

# Novelty and Anxiolytic Drugs Dissociate Two Components of Hippocampal Theta in Behaving Rats

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Hippocampal processing is strongly implicated in both spatial cognition and anxiety and is temporally organized by the theta rhythm. However, there has been little attempt to understand how each type of processing relates to the other in behaving animals, despite their common substrate. In freely moving rats, there is a broadly linear relationship between hippocampal theta frequency and running speed over the normal range of speeds used during foraging. A recent model predicts that spatial-translation-related and arousal/anxiety-related mechanisms of hippocampal theta generation underlie dissociable aspects of the theta frequency–running speed relationship (the slope and intercept, respectively). Here we provide the first confirmatory evidence: environmental novelty decreases slope, whereas anxiolytic drugs reduce intercept. Variation in slope predicted changes in spatial representation by CA1 place cells and novelty-responsive behavior. Variation in intercept predicted anxiety-like behavior. Our findings isolate and doubly dissociate two components of theta generation that operate in parallel in behaving animals and link them to anxiolytic drug action, novelty, and the metric for self-motion.

## Introduction

The hippocampal formation is held to play key roles in two very distinct brain functions: (1) context-dependent spatial and declarative memory (O’Keefe and Nadel, 1978; Squire, 1992; Aggleton and Brown, 1999; Eichenbaum, 2000), linked to novelty detection (Hasselmo et al., 1996; Lisman and Grace, 2005); and (2) anxiety (Gray and McNaughton, 2000; Kjelstrup et al., 2002; Bannerman et al., 2004; Engin and Treit, 2007; Oler et al., 2010), linked to stress/depression. The temporal organization of hippocampal processing is dominated by the septohippocampal theta rhythm of the local field potential (LFP) (Buzsáki, 2002; O’Keefe, 2006), implicated in anxiety/anxiolytic-drug action (Gray, 1982; Gray and McNaughton, 2000; Seidenbecher et al., 2003; Gordon et al., 2005; Shin et al., 2009; Adhikari et al., 2010; Cornwell et al., 2012), memory-related novelty processing (Hasselmo et al., 2002; Düzel et al., 2010; Lever et al., 2010; Rutishauser et al., 2010), and spatial

cognition (Jones and Wilson, 2005; Buzsáki, 2006; O’Keefe, 2006; Giocomo et al., 2007, 2011; Maurer and McNaughton, 2007; Huxter et al., 2008; Brandon et al., 2011; Jezek et al., 2011; Koenig et al., 2011).

Two mechanisms of theta generation have been identified: (1) “movement-related” type I relating to translational movement; and (2) “alert immobility-related” type II linked to arousal/anxiety (Kramis et al., 1975; Sainsbury et al., 1987; Buzsáki, 2002). A recent model (Burgess, 2008) links these two mechanisms to dissociable components of the relationship of theta frequency [ $f_{\theta}(t)$ ] to running speed [ $s(t)$ ]:

$$f_{\theta}(t) = f_0 + \langle\beta\rangle s(t),$$

where the increase with running speed ( $\langle\beta\rangle$ , slope) reflects the presence of “velocity-controlled oscillators” in the septohippocampal system (Burgess, 2008; Weldon et al., 2011): neurons whose firing shows theta-band modulation whose frequency increases with running speed, as seen in place (Geisler et al., 2007) and grid (Jeewajee et al., 2008a) cells. This slope component is identified with type I mechanisms in being movement related and entorhinal cortex dependent (O’Keefe, 2006); the second component ( $f_0$ , intercept) is identified with type II theta mechanisms in being independent of both movement and entorhinal cortex (Burgess, 2008, pp 1168–1169). Thus, the model predicts a dissociation between the factors affecting the intercept and slope of the relationship of theta frequency to running speed. Although work on theta typically focuses on theta power, two findings relate to these predictions for theta frequency.

All clinically effective anxiolytic drugs reduce average theta frequency, despite their substantial neurochemical dissimilarities

Received Oct. 4, 2012; revised March 13, 2013; accepted April 4, 2013.

Author contributions: C.E.W., D.P.A., V.D., J.R., N.B., and C.L. designed research; C.E.W., D.P.A., V.D., and C.L. performed research; C.E.W., D.P.A., A.J., V.D., N.B., and C.L. analyzed data; C.E.W., D.P.A., A.J., V.D., J.R., J.O., N.B., and C.L. wrote the paper.

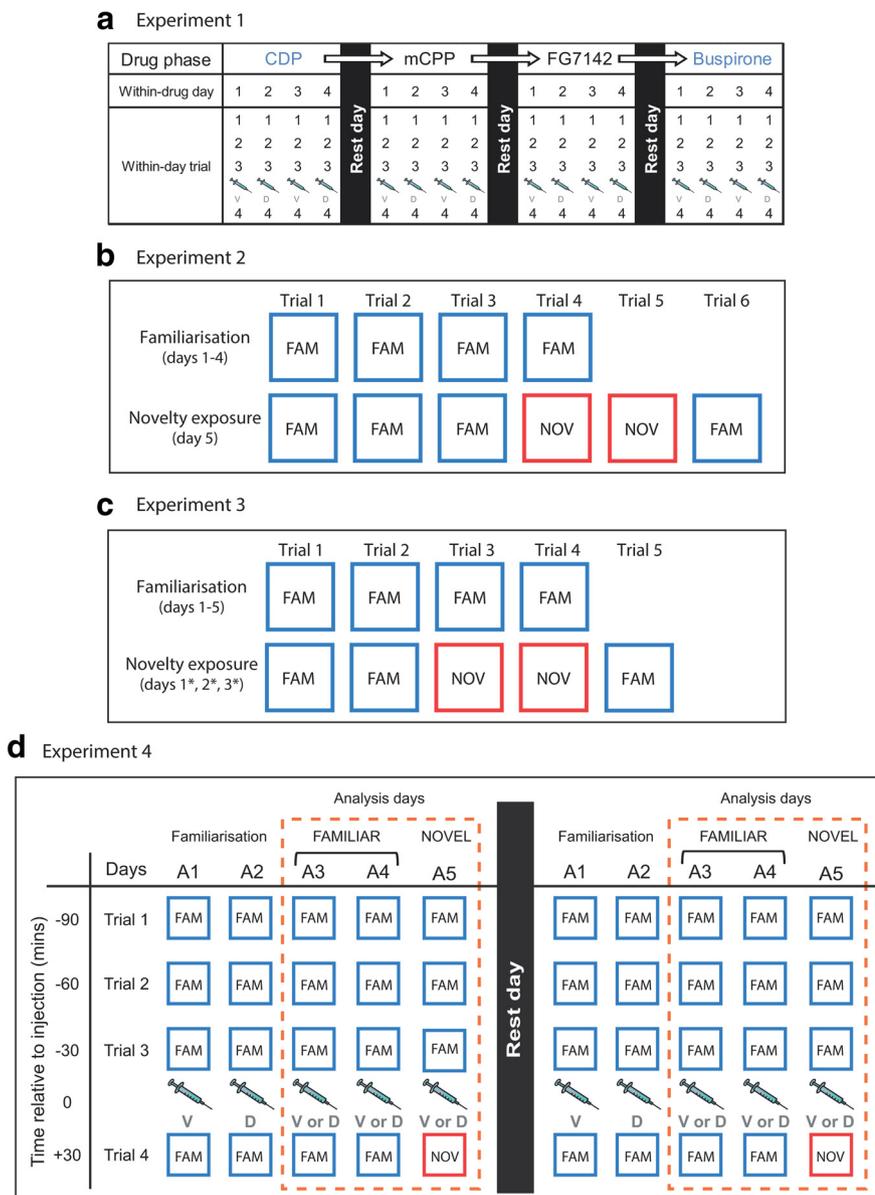
This work was supported by Biotechnology and Biological Sciences Research Council, Royal Society, Wellcome Trust, Medical Research Council, European Union SpaceBrain, and a research studentship (C.E.W.) and research funds (C.L.) from Institute of Psychological Sciences, University of Leeds. We thank Caswell Barry, Francesca Caccuci, and Peter Dayan for useful discussions.

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DOI:10.1523/JNEUROSCI.5040-12.2013

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**Figure 1.** Experimental setup and timelines. **a**, Experiment 1 procedure. Intraperitoneal injections were given immediately after the third trial of the day (V, vehicle; D, drug).  $n = 4$  rats. In addition, two additional drug-naïve rats (rats 5 and 6) were exposed to the testing environment of experiment 1 for 4 consecutive days and for five trials per day, with injections given immediately after the fourth trial of the day (days 1–3, saline; day 4, buspirone). **b**, Experiment 2 procedure.  $n = 6$  rats. **c**, Experiment 3 procedure.  $n = 5$  rats. **d**, Experiment 4 procedure.  $n = 6$  rats. Trials were 10 min in experiments 1, 3, and 4 and 15 min in experiment 2. ITIs ranged from 20 to 50 min. See Materials and Methods. FAM, Familiar; NOV, novel.

(Gray and McNaughton, 2000). In addition, immobility-related type II theta occurs during predator-elicited arousal/anxiety (Sainsbury et al., 1987) and during “anticipatory anxiety” (Gray and McNaughton, 2000) after standard footshock conditioning (Seidenbecher et al., 2003). Accordingly, because (1) arousal/anxiety is explicitly linked to type II theta, (2) anxiolytics reduce reticular-elicited theta frequency, and (3) the Burgess (2008) model links type II theta mechanisms to intercept, the model predicts that anxiolytics should specifically reduce the intercept  $f_0$ .

Like anxiolytics, environmental novelty reduces average hippocampal theta frequency (Jeewajee et al., 2008b). Because Burgess (2008) relates slope ( $\beta$ ) inversely to grid spatial scale and grid scale increases in novelty (Barry et al., 2012), we pre-

dicted that novelty would specifically decrease the slope ( $\beta$ ) of the frequency–speed relationship.

Parts of this paper have been published previously in abstract form (Wells et al., 2009b).

## Materials and Methods

### General methods

All subjects (experiment 1,  $n = 4 + 2$ ; experiment 2,  $n = 6$ ; experiment 3,  $n = 5$ ; experiment 4,  $n = 6$ ) were individually housed male Lister Hooded rats weighing 320–445 g at time of surgery. Rats were chronically implanted with microdrives in the hippocampus under deep anesthesia and were given 1 week for postoperative recovery, after which electrodes were lowered over several days/weeks. Rats were food restricted from ~1 week after surgery (target was 85% of initial bodyweight). Rats foraged for sweetened rice in the testing environments and were placed on a holding platform between trials in the same room as the testing environment. Across a given experiment, the first trials of the day were begun at a similar time of day. Trial durations were 10 min in experiments 1, 3, and 4, and 15 min in experiment 2. For additional details of the experimental setup and timelines, see Figure 1.

LFP data were recorded using an Axona data acquisition system. All electrode wire was heavy polyimide enamel-coated, 90% platinum–10% iridium (California Fine Wire). LFP was recorded single ended from one electrode tip of a 500  $\mu\text{m}$  separated stereotrode (i.e., effectively single electrode, 100  $\mu\text{m}$  diameter, three of four rats in experiment 1) or of a tetrode (25  $\mu\text{m}$  diameter, one rat in experiment 1 and all rats in experiments 2 and 3; 17  $\mu\text{m}$  diameter, plated, all rats in experiment 4). LFP signals were amplified 2000–8000 times, bandpass filtered at 0.34–125 Hz, and sampled at 250 Hz. Two light-emitting diode arrays attached to the head stage and viewed by an overhead camera tracked head position/orientation.

The LFP electrode sites for all rats in experiments 1, 2, and 4 were confined to dorsal, anterior CA1 (~3.0–4.6 mm posterior to bregma). The LFP electrode site ranged from stratum oriens to stratum lacunosum-moleculare: no effect of layer on the results was seen. The LFP electrode sites for experiment 3 (primarily a reanalysis of novelty-exposure days in

the study by Jeewajee et al., 2008b) were in different regions in the dorsal hippocampus: CA1 (two rats), subiculum (two rats), and dentate gyrus (one rat).

