Unconscious auditory priming during surgery with propofol and nitrous oxide anaesthesia: a replication

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Background. Priming during anaesthesia has been hard to replicate and the conditions under which it occurs remain poorly understood. We replicated and extended a recent study to determine whether intraoperative priming during propofol and nitrous oxide anaesthesia is a reliable phenomenon, whether it occurs due to awareness during word presentation and whether it is suppressed by a dose of fentanyl at induction.

Methods. Words were played through headphones during surgery to 62 patients receiving propofol and nitrous oxide anaesthesia. Thirty-two patients received fentanyl 1.5 μg kg⁻¹ at induction and 30 received no fentanyl. Neuromuscular blocking drugs were not used. Depth of anaesthesia was measured using the bispectral index (BIS). Anaesthetic variables were recorded at 1 min intervals during word presentation. On recovery, implicit and explicit memory were assessed using an auditory word-stem completion test and a yes–no word-recognition test, respectively.

Results. BIS, blood pressure, end-tidal carbon dioxide and heart rate during word presentation did not differ between the study groups. The infusion rate of propofol and the patients’ ventilatory frequency were significantly higher in the group not receiving fentanyl. No patient had unprompted explicit recall of surgery, although there was above-zero performance in six patients on the yes–no recognition task (P<0.05). There was no physiological evidence of awareness during anaesthesia (median mean-BIS=38 in the no-fentanyl group and 42 in the fentanyl group). There was evidence for priming (mean priming score=0.09, P<0.05 in the no-fentanyl study group; mean priming score=0.07, P<0.05 in the fentanyl group) even when patients with momentary light anaesthesia (maximum recorded BIS>60) and/or positive recognition scores were excluded from the analysis.

Conclusions. Existing knowledge can be primed by information presented during propofol and nitrous oxide anaesthesia. This priming is evidence of unconscious information processing and not the result of moments of awareness.

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Many studies have shown that auditory information presented to patients during anaesthesia can activate or prime existing knowledge and enhance performance on postoperative memory tests.¹,² This priming, often referred to as ‘learning’ in the anaesthetic literature, may have consequences for patients’ well-being on recovery, particularly if emotional memories or anxieties about surgery are primed.³ In a typical study, words are played to patients during anaesthesia and they are then tested for explicit and implicit memory, or priming, on recovery. Explicit memory is (by definition) conscious recollection of the learning episode and suggests awareness during anaesthesia. Implicit memory facilitates task performance in the absence of conscious awareness of the memory. Implicit memory may arise from awareness at the time of learning which, owing to the amnesic effects of anaesthetics or general forgetting, is not available to conscious recollection on recovery, or it may arise from information processing in the absence of awareness during anaesthesia. Evidence for truly unconscious priming during anaesthesia would suggest
that implicit memory for intraoperative events might be a general feature of surgery rather than something that could be avoided simply by better monitoring and control of anaesthetic depth.

Lubke and colleagues\textsuperscript{4} tested trauma patients anaesthetized with isoflurane and found that, although learning is more likely at lighter anaesthetic depths when awareness is more likely, it can also occur at deeper and clinically adequate depths of anaesthesia, defined as bispectral index (BIS) between 40 and 60. We recently demonstrated priming during day surgery with propofol anaesthesia, but not during equally deep anaesthesia prior to surgery.\textsuperscript{5} The median BIS during word presentation in the during-surgery group was 41.9, supporting the conclusion of Lubke and colleagues\textsuperscript{4} that priming occurs during deep anaesthesia. However, these findings do not necessarily mean that priming was unconscious. A moment of awareness immediately after word presentation might facilitate priming by that word, even if the word itself was presented during adequate anaesthesia. This problem is particularly pertinent for the study by Lubke and colleagues because their patients were undergoing trauma surgery during which relatively large fluctuations in anaesthetic depth were unavoidable. It is exacerbated by a lag of up to 1 min in BIS recordings.\textsuperscript{6} We tackle this problem in the present study by assessing priming as a function of the maximum BIS recorded at any point during word presentation. If patients show priming even though BIS never exceeds 60, this would be stronger evidence than we have at present for unconscious memory activation during anaesthesia.

