Lesions of the Vestibular System Disrupt Hippocampal Theta Rhythm in the Rat

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INTRODUCTION

The hippocampus is a brain structure that has been implicated in a variety of cognitive, affective, and mnemonic functions, including spatial navigation processes. A dominant feature of the hippocampal electroencephalography (EEG) is the rhythmic EEG signal known as theta. Theta activity is dependent on an animal’s behavioral state and has a dominant frequency between 6 and 9 Hz in the freely moving rat (see Bland 1986; Bland and Colom 1993; Buzsaki 2002; Vertes et al. 2004 for reviews).

Theta appears to have an important role in spatial information processing as the firing of hippocampal neurons is related to the phase of theta in a manner that indicates position in space (Huxter et al. 2003; O’Keefe and Recce 1993; Skaggs et al. 1996). Furthermore, theta frequency has been shown to positively correlate to the animal’s movement velocity (LS Leung 1984a; LW Leung 1984). This latter finding indicates that theta is modulated by self-movement signals and suggests that it may be part of the mechanism by which the hippocampus integrates allocentric and egocentric signals to construct a representation of the spatial environment (McNaughton et al. 1996; Wishaw 1998).

A number of different sensory systems could be capable of providing self-movement information to the hippocampus. The vestibular system in the inner ear is, however, particularly suited for this function as it contains specialized receptor cells that detect angular and linear acceleration of the head in different planes. These signals are then integrated in the brain stem vestibular nucleus to provide information about linear velocity, angular velocity and position (Raphan and Cohen 2002). These signals could then be made available to the hippocampus through several polysynaptic pathways (Cuthbert et al. 2000; Hayakawa et al. 1993; Horii et al. 2004b; Kirk and McNaughton 1991; Semba et al. 1988; Smith 1997; Taube et al. 1996; Vertes and Kocsis 1997).

The aim of the present study was to investigate the long-term effect of permanent bilateral lesions of the peripheral vestibular apparatus on theta activity in the hippocampus. The few previous studies that have addressed this question have used either reversible lesions of the labyrinth [i.e., using tetrodotoxin (TTX)] (Stackman et al. 2002) or animals with a congenital vestibular dysfunction (Frederickson et al. 1982) that would have denied them of any previous experience of normal vestibular input. Stackman et al. (2002) reported that bilateral intratympanic TTX had a minor effect on theta EEG activity, although these data were based on a small number of samples obtained from two animals and were of an observational nature only. Furthermore, it should be noted that some chemical injection procedures have been shown to produce neurochemical changes in the vestibular nucleus that are different from those induced by surgical vestibular lesions (Campos-Torres et al. 2005). In the only previous study of hippocampal activity that used a surgical intervention (Shoham et al. 1989), the EEG
was not fully quantified and the recordings were conducted at a time when vestibular compensation, the process by which the dynamic symptoms of peripheral vestibular deafferentation reach a steady state over ~60 days (Smith and Curthoys 1989), would still have been occurring. To the best of our knowledge, therefore, no previous study has examined the long-term effects of permanent and complete vestibular deafferentation on hippocampal theta activity. Some of these data have been presented previously in abstract form (Russell et al. 2001).

METHODS

Surgeries and recording procedure

Twelve naïve male Sprague-Dawley rats received either a bilateral labyrinthectomy (n = 6) or a sham surgical procedure (n = 6). Animals were anesthetized with pentobarbital (60 mg/kg ip), and before the labyrinthectomy procedure, the vestibular labyrinths were exposed using a retroauricular approach. Local anesthesia with lidocaine was also used in the wound margins. After removal of the tympanic membrane, malleus and incus, the vestibule just above the ampullae of the horizontal and anterior semicircular canals was drilled out. After aspiration of labyrinthine fluids and the membranous labyrinth from the drilled vestibule and the ventral portion of the oval window, the labyrinth was rinsed with 0.1 ml of absolute ethanol, and perfused through the ventral portion of the oval window and the drilled vestibule. This labyrinthectomy technique results in a complete and permanent destruction of the peripheral vestibular apparatus (Horii et al. 2003; Russell et al. 2003a) and has been confirmed by temporal bone histology (Fukushima et al. 2001; Horii et al. 2004a; Kitahara et al. 1995). For the sham operation, animals had their retroauricular skin incised in the same way as for the labyrinthectomy; however, the tympanic membrane, malleus, and incus were preserved. Behavioral testing (i.e., righting reflex function) was used to confirm the absence of vestibular function in the lesioned animals.

