Title:

Antidepressant suppression of REM and spindle sleep impairs hippocampus-dependent learning and memory but fosters striatal-dependent strategies

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ABSTRACT

REM sleep enhances hippocampus-dependent associative memory but has little impact on striatal-dependent procedural learning[1-3]. Antidepressant medications like desipramine (DMI) inhibit rapid-eye-movement (REM) sleep but it is little understood how antidepressant treatments affect learning. We found that DMI strongly suppressed REM sleep in rats for several hours and impaired reconsolidation of a familiar maze and consolidation of moved baited positions (reversal learning) in a sleep-dependent fashion. Unexpectedly, DMI also reduced the spindle-rich transition-to-REM sleep state (TR) and spatial memory changes were more related to TR than to REM sleep. Working memory was unaffected, but overnight reference memory was significantly impaired and subjects increased reliance on non-hippocampal strategies. Procedural memory performance was positively correlated with increases in non-REM sleep after DMI serving to offset memory declines, partially preserving performance. Our results suggest that familiar memories are reconsolidated during REM sleep, reversal memories consolidated during TR, and procedural memories consolidated during non-REM sleep.

We tested the involvement of REM sleep in the process by which memories are rendered stable (consolidation) by looking at REM sleep suppression using a commonly prescribed selective norepinephrine reuptake inhibitor and REM sleep suppressing antidepressant, desipramine. Studies have shown that short term REM sleep deprivation following new learning impairs hippocampus dependent spatial learning and memory, depending on the task and intensity of training[1-3]. Striatal dependent procedural learning is largely unaffected by REM sleep deprivation[1-5]. Arguments against the role of REM sleep in memory consolidation include the observation that instrumental REM sleep deprivation can be stressful[6,7] thereby confounding the effects of sleep manipulation with the negative effects of stress on memory[8,9]. Additionally, individuals using REM sleep suppressing antidepressants do not report marked reductions in memory function beyond those already associated with depression, although some have been documented[10]. We chose to address both issues using DMI as a non-instrumental REM sleep deprivation technique that is commonly employed in humans patients to treat depressive symptoms with a relatively low side effect profile[11].

DMI inhibits of the reuptake of noradrenaline (NA) thus elevating NA levels at all targets of the locus ceoruleus. Elevated levels of NA actively suppress REM sleep by acting in the pons to inactivate REM-ON cholinergic neurons[12]. Here we report for the first time that REM suppression impairs reconsolidation of a familiar recalled memory, and provide evidence that the often overlooked TR state is involved in the process of reversal learning[2] (learning a new response in a familiar context). Both tasks are dependent on normal hippocampal activity[13,14]. Finally, we contrasted hippocampal learning effects of DMI-induced sleep changes with striatal learning effects using a procedural fixed choice T-maze task.

RESULTS

DMI inhibits REM and TR sleep

DMI taken orally reduces REM sleep for 5-8 hours in patients being treated for depression[15]. A 10 mg/kg DMI dose p.o. ingested by our subjects suppressed REM sleep for 8 hours (Figure 1a, $p = 0.0006$ repeated measures MANOVA) with no discernable impacts on health or ambulatory activity. Continuous recordings in 8 subjects showed that time spent in REM sleep went from an average of 8.35 ± 1.24 % during the light phase (measured on the second day of baseline conditions) to 3.51 ± 1.22 % after administration of DMI ($p = 0.00038$, repeated measures ANOVA, baseline vs. DMI treatment day effect, Figure 1a). Within the first 6 hours post-training, i.e., the REM sleep critical window ascertained for this training regimen[1], REM sleep was reduced 95.6 +/- 0.22% ($p = 3.84 \times 10^{-8}$, Figure 1e) with no diminution across testing days (Figure 1f, $p = 0.95$, paired t-test on 1st vs. last day and Figure 1d). DMI administration also produced a significant ($p = 0.03$, paired 2-tailed t-test) decrease in the number of REM episodes relative to the same circadian time under baseline sleep conditions.

Insert Figure 1 here

DMI treatment does not impair activity or motivation

Twenty-four rats were tested under conditions of 10 mg/kg DMI or Control mash (Supplemental Figure 1) after performing a familiar and reversal (novel) 8-box mazes for 30 min each and 15 trials of a fixed choice T-maze task each day for 5 days. Both DMI and Control groups ran a similar number of laps on the 8-box mazes each test day and between days (Novel maze, Control 14.8 vs. DMI 14.2 laps, Wilcoxon Sign-Rank test $p = 0.9387$, 1st day 14.8 vs. last day 14.3 laps, $p = 0.365$; Familiar maze, Control 18.8 vs. DMI 18 laps, $p = 0.330$, 1st day 18.3 vs. last day 18.5 laps, $p = 0.461$). Controls and DMI treated rats also did not significantly differ in weight (day 5:

Control = 336 +/- 14.8 mean grams +/- SEM; DMI = 326.8 +/- 9.23; $p = 0.389$, t-test) indicating that maze sampling experience and hunger related learning motivation did not differ between groups.

DMI impairs novel spatial learning and familiar reconsolidation

Under DMI treatment, subjects showed significant performance deficits on novel reversal learning and familiar maze reconsolidation. A general linear model with repeated measures showed a performance effect of drug treatment: DMI performance was worse than controls ($p = 0.001$). Performance was better on the familiar vs. novel maze ($p = 0.000$), and DMI had the same negative effect on performance on both mazes as there was no drug by maze interaction ($p = 0.759$). Within subjects there was a powerful learning effect of training day ($p = 0.000$) that was dependent on whether the maze was novel (improved daily performance) or familiar (less marked daily improvement), as shown in a day by maze interaction ($p = 0.000$), and this day effect persisted independent of DMI administration (day by drug interaction $p = 0.143$) regardless of the maze run (day by drug by maze interaction $p = 0.705$).

Insert Figure 2 here

Reversal learning impaired by DMI treatment TR loss

For novel spatial learning on the reversal task (Figure 2a), the DMI group never reached criterion, always performing >1 mean error/lap even after 5 days (Figure 2a and Figure 4c). Interestingly, performance deficits under DMI treatment were not correlated with the level of REM sleep suppression on this reversal learning task (Supplemental Figure 2c) but instead were highly correlated with drops in TR under DMI treatment (Figure 2c and Table 1). Consistent with DMI's impact on REM sleep, DMI also attenuated the amount of TR sleep (Figure 2d) by a range of 13-75% in the first 12 h after treatment. TR and REM sleep can be independently regulated

(Supplemental Figure 5d,e); TR often occurs without a subsequent REM period and REM sleep can occur with an abbreviated TR state.

Within a single running session, both Control and DMI treatment conditions showed significant improvement of, on average, ~1 error/lap (Control 0.92 +/- 0.36, DMI 0.71 +/- 0.30, MANOVA, no group effect $p = 0.6423$, no day effect $p = 0.2031$, and no day by group interaction $p = 0.996$; supplemental Figure 3c and d). Thus, working and short term memory systems do not appear to be influenced by the prior day's DMI treatment.

The source of the learning deficit for DMI treated animals on the novel maze was poor retention across the night. Subjects given DMI committed 0.5 +/- 0.20 more errors on the first 3 laps than on the last 3 laps of the previous day. Controls, however, made 0.32 +/- 0.20 fewer errors than the last laps of the day prior (MANOVA drug effect $p = 0.0217$). There was no significant day effect ($p = 0.0825$) or day by drug interaction ($p = 0.1809$) in this retention measure, meaning that DMI affected reference memory retention on all days.

The DMI treatment condition also increased the utilization of non-hippocampal or procedural strategies to locate the positions of new food boxes, as revealed by a significant increase in errors after a 180° maze rotation between laps 10 and 11. For this rotation, baited boxes remained in the same allocentric positions but the boxes that held the food was changed, pitting non-hippocampus dependent local cue use against hippocampus-dependent allocentric spatial map strategies (Figure 2b; see methods for details). Reversal learning was thus attenuated by DMI treatment in a sleep-dependent fashion, with the most relevant sleep metric being the length of time spent in the transition to REM sleep state during DMI after training each day.

