Cortical and Hippocampal EEG Show Different Simultaneous Sleep States after Learning

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Abstract

Several lines of evidence have challenged the assumption that sleep is a whole brain phenomenon. The idea of covert rapid-eye-movement sleep (REMS) processes (Nielsen, 2000) suggests that activity in different areas of the brain participate in different behavioral states at once. Posited covert REMS processes are consistent with a model of brain organization that begins at a local, neuronal group level and eventually leads to sleep as defined on macroscopic levels through collective outputs (Krueger & Obál, 1993). Furthermore, the assumption of brain state homogeneity is methodologically convenient, but may lead to inconsistencies in observations of sleep states underlying learning processes. We found that that a hippocampal-dependent learning condition elicits heterogeneity in rapid-eye-movement sleep (REMS) and transition-to-REMS (TREMS) states in the hippocampus and cortex. These results depict that the states of these local structures differ when scored from respective EEG. Consequently, placement of electrodes influences the characterization of sleep states. Measuring sleep from the structures predicted to be affected by experimental manipulation may be a first step in reconciling inconsistencies in the extant literature.

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Introduction

Traditionally sleep has been considered to be a property of an organism as a whole. Consistent with this model, sleep scoring in humans (Rechtschaffen & Kales, 1968) and rats (Benington, Kodali, & Heller, 1994) assumes cortical state homogeneity and characterizes whole organism sleep state based on predominate EMG and cortical EEG. Recently the assumption that the brain as a whole must participate in the same state simultaneously has been challenged. Covert REMS processes have been suggested as a means to reconcile NREMS mentation through the suggestion that REMS processes "combine" with NREMS sleep processes (Nielsen, 2000). It remains to be determined whether these REMS processes could be isolated to a non-cortical site while NREM processes occur in the cortex. Directly challenging the traditional view of sleep, the neuronal group theory of sleep places the origin of sleep squarely at a local level within neuron groups (Krueger & Obál, 1993). REMS processes that occur covertly within NREMS may simply be the product of subcortical local, neuronal group activity.

Ample evidence in support of this alternate model of brain state regional heterogeneity exists at various levels of brain organization. Unihemispheric sleep has been shown in dolphins (Goley, 1999), seals (Lyamin, Mukhametov, & Siegel, 2004), and birds (Rattenborg, Amlaner, & Lima, 2001) under certain conditions. Cortical columns, the basic processing unit of the waking brain, have been shown to oscillate between waking and sleep-like states, with a minority of columns existing in a state *different* than that of the whole-animal (Rector, Topchiy, Carter, & Rojas, 2005). Serotonergic antidepressants have been shown to increase EMG activity in submental leads during tonic REMS (Winkelman & James, 2004) which is characterized by muscle atonia (Rechtschaffen & Kales, 1968; Benington, Kodali, & Heller, 1994) suggesting that

atonia centers in the brain are exhibiting an atypical state. Dissociated states of wakefulness and sleep, termed parasomnias (e.g., REM behavior disorder, sleepwalking, night terrors, and narcolepsy) have been reported in humans and described as mixtures of wakefulness, NREMS, and REMS (Mahowald & Schenck, 1991).

Dissociated state in some animals may be utilized when necessary. One idea is that regional difference in state could maintain synapses infrequently used during wakefulness (Krueger & Obál, 1993). Assuming slow wave sleep (SWS) reflects a reset of synaptic connectivity (Moruzzi & Magoun, 1949), heavy use of one area of the cortex during wakefulness results in an increase in slow wave activity in that area relative to others during sleep in mice, rats, chicken, pigeons, cats, and humans (Miyamoto, Katagiri & Hensch, 2003; Iwasaki, Karashima, Tamakawa, & Nakao, 2004; Vyazovskiy, Borbély, & Tobler, 2000; Cottone, Adamo, & Squires, 2004; Ferrara, De Gennaro, Curcio, Cristiani, & Bertini, 2002; Huber, Ghilardi, Massimini, & Tononi, 2004; Yasuda, Yasuda, Brown, & Krueger, 2005; Kattler et al., 1994), pointing to regional independence of intensity and, possibly, state. Songbirds in a migratory state have been reported to sleep nearly two-thirds less than when in a non-migratory state (Rattenborg, Mandt, Obermeyer, Winsauer, Huber, Wikelski, & Benca, 2004). Though this has been interpreted as evidence against sleep during flight, brain regions other than cortical sites have yet to be investigated and could prove to exist in a sleep-like state. In sleeping flocks, mallard ducks located at an edge of the group exhibit a 150% increase in unihemispheric slow-wave sleep (USWS) keeping one open eye as a means of predator detection (Rattenborg, Lima, & Amlaner, 1999). Fur seals display two fundamentally different patterns of sleep: bilaterally symmetrical slow-wave sleep (BSWS), the predominate pattern when sleeping on land; and SWS with a striking interhemispheric EEG asymmetry (ASWS), the predominate pattern when

sleeping in the water (Lyamin, Mukhametov, & Siegel, 2004). Furthermore, fur seals have shown an increase in BSWS when sleep deprived while on land (Lyamin, Kosenko, Lapierre, Mukhametov, & Siegel, 2008). Similarly, domestic chicks have been shown to spend more time in bihemispheric sleep in the recovery period after sleep deprivation (Bobbo, Nelini, & Mascetti, 2008).

The assumption of brain state homogeneity is methodologically convenient as characterization would require one pair of cortical leads, but may explain inconsistencies in the research that remain to be resolved. For example the involvement of REMS in learning processes remains controversial. Individuals taking antidepressant pharmaceuticals; monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors (SSRIs), and tricyclic antidepressants(TCAs); have shown significant reduction of REMS, but these classes of drug typically do not disrupt normal daily functioning (Vertes & Eastman, 2000). Yet in learning animals, sleep deprivation during the REMS window, a period after task training in which REMS has been shown to increase, causes performance deficits. The latency to onset and window duration have been shown to vary depending on the conditions of a task (e.g., 2-way shuttle avoidance task in rodents: 1-4 hr, 100 trials, single session [Smith, J. Young, & W. Young, 1980); 9-12 hr or 53-56 hr, 50 trials/day, 2 consecutive days (Smith & Lapp, 1986; Smith & MacNeill, 1993); 9-12 hr or 17-24 hr, 20 trials/day, 5 continuous days (Smith & Butler, 1982; Smith et al., 1980); 9-12 hr, 50 trials/day, 2 consecutive days (Smith, Tenn, & Annett, 1991)]. Additionally when animals learn there is a rise in sleep in only certain stages, and such higher percentage of that state is not consistently reported (e.g., pursuit rotor learning task in humans has shown statistically significant (Fogel, C. Smith, & Cote, 2007) and non-significant (Peters, V. Smith, & C. Smith, 2007) increases in Stage 2 sleep on acquisition night).

We suggest that brain state heterogeneity exists at the site level, contributing to the idea that sleep is not a whole brain phenomenon, and that we can observe such heterogeneities in the hippocampus and cortex for REMS and TREMS states in animals involved in a hippocampal-dependent learning condition. Additionally this work may serve as a first attempt to explain inconsistencies similar to those aforementioned in the sleep literature. In order to further test the model of regional heterogeneity against the current assumption of brain state homogeneity in all conditions, learning or otherwise, we examined hippocampal and cortical EEG of rats involved in a hippocampal-dependent spatial memory task for simultaneous differences in REMS and TREMS states between recording sites.

Methods

Five male Fisher 344 rats of weight and age, on average, 360.5 ± 44.54 g and 5.8 ± 1.89 months, performed a visual platform variation of the Morris water maze (Morris, 1984) (5 trials/d, 2 d) to test for visual acuity. Subsequently these animals were trained on a hippocampal-dependent spatial learning task, the Poe 8-box maze (Poe et al., 2002), for food reward. During training rats were food restricted, but maintained a minimum of 80% of their pre-training weight. Animals were observed during daily training sessions and scored for errors on each lap. Rats walked or ran around an elevated track in a clockwise direction for 30 minutes, feeding from three of eight baited boxes each containing 1 mL food (powdered LabDiet® 5001 Rodent Diet® pellets mixed with water) delivered from syringes through tubing. To avoid aiding animals with visual or scent cues, all boxes were attached to similar tubing and syringes and contained inaccessible food. After every 5 laps, animals were removed from the maze and placed on a platform to rest for 2 min. Animals were returned after resting to a random position on the maze

to begin the next lap. The maze was rotated 180º after every 10 laps to remove any egocentric cues from the animal. The location of baited boxes was adjusted in order to hold the 3 food-box configuration unchanged relative to allocentric cues. Possible error types were: commission, an investigation of an empty (non-baited) box with sniffing or a nose poke into the box; hesitation, a pause and 45º head turn toward an empty box; and omission, a failure to eat from or investigate a baited box. Animals participated in training until committing at maximum an average of one error per lap or fewer at a rate of 45 laps per hour.