After ≥3 wk recovery from the labyrinthectomy surgery, all animals were re-anesthetized (as in the preceding text) and implanted with single-unit recording electrodes (Russell et al. 2003a; see Bilkey and Muir 1999; Bilkey et al. 2003 for details) into the CA1 subfield of the hippocampus from which EEGs were recorded simultaneously with single-unit spiking during an open-field foraging task. Recordings were not made until ≥60 days postlabyrinthectomy (70 ± 3.0 days with no time difference between groups, t10 = 0.79, P > 0.1) at which time the symptoms of bilateral labyrinthectomy are considerably reduced and have reached a steady state (Smith and Curthoys 1989). The recording procedures have been described in detail previously (Russell et al. 2003a) but are briefly reiterated in the following text.

All rats were food-deprived to reduce their body weight to 85% of their free-feeding weight and were maintained at this weight throughout the remainder of the study. Recordings were made while the animals foraged for randomly scattered chocolate chips in a high walled black plastic cylindrical environment (75 cm diam) with a single dominant visual cue. After ≥5 min of habituation, recordings were made for either one 10-min session or three consecutive 10-min sessions (with no interruption between sessions) that were treated as independent for analysis purposes.

Animal movement was recorded by an infra-red sensitive video camera, which allowed the position of the animal to be monitored via an infrared light-emitting diode (LED) attached to the headstage of the animal. The output of the infrared camera was digitized and a dedicated PC calculated the animal’s position. It then generated analogue voltages corresponding to the x and y coordinates of the animal. These coordinates were continuously sampled by a digitizer at 60 Hz.

Single-unit recording

Single-unit spiking was recorded, and cells were classified electrophysiologically as either complex spiking (CS) or noncomplex spiking (non-CS) neurons based on the raw waveforms, the interspike interval histogram (ISI—histogram of times between adjacent pairs of spikes) and spike time autocorrelation (STA—histogram of times between every pair of spikes). CS cells were defined as those with a spike width of >400 μs and a spike autocorrelation with a clear peak at 3–8 ms, indicative of complex burst firing. When categorized on the basis of spike width and CS firing, there was a clear bimodal distribution that separated CS from non-CS cells.

The firing rate for each cell was then correlated to the animal’s velocity and acceleration. These movement variables were calculated from the position of the animal after an 80-ms (full width at half-maximum) Gaussian smoothing filter was applied to remove jitter and noise in the tracking system. Velocity was calculated as the distance between each pair of adjacent samples divided by the time between those samples and acceleration and the average cell firing rate for each bin was calculated. All results were found to be independent of the sign of the movement variable, hence results were recalculated using absolute values throughout. The number of bins, and hence the choice of bin size, was set to ensure the correlation coefficient reached an asymptote when plotted against bin size. This procedure is necessary as correlations in data sets with fewer data points can be artificially high.

The movement data had highly skewed distributions with very few data at high velocities and accelerations. Thus the firing rate variance increased at higher values, necessitating the use of a weighted regression analysis. This meant that no data had to be excluded due to undersampling. Furthermore, because the hyperkinesis evident in the lesioned animals provided a potential confound to the motion correlates, the use of a weighted regression analysis ensured that no arbitrary upper limit on the movement variables was required. Imposing such a threshold for inclusion would otherwise have resulted in different treatment of the data from the lesion versus control groups due to their different movement-variable distributions.

EEG recording

EEG was recorded simultaneously with single neuron activity but referenced to a ground screw on the animal’s skull. The power of theta rhythm varies considerably with the depth of the electrode within the hippocampus (e.g., Buzsaki 2002; Buzsaki et al. 1986; Leung et al. 1982; LW Leung 1984). Therefore to control for electrode depth, EEG recordings were made only from electrodes on which a CA1 neuron had been identified. This ensured all EEG recordings containing a CS cell were made from the CA1 cell layer; this is a more accurate method for determining electrode depth than postmortem histological verification for long-term recordings with moveable microdrives. Non-CS cells, however, are distributed across a wider range of depths in CA1 (Freund and Buzsaki 1996). Therefore a comparison was made between an analysis containing all sessions and an analysis that included only those recordings containing a CS cell. The results of these two analyses were found to be consistent with each other and therefore all sessions have been included during analysis unless stated otherwise.

The signal was passed through a Grass P511K amplifier with a single-order band-pass of 1–30 Hz and sampled continuously at f = 60 Hz by an Axon Instruments DigiData1200 series digitizer. Because the low-pass filter cut-off frequency was at the Nyquist limit, the possibility that corruption of the EEG signal could occur due to
Aliasing was initially of some concern. Because the effect of aliasing is to fold the spectrum about f/2, the major effects would be in the higher frequency components near 0-2. Therefore as a precautionary measure we also applied a further single pole band-pass digital filter at 1 and 20 Hz to the sample data. Then to test for any evidence of aliasing, we compared data from a recording session during which the hippocampal EEG was recorded from an animal with f = 60 Hz and then immediately after with f = 100 Hz. The filter settings were identical in the two recordings. Examination of the spectra confirmed that aliasing had not affected the EEG signal when recording was conducted at the lower rate (data not shown).