### Experiment-specific methods

**Experiment 1: effects of anxiolytic drugs.** The test environment was a black, square-walled open-field environment (dimensions, 60  $\times$  60  $\times$  50cm) with black Plexiglas flooring (65  $\times$  65cm). The experiment lasted for 19 d and consisted of four 4-d drug phases [in order: chlordiazepoxide hydrochloride (CDP); 1-(3-chlorophenyl)piperazine hydrochloride (mCPP); FG7142; and buspirone hydrochloride] (for experimental timeline, see Fig. 1a). Within each 4-d drug phase, days 1 and 3 were vehicle injection days, and days 2 and 4 were drug injection days; each drug phase was separated by a rest day. Having vehicle and drug injections on different days permitted full accommodation of the half-lives of the drugs. Fur-

**Table 1. Our doses of CDP and buspirone have been shown previously to be anxiolytic**

Test	Dose (mg/kg)	References
<b>Chlordiazepoxide</b>		
Elevated plus maze (anxiolytic increases proportion of open arm time)	2.5	Millan et al., 2001; Roy et al., 2009
	5.0	Pellow et al., 1985; Gentsch et al., 1987; Chaouloff et al., 1997; Frussa-Filho et al., 1999; Frussa-Filho and Ribeiro, 2002; Vendruscolo et al., 2003; Ramos et al., 2008; Roy et al., 2009
Social interaction (anxiolytic increases social interaction time)	2.5	Vale and Montgomery, 1997
	5.0	Baldwin and File, 1989; Vale and Montgomery, 1997; Millan et al., 2001; Haller and Bakos, 2002
Open field (anxiolytic increases center time, decreases defecation)	2.5	Angrini et al., 1998
	3.0	Sanger and Zivkovic, 1988
	3.5	Horváth et al., 1992
	5	Gentsch et al., 1987; Horvarth et al., 1992
Geller–Seifter conflict test (anxiolytic increases punished responding)	2.0	Britton et al., 1997
	4.0	Britton et al., 1997
	5.6	Paterson and Hanania, 2010
<b>Buspirone</b>		
Elevated T-maze (inhibitory avoidance task is time taken to leave the enclosed arm on three consecutive trials; reduction in latency compared with controls is anxiolytic)	1.0	Graeff et al., 1998; Poltronieri et al., 2003
	3.0	Graeff et al., 1998; Poltronieri et al., 2003
Open field (anxiolytic increases center time, decreases defecation)	0.3	Stefánski et al., 1992; Siemiakowski et al., 2000
	3.0	Lim et al., 2008
Social interaction (anxiolytic increases social interaction time)	0.16	Dekeyne et al., 2000
	0.63	Dekeyne et al., 2000
	1.0	Costall et al., 1992
Contextual fear conditioning (anxiolytic reduces freezing duration)	0.3	Kakui et al., 2009
	0.5	Wisłowska-Stanek et al., 2005
	1.0	Wisłowska-Stanek et al., 2005; Kakui et al., 2009
	2.0	Thompson and Rosen, 2006; Kakui et al., 2009

In experiment 1, CDP was injected intraperitoneally at doses of 2.5 and 5 mg/kg, and buspirone was injected intraperitoneally at doses of 1 and 2 mg/kg. The citations show that these doses have produced anxiolytic effects in previous studies.

thermore, the inclusion of rest days meant that there were 3 d between the administration of different drugs, further permitting return to baseline of any potential drug-related changes. Our focus was on drug effects on theta around the time of their peak concentration in the brain. The anxiolytic drugs CDP (a benzodiazepine agonist; Sigma-Aldrich) and buspirone (a 5-HT<sub>1A</sub> agonist; Tocris Bioscience) and the anxiogenic drug mCPP (a 5-HT<sub>2C</sub> agonist; Tocris Bioscience) were dissolved in a vehicle of physiological saline. The anxiogenic drug FG7142 (a partial inverse agonist; Tocris Bioscience) was suspended in saline containing Tween 80. All drugs were administered intraperitoneally at a volume of 1 ml/kg. Doses were as follows: CDP, 2.5 and 5 mg/kg; buspirone, 1 and 2 mg/kg; mCPP, 0.5 and 1 mg/kg; and FG7142, 5 and 10 mg/kg (for a summary of the literature that informed selection of the doses of CDP and buspirone, see Table 1). Within each drug phase, days 1 and 3 were vehicle injection days, and days 2 and 4 were drug injection days (lower and higher dose order counterbalanced across rats). The intertrial interval (ITI) was 30 min. Immediately after trial 3 (the preinjection trial), the subject was administered the vehicle/drug and placed on the holding platform until trial 4 (postinjection trial). Drug order was consistent across rats to conform to two principles. The first principle was to use an anxiolytic drug first, in case the effect of an anxiogenic drug was to elicit lasting aversion to the testing environment. An extension of this principle was to use the same anxiolytic drug in the first phase so that at least that anxiolytic drug could be tested on all four rats. In practice, there was no sign of any lasting aversion. CDP was selected first because the anxiolytic effects of benzodiazepines are very well established. The second principle was to alternate transmitter systems (GABAergic, serotonergic). Because buspirone was the last of the drugs administered in experiment 1, we tested the effects of a single administration of the low dose (1 mg/kg) in two additional, drug-naïve rats (rats 5 and 6) with CA1-implanted electrodes. These rats were exposed to the testing environment of experiment 1 for 4 consecutive days and for five trials per day (same trial length and ITI). Intraperitoneal injections were given immediately after trial 4 of the day (days 1–3, saline; day 4, buspirone).

Within each drug phase, dose order (high vs low) was counterbalanced across rats, with two rats experiencing drug stages in which the first administrations of CDP and mCPP were the high dose, and the first administration of FG7142 and buspirone were the low doses, with the other two rats first receiving low doses of CDP and mCPP and high doses of FG7142 and buspirone. Significant hypolocomotion occurred in three mCPP trials, precluding robust analysis of this drug. ANOVA for drug effects of the anxiogenic drug FG7142 showed no significant effects on slope ( $F_{(2,6)} = 0.69, p = 0.54$ ) or intercept ( $F_{(2,6)} = 2.37, p = 0.17$ ), and these results are not mentioned further. Although the doses of FG7142 used here were shown to be anxiogenic in rat models of anxiety (File and Pellow, 1984; Cole et al., 1995), we cannot rule out the possibility that the injections of FG7142 were not anxiogenic in our experiment.

*Experiments 2–4: effects of environmental novelty.* All experiments involving environmental novelty (experiments 2–4) shared the procedure that the rat was placed into a novel environment that was centered on the same position in the arena testing space (on a raised table) as the familiar environment (Fig. 1*b–d*). In all experiments, the familiar environment was a square-walled (60 × 60 or 62 × 62 cm) box with 50-cm-high walls. The novel environments were of two types, which were similar across experiments: (1) type A, which was a circular-walled box, with the wall being 50 cm high; and (2) type B, which was a wall-less square platform. Sets of visible cues external to the testing environments were also altered between the familiar-environment and novel-environment configurations. The diameter of the circular-walled box was as follows: 77 cm in experiment 2; 79 cm in experiment 3; and 80 cm in experiment 4. The sides of the wall-less square platform were as follows: 82 × 82 cm in experiment 2; and 100 × 100 cm in experiment 4. Novelty-exposure days involved trials in both the familiar and novel environments and occurred after many exposures over several days to the familiar environment, comprising at least 19 trials in experiment 2, 22 trials in experiment 3, and 29 trials in experiment 4. These exposures involved being repeatedly passively transported to and from the fixed locations of holding platform and familiar environment, which may further engender expectation of

the familiar environment within the testing-room setting. Thus, at least the very first exposures to novel environments present novelty that is “unexpected.” ITIs were as follows: experiment 2, 20–30 min; experiment 3, 20 min; and experiment 4, 20 min (T1–T2 and T2–T3 intervals) and 50 min (T3–T4 interval).

Slope and intercept data reported for experiment 2 were taken from saline-injected trials in novel environments described above. Rats were exposed to novel environments on days 5 or 11, with trials in familiar environments on days 1–4 and 7–10, with a rest day on day 6.

Experiment 3 examining novelty and refamiliarization reanalyzed the novelty-exposure days in the study by Jeewajee et al. (2008b). Subjects comprised the four rats 1–4 reported in that study, and an additional rat (rat 5) with good quality theta over days 1\*–3\*. As reported by Jeewajee et al. (2008b), the test trial series for rat 1 was terminated at the end of day 1\*, so ANOVA involving days 1\*–3\* exclude rat 1 but include rat 5 (thus, four rats in total). The frequency of rearing on hindlegs was counted using manual cell counters during each trial to index the behavioral response to environmental novelty (Anderson et al., 2006; Lever et al., 2006; Hunsaker et al., 2008).

For additional details of Experiment 4, see next section.

*Experiment 4: effects of drug (O-2545) and environmental novelty.* The water-soluble cannabinoid CB<sub>1</sub> receptor agonist O-2545 (Tocris Bioscience) (Martin et al., 2006) was dissolved at 100 μg/ml in a vehicle of physiological (0.9%) saline. Intraperitoneal injections were administered at a volume of 0.5 ml/kg. The dose was a “low” dose, which our pilot trials had shown to minimally reduce locomotion (for experiment 4 timeline, see Fig. 1e). The experiment consisted of two blocks of 5 d each, separated by a rest day, with four trials per day: block A (days A1–A5) and block B (days B1–B5). Trials 1, 2, and 3 occurred 90, 60, and 30 min before the injection, respectively (T3 is the “preinjection trial”), whereas trial 4 (T4 is the “postinjection trial”) began 30 min after the injection. On days 1–4 of each block, all trials were in the familiar environment. On day 5, the rat was introduced to the novel environment in trial 4: either NovA (A block) or NovB (B block). On days 1–2 of each block, vehicle was injected, and on days 3–5, injections alternated between drug and vehicle in a counterbalanced way across blocks and rats. Days 3–4 of each block provided the vehicle and drug data in a familiar environment, and day 5 provided the vehicle and drug data in a novel environment. For the 2 (vehicle, drug) × 2 (familiar, novel) repeated-measures ANOVA, the two T4–T3 data points for each drug condition in the familiar environment were averaged to provide one vehicle T4–T3 value and one drug T4–T3 value.

Thigmotaxis (“wall-hugging”) in novel walled environments, such as the traditional open field and elevated plus/zero-maze tests, is a well-established index of anxiety-like behavior in laboratory rodents. When placed into such environments, intact rats and mice prefer to stay near the walls, thereby avoiding the potentially dangerous open or “unprotected” areas of the apparatus. An extensive literature confirms that thigmotaxis (or open-area avoidance) is bidirectionally sensitive to a wide range of anxiolytic and anxiogenic drugs, with anxiolytic drugs increasing time spent in open, unprotected areas (Prut and Belzung, 2003; Rodgers, 2010).

*Experiment 4: CA1 place cell recording and analyses.* CA1 place cells were recorded simultaneously with the LFP recording described above. Tetrodes made from 17-μm-diameter, heavy polyimide enamel-coated, 90% platinum–10% iridium electrode wire with plated tips were loaded onto 16-channel microdrives, implanted above the dorsal hippocampus of one or two hemispheres per rat, and gradually lowered into the CA1 pyramidal layer. Tetrode signals were amplified (15,000–50,000) and bandpass filtered (500 Hz to 7 kHz). Each channel was continuously monitored at a sampling rate of 50 kHz, and action potentials were stored as 50 points per channel (1 ms, with 200 μs prethreshold and 800 μs postthreshold) whenever the signal from any of the four channels of a tetrode exceeded a given threshold. Cluster cutting of CA1 spike data was performed manually using custom-made software (TINT; Axona). Single units were classified into putative pyramidal cells and interneurons on the basis of the peak-to-trough interval of the mean waveform and the mean interspike interval in the first 20 ms of the spike train autocorrelogram (see Csicsvari et al., 1999). The inclusion criteria for pyramidal cells

were: peak-to-trough interval of at least 0.25 ms and mean interspike interval (0–20 ms) <13 ms. The inclusion criteria for interneurons were: peak-to-trough interval of <0.25 ms and mean interspike interval (0–20 ms) >9.5 ms. These criteria were used because they divided 681 units effectively into two classes (558 pyramidal cells, 115 interneurons) with only eight outliers.

Pyramidal cells were recorded during all four of the 2 (drug, vehicle) × 2 (familiar, novel) conditions across trial 3 (preinjection baseline trial) and trial 4 (postinjection probe trial) in six rats over 33 T3–T4 sessions. For each rat, there were four T3–T4 sessions comparing trials in the familiar environment and two T3–T4 sessions comparing trials in the novel environment, with the exception that three sessions from block B of rat 5 lacked pyramidal cell data.

Locational firing rate maps were constructed from 2.1 × 2.1 cm binned data and smoothed using a 5 × 5 bin boxcar filter. Spike count divided by dwell time gave firing rate per bin. To compare firing rate maps from trials in different environments in the assessment of remapping, corresponding bins in environments of different shape or size were defined as those with the same direction from the center and proportion of distance from the center to the edge along that direction, using nearest-neighbor interpolation (Wills et al., 2005). The degree of spatial remapping was assessed using the population vector method (Leutgeb et al., 2005). This measures how similar the firing activity of the pyramidal cell population is within each spatial bin across two trials. For each spatial bin of the rate map, the rates of all place cells in that bin were correlated across the two trials to give a Pearson’s correlation coefficient *r*. The mean of the correlation coefficients across all bins was taken as the estimate of the degree of remapping between the trials. When place cell ensembles were recorded from two CA1 hemispheres, the population vector was hemisphere weighted as follows. For *N<sub>L</sub>* cells from the left hemisphere with mean vector value *L* and *N<sub>R</sub>* cells from the right hemisphere with mean vector value *R*, the weighted population vector value was (*N<sub>L</sub>L* + *N<sub>R</sub>R*)/(*N<sub>L</sub>* + *N<sub>R</sub>*). Firing rate maps shown in the figures are autoscaled false color maps, with each color representing a 20% band of peak firing rate, from dark blue (0–20%) to red (80–100%). Peak rate (after smoothing) is the maximal firing rate in any bin.

*Experiments 1, 2, and 4: measuring aural temperature.* Aural temperature was measured using a Braun thermometer (experiments 1 and 2, ThermoScan 4020; experiment 4, ThermoScan IRT4520). In experiments 1 and 4, temperature was measured before (three times) and after (three times) every trial for two of four rats (experiment 1) and four of six rats (experiment 4). The highest value across the three measurements was taken as the most reliable reading, and only this value was used. The temperature for a given trial was the mean of the “before” and “after” values. In experiment 2, aural temperature was measured three times after every trial in all six rats, with the highest of the three measurements being taken as the trial value. All rats were habituated to temperature measurement in advance of the experimental phase.