Reconsolidation impaired by DMI treatment

Performance significantly worsened on the familiar maze when 10 mg/kg desipramine was administered after each training session (DMI negative slope curve fit 1st order polynomial $R^2 = 0.75$). By contrast, control performance held steady (Figure 3a, repeated measures ANOVA DMI vs. Control, $p = 0.005$). There was no performance difference between groups on the first day of the familiar maze ($p = 0.34$, t-test), but by day 5 the performance difference between groups was a full error per lap ($p = 0.001$, t-test). The relative preservation of performance was greatest for those DMI treated subjects that showed the least attenuation of REM sleep (Figure 3c). However, the strongest correlation between sleep measures and familiar maze performance was the percent time spent in TR during testing. Unlike the novel maze performance, this correlation was negative. That is, those subjects with the best performance had the least % TR during DMI treatment and those whose performance worsened most over the 5 days were those with the most %TR during DMI (Table 1 and Figure 3d). Thus, the more TR was preserved during treatment the better the consolidation of novel learning (Figure 2c), and the worse reconsolidation of familiar memory.

Insert Figure 3 here

As on the novel maze task, DMI treated subjects performing on the familiar maze were unable to effectively utilize reference memory to maintain performance overnight as evident by an increase in the number of errors on the first few laps from day 1 to day 5 (Figure 4a vs. 4c and Supplemental Figure 3a vs. 3b). Animals treated with DMI had more trouble retrieving correct reward box locations between days on the familiar maze than when not being treated with DMI (ANOVA $p = 0.0001$). DMI performance worsened by 1.19 +/- 0.29 errors in the first lap compared with the last lap of the prior day. Control performance improved by 0.16 +/- 0.14 errors overnight. Thus reference memory errors contributed to the degraded reconsolidation during DMI treatment.

Insert Figure 4 here

DMI treatment also resulted in more errors after the maze rotation in the familiar maze condition ($p = 0.0059$, Figure 3b) revealing increased use of non-hippocampal strategies. Animals also committed more errors on lap 11 than lap 10 under Control conditions ($p = 0.042$) but remained within criterion (<1 error/lap) performance throughout.

Under both treatments, animals improved by an average of about an error per lap between the first and final lap of the training session, showing the ability to improve performance based on working memory (DMI improved 1.13 +/- 0.20 errors/lap, Controls 0.96 +/- 0.23, mean +/- SEM) with no day effect (ANOVA $p = 0.7132$) and no group effect (ANOVA $p = 0.5762$).

DMI spatial memory related to REM and TR suppression, not QS

Together these results indicate that decreases in spatial maze performance under DMI treatment reflect memory consolidation or reconsolidation errors associated with REM and TR sleep changes. Neither familiar nor novel maze performance was related to non-REM sleep (here referred to as quiet sleep, QS). The Pearson correlations (R) of familiar and novel maze improvement vs. QS ranged from -0.46 ($p = 0.24$) to 0.01 ($p = 0.98$) for correlations with QS at baseline, during DMI and % change between conditions (data not shown). Consolidation of reversal learning was correlated with preservation of TR and reconsolidation of the familiar maze was associated with the preservation of REM sleep. On both familiar and novel mazes (Figure 4d), Control conditions showed significant improvement from day 1 (Figure 4b vs. 4d) and above criterion performance on all but the first two laps by day 5, whereas under DMI treatment, subjects were unable to achieve criterion performance (Figure 4c). Finally, on both familiar and novel mazes, alternative non-hippocampal performance strategies were utilized more heavily under DMI-induced REM sleep deprivation.

DMI procedural memory correlated to sleep parameters

In sharp contrast with the effects of DMI on sleep and hippocampus dependent learning performance, DMI treatment performance on the procedural T-maze task was significantly correlated to the level of QS demonstrated by each subject during testing (Figure 5e) and independent of changes in REM sleep during the testing period (Figure 5 b,c). Treated subjects with the largest % increases in QS during DMI administration performed well on the T-maze whereas those with declines in QS made far fewer correct choices (Figure 5f). Although REM sleep during testing was not related to procedural task performance during DMI treatment, the percent REM sleep obtained during the baseline recordings was related to later T-maze performance (Figure 5a). More surprising was that the amount of REM sleep during baseline was inversely correlated with the % change in QS during DMI treatment ($R = 0.8942$, $p = 0.0066$, Supplemental Figure 5b). That is, the more REM sleep a subject showed during baseline, the higher the percent of QS during DMI and the better the procedural performance. Little relationship has been found between the amounts of REM sleep and non-REM sleep under normal conditions[16,17], much less a relationship separated by days and experimental treatments.

Insert Figure 5 here

The number of correct choices and the latency to trial completion were highly correlated, as expected (Supplemental Figure 5a). Thus, those sleep parameters that were correlated with the number of correct T-maze choices were often also significantly correlated (inversely) with latency to trial completion (Table 1). T-maze performance was not correlated with the amount of waking or TR sleep obtained during baseline or testing periods (Supplemental Figure 4). DMI, then, increased procedural T-maze performance in a sleep dependent fashion according to the amount of REM sleep during baseline and QS during treatment.

DISCUSSION

Overall, our main hypothesis was strongly supported: DMI suppressed REM sleep and inhibited hippocampus-dependent learning during antidepressant treatment. Both spatial reversal learning and reconsolidation of a familiar maze were significantly impaired, although compensatory non-hippocampal strategies partially rescued performance within a session. Surprisingly, we found that impairments in reversal task learning were best correlated with declines in the amount of the TR state rather than decreases in REM sleep. The subjects with the least %reduction in TR following DMI treatment show reversal learning performances most similar to the control condition: improving performance by an error or more per lap across the 5 day period on the reversal learning task (Figure 2c). Subjects with declines in TR under DMI treatment showed substantially slower learning rates on the novel maze (0.5 errors/lap or less improvement). Conversely, reconsolidation of a familiar memory was poorest in those with the highest retention of the TR state during DMI testing (Figure 3d), as though processes during TR which best predicted successful incorporation of novel information were also unsupportive of the maintenance of an older schema. Reconsolidation of the familiar maze was, as predicted, also associated with the degree of REM sleep disturbance during antidepressants treatment (Figure 3c). Procedural learning, however, was enhanced with augmented QS during testing (Figure 5e,f) and, surprisingly, by the amount of pre-test baseline REM sleep (Figure 5a). Together, our findings suggest that the consolidation of novel reversal learning, reconsolidation of familiar memories, and consolidation of procedural memories may involve routines differentially called during different sleep states, with reversal consolidation occurring during TR (spindles), reconsolidation during REM sleep, and striatum-dependent procedural consolidation during QS.

REM sleep supports reconsolidation

In a human fMRI study, REM sleep deprivation is coincident with a decrease in hippocampal function[18]. Indeed, induced neural activity during REM sleep has been shown to drive changes in synaptic weights[19]. Reactivation of neurons encoding a memory network occurs during REM sleep[20] in a fashion supporting both LTP of new learning and the reversal of LTP for consolidated memories within the novelty encoding pathway[21]. Thus memories are likely revisited and reshaped during REM sleep outside of the original learning experience[22]. NA and DMI would impair the production of REM sleep through inhibition of REM-on cells in the pons. The prevention of REM sleep also prevents all REM sleep related processes including hippocampal reactivation, REM theta, and theta phase activity that support depotentiation (DP). These results for learning agree with those seen with REM sleep deprivation effects on hippocampal function *in vitro* through loss of induction or maintenance of long term potentiation (LTP)[23,24] and reductions in excitability[24]. Without timely opportunity to enter REM sleep and accomplish synapse-specific strengthening or weakening, consolidation and reconsolidation could be impaired. In the closest study to ours with a once daily 10 mg/kg dose testing rats on an 8-arm radial maze similar impairments in learning were found[25]; presumably REM sleep was also suppressed in their study.

