

**Can Medial Septal Stimulation that Elicits Hippocampal Theta
Rhythm Repair Cognitive and Emotional Deficits Resulting from
Vestibular Lesions?**

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Abstract

Bilateral vestibular lesions cause atrophy of the hippocampus and subsequent deficits in spatial memory and the processing of emotional stimuli. These problems are seen in both rats and humans. Vestibular lesions also impair hippocampal theta rhythm in rats. The aim of the present study was to investigate whether restoring theta rhythm to the hippocampus of a rat, via stimulation of the medial septum, would repair the deficits caused by vestibular lesions. It was hypothesised that the restoration of theta would repair the deficits and the vestibular rats would exhibit behaviour and EEG similar to that of the sham rats. Rats were given either sham or bilateral vestibular lesions followed in a later operation by electrode implants. Half of the lesioned rats received stimulation. Subjects were tested in spin, openfield, elevated T-maze and forced alternation tests. Except for the spin test which measured the natural production of theta, each test measured either cognitive or emotional functioning. BVD caused a deficit in hippocampal theta rhythm. Stimulation restored theta in the vestibular rats however the stimulation did not repair the cognitive and emotional deficits caused by the lesions. It was concluded that stimulation, at least in the form used here, would not be a viable treatment option for vestibular damaged humans. Further investigation is needed to determine if there is a true difference in the effects of vestibular lesions between rats and humans. The unique ability of humans to worry about future events and the role this has in the severity of symptoms experienced was another issue that could have contributed to the difference between the expected and observed findings.

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“I have no special talent. I am only passionately curious” (Albert Einstein).

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Dedication

To Mary Elizabeth Te Rua Hine Reece. The best Grandma in the world. I promised you when I was thirteen that if I ever wrote a book I would dedicate it to you. I do know how proud you would be.

To all the rats. Thank you for teaching me patience, for providing me with hours of entertainment, and most importantly for your great contribution to my thesis. You made everyday at the lab a good one. Rock on boys!

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List of Abbreviations

ATN: Anterior thalamic nuclei.

BIS: Behavioural Inhibition System.

BVD: Bilateral vestibular deafferentation.

DAT: Dopamine transporter.

DH: Dopamine β -hydroxylase.

DRN: Dorsal raphe nucleus.

DVN: Descending vestibular nucleus.

DTN: Dorsal tegmental nucleus.

EEG: Electroencephalography

HD Cells: Head direction cells.

IVN: Inferior vestibular nucleus.

LC: Locus coeruleus.

LVN: Lateral vestibular nucleus.

MRI: Magnetic resonance imaging.

MVN: Medial vestibular nucleus.

nNOS: Neuronal nitric oxide synthase.

PAG: Periaqueductal gray.

PBN: Parabrachial nucleus.

PHA: Posterior hypothalamic area.

PPT: Pedunculopontine tegmental nucleus.

PPV: Phobic postural vertigo.

ROb: Nucleus raphe obscurus.

RPa: Nucleus raphe pallidus.

RPO: Reticularis pontis oralis.

SDS: SSRI discontinuation syndrome.

SERT: Serotonin transporter.

SMD: Space and motion discomfort.

SSRI: Selective serotonin reuptake inhibitor.

SuM: Supramammillary nucleus.

SVN: Superior vestibular nucleus.

TH: Tyrosine hydroxylase.

TryH: Tryptophan hydroxylase.

UVD: Unilateral vestibular deafferentation.

VNC: Vestibular nucleus complex.

VOR: Vestibular ocular reflex.

VSR: Vestibular spinal reflexes.

