

Electrophysiological evidence for vestibular activation of the guinea pig hippocampus

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Vestibular information modulates hippocampal activity for spatial processing and place cell firing. However, evidence of a purely vestibular stimulus modulating hippocampal activity is confounded as most studies use stimuli containing somatosensory and visual components. In the present study, high-frequency electrical stimulation of specific vestibular sensory regions of the right labyrinth in anaesthetized guinea pigs induced an evoked field potential in the hippocampal formation

bilaterally with a latency of about 40 ms following stimulation onset. Field potentials localized in the hippocampal formation occurred with stimulus current parameters that were too small to produce eye movements. This provides direct electrophysiological evidence of vestibular input to the hippocampus. *NeuroReport* 11:1443–1447 © 2000 Lippincott Williams & Wilkins.

Key words: Hippocampus; Horizontal eye movement; Semicircular canal; Utricular; Vertical eye movement; Vestibular

INTRODUCTION

Information about self-movement and self-position is potentially useful for path integration (calculating present spatial position from a known starting point) and the hippocampal formation has been implicated in such spatial processing [1–3]. Neurochemical, electrophysiological and behavioural evidence suggests that the vestibular system is involved in providing sensory information for such spatial function and that this information may be processed by the hippocampus [4]. For example, in humans cold caloric vestibular stimulation selectively increases hippocampal neural activity as measured by fMRI [5]. In rats there is an increase in acetylcholine release in hippocampal CA1 and CA3 regions following caloric or electrical vestibular stimulation and that release is blocked by injection of a glutamatergic antagonist (DNQX) into the medial vestibular nucleus [6]. The activity of hippocampal place cells appears to be influenced by vestibular sensory input during rotation or translation of the animal [7–9]. Passive whole body rotations of rats in darkness modifies hippocampal theta activity [10]. However most previous studies of vestibular-hippocampal interaction have not used a purely vestibular stimulus: the rotational and translational stimuli typically used generate somatosensory as well as vestibular input. The caloric stimulus generates sound and somatosensory stimulation (and possibly pain) as well as vestibular stimulation. The stimuli used may also evoke

eye movements which have been shown to modulate hippocampal activity [11,12]. It is also possible that the stress associated with the vestibular stimulation could have been a factor in eliciting the hippocampal responses. Since the hippocampus may respond to stimuli of virtually any sensory modality (see [13] for a review) and stress and eye movements have not usually been measured or controlled for, these studies provide only indirect evidence that the hippocampus receives vestibular input.

Unequivocal evidence of the existence of a vestibular-hippocampal projection requires observation of a hippocampal response to a specifically vestibular stimulus. Furthermore procedures are required to eliminate stress and to identify whether the vestibulo-hippocampal response is indirectly mediated by the eye movements themselves. The present experiment aimed to satisfy these conditions by using specific electrical vestibular stimulation and recording extracellular field potentials from the guinea pig hippocampal formation while recording the eye movement responses. Stress was eliminated by conducting these measures in anaesthetized animals. The stimulating electrodes and the stimulus parameters of the specific vestibular stimulation were similar to those that have been used previously in studies of vestibulo-ocular pathways [14]. Specific vestibular stimulation eliminates the contribution of other, possibly confounding, sensory inputs but produces eye movements which may directly influence

hippocampal activity [11]. The role of eye movements was tested by identifying whether the response could still be obtained using stimulus parameters that did not produce an eye movement: we found that a change in the hippocampal field potential could still be evoked by specific vestibular stimulation too small to produce an eye movement.

A number of anatomical pathways for conveying vestibular information to the hippocampus have been identified. Vestibular nuclei project extensively to thalamic nuclei, both laterally and medially [15,16]. Subsequently, midline thalamic nuclei, particularly the reuniens nucleus have been shown to project to hippocampus and modulate electrical field potentials in area CA1 [17,18]. Alternatively vestibular hippocampal interactions may be mediated by thalamic-parietal projections [19,20], or via the pontine reticular formation, a structure possibly involved in integration of sensory inputs for storage of head velocity [21]. With such a potentially long neural pathway one would expect the hippocampal field potential changes to have a long and probably fluctuating latency.

MATERIALS AND METHODS

Recordings were made in seven pigmented guinea pigs, weighing 800–920 g at time of surgery. All procedures had been approved by the Animal Care Committee of the University of Sydney.