Previous literature demonstrates that, although the absolute temperature may differ somewhat across aural and brain sites, changes in aural temperature closely parallel changes in brain temperature, whereas temperature across brain regions is highly consistent. For example, Eshraghi et al. (2005) assessed normothermia (37°C), mild (33°C), and moderate (30°C) hypothermia and slow rewarming in anesthetized rats. They measured the temperature of the cochlea, brain, temporalis muscle, and rectum. Cochlear and brain temperature were highly correlated during induction of systematic hypothermia (*r* = 0.91, *p* < 0.001) and during rewarming (*p* = 0.89, *p* < 0.001). Dunscombe et al. (1980) recorded from the tympanic membrane (eardrum) while rats underwent brain heating (to 41°C). They confirmed that measuring tympanic membrane temperature could substitute for measuring intracranial temperature (mean brain – ear temperature = –0.1°C at 37°C and 41°C). Moser et al. (1993) used thermistors to record hippocampal temperature in freely moving rats, sometimes from opposite hemispheres. Bilateral activity-induced temperature changes were nearly identical (<0.15°C different). Kiyatkin et al. (2002, p 165) measured temperature in the cerebellum and dorsal and ventral striatum in freely moving rats exposed to a wide range of stimuli. They reported that brain temperatures “differed slightly but consistently between the regions sampled.”

In summary, at least in the rat, ear and regional brain temperatures are closely linked, and therefore measuring aural temperature provides a reasonably accurate reflection of temperature in brain regions contributing to septohippocampal theta.

#### Relationship between LFP theta frequency and running speed

To characterize the dynamic relationship between theta frequency and running speed, a cycle-by-cycle approach was used to estimate momentary LFP frequencies. The recorded LFP signal was filtered using a 6–12 Hz, 251-tap, Blackman windowed, bandpass sinc (sine cardinal) filter. Windowing the filter achieves good stop-band attenuation and small pass-band ripple. An analytic signal was then constructed using the Hilbert transform, taking the form  $S_a(t_k) = S(t_k) + iH[S(t_k)]$ , where  $H$  is the Hilbert transform,  $S(t_k)$  is the filtered LFP signal,  $t_k = k\Delta$ , where  $k = 1, \dots, K$  indexes the time step, and  $\Delta$  is the inverse of the sampling rate. The phase of the analytic signal  $\varphi(t_k)$  gives the phase of the LFP at  $t_k$ , and the difference in phase between each time point defines the frequency. Because the LFP sampling rate was five times that of position, instantaneous frequency was averaged over every five consecutive values corresponding to each position sample. Thus, concurrent measurements of speed and LFP theta frequency were produced every 20 ms.

To quantify the linear relationship between theta frequency and speed in a given trial, a regression line was fitted to the frequency–speed data points for speeds between 5 and 30 cm/s, with each speed bin being 2.5 cm/s in width, thus creating 10 speed bins for each trial. Speeds below 5 cm/s were excluded to avoid non-theta behaviors, such as stopping or sitting quietly. Speeds above 30 cm/s were excluded to avoid too few samples in some trials and occasional tracking errors or head movements producing apparent high speeds. The use of 10 narrow bins, within upper and lower limits, minimizes any differences in mean speed per bin between trials containing different distributions of speed (see below).

Theta intercept was defined as the intercept of the regression line with the  $y$ -axis (frequency) at 0 cm/s running speed, and theta slope was the gradient of the regression line. These values were used for statistical analyses. To best visualize these data across rats, we constructed plots of the theta frequency–speed relationship as described below.

We analyzed a subset of the data to investigate the possibility that drug-elicited changes in locomotion could systematically affect the average speed of the samples in the 10 matched bins in the 5–30 cm/s range. Buspirone was the only anxiolytic drug that caused a significant reduction in running speed (see Results). We analyzed the datasets for all rats 1–4 in experiment 1 for CDP and buspirone. The differences in average speed between vehicle and drug conditions in the 10 matched bins in the 5–30 cm/s range were vanishingly small. The net difference in average speed in drug versus vehicle trials in these 10 bins was  $-0.00015$  cm/s for CDP and  $-0.033$  cm/s for buspirone. These negligible values indicate that our speed-matching procedure works very well, even when whole-trial average speeds differ significantly.

We also analyzed these datasets to confirm that the 5–30 cm/s range is representative of the locomotion in this foraging paradigm. We found that the 5–30 cm/s range contains  $79.4 \pm 1.6\%$  of all behavior above 5 cm/s ( $n = 48$  trials, 12 per rat). In summary, our analysis approach combines being representative of the data with excellent speed matching.

#### Statistics

For drug effects on slope and intercept (experiments 1 and 4), we calculated difference values between the preinjection and postinjection trial as [postinjection value (trial 4)] minus [preinjection value (trial 3)] for vehicle and drug. In experiment 1, one-tailed  $t$  testing (for intercept reduction by anxiolytic drugs) was used to compare effects of vehicle versus drug (buspirone and CDP) on intercept. With the reduction being observed,  $t$  testing for intercept reduction by O-2545 in the subsequent experiment 4 was one tailed, and the ANOVA for a main effect of intercept reduction was unidirectional. All other  $t$  tests were two tailed, and all other ANOVAs were nondirectional.

#### Plots of the theta frequency–speed relationship

The absolute value of theta frequency varies systematically between rats and correlates with variables such as the rat's age (Wills et al., 2010). To display data across rats in the figures, we account for these differences

using a similar normalization principle for all summary plots of theta frequency versus running speed. Normalization was not applied to data for statistical analyses. A normalization value is calculated for each rat as the mean theta frequency over the relevant baseline trials. In experiment 1 (see Fig. 3*a–e*), the normalization value is the mean of the pre-vehicle and pre-drug trials. In experiments 2 and 3 (see Fig. 4, *a* and *b–d*, respectively) the normalization value is the mean of the trials in the familiar environment (i.e., trials 1, 2, 3, and 6 in experiment 2 and trials 1, 2, and 5 in experiment 3). In experiment 4, the normalization value is the mean of the pre-vehicle trials for vehicle-administration manipulations (see Fig. 6*a,c*) or the pre-drug trials for drug-administration manipulations (see Fig. 6*b,d*). The difference between each rat's normalization value and the mean normalization value over rats is then subtracted from each data point shown. This removes any effects of differences in the mean theta frequency over the relevant baseline trials across rats. In all plots, squares show the average over rats, and error bars are SEM. Each rat contributes one value to each speed bin in the range 5–30 cm/s, with that value being an average of the many data points for that bin. The size of each speed bin is 2.5 cm/s. Each rat contributes one value for intercept and one for slope, derived directly from the regression analysis.

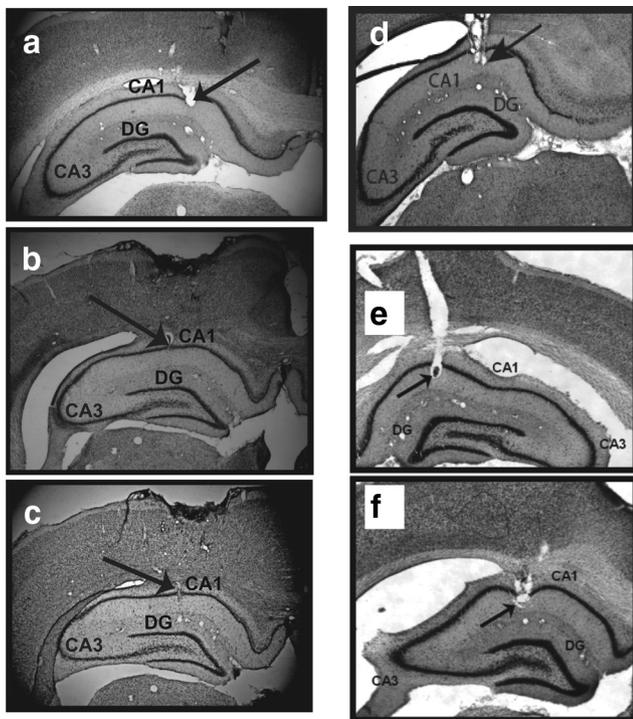
## Results

We tested our predictions (dissociation of the slope and intercept of the theta frequency–running speed relationship, with anxiolytics reducing intercept, novelty reducing slope) by recording naturally occurring hippocampal theta from rats freely foraging for scattered food rewards in open-field environments.

### Anxiolytic drugs reduce the intercept of the theta frequency–running speed relationship

The effects of two clinically well-established but neurochemically dissimilar anxiolytics, buspirone (a 5-HT<sub>1A</sub> agonist) and CDP (a benzodiazepine agonist), were tested in 4-d blocks (experiment 1). Four CA1-implanted rats experienced four trials daily for 4 consecutive days, receiving an intraperitoneal injection immediately after the third trial, of vehicle (days 1 and 3) or drug (days 2 and 4) (for experimental timeline, see Fig. 1*a*; for details of electrode locations for experiment 1, see Fig. 2*a–c*, Table 2). We used doses of buspirone and CDP shown previously to be anxiolytic in rats in a wide range of anxiety models (Table 1). As predicted, both anxiolytics significantly reduced the intercept of the frequency–speed relationship (buspirone,  $F_{(2,6)} = 22.16$ ,  $p = 0.002$ , Fig. 3*a,b*; CDP,  $F_{(2,6)} = 5.92$ ,  $p = 0.04$ , Fig. 3*c,d*) at both the higher (paired  $t_{(3)}$ : buspirone, one-tailed  $p = 0.004$ , 0.72 Hz mean reduction; CDP, one-tailed  $p = 0.04$ , 0.36 Hz mean reduction) and lower (paired  $t_{(3)}$ : buspirone, one-tailed  $p = 0.02$ , 0.50 Hz mean reduction; CDP, one-tailed  $p = 0.05$ , 0.25 Hz mean reduction) doses but had no effect on the slope (buspirone,  $F_{(2,6)} = 2.25$ ,  $p = 0.19$ ; CDP,  $F_{(2,6)} = 0.39$ ,  $p = 0.70$ ).

Buspirone was the final drug administered in experiment 1. To confirm that intercept reduction was not somehow attributable to previous injections, two additional, drug-naïve rats (rats 5 and 6) with CA1-implanted electrodes were exposed to the testing environment of experiment 1 for 4 consecutive days and for five trials per day (same trial length and ITI). Intraperitoneal injections were given immediately after the fourth trial of the day (days 1–3, saline; day 4, buspirone). As predicted, buspirone reduced intercept (mean reduction of 0.39 Hz), a result that even with just two rats approached significance ( $t_{(1)} = 4.08$ , Cohen's  $d = 5.78$ , one-tailed  $p = 0.076$ ; Fig. 3*e*). Buspirone did not affect slope ( $t_{(1)} = 0.22$ ,  $p = 0.86$ ).



**Figure 2.** LFP electrode tracks from experiments 1, 2, and 4. Cresyl-violet-stained sections of the dorsal hippocampus are shown for two rats (experiment 1, rats 1 and 2). Arrows indicate the track of the LFP electrode. **a, b**, The progression of the electrode track into CA1 stratum radiatum in rat 1. **b** shows the adjacent posterior section to **a**. **c** shows the bottom of electrode track in CA1 stratum radiatum of rat 2. **d, e**, Cresyl-violet-stained section of the dorsal hippocampus shown for experiment 2, rat 2. Arrow indicates the bottom of the LFP electrode track (i.e., the most medial) in stratum radiatum. **e**, Cresyl-violet-stained sections of the dorsal hippocampus are shown for two rats (experiment 4, rats 4 and 6). Arrows indicate the track of the EEG electrode. **e** shows the bottom of the LFP electrode track in CA1 stratum radiatum of rat 4. **f** shows the bottom of the electrode track in CA1 stratum radiatum in rat 6. DG, Dentate gyrus.

### Environmental novelty reduces the slope of the theta frequency–speed relationship

Experiment 2 tested the effect of environmental novelty. Six CA1-implanted rats experienced at least 4 d (four trials per day) in a familiar environment and then on the novelty-exposure day experienced an identically centered novel environment (trials T4 and T5) between exposures to the familiar environment (trials T1, T2, T3, and T6). Figure 4*a* shows that, as predicted, novelty robustly reduced slope, without affecting intercept [slope,  $t_{(5)} = 5.2$ ,  $p = 0.003$ ; intercept,  $t_{(5)} = 1.6$ ,  $p = 0.16$ ; mean familiar (T1–T3 and T6) vs mean novel (T4 and T5) trials].