Following training, animals were anesthetized with an intraperitoneal injection of 60 mg/kg sodium pentobarbital then implanted using stereotaxic surgery with a hyperdrive, a set of microdrives each capable of recording single cell activities from the hippocampus (Venkatachalam, Fee, & Kleinfeld, 1999). This device housed a dozen 12 μm wire tetrodes placed deep in the dorsal hippocampus and a reference tetrode placed 0.5 mm dorsal to the hippocampal cell layer in the deep white matter of the neocortex. The hippocampal EEG was 0-gain current amplified and obtained by referencing one of the twelve deep hippocampal tetrodes to the neocortical reference tetrode. The cortical EEG was obtained separately from a jeweler's screw electrode placed in the skull over the left parietal lobe (n=4) or the left frontal lobe (n=1), differentially referenced to a similar screw electrode placed in the skull over the frontal cortex. The EMG was differentially recorded from a pair of wires threaded through the nuchal muscles and the two EMGs were referenced together for one channel of EMG recording. After surgery animals were given an intramuscular injection of 1 mL Pro-Pen-G® (Penicillin G Procaine Injectable Suspension), orally administered liquid Children's Tylenol®, placed on a heating blanket and monitored until they had regained consciousness.

Each rat was habituated to recording conditions and resumed pre-surgical 8-box maze performance (45 laps/hr, <1 error/lap) during a minimum 10-day recovery period. After recovery the animals performed the 8-box maze daily at the beginning of the light period for food reward for 5 consecutive days. This task reused the 3 food-box configuration that rats had learned during previous training (familiar configuration) and included an initially novel configuration as well. The novel configuration was located on the opposite side of the room, previously hidden from the animals' visual field by a patterned divider. Learning trials consisted of 45 total laps: 15 on the familiar configuration, 15 on the novel configuration, and 15 again on the familiar configuration. Within a 15 lap sub-session, as during training, animals rested for 2 minutes after every 5 laps, and the maze was rotated 180º after the 10th lap to remove the predictive power of egocentric cues. Rats were scored for errors as previously described. The largest task performance gain across the learning sessions was calculated within each animal's error record. The largest performance gain, which typically occurred between day 2 and 3 or later, was a reduction in the errors on laps 11-15 after maze rotation on the novel maze. Errors on these post-rotation laps in days prior to the gain were committed at boxes that had contained food before the 180º rotation, suggesting that animals were not performing the task using allocentric cues that persist independently of maze rotation. The EEG and EMG were recorded after task performance for 4 hr roughly 60-90 minutes into the light period.

The EEG and EMG recordings were read into the *Sleepscorer* program (Mathworks) where states were manually scored in 10-second epochs for sleep/waking states. One of five sleep/waking states were assigned to each epoch (Bjorness, 2008):

Active waking (AW) = theta activity and high EMG activity

Quiet waking (QW) = low amplitude, desynchronized EEG and relatively little EMG activity

Quiet sleep (QS) [NREMS] = high amplitude synchronized EEG and low EMG activity

Transition-to-REMS (TR) [TREMS] = high amplitude spindle activity and low EMG activity rapid-eye-movement sleep **(RE) [REMS]** = clear, sustained theta activity and phasic muscle twitches on a background of low EMG activity

Recordings of sleep/waking activity prior to the most significant intersession task performance gain across the 5-day learning session were selected for further analysis since the largest increase of REMS intensity has been shown to occur at the day prior to the largest task performance gain (Smith, Nixon, & Nader, 2004). Custom Matlab programs (Mathworks; Gross, Walsh, Booth, & Poe, 2008) were used in the state analysis. Power spectral density values in the delta (0.4-4 Hz), theta (5-9 Hz), sigma (10-14 Hz), and beta (15-20 Hz) frequency bands for each epoch were calculated. Mean power values were found for each band in each 4 hr recording. Additionally power spectral density values ± SDM in dB were calculated from total RE, TR, and QS states for frequencies 0 - 20.04 Hz at 0.244 Hz intervals in each 4 hr recording.

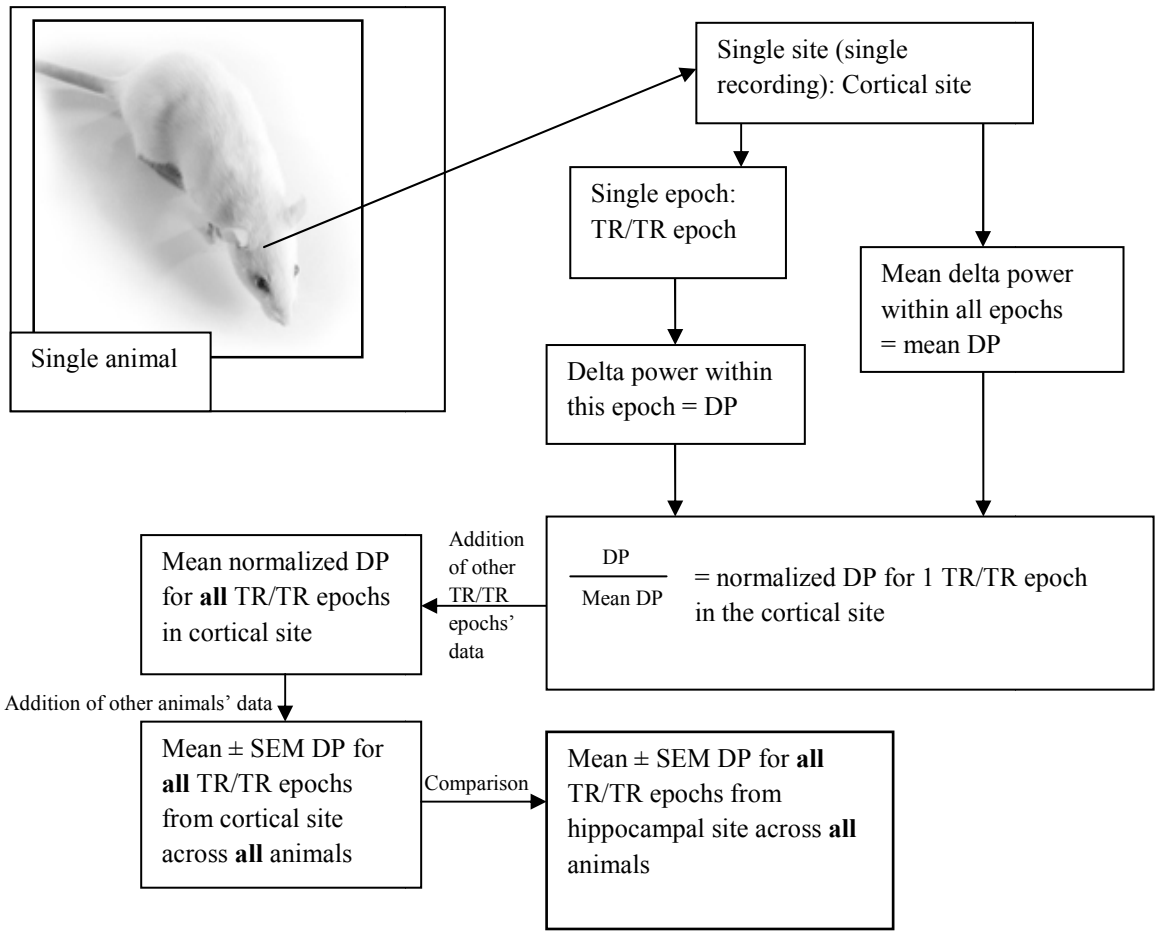
Normalized power spectral density values obtained from the frontal-frontal and hippocampal EEG represent one animal and were compared separately. Data from this animal were isolated in this power spectral density value analysis because relative wave powers for each state differ in the frontal and parietal cortices and cannot be directly compared. This data is not excluded from subsequent category analyses because recording site should not affect state if scored using accepted parameters.

RE and TR epochs were identified in hippocampal and, separately, cortical recordings. For any epoch scored as RE or TR the state at the alternative site was also noted. The denotation

of states in the two sites was assigned as hippocampal state/cortical state, e.g., TR/RE = (hippocampal state was TR and cortical state was RE, simultaneously). In this investigation we sought to explore the pressures for TR and RE states in particular as learning conditions have been shown to influence these states. Eight distinct categories including RE, TR, or 'n' (non-RE, non-TR) and describing both hippocampal and cortical state simultaneously were produced: TR/TR, RE/RE, TR/'n', 'n'/TR, RE/'n', 'n'/RE, TR/RE, and RE/TR. TR/TR and RE/RE categories were termed similar epochs as state was uniform between sites. The remaining six categories (TR/'n', 'n'/TR, RE/'n', 'n'/RE, TR/RE, and RE/TR) were termed dissimilar epochs as state was found to differ between sites. A mock example is provided below depicting the state of the two separate sites at simultaneous epochs, the resulting category denotation at each epoch, and the nature (dissimilar or similar) of the category:

Hippocampal state	QS	QS	QS	TR	RE	RE	RE	RE	QS
Cortical state	QS	TR	QS	TR	TR	RE	RE	QS	QS
Epoch Number	100	101	102	103	104	105	106	107	108
Category Denotation	none	**n/TR**	none	**TR/TR**	**RE/TR**	**RE/RE**	**RE/RE**	**RE/n**	none

In a single site's recording: each band (4 in total) of a category epoch had a spectral density (power) value. That single band power value was normalized to the mean of the respective band's power for all epochs in that single site's recording. The mean of the normalized band power for a category was then calculated within a site across all animals. Mean ± SEM power within each category and band was compared between sites. A diagram representing this process for delta power is provided on the following page:



Any category within a record was preceded or followed the same or another category. We analyzed only the category progressions which were the shifts from one category to the next. All non-category epochs, those without RE or TR in either site, were ignored. As stated above, in this investigation, we sought to explore the pressures for TR and RE states in particular as learning conditions have been shown to influence these states. Consecutive epochs of a single category were treated as one instance of that category so as to avoid overrepresentation of RE categories which frequently occur in sequence, e.g., 10 consecutive RE/RE epochs were treated as a single RE/RE epoch. Expected progression was based on total category prevalence, e.g., a category that appeared 10-fold more frequently should precede or follow another category 10-fold more often. Our mock example is revisited below, including category progressions.

