Ketamine disrupts theta synchrony across the septotemporal axis of the CA1 region of hippocampus.

Abbreviated title: Ketamine disrupts hippocampal synchrony

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Abstract

The hippocampal theta signal reflects moment-to-moment variation in the synchrony of synaptic input to hippocampal neurons. Consistent with the topography of hippocampal afferents, the synchrony (coherence) of the theta signal varies across the septotemporal axis. Septotemporal variation in the theta signal can also be observed in relation to ongoing and past experience. Thus, there is a systematic decrease in the relationship between locomotor speed and theta power across the septotemporal axis: septal hippocampus exhibiting the strongest relationship. Conversely, theta in temporal hippocampus decrements over repeated behavioral experience (running episodes), while theta in the septal hippocampus does not. Ketamine is an N-methyl-d-aspartate (NMDA) antagonist that can decrease theta power. The present study examined whether ketamine treatment could alter theta coherence across the long axis independent of changes in locomotor behavior. Rats were well trained to navigate a linear runway and outfitted with electrodes at different septotemporal positions within CA1. Locomotor behavior, theta coherence and power were examined following administration of 2.5 and 10 mg/kg of ketamine. Ketamine (2.5 mg/kg) decreased theta coherence between distant CA1 electrode sites without altering running speed or theta power. Both doses of ketamine also blunted and reversed the decrement in theta power observed at mid-septotemporal and temporal electrodes over repeated run sessions. The results demonstrate the sensitivity of global network synchronization to relatively low doses of ketamine and septotemporal differences in the influence of ketamine on hippocampal dynamics in relation to past experience.
Introduction

The hippocampal theta local field potential (LFP) reflects moment-to-moment variation in intrahippocampal and entorhinal excitatory (glutamatergic) inputs to hippocampal neurons. The LFP theta signal exhibits both laminar and areal variation across the somatodendritic fields of CA1 neurons (Bragin et al., 1995; Sabolek et al., 2009; Royer et al., 2010) reflecting the topographical organization of intrahippocampal (CA3) and entorhinal inputs (Ruth et al., 1982; Ishizuka et al., 1990; Dolorfo and Amaral, 1998). The signal is also sculpted by a broad network of GABAergic interneurons (Buzsaki et al., 1983; Brankack et al., 1993; Klausberger and Somogyi, 2008), as well as the septal GABAergic input and a number of subcortical modulatory inputs (Bland, 1986; Bland et al., 2007; Pignatelli et al., 2011).

During the awake state, CA1 theta reflects the processing of ongoing sensory inputs as filtered by neocortical associative networks and provided directly by the theta-related discharge of entorhinal cortical (EC) neurons (Alonso and Garcia-Aust E, 1987; Bragin et al., 1995; Chrobak et al., 1994; 1998; Deshmukh et al., 2010). The CA1 signal also reflects the theta-related output of the CA3 network (Kocsis et al., 1999), which is presumed to store and output “patterns” of activity in relation to prior experience (O’Reilly and McClelland, 1994). Both CA3 and EC inputs provide theta frequency synaptic potentials to the dendritic field of CA1 neurons and the precise timing of both inputs play a fundamental role in the amplitude modulation of the signal (Ang et al., 2005 as well as Sabolek et al., 2009 for discussion).

In association with a variety of sensory, associative and motor events, the theta signal clearly changes with regards to amplitude and frequency. These changes have
suggested an association between theta and the processing of sensory input, attention to novelty or meaningful stimuli, sensorimotor integration, as well as memory processing during both ongoing experience and subsequent REM sleep (Bland, 1986; Vinogradova, 1995; Buzsaki, 2002; Montgomery et al. 2008; Benchenane et al., 2010). The clearest quantifiable relationship in the rodent literature exists between locomotor speed and the amplitude and frequency of theta (Teitelbaum and McFarland, 1971; see Hinman et al., 2011 for additional references).

A goal of our studies is to understand the conditions that promote unitary activity (increased synchrony) across the long axis of the hippocampus (HPC) versus conditions that may isolate or dynamically differentiate septotemporal areas. Greater synchrony across the long axis may suggest integrated computational processing across the entire hippocampal network: while decreased synchrony may suggest segregate computational processing. We have described variation in the coherence of the theta signal across the long axis (Sabolek et al., 2009; Penley et al., 2011; see also Royer et al., 2010) and a systematic decrease in the relationship between running speed and theta power with distance from the septal pole of the HPC (Hinman, et al., 2011; see also Maurer et al., 2005). Such findings highlight the independent generation of theta at multiple laminar and areal sites across the somatodendritic field of HPC neurons (Montgomery et al., 2009), which nonetheless are interdependent and exhibit considerable coherence across the entire long axis (Buzsaki, 2002).

While examining how various manipulations might influence power and coherence, we discovered variation in the dynamics of theta power over the course of repeated trials of running on a linear runway (Hinman et al., 2011). Briefly, the
amplitude of theta LFP decreases over sessions of running within a daily session; this effect is quite prominent in temporal HPC sites, moderate at mid-septotemporal sites and generally negligible at septal HPC electrodes. Thus, theta at septal HPC sites exhibit the strongest relation to running speed and the amplitude is relatively stable over repeated running sessions. Theta at more temporal HPC sites exhibits weaker relationships with running speed and the amplitude of the theta signal decreases over daily run sessions. Such findings indicate the relative independence of theta generation across the long axis and highlight functional differentiation across the long axis of the HPC.