A Fourier analysis of the continuously sampled EEG voltage was conducted for each 10-min recording session for each animal. Spectrograms (the power at each frequency at each time), and power spectral densities (PSD, a spectrogram integrated across time) were obtained using Matlab. The spectrograms and PSDs were calculated using a discrete Fourier transform with a 256 sample, moving Hanning window. Each PSD was measured with a resolution of 0.25 Hz. Because the overall signal amplitude can vary across recordings and animals due to factors such as electrode impedance and location, the data from each session were normalized so that the integral of the PSD over the range of 3-30 Hz was equal to one. This allowed comparisons to be made across sessions and animals. The average PSD was then obtained over all sessions for each animal. To quantify any differences in spectral peaks against the underlying broadband activity, an exponential curve was fitted to the mean PSD for each animal using a least-squares method. The difference between the fitted curve and the PSD was then retained and the amplitude and frequency of the peaks in this difference curve were measured in both the 6- to 9.5-Hz and the 9.5- to 13-Hz ranges using an automated Matlab routine. The validity of this procedure was verified by manual checking of the data, and the overall pattern of results was also shown to be insensitive to changes in the parameters and algorithms used in the Matlab routine. The results for control and lesioned animals were then compared using t-test.

Because the presence and the frequency of theta rhythm are known to be movement-dependent (Bland 1986), a difference in theta rhythm frequency between the two groups might be expected because the lesioned rats were hyperkinetic with a positively skewed velocity distribution (Russell et al. 2003a). An epoch analysis was performed to compare theta in the two groups with this potential confound controlled. Theta waveforms were objectively identified in the raw EEG data and degree of rhythmicity was assessed using a fitting method similar to that described in Muir and Bilkey (2003). This procedure involves determining the best-fit between a sine wave and an epoch (usually 1 s) of EEG. The phase and amplitude parameters are free and the frequency parameter is limited to a range of 6-9 Hz. The mean correlation coefficient between the fitted sinusoidal wave and the one second samples of EEG, and the mean frequency of the fitted wave were then determined for each animal. This procedure is conceptually similar to wavelet analysis (Wyble et al. 2004) and is a temporal extension of that used by Huxter et al. (2003). To determine whether between-group differences in movement speed affected the results all one second epochs of theta generated when the animal’s average velocity was between 20 and 20.6 cm/s were compared. This particular velocity bin was chosen because the velocity distributions of the two groups were equal within this range of speeds (Russell et al. 2003a). A further comparison of the EEG data were made with all epochs binned according to animal velocity with same width bins generated for the range of velocities between 10 and 50 cm/s.

In a further analysis, the 1-s EEG epoch with the highest correlation (the most rhythmic sample) was then extracted from each recording session. These data were then averaged across all sessions for each animal and the fit parameters were compared between groups using a t-test. The behavioral state during this epoch of theta rhythm, defined by the animals’ linear and angular velocity and acceleration, and the firing rate of the simultaneously recorded single neuron, were also examined for any group-dependent biases. The distribution of the correlations between the sinusoidal waves, and the 1-s EEG epochs were plotted for each group. The distribution was first found for each 10-min recording session and then averaged across all sessions to produce a mean distribution for each animal. The group means were then calculated from these data. Finally, the automated theta-fitting procedure was validated and calibrated against the performance of an observer experienced in theta detection. The observer was instructed to identify theta where it occurred for >750 ms in each 1-s sample. These observer-generated data were then compared with the correlation coefficients generated by the fitting procedure.

**Blood corticosterone levels**

Anxiety levels are also known to have an effect on hippocampal theta rhythm (see Gray and McNaughton 2001 for a review). This is a potential confound in this study as it is possible that the lesioned animals were in a heightened state of anxiety. It is known, for example, that vestibular disorders and anxiety disorders coexist in a greater than chance percentage of the human population (Jacob and Furman 2001). To measure anxiety levels in the rats as objectively as possible, the strong correlation between anxiety and blood serum corticosterone levels was exploited and blood serum corticosterone (CORT) levels were assayed. Blood samples were taken from the renal artery immediately prior to perfusion. Handling of the animals prior to perfusion was minimized to avoid stressing the animal, and all animals were perfused at the same time of day to avoid diurnal variations in CORT levels. The blood samples were then frozen and analyzed at a later date. Serum CORT levels were assayed with the use of commercially prepared radioimmunoassay kits highly specific for rat CORT (Corticosterone RIA coat-a-count, Diagnostic Products). This is a solid phase RIA in which [125I]-labeled rat CORT competes with CORT for antibody sites immobilized to the walls of polypropylene tubes (Macleman et al. 2003). The minimum detectable level of CORT for this assay was 5.7 ng/ml. The rat CORT antiserum was highly specific for rat CORT; the amount of nonspecific binding was 3.2% and the CORT concentration coefficient of variation was 0.20. Although these CORT measurements provided an indication of the stress levels in the animals just prior to perfusion and were therefore likely to reflect any long-term stress associated with the effects of BVD, they did not necessarily reflect stress levels during the testing protocol. All animals were, however, well habituated to the experimenter and the testing protocol before recording commenced.