TR sleep supports reversal learning but impairs reconsolidation

Our data also suggest that consolidation of novel reversal learning and reconsolidation of familiar places are both proportional to the amount of TR sleep, though in opposite directions. Reactivation of hippocampal neurons and interaction with prefrontal cortex is uniquely intense during sleep spindles, possibly supporting memory consolidation[26,27]. Sleep spindles, which characterize the TR state, do not occur when NA, acetylcholine (ACh) or serotonin is present in the thalamus[28]. The DMI block of

NA reuptake during TR could decimate spindles and the hippocampal-forebrain reverberation that accompany them to incorporate novel information into established consolidated memory networks. Increases or a relative preservation of TR may maintain sufficient spindle-related reverberation to support remodeling of the synaptic memory network. Maintenance of familiar memories in the face of daily novel learning suffered in subjects with higher % TR during DMI. Finally, like REM sleep, TR is a state where P-waves are present, and P-wave density during TR and REM sleep have been strongly correlated with learning novel relationships[29,30]. Taken together, perhaps the strong correlation between novel learning and TR and the negative association with familiar memories through increased novel interference with familiar map memories, is not so surprising. These results also agree with recent findings on the function of sleep spindles[31] in humans. However, little is known about this transient sleep state and its interaction with mechanisms of learning[22].

QS during consolidation supports dorsal striatal learning

Procedural learning, was, in our hands, enhanced proportionately with the degree of increase in QS under DMI treatment. Quiet sleep (QS) dependent consolidation in the striatum may depend on other processes such as changes in dopamine or acetylcholine levels during QS. Increases in QS have been shown to correlate with other procedural learning tasks such as soccer playing and tumbling on a trampoline[32]. Also, acquisition of tasks that depend on the dorsal striatum (e.g. caudate and putamen), like learning to always turn left at a T-maze junction[33,34], often benefit when the competing hippocampal strategy is impaired[35,36]. In fact, striatal dependent learning strategies may be enhanced by REM sleep deprivation because they are enhanced by hippocampal inactivation.

Basal REM sleep supports dorsal striatal procedural learning

Another group also found a positive correlation between the amounts of baseline REM sleep and striatal learning performance, the swim path length on a visible platform Morris water maze. Interestingly, amounts of serotonin in the striatal caudate structure correlated with the amounts of baseline REM sleep and the numbers of 5HT-2 receptors in the caudate correlated with this visible platform water maze task, implicating some function for baseline REM sleep and caudate serotonin levels in the motor task[37] just as we found the relationship between baseline REM sleep and our striatal dependent fixed choice T-maze task. It would be interesting to test whether basal amounts of REM sleep correlate with all striatal-dependent learning tasks and whether serotonin is key to that involvement.

Unified theory of sleep-dependent memory consolidation

These results fit into a physiological conceptualization of sleep consolidation as depicted in Figure 6. The unifying principle underlying the TR- and REM–related reversal and reconsolidation learning benefits is based on literature demonstrating that the noradrenergic (NA) cells of the locus coeruleus (LC) are only off during the initialization of the characteristic spindles (10-15 Hz large amplitude waves that last 1-2 seconds) of TR and during REM sleep[38]. The absence of NA is necessary for synaptic depotentiation (DP), and DP is necessary alongside its opposite, LTP to efficiently incorporate newly learned information into previously established memory networks[39-41]. Specifically, NA acts at beta adrenergic receptors to block the action of calcineurin. The activation of calcineurin leads to the dephosphorylation of CAMK-II and MAPK, whose phosphorylation is thought to sustain LTP (see [42]). When NA blocks calcineurin, it blocks one half of the process of reshaping a synaptic network: the deconstruction side of remodeling, DP. Thus, DMI enhancement of NA levels supports synaptic strengthening (LTP), while preventing depotentiation in the hippocampus[43,44]. Striatal dependent tasks may be immune to REM and TR sleep

deprivation because the LC NA system does not project to the dorsal striatum[45] and the dorsal striatum does not respond to NA[46], and without NA, DP may always be possible. LC targets of the hippocampus and prefrontal cortex, however, may require the LC silence of TR and REM sleep as the only states that support efficient remodeling of memory networks for learning.

Insert Figure 6 here

DMI sleep effects parallel human antidepressant use

These findings support those found by Rasch et al.[47] who demonstrated that antidepressants enhanced procedural learning. Their study and ours indicate that the effects of REM suppression on striatum dependent procedural memory are quite different than on hippocampus dependent spatial memory. Some antidepressants, but not others, have been reported to improve memory function[48], which may depend on the sleep outcomes of the particular antidepressant and the memory function tested.

Antidepressant administration is an effective method of reducing REM sleep[49]. Stress is unlikely to mediate the changes seen under DMI treatment. Studies showing stress effects of REM sleep deprivation use a movement-restricting instrumental REM sleep deprivation continuously for many days[7]. This profile is quite unlike the partial-day non-instrumental REM deprivation method used in this study and unlike the 4-6 h REM low water under multiple platform restriction method we have used in the past[1,2], which likely avoids activation of the HPA axis. We were able to use a dose resulting in transient REM reduction that fully dissipated by 10 hours then returned to normal and produced this effect repeatedly across all 4 days of treatment. Both the pharmokinetics (despiramine's half-life of 4.6 h) [47] and the general effects on sleep (8-10 h) architecture based on our results indicate that DMI's action had ceased many hours before the following day's run and thus did not likely mediate the change in

daily performance directly, but rather worked during the post-learning consolidation period when DMI actively suppressed REM sleep.

Desipramine suppresses human REM sleep most strongly in the initial 3 weeks, whereafter REM sleep activity begins to increase slightly toward normal levels despite continued chronic administration [15]. If the REM sleep state itself is responsible for hippocampal memory consolidation, then consolidation related performance deficits may improve to somewhat several weeks into treatment coincident with the partial restoration of REM sleep: a hypothesis that remains to be tested. Additionally, we determined that although REM sleep suppressing antidepressant treatments impaired hippocampus-dependent learning, alternative strategies were augmented which improve task performance. Patients taking REM-suppressing antidepressants may also benefit from selecting alternative, REM-immune, non-hippocampal strategies to solve the learning problems at hand.

SUMMARY

Overall, these spatial and procedural learning results offer additional insight into our understanding of how different sleep states may suppress or augment the consolidation of memories during sleep. This is the first measure of reconsolidation memory under REM sleep deprivation of any kind, and the first test of antidepressant medication effects on the recall of familiar memories. Our results indicate that the sleep period following the recall of familiar memories is important for the reconsolidation process in a manner previously shown for the formation of an original memory.

Although the subjects used in the present study were normal rats rather than depressed humans, the profound effect we observed of this standard dose of desipramine on sleep and hippocampal dependent memory vs. striatal dependent memory suggests that further careful testing should be conducted on sleep-dependent

learning strategy selections in normal humans as well as those suffering with depression.

Future studies could offer insight into whether other antidepressants also negatively affect hippocampus dependent memories while benefiting procedural learning. Further, these results, taken together, lead us to newly propose the specific sleep stages that may be most important for consolidating different kinds of sometimes competing memory tasks, with REM sleep reconsolidating familiar memories, TR consolidating novel reversal learning at the expense of the old familiar memory, and non-REM quiet sleep enhancing procedural strategies.

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FIGURE LEGENDS

Figure 1. (**a**) 30 min after lights on rats were trained on the 8-box maze (familiar, followed by novel) and T-maze tasks. Testing on each apparatus took approximately 30 minutes. Animals were then given 10 mg/kg desipramine (DMI) in sweetened mash or Control sweetened mash, returned to their home cages, and their sleep recorded for 16 h. The % time spent in REM sleep was calculated for every 2 h time block, averaged per condition, and displayed with +/- SEM bars. The time the lights went off is depicted in a blue bar above the graph. (**b**) Hypnogram of sleep states (vertical axis) over 8 hours (horizontal axis) after training in one rat during baseline and (**c**) the same rat after training and 10 mg/kg desipramine ingestion. With 10 mg/kg DMI after training, mean REM sleep time suffered a mean 63.9 % reduction

(range 22.7% to 93.8%) in the 10-12 h light (sleep) period after the maze run compared with each animal's own baseline at the same circadian time. (**d**) % time of the sleep (light) period spent in REM sleep during baseline and sequential days of 10 mg/kg desipramine administration (n=10). (**e**) Percent recording time spent in REM sleep during the 1st 6 h critical REM sleep window (grey bar below 1st 6 h of (a)), after training with desipramine or control mash treatment ($p = 0.00000004$). (**f**) No REM sleep adaptation to desipramine was present during the critical window from the first to the last day of testing ($p = 0.95$, t-Test).