Many of the brain’s neurons use glutamate as their neurotransmitter. Glutamate receptors are thus important for sensory, motor and cognitive processes. N-methyl-d-aspartate glutamate receptors (NMDA-R) play a permissive role in synaptic change (Collinridge et al., 1983; Lynch et al., 1988) and exhibit distinct developmental and regional expression pattern (Monyer et al., 1984; Sanz-Clemente et al., 2012). NMDA receptors thus have a profound influence on the function and plasticity of association cortices including the HPC. Ketamine is an antagonist of NMDA receptors (Anis et al., 1983; Millan, 2005) that can produce a unique constellation of cognitive dysfunction including delay-dependent impairments on hippocampal-dependent memory tasks (see Morris et al., 1986; Robbins and Murphy, 2006; Bannerman et al., 2004 for review, as well as Chrobak et al., 2008). Systemic and intrahippocampal administration of NMDA-R antagonists decrease the theta signal but also produce subtle and significant changes in motor behavior (Leung and Shen, 2004). The presented study tested the hypothesis that ketamine treatment would decrease theta coherence across the long axis.
independent of speed related changes in the theta signal. Well-trained rats were
administered saline and two doses of ketamine (2.5 and 10 mg/kg; see Fig. 1A). The
effects of each treatment on theta power and coherence at distinct septotemporal
positions within CA1 were assessed over multiple five-minute run sessions (up to 120
minutes) post-injection.

Materials and Methods

Animals and Surgical Procedures

Six adult male Fisher-344 rats were individually housed in a temperature-
controlled room and maintained on a 12-h/12-h light-dark cycle. The guidelines set forth
by The University of Connecticut’s Institutional Animal Care and Use Committee and
NIH were strictly adhered to during all procedures presented in the present report.

The surgical procedures employed have been previously described (Hinman et
al., 2011; Penley et al., 2011). Briefly, burr holes were drilled in the rats’ skull over the
HPC following the induction of anesthesia with a ketamine cocktail (4 ml/kg consisting of
25 mg/ml ketamine, 1.3 xylazine mg/ml, and 0.25 acepromazine mg/ml). Several
electrode arrays, each consisting of four 50 µm tungsten wires (12 total electrodes;
California Fine Wire Co., Grover Beach, CA), were implanted in each animal at three
distinct sites across the septotemporal axis of the HPC (septal HPC: AP -3.0, ML 2.5,
DV 3.0; intermediate HPC: AP -5.0, ML 5.0, DV 5.0; temporal HPC: AP -6.5, ML 5.5, DV
7.0; all coordinates are relative to bregma (Swanson, 1998)). Stainless steel watch
screws positioned over the cerebellum served as indifferent and ground electrodes. Support screws were positioned in the anterior aspect of the skull, as well as the over the contralateral hemisphere and dental acrylic was used to bind the ensemble together. Rats were allowed to recover for one week following surgery.

**Ketamine Treatments**

Prior to electrode implantation, rats were food deprived to 85% of their ad libitum weight and extensively trained to shuttle between ends of a linear track (10 X 140 cm) for chocolate sprinkles. Ketamine hydrochloride (Ketaset, 100 mg/ml; Fort Dodge Laboratories, Fort Dodge, IA) was prepared in physiological saline and all injections were administered intraperitoneally in volumes of 1ml/kg. Each recording day started with a Baseline recording (5 minutes) at the end of which intraperitoneal injections of either ketamine (2.5 or 10 mg/kg) or saline were administered. The injections marked T₀. Four subsequent recordings were initiated at T₅, T₂₀, T₆₀ and T₁₂₀ minutes after the injection (Fig. 1A). During each recording rats were required to complete a minimum of 50 trials, each of which simply required the rat to move from one end of the track to the other. In between recording sessions, rats waited in their home cage on a table adjacent to linear track until the beginning of the next recording.

Doses of ketamine and saline were administered in counterbalanced order with each rat receiving each treatment once, with three or four days intervening between each treatment. Rats were run on the maze task for each intervening day between treatments. Doses of ketamine were chosen based on our experience giving ketamine
Ketamine disrupts hippocampal synchrony prior to radial water-maze performance (see Chrobak et al., 2008). Briefly rats receiving 2.5 mg/kg ketamine exhibit memory encoding deficits but do not exhibit any overt behavioral changes and experienced observers would be unable to detect ketamine treatment at this dose even when handling the rat. In contrast, at roughly 5 mg/kg and clearly at 10 mg/kg, an experienced observer/handler would detect some mild atonia (limb weakness) within 1-5 minutes while handling the animal. At these doses, however, rats will nonetheless run on a maze task, performed learned tasks, swim and the sensorimotor disruptions could be characterized as relatively mild. At doses of roughly 15-25 mg/kg, rats exhibit varying degrees of atonia with motor incoordination and mild head-weaving behavior for roughly 10 minute post treatment.