### The theta frequency–speed slope increases as a novel environment becomes familiar

To confirm that slope reduction results from environmental novelty per se and not intrinsic differences between environments, experiment 3 tested whether subsequent familiarization to a novel environment reverses the slope-reduction effect. After repeated exposures (four trials per day for 5 d) to a familiar environment, five rats received 3 d of novelty exposure (day 1\*, day 2\*, and day 3\*), each separated by a rest day. On these novelty exposure days, rats experienced an identically centered novel environment (trials T3 and T4) between trials in the familiar environment (trials T1, T2, and T5). As Figure 4*b* shows, exposure to the novel environment dramatically flattened the slope of the frequency–speed relationship, which then recovered over days (Fig. 4*c, d*) as the initially novel environment became famil-

**Table 2. Electrode recording sites for experiments 1–4**

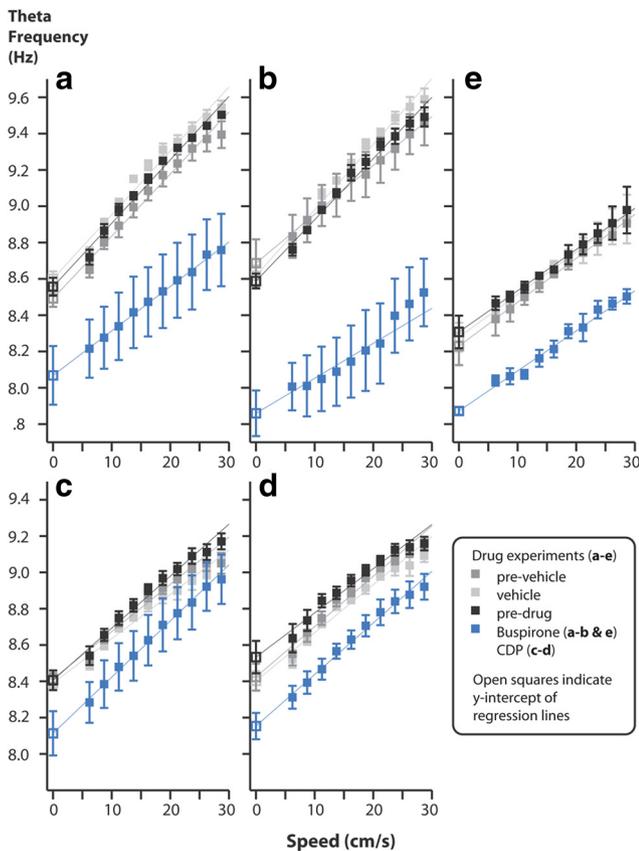
Rat	Location/layer
<b>Experiment 1</b>	
1	CA1 stratum radiatum
2	CA1 stratum radiatum
3	CA1 lacunosum moleculare
4	CA1 stratum radiatum/lacunosum moleculare
<b>Experiment 2</b>	
1	CA1 pyramidal layer/stratum radiatum
2	CA1 stratum radiatum
3	CA1 pyramidal layer/stratum radiatum
4	CA1 stratum radiatum
5	CA1 pyramidal layer/stratum radiatum
6	CA1 pyramidal layer/oriens
<b>Experiment 3</b>	
1	CA1 stratum radiatum
2	CA1 in/around pyramidal layer
3	Subiculum
4	Dentate gyrus
5	Subiculum
<b>Experiment 4</b>	
1	CA1 stratum radiatum
2	CA1 stratum oriens
	CA1 pyramidal layer/stratum radiatum
3	CA1 stratum radiatum
4	CA1 stratum radiatum
5	CA1 pyramidal layer/stratum oriens
6	CA1 pyramidal layer/stratum radiatum
	CA1 stratum radiatum

For each experiment, table indicates estimated LFP electrode recording sites. Most electrodes were in the pyramidal layer and stratum radiatum of CA1.

iar. This was confirmed by repeated-measures ANOVA of the first two trials in each environment (Fam = T1, T2; Nov = T3, T4) on each of the three novelty exposure days [environment (novel, familiar)  $\times$  day (1, 2, 3)  $\times$  trial (1, 2)]; this showed a main effect of environment ( $F_{(1,3)} = 22.68$ ,  $p = 0.018$ ), a main effect of day ( $F_{(2,6)} = 18.12$ ,  $p = 0.003$ ), and a day  $\times$  environment interaction ( $F_{(2,6)} = 125.98$ ,  $p = 0.00001$ ) on slope. In contrast, there was no reliable effect on intercept (main effect of environment,  $F_{(1,3)} = 3.22$ ,  $p = 0.17$ ; main effect of day,  $F_{(2,6)} = 0.21$ ,  $p = 0.82$ ; day  $\times$  environment interaction,  $F_{(2,6)} = 1.62$ ,  $p = 0.27$ ). The recovery of the slope suggests that there were no permanent effects or any intrinsic differences between the two environments. There was also a main effect of trial on slope ( $F_{(1,3)} = 29.73$ ,  $p = 0.012$ ) but not intercept ( $F_{(1,3)} = 0.07$ ,  $p = 0.81$ ), consistent with the observation that slope tends to increase on repeated within-day exposures to the same environment.

### Behavior was responsive to environmental novelty and predicted by slope

Was our novelty manipulation behaviorally salient? To examine this, we compared probe – baseline (T3–T2) counts of rearing on hindlegs on the last day of habituation (day 5, all trials familiar environment) to those on the first day of environmental novelty (day 1\*, novel environment on T3 and T4). As observed in a broadly similar study (Wells et al., 2009a), rats significantly increased their rearing in response to environmental novelty. On exposure to environmental novelty, rats greatly increased their rearing frequency (mean net increase, novel vs familiar,  $4.8 \pm 0.5$  rears/min, paired  $t_{(4)} = 8.9$ ; Fig. 5*a*). Thus, rearing on hindlegs, which is a hippocampus-dependent novelty-responsive behavior (Lever et al., 2006), was highly sensitive to our environmental novelty manipulation. The sharp reduction of slope in novelty



**Figure 3.** Anxiolytic drugs reduce the intercept of the theta frequency–running-speed relationship; environmental novelty reduces its slope. Plots show theta frequency versus running speed. **a–d**, Anxiolytic drugs (blue) reduce the 0 cm/s intercept of the frequency–speed relationship compared with control trials (gray/black) but has no significant effect on slope. Both low and high doses are shown (buspirone: **a**, 1 mg/kg and **b**, 2 mg/kg; CDP: **c**, 2.5 mg/kg and **d**, 5 mg/kg; all 1 ml/kg, i.p.;  $n = 4$  rats). See Experiment 1. **e**, Additional 1 mg/kg buspirone-only rats ( $n = 2$ ).

and its recovery with subsequent familiarization (Fig. 4*b–d*) closely paralleled the sharp increase in rearing frequency (Fig. 5*a*) and its habituation with subsequent familiarization (Fig. 5*b*, slope reduction expressed as a positive magnitude). Indeed, the rearing frequency in each of the four rats tested over the 3 novelty-exposure days was reliably predicted by the slope (Fig. 5*c*) but not the intercept (Fig. 5*d*; mean rearing vs slope,  $r = -0.73$ ,  $p$  values  $\leq 0.02$ ; for per-rat rearing vs slope and rearing vs intercept values, see Table 3).

#### Within-subjects dissociation of effects on theta frequency–speed slope and intercept

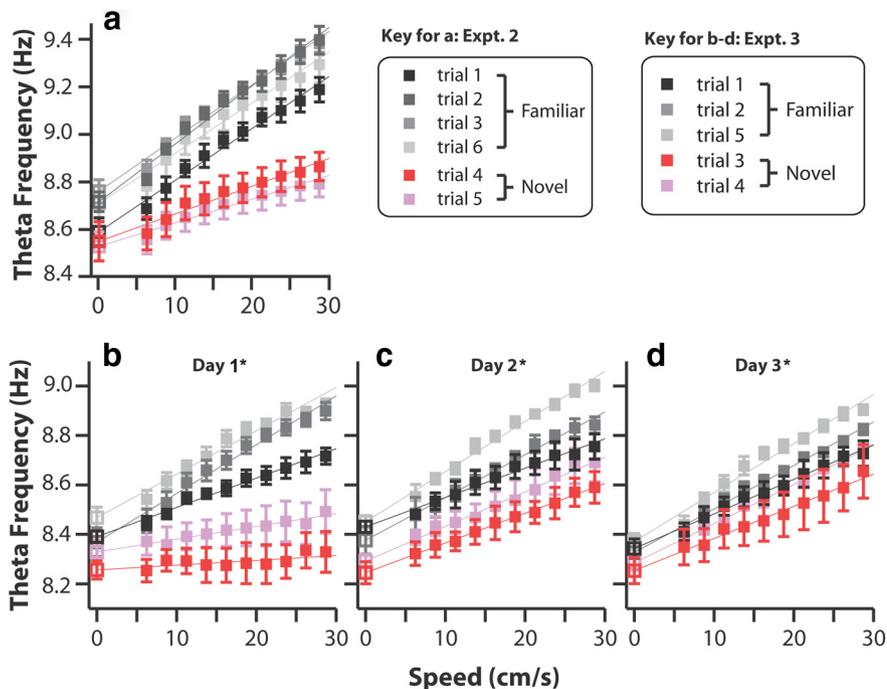
In separate experiments, anxiolytic drugs reduced intercept (experiment 1), whereas environmental novelty reduced slope (experiments 2 and 3). Experiment 4 combined the test for intercept reduction and slope reduction within the same experiment while targeting another neurotransmitter system to probe the generality of anxiolytic intercept reduction (see Materials and Methods and Fig. 1*d*). CB<sub>1</sub> receptor agonists (especially at low doses) are anxiolytic (Haller et al., 2004). Thus, we targeted the cannabinoid system with a low systemic (intraperitoneal) dose of the novel CB<sub>1</sub> receptor agonist O-2545. As predicted, O-2545 alone specifically reduced intercept (Fig. 6, *a* vs *b*; intercept, paired  $t_{(5)} = 4.8$ , one-tailed  $p = 0.002$ ; slope,  $t_{(5)} = 0.2$ ,  $p = 0.87$ ), whereas environmental novelty alone specifically reduced slope (Fig. 6, *a* vs *c*;

slope, paired  $t_{(5)} = 6.9$ ,  $p = 0.001$ ; intercept,  $t_{(5)} = 0.1$ ,  $p = 0.93$ ), and there was no interaction between the simultaneous effects of O-2545 and novelty (Fig. 6*a–d*; drug: intercept,  $F_{(1,5)} = 13.70$ ,  $p = 0.024$ ; slope,  $p = 0.83$ ; environment: slope,  $F_{(1,5)} = 449$ ,  $p = 4 \times 10^{-6}$ ; intercept,  $p = 0.77$ ; drug  $\times$  environment: intercept,  $p = 0.60$ ; slope,  $p = 0.83$ ). Thus, we demonstrate a double dissociation, with independent modifiability of both the intercept-related and slope-related theta components.

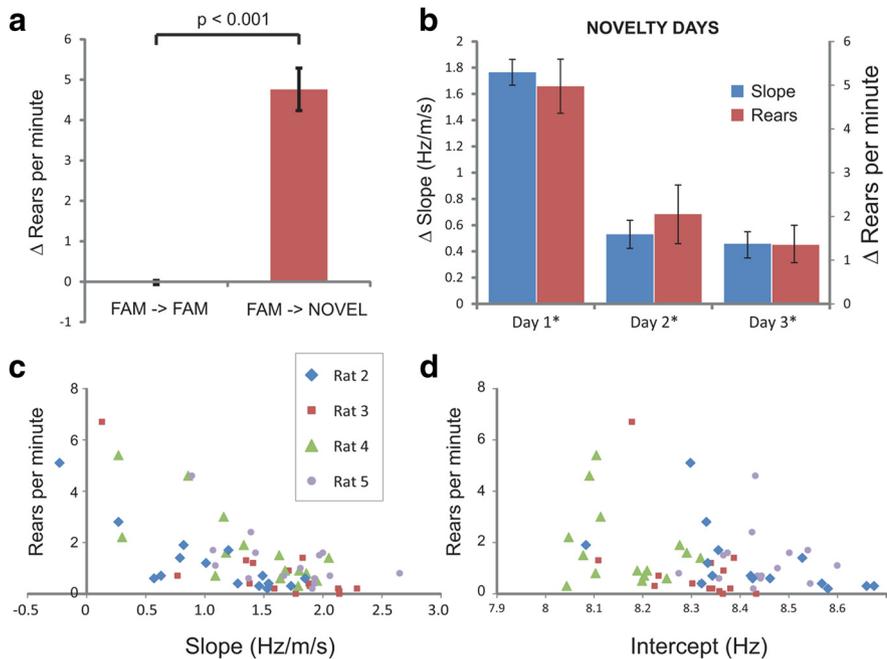
#### Reduction of theta slope between baseline and probe trials predicted the degree of remapping in CA1 place cells and place field size

In experiment 4, 558 CA1 place cells were recorded simultaneously with the CA1 LFP recordings from six rats over 33 sessions, at an average of  $16.9 \pm 2.2$  cells per session. We tested the prediction (Burgess, 2008) that theta slope reduction contributes to place cell remapping. Briefly, flatter slope expands grid scale, and grid scale expansion should cause a mismatch between the grid cell inputs to place cells and the environmental inputs mediated by boundary cells in subiculum (Lever et al., 2009) and entorhinal cortex (Solstad et al., 2008), which are unaffected by environmental novelty (Lever et al., 2009). If theta slope controls remapping (for quantification, see Materials and Methods), there should be a correlation between, on the one hand, the change in slope from the baseline trial (T3) to the probe trial (T4) and, on the other hand, the similarity of spatial firing patterns across the two trials. This prediction was confirmed (slope vs remapping,  $n = 33$ ,  $r = 0.72$ ,  $p = 0.000002$ ; vehicle-only sessions,  $n = 17$ ,  $r = 0.75$ ,  $p = 0.0005$ ; drug-only sessions,  $n = 16$ ,  $r = 0.69$ ,  $p = 0.003$ ). However, we cannot exclude the possibility that the slope is only predicting remapping because novelty elicits slope reduction and novelty elicits remapping.

