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Reduction in the occurrence of distressing involuntary memories following propranolol or hydrocortisone in healthy women

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ABSTRACT

Background. Pharmacological treatments targeting the neuroendocrine stress response may hold special promise in secondary prevention of posttraumatic stress disorder (PTSD). However, findings from clinical trials have been inconsistent and the efficacy of specific drugs, their temporal window of efficacy, effective doses and the characteristics of likely treatment responders remain unclear.

Method. Using an experimental human model of distressing involuntary memory formation, we compare the effects of two drugs that have theoretical or empirical support as secondary preventive agents in PTSD. Eighty-eight healthy women (average age: 23.5 years) received oral propranolol (80 mg), hydrocortisone (30 mg), or matched placebo immediately after viewing a 'trauma film'. They then completed daily, time-stamped intrusion diaries for 1 week, at the end of which, voluntary memory was tested.

Results. While neither drug affected voluntary memory for the trauma narrative, propranolol treatment was associated with 42% fewer, and hydrocortisone with 55% fewer intrusions across the week, relative to placebo. Additionally, propranolol reduced general trauma-like symptoms, and post-drug cortisol levels were negatively correlated with intrusion frequency in the hydrocortisone group.

Conclusions. Overall, this study shows substantial reductions in intrusive memories and preserved voluntary narrative-declarative memory following either propranolol or hydrocortisone in an experimental model of psychological trauma. As such, despite some inconsistencies in clinical trials, our findings support continued investigation of propranolol and hydrocortisone as secondary preventive agents for re-experiencing symptoms of PTSD. The findings also suggest that it is critical for future research to identify the conditions governing the preventive efficacy of these drugs in PTSD.

INTRODUCTION

Stressful experiences can produce extinction-resistant memory traces through 'over-consolidation' and subsequent strengthening via repeated retrieval-reconsolidation (de Quervain, Schwabe, & Roozendaal, 2017). However, overly-consolidated aversive memories can be maladaptive: their unintended, cue-driven retrieval in non-threatening contexts confers no survival advantage, but instead limits behavioural flexibility by promoting additional symptoms, such as avoidance and distress (Brewin & Holmes, 2003). Such involuntary ('intrusive') memories are a canonical symptom of posttraumatic stress disorder (PTSD) (Brewin, 2011; Brewin & Holmes, 2003). Their formation putatively relies on synaptic and systems-level neuronal adaptations interacting with psychological processes (e.g. rehearsal; emotion arousal). The time-course of 'early' (synaptic) memory consolidation (Dudai, 2004; Shadmehr & Holcomb, 1997) provides a window of opportunity to interfere with this process behaviourally, and this has been proposed as a means to protect trauma victims against developing re-experiencing symptoms in PTSD (Holmes, James, Kilford, & Deeprose, 2010; Horsch et al., 2017; Iyadurai, Blackwell, et al., 2018). The reliance of memory consolidation on protein synthesis also suggests pharmacological routes to such secondary prevention (e.g. using drugs that have indirect, downstream protein synthesis inhibiting properties; Sijbrandij, Kleiboer, Bisson, Barbui, & Cuijpers, 2015), especially if treatment is delivered soon after trauma. For example, propranolol, a non-selective β-adrenoceptor antagonist, which inhibits protein-synthesis dependent 'long-term potentiation' (a cellular/molecular mechanism proposed to underlie memory formation) and selectively impairs emotional memory (McGaugh, 2004), showed promise as a PTSD-preventive agent, in an early pilot study in emergency room trauma victims (Pitman et al., 2002). Early observational studies also suggested that the anti-inflammatory drug, hydrocortisone, lowered the incidence of intensive care treatment related PTSD (Schelling et al., 1999; Schelling et al., 2001). This prompted

several clinical trials examining hydrocortisone's potential as a preventive agent for PTSD, with promising results from these small-scale studies (Sijbrandij et al., 2015). However, such clinical findings are difficult to reconcile with the well-established consolidation *enhancing* effects of glucocorticoids on emotional memory (de Quervain et al., 2017) and the therapeutic mechanism of action of hydrocortisone in PTSD prevention therefore remains unclear.

Despite promising clinical evidence for hydrocortisone in secondary prevention of PTSD, and at least a theoretically compelling rationale for propranolol's use in secondary prevention, progress in translating these discoveries into highly effective secondary preventive treatments has been slow. This may be because the conditions that dictate the preventive efficacy of these drugs remain poorly understood. Clinical trials are not ideally suited to studying such conditions, especially given the highly variable treatment regimes common in the medical (often emergency) settings in which relevant trials have been conducted. Such variability might explain disappointing results with propranolol, which, on the basis of several extant trials, shows no overall preventive efficacy (incident rate ratio: IRR=0.95; see Sijbrandij et al., 2015).

These observations call for translational research that allows the effects of propranolol, hydrocortisone, and related drugs to be examined cleanly, and in the absence of clinical confounds (Iyadurai, et al., 2018). The sources of variability present in clinical settings are readily avoided or controlled in laboratory studies that model (mild) psychological 'trauma'. In addition, despite a number of clinical studies examining propranolol's preventive effects on *general* PTSD symptomotology, we are not aware of any existing experimental research examining the effects of a β-blocker specifically on *intrusive memories*. While such purely experimental studies do exist for hydrocortisone, these studies have involved either administering hydrocortisone before an analogue trauma (Rombold et al., 2016) or after a

long (~24 hr) delay (Graebener, Michael, Holz, & Lass-Hennemann, 2017). These conditions may be suboptimal for revealing *preventive* effects of hydrocortisone on involuntary memory formation, and do not allow effects on encoding (which is potentially *enhanced* by endogenous cortisol; van Ast et al., 2013), consolidation, and retrieval to be parsed. As such, further experimental studies are essential for improving our understanding of the specific PTSD symptoms affected by, and the parameters that govern the efficacy of, noradrenergic and glucocorticoid drugs as potential secondary preventive agents. In the current study, we therefore carry out the first head-to-head comparison between *single doses* of propranolol and hydrocortisone on intrusive (and voluntary) emotional memories using the 'trauma-film' model of PTSD (Holmes & Bourne, 2008). Comparing these drugs under identical experimental conditions is critical to understanding their apparently divergent effects in extant clinical trials (Sijbrandij et al., 2015). To maximise the clinical relevance of our findings, we tested the effects of '*post-trauma*' drug administration. Post-trauma treatment is important because it is usually not possible to prospectively treat *potential* trauma victims. In addition, treatments for PTSD should ideally selectively reduce the occurrence of 'decontextualised,' sensory, involuntary memories, while sparing voluntarily-accessible memories for spatial and temporal contextual and narrative details. The latter are thought to inhibit the occurrence of cue-driven sensory memories, and hence are required for recovery from psychological trauma (Brewin, 2001). As such, we also assessed whether voluntary trauma memory was spared after administration of these drugs.

METHODS

Participants

Medically and psychiatrically healthy women (n=88; 18-35 years old) received oral propranolol (80 mg), hydrocortisone (30 mg) or placebo in a randomised, double-blind manner. All procedures were approved by University College London ethics committee and

conducted in accordance with the Declaration of Helsinki. Participants provided written informed consent and received a £25 honorarium.

