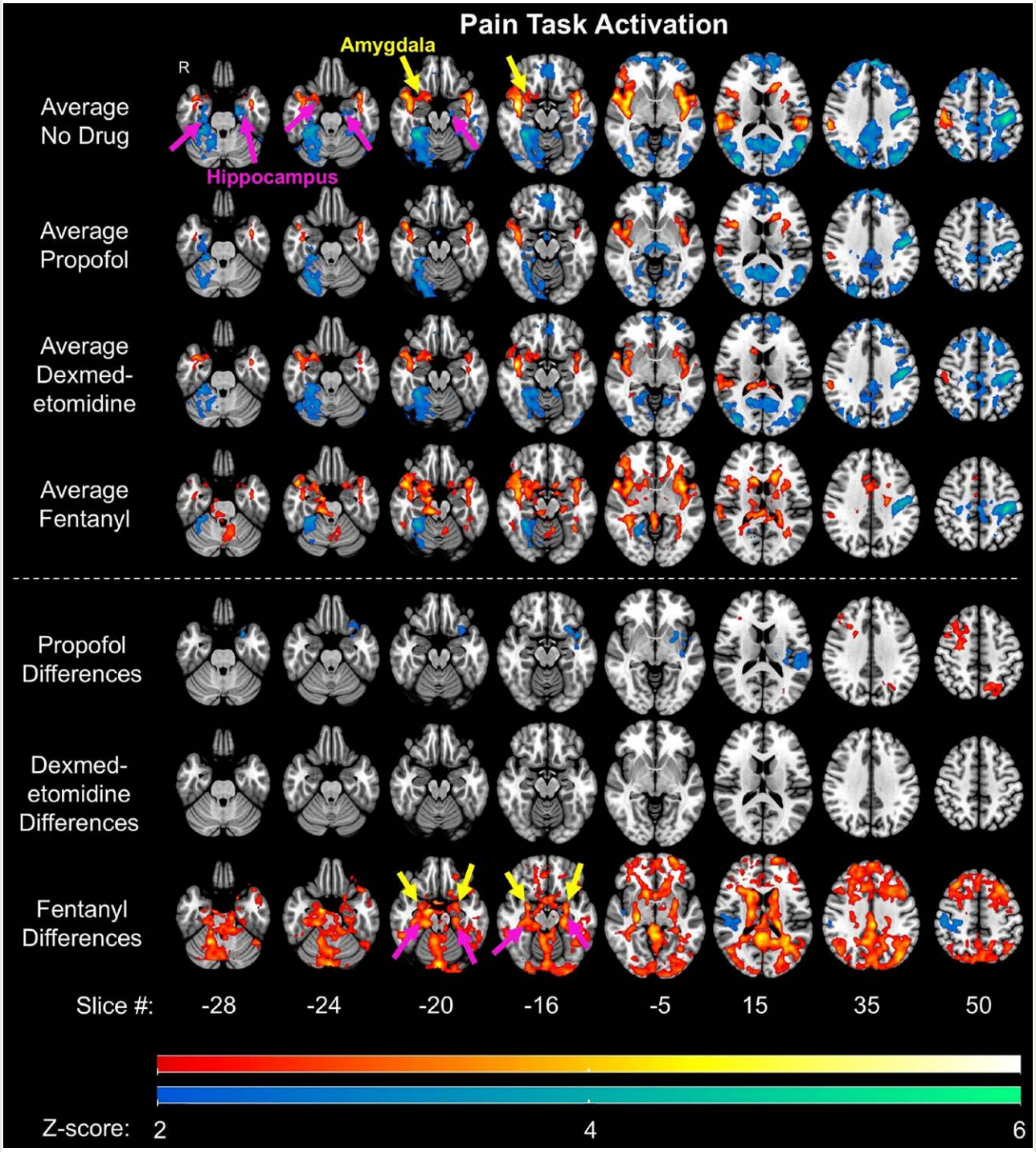

Fig. 6. Group average functional magnetic resonance imaging (fMRI) activation for block-design pain task scan for selected axial slices, with Monteal Neurologic Institute slice numbers shown. *Color bars* indicate Z-score, according to the scale shown. The *top rows* show brain areas with statistically significant positive (*warm colors*) or negative (*cool colors*) correlation to the task under the drug conditions. The *bottom three rows* display areas of statistically significant differences under each drug condition (the no-drug *vs.* drug contrast). *Warm colors* indicate drug greater than no drug and *cool colors* indicate no drug greater than drug differences. The amygdala is indicated with *yellow arrows*; the hippocampus (and/or parahippocampus) is indicated with *purple arrows*.

dexmedetomidine alone does not change subjective ratings of pain, including similar and higher doses,[47] and work showing that perception of electrical pain under moderate sedation is unchanged.[45]

On the cognitive task battery done in the scanner, only dexmedetomidine impaired performance on the three-back task (fig. 2). N-back tasks engage working memory[48] and depend on focused attention. Additionally, memory performance for images seen more than 1 min before (exceeding working memory temporal limits) was also decreased under dexmedetomidine and propofol. In this framework, both encoding and retrieval were done with potentially sedative effects of the drug making attention-demanding tasks more difficult. This may explain why next-day memory performance was not similarly reduced with dexmedetomidine, suggesting that encoding long-term memory is not as impacted as with propofol.