Animals were anaesthetized with i.m. injections of Hypnorm (fentanyl citrate 0.24 mg/kg, fluanisone 7.5 mg/kg; Janssen) and Nembutal (pentobarbitone sodium 30 mg/kg; Rhone Merieux) and placed in a modified guinea pig stereotaxic instrument. Bilateral hippocampal electrodes made from silver wire of 250 μ m diameter, insulated with Teflon except for an exposed recording tip were implanted stereotaxically at the following coordinates: 5 mm posterior, 3 mm lateral to bregma; 4.7 mm ventral to skull surface. Control histology showed that these coordinates located the electrode tip in the hippocampal formation. Two silver ball electrodes were implanted subcutaneously, close to the medial and lateral canthi of the right eye for recording EOG. A thin chlorided silver plate (20 \times 5 mm) was placed s.c. in the neck muscles and served as the indifferent electrode for recording the hippocampal response. The neural activity was amplified (\times 350) by a DC-coupled amplifier.

For placement of the stimulating electrode, the middle ear was exposed by opening the temporal bone. To stimulate the ampulla of the lateral or anterior semicircular canal a small section of the bony casing of the appropriate ampulla was drilled away, and the electrode tip was advanced to the nerve branch leaving the crista [22]. The stimulating electrode was a bipolar electrode made from two strands of stainless steel wire (California Fine Wire Company), each wire being 40 μ m in diameter and Teflon

insulated except at the tip. In all cases, the adequacy of the electrode placement and the effectiveness of the stimulation were verified by visual observation of the direction of the eye movements evoked by high frequency electrical stimulation using a high magnification operating microscope. Visualization of eye movement using the operating microscope has a resolution of $<0.5^\circ$ as confirmed by our objective search coil measures of eye movement [23]. To stimulate the utricular macula, the lateral and anterior semicircular canals were removed and the stimulating electrode tip was advanced directly onto the exposed macula at the region of the striola [24].

The electrical stimulation consisted of a number of constant current 0.1 ms pulses (usually 32) with 2.4 ms between each pulse [14]. The interval between the onset of each pulse train was variable and was \sim 5 s. During the stimulation testing the average current used to evoke a hippocampal response was 400 μ A, this was between \sim 2 and 6 times the threshold necessary to produce an eye movement.

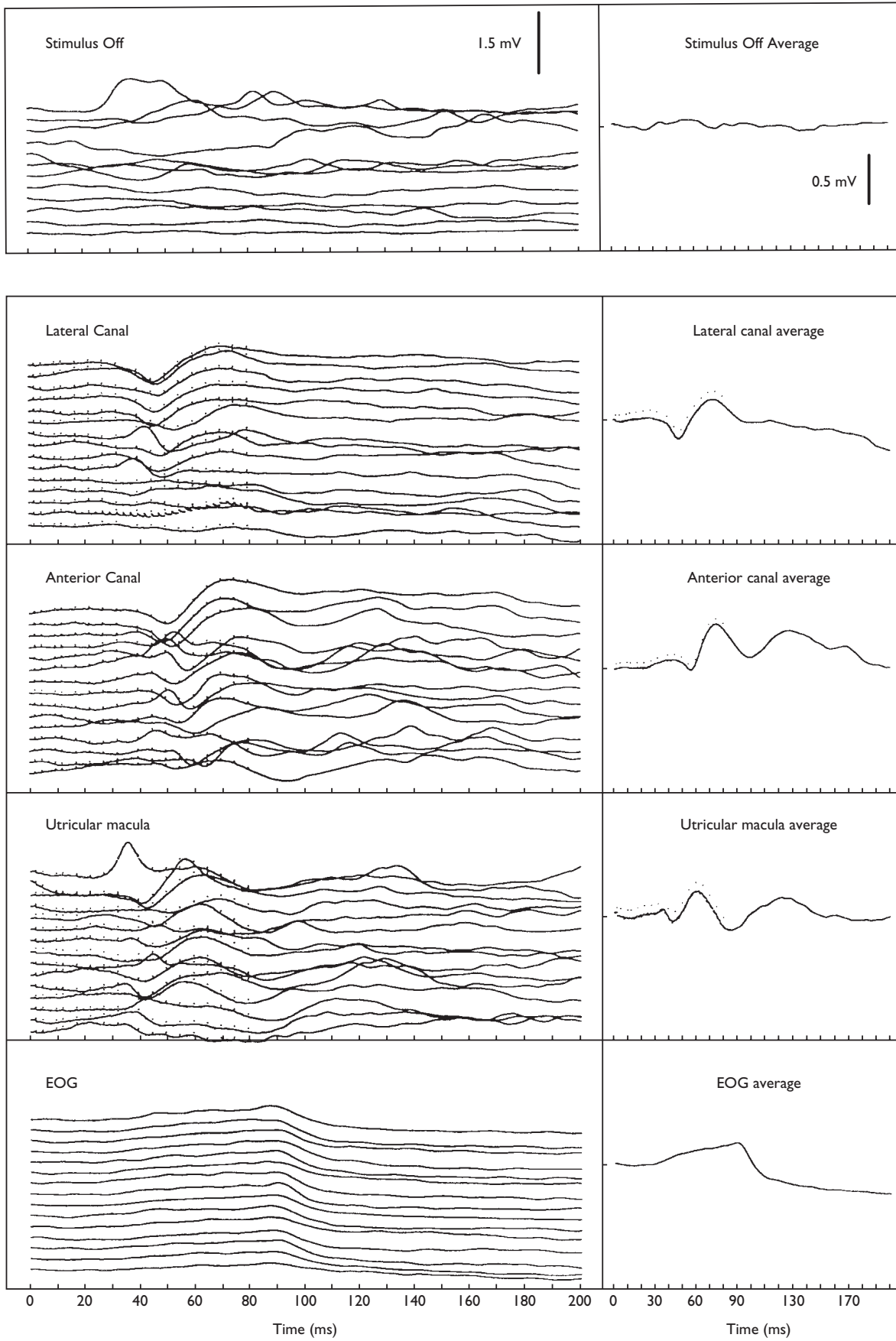
EEG and EOG signals, both DC-coupled, were amplified and either photographed on an oscilloscope screen or recorded, at a sampling rate of 5 kHz, via a 16-bit PCI-MIO data acquisition card (National Instruments, LabVIEW) on a separate computer.