Hippocampal state	QS	QS	QS	TR	RE	RE	RE	RE	QS
Cortical state	QS	TR	QS	TR	TR	RE	RE	QS	QS
Epoch Number	100	101	102	103	104	105	106	107	108
Category Denotation	none	**n/TR**	none	**TR/TR**	**RE/TR**	**RE/RE**	**RE/RE**	**RE/n**	none
Category Progressions		**n/TR →TR/TR**							
				TR/TR → RE/TR					
					RE/TR → RE/RE				
						RE/RE →RE/n			

All non-category epochs were ignored and consecutive category epochs were considered as a single epoch.

SPSS (SPSS Inc.) statistical software package was used for Student's t-test. Student's t-test was used to analyze differences in dissimilar and similar epochs, RE and TR epochs, and category band mean normalized power in hippocampal and cortical sites. Chi-squared test was used to analyze category progressions. Expected number of category progressions was based on relative category prevalence from all animals.

Results

Measurements of power spectral density values confirm that QS, TR, and RE states scored from the hippocampal, parietal, or frontal cortical EEG show typical characteristics (Figure 1). Regarding the hippocampal (left column) and parietal (middle column) EEG: RE contained greater theta band power relative to QS and TR states, TR contained greater sigma band power relative to QS and RE, and QS and TR contained comparable delta wave power that was greater in both relative to RE. In the frontal cortical EEG (right column): RE states consisted of moderate power in low frequency delta band and low power in theta, sigma, and beta bands, QS and TR contained comparable delta wave power that was greater in both relative to RE, and TR contained greater sigma band power relative to QS and RE. RE epochs from the frontal cortex contained the least theta power relative to other sites. In any site, TR epochs showed an increase in sigma power relative to QS and RE epochs. Cortical TR from either site showed greater sigma power relative to hippocampal TR.

Similar and dissimilar epochs were summed in each animal and normalized to the total number of epochs within each record (Figure 2A). Mean percentage of similar and dissimilar epochs were compared, $4.48 \pm 0.67\%$ and $7.91 \pm 1.04\%$, respectively, and found to be different with statistical significance.

Percentages of RE and TR were compared (Figure 2B). Mean percentage of hippocampal and cortical RE epochs, 5.63 ± 1.27% and 4.74 ± 0.77%, respectively, were compared and not found to be significantly different Similarly mean percentage of hippocampal and cortical TR epochs were compared, 2.85 ± 0.82% and 4.21 ± 0.78%, respectively, and not found to be significantly different.

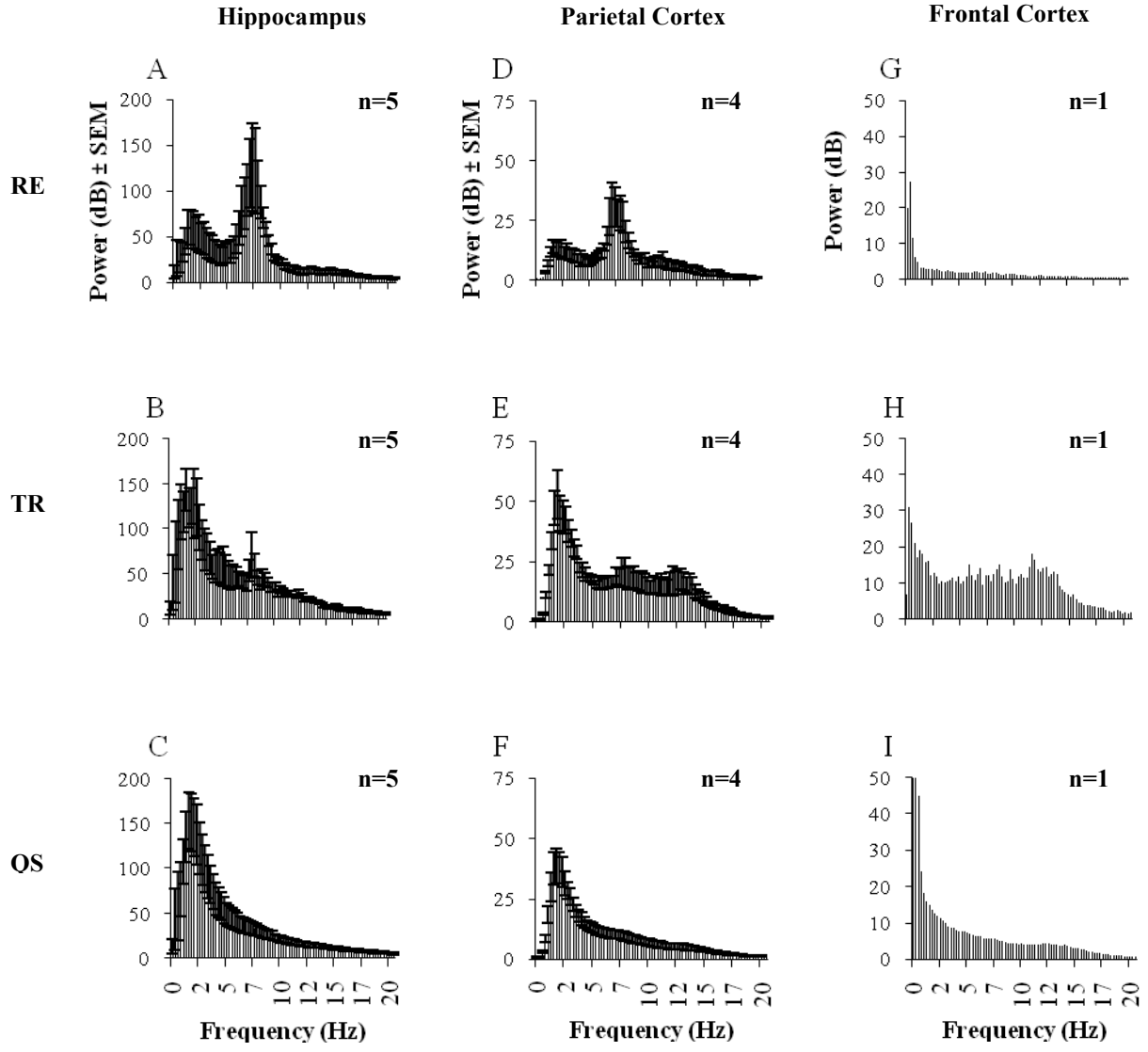


Figure 1. Mean power (dB) value ± SEM at frequencies from hippocampal electrode pairs (A, B, C), parietal-frontal cortical electrode pairs (D, E, F), and frontal-frontal cortical electrode pairs (G, H, I), epochs scored as RE (A, D, G), epochs scored as TR (B, E, H), and epochs scored as OS (C, F, I). n=number of rats included in the analysis.

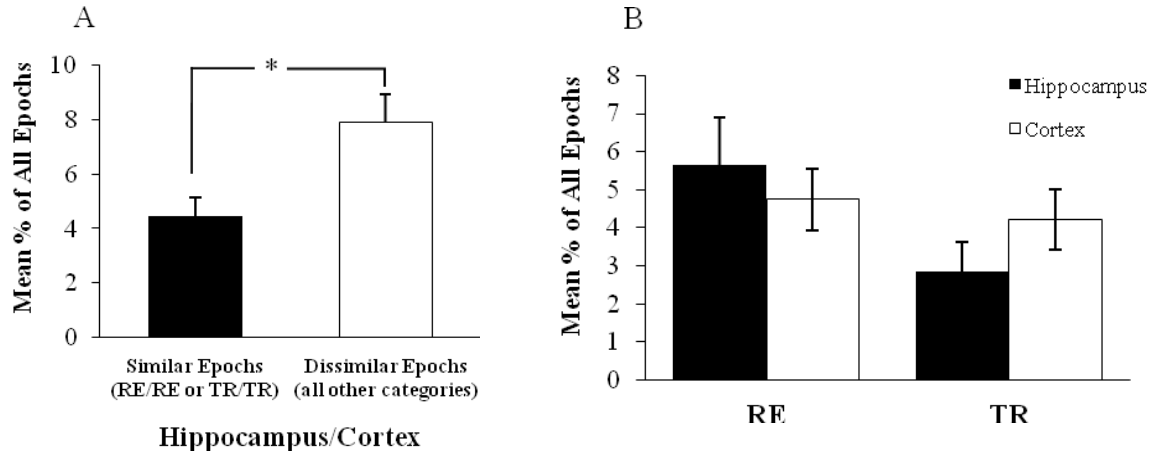


Figure 2. (A) Mean percentage ± SEM of similarly scored epochs between hippocampus and cortex compared to dissimilarly scored epochs when either hippocampus or cortex was in RE or TR, N=5. * indicates two-tailed significance p=0.02 using a Student's t-test. (B) Mean percentage ± SEM of RE and TR epochs scored from hippocampal and cortical sites, N=5. N = the number of animals included in the analyses. Two-tailed significance p=0.54 using a Student's t-test comparing RE mean percentage. Two-tailed significance p=0.32 using a Student's t-test comparing TR mean percentage.

