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## Midazolam and ketamine produce distinct neural changes in memory, pain, and fear networks during pain

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### Abstract

**Introduction:** 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. We hypothesized that both drugs would decrease recollection memory,

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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, we used behavioral measures and functional magnetic resonance imaging to study these two anesthetics, at sedative doses, in an experimental memory paradigm employing 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 pre-selected anatomical seed regions. All measures were determined under saline and steady-state concentrations of the drugs.

**Results:** —Recollection memory was reduced under midazolam ( $d' = 0.73$  [0.43, 1.02], median [95% confidence interval]), compared to saline ( $d' = 1.78$  [1.61, 1.96]) and ketamine ( $d' = 1.55$  [1.12, 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 to 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 to 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.

## Keywords

midazolam; ketamine; pain; memory; functional MRI; functional connectivity

## Introduction:

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 anesthetic agents differentially affect pain perception<sup>1–3</sup> and memory encoding at varying levels of sedation.<sup>4–6</sup> 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. While 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 psychological 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 underly 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.<sup>7</sup> Additionally, low frequency (< 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.<sup>8</sup> Functional connectivity is a measure of the coherence in these fluctuations over time, reflecting neuronal communication.<sup>9</sup> 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 to ketamine. Neuroimaging measures of brain activity and connectivity were evaluated as secondary outcomes. We predicted that, compared to saline, less 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.

## 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](https://clinicaltrials.gov/ct2/show/study/NCT02515890), NCT02515890.

### Participants

Healthy volunteer participants between the ages of 18 and 39 were recruited from the community and compensated up to \$200 for participation. Demographic information from all 26 participants is tabulated in Table 1. All 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 pre-anesthetic evaluation by a study anesthesiologist, urine pregnancy test for females, and MRI-compatibility screening confirmed no other contraindications to safe sedation inside a high-field magnet. In addition to following

American Society of Anesthesiologists guidelines for fasting,<sup>10</sup> participants abstained from tobacco and caffeine for 8 hours prior to the MRI sessions.

### Painful Stimulation and Monitoring

An electric nerve stimulator (EzStim II; Life Tech, Stafford, TX) 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 7/10 pain. The numerical rating scale for pain had anchors at 0 being no pain and 10 being the worst imaginable. Two 1-second test shocks were delivered once in the scanner, and a pain rating obtained, as listed in Table 2. The stimulator was re-adjusted 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 Figure 2).

### Infusions

Intravenous access was obtained in each participant's right hand with a 22-gauge catheter. Once positioned in the MRI scanner and connected to standard ASA monitors, a saline carrier infusion was run at 75 ml/hr 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 (<http://opentci.org/code/stanpump>). Brain effect site concentrations of 10 ng/ml for midazolam and 200 ng/ml for ketamine were targeted, using pharmacokinetic models<sup>11,12</sup> 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, Sharpsburg, PA). 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).<sup>13</sup> Ninety words were used in each segment, 30 of these were immediately followed by a 1-second electric shock. No more than two pain-paired words nor five non-pain words occurred consecutively. Word order was randomized between repetition blocks, though pain-pairing was kept consistent. To increase statistical power for detecting task events in the imaging data, 0–6 second periods of jitter were included between items.<sup>14</sup>

### 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 (version 1.0.153, <https://www.rstudio.com/>) running R version 3.2.5. The median absolute deviation was calculated,<sup>15</sup> and response time values more 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.

### Memory Testing

Explicit memory testing occurred the following day, 20–32 hours after the scanning session. Recognition testing used the Remember-Know-New scheme<sup>16</sup> (subject instructions were published previously<sup>13</sup>). Recollection (of specific details) was indicated by a Remember response, while 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,<sup>17</sup> memory performance was summarized using the signal detection metric,  $d'$ ,<sup>18</sup> which is calculated from the difference in Z-scores between cumulative Gaussian distributions:  $z(\text{hits}) - z(\text{false alarms})$ ; 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 Prisma 3 T scanner using a 32-channel head coil. Functional images were obtained using a gradient-echo echo-planar imaging sequence, with parameters: echo time = 30 ms, repetition time = 1 s, flip angle = 45 degrees, bandwidth 2004 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 inhomogeneities, 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, New York, NY). 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 to 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<sup>19,20</sup> suggesting that 24 subjects are adequate for task-based functional magnetic resonance imaging studies.

