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Propofol modulates early memory consolidation in humans

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Abstract

Maintenance of memory across time is crucial for adaptive behavior. Current theories posit that the underlying consolidation process depends on stabilization of synapses and reorganization of interactions between hippocampus and neocortex. However, the temporal properties of hippocampal-neocortical network reconfiguration during consolidation are still a matter of debate. Translational research on this issue is challenged by the paucity of techniques to transiently interfere with memory in the healthy human brain. Here, we report a neuro-pharmacological approach with the $GABA_A$-ergic anesthetic propofol and a memory task sensitive to hippocampal dysfunction. Patients undergoing minor surgery learned word lists before injection of an anesthetic dose of propofol. Results show that administration of the drug shortly after learning (~13 min) impairs recall after awakening but spares recognition. By contrast, later administration (~105 min) has no effect. These findings suggest significant changes in memory networks very early after learning that are decisive for later recall. Propofol general anesthesia provides an experimental tool to modulate the first steps of hippocampus-mediated memory consolidation in humans.

48 **Significance statement**

49 Consolidation of memories depends both on mechanisms at the synaptic and the systems
50 level. How and when these mechanisms interact is currently unclear. Here, we have used the
51 anesthetic drug propofol to create a transient pharmacological 'lesion' of the neural
52 substrates of memory consolidation in humans undergoing minor surgery. Our results show
53 that there is a brief time window after learning where hippocampus-dependent memories
54 are susceptible to GABA-ergic modulation with propofol. Later recall appears to depend
55 significantly on integrity of these first steps of memory formation. We infer that there is
56 significant rearrangement of memory networks during the first hours after learning. Propofol
57 general anesthesia provides an experimental approach to interfere with early memory
58 consolidation in humans.

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Introduction

A defining feature of memory is the creation of cerebral representations that bridge temporal gaps between experience and behavior. It has been known since the 19th century that memory is not a static mental image of the past but rather a dynamic and re-constructive process that alters memory traces and involves distinct neural substrates as time proceeds (Ribot, 1882; Ebbinghaus, 1885). The mechanisms that stabilize memories have been termed memory consolidation (Müller and Pilzecker, 1900). A key clinical finding that has shaped the currently prevailing view on the neural substrates underlying consolidation is that some patients with lesions affecting the hippocampus show a temporally graded amnesia with relative sparing of remote memories, i.e. memories that were acquired months to years before hippocampal damage (Scoville and Milner, 1957). The "standard model" posits that consolidation involves a re-distribution of memories between hippocampus and neocortical networks with a decreasing role of the hippocampus with increasing memory delays (Alvarez and Squire, 1994; McClelland et al., 1995; Squire et al., 2004). However, the time scales addressed in patient studies of memory consolidation are not easy to reconcile with results from more recent imaging studies, showing that interactions between hippocampus and neocortex during the seconds and minutes that follow memory encoding are predictive of later recall (Tambini et al., 2010; Ben-Yakov and Dudai, 2011). To account for the wide range of memory delays, it has been suggested that consolidation should be seen as a family of processes on multiple time scales that transform, stabilize and update memory traces according to contextual demands (Dudai et al., 2015). Drawing largely from results from experimental studies in animals, it has been proposed that processes on a synaptic level at a time scale of up to some hours may provide iterative

92 subroutines for consolidation on a systems level at much longer time scales (Dudai et al.,
93 2015; Kukushkin and Carew, 2017; Asok et al., 2019).

94 It has proven difficult to provide complimentary experimental data for humans. There are
95 virtually no studies that link clinical investigations in humans with hippocampal dysfunction
96 and synaptic accounts of memory consolidation. An ideal patient model for the investigation
97 of memory consolidation would consist of a transient brain lesion that acts selectively on a
98 distinct phase of memory consolidation. However, most brain lesions are permanent and
99 thus simultaneously affect encoding, consolidation and retrieval. Moreover, the
100 hippocampus and surrounding structures are not accessible for current transcranial brain
101 stimulation techniques. Modulation of long-term potentiation (LTP) by direct
102 microstimulation of the human entorhinal cortex during memory tasks is a promising tool in
103 this respect but limited to patients undergoing evaluation for epilepsy surgery (Titiz et al.,
104 2017).