Consistent with its known amnestic effect,[8,13] propofol reduced next-day memory performance (fig. 3), specifically for recollection, as familiarity was at chance performance in all drug conditions (Supplemental Digital Content 5, https://links.lww.com/ALN/D958). It seems participants had an all-or-nothing recollection response for the mental picture or story they generated. This interpretation is suggested by the relatively low Remember hit rates (0.35 $\pm$ 0.21) even under the no-drug condition, low and consistent false-alarm rates across drug conditions, and no specific memory for pain-pairing. This result sits in contrast to midazolam, which interferes with binding of experimental items to contextual details,[49] and has more impact on recollection, with preserved familiarity.[1] Unfortunately, participants were at floor performance for familiarity, making it impossible to directly assess propofol's specific effects on the recollection *versus* familiarity dimensions of memory, which would be better determined with a different memory task.

A previous fMRI study[9] of memory used propofol at an effect-site concentration of 0.9 mcg/ml (Schnider model[50]). When contrasting negative-arousing *versus* neutral images, the investigators found robust activation of the posterior hippocampus bilaterally along with (right greater than left) amygdala activation.[9] Interestingly, the amygdala remained responsive to the aversive images under propofol, while hippocampal activation was not observed. The authors posed a provocative possibility, that "amygdala-dependent fear systems may remain intact."[9] For a direct comparison to figure 3 in the previous work,[9] we show our Shock no-drug *versus* propofol difference maps in Supplemental Digital Content 13 (https://links.lww.com/ALN/D966) with the same coronal slices. We found propofol-associated decreases in both amygdala and anterior hippocampal activation. There are several key differences between the current and previous studies, including using painful stimulation rather than aversive images. We have a larger sample size, within-subject crossover design, and more sensitive fMRI technique. With all these advantages, our results do *not* suggest that propofol facilitates amygdala responses. Although fear responses under anesthesia in the setting of traumatic experiences are incompletely characterized,[51] our findings add reassurance that light sedation with propofol is unlikely to accentuate fear memory under clinically relevant conditions.

Both fentanyl and dexmedetomidine were associated with increased activation in the hippocampus, despite no memory performance differences. One interpretation is that increased neuronal activity in the hippocampus is needed to successfully overcome the sedative effects of these drugs. One interesting and unexpected finding is the differences in lateralization of hippocampus modulation with these two agents; increases were on the right with dexmedetomidine and on the left with fentanyl. This differs from a previous study that found right lateralized hippocampus increases with the same dose of dexmedetomidine.[22] However, the previous study showed only left lateralized increases in activation for subsequently remembered pictures[22] (analogous to fig. 4). Previous work has shown encoding of pictures tends to activate the posterior right hippocampus, with the anterior left hippocampus more engaged in encoding words[52] (although a gradient likely exists[53]). Our task of generating a visual story while hearing spoken words likely included overlapping features of both categories of psychologic tasks, and possibly some elements of memory retrieval (if participants drew upon previous memories in creating their mental picture). Thus, the laterality differences we found may represent drug-specific anatomical localization or an interaction between drug effects on specific cognitive effects predominantly mediated by distinct subdivisions of the hippocampus.

Brain response to acute painful electrical stimulation can be assessed in two distinct contexts within our data. The Memory and Conditioning scan had 30 widely spaced 2-s stimulations (the Shock contrast) that always overlapped the imagery task encouraging memory encoding. In the Pain task, five 10-s-long simulations occurred consecutively with approximately 20-s breaks. Despite these differences, we found similar activation for these tasks at baseline (top rows of figs. 5 and 6). Dexmedetomidine's effects on the brain responses to acute pain in humans is largely unknown, and we found no pain-related differences in activation to either task. Previous work using laser stimulation under propofol showed that thalamic and cortical responses to pain were maintained, even with dosing to loss of responsiveness.[54] Interestingly, propofol-associated changes differed between our two painful tasks; brief shocks showed decreased amygdala and hippocampus activation, while 30 s long stimulations in the Pain task had decreased left insula and secondary somatosensory cortex activation. This suggests context-dependent differences in propofol's effect on pain processing, which could depend on either competing cognitive function (mental imagery task coincident with brief shocks) or, less likely, the duration of the painful stimulation. A similar pattern for changes was seen with fentanyl, with more pronounced decreases in right

primary somatosensory cortex and right insula activation in the Pain task compared to the Shock contrast. Fentanyl was also associated with increased activation in bilateral amygdala and hippocampus during the Pain task. This could indicate that fentanyl has an effect in these memory/associative brain areas when experiencing pain without an accompanying cognitive task as an attentional distraction.