**Results**

**Behavioral observations and histology**

All of the lesioned animals exhibited clear deficits in the righting reflex test and displayed increased head weaving and hyperkinesis compared with the sham animals. A full description is provided in Russell et al. (2003a) including graphs of the velocity distributions. Histological analysis confirmed that all recording electrodes had been located in the CA1 cell layer of the dorsal hippocampus. Although histology was not performed on the vestibular lesions, we are confident that they were complete in all cases because temporal bone histological analysis has shown complete morphological destruction of the vestibular hair cells when an identical surgical procedure was performed by the same surgeon (Horii et al. 2004a).

**Power and frequency of theta rhythm**

The number of recording sessions per animal was 28.5 ± 4.9 (SD) for control animals and 32.2 ± 7.6 for lesioned animals. There was no significant difference between the two groups in...
terms of the number of sessions conducted ($t_{10} = 0.41$, $P > 0.1$). Initial examination of individual spectrogram and PSD plots revealed a disruption in the lesioned animal’s EEGs. This was most apparent as a reduction in energy in the 8-Hz theta band while a disruption in the 10- to 12-Hz band was also readily apparent in most recordings. Our previous findings indicate that this latter activity is probably not theta (Nerad and Bilkey 2005).

When all recording sessions were pooled to obtain the mean PSD for both lesioned and control animals, it became clear that there were smaller peaks at ~8 and 10–12 Hz (relative to the underlying broad band signal) in the lesioned animals. This indicated that there was less power in these bands. Furthermore, the peaks corresponding to theta and the 10- to 12-Hz activity occurred at lower frequencies (Fig. 1A). The difference in spectra between control and lesioned animals was quantified by measuring the power and frequency of the peaks above the background spectra. The frequencies of the spectral peaks were significantly lower in lesioned animals in both the 6- to 9.5-Hz band (control = 7.79 ± 0.077 Hz, lesioned = 7.42 ± 0.19 Hz, $t_{10} = 1.83$, $P < 0.05$) and 9.5- to 13-Hz bands (control = 11.3 ± 0.14 Hz, lesioned = 9.96 ± 0.29 Hz, $t_{10} = 4.15$, $P < 0.001$). The power is also significantly lower in the lesioned animals in both the 6- to 9.5-Hz band (control = 0.65 ± 0.15, lesioned = 0.25 ± 0.1, $t_{10} = 2.19$, $P < 0.05$) and 9.5- to 13-Hz band (control = 0.99 ± 0.10, lesioned = 0.52 ± 0.16, $t_{10} = 2.56$, $P < 0.05$) bands (Fig. 1B).

**Epoch analysis**

Careful examination of the raw EEG traces confirmed that the disruption to the spectra corresponded to a disruption in theta rhythm. Representative samples of raw EEG data recorded from control and lesioned animals are shown in Fig. 2. Although theta rhythm did occur in the lesioned animals, it was generally less rhythmic than in control animals. An epoch analysis was used to quantify this difference in an objective manner. In this analysis, blocks of EEG data were fitted to a sine wave (with phase and amplitude parameters of the sine free and the frequency parameter constrained to a range between 6 and 9 Hz). In an initial test, the fitting procedure was repeated 10 times for all EEG traces, with the epoch length varied systematically between 0.2 and 2 s in 0.2-s increments. A mean sine fit, as a measure of rhythmicity, was calculated for each epoch length for each animal. For all 10 epoch lengths tested, the data indicated that the EEG generated in the lesion-group animals was significantly ($P < 0.05$; $t$-test) less rhythmic within the theta-band compared with the EEG from control-group animals. On the basis of this initial analysis, 1-s epochs were chosen for further study on the grounds that this interval had good face validity.

The distributions of the mean correlation coefficients between all 1-s epochs of EEG and the fitted 6- to 9-Hz sinusoids are shown in Fig. 3A. A comparison of the distributions generated for lesion versus control animals indicates that the EEG was more likely to be rhythmic within the theta-band in the latter group of animals. Because the theta waveform is often sawtooth-shaped rather than sinusoidal, it was important to show that the sine-fitting procedures were a valid method of measuring the appearance of theta in the wave form. For this reason, the output of the fitting procedure was calibrated against the performance of an experienced observer instructed to identify theta where it occurred for ≥750 ms in each 1-s sample. The correlation coefficients generated by the fitting