1. General Introduction

Vestibular damage in both humans and animals has been seen to affect more than balance function and control of ocular movements. There is research that suggests that vestibular damage causes cognitive deficits especially relating to the processing and retention of spatial information (Smith et al., 2005a). An issue that will be further discussed in chapters 3, 4 and 5 is how far is this a deficit of spatial processing proper and how far it is a more general deficit in, e.g. paired associate learning. There is also a reported association between vestibular damage and emotional deficits (Zheng, Goddard, Darlington, & Smith, 2008; Kalueff, Ishikawa, & Griffith, 2008). An issue with this that will be further discussed in chapters 2 and 3 is how often vestibular dysfunction leads to the development of an anxiety disorder and how often the development of an anxiety disorder leads to vestibular dysfunction, and, if non-vestibular balance dysfunction plays a role. Vestibular damage causes the development of damage to the hippocampus as well (Brandt et al., 2005). This damage to the hippocampus has been implicated in the cognitive deficits observed in both vestibular lesioned animals and in vestibular deficient humans. Interestingly, the hippocampus and the vestibular system share a number of areas in the brain that influence their activity (Taube, 1995). The role of the hippocampus and its relationship with the vestibular system will be discussed in chapter 5.

2. The Vestibular System

The vestibular system responds to movement and gravitational stimuli (Angelaki & Cullen, 2008). This information is sent from the inner ear to the vestibular nuclei which then send it to the autonomic nervous system and various brain areas (Smith, 1997). The thalamus receives vestibular information and this is important because it sends this information to the hippocampus, via the head direction pathway, where it is used to update spatial memory and motor programmes (de Waele, Baudonniere, Lepecq, Tran Ba Huy, & Vidal, 2001; Taube, 1995). When the vestibular system is stimulated, cells in the hippocampus are activated which suggests the vestibular system and the hippocampus share some kind of relationship regarding spatial memory (Angelaki, Klier, & Snyder, 2009). The vestibular system also influences affective responses, serotonergic activity and noradrenergic activity through its involvement in a network that includes the parabrachial nucleus, raphe nucleus and locus coeruleus (Balaban, 2002). This relationship is important because bilateral vestibular lesions have been associated with anxiolytic effects in animals (Smith, Zheng, Horii, & Darlington, 2005b) and an increase in anxiety related behaviours in humans (Furman & Jacob, 1997).

2.1. What Does the Vestibular System Do/Where is It?

2.1.1. The Inner Ear

The vestibular system is located behind the ears of humans and other mammals. It is described as being an inertial sensor (Angelaki & Cullen, 2008). Specifically, the vestibular system encodes the motion of the head relative to the surrounding environment (Angelaki & Cullen, 2008). Two key structures of the vestibular system are the semicircular canals and the otolith organs (which include the

utricle and saccule; Snyder, 1999). There are three semicircular canals which respond to changes in angular acceleration and rotational movements (Snyder, 1999; Angelaki & Cullen, 2008). The otolith organs sense linear acceleration (Angelaki & Cullen, 2008). These organs consist of a matrix of loosely anchored calcium carbonate crystals, which are coupled to vestibular hair cells (Snyder, 1999). These crystals become displaced when there is a change in momentum and/or gravity. The direction of displacement of the crystals is dependent on head direction. If the head is tilted back, the crystals move and bend the cilia of the vestibular hair cells backwards, towards the back of the head (Snyder, 1999). Although gravitational and inertial stimuli produce the same effect on the otolith organs, the vestibular system is able to differentiate between the two (Snyder, 1999). This is because the canals reliably respond to rotational velocity that is equal to, or greater than 0.05Hz. When lower frequencies are experienced, the brain incorrectly interprets them as tilt (Angelaki et al., 2009).

2.1.2. The Vestibular Nuclei

Vestibular information, leaving the labyrinths of each ear, goes to the ipsilateral brainstem vestibular nucleus complex (Smith, 1997). The vestibular nucleus complex consists of four major nuclear groups, which are the medial vestibular nucleus (MVN), descending vestibular nucleus (DVN), lateral vestibular nucleus (LVN) and the superior vestibular nucleus (SVN; Barmack, 2003). The neurons in these nuclear groups receive different amounts of synaptic input from different parts of the labyrinths (Smith, 1997). Neurons in the MVN receive more input from the vestibular fibres that enter the horizontal canal ampulla. In comparison, the LVN neurons receive more input from fibres innervating the otoliths (Smith, 1997).