105 Here, we have taken a new neuro-pharmacological approach on human memory
106 consolidation. We tested whether general anesthesia with the anesthetic propofol (2,6-
107 diisopropylphenol) interferes with memory consolidation when applied shortly after learning
108 and whether these effects are time-dependent. Propofol is a short-acting anesthetic drug
109 that is broadly used for sedation during invasive diagnostic and surgical procedures and for
110 sedation in intensive care units (Sahinovic et al., 2018; Walsh, 2018). Propofol is both an
111 agonist on $GABA_A$ receptors and a partial antagonist on NMDA receptors. Studies in rat
112 hippocampal slices suggest that these properties account for reduction of LTP and affect
113 synaptic consolidation (Wei et al., 2002; Nagashima et al., 2005). Systemic administration of
114 propofol immediately after learning of a location in a water maze has moreover been shown
115 to affect consolidation of spatial memory in rats (Zhang et al., 2013).

Since ethical constraints limit experiments with anesthetic doses of propofol in healthy volunteers, we investigated patients undergoing minor ophthalmic surgery receiving propofol as a centrally acting drug during a short general anesthesia. Subjects performed a verbal learning and memory task that has previously proven to be sensitive to hippocampal dysfunction (Saury and Emanuelson, 2017). Verbal material was learned preoperatively at two different time points and tested postoperatively both for recall and recognition.

Materials and Methods

Participants. We included subjects between 18 and 60 years of age without any history of neuropsychiatric disorders, hearing disorders or substance abuse. Four groups with a total of 96 subjects were tested (4 x 24 age- and sex-matched subjects; 49 females; table 1). Two groups received general anesthesia with propofol for strabismus surgery at two different timepoints after learning ('early injection' and 'late injection', respectively; figure 1). In the 'early injection' group, we aimed to act on early steps of memory consolidation immediately following learning. We thus kept the delay between end of learning and injection of propofol as short as possible. In the 'late injection' group, it was aimed to act on a later phase of memory consolidation, i.e. clearly beyond the effects of propofol on maintenance of long-term potentiation (LTP) in rat hippocampal slices (Wei et al., 2002) and longer than the expected duration of surgery/anesthesia in the 'early injection' group (~60 min). We thus aimed at a delay of about 90 min between end of learning and injection of propofol. A third group consisted of healthy controls without any surgical procedure ('control' – no anesthesia; figure 1). A fourth group consisted of subjects undergoing local anesthesia for minor surgical procedures ('control – local anesthesia'; figure 1). This group was included to control for any pre-surgical arousal effects on our task (Pryor et al., 2010). All subjects spoke German fluently. Subjects undergoing surgery were recruited during preparatory visits in the outpatient departments of the Charité – Universitätsmedizin Berlin at least three days prior to surgery. Control subjects were recruited with advertisements via the intranet of the Charité-Universitätsmedizin Berlin. All procedures reported in this manuscript were approved by the ethics committee of the Charité – Universitätsmedizin Berlin. All subjects gave written informed consent before participation.

Behavioral Testing. Subjects were informed that they should perform a memory task prior to surgery and that they would receive a short additional testing after awakening from anesthesia. Subjects were not informed about the precise structure and purpose of the task and were not informed about the necessity to maintain to-be-remembered items across anesthesia. Subjects were tested with the "Verbaler Lern- and Merkfaehigkeitstest" (VLMT; Helmstaedter and Durwen, 1990), a German version of the widely used "Auditory Verbal Learning Test" (AVLT; Lezak, 1983). None of the subjects was familiar with the task. In the learning phase of this test, the examiner read a list of 15 semantically unrelated and emotionally neutral words (e.g. 'drum', 'coffee', 'river') to the subject at a rate of one word every two seconds. After each presentation, the subject was requested to recall as many words as possible and to report all recalled words orally to the examiner. This list was presented five times to the subject and was each time recalled. After the fifth recall, a distractor list with 15 other words was presented and recalled. Afterwards, the original word list had to be recalled again. Learning took about 15 to 20 minutes. Depending on the condition, subjects then received propofol general anesthesia, local anesthesia or were free to fill the delay until testing with intermediate activities. At testing, subjects were requested to recall the original word list and to report all recalled words orally to the examiner. Then, a recognition test was given. The examiner read a list which consisted of the 15 original words, the 15 words of the distractor list and 15 new words in pseudorandom order. For each word, the subject was requested to respond with "Yes" or "No" whether the word had been part of the original word list.