**FIG. 1.** Power spectrum analysis of electroencephalography (EEG) from control and vestibular-lesioned animals. A: mean power spectral density (PSD) for each animal in the control ($n = 6$) and lesion ($n = 6$) group. The integral of the PSD for each 10-min session was normalized to 1 before an average PSD was produced for each animal. The EEG recorded from lesioned animals has relatively more broadband power at lower frequencies but a smaller peak at the theta frequency (~8 Hz) relative to this background activity. This indicates that there is less coherent activity at ~8 Hz in the lesion group. B: mean ± SE normalized power and mean frequency relative to underlying broadband EEG at the energy peaks within the 6- to 9.5- and 9.5- to 13-Hz bands. Both peak frequency and peak power was significantly lower in the lesioned animals for both frequency bands.
procedure were found to be strongly related to the presence or absence of theta as determined by the observer. In an analysis of 594 1-s samples of EEG, the observer detected theta in 76% of samples with a correlation coefficient of ≥0.4 and in 93% of samples with a correlation coefficient of ≥0.6. In contrast, when the correlation was ≤0.2, theta was detected in only 12% of the samples (Fig. 3B). Thus from the distributions shown in Fig. 3A, we can conclude that the control group animals were over 3.8 times more likely to generate an EEG epoch with clear theta in the EEG (correlation of ≥0.6) than were lesioned animals.

When the 1-s epoch of EEG with the best fit was extracted from each recording session (as measured by the sine-fitting function) and then compared across groups, the EEG had a higher correlation to a sinusoidal wave in the control animals ($r = 0.83 \pm 0.01$), compared with the lesioned group ($r = 0.77 \pm 0.02, t_{10} = 3.1, P < 0.01$). In the lesioned animals, this sinusoidal wave also had a lower frequency (control, $f =$...
7.51 ± 0.06 Hz; lesioned, \( f = 7.36 \pm 0.04 \) Hz, \( t_{10} = 2.1, P < 0.05 \). The locomotion speeds and accelerations that occurred during the most rhythmic epochs were binned to create speed and acceleration distributions. The shape of these distributions were similar to the overall speed and acceleration distributions generated using all epochs available for each group of animals (data not shown). This indicates that the most rhythmic theta was not associated with any particular subset of speeds or accelerations for either group of animals.

Theta activity is movement-dependent. It was critical, therefore that the effects of the differences in behavior between the two groups be removed as a factor in any further analyses. EEG epochs were selected therefore from 1-s periods where the animals were moving at between 20 and 20.6 cm/s. This particular velocity bin was chosen because the mean velocity distributions of the two groups were identical within this range (Russell et al. 2003a). When this subsection of data was compared, the mean correlation coefficient obtained was significantly better in control animals than in the lesion group (control, \( r = 0.30 \pm 0.02 \), lesioned, \( r = 0.22 \pm 0.02, t_{10} = 3.0, P < 0.01 \).

To determine if the lesion-associated disturbance in theta activity relationship held across other velocities, the EEG data were binned into five bins covering speeds of between 10 and 50 cm/s. Sine fitting was conducted for all epochs within each bin, and these values were averaged to obtain a mean value for each bin for each animal (Fig. 4). When a two-way ANOVA was conducted on these data it revealed that there was a significant effect of group \( [F(1,10) = 5.77, P < 0.05] \), indicating that the EEG from the lesioned group of animals was significantly less rhythmic than that recorded from controls. There was also a main effect of velocity \( [F(4,40) = 5.00, P < 0.01] \), but no interaction. A Newman-Keuls post hoc test revealed that the EEG from the lesioned group of animals was significantly less rhythmic \( (P < 0.01) \), compared with the EEG from the control group, across all velocity bins.

Theta activity has been associated with movement initiation (Oddie and Bland 1998). Because the vestibular lesions may have altered movement transitions, we conducted a further analysis based around the 1-s epochs of EEG captured when the animals were moving at between 20 and 20.6 cm/s (the speed at which both groups had spent an equal proportion of time). We then broke these latter data down into eight subsets based on the average speed of the animal in the 1-s preceding the target epoch (limited to a maximum of 48 cm/s). Data were then matched so that the speed of the animal was the same, both during the target epoch and during the epoch preceding the target. The results of this analysis are presented graphically in Fig. 5. It is apparent that the lesion-group animals had reduced theta rhythmicity across the full range of behavioral transitions. A two-factor ANOVA revealed a significant effect of group \( [F(1,10) = 5.76, P < 0.05] \). There was no effect of
amplitude and wavelength (120 ms or 1/110 Hz) of the STA oscillations that occurs with increasing theta-frequency EEG observed in lesion-group animals, the firing of both CS and non-CS cells in this group is modulated with approximately the same interspike intervals as occurs in the control animals. This indicates that the coherence within the 6- to 9-Hz and 10- to 12-Hz range in the control and lesioned animals for both CS and non-CS neurons.