We also tackle another problem of research into learning during anaesthesia. The common failure to replicate significant results, even by the same research groups using the same stimuli, has been noted and casts doubt on the reliability of evidence for priming during anaesthesia.\textsuperscript{17}\textsuperscript{8} It is especially important to determine the reliability of priming occurring in the surgical period because this is when it could potentially do most harm. Thus in the present study we sought to replicate our previous demonstration of learning during surgery,\textsuperscript{5} using the same anaesthetic regimen, the same patient population and the same experimental stimuli and memory tests.

We extended our previous study by testing the effect on intraoperative priming of fentanyl, commonly used to supplement the hypnotic agent at induction of anaesthesia. We know of no evidence that fentanyl impairs implicit memory formation and it has almost no effect on explicit recall in sedated volunteers.\textsuperscript{9} We have previously proposed that surgical stress facilitates priming during anaesthesia.\textsuperscript{5} It is conceivable that fentanyl may reduce priming in patients who are being surgically stimulated because it suppresses the stress response.\textsuperscript{10} In the present study we tested the effect on intraoperative priming of a dose of fentanyl at induction. All patients were played words during surgery, but only half received fentanyl at induction as their conditions were similar to those of the during-surgery group in the previous study, and predicted more priming in the patients without fentanyl because they would have an unsuppressed stress response to surgery.

**Methods**

Approval for the study was obtained from North Sheffield Medical Ethics Committee. Adults (ASA I) undergoing orthopaedic day surgery and who spoke English as their first language were considered for inclusion in the study. Exclusion criteria were any known visual or hearing impairments, language difficulties, neurological disorders, contraindications to the proposed anaesthetic or use of medication known to affect the central nervous system.

**Construction of experimental stimuli and memory tests**

The stimuli and memory tests were identical to those used in our previous study.\textsuperscript{5} We used word-stem completion to test implicit memory and yes–no recognition to test explicit memory. Most memory tests can be performed using a combination of implicit and explicit memory. However, our piloting of these tests with an undergraduate sample, as reported in detail in the previous study, indicated that these tests offered reasonably pure and sensitive measures of the type of memory they were designed to test.

Twenty-eight five-letter relatively common English words were recorded onto a Macintosh Powerbook (1400cs/133, Apple Computer Inc., California) at a sample rate of 44.1 Hz and 16-bit sample size using a microphone and SoundEdit software (16 Version 2, Macromedia, USA). In a copy of these word files, SoundEdit was used to remove the tail of each word, leaving word stems typically three phonemes long. Each word stem was unique in that, although it was possible to complete it by words not used in this study, it could be completed by only one of the 28 words used here as targets and distractors. The mean spontaneous (unstudied) word-stem completion rate was 0.32 in our pilot study with 48 undergraduate subjects.\textsuperscript{5}

The 28 words were assigned to four lists, as shown in the Appendix. Two of these lists were presented to each patient during surgery, with the words from the two lists combined and presented in random order. One of the lists provided targets for the subsequent recognition test. The stems corresponding to the words in the other list were the target items for the word-stem completion test. The two remaining lists provided distractor stimuli, one list per memory test. Each word list appeared equally often as target and distractor stimuli on both the implicit and explicit tests. The words in the study lists and the stimuli on the test lists were presented in a different random order to each patient.

**Anaesthetic technique and experimental procedures**

The anaesthetic technique differed from that of the previous study only in that one group received no fentanyl.
at induction and that intraoperative analgesia was given after
word presentation if deemed clinically necessary. None of
the patients were premedicated. Patients were assigned ran-
domly to a fentanyl or no-fentanyl study group to which the
experimenter (CD) was blinded. Patients in the fentanyl
group received fentanyl 1.5 μg kg⁻¹ followed by a
‘sleep’ dose of propofol. Patients in the no-fentanyl group
received only propofol for induction of anaesthesia. Anaes-
thetia was maintained in all patients with a target-controlled
infusion at a rate between 3 and 9 mg kg⁻¹ h⁻¹ depending on
the anaesthetist’s clinical judgement. Patients breathed
nitrous oxide 66% and oxygen 33% spontaneously through
a laryngeal mask.