In addition to the eight subjects that 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 (SPM12 v7219, <http://www.fil.ion.ucl.ac.uk/spm/>) using a Windows 10 computer running MATLAB 2018b (Mathworks, Natick, MA). 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. Prior to further analysis, the CompCor algorithm<sup>21</sup> 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 CSF 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, as 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  $P < 0.001$ , 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<sup>22</sup> version 18b (<https://www.nitrc.org/projects/conn/>). As part of data pre-processing, ART-based outlier detection was employed. Data was band-pass filtered (0.008 – 0.09 Hz); linear detrending was applied. A seed-to-voxel analysis was employed, using 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, 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 timecourse. This allows background connectivity to be assessed with minimal contamination from task events.<sup>23,24</sup> Group-level connectivity contrasts for saline > midazolam and saline > ketamine were calculated and thresholded within Conn toolbox, correcting the overall significance for a cluster false-discovery rate of  $p < 0.05$ .<sup>25</sup> Complete lists of all clusters showing statistically significant connectivity change with drug are available (see Tables, Supplemental Digital Content 1, for saline > midazolam and Supplemental Digital Content 2, for saline > 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<sup>26</sup>). 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 temporal 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 0–10 numerical rating scale,  $P < 0.001$ ), but not midazolam ( $P = 0.194$ ). Data noted as missing in Table 2 also reflects that 8 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 (Figure 3). Items with no recorded response were infrequent (449/23220), at  $< 2\%$  of all trials (see Table, Supplemental Digital Content 3, 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, for saline; Supplemental Digital Content 5, for midazolam; Supplemental Digital Content 6, for ketamine). False Alarm responses were also tabulated (see Table, Supplemental Digital Content 7). Figure 4 displays the results for recollection and familiarity from next-day recognition testing, using the summary statistic  $d'$ . Consistent with previous results<sup>13</sup> demonstrating that painful stimulations may affect memory for non-pain items within the same experimental block, no differences between pain-paired vs non-pain items were detected, ( $P = 0.813$ , Figure 4, Panel A). Collapsing across pain-pairing (Figure 4, Panel B), recollection under saline was  $d' = 1.78$  (median [1.61, 1.96] (95% confidence interval). Recollection performance under midazolam ( $d' = 0.73$  [0.43, 1.02]) was significantly reduced compared to saline ( $P < 0.0001$ ) and ketamine ( $d' = 1.55$  [1.12, 1.97],  $P < 0.001$ ), representing a meaningful performance difference of more than 0.75 standard deviation units. Comparisons of recollection between saline ( $d' = 1.78$  [1.61, 1.96]) and ketamine ( $d' = 1.55$  [1.12, 1.97]) were not considered different ( $P = 0.081$ ). Aggregate performance for familiarity, indicated by correct Know responses, did not significantly differ between saline ( $d' = 0.36$  [0.18, 0.53]), midazolam ( $d' = 0.31$  [0.03, 0.59]), and ketamine ( $d' = 0.31$  [0.02, 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.<sup>27</sup>

### fMRI task-related activation

A whole-brain annotated group average fMRI 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) and items specifically paired with painful stimulation (see Figure, Supplemental Digital Content 9). 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 and 9) 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 Table in Supplemental Digital Content 1). The direction of change was predominantly increased in this data (20/28 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. Though 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 in Table 4 (complete list of clusters and coordinates of local maxima in Table, Supplemental Digital Content 2). 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, activation in the hippocampus, parahippocampus, and temporal gyrus, in combination with the lateral prefrontal cortex and posterior parietal cortex, represent the core memory network.<sup>28,29</sup> Activation in the amygdala and anterior cingulate demonstrate engagement of core elements in fear response.<sup>30–33</sup> These function in a larger network involved in classical conditioning that include the hippocampus,<sup>34</sup> parahippocampus,<sup>35</sup> and connections to the ventromedial prefrontal cortex.<sup>34</sup> The somatosensory network, which most prominently features the thalamus and primary and secondary somatosensory cortices, is commonly activated in pain processing.<sup>36</sup> Activity seen in the insula represents a structure with specificity for pain processing,<sup>36</sup> though the insula is also active in fear conditioning.<sup>30,33</sup> Additional activation in the posterior cingulate and precuneus (core components in the default-mode network<sup>37–39</sup>) 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 Figure 5 for familiarity and Figure, Supplemental Digital Content 8, 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). A relatively larger magnitude of signal change following 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 following non-painful 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.<sup>13</sup>