Temporal firing patterns of individual neurons

Theta rhythm could be altered in the lesioned animals by a disruption in the intrinsic firing properties of single neurons or by a loss of coherence in the activity of groups of neurons. An examination of the ISIs and STA plots for both CS and non-CS neurons was made. Figure 6 shows the mean STA plots for all CS and non-CS cells for both lesioned and control animals (CS cells: control n = 41, lesioned, n = 39; non-CS cells: control n = 11, lesioned, n = 22). Note that a description of the spatial firing properties of these neurons has been provided elsewhere (Russell et al. 2003a). The cause of this hyperkinesis is unknown although it occurs after lesions of both the vestibular

Neural firing, velocity, and acceleration

Overall, the firing rates of both CS and non-CS cells had a statistically significant positive correlation with the animal’s linear velocity in both lesioned and control groups. These correlations were much larger for non-CS cells (control = 0.47 ± 0.09, lesion = 0.58 ± 0.05) as compared with CS cells (control = 0.12 ± 0.05, lesion = 0.10 ± 0.05). There was no significant difference in these correlations, for both CS or non-CS cells, when control and lesioned animals were compared (all P > 0.1). There were no significant correlations between the animal’s linear acceleration and cell firing rate in any group.

Finally, there was no significant difference between the control and lesioned animals’ blood CORT levels, which were 281 ± 66 ng/ml for the control animals and 298 ± 55 ng/ml (P > 0.05) for the lesioned animals.

DISCUSSION

The present study demonstrates that a permanent bilateral vestibular labyrinthectomy, made using a surgical-chemical procedure, is associated with a disruption of the 6- to 9-Hz and 10- to 12-Hz hippocampal EEG activity. This effect was apparent, in an analysis of the whole recording session, as a labyrinthectomy-associated reduction in the size and frequency of the theta-related PSD energy peaks. Furthermore, in an automated analysis conducted by examining the data on a second-by-second basis, the EEG was shown to be less rhythmic within the 6- to 9-Hz and 10- to 12-Hz range in the vestibular-lesioned animals.

Theta rhythm activity is known to depend on the behavioral state of the animal and, in particular, is strongly associated with movement (Bland 1986). It is critical therefore that any analysis of theta activity controls for lesion-induced changes in movement. Hyperkinesis was observed in the lesioned animals (Russell et al. 2003a). The cause of this hyperkinesis is unknown although it occurs after lesions of both the vestibular
system (Basile et al. 1999; Schaeppi et al. 1991) and the septo-hippocampal system (Andersen et al. 1997; Bannerman et al. 2001; Decker et al. 1995; Gerhardt and Boast 1988 for example). Although the power and frequency of theta normally increases rather than decreases with more rapid movements (Terrazas et al. 2005; see Buzsaki 2002 for a review), which should lead to an increase in theta activity in the lesioned group rather than the opposite, it is possible that some more subtle aspect of the lesion-induced movement change could affect theta generation. For this reason, we were careful to match data, both for the running speed of the animal and for behavioral transitions as the animal either accelerated or decelerated across a 2-s analysis period. In each case, it was apparent that theta was less rhythmic in the lesion group even when movement differences were controlled for. This suggests that the hyperkinetic behavior of the lesioned animals is unlikely to account for the disruption to theta rhythm.

Alternative explanations of the change in theta rhythm relate to lesion-induced changes in internal states. For example, a disruption to theta rhythm could be an indirect consequence of the lesioned animals being in a state of heightened anxiety, which could be mediated by, or have an effect on, the hippocampus. It is known, for example, that both classical and novel anxiolytic drugs increase the threshold for septic driving of theta rhythm, reduce the frequency of both reticulotectal and spontaneous theta rhythm and share the same behavioral effects as septo-hippocampal lesions (Gray and McNaughton 2001). This explanation for the observed reduction in theta activity in the current study is unlikely, however, as on the basis of previous studies an increase in power and frequency of theta rhythm would be expected in more anxious animals. Furthermore, it was shown that lesioned and control animals’ blood serum CORT levels did not differ significantly. This result is consistent with previous studies in guinea pigs and rats which have shown that the increase in salivary cortisol or blood CORT (respectively) that is associated with labyrinthectomy subsides within 2 days of the lesion (Gliddon et al. 2003; Lindsay et al. 2005; Zhang et al. 2005). A further explanation is that lesion-induced changes in body temperature might have indirectly affected the results. Previous data show that as cortical temperature increases, so do theta frequency and power (Deboer 2002; Pan and McNaughton 1997). One might expect, therefore, that the increased hyperactivity of the lesioned-group animals would, if anything, lead to increased body/brain temperature and, as a result, increased theta power and frequency, rather than the opposite. Recent evidence suggests, however, that, in mice at least, the vestibular system may have some role in modulating body temperature (Fuller et al. 2002). Because we did not measure body or brain temperature in the group of animals used in the present study, we cannot be certain that theta was not altered because of a temperature change. We have, however, measured the rectal temperature of a large group of awake rats (n = 18) that had identical vestibular lesions and compared them to a matched group (n = 17) with sham surgeries. In these animals, there was no evidence of differences in temperature t(34) = 0.36 (P > 0.1). On this basis, we conclude that it is unlikely that lesion-induced changes in body temperature produced the EEG effects that we observed.