Electrophysiological Data Acquisition and Analyses

Neuralynx data acquisition system (Bozeman, MT) was used to record wide-band electrical activity (3787 samples/sec). An overhead camera recorded light emitting diodes attached to the headstage, which provided the animals position on the track over time and allowed for the calculation of locomotor speed by getting the positional difference between successive tracking samples (33 samples/sec).

All data analysis was conducted using custom written programs in MatLab (The MathWorks, Natick, MA) or in SPSS (SPSS Inc., Chicago, Illinois). Movement related data was visualized as a state-space plot (position versus velocity; Fig. 1B). All analyses were restricted to periods of locomotion by excluding data recorded during the consumption of sprinkles and turning behavior. A physical threshold 14 cm from each
end of the maze was set in order to exclude the just mentioned behaviors and any trial during which the rat’s speed decreased below 5 cm/sec was discarded from further analysis.

Spectral Indices

The average power in the 6 – 12 Hz band was calculated from the power spectral density estimates obtained using Welch’s averaged modified periodogram method (Welch, 1967) for each trial. In order to calculate coherence, EEG signals from individual trials were concatenated into continuous twenty second long strings of data (Roark and Escabi, 1999; see also Sabolek et al., 2009) with each recording generating a series of such strings with different associated mean speeds. In order to achieve this, trials were sorted based on mean speed and then the slowest trials totaling twenty seconds were concatenated, then the next slowest twenty seconds worth of data and so forth for the rest of the data. Coherence values (Bullock et al., 1990) for each channel pair were computed using the Welch periodogram estimation procedure with a spectral resolution of ~ 2 Hz obtained through the use of a b-spline window with a temporal half width of 0.48 sec..

Statistics: Coherence Analysis

A significance estimation procedure was devised in which the coherence estimate was compared to that of signals with identical magnitude spectrum but with
zero phase coherence. For each channel pair, the cumulative distribution of the
frequency-dependent coherence values was created by randomizing the phase
spectrum of the signals while preserving the magnitude spectrum, calculating the
coherence for the phase randomized signals, and bootstrapping the procedure 250
times (Efron and Tibshirani, 1993). This procedure guarantees that the signal
magnitude spectrums are identical but have no linear association, because the phase or
time information has been removed. The coherence distribution obtained via
bootstrapping the procedure was used to determine a significance threshold for each
frequency band (2 Hz resolution), below which 95% of the shifted null hypothesis
coherence values fell (i.e., the Null hypothesis; see also Sabolek et al., 2009).

Only regions of the experiment coherence spectrum falling above the 95%
threshold were considered statistically significant. For each channel pair, the statistically
significant area between the experiment coherence spectrum and the bootstrapped
coherence spectrum in the theta (6-12 Hz) range was calculated and normalized by
themaximum possible coherence within the theta range, which consists of the total area
above the bootstrapped coherence spectrum. Finally, the normalized coherence area
was divided by the frequency bandwidth, thus yielding values expressed as average
coherence value per Hz.

Statistics: Linear Regression

In order to control for any changes in the locomotor speed of the rats’, a linear
regression analysis was utilized. The mean speeds and four orthogonally coded
dummy categorical variables for the five recording timepoints (e.g., $T_5$, $T_{20}$ etc) were included as explanatory variables. Each electrode site (or electrode pair) yielded a single standardized regression coefficient ($\beta$-value, where $\beta = b \frac{SD_x}{SD_y}$) for each of the explanatory variables. Thus the regression equation takes the form: $y = \beta_{T5}X_1 + \beta_{T20}X_2 + \beta_{T60}X_3 + \beta_{T120}X_4 + \beta_{\text{speed}}X_5 + \epsilon$. The resulting $\beta$-values for the different time points indicate how theta power changes in relation to the baseline recording while controlling for speed. Thus a negative $\beta_{T5}$ value indicates that theta power for a particular electrode was decreased during the recording five minutes after an injection compared to the baseline recording having controlled for speed. Distributions of $\beta$-values were assessed using a repeated measures ANOVA followed by post hoc Dunnett T-tests (Lorch and Myers, 1990).

**Histology**

After the final recording, rats were anesthetized with Euthasol (sodium pentobarbital solution) before being transcardially perfused with saline and then 4% paraformaldehyde in 0.1M phosphate buffer. Brains were removed from the skull and sliced (50 µm sections) using a vibratome (Vibratome Series 1500). Sections were then mounted and Nissl stained using thionin. Electrode placements were then verified and categorized based on laminar and septotemporal position. Electrodes positioned in all lamina of CA1 were grouped together and thus all subsequent references to CA1 electrodes includes those positioned in all lamina of CA1. All final placements were indicated on a flatmap representation of the hippocampus (Swanson et al, 1978) and
were documented with photomicrographs using a Nikon microscope connected to a Spot RT camera system, digitized and prepared for presentation using Adobe Photoshop 7.0.