Following completion of the recording session an electrolytic lesion was produced at the tip of the recording electrode (25 μ A for 10 min, electrode positive). The animal was perfused with Karnovsky's fixative (4% w/v paraformaldehyde/5% w/v glutaraldehyde/phosphate buffer), the brain removed and sliced into 25 μ m sections, stained with cresyl violet and examined under light microscopy for EEG electrode location.

RESULTS

High frequency pulse train stimulation of vestibular sensory regions produces eye movements with directions characteristically dependent on the particular sensory region stimulated [22,24,25]. In all seven animals stimulating the nerve of the lateral canal produced conjugate eye movements with both eyes moving in a horizontal direction away from the stimulated side. The presence of the eye movement and its direction confirmed the adequacy of the stimulating electrode placement and current strength. Such specific vestibular stimulation also produced consistent, long latency evoked potentials (40–50 ms after stimulus onset) in the hippocampus as shown by the data from individual trials (Fig. 1). This result was obtained in all six animals in which the recording electrode tip was located in the hippocampus. The evoked field potential in the hippocampus was present at threshold-straddling currents for producing eye movements. The hippocampal evoked potential occurred during the initial movement of the eye away from its resting position (Fig. 1). It should be

Fig. 1. Successive field potentials from the hippocampus in one animal during stimulation at specific sites of the vestibular apparatus. The top trace shows the potentials that occur without any stimulation and includes the average evoked response over 200 ms for 12 trials. The middle traces show field potentials during pulse train stimulation of the nerve from the ampulla of the lateral and anterior canals, as well as from the surface of the utricular macula. The stimulus artifact defines the pulses during the first 80 ms. The evoked response is as clear in the individual traces as it is in the average (right panels). There is a consistent biphasic change in the field potential \sim 40 ms following the onset of a 32-pulse, 400 μ A, 80 ms stimulus train. The electro-oculographic (EOG) trace at the bottom represents the eye movements produced by stimulation of the lateral canal. Traces are shown in order of decreasing amplitude between 20 and 80 ms.