The vestibular nuclei are mainly involved in the control of balance. This control exists via the influence the nuclei have on the discharge of motor and pre-motor neurons (Barmack, 2003). The firing of the vestibular nuclei neurons is largely dependent on vision and neck movement signals. All of the vestibular nuclei neurons have bidirectional connections with the cerebral and cerebellar cortices (Barmack, 2003). Skeletal muscles and the autonomic nervous system receive inputs from the medial and inferior vestibular nuclei. These vestibulo-sympathetic pathways influence changes in blood flow, heart rate and respiration (Barmack, 2003; Balaban, 2004).

2.2. Main Inputs/Outputs to/From the Vestibular Nuclei

2.2.1. Vestibular Projections to the Cortex

Within the vestibular network, visual and proprioceptive information is integrated with vestibular information and processed via the central vestibular pathways (Angelaki & Cullen, 2008). At the first synapse, interactions between the brain stem, cerebellum and canal/otoliths all take place. The vestibular nuclei send large projections to the prefrontal and/or frontal lobes, the ipsilateral temporoparietal cortex, the anterior part of the supplementary motor area and the contralateral parietal cortex (de Waele et al., 2001). In anaesthetised vestibular patients, the vestibular nerve was stimulated which resulted in an evoked potential with a six millisecond latency from the time of stimulation to the time of activation of the cortical areas (de Waele et al., 2001). This 6ms latency was broken down into a trisynaptic model which showed that it took 2ms for the vestibular nuclei to activate after stimulation of the vestibular nerve. Once the vestibular nuclei were activated it then took 1.5ms for the thalamus to react and 2.5ms for the cortex to show activity after the thalamus did.

Based on this trisynaptic activity, de Waele et al. (2001) concluded that vestibular information was processed simultaneously by the various cortical areas and that a primary vestibular cortex does not exist.

A direct projection between the MVN and the supragenual nucleus plays an important role in the generation of the head direction signal. This signal provides information about the location and the direction of the animals head in its environment (see below; Stackman & Taube, 1997; Biazoli Jr., Goto, Campos, & Canteras, 2006). The supragenual nucleus works as a relay station for vestibular inputs to areas of the cortex involved in the generation of head direction signals, which come from head direction (HD) cells (Taube, 1995).

2.2.2. Areas of the Brain Activated by Vestibular Stimulation

The vestibular system, including the vestibular nuclei, is multimodal, therefore when it is stimulated, cells in other brain areas are also activated (Angelaki & Cullen, 2008). These areas include the parieto-insular vestibular cortex, the visual temporal sylvian area, the superior temporal gyrus, the inferior parietal lobule, the anterior cingulate cortex, and the hippocampus (Dieterich & Brandt, 2008). In particular, within six milliseconds of the vestibular system being stimulated, the thalamus is activated: the nucleus ventralis posterior inferior, the magnocellular division of the medial geniculate body and the intralaminar nuclei (de Waele et al., 2001). Vestibular responses were also recorded in the caudate nucleus and globus pallidus when the evoked potential technique was used (Fukushima. 1997).

Some more caudal areas of the subcortex that are known to process sensory stimuli also respond to vestibular stimuli. These areas include the interstitial nucleus of Cajal, the rostral interstitial nucleus of the medial longitudinal fasciculus, the paramedian pontine reticular formation, the nucleus prepositus hypoglossi, cerebellum

and putamen (part of the basal ganglia; Dieterich & Brandt, 2008; Angelaki et al., 2009). Vestibular stimuli can simultaneously activate other sensory systems, so the noted areas, which respond to vestibular stimulation, could also be responding to other sensory inputs at the same time (Angelaki et al., 2009).

2.2.3. Important Vestibular System Circuits

The vestibular system belongs to a number of pathways that work as a circuit that is involved in the control of balance and the modulation of the anxiety response Balaban (2002). The vestibulo-parabrachial, the coeruleo-vestibular, and the raphe nucleus-vestibular are the main pathways in this circuit.