Results

Electrodes were implanted at multiple septotemporal levels of CA1 (Fig. 3A) and local field potentials were recorded while rats shuttled between ends of a linear track before and after i.p. injections of ketamine (2.5 or 10 mg/kg) or saline (Fig. 1A). A series of five recording sessions were obtained for each dose, which included a baseline recording and four post injection recordings (Fig. 1A). Injections were administered at the end of the baseline recording and post injection recordings were initiated 5, 20, 60 and 120 minutes after the injection. Each recording session required the rat to complete 50 trials, where a single trial simply constituted a traversal from one end of the maze to the other.

Effect of ketamine on locomotion

Since ketamine is known to acutely alter locomotive behavior (eg, Littlewood et al., 2006), we used the tracking data obtained during each recording to investigate whether the animals running performance changed as a result of either dose of ketamine or across the multiple recording sessions. Both the maximum speed per trial and the mean speed per trial were dose dependently affected by ketamine (see Fig. 1B,
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C, D). RMANOVA’s on dose x time trial speed data indicated a significant interaction between dose and time on both measures [F’s(8, 40) > 5.57 p < 0.001; Fig. 1C, D]. Post hoc analyses indicated that only the 10 mg/kg dose of ketamine decreased (~ 20%) running speed at both the 5 and 20 minute time points (t’s(5) > 3.9; p < 0.01). By 60 and 120 minutes post-injection, rats receiving 10mg/kg were running at the same average and maximum speeds as during baseline. Note there were no significant changes in the maximum speed per trial or the mean speed per trial as a function of the multiple recording sessions following either saline or 2.5 mg/kg ketamine injections. The latter indicates that rats did not become fatigued or less motivated after having run multiple sessions within a single day. This is notable because decreases in theta power are observed at certain electrode sites as a function of multiple run sessions (see Fig. 3B, as well as Hinman et al., 2011) and this is not obviously related to decreased running speed or the motivational level that speed indicates.

**Theta coherence**

Our main finding was that 2.5 mg/kg ketamine decreased theta coherence across the long axis as a function of the septotemporal distance between electrodes (Fig. 2A, B) during the first five minute post-injection recording session. A decrease in theta coherence was also observed following 10 mg/kg ketamine, but the decrease was relatively constant with respect to septotemporal distance between electrodes. Figure 2A shows coherence before (blue) and five minutes after (orange) saline and each dose of ketamine for two pairs of electrodes within a single animal with the inset showing the
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theta frequency range. The three electrodes are positioned in quartile 1 (Q1), quartile 2 (Q2) and quartile 3 (Q3) of CA1 and thus the two pairs span different distances of the septotemporal axis. It can be seen that regardless of the distance between the electrodes there is no change in theta coherence following a saline injection, but that there are decreases following injections of both 2.5 and 10 mg/kg of ketamine. In particular, following 2.5 mg/kg ketamine the decrease is larger for the pair positioned farther apart than the pair located closer together (top vs bottom of middle column Fig. 2A; see also Fig. 2B, 2C), while both pairs are similarly decreased following 10 mg/kg ketamine (top vs bottom of right column Fig. 2A).

A simple linear regression analysis on the change in theta coherence from baseline in relation to the distance between electrodes is plotted in Figure 2B. In the first five minutes following saline injection there was no change in theta coherence regardless of the distance between the electrodes as indicated by the non-significant intercept and slope (intercept = 0.01, p = 0.18; slope = -0.0003, p = 0.94; Fig. 2B left). The low dose of 2.5 mg/kg ketamine resulted in a septotemporally differential change in theta coherence, such that electrodes positioned close together underwent a small decrease in theta coherence while electrodes positioned farther apart decreased by greater amounts (intercept = -0.028, p < 0.01; slope = -0.015, p < 0.0005; Fig. 2B middle). The significant change in intercept indicates that even pairs of electrodes positioned at the same septotemporal level displayed decreased theta coherence; the significant negative slope indicates that theta coherence decreased by greater amounts the farther apart the electrodes were separated. This graded effect was not observed following administration of 10 mg/kg ketamine, but instead theta coherence was
decreased similarly regardless of the distance between the electrodes (intercept = -0.10, p < 0.001; slope = -0.001, p = 0.89; Fig. 2B right).

As noted in the introduction, theta power is highly correlated with locomotor speed particularly at septal CA1 sites (Fig. 4A). In order to ensure that the changes in coherence did not result from the changes in running speed following 10 mg/kg, a multiple regression was conducted regressing locomotor speed on theta coherence between each electrode pair across the multiple recordings of each dose. The regression analysis confirmed each change observed in theta coherence described above with no changes resulting following saline injection (intercept = 0.07, p = 0.21; slope = -0.005, p = 0.87; Fig. 2C left), a significant graded decrease following 2.5 mg/kg ketamine (intercept = -0.16, p < 0.01; slope = -0.10, p < 0.001; Fig. 2C middle) and a uniform decrease in theta coherence regardless of the distance between the electrodes following 10 mg/kg (intercept = -0.93, p < 0.001; slope = -0.03, p = 0.39; Fig. 2C right).

The latter analyses in fact mirrored the changes observed independent of the speed measures indicating that the changes in theta coherence at either dose were independent of any speed related differences.