BIS monitoring commenced in the operating theatre, prior
to surgery, using an Aspect-1000 monitor (software version
2.51, Aspect Medical Systems, Framingham, MA, USA)
with bifrontal montage (F7, F8, reference Fp2). The anaes-
thetist was not blinded to BIS, but the anaesthetic was not
BIS guided.

For all patients, word presentation began at first surgical
incision and ended before the completion of surgery. In each
case, 14 words were presented by experimenter CD in
random order using a Macintosh PowerBook (1400cs/133,
Apple Computer Inc., California) and closed headphones
(KOSS TD/80). Each word was repeated consecutively 15
times over a period of 1 min, including a 5 s period of silence
at the end of each word series; thus the total presentation
time was 14 min. Twenty-eight BIS readings were recorded,
one at the beginning and one at the end of each word series.
Anaesthetic variables (heart rate, mean blood pressure, end-
tidal carbon dioxide concentration, ventilatory frequency
and infusion of propofol) were recorded at 1 min intervals
during word presentation.

Intraoperative analgesia following word presentation and
postoperative analgesia were given as clinically appropriate.

Memory testing

When patients were able to sit up in bed following recovery
from anaesthesia and were agreeable to completing the tests,
they were asked a series of open-ended questions as used by
Russell and Wang¹¹ to probe for any explicit recollection of
intraoperative events. Patients were asked: ‘What is the last
thing you remember before falling asleep’?, ‘What is the first
thing you remember about waking up’?, ‘Did you dream
while you were asleep’? and ‘Did you hear any words while
you were asleep’? Patients then completed the explicit and
implicit memory tests in the order determined by counter-
balancing. In yes–no recognition, patients were presented
with seven target words (presented during anaesthesia) and
seven distractor words (not presented during anaesthesia) in
random order and were asked to state after each whether or
not they recalled hearing that word while they were asleep.
The word-stem completion test consisted of word stems, in
random order, for seven target and seven distractor words.
Patients were played each stem and asked to complete it
‘with the first complete word which comes to mind’. In each
test, the number of distractor ‘hits’ (‘yes’ responses on the
recognition test or correct completions on the word-stem
completion test) was subtracted from the number of target
hits and expressed as a proportion of the total number of
items studied in the test (i.e. seven).

Statistical methods

Two BIS scores were analysed for each patient: the mean of
the 28 BIS scores recorded during word presentation (mean-
BIS), and the highest of those 28 scores, representing the
lightest anaesthetic depth reached during word presentation
(max-BIS). These scores were compared across groups using
the Mann–Whitney test for independent samples. The other
anaesthetic variables were compared across groups using
two-tailed t-tests for independent samples. One-sample t-
tests were used to test whether the memory scores in the
fentanyl and no-fentanyl study groups exceeded zero, and
to test whether memory scores exceeded zero in patients
with max-BIS<60. These tests were one-tailed because we
were attempting to replicate previous evidence for priming
during surgery⁵ and predicted that the priming effect would
be at least as large when fentanyl was omitted from the anaes-
thetic regimen. We report two-tailed confidence intervals for
further comparison. Pearson’s correlations were used to test
the relationship between memory scores and anaesthetic vari-
ables. Spearman’s rank correlations were used to test the
relationship between BIS (mean-BIS and max-BIS) and
memory scores on recovery. For these analyses, we report
p values corrected for ties. Statistical significance was
assessed with α=0.05 unless otherwise stated. All analyses
were performed using StatView 5.0 (SAS Institute Inc.,
Cary, NC).

Results

Data from 62 patients are included in the analysis, although
we obtained written informed consent from 64. One patient
had the scheduled surgery cancelled following brief exam-
ination by the surgeon under anaesthetic. Another patient
required drugs not included in the study protocol, owing to
complications, and was also withdrawn. This gave 32
patients in the fentanyl group and 30 in the no-fentanyl
study group. Mean patient age, weight, duration of surgery
and time to test are shown in Table 1 and were similar
between study groups. Values are similar to those in our
previous study,⁵ except that time to test was slightly longer
(mean 82 min compared with 69 min in the previous study).