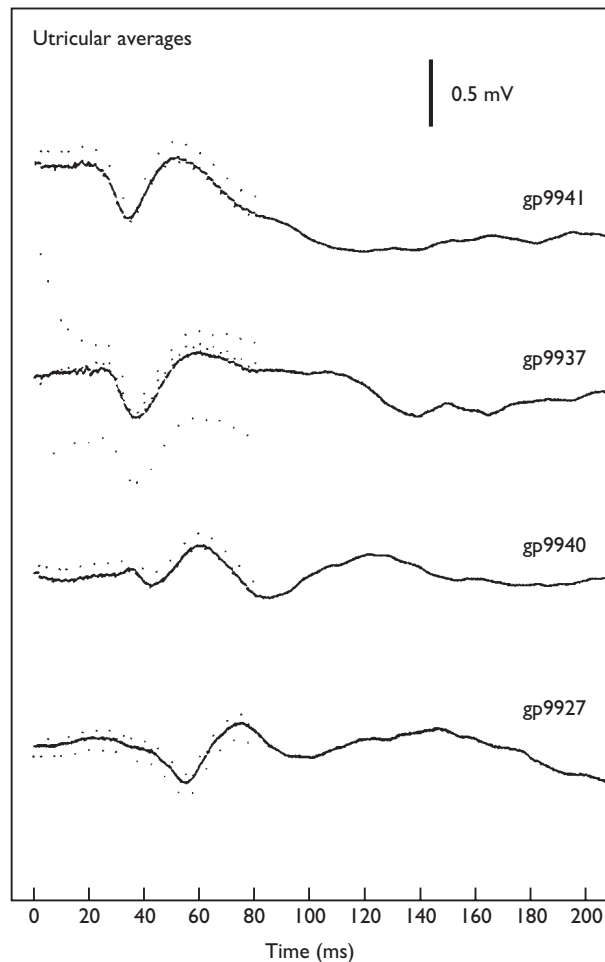


Fig. 2. The averaged traces of hippocampal field potentials of four animals during direct electrical stimulation to the utricular macula. The 80 ms stimulus is composed of a 32-pulse, 400 μ A train. The field potentials display the characteristic biphasic change at around 40 ms after the stimulus onset.

noted that the evoked hippocampal response could not be obtained until ≥ 30 min after electrode implantation, presumably due to a recovery period following the trauma of electrode placement.

Stimulation of the anterior canal ampulla and utricular macula produced upward movement of the eye ipsilateral to the stimulating electrode and downward movement of the contralateral eye in all seven animals [24]. This stimulation produced a similar evoked potential to that observed during lateral canal stimulation with a biphasic change in potential with a latency also of ~ 40 ms following stimulus onset (Fig. 2).

The magnitudes of the evoked responses and the latencies were similar in both hippocampi, irrespective of which vestibular sensory region was stimulated. For two of the animals the stimulating current was systematically reduced below the threshold for observing eye movements. In such cases the evoked field potential was attenuated but still clearly present (Fig. 3).

Histology indicated that the effective locations for recording an evoked hippocampal response were in regions