Figure 2. Effect of 10 mg/kg desipramine (DMI, filled squares) on reversal learning (Novel). (**a**) Average number of errors committed per lap declined half as quickly under DMI as compared to control treatment and did not reach criterion within the 5 days of training. (**b**) Errors after maze rotation reveal use of simple cues rather than allocentric hippocampal strategies to solve the spatial task. DMI treated rats showed increased errors after maze rotations on the Novel maze (* = $p < 0.05$). (**c**) The highest performance gains on the novel maze during DMI treatment were made by those animals with the smallest declines in the transition to REM sleep (TR) state compared to their baseline amounts. (**d**) Mean losses in the TR state under DMI were 30.8% on average (paired t-test p=0.016) with all animals expressing a loss between 75% to 13% TR with the exception of a single animal showing an increase of 23% more TR state under DMI.

Figure 3. Effect of 10 mg/kg desipramine (DMI, filled squares) on familiar spatial maze reconsolidation. (**a**) Desipramine worsened performance on the already consolidated familiar maze configuration whereas non-treated animals continued to make small improvements on the Familiar maze over the same time period. (**b**) DMI treated rats showed increased errors after rotations of the Familiar maze revealing increased use

of non-hippocampal strategies. Controls also had significantly more errors after maze rotation on the familiar maze but even so performed better than the 1 error per lap criterion (** = $p < 0.01$). (**c**) The positive correlation between a change in REM amounts and performance on the familiar maze reconsolidation task ($R = 0.8326$, $p = 0.0103$) was driven by the two subjects least responsive to DMI. The subject with highest improvement was eliminated from Familiar maze comparisons due to an inadvertent 5 day prior reversal task without familiar rehearsal. (**d**) The largest performance losses on the familiar maze were made by animals with the highest amounts of TR sleep during DMI treatment.

Figure 4. Lap-by-lap performance on the first (**a, b**) and last (**c**,**d**) days of 8-box training. The dashed line represents criterion performance. The increase in errors/day between **a** and **c,** especially in the first few laps, reveal deficits in reference memory between days, while a drop in errors across laps within each day suggests normal within session learning based on working memory. Rats were removed from the maze to a resting pot for 2 min between laps 5 and 6, and removed for rotation of the maze between laps 10 and 11. Increased errors on lap 6 vs. 5 reveal a short term memory deficit and increased errors on lap 11 vs. lap 10 reveal use of local or egocentric cues rather than hippocampal dependent allocentric cues. All groups showed intact working and short term memory as there were always declines in errors across the first 10 laps without consistent increases in errors after the lap 5 rest.

Figure 5. Performance on the T-maze procedural task correlated with the amount of REM sleep during the baseline sleep period (**a**) the amount of QS during the DMI testing period (**e**), and the % change in QS from baseline to DMI testing (**f**), but not with any other sleep parameter measured. See Supplemental Figure 5 for QW and TR comparisons. Performance on the procedural task was not correlated with % REM

sleep during testing, the % change in REM sleep from baseline to testing, or the % QS during baseline (**b**, **c, d**).

Figure 6. Model schematic of our results (outlined yellow arrows and lines) in a physiological conceptualization of sleep-dependent memory consolidation. T-capped lines show inhibitory relationships. Locus ceoruleus (LC) noradrenergic (NA) cells inhibit production of thalamic spindles and therefore TR and inhibit production of REM sleep in the pontine reticular formation (PRF). DMI (yellow molecules) prolongs NA effects at synapses and potentiate NA effects to inhibit TR and REM. Reduced consolidation of reversal learning was proportional to the reduction of TR (yellow arrows with purple outlines). DMI would enhance NA prevention of spindles and depotentiation (DP) processes (yellow "x"). Information transfer from the hippocampus to the prefrontal cortex (PFC), important for reversal learning, is uniquely strong in TR sleep. DMI would impair REM sleep and all REM sleep related processes including reactivation and theta-associated bidirectional plasticity (LTP and DP). Reconsolidation was impaired proportionate to REM sleep reduction from baseline (yellow arrows with red outlines). In the striatum, NA has no effect[46] and DP may occur any time. Procedural learning is proportionate to the amount of Quiet sleep (QS) (yellow arrow, blue outline) which is inversely proportionate to the amount of REM sleep and correlated with the amount of TR suppression. Mutually competing striatal and hippocampal strategy selections are shown in the T-capped line between structures.

METHODS

Animals and materials

Thirty male Fisher 344 rats aged 6 months and weighing approximately 250-350g (Simonsen: Gilroy, CA) were individually housed in plastic cages (45.7 x 24.1 x 20.3 cm) with shaved wood bedding in a climate controlled (22 ± 3C°, and 73 ± 5% humidity) chamber. The chambers ran on a 12:12h light/dark cycle. Food and water were available *ad libitum*. The visible platform version of the Morris water maze task was administered to test visual acuity (Supplemental Figure 4c). For this task, animals were placed in a circular tank of 24° C water and had to locate and swim to a highly visible platform 2 cm above the surface of the water to escape. The animals swam 10 trials a day for two consecutive days, and all but 1 were selected to continue the study as their performance was within 2 SD of the average latency to reach the platform. Proficient performance on this visible platform version of the water maze was essential for their continuation in the study due to the need to use visible distal spatial cues to successfully solve the 8-box maze.

Electrode implantation

All 30 animals were given antibiotic an analgesic and anesthetized with 60 mg/kg sodium pentobarbital and then placed in a stereotaxic apparatus. Four miniature screw electrodes were bilaterally implanted for electroencephalography (EEG) monitoring: 2 frontal cortex screw electrodes and 2 hippocampal screw electrodes. Two electrodes were threaded into the dorsal neck muscles to record electromyography (EMG). All electrodes were crimped to gold pins threaded to a plastic pedestal head stage (Plastics One, Roanoke, VA) and the entire assembly was affixed to the cranium with dental acrylic. Sutures closed the skin around the implant. Animals were monitored until they were responsive and mobile then returned to their home cage to recover, continuing on antibiotic and analgesic for 3 days then left undisturbed an additional 4 with *ad libitum* food and water. All animal

procedures were carried out in accordance with the National Institute of Health guide for the care and use of laboratory animals and in accordance with the University of Michigan Committee on the Use and Care of Laboratory Animals.

Nine rats were used for an initial DMI dose-response study. Rats were given DMI p.o. at either 8 (n=3), 10 (n=3) or 23 (n=3) mg/kg within 20 min after running three mazes (Familiar spatial, reversal spatial, and procedural T-maze). At 10 mg/kg, mean REM sleep time suffered a mean 63.9 % reduction (range 22.7% to 93.8%) in the 10-12 h light (sleep) period after the maze run compared with each animal's own baseline at the same circadian time (Supplemental Figure 1a vs. b). As the 10 mg/kg reduction in REM sleep time was comparable to that of human whereas the 8 mg/kg dose had little effect on sleep and the 20 mg/kg dose showed some adverse health consequences (increased heart rate, slowed maze performance), the 10 mg/kg dose was used for the remaining experiments. Data from the 3 pilot rats run at 10 mg/kg were folded in with another 21 rats remaining tested under Control and DMI treatment conditions in pairs run in semi-counterbalanced treatment order for familiar maze performance, reversal learning, and T-maze performance (Supplemental Figure 4c). Eight of the DMI-treated rats were recorded during all non-training times and their records analyzed in detail for baseline vs. DMI sleep characteristics and task performance relationships.