Anaesthetic variables are shown in Table 2. Only the
ventilatory frequency and mean infusion rate of propofol
differed between the groups. There were no significant dif-
fferences in anaesthetic depth (median mean-BIS or max-
BIS). The values were very similar to those obtained for
the during-surgery group in our previous study. For compa-
rison with previous research¹², we calculated the percentage
of BIS readings that were >60 for each patient. The mean of
these percentages was 11.9 (SD 24.4).
Seventeen patients in the fentanyl group and 18 patients in the no-fentanyl group received morphine 3–5 mg intraoperatively after word presentation. Five patients in the fentanyl group and 11 patients in the no-fentanyl group received morphine 4–10 mg postoperatively.

Preliminary analyses showed no effect of test order (word-stem completion followed by yes–no recognition, or vice versa) on task performance. No patient revealed spontaneous or prompted recall on the structured interview for intraoperative events. Performance on the yes–no recognition task ($P < 0.05$) was above chance for the sample as a whole, as shown in Table 3. When split by group, this was clearly non-significant in the fentanyl study group ($P=0.18$) and just beyond significance in the no-fentanyl study group ($P=0.051$). Examination of the unprocessed data revealed six patients overall with non-zero scores, five of whom also made two or more false alarms (i.e. ‘yes’ responses to distractor words).

Performance on the word-stem completion test exceeded zero for the sample as a whole (mean priming score=0.08, 95% CI 0.03–0.13, $P<0.002$) and for each group independently as shown in Table 4 ($P<0.05$ in each group). There was slightly more priming in the no-fentanyl group, but the difference between the groups was not statistically significant. For the sample as a whole, the priming effect size was 0.39. Priming remained above zero when data from the six patients with above-chance performance on the yes–no recognition test and the 25 patients with max-BIS $>$60 were excluded from the analysis (mean=0.08, $n=34$, 95% CI 0.01–0.17, effect size=0.40, $P<0.02$) as shown in Figure 1. Patients who received intraoperative morphine after word presentation had no less implicit memory than those who did not receive morphine.

As the fentanyl and no-fentanyl study groups did not differ reliably in terms of their demographic, anaesthetic or memory data, we conducted correlation analyses with the scores on each memory test from the whole sample to maximize power. Yes–no recognition performance did not correlate with time to testing ($r=0.21$), length of surgery

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**Table 1** Mean patient characteristics, surgery duration and time from end of surgery to memory testing (range or SD)

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>Operations</th>
<th>Sex ratio (M:F)</th>
<th>Surgery duration (min)</th>
<th>Time to test (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fentanyl ($n=30$)</td>
<td>42 (16–72)</td>
<td>76.2 (14.3)</td>
<td>Arthroscopy (16) Bunionectomy (1) Excision neuroma (7) Osteotomy (2) Pip fusion (3)</td>
<td>15:15</td>
<td>34 (12)</td>
</tr>
<tr>
<td>Fentanyl ($n=32$)</td>
<td>42 (16–72)</td>
<td>73.8 (18.3)</td>
<td>Tendon excision (1) Abscess excision (1) Arthroscopy (18) Bunionectomy (2) Excision neuroma (2) Osteotomy (6) Pip fusion (1) Tennis elbow release (1) Ulnar release (1)</td>
<td>17:15</td>
<td>41 (22)</td>
</tr>
</tbody>
</table>

**Table 2** Median mean-BIS (interquartile range) and median max-BIS (interquartile range), and mean (SD) pulse, blood pressure (BP), end-tidal carbon dioxide $E_{CO_2}$, ventilatory frequency and target controlled infusion (TCI). Mean-BIS is the mean of the 28 BIS values recorded during word presentation for each patient; max-BIS is the highest of the BIS values recorded during word presentation). $***P<0.001$.