Theta power: habituation across sessions following saline

As noted in the introduction, minimally three key factors predict variability in theta power at a given electrode site over multiple run sessions: 1) running speed, 2) the septotemporal position of the recording electrode and 3) the number of run sessions. In line with previous observations, mean theta power in the second and third quartiles
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significantly decreased across the multiple recording sessions following saline injections: a RMANOVA on all data obtained prior (baseline) and subsequent to saline treatment (+5, +20, +60 and +120 minutes) indicated a significant Time x Quartile interaction (F(8,88) = 3.97, p < 0.001). Post-hoc analyses at each time interval (eg, +5 min) indicated that significant decreases (p < 0.05) were observed in the second and third quartiles by the first session (+5 min) and all subsequent sessions (Fig. 3B, left column). Note no changes were observed among Q1 electrodes even by the fifth run session (+ 120 min; Fig. 3B, top left).

Despite the decreases in theta power in Q2 and Q3 during the second run session, there was no decrease in theta coherence at any electrode pair across the septotemporal axis. This illustrates the important point that changes in the power of a signal(s) does not necessitate changes in the coherence of those signals; changes in theta power can alter theta coherence but the biological mechanisms mediating changes in power at single sites and coherence across sites can be relatively independent.

**Theta power: Acute decrease following only 10 mg/kg ketamine dose**

As evidenced in Figure 3B, the effects of ketamine treatment on theta power varied as a function of dose, time and quartile. An omnibus Dose x Quartile x Session RMANOVA indicated significant interactions across all main factors (p < 0.05). Surprisingly, we observed a clear alteration of the habituation in theta power observed at Q2 and Q3 electrodes (compare time series of changes following saline versus doses
of ketamine in Fig. 3B). Given this alteration of the habituation effect, we ran separate RMANOVA’s (Dose x Quartile) for each run session (+5, +20, etc) with subsequent post-hoc comparisons to the saline treatment within each quartile (eg, +5 saline in Q3 vs +5 min 2.5 mg/kg ketamine in Q3).

The 2.5 mg/kg dose had no effect on theta power as compared to saline treatment during the initial +5 and +20 minute run sessions (p > 0.05; Fig. 3B, middle column) regardless of electrode position. This is particularly noteworthy given that theta coherence is altered during these initial run sessions (Fig. 2). The higher dose of ketamine (10 mg/kg) did decrease theta power relative to saline during the +5, +20 and + 60 min run sessions in Q1 and Q2 and during the first run session in Q3 (p < 0.01; see Fig. 3B, right column).

**Theta power: blunting of habituation**

The only effect of 2.5 mg/kg ketamine on theta power was to decrease the degree of habituation observed during the later run sessions. In Q2 during the last run session and Q3 during the last two run sessions, theta power was elevated relative to the time matched sessions following saline treatment (p < 0.01; see Fig. 3B, middle column). A similar pattern was observed following 10 mg/kg, with Q3 electrodes having theta power elevated relative to saline treatment during the last run session (p < 0.005; Fig. 3B lower right). The data suggest that NMDA receptors directly contribute in some way to the theta habituation effect. Given that the effect is observed later in the course of repeated runs (eg, +60, +120 minutes), it may be that this effect relates to alterations
Ketamine disrupts hippocampal synchrony in the rats sensory experience as the effects of ketamine wear off. It is certainly the case that the encoding of spatial memory is impaired within the first 10-15 minutes following ketamine administration (Chrobak et al., 2008) and it may be the case that the altered habituation reflects an altered recognition of recent experience.

**Relationship between speed and theta power under ketamine**

Consistent with previous findings (Hinman et al., 2011; Maurer et al., 2005), the relationship between theta power and running speed varied as a function of the septotemporal position of the electrodes during the baseline recording session (Fig. 4). In order to examine what, if any, effect ketamine treatment had on the relationship between locomotor speed and theta power, we examined the correlations between speed and theta power in each quartile during the baseline period and during subsequent run sessions. Figure 4A (first column) illustrates the decrease in this relationship across a set of CA1 electrodes at different septotemporal positions in a single animal during the baseline and first post-injection saline run session. Theta recorded from a septal electrode (Q1; Fig. 4A, top row) increased as a function of running speed, while theta recorded from a mid-septotemporal (Q2; Fig. 4A, middle row) and a more temporal site (Q3; Fig. 4A, bottom row) exhibited relatively flatter slopes. Mean r-values for all electrodes, in all animals, during the baseline recording session were 0.63 +/- 0.07, 0.31 +/- 0.08 and 0.21 +/- 0.08 for Q1-3, respectively. Ketamine (10 mg/kg) decreased the speed to power correlation at all CA1 electrode sites (Fig. 4B) and decreased the slope value only at Q3 electrodes (Fig. 4C).
A RMANOVA on the r-values indicated a significant dose effect ($F(2,44) = 17.44$, $p < 0.001$), subsequent post-hoc tests indicated that only 10 mg/kg dose was significant in all quartiles (see Fig. 4B). The decrease in the speed to power relationship was most prominent following the 10 mg/kg dose particularly at mid-septotemporal and temporal sites (Fig. 4A, right column). There was also a significant decrease in the slopes of the speed to power relationship following 10 mg/kg at more temporal electrodes sites (Q3), while the speed to power slopes were relatively constant at all sites following saline and 2.5 mg/kg ketamine (Fig. 4A, B). Thus, the higher dose of ketamine (10 mg/kg) decreased the variability in theta power explained by speed at all septotemporal sites and altered the slope of the speed to power relationship at the most temporal electrode sites (Fig. 4A, B). Such findings minimally indicate that NMDA receptors play a more prominent role in the speed to power transformation within hippocampal circuits at more temporal electrodes, despite the fact that these electrodes exhibit a much more limited relationship to speed in general. While speculative, these findings hint that sub-cortical inputs responsible for the speed to power relationship in the septal hippocampus do not directly engage more temporal levels of the HPC, rather intrahippocampal glutamatergic circuits may convey speed-modulated information down the long axis.