Procedure

Participants were telephone-screened to determine eligibility (see Supplement). Testing commenced between 2-5pm on both testing sessions (days 1 and 8). After ECG electrodes were attached, baseline questionnaires were completed in the following order: Beck Depression Inventory-II (BDI), State-Trait Anxiety Inventory (STAI) and Dissociative Experiences Scale-II (DES). These were followed by the first blood pressure (BP) reading and saliva (cortisol) sample at the baseline (T1) timepoint (Figure 1). Heart rate (HR) was assessed continuously with the 5-min pre-film period forming the T1 HR measure. Baseline (T1) state measures – Positive-Negative Affect Schedule (PANAS), then the Bodily Symptoms Scale (BSS) – were then taken. See Supplement for further details on self-report and physiological measures.

The trauma-film (formed of two scenes from the commercial film 'Irreversible', Studio Canal lasting approximately 15 min, including audio descriptions that introduced and linked the scenes) is described in detail elsewhere (Das et al., 2016). Participants were seated comfortably in front of a computer monitor and head movements were minimised using a chin rest while eye-tracker calibration was performed. While remaining in the chin rest, participants then viewed the trauma-film in a darkened room via a 15 inch laptop monitor with audio presented through headphones. The exact start time of the film was event-marked.

Eye-tracking was performed throughout the film viewing period to determine whether engagement during film viewing differed between groups at 'baseline' (i.e. before drug administration) with the aim of ruling this out as a potential explanation for any observed group effects on intrusion. Eye-movements metrics (dwell-time and number of

fixations) were recorded continuously during film viewing over 44 pre-defined areas of interest (GP3 eyetracker, Gazepoint, Vancouver, Canada). Analysis was conducted offline using Gazepoint software.

HR during the entirety of the film served as the T2 measure of cardiac activity and the other T2 measures/samples were taken immediately after the film in the following order: BP, saliva, subjective measures (PANAS, BSS). Note: given the duration of the film and time-course of salivary cortisol changes, the cortisol sample taken immediately after the film (T2) was considered to reflect peri-film cortisol levels.

Drug capsules containing propranolol, hydrocortisone or placebo were then administered and participants sat quietly while completing a 'filler' task for 1 hr. This involved rating 25 clips of classical music for pleasantness. A 10 min rest break occurred midway through the filler task, during which participants were discouraged from using electronic devices. After the 1 hr period, participants and experimenters made independent guesses on treatment followed by final BP, saliva, PANAS and BSS measures at T3 (T3 HR was taken during the final 5-min of the filler task).

Detailed instructions on remote recording of intrusive memories were provided before participants left the laboratory on day 1 and participants received daily reminders at 8 pm to record (as close to bedtime as possible) the number of intrusions (with brief descriptions to allow verification of relevance to the trauma film contents). Vividness and distress were also recorded, using a 5-point rating scale (1='not at all'; 5='extremely') and averaged across intrusions for each participant on each day. All intrusion variables were recorded using the online/smart device-ready survey tool, Qualtrics (Provo, UT). On day 2, participants also completed online questions on sleep quality (rated very good, fairly good, fairly bad, very bad) and quantity (hours of sleep) relating to the previous night.

Participants returned to the study centre on day 8 (at approximately the same time as day 1) and completed an adapted version of the Impact of Events Scale (IES), followed by free-, then cued recall. Upon completion, participants were debriefed and compensated.

Statistical analysis

One-way ANOVAs were used to analyse baseline variables, voluntary memory and (log transformed) IES scores. Day 1 physiological and subjective data were analysed using 3 (Timepoint: T1, T2, T3) x 3 (Group: Placebo, propranolol, hydrocortisone) repeated measures ANOVAs. Mixed effects and generalized linear mixed models were used to analyse intrusion data (counts, vividness and distress), with participant and Day as random factors, and model-fit assessed using Akaike's Information Criterion (AIC). Peri-film (T2) HR and cortisol were included as covariates in the analyses of intrusions and IES, based on previous research demonstrating their association with trauma-related symptoms (Chou, La Marca, Steptoe, & Brewin, 2014a; Chou, La Marca, Steptoe, & Brewin, 2014b). The main outcome of interest was intrusion frequency; secondarily we examined intrusion vividness and distress, IES scores and voluntary memory recall. The threshold for statistical significance was p=0.05 and post-hoc p values were Bonferonni-corrected. Two tailed tests are reported throughout. See Supplement for further details on statistical analyses.

RESULTS

Baseline characteristics

Groups were well matched on demographic and psychological variables (Table 1; see Supplementary Results).

Subjective and physiological response to film and drugs

PANAS-negative scores only showed a main effect of Time (F(1.47, 124.56)=205.062, p<0.001, η_p^2=0.707), reflecting an increase in negative affect from T1 to T2, followed by return to baseline at T3. PANAS-positive also only showed a main effect of Time (F(2,170)=93.566, p<0.001, η_p^2=0.524), with deterioration in positive affect between T1 and T2, maintained at T3. No Group or Time effects were observed on BSS items, which were generally at floor level (i.e. <10 on a 0-100 scale) across time-points. The absence of reported changes in bodily symptoms on the BSS was consistent with chance level correct guessing on treatment by participants (29.54%; χ^2(4)=2.513, p=0.642). Experimenters also guessed at chance level (37.5%; χ^2(4)=3.206, p=0.524), suggesting that blinding was successful.

A Time x Group interaction on HR (F(3.20, 132.71)=7.730, p<0.001, η_p^2=0.157; Figure 2A) was driven by a drop between T2 and T3 only after propranolol (p<0.001). Systolic BP also showed a Time x Group interaction (F(4,168)=3.766, p=0.006) driven by a reduction in systolic-BP only in the propranolol group between T2 and T3 (p<0.001). Baseline salivary cortisol levels (0.144 µg/dl) were in the expected range (Miller et al., 2016). A Time x Group interaction on cortisol levels (F(2.01, 80.37)=16.78, p<0.001, η_p^2=0.296), reflected a T2 to T3 increase only after hydrocortisone (p<0.001; Figure 2B).

Involuntary memory

As shown in Figure 3A, there was a steady decline in intrusions across Days (*IRR*=0.636, *SE*=0.024, z=12.02, p<0.001; T2 HR and cortisol were covariates in all intrusion analyses). The effect of Group was also significant (χ^2(2)=9.81, p=0.007): relative to placebo, the propranolol group experienced fewer intrusions (*IRR*=0.580, *SE*=0.147, z=2.16, p=0.031), as did the hydrocortisone group (*IRR*=0.452, *SE*=0.118, z=3.04, p=0.002). The IRR values

correspond to ~42% and ~55% fewer intrusions in the propranolol and hydrocortisone groups respectively (see Supplement for full details on model building and testing).

Descriptively, the differences between placebo and the drug groups were most evident on days 1-2, whereas the number of intrusions was close to 0 in all groups by day 7 (Figure 3A). However, whereas intrusions were abolished by day 7 in 87% and 76% of participants in the propranolol and hydrocortisone groups respectively, only 55% of participants in the placebo group had no intrusions on day 7 ($\chi^2(2)=7.59$, $p=0.023$).