Epochs of each category were summed within each record and compared to the total number of epochs in that record (Figure 3). The most prevalent category and also similar category, on average, was RE/RE (3.48 ± 0.81%). n/TR was the most prevalent dissimilar category on average (2.84 ± 0.70%). TR/RE was the least prevalent category on average (0.19 ± 0.16%) followed by RE/TR (0.37 ± 0.10%). RE/n (1.77 ± 0.93%) was less abundant and n/RE (1.07 ± 0.38%) was least abundant compared to RE/RE. TR/TR (1.00 ± 0.35%) was less abundant and TR/n (1.66 ± 0.71) was least abundant relative to n/TR.

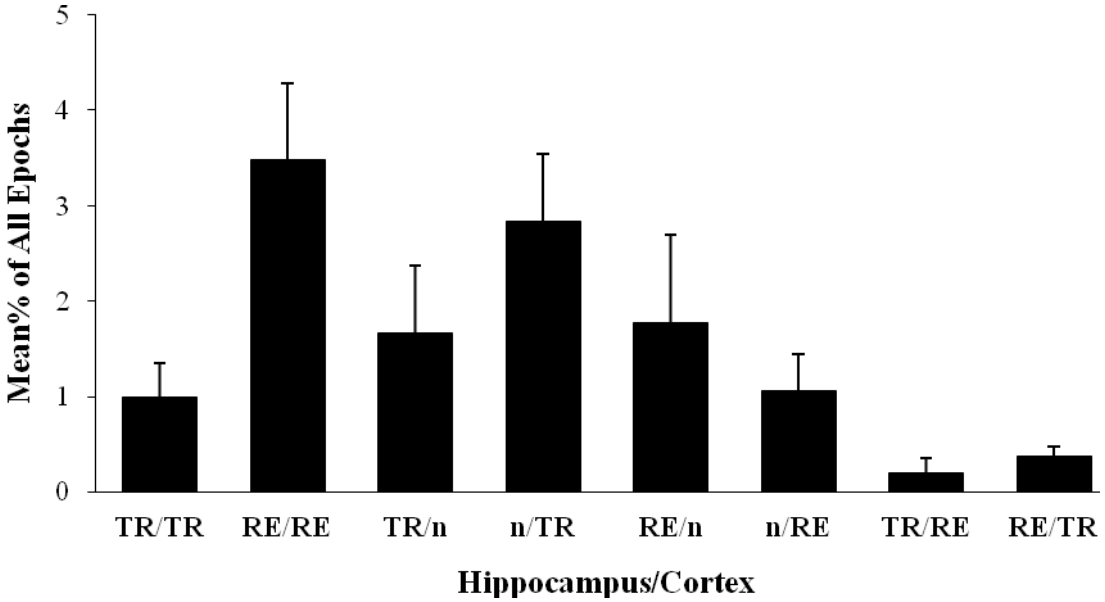


Figure 3. Comparison of hippocampal and cortical RE and TR epochs resulted in eight distinct categories denoted by: "hippocampal state/cortical state," N=5. N = the number of animals included in the analysis. Bars represent mean percentage ± SEM.

Mean normalized hippocampal and cortical (from parietal-frontal cortices) EEG power within each category and frequency band were compared (Figures 4 and 5). When comparing hippocampal and cortical normalized delta band power within each category there was not a statistically significant difference in mean power for any category (Figure 5A). When comparing hippocampal and cortical normalized theta band power within each category there was a statistically significant difference in mean power for categories: TR/TR, TR/'n', 'n'/TR, RE/'n', TR/RE, and RE/TR. Two-tailed significance *p*-values were 0.001, 0.007, 0.001, 0.028, 0.046, and 0.004, respectively (Figure 5B). When comparing hippocampal and cortical normalized sigma band power within each category there was a statistically significant difference in mean

power for categories: TR/TR, TR/'n', 'n'/TR, and RE/TR. Two tailed significance *p*-values were 0.013, 0.05, 0.002, and 0.022, respectively (Figure 5C). When comparing hippocampal and cortical normalized beta band power within each category there was a statistically significant difference for categories: TR/TR, TR/'n', 'n'/TR, and RE/TR. Two-tailed significance *p*-values were 0.10, 0.042, 0.04, and 0.005, respectively (Figure 5D).

When the cortex was scored as TR there was a consistent increase in the relative sigma power roughly between 300% and 475% that did not occur with similar magnitude in the hippocampus. This increase, apparent in any category that included TR scored in the cortex (n/TR, TR/TR, and RE/TR), was termed the sigma surge. Similarly, when the cortex was scored as TR, a relative increase in theta power was observed. This theta increase alone was not sufficient to characterize a TR epoch in the cortex as evident in the category TR/RE. In TR/RE epochs the cortex was scored RE due to an increase in theta power with the sigma power being variable (denoted by the large error bars) without a significant difference from the hippocampal sigma power. Cortical beta power showed relative power increases in the same categories that had showed the cortical sigma surge. These cortical beta power increases were not of the same magnitude and were roughly between 200% and 300%. TR/n epochs showed significant increases in cortical theta, sigma, and beta power relative to the hippocampus, but the cortex was scored as non-RE, non-TR. When the hippocampus was scored RE there was a marked increase in hippocampal theta power without increases in other power bands. Hippocampal TR epochs displayed a notable increase of 200%, roughly, in hippocampal sigma power, but these increases were smaller relative to the sigma surge in the cortex.

Mean normalized hippocampal and cortical (from frontal-frontal cortices) EEG power within each category and frequency band were compared (Figures 6 and 7). In the frontal-

frontal EEG we observed a greater sigma surge than in the parietal-frontal EEG when the cortex was scored TR with the sigma power increasing roughly between 400% and 500%. In the same TR epochs we observed cortical theta and beta power increases roughly between 200% and 325% that accompanied the frontal-frontal cortical sigma increase. As expected from a frontal-frontal EEG, when the cortex was scored RE, all cortical band powers showed a decrease. We observed this decrease when the hippocampus was scored as RE in hippocampal power bands other than theta; hippocampal theta showed a 275% to 425% increase in power, roughly. When the hippocampus was scored TR, we observed increases of 200% to 300% in sigma and 150% to 200% in beta power, roughly. The category TR/n shows a similar relative power profile to n/TR, but the 200% increase in delta band power that is unique to TR/n may explain the non-RE, non-TR state of the cortex in these epochs.