**Delta coherence and power**

Previous reports have demonstrated that NMDAR antagonism can increase the prevalence of delta frequency (1 – 4 Hz) activity (Leung and Desborough, 1988; Zhang et al., 2012) and the presence of delta frequency oscillations may disrupt theta
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coherence in the stationary rat (Leung et al., 1982). Since all of the data included in the current analysis was restricted to periods of locomotion, we first determined whether delta power varied as a function of running speed. Across all three quartiles of CA1, delta power decreased as a function of running speed (p < 0.05; Fig. 5A, B). Delta power was not altered at any time point at any septotemporal position following 2.5 mg/kg dose (p > 0.05), while delta power did increase in Q2 and Q3 positions following the 10 mg/kg dose (p < 0.05). Multiple regression analysis indicated that when running speed is taken into consideration delta power actually decreased in Q1 (p < 0.05), but remained unchanged in Q2 and Q3 following 10 mg/kg ketamine (p > 0.05) indicating that the increases in delta power observed in Q2 and Q3 were a consequence of the slower running speeds following 10 mg/kg ketamine. Coherence within the delta frequency range remained unchanged following injections of saline and 2.5 mg/kg ketamine regardless of the distance between the electrodes (intercepts: p > 0.05; slopes: p > 0.05; Fig. 5C, D). Following injections of 10 mg/kg ketamine delta coherence actually increased among nearby electrodes, while delta coherence approached zero for electrodes positioned farther apart as indicated by the negative slope (change in delta coherence: intercept = 0.12, p < 0.05; slope = -0.02, p < 0.05; standardized regression coefficient: intercept = 0.56, p < 0.05; slope = -0.09, p < 0.05; Figure 5C, D). Thus the decreases in theta coherence described above (especially after 2.5 mg/kg ketamine) are unlikely to be a result of increased delta frequency oscillations.

Discussion
The present study tested the hypothesis that ketamine treatment would decrease theta coherence across the septotemporal axis of the HPC independent of alterations in running speed. Our main finding is that a relatively low dose of ketamine (2.5 mg/kg), a dose that produces no overt changes in sensorimotor performance, decreases theta coherence across the septotemporal CA1 network. Ketamine at 2.5 mg/kg did not alter running speed, or the power of theta, but decreased the coherence of the theta signal across the long axis. The decrease in coherence across the long axis was greater with increasing distance between CA1 electrode pairs. The latter suggests that long-range intrahippocampal synchronization, and perhaps hippocampal-entorhinal or hippocampal-prefrontal communication, may be highly dependent on NMDA receptors. We suggest that the dynamic and presumably highly plastic interactions among large ensembles of hippocampal and neocortical neurons are highly sensitive to low doses of NMDA blockade and that such disruption likely mediates the cognitive and memory dysfunctions consequent of NMDA receptor antagonist administration.

Two other observations were noteworthy. First, ketamine (10 mg/kg) not only decreased running speed and theta power, but also significantly altered the speed to power relationship (altering the slope of the linear relationship between speed and theta power) prominently at mid-septotemporal and temporal electrode positions. The latter suggests that there is a differential contribution of NMDA receptor activation to the speed to power relationship across the septotemporal axis. In this regard, we have observed a decrease in theta power at sites distal from the septal pole over repeated running sessions (habituation). This characteristic “habituation” in theta power increases
Ketamine disrupts hippocampal synchrony in magnitude with distance from the septal pole (see Figure 6 in Hinman et al, 2011). The septotemporal variation in habituation, and the sensitivity of speed to power relationships to ketamine treatment, suggests that the speed to power relationship may be highly variable or exhibit short-term “plasticity”. We are currently exploring the consistency of the speed to power relationship at individual electrode sites over multiple sessions and days of recording.

More surprising was our second observation that the habituation of theta power observed at more temporally located CA1 sites were blunted by ketamine treatment at both 2.5 and 10 mg/kg. The habituation (degree of decrease) of theta power at CA1 sites increases (theta decreases more) over multiple repeated running sessions (5 minutes of running spaced tens of minutes apart). The greatest habituation (decrease) was observed during the last session of the day (two hours after an initial baseline recording). The largest decrement (blunting or reversal) of this habituation was also most evident during latter sessions, hours after ketamine treatment.