Since drug effects were already evident on day 1, it was of interest to determine whether biomarkers of drug response were associated with sub-acute (day 1) intrusion counts. Correlations were therefore conducted between log-transformed intrusion frequency and (i) T3 salivary cortisol, with a primary focus on the hydrocortisone group, and (ii) T3 HR, focusing on the propranolol group. There was a significant negative correlation between T3 cortisol and day 1 intrusion frequency in the hydrocortisone group ($r(28)= -0.480$, $p=0.01$; see Supplementary Figure 1), but no correlation between T3 HR and intrusion frequency in the propranolol group ($r(24)= 0.128$, $p=0.499$).

For vividness of intrusions, a Day x Group interaction ($\chi^2(2)=6.62$, p=0.037) reflected a shallower slope in the hydrocortisone relative to placebo condition ($p=0.011$; Figure 3B), but not propranolol relative to placebo ($p=0.181$). Bonferroni corrected post-hoc pairwise comparisons of vividness ratings showed that on day 1, only the propranolol versus placebo comparison was significant ($p=0.04$). In contrast, groups did not differ significantly on vividness by day 7 ($ps>0.1$). Distress ratings showed a similar declining pattern across days ($B=-0.311$, $SE=0.046$, z=6.76, $p<0.001$). However, based on the same model specifications as used to analyse vividness (which also produced the best fitting model of distress), the effect of Group did not reach statistical significance ($\chi^2(2)=5.25$, $p=0.072$) and neither did the Day x

Group interaction ($\chi^2(2)=4.86$, $p=0.088$; see Supplementary Results; Figure S2). It is important to note that average distress ratings on day 1 were 3.0 on a 1-5 scale, suggesting at least moderate sub-acute levels of intrusion-related distress. It should be noted that dwell-time and number of fixations on pre-specified areas of interest during film viewing did not differ between groups (Supplementary Results) and therefore attentional differences are unlikely to explain the reported drug effects.

Voluntary memory: free and cued recall

In contrast to the effects on intrusions, voluntary memory tested on day 8 was unaffected by Group. Participants in the three groups performed similarly on cued recall (*Mean* $\pm$ *SD*): placebo: 7.76 $\pm$ 1.91; propranolol: 8.17 $\pm$ 1.96: hydrocortisone, 7.86 $\pm$ 2.61, free recall-gist: placebo: 13.09 $\pm$ 5.24; propranolol: 14.47 $\pm$ 4.65; hydrocortisone: 13.29 $\pm$ 4.72, and free recall-detail: placebo: 16.91 $\pm$ 8.60; propranolol: 18.33 $\pm$ 5.87; hydrocortisone: 17.19 $\pm$ 7.44 (F values $\leq$0.692, p values $\geq$0.503).

Adapted Impact of Events Scale

The effect of Group, co-varying T2 HR and cortisol, on IES scores was marginally significant ($F(2,78)=3.129$, p=0.049, $\eta^2=0.074$). Although both propranolol and hydrocortisone showed lower scores relative to placebo, only the pairwise Bonferroni-corrected comparison between placebo and propranolol was significant ($p=0.044$; Figure 4).

DISCUSSION

The current study is the first that we are aware of to directly compare effects of hydrocortisone and propranolol on voluntary and involuntary memory for emotional material intended to simulate re-experiencing symptoms (intrusive memories) in PTSD. We showed that both propranolol and hydrocortisone administered immediately after an analogue 'trauma' produced similar substantial reductions (by 42% and 55% respectively) in intrusive memories, starting on the day of the trauma-film. Drug effects on intrusions did not reflect generalised memory impairment, as long-term *voluntary memory* was intact in both drug groups.

The effects of propranolol reported here are consistent with a large body of neurobiological and behavioural research on the role of the noradrenergic system in *long-term* emotional memory in rodents and humans (Lonergan et al., 2013; Van Stegeren, 2008) and extend this to involuntary (episodic) emotional memory. Although not often reported, impairing effects of propranolol on pre-sleep, *short-term* memory performance – as seen here - have also been reported (Maheu, Joober, Beaulieu, & Lupien, 2004). The propranolol group also showed lower general trauma-like symptoms (IES scores) after one week.

Although our findings support the idea that propranolol can reduce the occurrence of intrusive memories, meta-analysis of randomised clinical trials of propranolol in trauma victims have not shown reduced incidence/severity of symptoms of PTSD (Sijbrandij et al., 2015), and therefore the clinical evidence does not currently support its use in secondary prevention. Clearly, lab studies of simulated trauma, and clinical studies involving actual trauma, are not directly comparable. It is possible, for example, that the severity of the stress reaction in real-life traumas results in a level of noradrenergically-mediated hyper-consolidation that is not readily constrained by propranolol. This might suggest that larger

acute (rather than cumulative) doses of propranolol might need to be tested in clinical studies, although this must be balanced against tolerability.

In studies reviewed by Sijbrandij et al (2016), the interval between trauma and propranolol treatment varied between 6 and 48 hr with 4/6 studies administering propranolol >6hr after trauma. If rapid treatment is critical, future clinical research with β-blockers may need to consider the clinical *context* in which such research is conducted. For example, first-line medical settings (e.g. ambulance or emergency triage) may be more appropriate treatment contexts than later in the healthcare chain.

The effects observed here with hydrocortisone are consistent with a number of small-scale clinical studies in recently traumatised individuals. In contrast to studies with propranolol, it is noteworthy that 4 out of 5 studies of hydrocortisone in Sijbrandij et al's meta-analysis administered the drug within the putative period of synaptic consolidation (<6h after trauma; overall IRR=0.38). Again, while this might suggest that rapid treatment is critical, the effects of endogenous/exogenous cortisol on memory are complex, with opposing effects on working memory *versus* consolidation (van Ast et al., 2013) and retrieval *versus* (re)consolidation (de Quervain et al., 2017). The effects reported here are not readily reconciled with existing experimental behavioural studies showing *enhancement* of consolidation of emotional memories by endogenous and exogenous glucocorticoids (Buchanan & Lovallo, 2001; Roozendaal, 2000). Such enhancing effects are usually most evident when there is a long delay (≥24 hr) between training (and drug administration) and the retention test (Roozendaal, 2002). However, after showing lower intrusion counts than placebo on day 1, there was no sign of a rebound increase in intrusive memories on day 2 in the hydrocortisone group, suggesting that there was minimal effect of hydrocortisone on consolidation of sensory memories underlying intrusion.