The current data show that a loss of vestibular input produces a decrease in the rhythmicity of theta activity in the hippocampus. Because these findings appear unlikely to be due to the confounding effects of changes in behavior or body state, they indicate that vestibular signals may be important for normal hippocampal function. Vestibular signals could potentially be routed to the hippocampus through one or more of several different polysynaptic pathways. One possibility is that it may ascend via a thalamo-cortical route (Smith 1997), through theta rhythm generating structures leading to the medial septum (Hayakawa et al. 1993; Kirk and McNaughton 1991; Semba et al. 1988; Vertes and Kocsis 1997) or via the “head direction” system (Taube et al. 1996; Vertes et al. 2004). In regard to the latter hypothesis, it is of interest to note that head direction cell activity is disrupted after vestibular damage (Stackman and Taube 1997).

The current finding is consistent with the results of previous studies showing that hippocampal place cells respond to vestibular signals (Sharp et al. 1995) and that vestibular stimulation can induce theta rhythm (Arnolds et al. 1984; Gavrilov et al. 1995, 1996) and increase acetylcholine release in the hippocampus (Horii et al. 1994). In addition, when rats are deprived of normal vestibular cues and are provided with optic flow signals only, theta rhythm is significantly reduced in power (Terrazas et al. 2005). Although most of this reduction appears to be attributed to an absence of ambulation, we should note that the effects of a vestibular lesion are likely to be quite different to the effects of a visual-vestibular conflict. The current finding may be a factor in explaining recent findings that vestibular lesions disrupt the spatial firing of principal neurons within the hippocampus (Russell et al. 2003a; Stackman et al. 2002). Interestingly, it has recently been reported that bilateral vestibular lesions in humans result in hippocampal atrophy; whether this is due to cell death remains to be determined (Brandt et al. 2005).

Few previous studies have investigated the effects of peripheral vestibular deafferentation on hippocampal EEG. Frederickson et al. (1982) reported that hippocampal theta activity related to movement was still present in mice with congenital vestibular defects. However, because such mice have never had normal vestibular function, it is difficult to exclude the possibility that they compensated for the congenital defect. Shoham et al. (1989), using chemical or surgical deafferentation of the inner ear in rats, reported that theta activity was diminished somewhat during active movement. This latter effect was not sensitive to atropine and therefore the authors suggested that it reflected changes in the atropine-insensitive type I theta that occurs during movement. Although the theta disturbances described in the present study appear to relate to type I movement-related theta, it is unclear whether atropine-sensitive, type II theta is also affected as both type I and type II theta are typically present simultaneously in the awake, moving animal (see Buzsaki et al. 1986; Leung 1984b; Leung et al. 1982 for reviews). The use of chemical deafferentation has been shown to result in neurochemical changes in the vestibular nucleus which are distinct from those produced by surgical labyrinthectomy (Campos-Torres et al. 2005). However, Shoham et al.’s use of surgical lesions is more comparable to the technique used in the current study. Our study extends these previous findings by quantifying the EEG characteristics and by conducting the recordings ≥60 days after the vestibular lesions by which time any effects of vestibular compensation should have stabilized (Smith and Curthoys 1989). In the most
recent study of vestibular influences on hippocampal EEG, Stackman et al. (2002) reported that hippocampal theta was generally intact during, and 24 h after, vestibular inactivation produced by bilateral intratympanic injections of TTX, but with some irregularity in the rhythm at 24 h. They may therefore have been describing a similar effect to that observed in the present study, with the small size of the effect possibly being due to their inactivation procedure, which may not completely eliminate vestibular function, and could produce different effects in the vestibular nucleus compared with surgical lesions (Campos-Torres et al. 2005). Because the focus of the Stackman et al. (2002) study was on cell activity in the hippocampus, EEG data were not quantified.

As regards cell activity, there were two clear differences between the current study and that of Stackman et al. (2002). First, they reported that the firing rates of both place cells (CS) and theta cells (non-CS) were unchanged during vestibular inactivation, whereas an increase in firing rate was observed for both groups of cells in the current study. Second, in the previous study a disruption to the periodicity in the autocorrelograms was observed in around half of the theta cells recorded. We did not observe this effect. These differences may be due to the compensation process that follows labyrinthectomy. Bilateral vestibular loss results in a collapse of spontaneous resting activity in the two vestibular nuclei that then recovers to some degree over a period of several days (Ris and Godaux 1998). Although the exact mechanisms are unclear, it is possible that this recovery process has many downstream consequences that could change both the long-term firing rates and firing patterns in the hippocampus.