Procedure. In the 'early injection' group (table 1), subjects learned the word lists in a preparatory room adjacent to the operating theatre, while being in a supine position. The time between end of learning and induction of anesthesia (Mdn 13.0 min, IQR 10 - 17) was

filled with small talk with the examiner and explanatory remarks of the anesthesiologist. Then, subjects were pre-oxygenated with a face mask and received a bolus of propofol for induction of anesthesia (Mdn 200 mg, IQR 200 – 215) followed by a continuous infusion of propofol for maintenance of anesthesia (Mdn 6 mg/kg/h, IQR 6 – 6) and remifentanil for analgesia (Mdn 0.2 µg/kg/min, IQR 0.15 – 0.2). After loss of consciousness, the airway was managed with a laryngeal mask and subjects were mechanically ventilated. During anesthesia, subjects underwent surgery for ocular misalignment with recession, plication or resection of eye muscles according to established surgical standards (von Noorden and Campos, 2001). Anesthesia was continued for about one hour (Mdn 58 min, IQR 53 - 65). After surgery, patients remained in a supine position until the end of testing. Apart from occasional communication with nurses and physicians, the postsurgical period was free of any specific activities. Post-surgical pain was treated with Ibuprofen and Paracetamol. Testing for delayed recall and recognition was conducted about two hours after recovery from Anesthesia (Mdn 113.5 min, IQR 106.5 – 128) and about three hours after learning (Mdn 189.5 min, IQR 175.75 - 205).

In the 'late injection' group (table 1), subjects learned the word lists in a room on the ward, while being in a supine position. The time between end of learning and induction of anesthesia was filled with periods of rest, small talk with nurses and the examiner and explanatory remarks of the anesthesiologist (Mdn 105 min, IQR 95.25 - 115; $U = 0$, $p < 0.001$ difference with 'early injection). Subjects maintained a supine position during the entire delay between learning and anesthesia. Subjects underwent the same surgical procedure and received a comparable dose of propofol (bolus: Mdn 200 mg, IQR 155 – 237.5; maintenance: Mdn 6.0 mg/kg/h, IQR 6 – 6.75; $U = 272$, $p = 0.723$ and $U = 235$, $p = 0.108$ difference with 'early injection' group) and remifentanil as the 'early injection' group (Mdn

194 0.2 µg/kg/min, IQR 0.2 – 0.2; U = 218.5, p = 0.093 difference with 'early injection' group).
195 Duration of anesthesia and post-anesthesia recovery was like in the 'early injection' group
196 (Mdn 56 min, IQR 46.25 – 64.75; Mdn 113 min, IQR 108.5 – 116.75; U = 251, p = 0.445 and U
197 = 250, p = 0.433 difference with 'late injection' group). General post-surgical management
198 was like in the 'early injection' group.

199 In the 'control - local anesthesia' group (table 1), subjects learned the word lists in a
200 preparatory room adjacent to the operating theatre, while being in a supine position. The
201 minutes between end of learning and local anesthesia (< 10 minutes) were filled with small
202 talk with the examiner and explanatory remarks of the surgeon. Depending on the surgical
203 procedure, subjects then received local injections of lidocaine close to the region of surgery.
204 Memory was tested after a three-hour delay (Mdn 180 min, IQR 150 - 180). General post-
205 surgical management was like in the two propofol groups.