Motivation

Food was withheld for 2 d prior to training sessions. The food received during testing was a mash of ground rat chow pellets mixed with water. A 2 ml mash supplement was given p.o. to all rats after training each day as the vehicle for the DMI (or no-DMI control). Consumption of DMI-treated or control food was voluntary in most cases and was checked by examining the contents of the supplement bowl after 20 min. Any remaining food was placed at the back of the animal's mouth to insure consumption. If body weight dropped to near 80% of free

feeding weight, the rats were offered further supplement mash in a bowl in their home cage post-training and post DMI to maintain the 80% minimum free feeding weight. Total daily intake of food was equivalent to ~40 cc mash/day.

Place learning task description

The raised 8 box maze spatial memory task[50] is a rectangular track with 8 boxes oriented symmetrically around the perimeter of the track. Three of the eight positions contained 0.2 cc of available food in a shallow dish behind a hinged door. All boxes were baited with unreachable food behind a wire mesh just below the door to prevent the successful use of odor-cue location strategies. The familiar maze was configured with the same baited box positions every day of pretraining (min 10 days) and the 5 days of testing. The novel maze was a reversal task: the maze remained in the same room and one of the boxes of the familiar configuration remained baited but the other two formerly baited boxes where changed to new box locations on the maze. To encourage forward motion, the animals were required to make one full clockwise lap before all 3 baited boxes were refilled. Each training session on each 8-box maze lasted 30 min. Every 5 laps, rats were removed from the track and placed in a towel-lined pot for 2 min to encourage reliance on short term reference memory (hippocampally mediated) over working memory. Errors committed on each lap were counted as follows: ambulating past a baited box without stopping was considered an error of omission, inspecting a box with no accessible food (unbaited) was an error of commission, and hesitating in front of unbaited boxes was considered an error of hesitation. The experimenter running and scoring the performance of the animal was blind to the drug treatment.

The main outcome measure on the 8-box maze was the number of errors committed on each lap and the total errors per lap per maze and per training day. An average of less than 1 error per lap over the session was considered performance

criterion. Once each animal met criterion during the pre-training period, the 5 day testing period began.

Food locating strategies

Without the hippocampus, animals rely more heavily on intramaze cues to successfully perform the task [1]. In order to test the use of non-hippocampally dependent solution strategies, the maze was rotated 180º after the 10th lap, the boxes cleared of any accessible food and new boxes in the old positions baited, maintaining the same functional reward positions within the room. A heavy reliance on non-spatial cues to find baited boxes is accompanied with a higher number of errors per lap on lap 11 (after rotation) than on lap 10. Working memory was assessed by comparing the decline in errors (or maintenance of low errors) over each 5 lap set since there were no interruptions in maze running within each set. Dependence on reference memory was assessed by retrieval efficiency between days, which was calculated as the number of errors in the first 3 laps each day minus the number committed the last 3 laps of the day before.

T-maze task description:

All animals were tested on 15 trials of the fixed choice T maze task immediately after training on the 8-box maze, and in a different room from the 8-box maze. The T-maze consisted of four arms joined together to form a "plus" sign. Each arm is 21"x 6"x 9" (length x width x height). On each trial one of the arms, chosen semi-randomly, was blocked off by a wooden barrier and the opposite arm was the starting position. From the start position rats had to advance to the center choice point and either turn right or left to go to the end of the arm for a reward. Rats were rewarded only if they consistently turned in the same direction from the start arm no matter the position of the start and choice arm in the room. The correct turn direction for each rat was determined by their own first choice on the first trial. The rats were allowed 60 s to traverse to the end of the chosen arm, then placed in a

towel-lined clay pot to rest for another 60 s. At the completion of 15 trials, the average latency to reward and percent correct turn direction choices were calculated.

DMI dose treatment

An initial dose-response pilot study was undertaken where 8 (n=3) 10 (n=3) and 23 (n=3) mg/kg was given and sleep responses measured. In addition 4 rats were given DMI twice a day, just after running and 12 h later. Their performance results were compared to those given DMI once per day just after training (Supplemental Figure 1a, b). No difference was found under the two conditions (repeated measures ANOVA, $p = 0.505$) so data were collapsed across methods for statistical analyses and all further experiments were conducted with once per day administration after training. Fifteen rats were given 10 mg/kg DMI in solution added to 2 ml of food mash or no DMI in 2 ml mash after each day's testing session just before returning to their home cages.

Sleep recording/scoring/analysis:

A cable from the headstage was connected to a commutator within the home cage to allow free range of motion while recording EEG and EMG signals. The commutator was connected via a shielded cable (Plastics One, Roanoke, VA) to the Lynx 8 patch panel and amplifier units (Neuralynx, Tuscon, AZ) and to the AD data acquisition system (Wilson, M. MIT). After three days habituation to the recording conditions, training began on the 8-box and T-mazes at circadian time 0.5 h after lights on for 1 h each day. EEG and EMG recordings began during the second baseline training day within 1.5 h of the training session and continued for 24 hours on the last two days of the pre-training period, (baseline) and within 1.5 hours of the testing session on day 1-5 of testing, recording for 22.5 hours each day. Eight rats were recorded 22.5 h /day (non-training time) for comparisons of sleep and performance parameters.

Sleep/waking states were first scored automatically from EEG and EMG recordings. Every 10-s epoch was assigned a state of active waking, quiet waking, quiet sleep, transition to REM, or REM sleep by an in-house automatic sleep scoring program (Gross et al., 2009), then the scored states were examined and confirmed or corrected after visual inspection by an expert human scorer blind to the experimental condition of the animal.

Statistics

Parametric tests such as t-tests and ANOVAs were run after tests for Normality of the data showed no violations. The non-parametric Wilcoxon sign-rank test was run on the comparison of number of laps run for DMI and Control rats after the DMI lap distribution failed the normality test (median =15, range =11-27 laps, skewed high $p = 0.0003$). Paired t-tests were used to evaluate significance ($n=8$, $\alpha = 0.05$) of sleep state changes from baseline to DMI conditions in the same animals (Figure 1) and to compare lap 10 vs. lap 11 errors within each group ($\alpha = 0.05$) (Figure 4). A t-test was used to compare weights between DMI and control groups on the last of the 5 day test period. Repeated measures MANOVAs ($\alpha = 0.05$) were performed on the number of errors/lap over testing days 1-5 and between groups (Figure 2a and Figure 3a). Familiar maze data from the first day (only) of 2 DMI/Control pairs was discarded because a short break between the last pretraining and first testing day increased familiar day 1 errors to over criterion (<1 error/lap). Familiar DMI week data were not analyzed from one rat as 1 familiar box had been inadvertently reversed during the prior week in that one animal, putting the familiarity of the familiar maze during DMI test week into question. Thus the statistic of the Familiar DMI group were run with an n=14 rather than 15. Percent correct choices and latency on the T-maze task over each day for each rat and improvements across the testing days for the familiar and reversal mazes were the performance metrics correlated to % sleep state parameters during the baseline period of the same subject and testing conditions and

against the change in each of those states from baseline to DMI conditions (n=8, α = 0.01 to correct for multiple comparisons). Normality tests were run using JMP (SAS, Carey, NC). Paired T-tests were done using Microsoft Excel 2004 Data Analysis Add-In pack (Microsoft, Redmond, WA). MANOVAs were run with SPSS (IBM, Armonk, NY) and Pearsons's correlation matrix statistics were run using StatPlus (AnalystSoft).