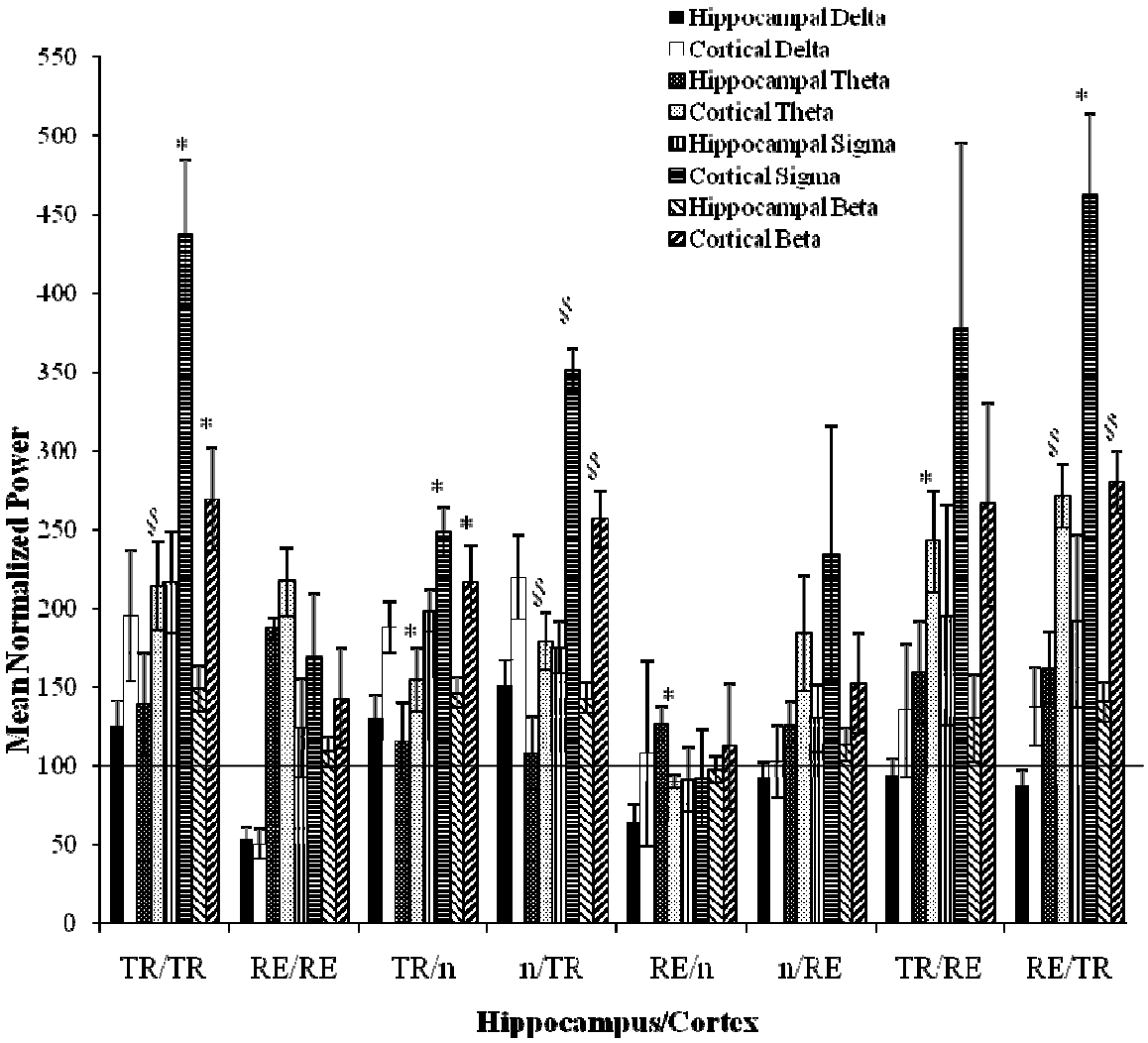
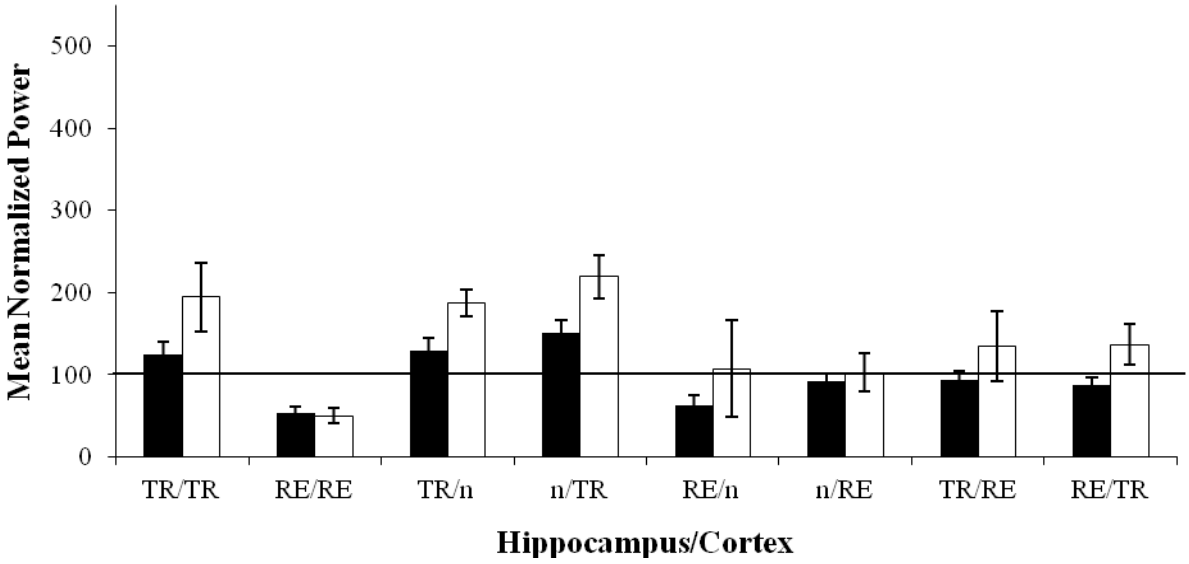


Figure 4. Mean % power ± SEM in frequency bands delta, theta, sigma, and beta relative to the mean (denoted by a solid black line) across 4 hr sleep recording. RE/'n', TR/RE N=3, all other categories, N=4. N= the number of animals included in the analyses. Categories denoted as in Figure 3 above. * indicates two-tailed significance $p<0.05$ using Student's t-test, § indicates two-tailed significance $p<0.005$ using Student's t-test. Statistical comparisons were conducted between mean normalized power hippocampal and cortical EEG power within each category and frequency band.

A


Mean Normalized Power
500
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300
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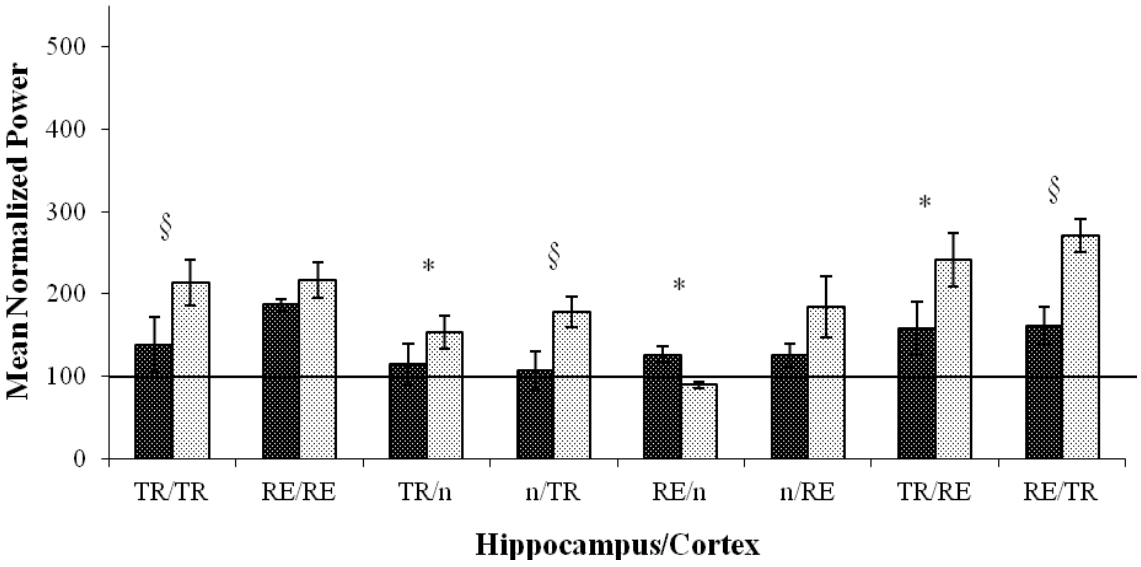
Hippocampus/Cortex

B


Mean Normalized Power
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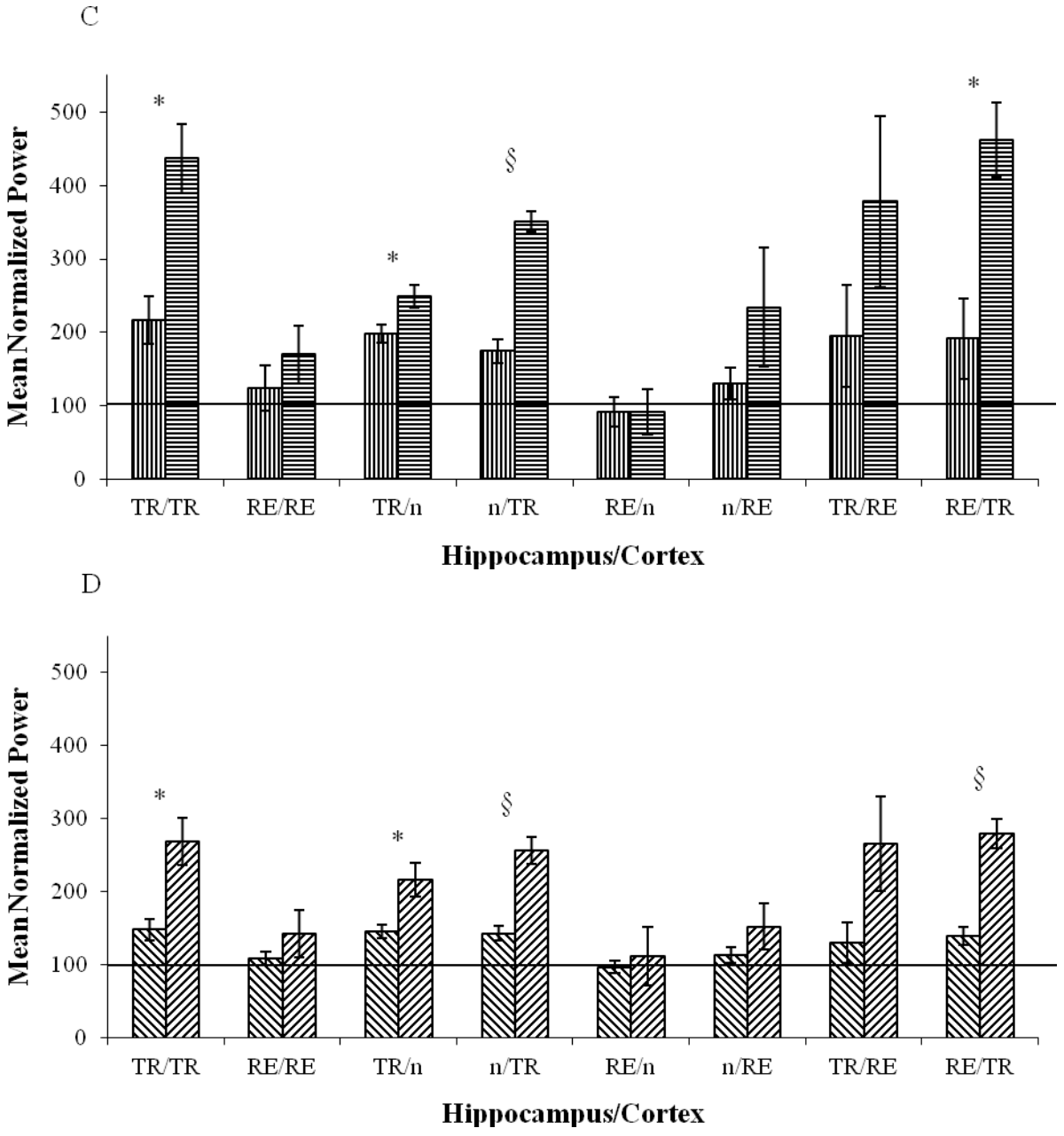