206 In the 'control – no anesthesia' group (table 1), subjects learned word lists in a seated
207 position in a room on the ward. After learning, subjects were free to walk in the hospital, but
208 were requested to return after about 170 min. Testing was performed about three hours
209 after end of learning (Mdn 180 min, IQR 180 - 180).

210 *Experimental Design and Statistical Analyses.* All data obtained in this study are openly
211 available at the Open Science Framework (osf) at https://osf.io/3x95n/. Data were analyzed
212 by using IBM SPSS, Version 25. Performance was described as percent correct responses in
213 each subject. For initial learning, we analyzed the number of correctly recalled items from
214 the original word list after presentation of the distractor list. For delayed recall, we analyzed
215 the number of correctly recalled items from the original word list after the delay. For
216 delayed recognition, we analyzed the number of correctly recognized items (hits) minus the
217 number of erroneously recognized items (false alarms), thus yielding a 'corrected

recognition' value for each subject (Helmstaedter & Durwen, 1990). In order to analyze possible subtle impairments in source memory, we further separately analyzed false alarms to items from the distractor list and false alarms to new items. Group averages are given as medians (Mdn) and interquartile ranges (IQR). Since accuracy in behavioral tests is rarely normally distributed and since Kolmogorov-Smirnov-testing showed that the assumption of a normal distribution had to be rejected ($p < 0.05$ for at least one subject group in learning, recall and recognition conditions), non-parametric statistical testing was used for statistical analysis (Altman, 1991; Altman and Bland, 2009). Kruskal-Wallis ANOVA was used for analysis of group differences and two-tailed Mann-Whitney tests were used for post-hoc comparisons between groups. Spearman rank order correlation was used for correlation analysis. Significance was accepted at a $p < 0.05$ level.

Results

After five repetitions of the original word list and presentation of the distractor list, all four groups showed similar retention of original word lists, with no significant differences between groups (Figure 2; X^2 (3) = 6.204, p = 0.102). This suggests that pre-surgical arousal did not significantly affect initial learning of verbal stimuli. However, after the memory delay, significant group differences were found for recall of word lists (Figure 2; X^2 (3) = 19.459, p < 0.001). Compared to 'control – no anesthesia' and 'control – local anesthesia' subjects, 'late injection' patients showed unimpaired performance with no significant differences in recall of word lists (Figure 2; 'late injection', Mdn 93.3 %, IQR 86.7 – 93.3; 'control – no anesthesia', Mdn 86.7 %, IQR 81.7 – 93.3; 'control – local anesthesia', Mdn 86.7 %, IQR 80.0 – 93.3; U = 261.0, p = 0.565 and U = 241.5, p = 0.332 respectively). Any hangover effects of general anesthesia or the surgical procedure on recall are thus unlikely. By contrast, 'early injection' patients showed a significant decrease in recall of word lists compared to 'control – no anesthesia' subjects and 'late injection' patients (Figure 2; 'early injection', Mdn 66.7 %, IQR 60 - 85; U = 123.0, p = 0.001 difference with 'control – no anesthesia'; U = 107.5, p < 0.001 difference with 'late injection'). Importantly, recall in 'early injection' patients was also significantly different from the 'control - local anesthesia' group (Figure 2; U = 129, p = 0.001 difference). This result and the almost identical performance in both control conditions (Figure 2; U = 274, p = 0.767 difference) suggest that pre-surgical arousal or some other direct reaction to the surgical procedure did not significantly affect initial consolidation of word lists.

Similar to previous observations of a differential susceptibility of delayed recall and recognition of word list learning to hippocampal dysfunction (e.g. Schoenberg et al., 2006;

Finke et al., 2017), corrected recognition of word lists did not differ significantly between groups, although a statistical trend might have been present (X^2 (3) = 7.363, p = 0.061). Comparison of corrected recognition scores shows that this trend was mainly driven by a slightly lower performance of the 'control – local anesthesia' group rather than by subtle performance deficits in the propofol groups (Figure 2; 'late injection', Mdn 93.3 %, IQR 86.7 – 100.0; 'early Injection', Mdn 90 %, IQR 81.7 - 93.3; 'control – no anesthesia', Mdn 93.3 %, IQR 86.7 – 93.3; 'control – local anesthesia', Mdn 86.7 %, IQR 80 – 93). Moreover, when hit rates and false alarms were analyzed separately, no significant differences were found between groups (hit rate, X^2 (3) = 4.733, p = 0.192; false alarms to items from the distractor list, X^2 (3) = 1.626, p = 0.653; false alarms to new items, X^2 (3) = 2.926, p = 0.403).