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Variable vs. Variable	*R*	*p*
REM Baseline vs. % Correct	0.923	0.0011
QS (DMI) vs. % Correct	0.910	0.0017
% change in QS vs. Latency	-0.895	0.0027
QS (DMI) vs. Latency	-0.879	0.0041
REM Baseline vs. Latency	-0.872	0.0047
% change in TR vs. Nov improvement	0.867	0.0053
TR (DMI) vs. Fam improvement	-0.865	0.0119

Table 1. Correlation Coefficient Matrix. All significant sleep vs. performance comparisons are shown for the T-maze (top 5 rows) and spatial mazes (bottom two). To correct for multiple comparisons an alpha = 0.01 was selected. No waking parameters were significantly correlated with T-maze or 8-box maze performance metrics. The amount of REM sleep during the baseline recording was most correlated to the % of correct choices and speed of trial completion on the T-maze. The amount of QS during desipramine administration was correlated with both the % correct choice and speed of completing the T-maze trial. The larger the increase in QS after desipramine, the faster the T-maze trial completions. On the 8-box maze the degree of improvement in the Novel maze under desipramine treatment was most correlated with the preservation of the transition to REM sleep state as shown in Figure 2b. Improvement on the Novel maze was not significantly correlated with any other sleep parameter. Those animals whose performance on the Familiar maze was best preserved under desipramine treatment were those with the smallest amount of TR sleep, as shown in Figure 3c.