Although the mechanism of hydrocortisone's protective effects against PTSD remains unclear, we speculate on two indirect routes to reduced intrusions. One explanation for our findings (and potentially, previous clinical studies with hydrocortisone as well) is that they reflect an impairment of involuntary *retrieval* which indirectly affects early consolidation due to limited retrieval-rehearsal. This explanation requires that such acute retrieval impairment (and consequent, indirect *reductions* in consolidation) precedes and/or exceeds any concurrent consolidation *enhancing* effects of hydrocortisone. An alternative explanation, drawing on dual representation theories of PTSD, is that voluntarily-retrievable, contextually-based memories - which ordinarily down-regulate the expression of sensory-based involuntary memories (Brewin, 2001, 2014; Brewin & Holmes, 2003) - might be selectively over-consolidated by hydrocortisone, and hence exert increased top-down control over cue-driven retrieval of sensory representations. In contrast to the retrieval/indirect consolidation-impairment explanation, this would require that such effects of hydrocortisone exceed its retrieval impairing effects, and are relatively selective for declarative, contextual (rather than sensory-) representations of the traumatic event. However, as we found no evidence for improved voluntary memory for narrative aspects of the trauma film on day 8 in the hydrocortisone group, this latter explanation is not supported by our data. In addition, as with propranolol, hydrocortisone's effects were observed before sleep. This therefore also raises some problems for the former explanation, as the "retrieval as a route to memory consolidation" account (p573; Antony, Ferreira, Norman, & Wimber, 2017) proposes a major role for memory reactivations during slow wave sleep (Antony et al., 2017). Nonetheless, the idea that pre-sleep replay of events shortly after encoding could also contribute to consolidation, and be disrupted by hydrocortisone, has not, to our knowledge, been tested empirically. Regardless, other studies have also shown memory performance decrements due to endogenous/exogenous cortisol before sleep (Diamond, Fleshner, Ingersoll, & Rose, 1996;

Kirschbaum, Wolf, May, Wippich, & Hellhammer, 1996; Kuhlmann, Kirschbaum, & Wolf, 2005). Future studies should seek to test these ideas by, for example, testing declarative memory and behaviourally manipulating retrieval/rehearsal on day 1.

While the hydrocortisone group showed fewer intrusions, this group also showed a slower rate of decline in vividness of intrusions relative to placebo. The reasons for this are unclear, but may reflect enhanced sensory processing, as previously demonstrated with hydrocortisone (Born, Hitzler, Pietrowsky, Pauschinger, & Fehm, 1988). However, no drug was present during film viewing, and initial (day 1) vividness was (non-significantly) lower in the hydrocortisone relative to placebo group. As such, any effects on sensory processing would have to have occurred *after* day 1 (e.g. through interaction between working memory and consolidation processes). It should also be noted that while vividness of residual intrusions on day 7 appeared to be higher in the hydrocortisone group, group differences were not statistically significant. As such, and in the absence of any relevant outcomes directly related to sensory processing performance following drug, we are unable to comment further on this finding at this stage.

A previous experimental study that examined the effects of hydrocortisone on intrusive memories in humans showed different effects to those described here (Rombold et al., 2016). That study had a number of similarities with the current study, including use of the same trauma stimuli and participants with similar baseline demographic and mood characteristics. However, that study failed to show differences relative to placebo. Despite some methodological similarities, it is important to consider divergent procedures in the two studies, as these could point to critical boundary conditions that determine hydrocortisone's effects on intrusive memories. Three methodological differences stand out. Firstly, we administered hydrocortisone immediately *after* the trauma-film, whereas drug administration *preceded* film presentation by 1 hr in the study by Rombold et al (2016). Secondly, our

hydrocortisone dose (30 mg) was higher than used by Rombold et al (2016; 20 mg). Given the relatively short $t_{1/2}$ of hydrocortisone, these differences might have been particularly consequential in terms of the presence or persistence of adequate levels of cortisol during critical plasticity-related processes. Finally, although both studies tested healthy young women, participants differed in the use of contraceptive medication. Only 40% of participants in Rombold et al's (2016) study were using oral contraceptives (compared to 100% in the current study), and such participants might show a distinct response to the trauma film (Roche, King, Cohoon, & Lovallo, 2013) and to hydrocortisone (Gaffey, Wirth, Hoks, Jahn, & Abercrombie, 2014) relative to non-users.

In another study, hydrocortisone was administered on three consecutive days (10 mg twice a day), starting ~24-hr after the trauma film (Graebener et al., 2017). Given this delayed treatment, the latter study might have less direct relevance for experimental investigation of secondary prevention. However, by starting treatment outside of the putative early consolidation period, that study (Graebener et al., 2017) does address the specific effects of hydrocortisone on retrieval, rather than encoding and/or consolidation. Regardless, no difference between hydrocortisone and placebo was found in the latter study either. Comparison of that study with the current one suggests that timing (immediacy) of treatment might be a critical boundary condition for hydrocortisone's effects on intrusive memories.

A number of limitations of the current study must be acknowledged. Firstly, given the continued presence of circulating propranolol ($t_{1/2}$=4-6 hr) and hydrocortisone ($t_{1/2}$~100 min) during the interval between the film and intrusion recording on day 1, we cannot rule out additional non-memory related drug effects on day 1 (e.g. changes in peripheral arousal, interoception, meta-cognition). Since the memory-based explanations proposed here were not tested mechanistically, they must remain speculative, but should be the subject of future research. The effects were also only tested in young women taking hormone-based

contraceptives, raising questions about generalisability. However, it should be noted that use of hormone-based contraceptive pill is common in the United States and many European countries, with approximately a quarter of women using them (Enewold et al., 2010). The alternative approach of testing non-contraceptive using, regularly cycling women at a specific phase in their menstrual cycle (Kamboj, Krol, & Curran, 2015; Soni, Curran, & Kamboj, 2013) is cumbersome, and does not improve generalisability. Furthermore, studies limited to men are also problematic, especially given that there is a >2-fold higher lifetime prevalence of PTSD among women (Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995).

Another issue, which is common to most studies of the trauma-film paradigm, is that recording of memory events did not occur 'on line' or very shortly after the memory events occurred, but rather, was retrospective. Although recent evidence suggests minimal differences between ecological momentary assessments using frequent probes (trauma reminders) on smartphones *versus* continuous recording (i.e. as and when intrusions occur), *versus* single episode recording (as used here) (Rattel et al., 2018), it is important to recognise that retrospectively recorded intrusion frequency must reflect, at least in part, metacognitive (meta-memory) processes. Although the role of noradrenergic and glucocorticoid pathways in modulating meta-memory remains poorly understood, it is worth noting that propranolol seems to *enhance* some aspects of metacognition (Hauser et al., 2017). On the other hand, emotional arousal is associated with increased memory confidence (Talarico & Rubin, 2003), an aspect of meta-memory that could conceivably be down-regulated by arousal reducing drugs. This may therefore be a partial explanation for our observations with propranolol, but seems less likely for hydrocortisone.

As with any non-clinical, lab-based model of traumatic stress, we can never ethically simulate actual psychological trauma. The extent to which such experimental models reflect actual vulnerability to PTSD has yet to be determined, and as such, extending our conclusions

to clinical treatment would be premature. However, clinical researchers might benefit from considering some of the methodological characteristics (dose, timing, participant characteristics) of this study in future testing of propranolol and hydrocortisone for secondary prevention. Finally, to enhance the credibility of future experimental studies, hypotheses, methods and statistical plans should be pre-specified and published on a suitable open science platform.

In summary, the current study is the first to demonstrate rapid reductions in involuntary memories with propranolol or hydrocortisone. The findings are consistent with previous clinical findings with hydrocortisone and also support continued investigation of propranolol in secondary prevention, especially if treatment can be delivered rapidly.

SUPPLEMENTARY MATERIAL

Submitted file name: Other supplementary material_Kamboj et al_R1_Final (see end of this file)

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CONFLICT OF INTEREST

None.