In order to analyze the selectivity of the recall – recognition dissociation in 'early injection' patients, we next compared difference between recall and corrected recognition in all four groups. ('Δ-R-R'). As expected, there was a significant difference of Δ-R-R between groups ('early injection', Mdn 20 %, IQR 6.67 – 33.33; 'late injection' Mdn 0 %, IQR -5.0 – 6.67; 'control – no anesthesia', Mdn 0 %, IQR -5.0 – 13.33; 'control – local anesthesia Mdn 0 %, IQR 0 – 0; X^2 (3) = 25.111, p < 0.001 difference between groups). Post hoc testing further showed that there was a significant difference of Δ-R-R between 'early injection' and all other three groups but not between the other three groups ('early injection' vs. all other groups, $U \leq 146$, $p \leq 0.003$; all other comparisons, $U \geq 221$, $p \geq 0.157$). This analysis shows that the difference between recall and corrected recognition is selective for the 'early injection group'.

Due to the clinical setting, subjects both in the 'early injection' and 'late injection' condition showed some variability in time between end of learning and injection of propofol (ranges: 'early injection', 6 – 21 min., 'late injection', 72 – 140 min.). To more precisely infer on a possible time window for propofol effects on word list consolidation, we tested whether recall performance showed a relationship with time to injection in both groups. However, we found no significant correlation between these variables when calculated separately for both groups ('early injection', $r = 0.286$; $p = 0.175$; 'late injection', $r = -0.007$; $p = 0.975$), thus suggesting that susceptibility of word list consolidation to propofol general anesthesia ends at some time point between 21 and 72 minutes following learning.

289 **Discussion**

290 The findings of our study show that propofol general anesthesia interferes with declarative
291 memory in a task that is commonly used to assess integrity of the human hippocampus. The
292 amnesic effect of propofol general anesthesia is critically time-dependent and appears to be
293 limited to a brief time window following learning. We infer that propofol general anesthesia
294 modulates presumably hippocampus-dependent initial steps of memory consolidation.

295 Since its approval at the end of the eighties of the last century, propofol has become a
296 dominant anesthetic agent for induction and maintenance of general anesthesia,
297 ambulatory surgical procedures and sedation in intensive care patients (Sahinovic et al.,
298 2018; Walsh, 2018). Propofol has a rapid onset and is quickly eliminated. With infusions of a
299 duration of one hour, the context-sensitive half-time of propofol is less than 10 minutes
300 (Hughes et al., 1992; Sahinovic et al., 2018). Clinically, this accounts for rapid recovery times
301 compared to other anesthetics (10 - 30 minutes). Apart from its clinical applications,
302 propofol has increasingly been used as a recreational drug (Xiong et al., 2018). Since soybean
303 oil is used as a solubilizer, propofol has a milk-like appearance and has thus been nicknamed
304 the 'milk of amnesia' (Walsh, 2018). Although this sobriquet implies some interference of
305 the drug with memory processes, there are surprisingly few experimental investigations of
306 propofol effects on the neural substrates underlying memory formation.

307 Long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission are
308 thought to represent key mechanisms underlying transformation of labile representations of
309 perceptual input into longer-lasting memories (Martin et al., 2000; Takeuchi et al., 2014).
310 Recordings of excitatory postsynaptic potentials (EPSPs) from the CA1 region of the rat
311 hippocampus have shown that an injection of propofol transiently (< 60 min) inhibits field
312 EPSPs in CA1 and affects maintenance of LTP, if given after LTP induction (Wei et al., 2002).