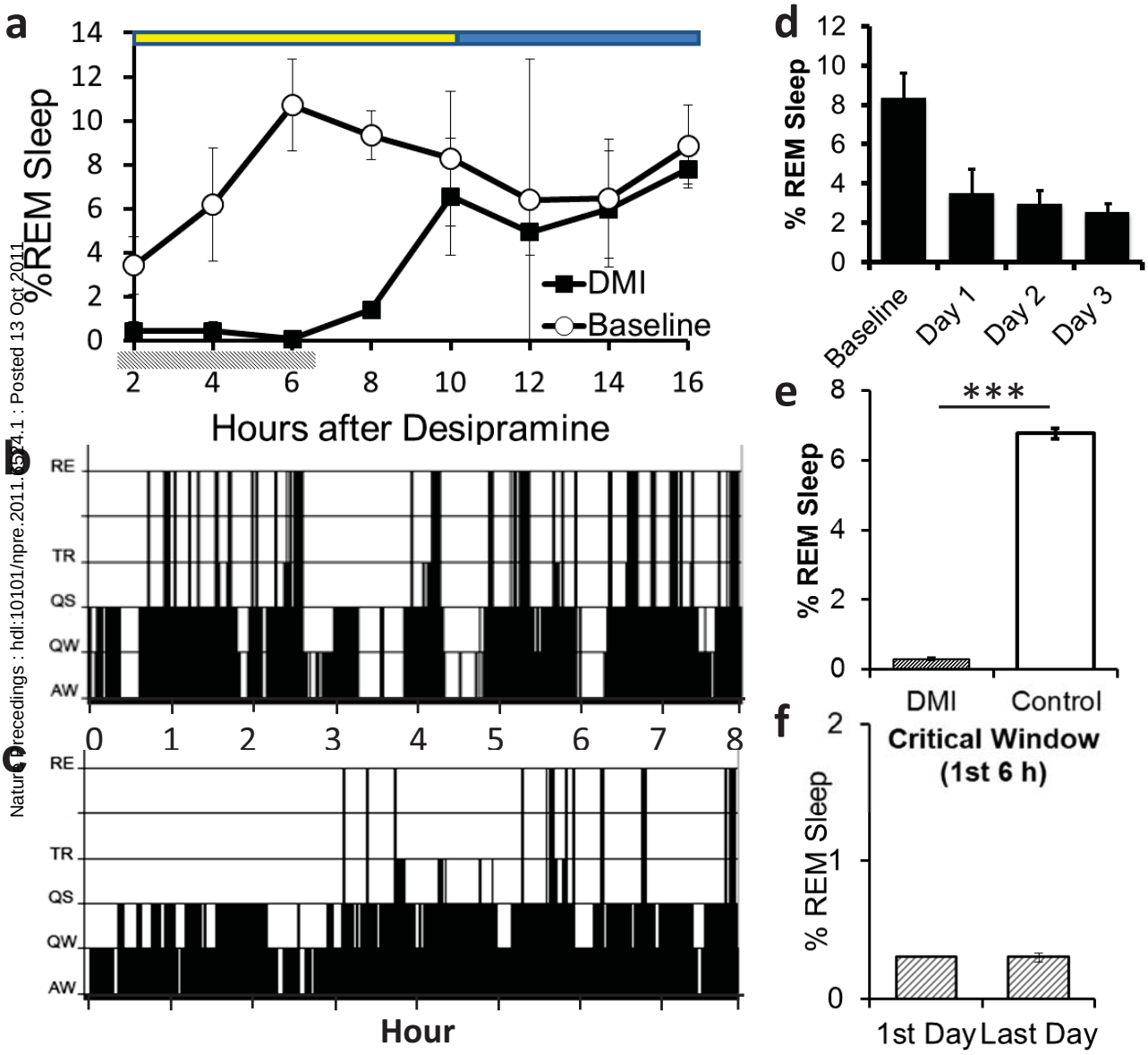

Figure-1 Poe

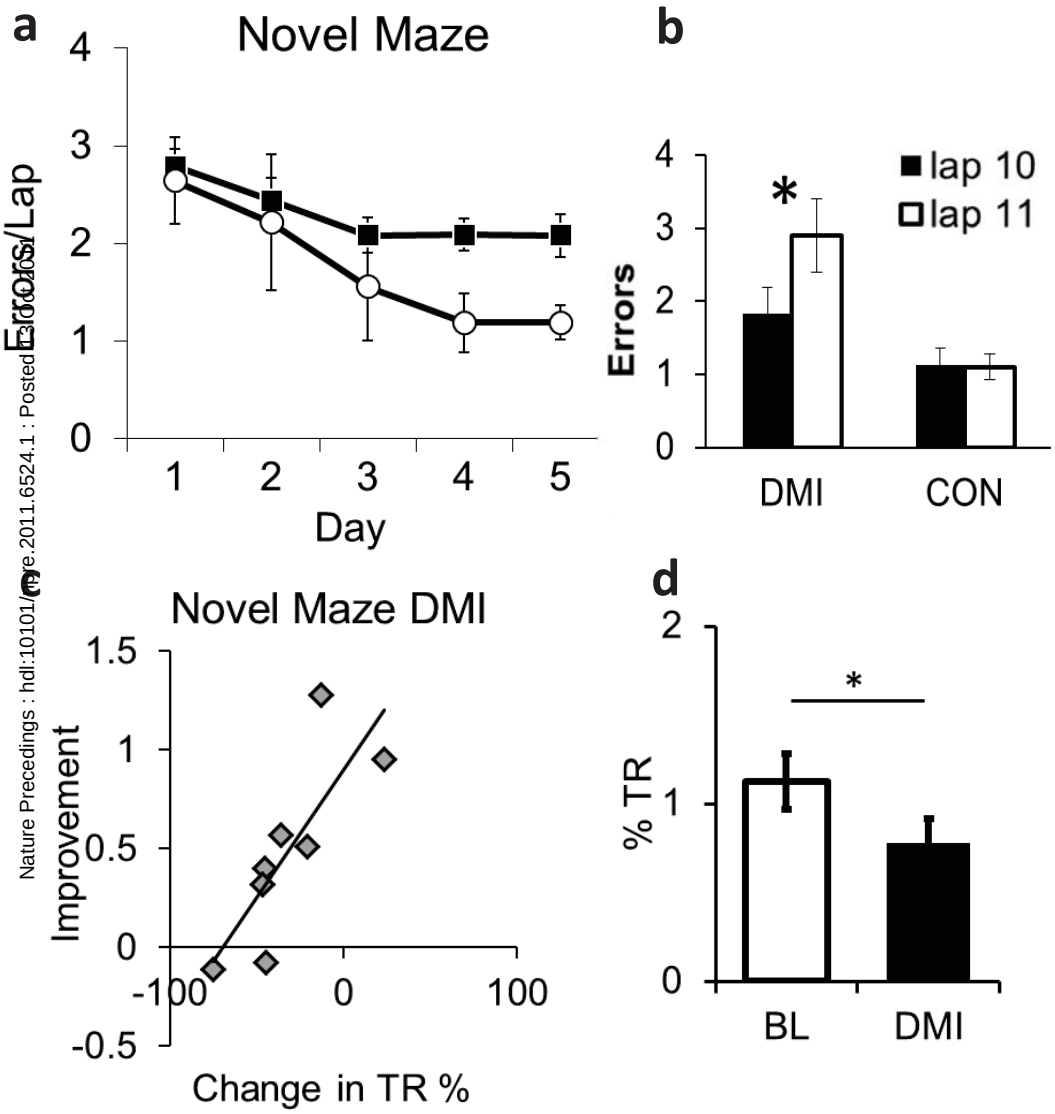

Figure-2 Poe

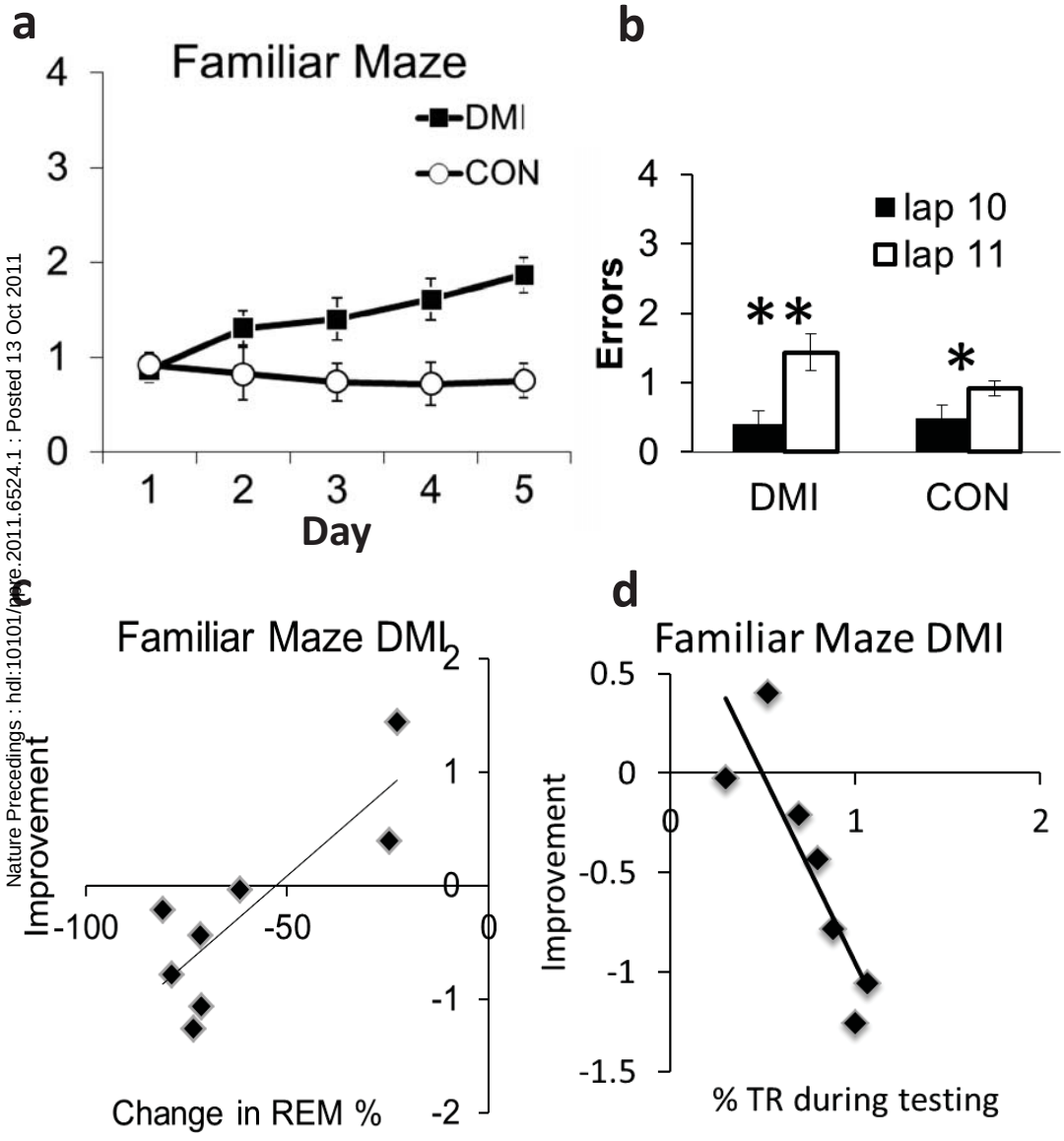

Figure-3 Poe