ETHICAL STANDARDS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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Table 1: Baseline variables (*Mean ± SD*). The p values relate to the outcome of a one-way ANOVA.

	Placebo	**Propranolol**	**Hydrocortisone**	***P value***
Age (years)	23.76 (3.64)	23.20 (3.46)	23.65 (3.12)	0.799
BMI (kg/m^2)	22.01 (2.60)	22.42 (2.91)	22.72 (2.09)	0.578
Education (years)	16.24 (2.20)	15.83(1.60)	16.34 (1.37)	0.498
BDI	6.69 (4.09)	4.33 (5.14)	5.10 (4.69)	0.120
STAI	38.62 (7.82)	33.03 (8.73)	36.34 (9.06)	0.057
DES	9.37 (6.65)	6.81 (6.43)	9.57 (9.51)	0.306
Systolic BP (mmHg)	110.57 (10.80)	114.67 (13.64)	109.66 (13.58)	0.279
Diastolic BP (mmHg)	71.52 (7.93)	70.83 (7.41)	68.45 (8.23)	0.300
Post-film period on day 1 (film- to diary completion time; hr)	7.70 (4.79)	7.26 (5.66)	7.64 (5.47)	0.942

Figure 1: Procedure. On day 1, pre-film measures was taken at T1; T2 are post-film measures (T2 HR was measured continuously during the film) and a final set of post-drug assessments were taken at T3. Participants then completed a 7-day online memory monitoring procedure and returned to complete a general PTSD-symptom measure (adapted Impact of Events Scale; IES) and voluntary declarative memory assessments on day 8.

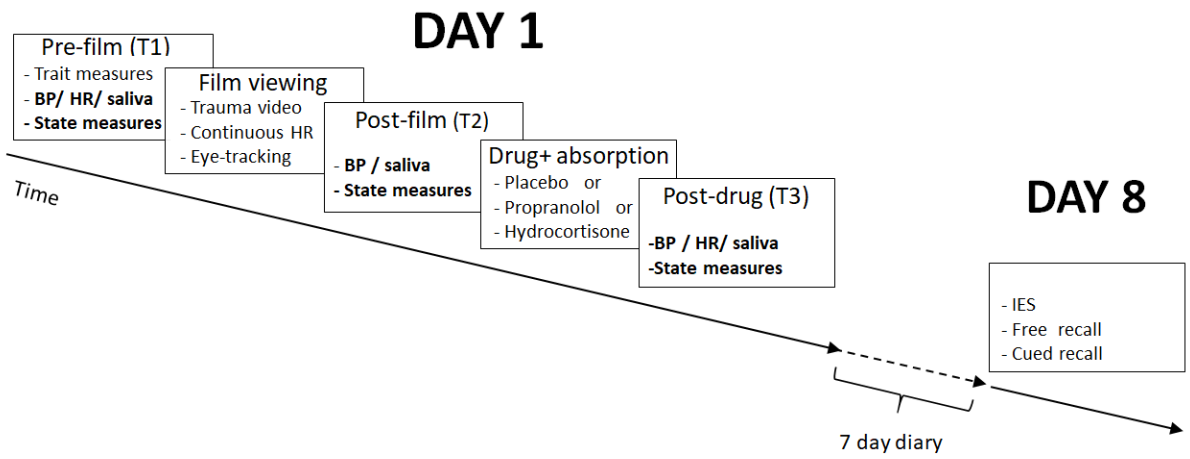


Figure 2A: Time by Group effects on (*Mean* ± *SEM*) heart rate (beats/min) and **B:** salivary cortisol (µ/dL) on day 1. T1=pre-film; T2=post-film; T3=1 hr post drug.

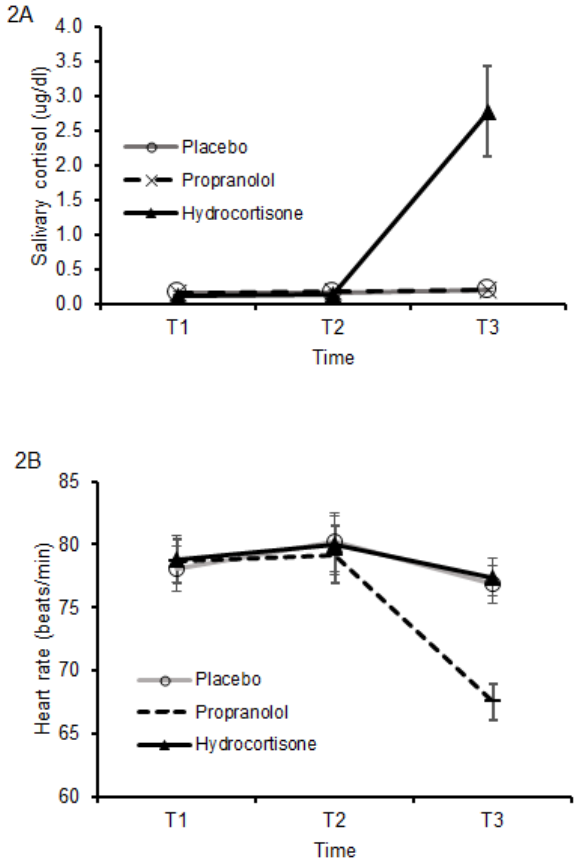


Figure 3A: Intrusion counts. Predicted mean ± SEM number of intrusion based on negative binomial regression results. The main effect of Group reflects fewer intrusions relative to placebo in the propranolol (p=0.031) and hydrocortisone (p=0.002) groups. **B: Vividness of intrusions.** Predicted mean ± SEM vividness. *p=0.04 for post-hoc Bonferroni corrected pairwise comparison on Day 1. HR and salivary cortisol at T2 were covariates in both models.