Further experiments on rat hippocampal slices showed that propofol can also inhibit induction of LTP and that this effect can be blocked by agents that block $GABA_A$-receptors, but not by agents that block NMDA-receptors (Nagashima et al., 2005). $GABA_A$-receptors are densely expressed in the hippocampus and the deep layers of the cortex where they are pivotal for learning and memory, with some isoforms being particularly important for memory formation (Engin et al., 2018). Pharmacological modulation of $GABA_A$-receptors has moreover been shown to affect memory consolidation-related sharp wave-ripple complexes in hippocampal networks. For example, at clinical concentrations, the anesthetic thiopental affects the incidence, rhythmicity and synchrony of sharp waves and the quantity of ripple oscillations in the CA1 region of hippocampal slices (Papatheodoropoulos et al., 2007). These effects appear to be mediated by distinct subunits of $GABA_A$-receptors. In particular, $\alpha5GABA_A$-receptors appear to reduce hippocampal excitability and may inhibit memory formation (Engin et al., 2018). Accordingly, stimulation of $\alpha5GABA_A$-receptors with therapeutic concentrations of diazepam has been shown to reduce the number, duration and power of ripple oscillations and to produces a partial temporal dissociation between ripples and sharp waves (Koniaris et al., 2011). Application of high concentrations of diazepam can also reduce the frequency of sharp waves (Viereckel et al., 2013). Computational modelling of the effects of various GABA-ergic drugs suggests that changes in power and duration of ripple oscillations reflects altered dynamics of interneuron networks in the CA1 region of the hippocampus (Donoso et al., 2018). Correspondingly, when propofol is systemically administered to rats immediately after learning of a location in a water maze, memory retention 24 hours following learning is impaired in a dose-dependent way (Zhang et al., 2013).

While these findings suggest that propofol should act on consolidation of human memory too, a transfer of these results on clinical settings has not been successful so far. It has been controversial whether it is possible to induce deficits in preoperatively learned material by subsequent administration of anesthetic agents (Veselis, 2018). Early experiments showed that sedative doses of propofol, i.e. doses that leave subjects able to communicate and breathe spontaneously, may affect memory of visual and verbal material, when stimuli are learned and tested during a continuous infusion of the drug - with effects being largely independent of the level of sedation (Veselis et al., 1997). Subsequent experiments with event-related potential recordings (ERPs) from subjects performing a continuous picture recognition task during propofol infusion showed a selective drug effect on pictures that were tested after 27 seconds, but not after six seconds (Veselis et al., 2009). ERP amplitudes during recognition decreased in parallel. More recently, functional magnetic resonance imaging (fMRI) during encoding of emotional pictures and continuous propofol infusion showed suppression of hippocampal responses that correlated with the degree of memory impairment for the stimuli (Pryor et al., 2015). While these studies make a strong point for modulation of memory-related neural activity in the human hippocampus by sedative doses of propofol, their focus was on revealing the mechanisms and the prevention of surgery-induced post-traumatic stress disorder (Pryor et al., 2015). Thus, it is difficult to disentangle the relative contributions of encoding, consolidation and retrieval to the antegrade amnesia induced in these experiments.

A critical prerequisite for studies of the time course of consolidation with anesthetic agents like propofol is the induction of retrograde memory effects, i.e. effects on material that is learned before infusion of the drug and tested after discontinuation. So far, there has been no convincing evidence for anesthetic-induced retrograde amnesia (Veselis, 2018). A

previous study on patients with depression however successfully used electroconvulsive therapy (ECT) in deep anesthesia as an intervention to study reconsolidation of emotionally negative stories learned one week before treatment (Kroes et al., 2014). Recall of these stories was impaired, when memory of the story was cued immediately prior to ECT and tested 24 hours afterwards. By applying the same behavioral paradigm to patients receiving sedation for endoscopy, a recent study showed that propofol at sedative doses may induce similar - albeit slightly weaker - effects on reconsolidation of emotional story contents (Galarza Vallejo et al., 2019).