Figure 5. Mean % power ± SEM data from Figure 4 broken up by frequency band (A) delta, (B) theta, (C) sigma, (D) beta relative to the mean (denoted by a solid black line). RE/n, TR/RE N=3, all other categories, N=4. N = the number of animals included in the analyses. Categories denoted as in Figure 3 above. * indicates $p<0.05$, § indicates $p<0.005$. Statistical comparisons were between hippocampal and cortical EEG normalized power within each category and frequency band.

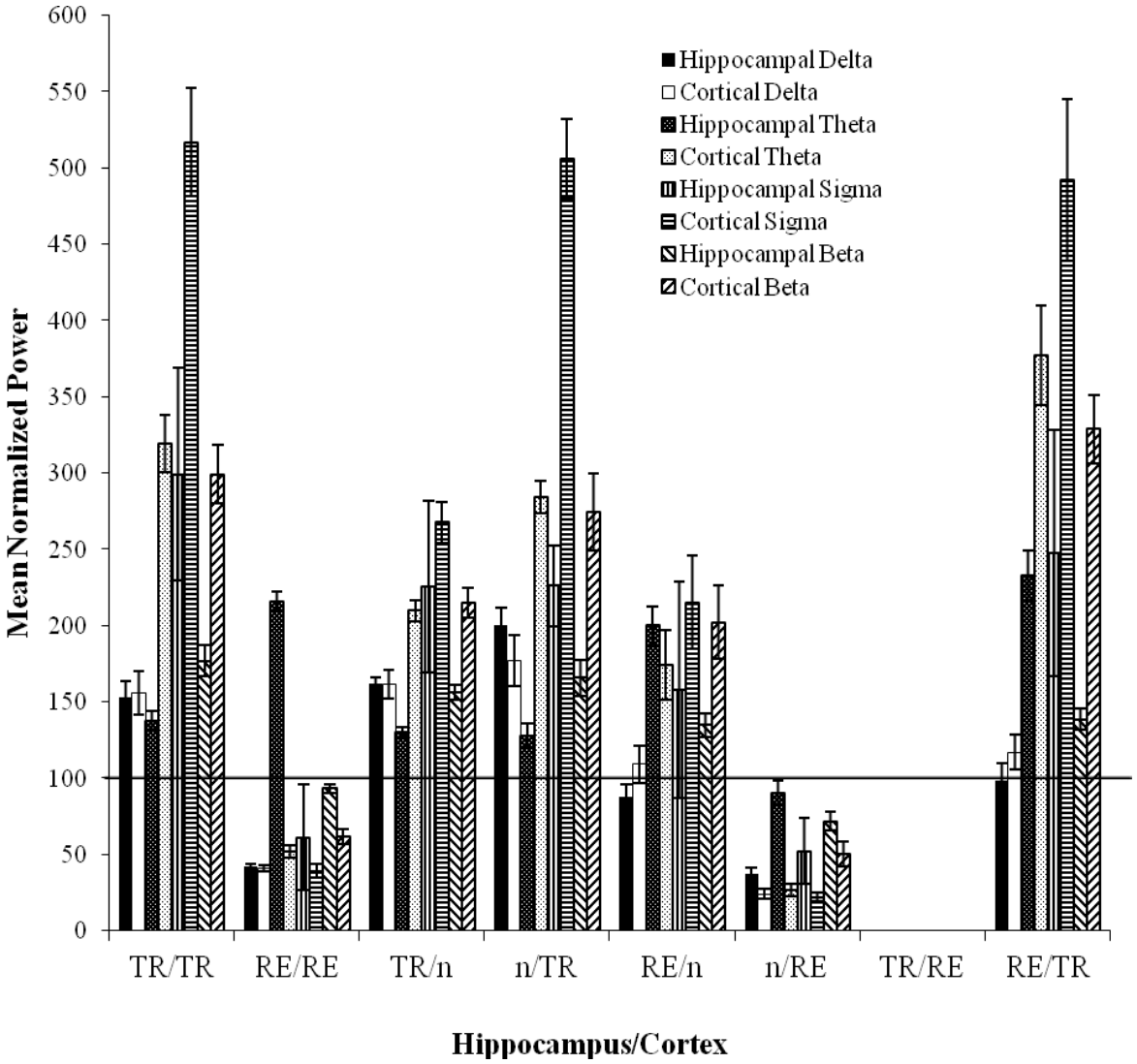
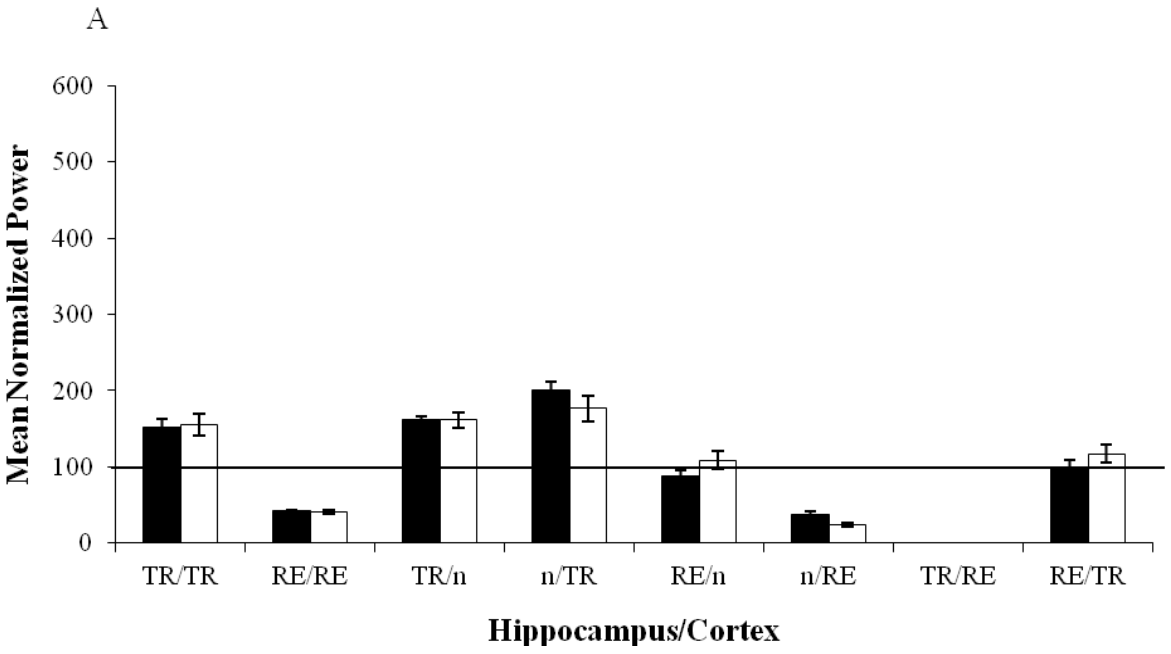