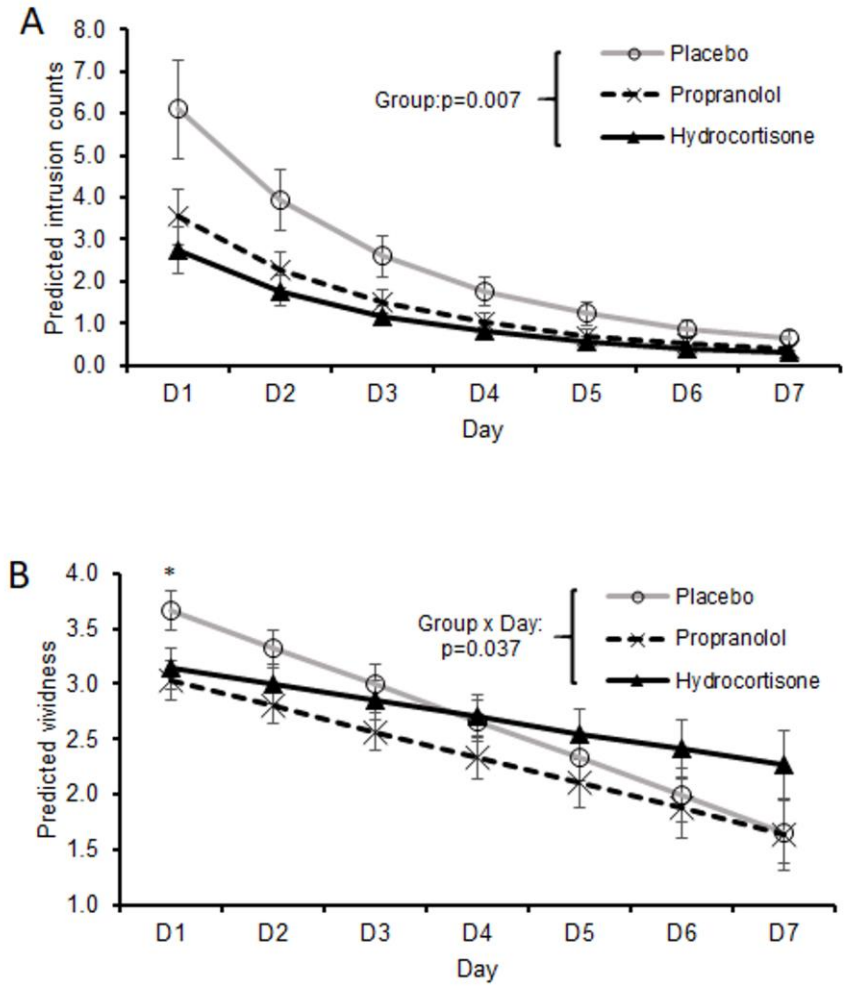
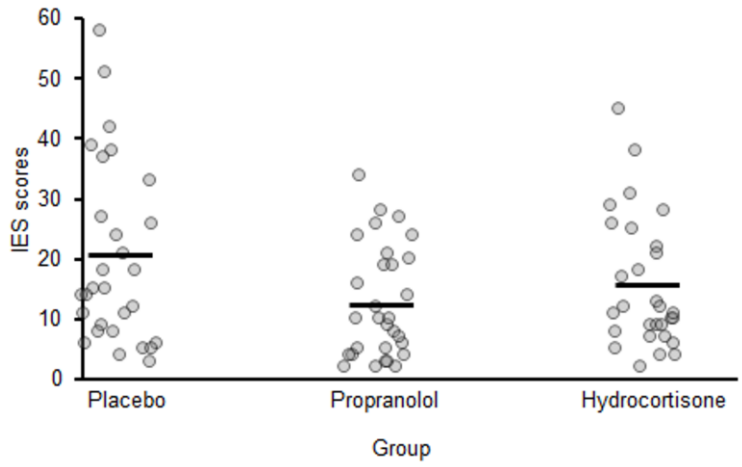


Figure 4: Participant (filled circles) and group-level (solid black lines) scores on the adapted Impact of Events Scale (IES). For clarity, values are untransformed. Solid black horizontal lines represent estimated marginal means (T2 HR and cortisol levels as covariates).



OTHER SUPPLEMENTARY MATERIAL

SUPPLEMENTARY METHODS

Participants

Participants were recruited from the university locale. Inclusion criteria were: age 18-35 years; infrequent (≤2/month) recreational drug use (except caffeine, alcohol and tobacco), ≤112 g weekly alcohol, BMI 18.5-30 kg/m^2, normal blood pressure, normal or corrected to normal vision and hearing, access to a smart/internet device and ability to complete the remote intrusion monitoring task on the first three days, and at least five of the seven monitoring days. To limit the effects of ovarian hormone fluctuation on intrusions or emotional responding, participants also had to be daily users of a hormone-based oral contraceptive, confirmed using their most recent personal (named) prescription or drug pack. Exclusion criteria were: known memory impairments, significant sleep problems, asthma, diabetes, chronic obstructive pulmonary disease, cardiac disease, low blood pressure, history of seizures or neurosurgery, impaired liver or kidney function, and history of severe anaphylactic reaction to a variety of allergens, current use of glucocorticoid/ cardiovascular/psychiatric medication use; self-declared history of psychiatric disorder; (in)direct experience of significant interpersonal violence; history of fainting; previous exposure to the trauma-film ('Irreversible', Studio Canal). Note, since only the general nature of the film, rather than its title, were disclosed during the screening procedure, we could only ascertain whether participants had previous exposure to the film on day 1, after film viewing.

Related intrusion-interference studies have shown large effect size estimates (d>0.8) for intrusion counts (Holmes, James, Coode-Bate, & Deeprose, 2009; James et al., 2015). Given uncertainty about the effects of propranolol and hydrocortisone on intrusive memories, we assumed a more conservative, medium effect (f=0.25). With α=0.05 and 1-β=0.8, n=93 was required to detect a main effect of drug (Faul, Erdfelder, Lang, & Buchner, 2007). Of n=186 screened participants, n=111 reported meeting inclusion criteria and n=92 attended day 1. Of these, four participants could not be included because: n=1 had previously seen the film; n=1 found film too distressing; n=1 disclosed severe depression on day 1, n=1 inaccurately reported use of contraceptive at screening. This left n=88 who completed day 1 and the intrusion diaries (achieved power=0.78). All those who completed the diaries attended day 8.

Randomisation was performed using an online random number generator (Randomizer.org), using 30 consecutive 'blocks' of triplets, corresponding to each of the three conditions. Participants were assigned to condition at screening and coded envelopes containing the capsules were retained by the PI (SKK) until day 1. No experimenter had access to the treatment-participant code, which was held by the medical consultant and senior investigators (none of whom were involved in any data collection and had no access to the data during the study).

Questionnaire and physiological assessments

Baseline mood and trait measures were the Beck Depression Inventory-II (BDI; (Beck, Steer, & Brown, 1996), the Trait version of the State-Trait Anxiety Inventory (STAI; (Spielberger, 2010) and the Dissociative Experiences Scale-II (DES; (Carlson & Putnam, 1993). State positive and negative affect was assessed using the Positive-Negative Affect Schedule (PANAS; (Watson & Clark, 1999), with instructions to indicate *current* ("right now") feelings. In addition, numerical rating scales of negative emotions were used (prior to PANAS). However, since these loaded onto a single factor which correlated highly with PANAS-negative scores (r(88)=0.769, p<0.001 – correlation between T1 scores), only

PANAS-negative scores are reported. Similarly a single NRS item for happiness was strongly correlated with total PANAS-positive ($r(88)=0.506$, $p<0.001$), and so only PANAS-positive scores are reported.

The Bodily Symptoms Scale (BSS; (Bond & Lader, 1974), was used to assess drug-related psychological and physical states. Thirteen bodily/mental state items are included in the BSS: anxiety, depression, memory impairment, palpitations, nausea, emotional numbness, euphoria, drowsiness, muscle-tension, headache, loss of concentration, shaking/trembling and confusion). Each is scored on a 0 (no symptom) to 100 (very strong/severe) range.

General trauma symptoms (previous 7 days) were assessed using the 22-item Impact of Events Scale (IES; (Weiss, 2007) adapted for the trauma-film (Holmes et al., 2009). Heart rate (HR) was recorded using a BodyGuard-2 ECG device (FirstBeat Technologies, Finland), and episodic blood pressure with a BM40 XL device (Beurer UK) as outlined elsewhere (Kamboj et al., 2017). Event markers corresponding to the start of the film and end of the 1 hr filler period were recorded to identify the 5-min pre-film and post-drug periods (T1 and T3 as described in the main paper and Figure 1).