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 non-definitive 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,<sup>40</sup> but previous qualitative results show reduced hippocampal activity under propofol in a similar paradigm.<sup>17</sup> Previous task-based functional studies under ketamine have shown reduced activation related to memory<sup>41</sup> and pain<sup>42</sup> 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,<sup>26</sup> 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 to 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) which showed reduced connectivity from the posterior cingulate to other areas.<sup>43</sup> In contrast, our findings of predominantly increased connectivity with midazolam diverges from some previous resting-state studies. A recent study, which showed reduced connectivity across many predefined brain networks, used a comparable dose (2 mg) of midazolam to our study. However, this was done in a smaller number of much older subjects, ranging from 55–73 years.<sup>44</sup> Our midazolam connectivity findings align best with a previous study<sup>45</sup> that used a similar dose administered as a bolus 5 minutes before a 7-minute long resting-state scan. Though 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).<sup>45</sup> In the same study, decreased connectivity under midazolam was found for other networks investigated.<sup>45</sup> 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.<sup>46</sup> Further, increases in connectivity between functionally distinct brain regions have been demonstrated with sedative doses of other anesthetics that similarly act as gamma-aminobutyric acid receptor agonists.<sup>47–49</sup>

Under ketamine, connectivity changes between brain regions within our networks of interest were predominantly decreased, compared to saline. Notable between-systems decreases in connectivity under ketamine included 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 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<sup>50</sup> 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<sup>51</sup> can be seen, as can relative increases in default-mode network connectivity at higher doses.