<table>
<thead>
<tr>
<th>Mean-BIS</th>
<th>Max-BIS</th>
<th>Heart rate (beats min$^{-1}$)</th>
<th>BP (mm Hg)</th>
<th>$E_{CO_2}$ (kPa)</th>
<th>Ventilatory frequency*** (min$^{-1}$)</th>
<th>TCI*** (mg kg$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fentanyl</td>
<td>37.9 (16.6)</td>
<td>48 (28)</td>
<td>79 (12.5)</td>
<td>84.6 (10.7)</td>
<td>5.59 (1.54)</td>
<td>21.1 (6.7)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>41.8 (14.3)</td>
<td>53 (21)</td>
<td>75 (12.2)</td>
<td>82.3 (11.8)</td>
<td>8.48 (12.9)</td>
<td>15.2 (6.1)</td>
</tr>
</tbody>
</table>

**Table 3** Mean distractor, target and recognition scores as proportions (SD) on the yes–no recognition test for patients receiving stimulus words during surgery with propofol anaesthesia with or without fentanyl at induction

<table>
<thead>
<tr>
<th>Mean distractor score</th>
<th>Mean target score</th>
<th>Mean yes–no recognition score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fentanyl</td>
<td>0.06 (0.18)</td>
<td>0.09 (0.10)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.02 (0.08)</td>
<td>0.03 (0.13)</td>
</tr>
</tbody>
</table>

**Table 4** Mean distractor, target and priming scores as proportions (SD) on the word-stem completion test for patients receiving stimulus words during surgery with propofol anaesthesia with or without fentanyl at induction

<table>
<thead>
<tr>
<th>Mean distractor score</th>
<th>Mean target score</th>
<th>Mean word-stem completion score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fentanyl</td>
<td>0.21 (0.14)</td>
<td>0.30 (0.22)</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.24 (0.16)</td>
<td>0.31 (0.22)</td>
</tr>
</tbody>
</table>
In the study by Kerssens and colleagues, data were monitored propofol anaesthesia). The maximum BIS values in previous studies of intraoperative priming during BIS-anaesthesia because it suppresses the stress response to surgery that we hypothesized facilitates intraoperative priming. We had predicted that fentanyl would reduce priming during anaesthesia because it suppresses the stress response to surgery or explicit recall. This is consistent with the lack of correlation between implicit memory scores and BIS. Our patients did not receive neuromuscular blocking drugs and therefore were free to move if they became conscious. None did so.

No patient had spontaneous or convincing prompted recollection of intraoperative events evidenced by the ability to report specific details. However, one patient, who also performed above chance on the yes–no recognition task, did report a ‘dreamlike’ memory of hearing a voice. Performance on the yes–no recognition task slightly, but not significantly, exceeded chance in the no-fentanyl group.

Inspection of the data showed that just six patients responded ‘yes’ to one or more target words. Patients were not told how many of the words had been presented during anaesthesia, but were asked to say ‘yes’ to any words they remembered hearing during anaesthesia. Five of these patients also responded ‘yes’ to two or more distractor words (of a possible seven). No other patient made false alarms. This pattern of responding suggests a general bias towards making a ‘yes’ response in the five patients. This bias may have facilitated implicit memory influences, such as familiarity in the absence of explicit recollection, on yes–no recognition performance. Three of these patients also had above-zero scores on the implicit memory test. Thus the sensitivity of the yes–no recognition task may compromise its purity, allowing implicit memory to contaminate performance. Given the fact that our patients did not receive neuromuscular blocking drugs and that BIS recordings indicated deep anaesthesia, we suggest that this performance on the yes–no recognition task is spurious rather than an indication of awareness during surgery or explicit recall. This is consistent with the lack of correlation between our measure of awareness (BIS) and yes–no recognition scores.

The mean implicit memory score was slightly higher for the group who received no fentanyl than for the group who did receive fentanyl (mean 0.09 compared with 0.07), but the difference between groups was not statistically significant. We had predicted that fentanyl would reduce priming during anaesthesia because it suppresses the stress response to surgery that we hypothesized facilitates intraoperative priming. This study provided no evidence for an effect of fentanyl, although our dose was relatively small and the memory scores were in the predicted direction. From the present findings and those of our previous study, it would appear that any lingering effect on the surgical stress response of a propofol anaesthesia. Even when the anaesthetist aimed to keep depth within the BIS range 40–60, 20% of BIS readings were >60. When depth was not BIS-guided 26% were >60. In our study, only 12% of BIS readings were >60 even though BIS was not deliberately used to guide maintenance of a stable anaesthetic depth. Therefore we suggest that the anaesthetic used in our study was deep and relatively well controlled. Priming remained above chance even when patients whose max-BIS was >60 were excluded from the analysis. There was no suggestion that priming was occurring at lighter anaesthetic depths when awareness was more likely; thus there was no correlation between implicit memory scores and BIS. Our patients did not receive neuromuscular blocking drugs and therefore were free to move if they became conscious. None did so.