Our brief Shock task and block-design Pain task both show activation of a portion of the right amygdala. Amygdala laterality has historically favored left-sided activation for emotional stimuli (most commonly aversive pictures) in humans, with right laterality most commonly seen in fear responses in animals, but modality and context of experimental items may influence amygdala response laterality in human studies,[55,56] including the amygdala's role in pain processing.[57] However, a more recent appraisal questions amygdala laterality.[58] Decreases in amygdala activation under propofol were bilateral for brief shocks, and drug-based amygdala increases were seen bilaterally under fentanyl during Pain blocks. Given the paucity of real-time human neuroimaging focused on drug modulation of the amygdala during acute experimental painful stimulation, our results add to the growing body of knowledge surrounding systems-level neuroanatomical targets for anesthetic and analgesics.

With recent evidence that sex hormones may affect anesthetic sensitivity, specifically including cognitive function in humans,[59] we sought to characterize differences between male and female subjects in our study. For successful memory encoding, male participants showed greater activation compared to women in the left amygdala. Men also showed greater activation from shocks in the left hippocampus. These lateralized differences could align with previous data showing differences in laterality of activation in the hippocampus between sexes[60] and changes in laterality of both the hippocampus and amygdala throughout the menstrual cycle, suggesting hormonal influence.[61] For both memory and shock contrasts, there were minimal sex-related differences under any drug in regions of the medial temporal lobe memory systems. This indicates that drug-associated modulation of memory does not show differences based on biological sex in our experiment. However, as dividing our data into sex-based cohorts decreases power by half, these findings (especially the absence of differences) should be further characterized by future experiments.

In conclusion, our study describes the effects on memory encoding and pain perception during light sedation with propofol, dexmedetomidine, and fentanyl. Propofol impaired recollection memory, with corresponding decreases in activation of the hippocampus and amygdala during encoding. Propofol also decreased pain intensity ratings and was associated with statistically significant decreases in activation in the insula and anterior cingulate, as well as the hippocampus and amygdala, during painful stimulation. We found no differences for dexmedetomidine in long-term memory performance, despite decreased activity in the hippocampus during encoding. Dexmedetomidine was not associated with differences in pain ratings or drug-based activation differences in any key pain-processing areas. Fentanyl showed decreased activity in the hippocampus and amygdala, despite no difference in memory performance. During painful stimulation, fentanyl showed decreased activity in the primary somatosensory cortex and insula, while paradoxically showing increased activation in the anterior cingulate, hippocampus, and amygdala. Taken together, these findings show differential action of distinct anesthetics on important targets in the brain, demonstrating some neuroanatomical underpinnings of observed differences in behavior. This adds to our growing body of knowledge about how anesthetics work under experimental conditions that model some important aspects of clinical anesthesia practice. Future research should examine the dose dependence of these effects, as we expect differences with deeper levels of sedation.

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Competing Interests

Dr. Pryor receives unrelated research funding from the Patient-Centered Outcomes Research Institute

(Washington, D.C.). Dr. Shafer is a consultant for and has an equity interest in Concentric Analgesics (San Francisco, California). Dr. Ibrahim is a consultant for DxTx medical (Pittsburgh, Pennsylvania). The other authors declare no competing interests.

Reproducible Science

Full protocol available at: keith.vogt@pitt.edu. Raw data available at: keith.vogt@pitt.edu.

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Supplemental Digital Content

1. Consolidated Standards of Reporting Trials diagram, https://links.lww.com/ALN/D954
2. Drug bolus and infusion dosing summary, https://links.lww.com/ALN/D955
3. Intervals between sessions, by drug group, https://links.lww.com/ALN/D956
4. Next-day memory performance by pain-pairing, https://links.lww.com/ALN/D957
5. Detailed next-day memory testing results, https://links.lww.com/ALN/D958
6. Violin plots of numerical behavioral data, https://links.lww.com/ALN/D959
7. Mixed model analysis of numerical data, https://links.lww.com/ALN/D960
8. fMRI activation maps without labeling arrows, https://links.lww.com/ALN/D961
9. fMRI results table for successful memory, https://links.lww.com/ALN/D962
10. fMRI results table for shock events, https://links.lww.com/ALN/D963
11. Sex differences in task activation, https://links.lww.com/ALN/D964
12. fMRI results table for Pain task, https://links.lww.com/ALN/D965
13. Coronal slice fMRI activation for shock events, https://links.lww.com/ALN/D966

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