<sup>52</sup> It should be noted, that variability across studies in choosing how to group anatomically disparate seed and target ROIs for connectivity analyses makes comparison to prior work not straightforward. It is our hope that granular reporting of connectivity changes for individual anatomically described ROIs 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. Though 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 this 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 (see Table 2) and amnesic response from midazolam (compare Tables in Supplemental Digital Content 4 and 5). 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). This concern is ameliorated, however, by the similar numbers of saline and drug experimental items analyzed in the familiarity (Figure 5) and pain-paired (see Figure, Supplemental Digital Content 9) 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 un-sedated non-MRI cohort, that our pain stimulation paradigm causes robust skin responses with shock delivery,<sup>53</sup> 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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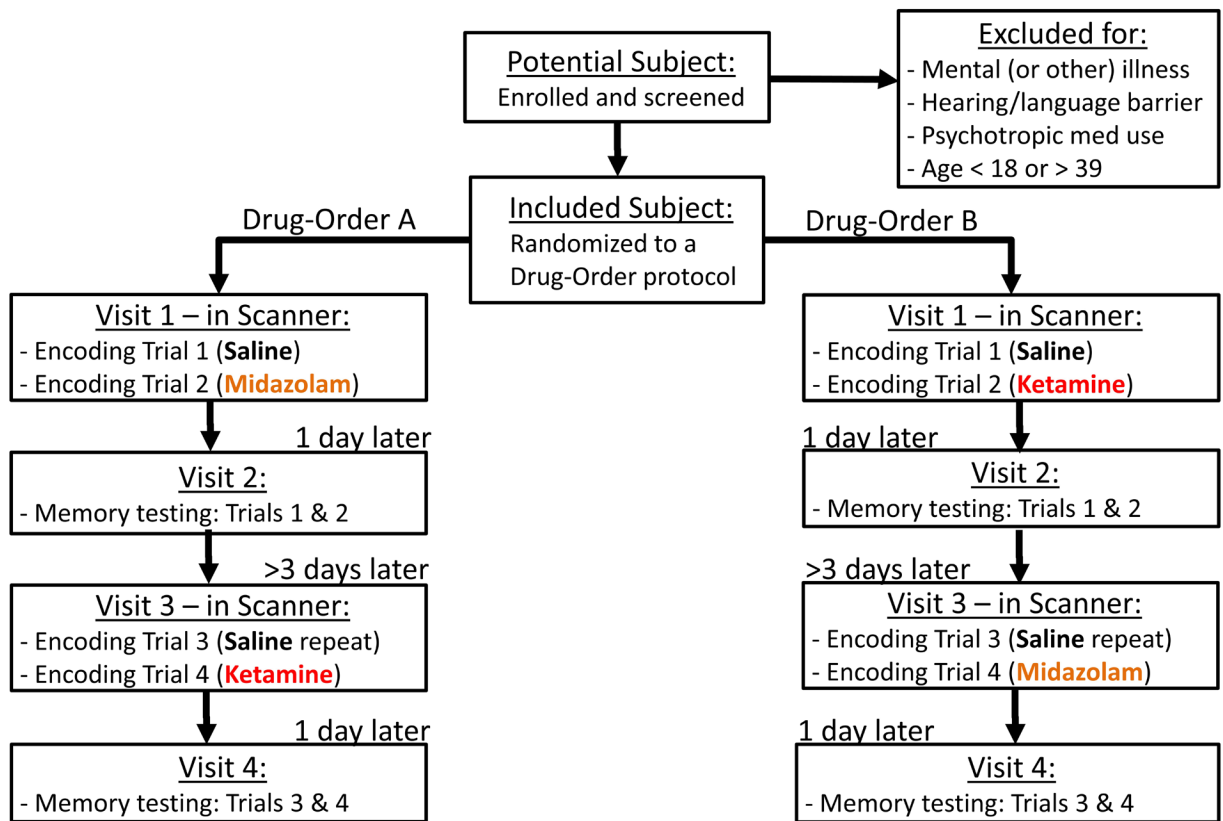
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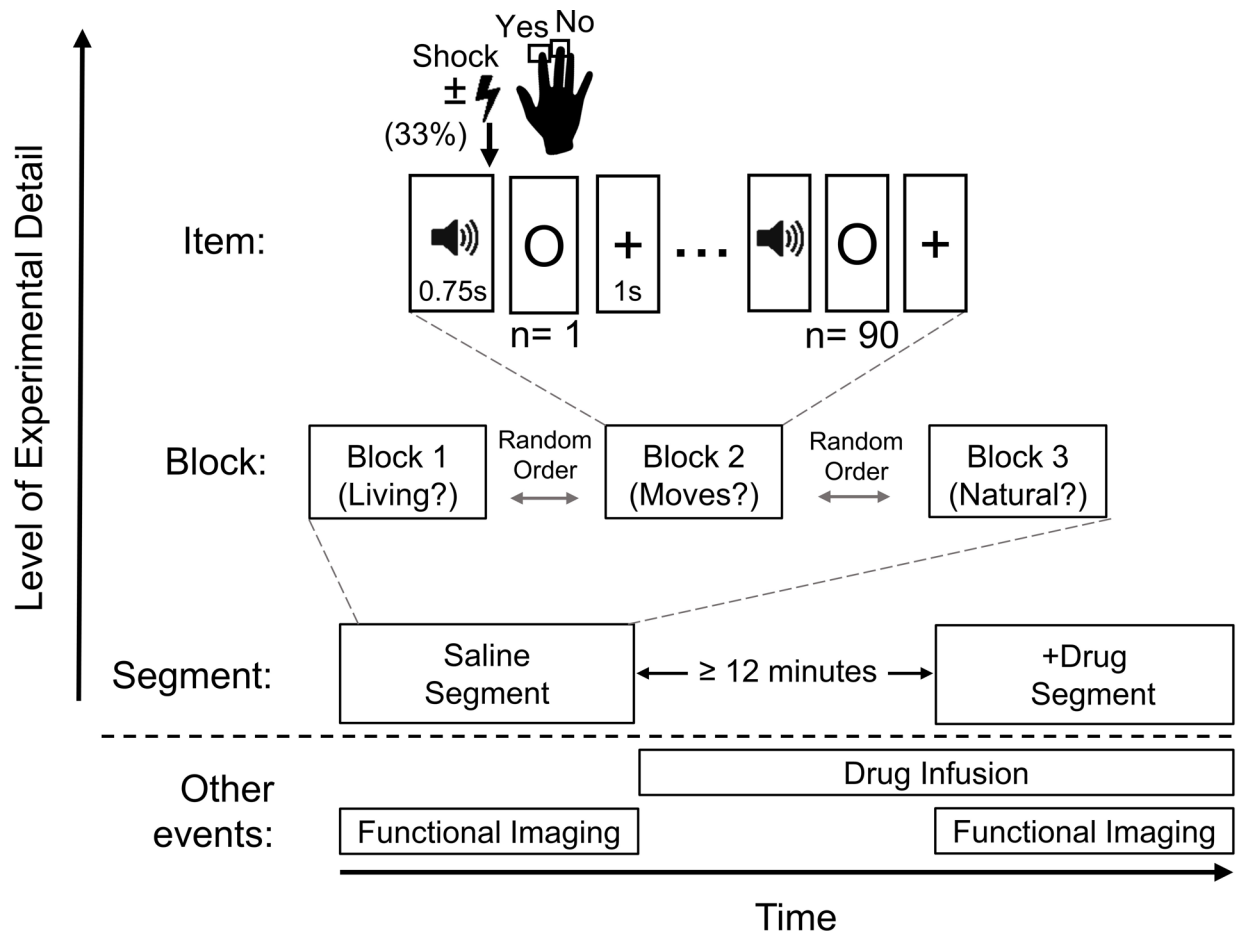
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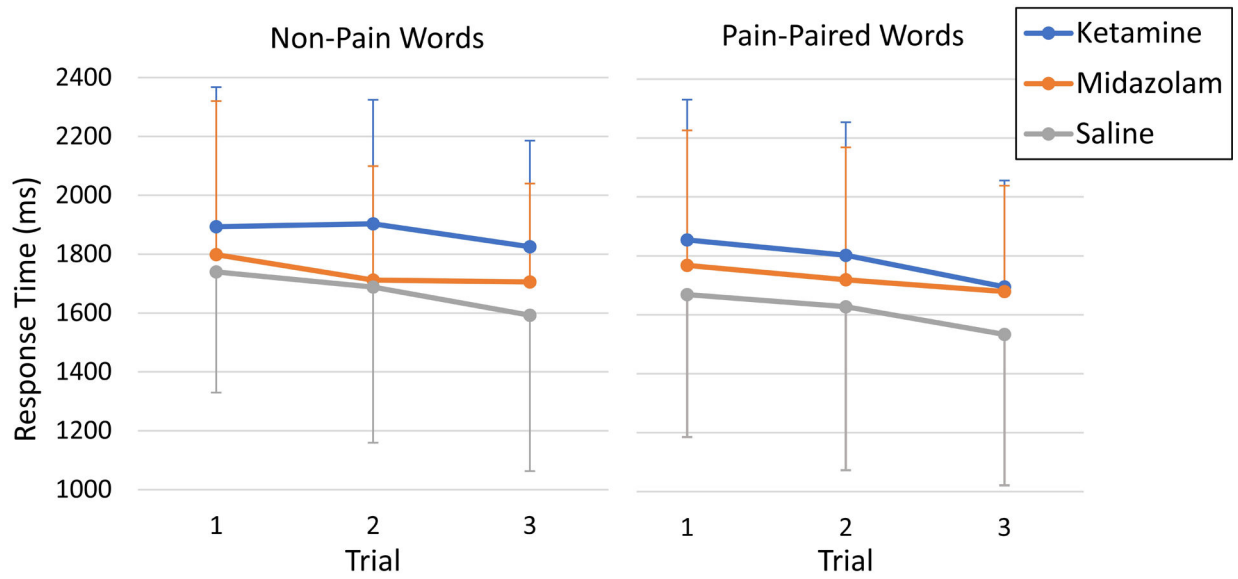