Discussion
The aim of this study was to replicate our previous finding of priming in patients receiving propofol and nitrous oxide anaesthesia and undergoing surgery during word presentation, and to provide a stronger test of the hypothesis that priming occurs during moments of light anaesthesia or awareness. We also tested whether using fentanyl at induction suppresses intraoperative priming. We played words at the onset of surgical incision to two groups of patients, one receiving propofol and fentanyl at induction, and the other receiving just propofol. Implicit memory was present in both groups. The priming effect size overall was 0.39, comparable to the effect size (0.40) observed in our previous study. Conditions for the group who received fentanyl in the present study exactly replicated those of the during-surgery group in the previous study. The mean implicit memory score was slightly higher for patients with no evidence of light anaesthesia (max-BIS<60 and a score of zero on the yes–no recognition test).

\((r=0.15)\), dose of propofol \((r=-0.12)\), mean-BIS \((r=-0.05)\) or max-BIS \((r=-0.09)\). Word-stem completion performance did not correlate with time to testing \((r=0.01)\), length of surgery \((r=-0.01)\), dose of propofol \((r=0.08)\), mean-BIS \((r=0.01)\) or max-BIS \((r=0.013)\).

\(\text{Fig 1} \quad \text{Histogram of implicit memory scores for patients with no evidence of light anaesthesia (max-BIS<60 and a score of zero on the yes–no recognition test).}\)
dose of fentanyl at induction is insufficient to prevent priming during surgery.

Previous studies of intraoperative priming during BIS-monitored propofol anaesthesia have provided mixed results. As already discussed, our own previous study \(^5\) using a word-stem completion test found evidence for intraoperative priming when mean BIS was 44. Struys and colleagues \(^12\) played patients the Robinson Crusoe story during surgery and found that three of 58 patients associated Robinson Crusoe with the cues ‘Friday’ or ‘desert island’ on recovery. Although they do not report a baseline associated with Friday in a control group of 15 patients who had not been played the story, so it is conceivable that the three positive responses represent implicit memory for the story. Kerssens and colleagues \(^13\) presented repetitions of the phrase ‘yellow banana green pear’ during surgery with BIS between 40 and 60, and measured implicit memory with a category generation task that required patients to name the first three exemplars of fruits and colours that came to mind. They found no difference in the hit rate (‘banana’, ‘pear’, ‘yellow’ or ‘green’ responses) between the experimental group and a control group that heard bird sounds during surgery. We suggest that previous failures to find priming during propofol anaesthesia may lie in the type of memory test used. Anaesthesia leaves low-level auditory processing intact, and perceptual implicit memory tests such as the word-stem completion task, which rely upon the same neural networks that subserve initial processing of stimuli, \(^15\) are more likely to detect priming during anaesthesia than conceptual tests. Conceptual tests such as category generation and word association tasks demand higher-level semantic processing of stimuli and priming of links between stimuli, probably in the association areas of the frontal and temporal lobes to which the flow of information is disrupted by anaesthesia. \(^16\)

To conclude, we replicated our finding of auditory priming in patients undergoing surgery with propofol and nitrous oxide anaesthesia. Priming exceeded chance even when patients who experienced moments of light anaesthesia (BIS=60) were excluded from the analysis. Thus memories can be primed even when patients are unconscious.

### Appendix

<table>
<thead>
<tr>
<th>Table A1 Word lists</th>
</tr>
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<tbody>
<tr>
<td><strong>A1</strong></td>
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<td>Reach</td>
</tr>
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<td>Glass</td>
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<tr>
<td>Merit</td>
</tr>
<tr>
<td>Flute</td>
</tr>
<tr>
<td>Store</td>
</tr>
<tr>
<td>Shape</td>
</tr>
<tr>
<td>Snail</td>
</tr>
</tbody>
</table>

### Acknowledgements

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