Figure 6. Mean % power ± SEM in frequency bands delta, theta, sigma, and beta relative to the mean (denoted by a solid black line at 100%) across 4 hr sleep recording. N displayed below each category on the x-axis. N = the number of normalized band power values (epochs) for categories within an animal that were included in the analysis.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Mean Normalized Power
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RE/n
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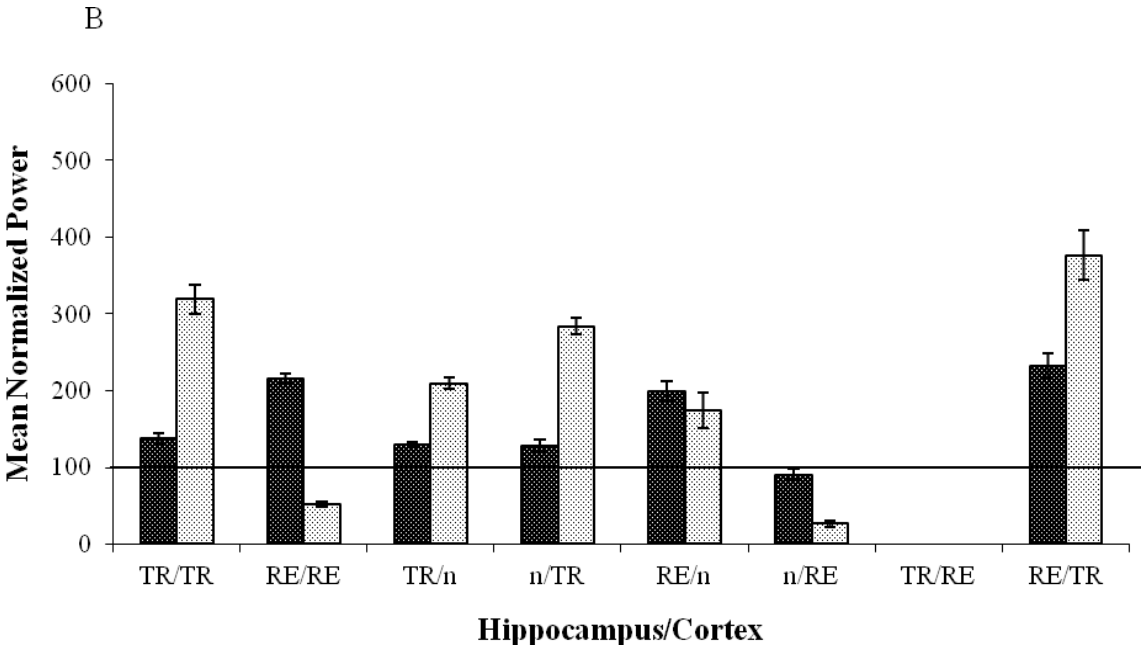
RE/TR
Hippocampus/Cortex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RE/TR
Hippocampus/Cortex

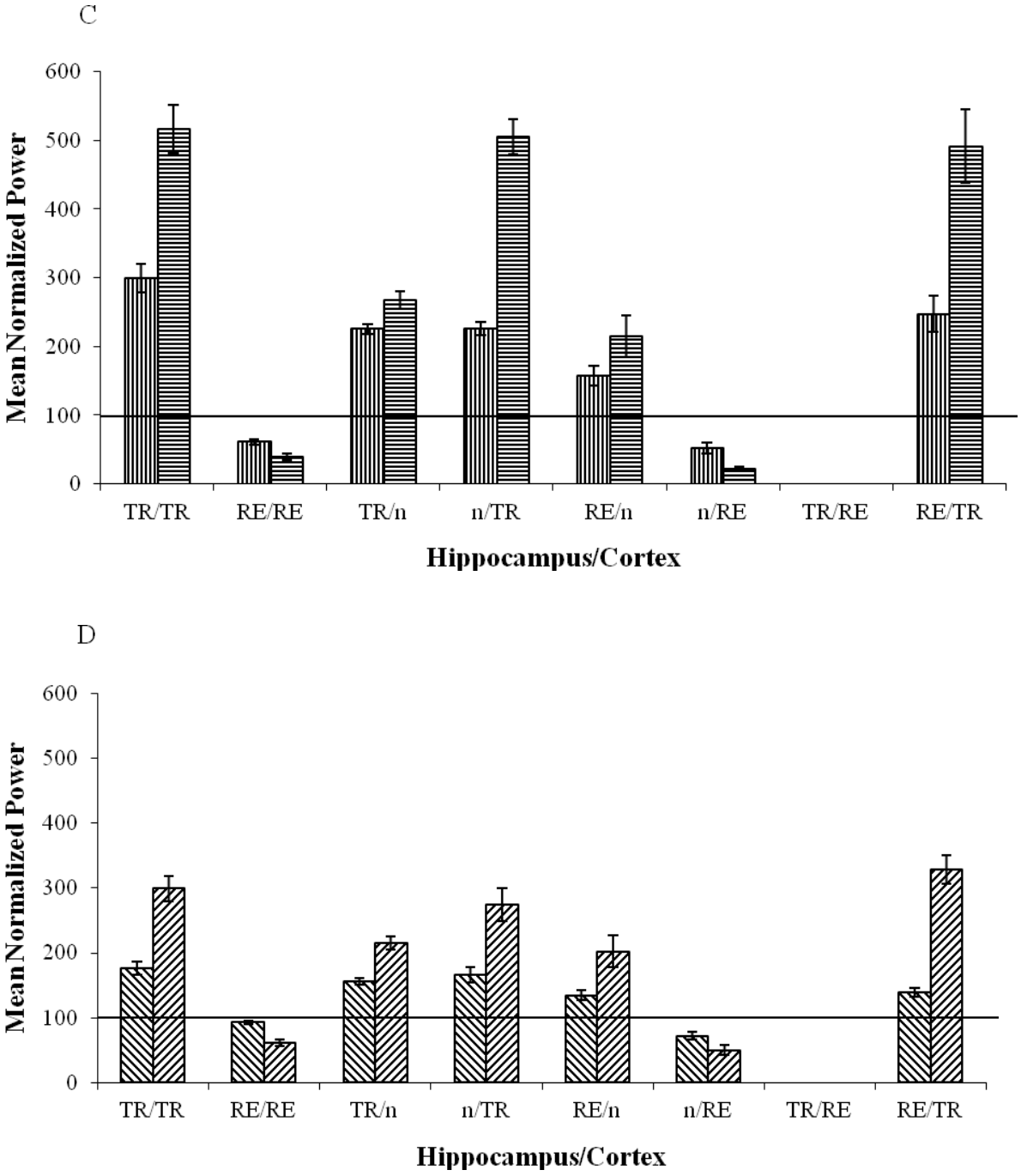

Figure 7. Mean % power ± SEM data from Figure 6 broken up by frequency band (A) delta, (B) theta, (C) sigma, (D) beta relative to the mean (denoted by a solid black line). Categories denoted as in Figure 3 above. N as shown in Figure 6 above.

We observed statistically significant observed progressions, those present more or less often than predicted as due to chance (Figure 8A and 8B). Dashed lines represent particular progressions of interest that were not observed more often than chance would predict (Figure 8A). RE/n represented an event after RE/RE, when the hippocampus remained in REMS after the cortex had exited (p=0.00). n/RE represented an event before RE/RE when the cortex was entering REMS prior to the hippocampus entering REMS (p=0.02) or after RE/RE when the cortex remained in REMS after the hippocampus had exited (p=0.00). TR/n often preceded n/TR (p=0.03), but the opposite progression was not significant. TR/TR followed n/TR (p=0.00), but not TR/n with significance. This indicates that the hippocampus followed the cortex into TREMS, but not the opposite. RE/n also followed TR/n (p=0.04) signifying progression of the hippocampus from TREMS to REMS independent of the cortex. A category progression from n/TR to n/RE was not observed with significance, thus the cortex's progression from TREMS to REMS was not independent as in the hippocampus. Most often n/RE was reached from n/TR through a series of probable progressions: n/TR to TR/TR (p=0.00), TR/TR to TR/RE (p=0.03), TR/RE to n/RE (p=0.01). RE/TR and TR/RE were shown to precede RE/RE (p=0.00 and p= 0.01 respectively). In general, progressions occurring more often than expected with significance, state progressed in only one site.

Progressions that occurred less often than expected due to chance with statistical significance were also observed. Progressions RE/RE to TR/TR (p=0.01) and RE/TR to TR/TR (p=0.04) rarely occurred indicating that REMS was not preceding TREMS. RE/n was never observed following n/RE (p=0.01). The progressions TR/n to RE/RE (p=0.00), TR/n to n/RE (p=0.00), TR/TR to n/RE (p=0.03), and n/TR to RE/RE (p=0.00) were observed less often than expected as well.