An additional prediction from the model is that slope change should predict changes in place field size. Because entorhinal grid cells form an important input to place cells, any expansion in grid scale in novelty (Barry et al., 2012) would be expected to increase place field size, consistent with previous reports of novelty-induced place field expansion (Karlsson and Frank, 2008; Barry et al., 2012). We tested this prediction using place cells that fired sufficiently robustly for field size measurement ( $\geq 120$  spikes, spatial peak rate  $\geq 1$  Hz,  $n = 360$  total cells). As predicted, average cumulative place field size (i.e., sum of areas of firing  $\geq 20\%$  of peak rate) significantly increased in the novel environments (main effect of novelty,  $F_{(1,4)} = 71.2$ ,  $p = 0.001$ ; mean field size change: familiar,  $-8.2 \pm 27$  cm<sup>2</sup>; novelty,  $+967 \pm 133$  cm<sup>2</sup>). The increase in place field size appeared somewhat blunted by O-2545 (drug  $\times$  novelty,  $F_{(1,4)} = 3.3$ ,  $p = 0.14$ ), consistent with a CB<sub>1</sub> receptor agonist-specific reduction of field size, shown previously with a different CB<sub>1</sub> agonist (Robbe and Buzsáki, 2009). Accordingly, to better isolate the influence of slope on place field size, we tested this relationship in vehicle and drug sessions separately and together. As predicted, change in slope robustly predicted change in average place field size in vehicle-only sessions ( $r = -0.81$ ,  $p = 0.00009$ ;  $n = 17$  sessions), drug-only sessions ( $r = -0.73$ ,  $p = 0.001$ ,  $n = 16$  sessions), and combined sessions ( $r = -0.73$ ,  $p = 0.000001$ ,  $n = 33$  sessions), indicating that the result is robust. We acknowledge the caveat that our novel environments were larger than the familiar environment, but place field area typically expands much less than total environmental area (Muller and Kubie, 1987; Fenton et al., 2008). The familiar environment was approximately half the size (3844 cm<sup>2</sup>) of the novel environments (average of 7513 cm<sup>2</sup>: novel A = 5026 cm<sup>2</sup>; novel B = 10,000 cm<sup>2</sup>). The novelty-elicited



**Figure 4.** Environmental novelty reduces theta slope, which recovers as the novel environment becomes familiar (experiments 2 and 3). **a**, Environmental novelty (red, pink) reduces frequency–speed slope; see experiment 2. Absolute frequency values are normalized across rats. Squares, mean across rats; dashed lines, linear regression. Error bars are SEM. For details, see Materials and Methods. Plots show theta frequency versus running speed. **b**, The first exposures to a novel environment in experiment 3 (trial 3, red; trial 4, pink) on the first novelty day elicits a dramatic reduction in slope compared with exposure to the familiar environment (trial 1, black; trial 2, dark gray; trial 5, light gray). Slope increases as the initially novel environment becomes more familiar on subsequent days (**c**, day 2\*; **d**, day 3\*); see experiment 3. Conventions as in Figure 3. In both experiments 2 and 3, the same novel environment is experienced twice (experiment 2, trials 4 and 5; experiment 3, trials 3 and 4).



**Figure 5.** Exploratory behavior (rearing) elicited by environmental novelty is predicted by theta slope (experiment 3). The first exposure to a novel environment on the first novelty day (experiment 3) elicits a dramatic increase in rearing frequency (**a**). Probe trial (T3) — baseline trial (T2) values shown for FAM → FAM on day 5 and FAM → NOVEL on the next day, day 1\*. **b**, Rearing frequency parallels slope reduction over the 3 novelty days (see Fig. 4*b–d* and experiment 3). For ease of comparison, slope reduction is shown as positive in magnitude [i.e.,  $-(T3 - T2)$ ]. **c, d**, Rearing frequency over 3 novelty days is reliably correlated with slope (**c**) but not intercept (**d**).

mean increase of  $967 \pm 133 \text{ cm}^2$  that we observed represents more than a doubling in area; this is well in excess of an increase in field area by a factor of only  $\sqrt{2}$ , which would be predicted by the change in environmental size alone (Muller and Kubie, 1987).

Does slope change or the novelty/familiarity of the probe environment best predict changes in field size across baseline and probe trials? To address this, we averaged the two values from the two trials in the familiar environment in each condition (Fig. 1) so that each rat contributed an equal amount of data from the familiar and novel probe sessions. ( $n = 23$  total sessions, comprising 2 (novel vs familiar)  $\times$  2 (vehicle vs drug) conditions for each of the six rats, minus one novelty-under-drug condition lacking cells). To take account of the novelty condition, we divided field expansion in the novel environments by  $\sqrt{2}$  and assigned novelty status value 1 and 2 for familiar and novel environment probes, respectively. We then performed partial correlation analysis to test which of the two directional hypotheses had primacy: did slope reduction or novelty status best predict field expansion? As predicted by the model by Burgess, 2008, slope change predicted field size change controlling for the effect of novelty status ( $r = -0.49$ , one-tailed  $p = 0.01$ ,  $n = 23$ ,  $df = 20$ ), whereas novelty status did not predict changes in field size controlling for the effect of slope ( $r = 0.11$ , one-tailed  $p = 0.32$ ) (results were very similar for uncorrected field sizes). Figure 7 shows the simple correlation between change in slope and change in field size ( $r = 0.72$ , one-tailed  $p < 0.0001$ ,  $n = 23$ , slope reduction expressed as positive magnitude). In summary, novelty elicited field expansion, and, moreover, the theoretically predicted relationship between slope and field size change was observed.

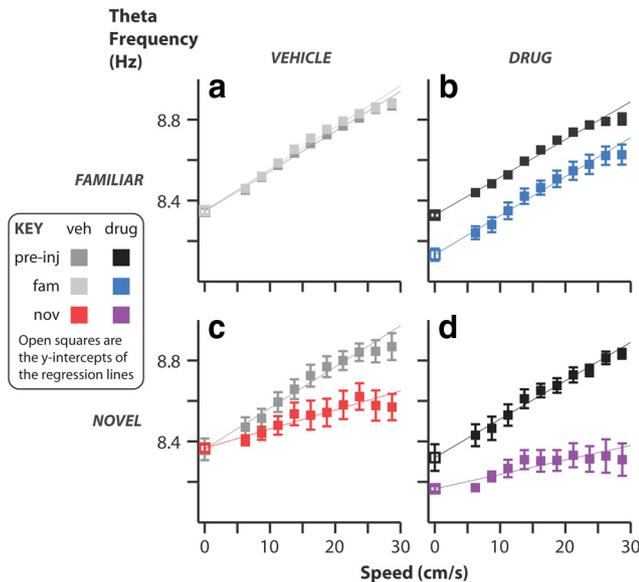
#### O-2545 was anxiolytic, and theta intercept predicted anxiety-related behavior

A classic rodent test of anxiety measures the rodent's thigmotaxis in a novel, walled, open field. A voluminous literature shows that anxiolytic drugs increase the time spent by rodents in the central open area (see Materials and Methods). Because the putative anxiolytic properties of O-2545 have not been demonstrated, we measured its effects in the walled novel environment probe trial (T4, novel type A; three rats saline, three rats O-2545). As predicted for an anxiolytic, O-2545

**Table 3. Slope but not intercept predicted novelty-responsive behavior**

Rat	Rears versus slope		Rears versus intercept	
	<i>r</i> value	<i>p</i> value	<i>r</i> value	<i>p</i> value
5	−0.584*	0.022*	−0.003	0.991
2	−0.810*	0.0002*	−0.525*	0.045*
3	−0.791*	0.0004*	−0.499	0.058
4	−0.754*	0.001*	−0.305	0.269

In experiment 3, rears per trial was reliably predicted by the slope (mean *r* value across 4 rats = −0.73, *p* values ≤ 0.02), but not the intercept, of the theta frequency–speed relationship across the 3 d of combined exposure to the familiar environment and novel environment. For each correlation test, *n* = 15 (3 d × 5 trials/d). The 3 d of testing are the novelty-exposure days 1\*–3\* of experiment 3, with 3 trials/d in familiar environment and 2 trials/d in novel environment. \**p* < 0.05, statistically significant correlations.



**Figure 6.** Within-subjects dissociation of anxiolytic and novelty effects on theta-speed intercept and slope (experiment 4). Plots show theta frequency versus running speed in the various conditions of experiment 4 (*n* = 6 rats). *a, b*, Putative anxiolytic cannabinoid CB<sub>1</sub> receptor agonist (O-2545) reduces intercept. *a, c*, Environmental novelty reduces its slope. Simultaneous drug and novelty reduces both intercept and slope independently (*a, d*). O-2545 dose: 100 μg/ml, 0.5 ml/kg intraperitoneally. Conventions as in Figures 3 and 4. pre-inj, Pre-injection; fam, familiar; nov, novel.

significantly increased the proportion of time that rats spent in the central area in the novel probe trial (Fig. 8*a, b*). Moreover, as predicted by the identification of intercept-related mechanisms with arousal/anxiety, the intercept predicted thigmotaxis. The absolute level of the intercept predicted the absolute center time in the novel probe trial (T4 intercept vs T4 center time, *r* = −0.87, *p* = 0.02, *n* = 6; Fig. 8*c*), and the change in intercept predicted the change in center time (T4–T3 intercept vs T4–T3 center time, *r* = −0.83, *p* = 0.04). Lower intercept values were associated with lower anxiety, that is, higher occupation of the central region. (The absolute levels of occupation of the central region are of course much higher than in the novel open-field paradigms typical in anxiety screening, but our rats are extensively handled and have run many trials in the testing room.)

In summary, in addition to showing in experiment 1 that well-established anxiolytic drugs reduce intercept, we identified a novel drug as putatively anxiolytic and then showed that it too reduces intercept and has anxiolytic effects behaviorally. We also found suggestive evidence, albeit across vehicle and drug conditions in a small sample (*n* = 6), for a positive correlation between intercept and anxiety-like behavior. The positive relationship be-

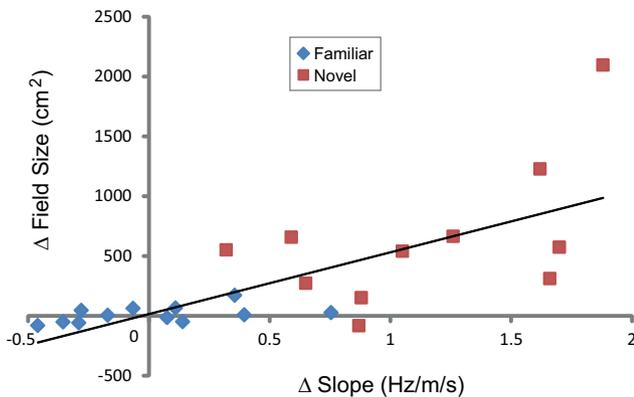
tween intercept and behavioral anxiety should be tested in other paradigms with a larger sample.

### Theta power is unaffected by anxiolytic drugs but can increase in novel environments

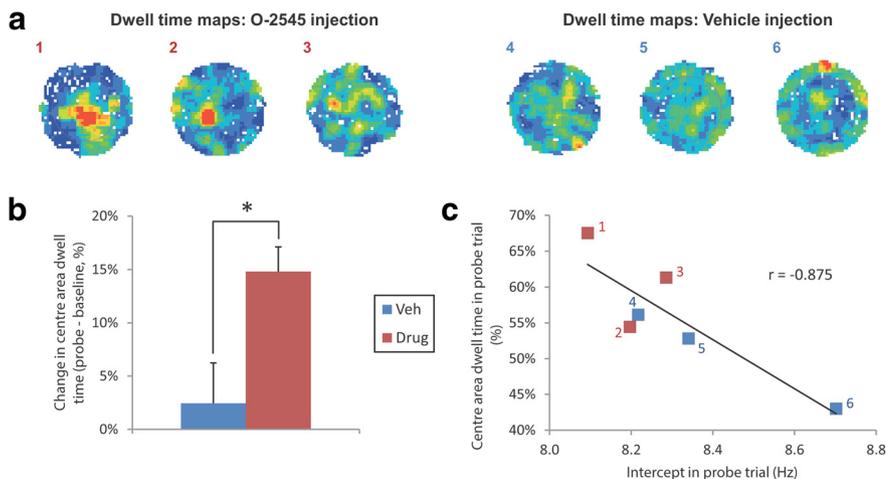
We had no a priori model-based predictions regarding theta power but tested for theta power changes elicited by the anxiolytic drugs and environmental novelty. To control for differing behavior across conditions, we calculated peak theta power based on subsets of data from each trial, chosen such that median running speed was constant in the data from each trial for a given rat. There was no effect of either the anxiolytic drug (CDP,  $F_{(2,6)} = 0.07$ , *p* = 0.93; buspirone,  $F_{(2,6)} = 1.08$ , *p* = 0.40; experiment 4: O-2545, drug (T4) – baseline (T3) vs vehicle (T4) – baseline (T3),  $t_{(5)} = 0.03$ , *p* = 0.98). Theta power is often reported to increase under novelty; however, previous work has typically failed to control for the different behavior accompanying the response to novelty (see discussion by Lever et al., 2009). To test the effects of environmental novelty with increased statistical power, results from experiments 2 and 4 were pooled and the baseline trial compared with its immediately following novel environment trial (*n* = 12, all CA1, drug-free, T3 vs T4). The results showed that, overall, theta power increased under novelty, but this was not reliable (baseline,  $0.65 \pm 0.10 \times 10^{-6} \text{ V}^2/\text{Hz}$ ; novelty,  $0.75 \pm 0.13 \times 10^{-6} \text{ V}^2/\text{Hz}$ , paired  $t_{(11)} = 1.5$ , *p* = 0.16). In fact, closer inspection showed that theta power increased significantly in the novelty of experiment 2 (baseline,  $0.69 \pm 0.11 \times 10^{-6} \text{ V}^2/\text{Hz}$ ; novelty,  $0.86 \pm 0.16 \times 10^{-6} \text{ V}^2/\text{Hz}$ , paired  $t_{(11)} = 2.7$ , *p* = 0.04) but negligibly in the novelty of experiment 4 (baseline,  $0.62 \pm 0.18 \times 10^{-6} \text{ V}^2/\text{Hz}$ ; novelty,  $0.65 \pm 0.20 \times 10^{-6} \text{ V}^2/\text{Hz}$ , paired  $t_{(11)} = 0.3$ , *p* = 0.78). The pooled analysis also confirmed that environmental novelty reliably and specifically reduced slope (slope baseline,  $2.14 \pm 0.15 \text{ Hz} \cdot \text{m}^{-1} \cdot \text{s}^{-1}$ ; slope novelty,  $0.97 \pm 0.13 \text{ Hz} \cdot \text{m}^{-1} \cdot \text{s}^{-1}$ , paired  $t_{(11)} = 6.4$ , *p* = 0.00005; intercept baseline,  $8.56 \pm 0.07 \text{ Hz}$ ; intercept novelty,  $8.45 \pm 0.07 \text{ Hz}$ , paired  $t_{(11)} = 0.6$ , *p* = 0.13). In summary, our results indicate a dissociation in the effect of environmental novelty on theta power and frequency: power shows a variable increase, whereas frequency (slope) shows a reliable decrease.