**Figure 1:**  
Study flowchart showing participant flow through the four experimental visits.

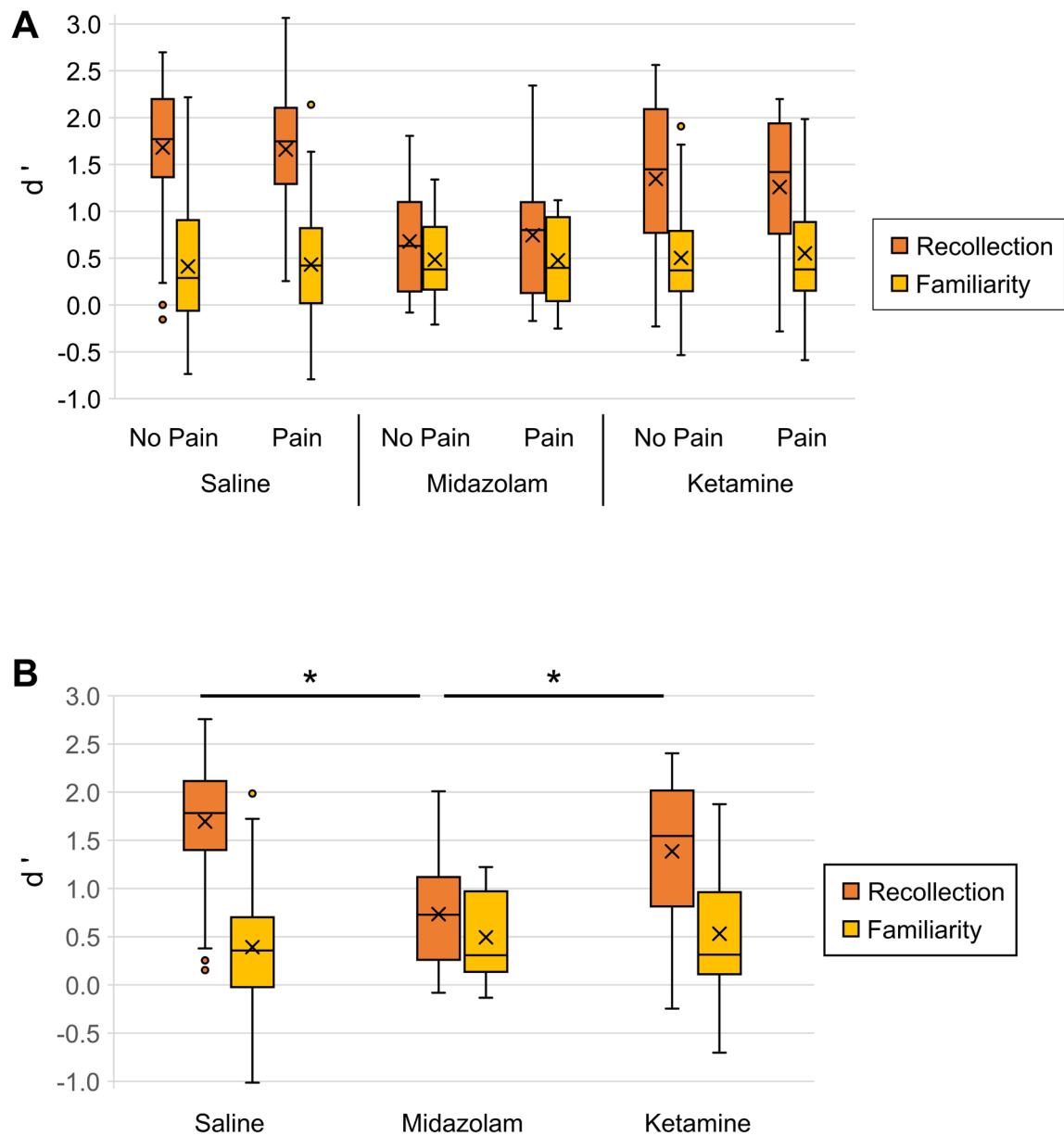