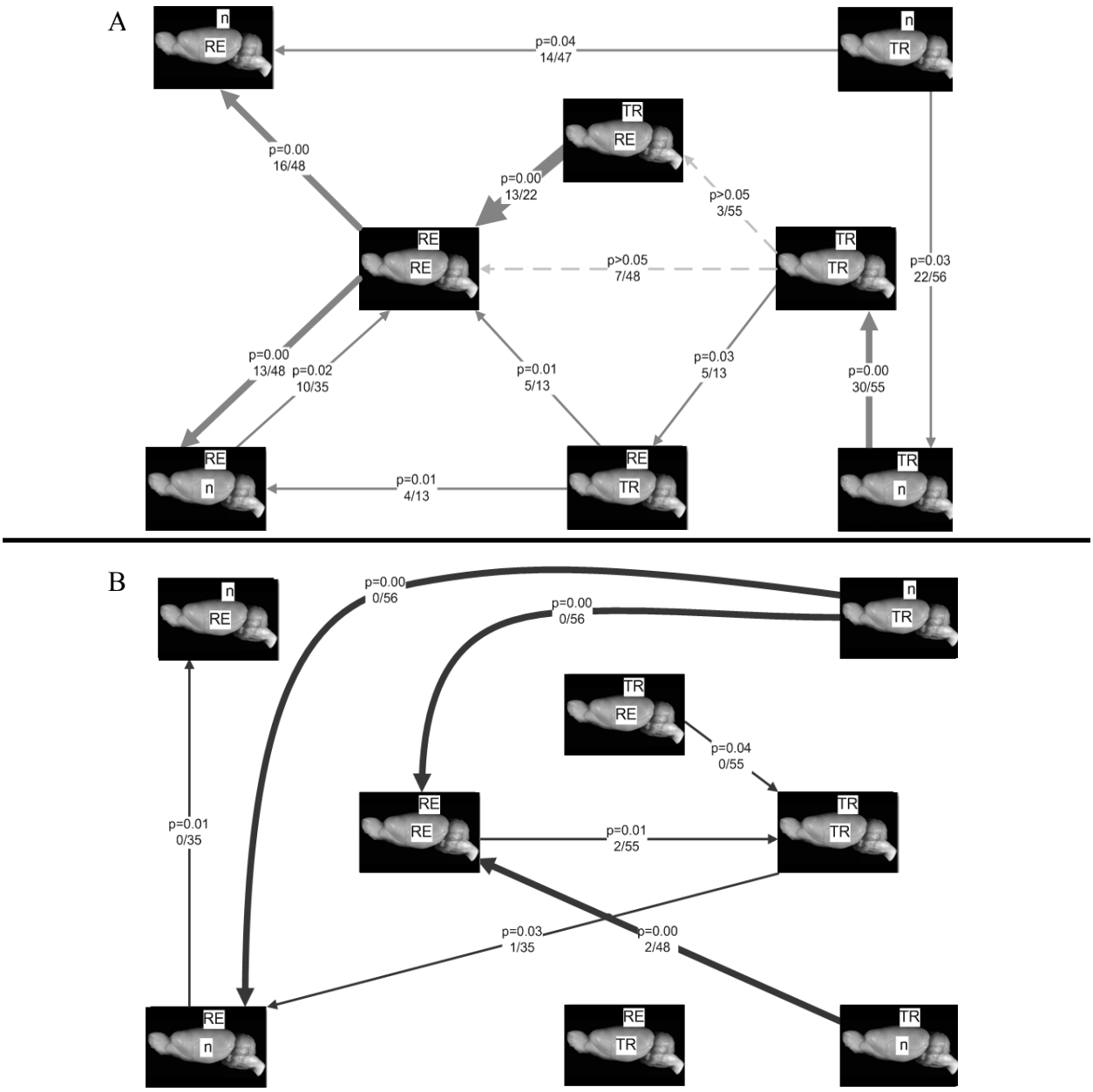

Figure 8. Positive (A) and negative (B) statistically significant category progressions based on expected and observed values. Arrows signify directionality. Arrow weight is inversely proportional to the calculated *p*-value for each shift. *p*-values were calculated for each possible progression based on chi-squared values. Fractions denote "number of observed shifts/total possible shifts." Each sub-image shows simultaneous cortical (top) and hippocampal (middle) state.

Discussion

Power spectral density values from epochs scored as QS, RE, and TR verified that these states were scored according to accepted parameters for the EEG recording (Benington et al., 1994). Average relative band powers within QS, RE, and TR were also as predicted based on location of recording electrode. For example, recording electrodes used to obtain hippocampal and frontal-parietal EEG were in proximity to the hippocampus, where a slow (4-9 Hz) theta rhythm is expressed via modulation by medial septal nucleus and the nucleus of the diagonal band (Vinogradova, Kitchigina, & Zenchenko, 1998). Conversely frontal-frontal EEG electrodes were located most distal of all recording electrodes from the hippocampus. Spindle activity by definition is within the sigma band (10-14 Hz) (Rechtschaffen & Kalen, 1968) and due to thalamocortical projections (Steriade, 2000). This taken with the observation that peak sigma power marks the transition from NREMS to REMS (Benington et al., 1994) is consistent with the observation of greatest relative sigma power from cortical sites.

Categories were created by comparing RE or TR epochs to the simultaneous state at the opposing site within the animals. RE epochs were chosen for analyses as REMS represents a common dependent variable manipulated with learning conditions. TR epochs were chosen as another state for analyses as some studies have shown increases in TREMS in learning conditions.

Normalized power spectral density values obtained from the frontal-parietal and hippocampal EEG were compared at epochs designated as categories. This resulted in significant differences. As previously described any differences were not due to abnormal scoring, so we suggest that these are due to state heterogeneity and are verified by the power spectral density value comparison data. TR and RE epochs were characterized by unique

profiles in both the hippocampus and cortex. The sigma surge, the increase in the relative sigma power roughly between 300% and 475%, appears to be signature of cortical TREMS; TR/n, TR/TR, and TR/RE epochs exhibited relative power increases of lesser magnitude, 100% to 300%. TR and RE are observed in the cortex only with large increases in relative band powers, e.g., the sigma surge, whereas TR and RE occur in the hippocampus with smaller relative increases in power bands. These observations are consistent with hypothesized covert REM processes (Nielsen, 2000) as well as brain organization at a neuronal, local group level leading to sleep on a macroscopic level (Krueger & Obál, 1993). In this case the hippocampus and cortex may represent structures receiving separate inputs from local neuron groups, the septal nucleus and nucleus of the diagonal band and thalamocortical projections, respectively.

Categories were useful in making a broad comparison of state between sites as each category describes a spatial and temporal relationship of state. The percentage of dissimilar epochs, that is, epochs in which state was not homogenous between sites, was greater than the percentage of similar epochs with significance. These findings provide evidence that brain state is not necessarily homogenous throughout all brain sites. Total percentage of RE and TR epochs did not differ significantly, suggesting that any difference in state that was observed in individual epochs did not affect the overall percentage of state of a longer recording session. This may affect the overall percentage of RE and TR epochs in shorter recording periods or in recordings obtained from later in sleep episodes, where REMS density is greater (Aserinsky, 1971).

If the hippocampus and cortex do not necessarily exhibit TREMS and REMS simultaneously, then assumptions about the sleep-wake state of the hippocampus based on the cortical sleep-wake state may not be valid. This may provide an explanation for contradictions in the literature. Antidepressant pharmaceuticals (MAOIs, SSRIs, TCAs) may show significant

reduction of REMS when scored from the cortex (Vertes & Eastman, 2000), but not from a separate site in the brain, thus failing to disrupt normal daily functioning. The various REMS windows that have been reported may show variation (Smith et al., 1980; Smith & Lapp, 1986; Smith & MacNeill, 1993; Smith & Butler, 1982; Smith et al., 1980; Smith et al., 1991), but recording from other affected brain structures may reveal a common window, with consistent duration and latency to onset. Additionally consistent rises in a given sleep stage may reside in structures other than the cortex and reconcile the variable evidence reported thus far (e.g., pursuit rotor learning task in humans has shown statistically significant (Fogel et al., 2007) and non-significant (Peters et al., 2007) increases in Stage 2 sleep on acquisition night).

Combining all categories of regionally heterogeneous state masked which individual categories constituted the total percentage of dissimilar epochs. Thus Figure 3 shows the individual category profiles; these data taken with the category progression data allow useful interpretations to be made. The high prevalence of RE/RE was expected as REMS is a sustained state, generally, so overlap is relatively common. We interpret RE/RE as an overlap in REMS periods in each site, not a direct shift from the similar epoch TR/TR, which supports our finding of heterogeneities. The prevalence of n/TR is consistent with recent work that has shown that learning animals spend 180.6% more time in a transitional state between slow-wave sleep and REM sleep (tS-R) (Datta, 2000). Our evidence indicates that, in these conditions, the hippocampus may progress in state independently of the cortex as we observed that: RE/n followed TR/n. Lower relative band power in RE and TR states observed in the hippocampus is consistent with the relative independence of the hippocampus. The progression n/TR to n/RE was not observed directly, but through intermediate categories. Arrival at n/RE through intermediate categories is consistent with the idea of a temporally discontinuous hippocampo-

neocortical dialogue (Buzsáki, 1996), though occurs in a far shorter timeframe than previously suggested. Under these conditions RE/TR preceding RE/RE with greater significance than TR/RE preceding RE/RE suggests again that the cortex shows greater dependence on the hippocampus, than the reverse. Cortical dependence on the hippocampus is consistent with the posited main function of the hippocampus as transferring stored representations to the neocortex (Buzsáki, 1996). The observation that TR/TR followed n/TR, but not TR/n, may reflect, not hippocampal dependence on the cortex, but that the relative power necessary for TREMS in the cortex (e.g., the sigma surge) could not be supplied by the lesser increase in relative sigma power of the hippocampus.

The lack of RE/RE to TR/TR progressions may be interpreted in two ways: as violation of the TREMS to REMS progression or as support of categories occurring between RE/RE and the following TR/TR. The latter supports our evidence that brain state is not necessarily homogenous throughout all brain sites.

Conclusion

Our findings suggest that the model of brain state homogeneity is inadequate to describe the brain in all conditions since we have shown brain state regional heterogeneities during learning; that is, based on categories, dissimilar epochs were greater than similar epochs. Overall RE and TR state percentages did not vary between sites within the 4 hr recording period, but differences in RE and TR state percentages may surface within shorter recording periods or recordings obtained from a later portion of sleep episodes, where REMS density is greater (Aserinsky, 1971). Furthermore, as we have shown that placement of electrodes influences the

characterization of sleep states, recording sleep from the structures predicted to be affected by experimental manipulations may begin to reconcile state controversies in the sleep literature.

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