Salivary cortisol levels

Participants were instructed to avoid consuming any foods for >2 hr prior to the session and any drinks other than water for >1 hr. After a mouth rinse with water, passive drool (approximately 500 µl) was collected into cryovials. Samples were frozen immediately and stored at -80°C until analysis.

Prior to assay, samples were thawed completely at room temperature, vortex mixed, and centrifuged at 1500 x g for 15 minutes to remove precipitated mucins and particulate matter. Samples were later analysed using the Expanded Range, High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics LLC, CA, USA), according to the manufacturer's instructions.

Drugs

Drug doses were based on previous research demonstrating reliable effects on hormonal/physiological indices (Kuhlmann, Kirschbaum, & Wolf, 2005), which might not be observed at lower doses (Maheu, Joober, Beaulieu, & Lupien, 2004). Propranolol (2x40 mg; Accord Healthcare, Middlesex, UK) and hydrocortisone (1x10 mg + 1x20 mg; Auden Mckenzie Pharma Division, Ruislip, UK) tablets were mechanically crushed and re-encapsuled in opaque gelatine capsules, with additional lactose powder. Identical placebo capsules contained only lactose. Capsule preparation was performed by two trained researchers who were not otherwise involved in the study. Participants took these with water. Participants were aware of the side effect profile of the active drugs (based on information provided in the study information sheet) and provided a guess on treatment at the end of day 1, entered directly onto the computerised survey tool, allowing the experimenter to make independent guesses. This occurred before BP assessment at T3 to limit the influence of interoceptive signals (e.g. from the tightened BP cuff among participants) and device readings (experimenters).

Intrusive memory recording

At the end of the day 1 lab session, the importance of completing diary entries on each day was stressed to participants. They were provided with a detailed description of the nature

of intrusive memories as '…*spontaneously occurring memory. By 'spontaneous' we mean memories of the film that suddenly pop into your mind automatically….not .....when you deliberately think about it. The spontaneous memories may pop into your mind when you are doing or thinking about something completely unrelated. The main thing is that you didn't mean to think about the film but recalled something about it out of the blue.*'

Voluntary memory assessment

For free recall, participants were asked to recall "as much information and detail as possible including information about where things happen, when they happen, who they happen to, what the people and scenes look like, etc.," typing this directly into a text box with no time limit. Performance was determined by counting the number of recalled 'idea units'. For cued recall task (11), participants provided responses to 19 questions about events in the two clips.

Free and cued recall were independently scored either 0 (inaccurate), 0.5 (partially accurate), or 1 (highly accurate) by two treatment-blind researchers. Freely recalled items were classified as gist or detail (Kamboj & Curran, 2006). High interrater agreement was achieved (>90%) across recall metrics, with disagreements resolved through discussion.

Statistical analysis

Analyses were conducted on IBM SPSS (version 24) and Stata (version 14). Descriptive statistics are *means* $\pm$ *SDs* (except where indicated). Outliers (studentised residuals >|3|) were winsorised $\left(x_{(highest\ non-outlier)} + 1\right)$. Data was checked for normality and homogeneity of variance, and where appropriate (IES score), log transformation applied prior to analysis (untransformed data are presented in the results for clarity). One-way ANOVAs were used to analyse baseline variables, voluntary memory and IES. Physiological and subjective data with limited missing values (due to recording failures, as reflected in lower-than-expected *dfs*: HR, BP, state affect/ bodily symptoms) were analysed using repeated measures ANOVA. Where assumptions of sphericity were violated, Greenhouse-Geisser correction was applied to *dfs* and *ps* adjusted accordingly. Correlation coefficients are Pearson's *r* and case-wise diagnostics were used to determine the presence of outliers that might have influenced any significant effects. Day 1 intrusion counts were transformed $(\mathrm{Log}_{10}x_i + 1)$ for correlation analyses.

Given the absence of valid vividness and distress ratings later in the week, when intrusion counts tended towards 0, linear mixed models were used to analyse these variables. Missing intrusion counts were rare (1.1%) and conservatively replaced with the next day's value (usually '0'). Given the non-zero inflated, over-dispersed nature of the intrusion counts (dispersion parameter $\alpha>0$, $p<0.001$), mixed effects negative binomial regression was used to analyse counts. 'Participant' and Day were random factors and day was treated as continuous. Random intercept models were compared to random slopes models, with and without covariates, and interaction terms. Sequential change in model-fit was assessed using Akaike's Information Criterion (AIC). Peri-film (T2) HR and cortisol were of special interest as covariates based on their demonstrated influence on intrusion characteristics(Chou, La Marca, Steptoe, & Brewin, 2014; Chou, Marca, Steptoe, & Brewin, 2014) and their potential interaction with subsequent propranolol and hydrocortisone treatment respectively. The influence of baseline anxiety (STAI) and depression (BDI) were also explored, given their reliable association with intrusive memories (Marks, Franklin, & Zoellner, 2018). Since STAI and BDI scores did not improve model fit, the effects of these are not discussed further.

SUPPLEMENTARY RESULTS

Baseline characteristics

Groups were well matched on demographic and psychological variables (Table 1 in the main paper). Due to the large number of cells, ethnicity data (South Asian, (South) East Asian, Black, White or mixed race/other) is not presented in Table 1 (main paper). However, ethnicity was balanced across groups (Fisher's exact test=12.150, p=0.099).

Critically, the number of hours between film viewing and diary completion on day 1 (i.e. the period over which intrusive memories could be retrieved and rehearsed; 'post-film period on day 1' in Table 1) did not differ between groups. Neither was there a difference in quality (Fisher's Exact Test=4.387, p=0.654) or amount of sleep (F(2,86)=0.196, p=0.822) on the night of day 1.

Subjective and physiological response to film and drugs

As in most previous studies with these drugs(Sijbrandij, Kleiboer, Bisson, Barbui, & Cuijpers, 2015), no adverse effects were reported. PANAS-negative scores showed no Group effects (F values ≤1.70, p values≥0.153), but a main effect of Time (F(1.47, 124.56)=205.062, p<0.001, η_p^2=0.707), reflecting an increase from T1: 12.51 ± 3.19 (M ± SD) to T2: 24.61 ± 7.87 (p<0.001), and return to baseline at T3 (13.48 ± 4.32). PANAS-positive also showed no Group effects (F values ≤0.358, ps≥0.716), but a main effect of Time (F(2,170)=93.566, p<0.001, η_p^2=0.524), with deterioration in positive affect between T1 (28.36 ± 7.80) and T2 (19.59 ± 5.99; p<0.001), maintained at T3 (20.74 ± 7.85; p=0.247). No significant Group x Time interactions were found on any BSS items, which, with the exception of drowsiness (which increased equivalently in all groups), were generally at floor level (i.e. <10 on a 0-100 scale) across T1-T3. The absence of reported changes in bodily symptoms was consistent with chance level correct guessing on treatment by participants, (29.54%; χ^2(4)=2.513, p=0.642) and experimenters (37.5%; χ^2(4)=3.206, p=0.524).