**Figure 2:**

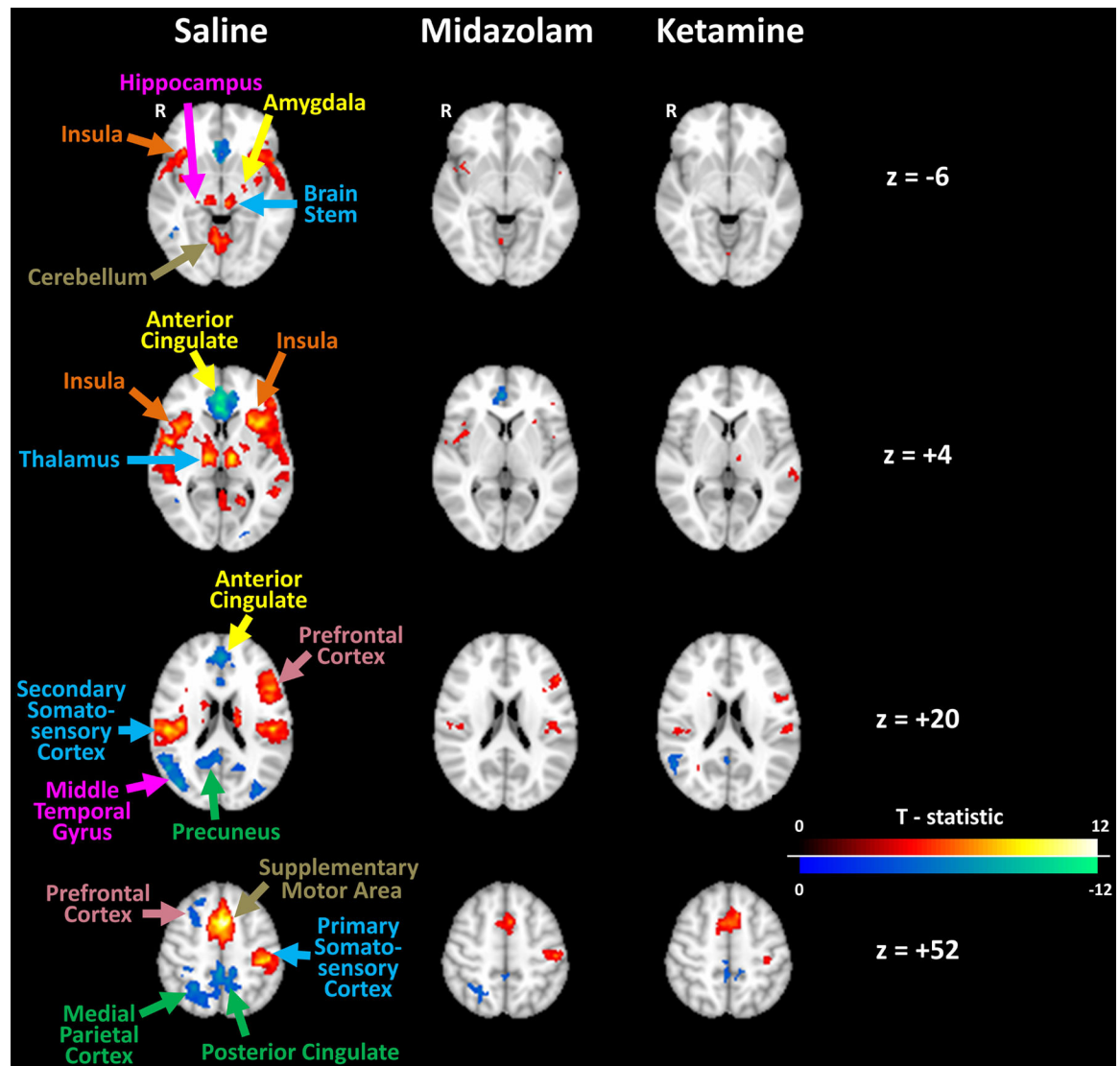
Graphical representation of detailed timeline for experimental procedures performed in the scanner (during visits 1 and 3).