Previous research suggests that theta rhythm results from an external synchronizing input that interacts with the intrinsic tendency of hippocampal neurons to generate membrane potential oscillations at around theta frequency (Bland and Colom 1993). As a result, a large proportion of both CS and non-CS cells in the hippocampus typically fire in synchrony with theta rhythm EEG (Bland and Colom 1993; Buzsaki 2002; Fox et al. 1986). The contrast between the effects of the vestibular lesion on the EEG, and its lack of effects on periodicity in single CA1 cells is, therefore an interesting aspect of the current study. Furthermore, this finding suggests several possible explanations for the observed disturbance in EEG. First, it is possible that the vestibular lesion disrupts the coordination of neuronal firing within the hippocampus such that these neurons oscillate in accord with intrinsic dynamics but do not synchronize with their neighbors to generate population-based EEG effects. A second alternative is that the currents that generate theta may be shunted to some degree after the vestibular lesion, perhaps because of the removal of an inhibitory input onto hippocampal inhibitory interneurons (e.g., Matyas et al. 2004). As the medial septum inputs play a major role in synchronizing hippocampal neuronal activity during theta, possibly via inhibitory modulation of interneurons (Bilkey and Goddard 1985; Freund and Antal 1988; Toth et al. 1997), it may be hypothesized that one consequence of the vestibular lesion is to disrupt this input.

Reversible septal inactivation with local lidocaine injections and permanent excitotoxic lesions do not disrupt the firing rate or the spatial firing correlates of CA1 pyramidal neurons (Leutgeb and Mizumori 1999; Mizumori et al. 1989), but they do reduce the firing of CA1 inhibitory interneurons (Mizumori et al. 1989). By comparison, selective blocking of cholinergic neurons in the septum has the opposite effect, reducing the firing rate and spatial firing correlates of pyramidal neurons but leaving interneuron firing rates intact (Brazhnik et al. 2003, 2004). In addition, selective destruction of septal cholinergic neurons reduces theta rhythm power but not frequency (Lee et al. 1994). These results suggest that the GABAergic and the cholinergic septal projections to the hippocampus may have opposing roles in normal hippocampal function so that removing one has a larger effect than removing both. It is possible that the vestibular lesion leads to abnormal septal activity either via influences on the GABAergic or the cholinergic system, which then results in a disruption of CA1 neuron firing. This could interfere with the coordination of neural activity in the hippocampus and/or alter current flow in the dendrites of the principal cells of this latter region, either of which could lead to a disruption in theta rhythm EEG.

It is well known that the firing rate of both CS and non-CS cells in the hippocampus is positively correlated with the animal’s movement velocity (McNaughton et al. 1983). This suggests that motion signals may contribute to hippocampal function, possibly by allowing the processing and integration of allocentric and self-motion cues (Bures et al. 1997; McNaughton et al. 1996; Whishaw 1998). In the present study, it was shown that lesions of the vestibular system do not alter the firing rate-velocity relationship, indicating that nonvestibular sensory systems provide the signals that modulate hippocampal cell firing rate. This is consistent with the results of a previous finding that showed intact firing-rate-velocity relationships in rats trained to run in a stationary wheel (Czurko et al. 1999). One possible alternative source of velocity information is optic flow derived from the visual system because there is some evidence that optic flow information reaches the hippocampus via the accessory optic system in pigeons and possibly in rats (Wylie et al. 1999). There are, however, other possible sources, including signals derived from olfactory or tactile sensory flow and from proprioception and motor efference copy systems. Given that vestibular lesions disrupted spatial firing correlates in the cells described in the current study (Russell et al. 2003a), the intact velocity correlates indicate that these neurons are capable of encoding multiple, independent signals.

The finding that vestibular lesions affect theta activity raises the question of what role vestibular input plays in normal hippocampal function. One possibility is that theta activity provides information about spatial orientation (Oddie and Bland 1998). For example, the phenomenon of phase precession describes how place cell firing varies systematically against theta as the animal moves through a place field (Huxter et al. 2003; O’Keefe and Recce 1993; Skaggs et al. 1996). Phase precession mechanisms could potentially separate the firing of an active cluster of place cells into those that represent the region that the animal’s head is pointing toward from those that represent the region behind the animal (Bilkey and Clearwater 2005; Skaggs et al. 1996). Such information could be important for planning trajectories or for determining the direction to a goal and vestibular input would be particularly relevant as the animal moved its head back and forth to “sample” the environment. A disruption in this type of mechanism could potentially underlie the deficits in path integration behavior and spatial memory that accompany vestibular lesions in rats (Russell et al. 2003b; Wallace et al. 2002) and humans.
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