### Intercept as a physiological measure: intercept reduction cannot be predicted from the firing rates of CA1 pyramidal cells and interneurons

We have shown that the intercept of the hippocampal theta frequency–running speed relationship is an important physiological measure. Intercept reduction is common to three anxiety-reducing drugs tested in our study and appears to predict anxiety-like behavior. However, to what extent is intercept predictable from other variables? Do anxiolytic drugs set hippocampal excitation–inhibition dynamics toward a mode of inhibition whose global physiological signature is the reduction of the theta frequency–speed intercept, or do they share another effect for which intercept reduction is only a useful proxy? We have shown that CDP, buspirone, and O-2545 reduce intercept without changing slope or theta power. Here we demonstrate that O-2545 does not reliably affect firing rates of CA1 pyramidal cells and interneurons. In experiment 4, 115 interneurons and 558 CA1 pyramidal cells were recorded simultaneously with the CA1 LFP recordings. Interneuron firing rate did not increase after injection of the putative anxiolytic drug O-2545 (e.g., in familiar environment, mean rate in preinjection and postinjection trials, 10.9 and 10.8



**Figure 7.** Change in theta slope between baseline and probe trials predicts change in place field size across those trials in CA1 place cells (experiment 4). Scatter plot showing the change in theta slope versus change in place field size. Reduction in theta slope is expressed as positive in magnitude [i.e.,  $-(T4 - T3)$ ]. Familiar environment probe trials are shown as blue diamonds, and novel environment probe trials are shown as red squares. Field size is average place field size of all cells firing  $\geq 120$  spikes, the place field of each cell is defined as the sum of all bins with firing at  $\geq 20\%$  of peak rate. Field size expansion values in novel environments are corrected by dividing by  $\sqrt{2}$  (i.e., the increase expected because of the larger novel environment).



**Figure 8.** Anxiety-related behavior (thigmotaxis) is increased by the putative anxiolytic O-2545 and predicted by theta intercept (experiment 4). *a*, Dwell time maps showing where rats spent their time in the novel walled open-field probe trial (T4, circular-walled novel environment) after injection with O-2545 (3 rats, maps 1–3) and vehicle (3 rats, maps 4–6). Rats injected with O-2545 showed less thigmotaxis, i.e., spent more time in the center, indicating anxiolysis. Dwell time is indicated by color (10 equal bands: dark blue bins, least time; red bins, most time, occupied by the rat for  $\geq 1.5$  s). White squares denote unvisited bins. *b*, O-2545 injection produced significantly less thigmotaxis than vehicle injection (Veh).  $*t_{(4)} = 2.79, p < 0.05$ , O-2545 versus vehicle, T4–T3 center time percentage. *c*, Theta intercept predicts anxiety-related behavior in the novel walled open field. Scatter plot shows occupancy of central area versus intercept value. Numbers refer to dwell time maps shown in *a*.

Hz, respectively). For each of the five rats from which interneurons were recorded in all of the 2 (drug, vehicle)  $\times$  2 (novel, familiar) conditions, we averaged firing rates over all interneurons such that each rat contributed one value to each of the four conditions. There was no main effect of drug ( $F_{(1,4)} = 0.2, p = 0.67$ ) or interaction between drug and novelty ( $F_{(1,4)} = 0.7, p = 0.45$ ).

The firing rate of putative pyramidal cells did not decrease after injection of the putative anxiolytic drug O-2545 (e.g., in familiar environment, mean rate in preinjection and postinjection trials, 0.6 and 0.6 Hz, respectively). For each of the five rats from which pyramidal cells were recorded in all of the 2 (drug,

vehicle)  $\times$  2 (novel, familiar) conditions, we averaged firing rates over all pyramidal cells such that each rat contributed one value to each of the four conditions. There was no main effect of drug ( $F_{(1,4)} = 2.9, p = 0.17$ ) or interaction between drug and novelty ( $F_{(1,4)} = 3.0, p = 0.16$ ). Similar results were seen when restricting analysis to pyramidal cells that fired with a spatial peak rate of at least 1.0 Hz in both the baseline and probe trials: there was no main effect of drug ( $F_{(1,4)} = 3.3, p = 0.15$ ) or interaction between drug and novelty ( $F_{(1,4)} = 3.6, p = 0.13$ ). Together, these results argue for the singular utility of intercept as a physiological measure and for the apparently unique utility of intercept reduction as a physiological signature of the effectiveness of an anxiolytic drug.

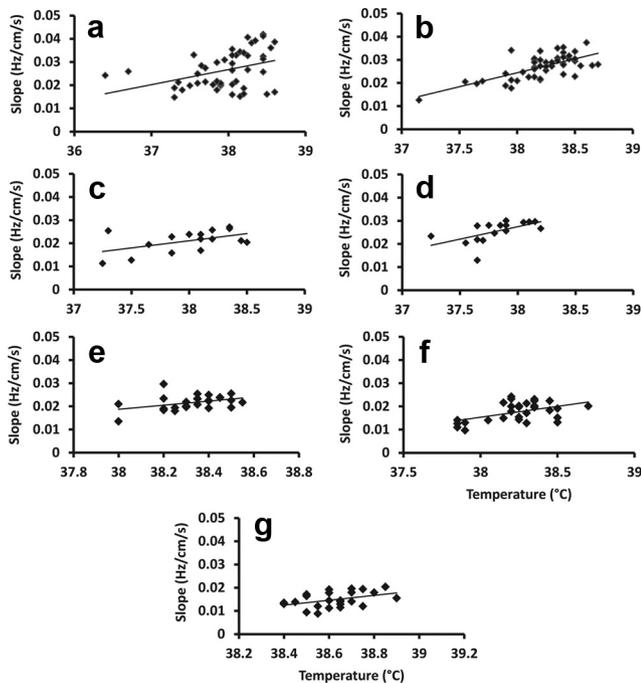
### Confirmatory analyses: intercept reduction by anxiolytic drugs was not a secondary consequence of changes in cranial temperature

Previous studies have indicated a positive relationship between temperature and theta frequency (Whishaw and Vanderwolf, 1971; Deboer, 2002). We measured aural temperature in a subset of eight rats (experiment 1, two of four rats in the main study and the two rats administered buspirone only; experiment 4, four of six rats) to ask how temperature affected the intercept and slope of the theta frequency–speed relationship in non-drug conditions and whether these were affected by anxiolytic drugs. We conclude that the intercept-reducing properties of the anxiolytic drugs cannot be attributed to secondary effects of temperature because (1) temperature does not affect intercept, although it does affect slope, (2) temperature was reduced by buspirone and O-2545 but not by CDP, and (3) correcting for temperature reduction showed that intercept was still reliably reduced by anxiolytic drugs. These three findings are addressed below.

Importantly, there was no sign that the measurement process itself increased temperature in the rats. Over the anxiolytic drug phases of experiment 1 and all of experiment 4, the average difference between the first and last measurement was  $+0.008 \pm 0.02^\circ\text{C}$ .

### Temperature showed no relationship to intercept but was positively correlated with slope

We examined the relationship between temperature and slope and intercept in drug-free conditions and found no significant correlation between temperature and intercept in any of the eight rats, whereas slope and temperature were significantly positively correlated in seven of eight rats. In experiment 1 (rats 3 and 4), we analyzed all the preinjection trials (trials 1–3) on all 16 test days ( $n = 48$ ). There was no correlation between temperature and intercept in either rat (rat 3,  $r = -0.23, p = 0.13$ ; rat 4,  $r = 0.21, p = 0.15$ ). In contrast, temperature and slope were positively correlated in both rats (Fig. 9*a,b*; rat 3,  $r = 0.38, p < 0.01$ ; rat 4,  $r = 0.68, p < 0.01$ ). In experiment 1 (rats 5 and 6), we analyzed all preinjection trials ( $n = 16$ , trials 1–4, days 1–4) and found that there was no correlation between temperature and intercept in either rat (rat 5,



**Figure 9.** Aural temperature showed no relation to intercept but was positively correlated with slope (experiments 1 and 4). In experiment 1, temperature was measured in rats 3–6 (**a–d**) and was positively correlated with slope. In experiment 4, slope and temperature were positively correlated in three of four rats: rat 3 (**e**), rat 5 (**f**), and rat 6 (**g**).

$r = 0.18$ ,  $p = 0.49$ ; rat 6,  $r = 0.17$ ,  $p = 0.52$ ). In contrast, again, temperature and slope were positively correlated in both rats (Fig. 9*c,d*; rat 5,  $r = 0.51$ ,  $p = 0.04$ ; rat 6,  $r = 0.58$ ,  $p = 0.02$ ).

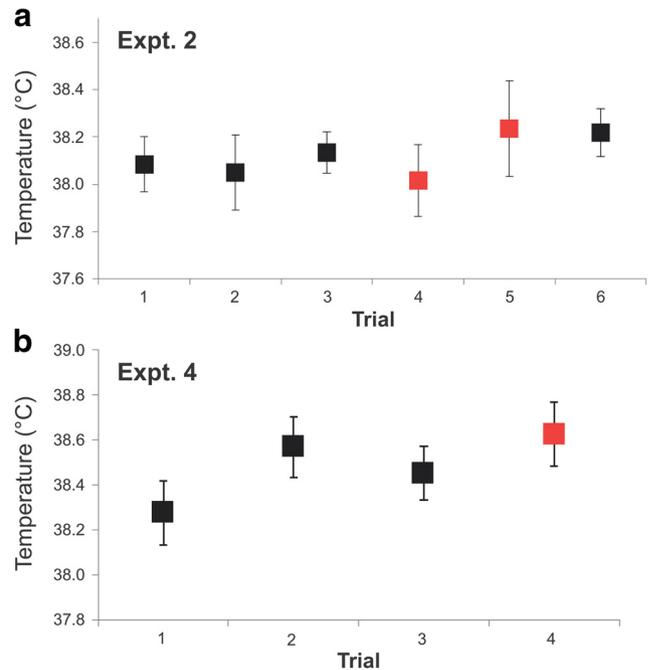
In experiment 4, we used all three preinjection trials on both sets of 5 d blocks [rat 5,  $n = 30$ ; rats 2 and 6,  $n = 24$  because two familiarization days (A1, A2) were excluded as a result technical issues; rat 3,  $n = 27$  because 1 d (B5) was excluded as a result of missing temperature data]. There was no correlation between temperature and intercept in any of the rats (rat 2,  $r = -0.16$ ,  $p = 0.47$ ; rat 3,  $r = +0.35$ ,  $p = 0.07$ ; rat 5,  $r = +0.36$ ,  $p = 0.054$ ; rat 6,  $r = +0.21$ ,  $p = 0.32$ ). In contrast, again, temperature and slope were significantly positively correlated in three of four rats (rat 2,  $r = 0.23$ ,  $p = 0.28$ ; rat 3,  $r = 0.40$ ,  $p = 0.04$ ; rat 5,  $r = 0.50$ ,  $p < 0.01$ ; rat 6,  $r = 0.43$ ,  $p = 0.04$ ; Fig. 9*e–g*).

### Temperature was reduced by buspirone and O-2545 but not by CDP

The data were analyzed in the same way that we analyzed drug effects on slope and intercept. We compared the difference values (postinjection temperature – preinjection temperature) on the drug days (regardless of dose) to those of vehicle days. This gave net temperature changes resulting from drug administration. In experiment 1 (rats 1–4), there was no evidence of temperature reduction by CDP in either rat (mean net change: rat 3,  $0.3^{\circ}\text{C}$ ; rat 4,  $0^{\circ}\text{C}$ ). Buspirone appeared to mildly reduce temperature in both rats 3 and 4 (mean net change: rat 3,  $-0.1^{\circ}\text{C}$ ; rat 4,  $-0.4^{\circ}\text{C}$ ) and in the two drug-naïve rats (mean net change: rat 5,  $-0.25^{\circ}\text{C}$ ; rat 6,  $-0.6^{\circ}\text{C}$ ). In experiment 4, O-2545 reduced aural temperature in all temperature-measured rats (net temperature change in familiar: rat 2,  $-0.32^{\circ}\text{C}$ ; rat 3,  $-0.27^{\circ}\text{C}$ ; rat 5,  $-0.27^{\circ}\text{C}$ ; rat 6,  $-0.43^{\circ}\text{C}$ ).