**Figure 3:**  
Response times for each experimental block during encoding segments of experiment. Note:  
Error bars reflect standard deviation.

**Figure 4:**

Overall memory performance shown as box & 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 1.5x the interquartile range from the 1st and 3rd quartile values (limits of the box). Panel A shows performance for items separated by pain pairing. As there was no significant effect of pain on memory performance, Panel B shows performance collapsed across pain pairing, with significant differences indicated with an asterisk (\*).



**Figure 5:**

Group average fMRI 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 standard space brain was used as the underlay, with axial slices locations listed. 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)

**Table 1:**

Subject demographic data and total drug doses received

| Subject | Age (years) | 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     |

“-” indicates participant did not participate in experimental session

**Table 2:**

Nerve stimulator intensities and pain scores for each subject, by session

| Ketamine Session |                   |                      |                     |                     |                     |                  |                   |                      |                     |                     |                     |     |     |
|------------------|-------------------|----------------------|---------------------|---------------------|---------------------|------------------|-------------------|----------------------|---------------------|---------------------|---------------------|-----|-----|
| Subject          | Midazolam Session |                      |                     |                     |                     | Ketamine Session |                   |                      |                     |                     |                     |     |     |
|                  | Pain Scores       |                      |                     |                     |                     | Intensity (mA)   | Pain Scores       |                      |                     |                     |                     |     |     |
|                  | After Test Shocks | After Block 3 Saline | After Block 1 +Drug | After Block 2 +Drug | After Block 3 +Drug |                  | 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                   | 7   | 7   |
| 2                | 16                | 5                    | 6.5                 | 7                   | 7                   | 19               | 7                 | 7                    | 7                   | 5.5                 | 5.5                 | 5.5 | 5.5 |
| 3                | 13                | 5                    | 5                   | 7                   | 7                   | 11               | 7                 | 7                    | 5.5                 | 2.5                 | 0                   | 1.5 | 1.5 |
| 4                | 13                | 6                    | 7                   | 6                   | 5.5                 | 11               | 6.5               | 6                    | 6                   | 4                   | 3.5                 | 3.5 | 3.5 |
| 5                | 13                | 7                    | 8                   | 7.5                 | 7.5                 | 13               | 7                 | 7                    | 7                   | 5.5                 | 6                   | 7.5 | 7.5 |
| 6                | -                 | -                    | -                   | -                   | -                   | 9                | 6.5               | 5.5                  | 5.5                 | 5.5                 | 5.5                 | 5.5 | 5.5 |
| 7                | 22                | 5.5                  | 7                   | 7                   | 7                   | 23               | 7                 | 7                    | 7                   | 6                   | 6                   | 6.5 | 6.5 |
| 8                | 10                | 6                    | 6                   | 5                   | 4                   | -                | -                 | -                    | -                   | -                   | -                   | -   | -   |
| 9                | 13                | 7                    | 7                   | 7                   | 7                   | 11               | 6                 | 7                    | 7                   | 7                   | 7                   | 7   | 7   |
| 10               | 25                | 6                    | 7                   | x                   | 7                   | 21               | 5.5               | 6                    | 6                   | 6                   | 6                   | 5.5 | 5.5 |
| 11               | 14                | 7                    | 7                   | 6.5                 | 7                   | 10               | 7                 | 7                    | 7                   | 5                   | 5.5                 | 6   | 6   |
| 12               | 14                | 6                    | 7                   | 7                   | 6                   | -                | -                 | -                    | -                   | -                   | -                   | -   | -   |
| 13               | 25                | 7                    | 7                   | 7                   | 7                   | 20               | 7                 | 7                    | 7                   | 3                   | 1                   | 0   | 0   |
| 14               | 14                | 7                    | 7                   | 7                   | 6                   | 42               | 7                 | 7                    | 7                   | 5                   | 5                   | 5   | 5   |
| 15               | 11                | 7.5                  | 7.5                 | 7.5                 | 7.5                 | 11               | 7                 | 7                    | 7                   | 2                   | 2                   | 2   | 2   |
| 16               | 11                | 7                    | 7                   | 5.5                 | 6                   | 17               | 5.5               | 6                    | 6                   | 5.5                 | 4.5                 | 4   | 4   |
| 17               | 23                | 7                    | 7                   | 7                   | 7                   | 24               | 7                 | 7                    | 7                   | x                   | 5                   | 4.5 | 4.5 |
| 18               | 23                | 7                    | 7                   | 7                   | 6                   | 18               | 7                 | 7                    | 7                   | 3                   | x                   | 3   | 3   |
| 19               | -                 | -                    | -                   | -                   | -                   | 19               | 7                 | 7                    | 7                   | 7                   | 7                   | 7   | 7   |
| 20               | 24                | 7                    | 7                   | 6                   | 7                   | -                | -                 | -                    | -                   | -                   | -                   | -   | -   |
| 21               | -                 | -                    | -                   | -                   | -                   | 18               | 7                 | 7                    | 7                   | 7                   | 7                   | 7   | 7   |
| 22               | 12                | 7                    | 7                   | 7                   | 7                   | -                | -                 | -                    | -                   | -                   | -                   | -   | -   |
| 23               | 21                | 7                    | 7                   | 7                   | 7                   | 16               | 7                 | 6                    | 6                   | 4                   | 4                   | 3.5 | 3.5 |
| 24               | 18                | 7                    | 5                   | 5                   | 4                   | 16               | 7                 | 5.5                  | 5.5                 | 4                   | 4                   | 3   | 3   |
| 25               | 22                | 7                    | 7                   | 7                   | 7                   | 22               | 7                 | 7                    | 7                   | 6                   | 7                   | 7   | 7   |

| Subject | Midazolam Session |                   |                      |                     |                     |                     | Ketamine Session |                   |                      |                     |                     |                     |
|---------|-------------------|-------------------|----------------------|---------------------|---------------------|---------------------|------------------|-------------------|----------------------|---------------------|---------------------|---------------------|
|         | Pain Scores       |                   |                      |                     |                     |                     | Pain Scores      |                   |                      |                     |                     |                     |
|         | 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 |
| 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                 |

“.” indicates subject did not participate in experimental session, “x” indicates missing data

**Table 3:**

Saline versus midazolam functional connectivity contrast, results summary

| Seed Region     |                     | Target Identified      |                                                   | Connectivity Change |
|-----------------|---------------------|------------------------|---------------------------------------------------|---------------------|
| Network (Role)  | Specific Area       | Network (Role)         | Specific Area                                     |                     |
| 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           |

Notes: Increases in the Connectivity Change column indicates a greater connectivity value seen under midazolam, compared to saline. Anatomic location labels were systematically determined using mutual information from three atlases (described in 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      |                                                    | Connectivity Change |
|--------------------|------------------------------|------------------------|----------------------------------------------------|---------------------|
| Network (Role)     | Specific Area                | Network (Role)         | Specific Area                                      |                     |
| 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    | Para-hippocampus             | Motor                  | Motor cortex                                       | Decreased           |
| Memory encoding    | Para-hippocampus             | 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           |

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