NMDA receptors may contribute to speed to power relationships

Glutamatergic input clearly contributes to the generation of the theta LFP and glutamatergic antagonists administered peripherally or centrally decrease characteristics of the theta as well as gamma signals (Leung and Shen, 2004; Zhang et al., 2012). Many studies examining the effects of pharmacological manipulations on theta have not necessarily controlled for changes in motor performance while examining changes in either theta power or frequency. Subtle modifications in the behavior of the
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rat (eg, changes in speed) following both central or peripheral drug administration may contribute to changes in theta as opposed to any direct effect on the intrahippocampal or extrahippocampal circuits generating theta (see Buzsaki et al., 1979; 1981 for discussions). The results reported in the current study illustrate how ketamine at fairly low dose (10 mg/kg) can alter theta power at specific electrode sites likely as a consequence of changes in locomotor speed, while lower doses (2.5 mg/kg) alter theta indices independent of any changes in locomotor speed.

It is important to note that the present results, obtained following peripheral drug administration, do not directly speak to modulation of intrahippocampal NMDA receptors. Rather, they speak more directly to ketamine-induced changes in hippocampal physiology that likely result from actions of ketamine at multiple NMDA receptors in the brain, and as would occur in relation to memory/cognitive dysfunction in association with peripheral ketamine treatment (eg, Chrobak et al., 2008).

Ketamine treatment and memory dysfunction

Peripheral NMDAR blockade can disrupt the encoding, retention, and retrieval of memories (Newcomer and Krystal, 2001; Bannerman et al., 2006; Robbins and Murphy, 2006) including performance on hippocampal-dependent memory tasks (see Chrobak et al., 2008 for review and references). Given the widespread distribution of NMDAR in cortical areas, it is likely that multiple memory systems (eg, procedural and declarative) and processes (eg, encoding and retrieval) contribute to task-related deficits. The information processing functions of the HPC and prefrontal cortex, as well as many
areas of limbic cortex, are adapted to provide maximal plasticity to allow for flexibility in sensori-sensory and sensori-motor circuits. Such flexibility may be highly sensitive to pharmacologic manipulation of NMDA receptors.

In this regard, we have observed that the lower dose of ketamine (2.5 mg/kg) used in the present study can impair the encoding of one-trial, episodic memories (hippocampal-dependent) in a task in which the performance and procedural memory skills are well-learned (Chrobak et al., 2008) when drug is administered just prior to-be-remembered events. Such studies suggest that low doses of ketamine can selectively weaken the “strength” of encoded representations (see also Kentros et al., 1998). Our current study highlights that long-range coherence is likely the most sensitive measure of neurophysiological activity altered by peripheral ketamine administration. A few recent studies have highlighted hippocampal-prefrontal coherence in relation to behavioral/cognitive performance in rodents (Sigurdsson et al., 2010). The present findings illustrate that long-range coherence is disrupted by ketamine treatment and we suggest that future studies should examine whether lower doses disrupt hippocampal-prefrontal coherence.

**Putative neurophysiological mechanisms**

Ketamine induces a range of physiological effects consistent with the wide distribution of NMDAR in the brain. The present data make no statement about the underlying mechanisms, although we suggest the alterations of hippocampal theta may depend on physiological changes subsequent to NMDAR blockade in both hippocampal
and widespread brain regions. Our evidence indicates that fairly low doses (2.5 mg/kg) disrupt theta coherence most prominently at millimeter distant electrode sites in septal and temporal HPC. In this regard, our studies highlight the disruption of long-range communication as a central feature of ketamine-induced neurophysiological events in contrast to a focal site of action at any of the numerous NMDA receptors in the brain.

**Summary**

The hippocampal theta (local field potential) signal reflects moment-to-moment variation in synaptic inputs to hippocampal neurons. One source of variation in the amplitude of theta is locomotor speed and the relationship of this variable to theta power varies across the septotemproral axis. The theta signal also habituates in amplitude in relation to “experience”, thus simply running across a linear track over time decreases the amplitude of theta. This phenomenon also exhibits variability across the septotemporal axis. The present studies demonstrate that doses of the NMDA antagonist ketamine that alter memory and cognitive performance in the rat can alter theta indices independent of overt changes in behavior (locomotor speed). Further they illustrate dynamic variability in the relationship between locomotor speed to theta power and that this variability is sensitive to alteration by ketamine treatment. Most importantly, the study demonstrates that long-range synchronization of the theta signal across the septotemporal or long axis of the HPC is highly sensitive to the lowest dose of ketamine. We suggest that a neural network account of the effects of ketamine includes a disruption of long-range communication in cortico-cortico networks.
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References