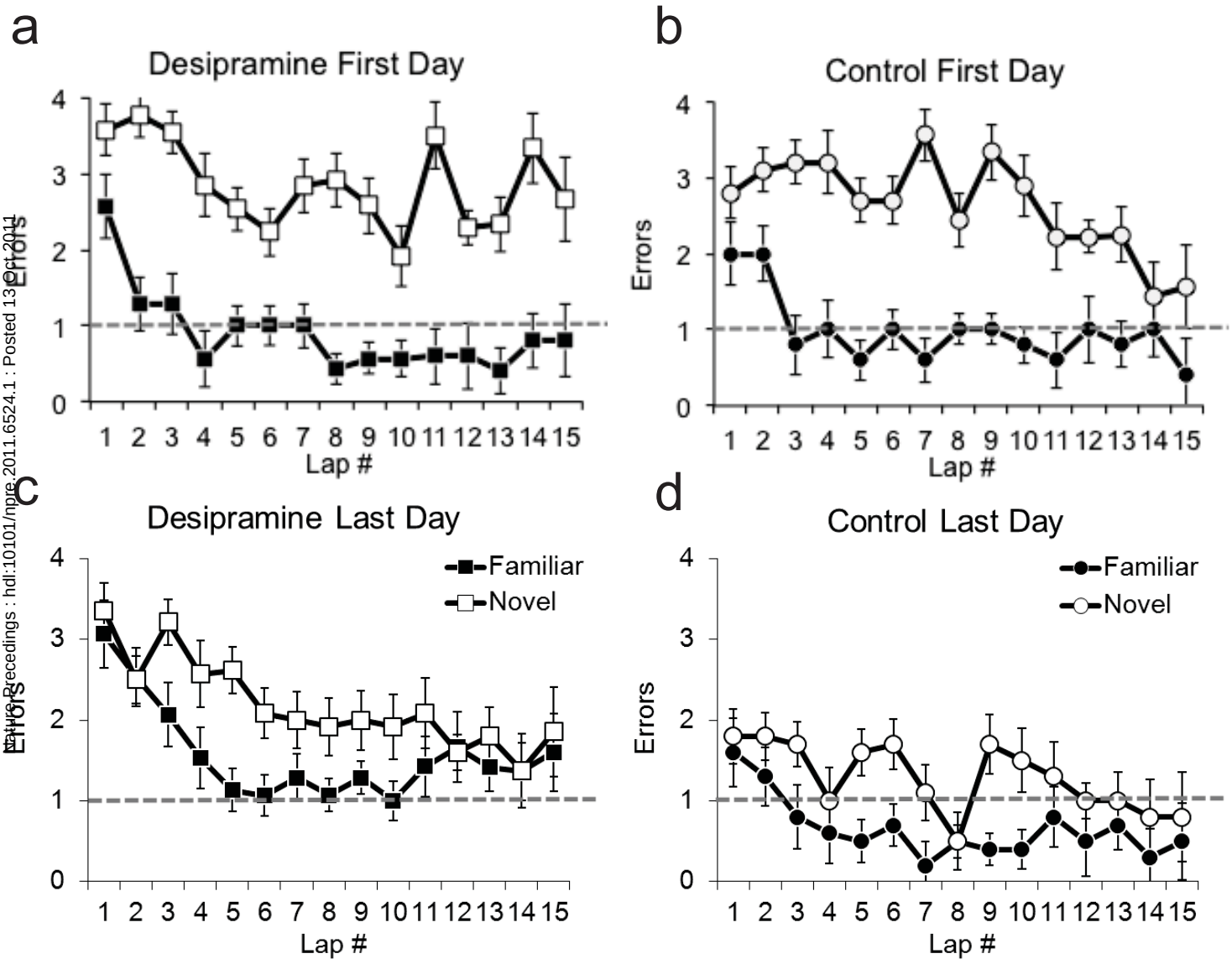

Figure-4 Poe

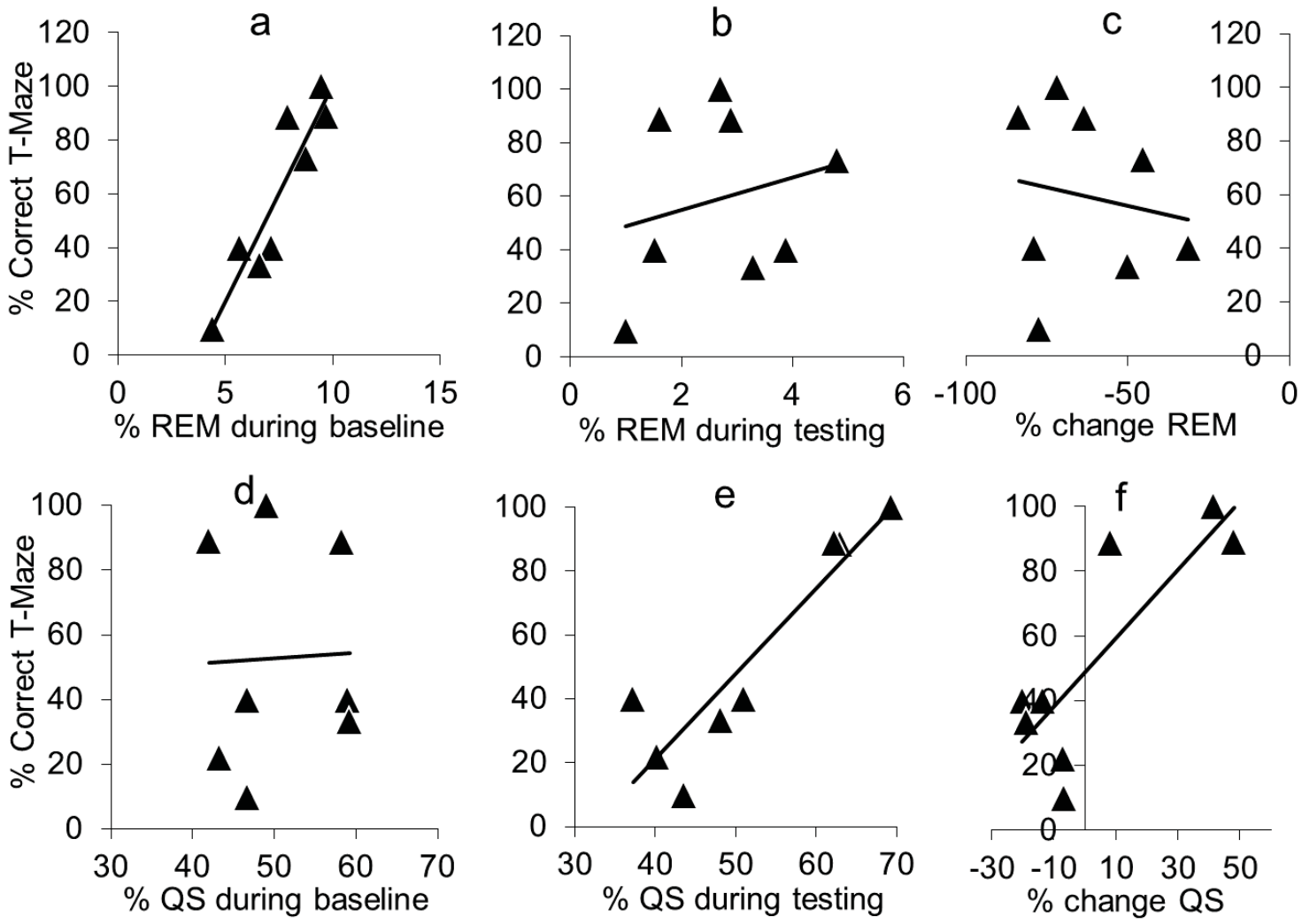

Figure-5 Poe

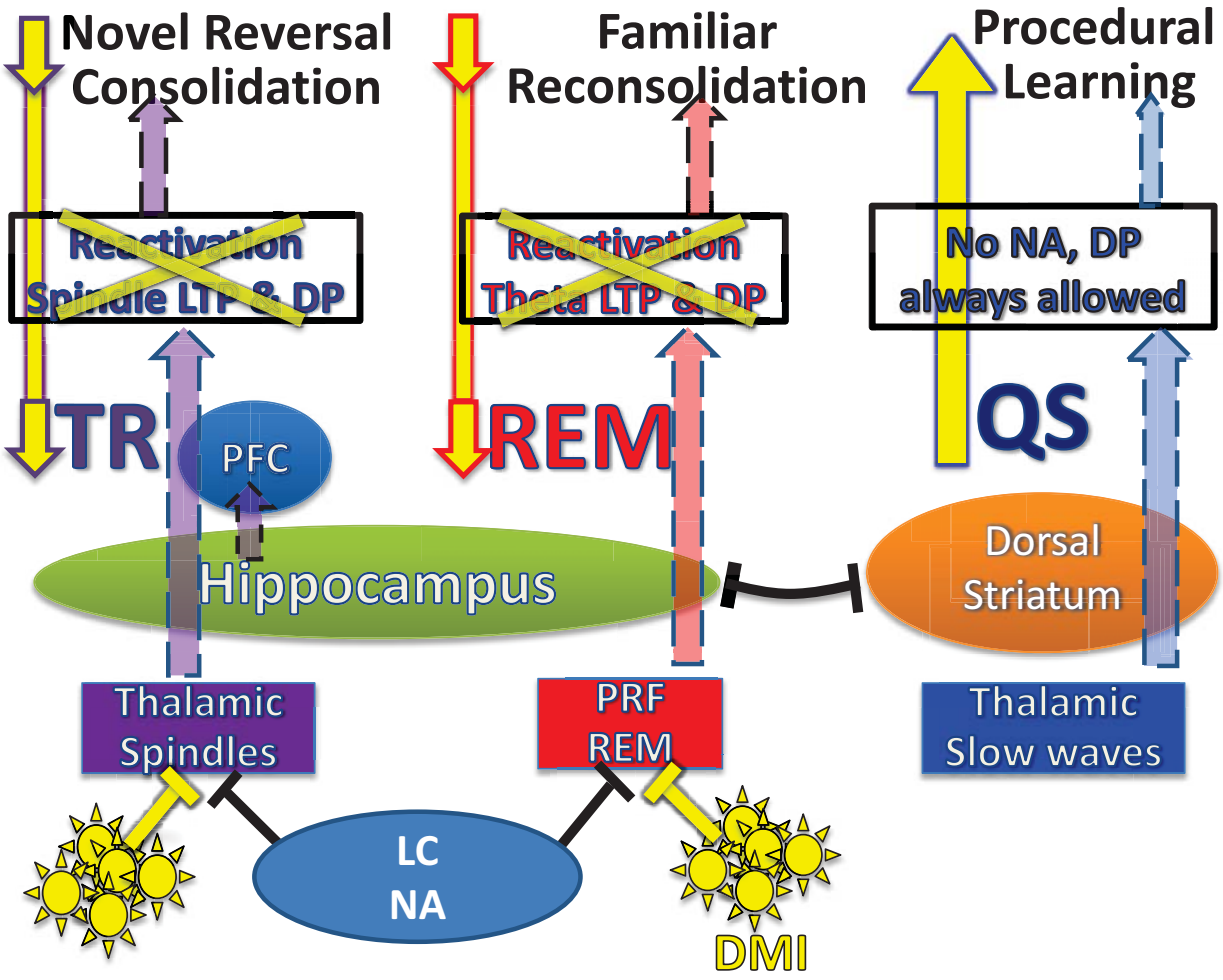

Figure-6 Poe