Our results add significantly to previous work by showing that propofol general anesthesia can indeed exert retrograde amnesia for emotionally neutral declarative to-be-remembered items. Normal performance in the late injection condition of our study suggests that the amnesic effect of propofol general anesthesia may extend up to about 30 to 60 minutes prior to injection. This new finding suggests that propofol general anesthesia acts on post-encoding processes that are decisive for initial consolidation and later recall. Electrophysiological signatures of early memory formation have been found in direct recordings of ERPs from the hippocampus of patients undergoing evaluation for epilepsy surgery. ERPs recorded during learning of word lists separated subsequently recalled from unrecalled words (Fernandez et al., 1999). Studies with fMRI have further shown that interactions between hippocampus and neocortex during the minutes that follow encoding of visual associative stimuli are predictive of later recall (Tambini et al., 2010). Similarly, activity in hippocampus and caudate nucleus following stimulus offset can predict memory of audiovisual epsiodes (Ben-Yakov and Dudai, 2011). Despite the heterogeneity of approaches, these and related studies therefore provide evidence for a pivotal role of the hippocampus for the very first steps of declarative memory consolidation.

384 It must be conceded that clinical propofol anesthesia is always administered in the context
385 of invasive procedures, mostly in combination with intravenous opioid analgesia. Whether
386 this might have contributed to the deficits in the 'early injection' condition of our study
387 remains elusive. A recent review concluded that opioid signaling is not required for, but can
388 sometimes act to constrain, hippocampus-dependent memory (Thomas, 2015). Likewise, it is
389 possible that arousal before a surgical procedure may influence memory consolidation
390 (Pryor et al. 2010; Chen et al., 2016). We deem this factor not to be decisive - at least for the
391 task in our study - as surgery in local anesthesia did not produce a memory impairment. We
392 are therefore confident that the effects on early consolidation observed here are mainly
393 attributable to pharmacological actions of propofol.

394 One reason why previous pharmacological studies did not reveal the same retrograde effects
395 observed here may be lower serum concentrations of propofol in experiments with sedative
396 doses of propofol in cooperative and spontaneously breathing normal subjects. In a study on
397 reconsolidation of emotional story contents, retrograde propofol effects on reactivated
398 memory of stories prior to propofol sedation were observed when subjects were tested 24
399 hours after anesthesia, but not when tested after a delay of up to 106 minutes (Galarza
400 Vallejo et al. 2019). Compared to this study, the anesthetic doses applied to our patients are
401 significantly higher. At least in animal experiments, propofol effects on LTP are critically
402 dose-dependent (Wei et al., 2002; Nagashima et al., 2005). fMRI studies on pain processing
403 at different propofol concentrations have moreover shown that connectivity changes within
404 cerebral large-scale networks are critically dose-dependent (Lichtner et al., 2018). A further
405 point may be a differential sensitivity of the mnemonic representations across tasks to
406 $GABA_A$-ergic drugs and to altered neuronal activity in distinct brain regions. The task used
407 here has proven to be a reliable marker of hippocampal integrity, particularly for its recall

408 component (Saury and Emanuelson, 2017). Thus, although propofol general anesthesia is
409 likely to act on a wide network of brain regions, the pattern of results is most consistent with
410 modulation of hippocampal neural activity (Finke et al. 2017; Esfahani-Bayerl et al., 2019).
411 Predominant effects on recall in our experiments and the abovementioned reconsolidation
412 study (Galarza Vallejo et al., 2019) further show that application of propofol after memory
413 encoding does not lead to an unselective impairment but rather tends to affect some
414 memory domains more than others, presumably sparing less hippocampus-dependent
415 routes of memory consolidation.

416 Which level of consolidation has been modulated in our experiment? The time window
417 identified in our study is suggestive of propofol actions on synaptic memory consolidation
418 (Dudai et al., 2015; Asok et al., 2019). Systems and synaptic levels of memory consolidation
419 have traditionally been considered separately and with distinct experimental approaches. It
420 is only recently that the interaction between these two levels has been discussed within a
421 common conceptual framework (Dudai et al., 2015; Asok et al., 2019). Current models of
422 synaptic consolidation propose mechanisms by which synaptic plasticity impacts on
423 memory-guided behavior at various timescales – including the short delays addressed here
424 (Ziegler et al., 2015). Complimentary data from humans have been scarce so far. While it is
425 of course not possible to infer from our behavioral results on modulation of synaptic and/or
426 systems levels of memory consolidation, combination of the neuropharmacological
427 approach of our study with imaging techniques may provide a way to link synaptic and
428 systems consolidation in humans.