### Correcting for temperature reduction showed that intercept was still reliably reduced by anxiolytic drugs

Although temperature and intercept were not significantly correlated in any of the rats, the linear effects of temperature on



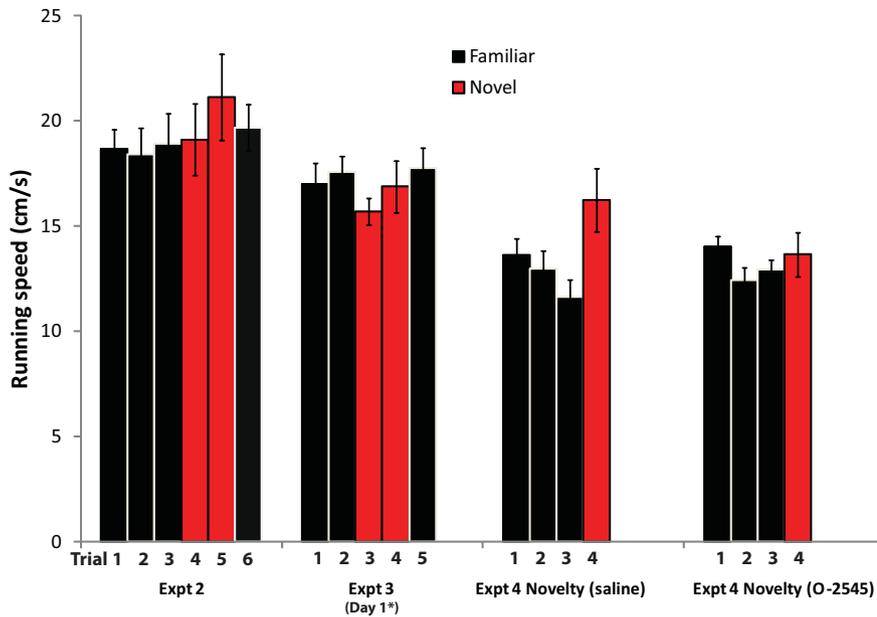
**Figure 10.** Slope reduction by environmental novelty is not attributable to temperature reduction (experiments 2 and 4). **a**, Experiment 2: temperature was similar in the familiar (black squares) and novel (red squares) environments. **b**, Experiment 4: temperature was similar (slightly increased) in the novel environments (red square) relative to the familiar environment (black squares).

intercept were subtracted from the raw intercept values, and these corrected values were retested. In experiment 1 (rats 1–4), the relationships between aural temperature and intercept in the two temperature-measured rats in experiment 1 were similarly weak and opposite in sign (rat 3,  $r = -0.23$ ,  $p = 0.13$ ; rat 4,  $r = 0.21$ ,  $p = 0.15$ ). Raw intercept values were not temperature corrected in analyses of the effect of drug. The temperature-corrected results for rats 5 and 6 (mean change,  $-0.36$  Hz,  $t_{(1)} = 3.18$ , one-tailed  $p = 0.097$ ) were similar to the uncorrected results (mean change,  $-0.39$  Hz,  $t_{(1)} = 4.08$ , one-tailed  $p = 0.076$ ).

To run temperature-corrected analyses for comparison with the original dataset from experiment 4 (rat  $n = 6$ ), we estimated corrected values for the two temperature-unmeasured rats from averaged data in the four temperature-measured rats. From these four rats (individual results above), we obtained the average temperature change for each condition and the averaged linear relationship between temperature and intercept. Then, for each of the two temperature-unmeasured rats, we corrected raw intercept values by assuming condition-specific average temperature change and subtracting the average linear effect of temperature on intercept. A  $2 \times 2$  repeated-measures ANOVA was run exactly as before. The predicted intercept reduction of O-2545 remained (main effect of drug on intercept,  $F_{(1,5)} = 9.14$ ,  $p = 0.043$ ).

### Confirmatory analyses: slope reduction by environmental novelty was not a secondary consequence of changes in cranial temperature

Because aural temperature and the slope of the theta frequency–speed relationship were significantly correlated during baseline (preinjection) trials in experiments 1 and 4, we checked whether the slope reduction by environmental novelty was attributable to lower temperatures in the novel environments. In experiment 2, a paired  $t$  test confirmed that temperature was very similar across



**Figure 11.** Slope reduction by environmental novelty is not attributable to changes in running speed (experiments 2–4). Environmental novelty elicited inconsistent average running speed responses. Relative to baseline trials, average running speed in novelty probe trials (experiments 2 and 4, T4 vs T3; experiment 3, T3 vs T2) were similar (experiment 2, experiment 4 under drug), lower (experiment 3), or higher (experiment 4 under saline). In all cases, novelty reduced slope (cf. Figs. 4, 6).

the novelty and familiarity conditions [paired  $t_{(5)} = 0.03$ ,  $p = 0.97$ , average of novelty (T4 and T5) vs average of familiar (T1–T3 and T6) trials; Fig. 10a]. Accordingly, the slope reduction observed during exposure to unexpected environmental novelty was not attributable to temperature reduction. In experiment 4, the equivalent comparison with that for experiment 2 is shown by Figure 10b: temperature increases somewhat under unexpected novelty on the vehicle novelty day. The mean net effect of unexpected novelty under vehicle was a mild increase of  $0.16^{\circ}\text{C}$  (vehicle novelty T4–T3 vs vehicle familiar T4–T3,  $t_{(3)} = 0.71$ ,  $p = 0.53$ ; the mean net effect of unexpected novelty under drug was  $+0.04^{\circ}\text{C}$ , n.s.). Given the evidence from baseline trials in experiment 4 (and experiment 1) that slope and temperature are positively correlated (Fig. 8), increases in temperature could only have worked against the observed effect of novelty-elicited slope reduction.

#### Confirmatory analyses: coverage of the environments cannot explain the reduction of slope of novelty and the reduction of intercept of anxiolytic drugs

We examined whether differential coverage of the environments could explain the observed effects. We saw no sign of this. Regarding average running speed, we note that our analysis purposely minimizes the effect of any consistent changes in average running speed by analyzing samples of locomotion binned into 10 small (2.5 cm/s wide) bins and by applying both lower limits (no samples  $<5$  cm/s) and upper limits (no samples  $>30$  cm/s). Across-trial differences in the average speed in each bin are negligible. Nevertheless, to provide a richer characterization of the response to novelty and anxiolytic drugs, we consider average running speed further here.

Figure 11 depicts the effect of the first exposures to novelty on average running speed in experiment 2 (left columns) in the first novelty day of experiment 3 (left middle columns) and in the novelty exposures of experiment 4 [divided into novelty under saline (right middle columns) and novelty under drug (right col-

umns)]. There is no consistent change in running speed. Comparing novelty probe relative to baseline trials, average running speed did not change in experiment 2 (paired  $t_{(5)} = 0.12$ ,  $p = 0.91$ , T4 vs T3 net change,  $+0.19$  cm/s) or in experiment 4 under O-2545 (paired  $t_{(5)} = 0.68$ ,  $p = 0.53$ , T4 vs T3 net change,  $+0.70$  cm/s), significantly decreased in experiment 3 (paired  $t_{(4)} = 4.05$ ,  $p = 0.02$ , T4 vs T3 net change,  $-1.87$  cm/s), and significantly increased in experiment 4 under saline (paired  $t_{(5)} = 3.00$ ,  $p = 0.03$ , T4 vs T3 net change,  $+4.62$  cm/s). Thus, all three possible responses were seen: no change, decrease, and increase in average running speed. Of course, as presented above (cf. Figs. 4, 6) in all four conditions of environmental novelty, the novelty consistently, powerfully reduced slope. In summary, it is implausible that changes in average running speed can explain novelty-elicited slope reduction.

Regarding anxiolytic drugs, CDP did not affect average whole-trial running speed ( $F_{(2,6)} = 1.29$ ,  $p = 0.34$ ; mean net effect of CDP doses vs saline =  $+1.08$  cm/s); Buspirone did reduce average whole-trial running speed ( $F_{(2,6)} = 25.1$ ,  $p = 0.001$ ; mean net effect of buspirone doses vs saline,  $-7.22$  cm/s), and O-2545 had no significant effect on average whole-trial running speed (two-way repeated-measures ANOVA; main effect of O-2545,  $F_{(1,5)} = 4.8$ ,  $p = 0.08$ ; mean net effect of O-2545 vs saline,  $-2.03$  cm/s). Thus, for two of three anxiolytic drugs, there was no significant reduction in running speed.

Although it is conceivable that average running speed might be associated with a variable that is not directly controlled for by matching speeds across different trials and conditions and that variable might be linked to slope or intercept, the only likely candidate for such a mediating variable is temperature, which tends to positively correlate with running speeds. We have already shown that variation in temperature cannot explain the reduction of slope by novelty or the reduction of intercept by anxiolytic drugs.

Finally, we could see no qualitative differences in paths taken by the rats under anxiolytic drugs or in environmental novelty (Fig. 12). Figure 12a shows examples in which rats take longer paths under anxiolytic drugs (i.e., run faster on average) and intercept is reduced. Figure 12b shows examples of longer and shorter path lengths in environmental novelty, and slope is consistently reduced.

#### Statistical power analyses: the independence of slope and intercept

Our theoretical framework predicted, and we observed, intercept reduction by anxiolytic drugs and slope reduction by environmental novelty. Furthermore, the model by Burgess, 2008 predicts dissociability between the slope and intercept components of theta frequency. We observed reduction of intercept without slope reduction by anxiolytic drugs in experiments 1 and 4 and reduction of slope without intercept reduction in environmental novelty in experiments 2–4, thus, a double dissociation over the study. Additionally, in experiment 4, we observed a within-

subjects double dissociation of slope and intercept. To what extent does this show that the slope and intercept are truly dissociable? We do not argue that anxiolytic drugs are invariably without effect on slope or that unexpected environmental novelty conditions are invariably without effect on intercept, but that the two components are entirely dissociable in some conditions. To investigate this issue further, we consider effects of size, power, and sample size to show that, in some conditions, as predicted, the components are entirely dissociable. In what follows, power-related calculations were performed using G\*Power, version 3.1.3, with  $\alpha$  set to 0.05, two tailed.

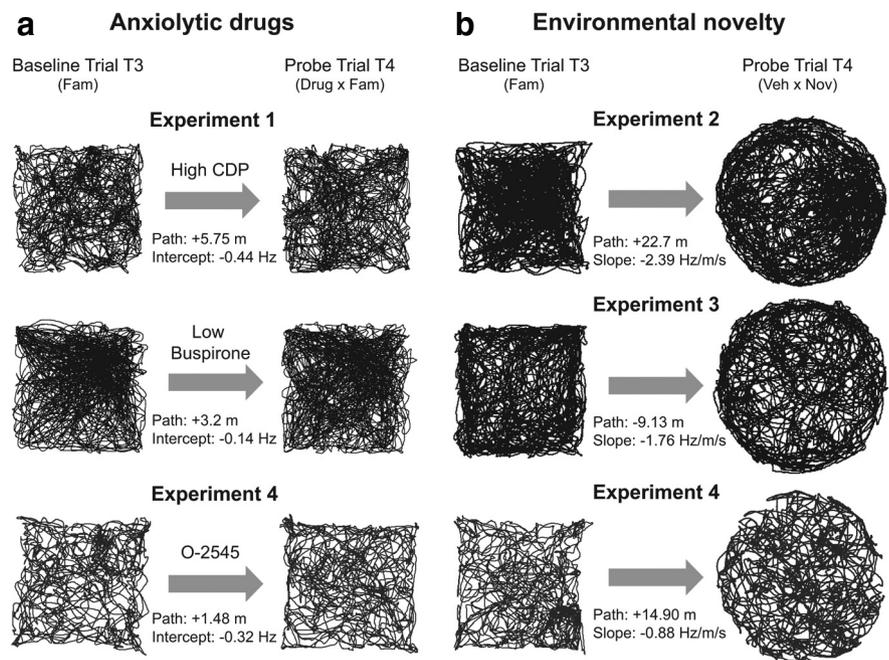
### Anxiolytic drugs can reduce intercept with negligible effects on slope

We tested three drugs: CDP and buspirone in experiment 1 and O-2545 in experiment 4. The effect size of intercept reduction for the low dose of CDP was 2.09, with observed power of 0.93. The effect size of intercept reduction for the low dose of buspirone was 2.06, with observed power of 0.92. The appropriate sample sizes to detect these effects with minimum power of 0.8 (in fact, the closest minimum power is 0.93 and 0.92, respectively, i.e., the observed power) would be  $n = 4$  for CDP and  $n = 4$  for buspirone. In clear contrast, the effect size of slope reduction for the low dose of CDP was 0.037 (observed power of 0.06). The effect size of slope reduction for the low dose of buspirone was 0.56 (observed power of 0.22). The appropriate sample sizes to detect these effects at minimum 0.8 power would be  $n = 4577$  for CDP and  $n = 22$  for buspirone. Thus, detecting an effect of low-dose CDP on slope would require three orders of magnitude more subjects than to detect an effect of the same dose of CDP on intercept. In our view, practically speaking, this equates to a complete (single) dissociation. The equivalent analysis on the drug O-2545 (familiar environment vehicle vs familiar environment drug) showed that the effect size of intercept reduction by O-2545 was 3.06 (observed power approaching unity). In clear contrast, the effect size of slope reduction by O-2545 was 0.07 (observed power of 0.05).

The appropriate sample sizes to detect these effects at minimum 0.8 power would be  $n = 4$  for intercept reduction and  $n = 1604$  for slope reduction. Thus, detecting an effect of our dose of O-2545 on slope would require 400 $\times$  more subjects than to detect an effect of the same dose of O-2545 on intercept. In our view, again, this equates to a complete (single) dissociation.