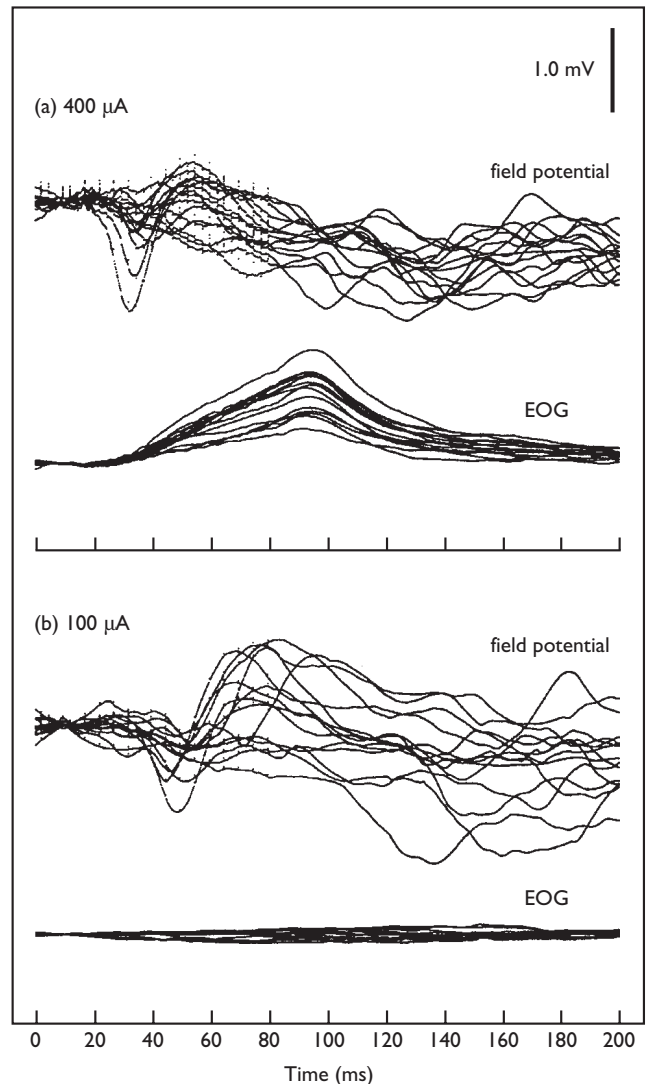


Fig. 3. A comparison of the hippocampal field potential and the corresponding eye movement response (EOG) in the same animal during direct electrical stimulation of the utricular macula with different current strength. (a) For a higher stimulus value (32-pulse, 400 μ A, 80 ms stimulus train) there was a clear eye movement evident in the EOG trace and a characteristic change in the hippocampal field potential. (b) For a stimulus value below the threshold to produce an eye movement (32-pulse, 100 μ A, 80 ms stimulus train) the characteristic change in the hippocampal field potential persisted. The stimulating electrode remained in the same position for (a) and (b).

CA1, CA2 and the fimbria of the hippocampal formation. As a control, three animals had at least one of the bilaterally implanted electrodes located outside the hippocampus (in one case the ventricles, in one case the corpus callosum and in one case the caudate nuclei). In such cases no field potential could be recorded, although the stimulation-induced eye movements confirmed that the vestibular stimulation was effective.

DISCUSSION

High frequency electrical stimulation of the specific vestibular sensory regions causes characteristic eye movements

and induces a reproducible hippocampal field potential with a latency of 40–50 ms after stimulus onset. This evoked response is evident in a number of areas of the hippocampal formation, including at least the CA1 and CA2 regions and the fimbria but is not recorded when the electrode tip is outside the hippocampal formation. The latter control shows that the evoked potential is not an electrical artefact produced by the pulse train itself, nor an artefact generated as a result of movement of the eye, nor a widespread neural response to the stimulus, nor due to the activation of the neck muscles as recorded by the indifferent electrode.

There was some variability between animals in the latency of the hippocampal evoked response, presumably due to a polysynaptic pathway and the exact location of the electrode. For example Fig. 2 shows one animal displaying an evoked response ~15 ms later than other animals for the same stimulus. Histology revealed that this electrode was positioned in the dentate gyrus, while the other animals had electrodes in area CA1/CA2. With thalamo-hippocampal projections directly to CA1 this difference in latency may be expected.

These results show a hippocampal response to a specific vestibular stimulus without confounding sensory input, eye movements or stress-evoked responses, demonstrating the existence of a pathway from the vestibular periphery to the hippocampal formation. The observed field potential may be the initiation of hippocampal theta. It is well documented that changes in hippocampal theta activity occur during a variety of spatial processing tasks [13]. Indeed we found that later waves of the EEG trace, up to a second after vestibular stimulus onset, displayed peak to peak intervals of 150 ms or 6.5 Hz, which approximates the period of hippocampal theta.

The magnitudes of the evoked responses and the latencies are very similar in both hippocampi, suggesting hippocampal processing of vestibular input is not lateralised according to side of stimulation. The partial lateralization of hippocampal activation observed by Vitte *et al.* [5] was not confirmed by the present study.

Lateral canal, anterior canal and utricular stimulation each produced a hippocampal response, suggesting the hippocampus may be responsible for processing of vestibular stimuli associated with body movement in vertical planes as well as for horizontal plane processing [7,10]. Interestingly, this compliments a recent demonstration that rat head direction cells located in the anterodorsal thalamic nuclei are sensitive to locomotion in the vertical plane [26].

There may be differences between the responses to canal and utricular stimulation but such differences await more detailed and systematic mapping of the hippocampal responses.

CONCLUSION

Many studies suggest that vestibular activity may modulate hippocampal activity for spatial processing and place cell firing. However this is the first study that shows a change in hippocampal field potentials following specific stimulation to the vestibular labyrinth itself. The result is a long-latency evoked potential indicative of a polysynaptic pathway from the vestibular nucleus to the hippocampus.

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