429 *Conclusion*

430 The results of our study show that propofol general anesthesia may create a transient
431 pharmacological 'lesion' of the neural substrates supporting early memory consolidation.

The lack of effect beyond this time window further suggests rapid subsequent reconfiguration of hippocampus-dependent memory networks. While our approach is spatially not selective, it nevertheless circumvents restrictions of traditional patient-based approaches and makes the initial steps of memory consolidation accessible to experimental modulation - without affecting encoding or memory retrieval. Importantly, it allows for the study of memory consolidation in human subjects with brains that are unaltered by neuropsychiatric disorders or brain surgery. Combination of propofol general anesthesia with subsequent functional imaging of memory replay in the hippocampus may ultimately reveal how transient modulation of GABA-ergic neurotransmission affects mechanisms of memory consolidation in humans.

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579 **Table 1.** Demographic and clinical data of the investigated patient groups; values are
580 medians and interquartile ranges

	Early injection	Late injection	Control – no anesthesia	Control - local anesthesia
n	24	24	24	24
Female / male	13 / 11	13 / 11	12 / 12	11 / 13
Age (years)	35.5 (27-45)	36.5 (31-47)	38.5 (25-46.25)	35 (29.25-46)
Years of Education	13.75 (12.25-18)	14 (12-16)	16 (15-18)	15 (12-17)
Medical Procedure	Strabismus Surgery (n = 24)	Strabismus Surgery (n = 24)	n.a.	Nevus excision (n = 13); Muscle/nerve biopsy (n = 6); Removal of ostheosynthetic material (n = 5)
Propofol Bolus dose (mg)	200 (200-215)	200 (155-237.5)	n.a.	n.a.
Propofol Maintenance dose (mg/kg/h)	6 (6-6)	6 (6-6.75)	n.a.	n.a.
Remifentanil dose (µg/kg/h)	0.2 (0.15-0.2)	0.2 (0.2-0.2)	n.a.	n.a.
Delay end of learning and Propofol (min)	13 (10-17)	105 (95.25-115)	n.a.	n.a.
Duration anesthesia (min)	58 (53-65)	56 (46.25-64.75)	n.a.	n.a.
Delay end of anesthesia and testing (min)	113.5 (106.5 - 128)	113 (108.5-116.75)	n.a.	n.a.
Delay end of learning and testing (min)	189.5 (175.75-205)	271 (261.25-289.5)	180 (180-180)	180 (150-180)

581

582

583 **Figure legends**

584

585 **Figure 1:** Task and experimental conditions. First row, early injection condition; second row,
586 late injection condition; third row, control condition; fourth row, local anesthesia condition.
587 In all conditions, subjects learned a list of semantically unrelated and emotionally neutral
588 words. In the early injection condition, subjects received general anesthesia with propofol
589 about 13 minutes following learning and were tested for recall and recognition about three
590 hours after learning. In the late injection condition, subjects received general anesthesia
591 about 105 minutes after learning and were tested about 4.5 hours after learning. In the
592 control condition, subjects received no anesthesia and were tested three hours after
593 learning. In the local anesthesia condition, subjects received local anesthesia and were
594 tested three hours after learning.

595

596 **Figure 2:** Results. A, free recall immediately after initial learning of target word list and after
597 learning of a distractor word list; B, delayed free recall of target word list; C, delayed
598 corrected recognition of target word list (hits – false alarms). Bars show median percent
599 correct responses in four experimental conditions. Purple, propofol injection conditions;
600 gray, control conditions. No A., no anesthesia; Loc. A., local anesthesia. *** $p \leq 0.001$
601 difference between conditions, two-tailed Mann-Whitney test. Note selective performance
602 decrease for recall in the early injection condition.

603

604