### Environmental novelty can reduce slope with negligible effects on intercept

To increase the power of our analyses, we combined the results of experiments 2 and 4 ( $n = 12$ , all electrodes in CA1, drug-free, baseline familiar environment trial 3 vs novel environment probe trial 4). The effect size of slope reduction by environmental novelty was 2.42, with observed power approaching unity. The effect size of intercept reduction by environmental novelty was 0.45 (observed power of 0.43). The appropriate sample sizes to detect these effects with minimum power of 0.8 would be  $n = 3$  for slope (closest



**Figure 12.** Anxiolytic drug-elicited intercept reduction and novelty-elicited slope reduction are not attributable to changes in environmental coverage. **a**, Anxiolytic drugs reduce intercept even when path length increases (i.e., average speed increases). **b**, Environmental novelty reduces slope regardless of changes in path length. All values shown are T4–T3 difference values. Paths shown are cumulative path taken by rat over whole trial. There are no obvious qualitative difference in paths across baseline and probe trials in either condition. Fam, Familiar; Nov, novel; Veh, vehicle.

minimum power is 0.83) and  $n = 32$  for intercept. Thus, detecting an effect of environmental novelty on intercept would require one order of magnitude more subjects than to detect an effect of the same novelty on slope. In our view, this equates to a strong dissociation.

Even this low level of intercept reduction is not inevitable on exposure to unexpected environmental novelty. Taking experiment 4 on its own ( $n = 6$ ), the effect size of slope reduction by environmental novelty was 2.24 (observed power of 0.99). In clear contrast, the effect size of intercept reduction by environmental novelty was 0.07 (observed power of 0.05). The appropriate sample sizes to detect these effects at minimum 0.8 power would be  $n = 4$  for slope reduction (closest minimum power is 0.83) and  $n = 1604$  for intercept reduction. Thus, detecting an effect of environmental novelty on intercept would require 400 $\times$  more subjects than to detect an effect of the same novelty on slope. In our view, this equates to a complete (single) dissociation.

Together, the results show a double dissociation in which both intercept and slope can vary independently. Experiment 4 shows this double dissociation within subjects: intercept was reduced by a drug while negligibly affecting slope, and slope was reduced by a novelty condition while negligibly affecting intercept. We conclude that, although some probe conditions will involve changes in both the intercept and slope relative to a baseline condition, these two contributions to theta frequency are entirely dissociable.

### Summary

We hypothesized a dissociation between the factors affecting the intercept and the slope of the theta frequency–running-speed relationship seen in hippocampal theta. Using two clinically well-established anxiolytic drugs (CDP and buspirone) and one putative anxiolytic drug (O-2545), which did indeed elicit anxiolytic effects in our walled open-field model, we have shown that three

anxiolytic drugs have the common effect of reducing the intercept of the frequency–speed relationship, despite their neurochemical dissimilarity. In contrast, we have shown that environmental novelty reduces the slope of the frequency–speed relationship, which then increases as the novel environment becomes more familiar. The change in slope from baseline trial to probe trial predicts changes in the average place field size of hippocampal place cells and predicts changes in rearing, a novelty-responsive behavior linked to the hippocampal response to novelty. We also observed an unpredicted dissociation: aural temperature (and thus presumably brain temperature) positively correlates with slope but not intercept. We ruled out the possibility that uncontrolled changes in temperature explain our findings that anxiolytic drugs reduce intercept and environmental novelty reduces slope.

## Discussion

### Theta frequency–speed slope: spatial representation and novelty

The flatter frequency–speed slope in environmental novelty is a surprising result and implies a disruption to running speed-based hippocampal calculations of distance traveled, which will affect models of spatial coding relating theta to spatial metrics (Burgess et al., 2007; Geisler et al., 2007; Maurer and McNaughton, 2007; Burgess, 2008; Jeewajee et al., 2008a; Brandon et al., 2011; Koenig et al., 2011; Navratilova et al., 2012). Briefly, the predicted link under the oscillatory-interference model between theta slope ( $\beta$ ) and the spatial scale of grid cell firing patterns implies an expanded spatial metric for novel environments. The basic idea that increasing frequency with running speed allows the spatial representation to remain constant despite changes in running speed (Lengyel et al., 2003; Burgess et al., 2007) is seen in the theta-band modulation of place (O'Keefe and Recce, 1993; Geisler et al., 2007) and grid cell firing (Jeewajee et al., 2008a). Intuitively, the reduced slope in a novel environment, i.e., the reduced gain in converting velocity increases into frequency increases, suggests that the rat's physical displacement will be underestimated in the hippocampal formation. The prediction of the model of a novelty-induced expanded metric concomitant with slope reduction has been observed in grid (Barry et al., 2012) and to a lesser degree place (Karlsson and Frank, 2008; Barry et al., 2012) cells, consistent with the consensus that grid cells provide an important metrical input to place cells (McNaughton et al., 2006). We now show here that changes in slope predict changes in place field size across baseline and probe trials even when the novelty status of the probe environment is controlled for.

What is the function of the striking reduction of slope under novelty? Grid scale expansion should cause a mismatch between the grid cell inputs to place cells and the environmental inputs mediated by boundary-related cells in subiculum (Lever et al., 2009) and entorhinal cortex (Solstad et al., 2008), which are essentially unaffected by environmental novelty (Lever et al., 2009). This mismatch could be an important trigger of the novelty-elicited remapping of place cell representations (Burgess, 2008; Barry et al., 2012; the present study) and a trigger for novelty-elicited exploration. Correspondingly, the frequency of rearing, a novelty-responsive, hippocampal-dependent, exploratory behavior (Lever et al., 2006), was also strongly correlated with theta slope.

Our findings also help to clarify the hippocampal response to novelty. We previously observed a novelty-elicited theta frequency reduction (Jeewajee et al., 2008b). We now show that this effect is driven by reduced theta slope, with interesting implications for computations of spatial scale, and this reduction is dis-

sociated from the frequency reduction with anxiolytic drugs, which we now show corresponds to reduced theta intercept in freely moving rats. These findings were predicted previously (Burgess, 2008) but may also be consistent with other models (Navratilova et al., 2012). We have also observed a later theta phase of firing in CA1 place cells in novelty (Lever et al., 2010), consistent with models of encoding-retrieval dynamics within the theta rhythm (Hasselmo et al., 2002).

### Theta intercept, anxiolysis, and immobility theta

It is well established that a neurochemically wide range of anxiolytic drugs reduce the frequency of reticular-elicited hippocampal theta in the anesthetized rat (for review, see McNaughton et al., 2007; Engin et al., 2008; Siok et al., 2009; Yeung et al., 2012). Indeed, Gray and McNaughton (2000) have argued that theta-frequency reduction remains the best preclinical (i.e., animal-based) test for predicting a clinically efficacious anxiolytic drug. Conversely, drugs that are antipsychotic or sedative but not anxiolytic (e.g., haloperidol and chlorpromazine) do not reduce reticular-elicited theta frequency. Currently, as an animal-based test for efficacy in treating human generalized anxiety disorder, the theta-frequency reduction test for anxiolytics has no false positives and no false negatives and has predicted the anxiolysis of several drug classes introduced since the original idea, e.g., 5-HT<sub>1A</sub> agonists (e.g., buspirone), serotonin-reuptake inhibitors (Munn and McNaughton, 2008), and the calcium-channel blocker pregabalin (Siok et al., 2009).

Theta intercept is the frequency of theta during immobility, as extrapolated from theta during movement. Theta during alert immobility is characterized as type II as opposed to movement-related type I theta. Accordingly, the power of immobility theta has been shown to depend on arousal and be atropine sensitive (Green and Arduini, 1953; Kramis et al., 1975; Sainsbury et al., 1987). The frequency of immobility theta has been less studied. In contrast to the low (4–6 Hz) theta frequencies reported in young (Wills et al., 2010) or anesthetized (Kramis et al., 1975; Klausberger et al., 2003) rats, aroused, immobile adult rats often show higher frequencies consistent with our intercept values (e.g., ~8.8 and 8 Hz during avoidance paradigms: Bland et al., 2006, 2007; 8 Hz during fixation in nose-poke paradigms: Takahashi et al., 2009). Our proposed identification of intercept mechanisms with type II theta mechanisms remains to be confirmed. Moreover, although we show that anxiolytic drugs robustly reduce intercept, we cannot currently distinguish between three possibilities regarding the potential bidirectionality of anxiety and intercept: (1) anxiety increases intercept but our anxiety paradigms were too mild to elicit this; (2) relationships between intercept and anxiety reflect individual differences mediated by the hippocampus (Oler et al., 2010) and altered by anxiolytic drugs, such that intercept is higher in high-anxious trait rats exposed to anxiogenic stimuli than similarly exposed low-anxious trait rats, as would be consistent with Meyza et al. (2009); and (3) intercept does not increase with anxiety, with the level of intercept perhaps reflecting a homeostatic set point that is disrupted by anxiolytic drugs (see also point 2, above).

Here we have shown that the intercept frequency extrapolated from freely moving rats shows the same response to anxiolytic drugs as reticular-elicited theta in anesthetized rats. This allows concurrent examination (e.g., during preclinical screening) of the effects of anxiolysis on theta and behavior during classic anxiety tests (and potentially novel ones), in which the rodent moves naturally (and is not anesthetized, freezing, or otherwise immobile). Our experiment 4 demonstrated the feasibility of this ap-

proach. We identified a recently introduced drug (O-2545) as a putative anxiolytic on the basis of its CB<sub>1</sub> receptor agonism. We then demonstrated that it reduces anxiety-like behavior and reduces the intercept of the theta–speed relationship, as predicted by our model. Thus, altogether, we refine the theta frequency–anxiolysis relationship, expand its scope, show its specificity (neither slope nor power being affected), and extend its range beyond drugs already known to reduce theta frequency (albeit reticular-elicited and under anesthesia; Yeung et al., 2012).

### Theta and anxiolytic drug action

There is currently no comprehensive understanding of the mechanisms underlying anxiolysis. One challenge here is the diversity of initial receptor targets of anxiolytic agents, which include barbiturates, benzodiazepines, 5-HT<sub>1A</sub> agonists, serotonin reuptake inhibitors, tricyclic antidepressants, ethanol, somatostatin, pregabalin, phenytoin, and CB<sub>1</sub> agonists. The diverse pathways to anxiolysis are exemplified by comparing the benzodiazepines with buspirone, which is not anticonvulsant, hypnotic, muscle relaxant, or addictive, and has no known clinical effect in common with benzodiazepines other than anxiolysis. Thus, benzodiazepine-mediated anxiolysis is blocked by benzodiazepine receptor antagonists (Menard and Treit, 1999) but not by 5-HT<sub>1A</sub> receptor antagonists (Schreiber and De Vry, 1993). Gray and McNaughton (2000) argue that the shared effect of anxiolysis involves the reduction of the frequency of hippocampal theta rather than the diverse pathways leading to this effect. Accordingly, in parallel to its anxiolytic effects, the ability of buspirone to reduce theta frequency is blocked by the 5-HT<sub>1A</sub> receptor antagonist pindolol (Coop and McNaughton, 1991) but not by the benzodiazepine receptor antagonist Ro 15-1788 (Zhu and McNaughton, 1991), whereas the frequency reduction effect of benzodiazepines is blocked by Ro 15-1788 (Coop et al., 1992) but not by pindolol (Zhu and McNaughton, 1994).

### The dorsoventral axis of the hippocampus

We recorded theta from dorsal hippocampus, the site of anxiety-elicited increased theta power in 5-HT<sub>1A</sub> receptor knock-out mice (Gordon et al., 2005) and of all the studies on the theta-frequency reduction anxiolysis model (McNaughton et al., 2007; Engin et al., 2008; Siok et al., 2009; Yeung et al., 2012). Because our intercept-reduction paradigm in the locomoting rat extends this model, dorsal recording locations were the most appropriate, and our double dissociation is strengthened by coming from a single neural region. Nevertheless, future investigations of dorsoventral differences will be of interest for three reasons. First, the dorsal hippocampus is associated with anticipatory anxiety (Gray and McNaughton, 2000), that is, conditioned anxiety/fear (Phillips and LeDoux, 1992), whereas the ventral hippocampus is associated with both conditioned and unconditioned anxiety. Second, the dorsal hippocampus is associated with spatial memory (Moser et al., 1995) and small-scale spatial representations (Jung et al., 1994; Kjelstrup et al., 2008; corresponding to high frequency-speed slope under the oscillatory-interference model, Burgess, 2008), whereas ventral hippocampus is more associated with unconditioned anxiety (Kjelstrup et al., 2002; Bannerman et al., 2004; Pentkowski et al., 2006; Fanselow and Dong, 2010; McHugh et al., 2011) and large-scale spatial representation (Jung et al., 1994; Kjelstrup et al., 2008; corresponding to low frequency-speed slope under the oscillatory-interference model, Burgess, 2008). Finally, movement-related theta is more prominent in the dorsal hippocampus (Maurer and McNaughton, 2007; Royer et al., 2010; Hinman et al., 2011; Patel et al., 2012),

whereas ventral hippocampal theta shows an anxiety-dependent coherence with medial–prefrontal theta (Adhikari et al., 2010).

### Conclusion

Overall, these findings corroborate a two-component model of the generation of the theta rhythm in freely moving rodents, with specific focus on theta frequency (Burgess, 2008), extending previous work isolating type I versus type II theta (Kramis et al., 1975; Shin et al., 2005; Korotkova et al., 2010). These findings suggest novel ways to isolate spatial translation- and arousal/anxiety-related theta mechanisms in behaving animals and have wide-ranging implications for the role of hippocampal theta in novelty detection and memory, path integration and spatial mapping, and anxiolytic drug action.

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