Buzsáki G, Leung L, Vanderwolf CH (1983) Cellular basis of hippocampal EEG in the
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Welch PD (1967) The use of fast fourier transform for the estimation of power spectra:
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Figure 1. **Experimental procedure and locomotor behavior** A) Timeline of the recording procedure with a baseline recording followed by an injection of saline, 2.5 or 10 mg/kg ketamine and post-injection recordings starting 5, 20, 60 and 120 minutes after the injection. Each recording required the rat to run fifty trials on a linear track. B) Each plot shows a single rat's velocity as a function of position on the linear track during Baseline (*left column*) and five minutes (*right column*) after 2.5 (*top row*) and 10 mg/kg ketamine (*bottom row*). Track traversals from left to right and from right to left are represented as positive and negative velocities, respectively. Note that there was no change in locomotor behavior following 2.5 mg/kg ketamine, but that maximum and average velocity was reduced following 10 mg/kg. C) As a group, rats (N=6) ran at slower mean speeds during the +5 and +20 minute post-injection recordings following 10 mg/kg ketamine, but showed no change in mean running speed at any time point following 2.5 mg/kg. D) The same pattern of changes was observed for the maximum speed achieved per trial with rats achieving lower maximum speeds following 10 mg/kg ketamine, but not following either saline or 2.5 mg/kg ketamine.

Figure 2. **Ketamine disrupts theta coherence across the septotemporal axis.** A) Coherence within the theta range is shown for two pairs of electrodes from a single animal (*rows*) differing in the distance across the septotemporal axis between the electrodes (*right*). No change in theta coherence is observed between either pair of electrodes following the saline injection, but there is a selective decrease in theta coherence between the more distant pair of electrodes following 2.5 mg/kg ketamine. Theta coherence is similarly decreased between both electrode pairs following 10 mg/kg ketamine despite the difference in distance between the electrodes. B) Change in mean theta coherence during the +5 minute post-injection recording from Baseline is shown as a function of the distance between the electrodes. There is no change in mean theta coherence following saline injection, but theta coherence decreased as a function of the distance between the electrodes across the septotemporal axis following 2.5 mg/kg ketamine and decreased similarly regardless of the distance between the electrodes following 10 mg/kg. C) Same as in B), but for the standardized regression coefficients. The same pattern of changes was observed when controlling for the speed of the animal.

Figure 3. **Ketamine acutely decreases theta power and blocks experience dependent theta power habituation.** A) The location of all electrode recording sites are marked with stars on the flatmap representation of CA1. The bold lines mark the boundaries between quartiles. B) Theta power as a percentage of Baseline is shown...
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for each quartile (rows) following each dose of ketamine and saline (column). Theta power habituated as a function of repeated experiences on the track in the second and third quartiles following saline administration (left column). There were no acute effects on theta power following 2.5 mg/kg compared to saline, but the experience dependent habituation observed following saline was blocked by 2.5 mg/kg ketamine (middle column). 10 mg/kg ketamine both decreased theta power acutely and blocked theta power habituation (right column).

Figure 4. **Ketamine alters the relationship between speed and theta power.** A) The relationship between speed and theta power for three electrode sites from a single animal is shown for the Baseline (blue) and 5 minute post injection (orange) recording for both doses of ketamine and saline. Right, a flatmap representation of CA1 is shown with the three electrode sites marked by stars and their distance from the septal pole indicated. Note particularly that the slope of the relationship between speed and theta power show a septotemporally differential decrease following 10 mg/kg ketamine, but remain unchanged following 2.5 mg/kg ketamine and saline. B) Ketamine dose-dependently decreased the amount of variability in theta power explained by running speed in all three quartiles. C) The slope of the speed to theta power relationship is decreased selectively in the third quartile, without a change to the slopes in the first and second quartile.

Figure 5. **The effect of running speed and ketamine on delta.** A) A clear negative relationship between running speed and delta power is shown for a single electrode. B) Mean correlation coefficients for each quartile of CA1 investigated. Note that the relationship is negative in each quartile, thus indicating that delta power decreases as a function of running speed. C) Change in delta coherence 5 minutes post injection as a function of the distance between the electrodes is shown for both doses of ketamine and saline injection. No change in delta coherence is observed following saline and 2.5 mg/kg ketamine, but 10 mg/kg ketamine increases delta coherence between closely positioned electrodes. C) Same as in B), but for standardized regression coefficients.
Figure 1

A

Home Cage

Maze

Time

Base +5 +20 +60

T0 Saline
2.5 mg/kg
10 mg/kg

B

Baseline +5 minutes post injection

2.5 mg

10 mg

Position on track (cm)

Velocity (cm/sec)

D

Percent of Baseline

Minimum Speed

Position on track (cm)

Minutes post injection

2.5mg Saline 10mg

Baseline +5 +20 +60 +120

* *
Figure 2

A

Saline

2.5 mg

10 mg

Coherence

Coherence

Baseline

+5 minutes post injection

Frequency (Hz)

Frequency (Hz)

Distance between electrodes (mm)

Change in theta coherence from baseline

Standardized Regression Coefficient (g)

B

Saline

2.5 mg

10 mg

C

Distance between electrodes (mm)

Distance between electrodes (mm)

Distance between electrodes (mm)
Figure 4

A

Saline  2.5 mg  10 mg

Theta Power (dB)  Theta Power (dB)  Theta Power (dB)

Mean Trial Speed (cm/sec)  Mean Trial Speed (cm/sec)  Mean Trial Speed (cm/sec)

Baseline  +5 minutes post injection

2.6 mm  4.8 mm  7.0 mm

B

C

Figure 4