A Time x Group interaction on HR (F(3.20, 132.71)=7.730, p<0.001, η_p^2=0.157; Figure 2A), was driven by a drop between T2 and T3 (p<0.001) only after propranolol. Systolic BP also showed a Time x Group interaction (F(4,168)=3.766, p=0.006) driven by a reduction in systolic-BP only in the propranolol group between T2 and T3 (p<0.001). Baseline salivary cortisol levels (0.144 µg/dl) were in the expected range (Miller et al., 2016). A Time x Group interaction on cortisol levels (F(2.01, 80.37)=16.78, p<0.001, η_p^2=0.296), reflected a T2 to T3 increase only after hydrocortisone (p<0.001; Figure 2B).

Attention to film: Eye tracking

There were no significant group differences in (pre-drug) attentional parameters during film viewing. The averaged dwell-time on AOI for placebo (2.54 ± 1.84 s) was not significantly different to that for propranolol (1.88 ± 2.18 s) or hydrocortisone (2.59 ± 2.35 s; F(2,83)=0.983, p=0.379). Similarly, the number of fixations per AOI in the placebo group (8.02 ± 5.99) did not differ relative to propranolol (5.79 ± 6.29) or hydrocortisone (7.72 ± 6.33; F(2,85)=1.132, p=0.327).

Involuntary memory: intrusion frequency

The main results section (Figure 3A) illustrates the predicted intrusion values based on a mixed effects model. Supplementary Table 1 shows the raw means (± SEM) for the three groups (mean number of intrusions by Day).

	Day						
	D1	D2	D3	D4	D5	D6	D7
Placebo	4.97 ± 0.70	5.10 ± 1.21	2.62 ± 0.63	1.59 ± 0.40	1.31 ± 0.32	1.03 ± 0.27	1.07 ± 0.28
Propranolol	3.00 ± 0.62	2.97 ± 0.68	1.73 ± 0.43	0.97 ± 0.23	0.63 ± 0.21	0.43 ± 0.15	0.23 ± 0.12
Hydrocortisone	2.21 ± 0.33	2.28 ± 0.58	1.48 ± 0.41	0.69 ± 0.24	0.62 ± 0.16	0.31 ± 0.10	0.28 ± 0.10

Table S1. Raw means ± SEMs for intrusion counts per Group by Day.

A compact, random intercept model, with Day and Group as fixed factors was tested first (AIC=1788.57). In the absence of any covariates, this baseline model showed main effects of Group and Day (*p* values <0.01). Model fit was improved by inclusion of a random slope (AIC=1780.23; Group and Day effects: *p* values <0.0183) and addition of peri-film cortisol and HR (but not their interaction, nor the interaction between Group and Day) substantially further improved this model (AIC=1660.21). Predicted intrusion counts based on this model are shown in Figure 3A and parameter estimates, described in the main results section of the paper.

The correlation between intrusion frequency (log transformed) and salivary cortisol at T3 was significant (r=0.48, p=0.01). This illustrated in Figure S1. Note, case-wise regression diagnostics showed that no residuals exceeded |1.88| suggesting an absence of influential cases.

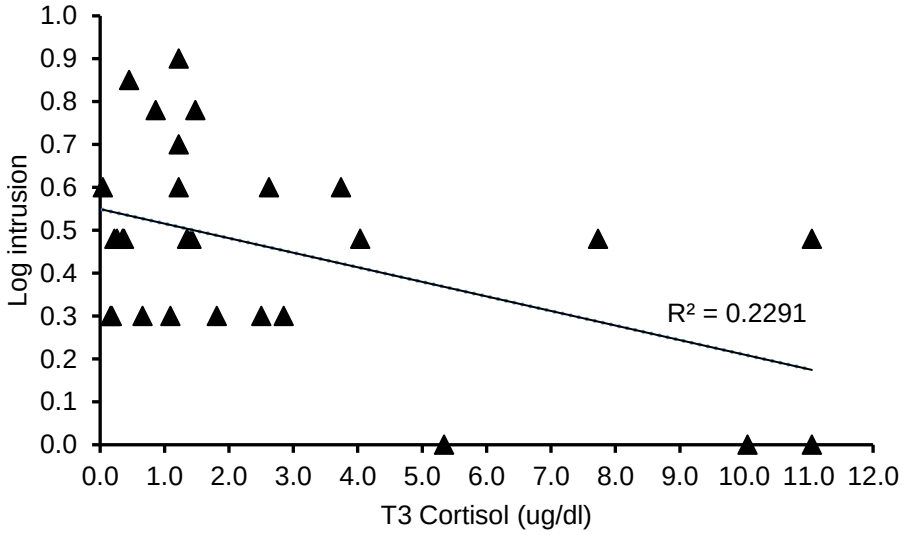


Figure S1: Relationship between intrusion counts (log transformed) and salivary cortisol.

Involuntary memory: vividness

The baseline model for vividness also included Day and Group as fixed factors, with random intercepts for Participant (AIC=889.91). The model was improved by including a random slope and a Day x Group interaction (AIC=883.81; Day and Group main effects and their interaction were significant: $p<0.037$). However, the best fitting model, with random slopes, again also included peri-film HR and cortisol as covariates (as above for intrusion frequency), along with the Day x Group interaction term (AIC=827.758). Parameter estimates for this model are described in the main text.

Involantary memory: distress

The same model specifications as used in the vividness mixed effects model above were used to analyse intrusion-related distress. Again, the inclusion of a Day x Group interaction and covariates improved the model (AIC=775.44) relative to the fixed effects model without covariates (AIC=834.59). However, as noted in the main results section, the interaction was not significant. The best fit model is shown in Figure S2.

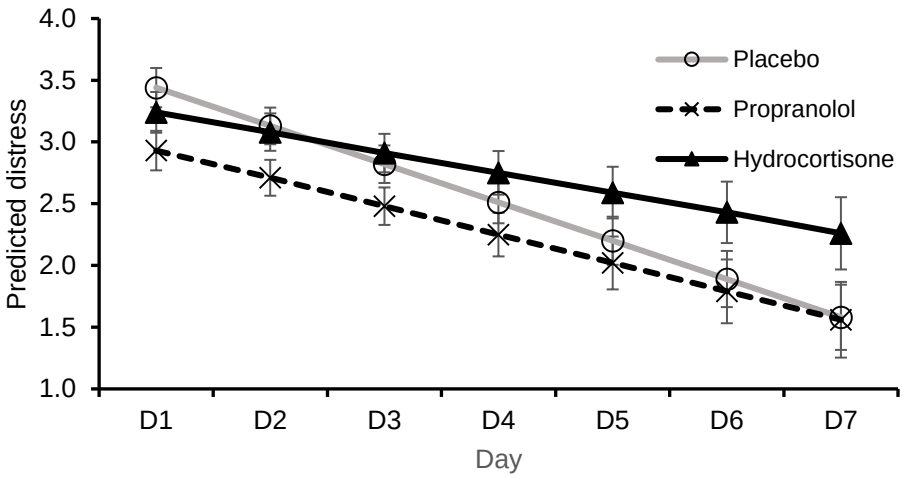


Figure S2: Predicted intrusion-related distress (mean ± SEM). The fitted model included T2 HR and cortisol as covariates.

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