One pathway linking the vestibular system to areas of the brain involved in the control of balance and anxiety is the vestibulo-parabrachial pathway. The vestibulo-parabrachial pathway appears to relay information about body motion to neural tracts which mediate autonomic and affective responses (Balaban, 2002). The vestibular nuclei have dense, ascending projections to the parabrachial nucleus (PBN) which has regions known to contain cells that are vestibulo-recipient. These regions include the medial, external medial and external lateral sub-nuclei of the caudal PBN. These recipient areas of the PBN may send information, regarding body movement and motion, to pathways known to mediate autonomic and affective responses which also included anxiety. The vestibulo-recipient cells send sparse, but spatially extensive projections back to the vestibular nuclei, which led to the conclusion that communication between the vestibular nuclei and the PBN is bidirectional (Balaban, 2002). This bi-directionality suggests that the discharge of some vestibular nucleus neurons may represent contextual information regarding the level of danger of incoming gravito-inertial information (Balaban, 2002). Descending vestibulo-autonomic projections, to areas such as nucleus ambiguus, rostral ventrolateral

medulla and lateral medullar tegmentum, can all be influenced by parabrachial-vestibulo projections to the inferior vestibular nuclei (IVN) and caudal MVN (Balaban, 2004).

Retrograde tracing labelled the inputs and outputs of the vestibular nuclei and PBN processing unit. The tracer showed that the areas of the PBN that received vestibular nuclei inputs, also shared bidirectional pathways with the amygdala, infralimbic cortex and hypothalamus (Balaban, 2002; Balaban, 2004), and the posterior hypothalamic area (PHA) was connected to the amygdala, and the septal regions. Retrograde tracing also showed that labelled fibres were seen to leave the MVN and project to the mammillo-thalamic tract before bilateral termination in the PHA. Labelled neurons were also found in the periaqueductal gray (PAG), dorsal raphe nucleus (DRN), locus coeruleus (LC) and the nucleus of the solitary tract (Matsuyama, Kayahara, Nomura, & Nakano, 1996). This network is important because the connections the vestibular nuclei (specifically the MVN) share, with the hypothalamus and amygdala, allow them to influence the activity of the limbic system (a processor of emotional stimuli). The connections also influence the processing of convergent vestibular, somatic and visceral information which can mediate avoidance and fear conditioning and anxiety (Matsuyama et al., 1996; Balaban, 2002).

A second pathway linking the vestibular system to areas of the brain involved in balance control and anxiety is the coeruleo-vestibulo pathway. The activity in the LC is linked to changes in vigilance, activation and arousal; and so may be an initiator of the anxiety response and a modulator of vestibular function (Balaban, 2002). The LC sends projections to the vestibular nucleus with multiple collateral projections to the PBN, motor pathways, spinal cord, hypothalamus, hippocampus, neocortex and cerebellum. The caudal pole of the LC and the adjacent nucleus sub-coeruleus are

sources of noradrenergic connections to the vestibular nuclei. The role of this noradrenergic innervation is to coactivate the vestibular nucleus and other structures that are known to be involved with motor activity. The noradrenergic innervation also acts as a mediator of the effects arousal has on the vestibular reflex (Balaban, 2002).

A third pathway, linking the vestibular system to areas of the brain associated with anxiety, is the raphe nucleus-vestibular pathway (Balaban, 2002).

Immunohistochemical reactions for serotonin and serotonin transporters showed that both shared a similar axon plexus in the vestibular nuclei of the rat. Axons appeared to come from the DRN and an area which included the nucleus raphe pallidus (RPa) and the nucleus raphe obscurus (ROb; Balaban, 2002). The neuron fibres coming from the raphe nuclei are believed to be collateralised. Because of this collateralisation, the projections which come from the DRN, ROb and the RPa, and go to the vestibular nuclei, are thought to be both serotonergic and non-serotonergic. The role of these serotonergic and non-serotonergic neurons is to co-modulate the vestibular pathways. Serotonergic neurons, coming from the DRN, send collateralised inputs to the vestibular nuclei and the amygdala. This shared input is important because the vestibular nucleus has 5HT_{2A} neurons in all four nuclei, while both the amygdala and the cortical targets of the PBN have extensive 5HT_{2A} receptor expression (Balaban, 2002). The importance of the role of the raphe nucleus-vestibular pathway is two-fold. The serotonergic DRN and ROb efferents have a coordinated effect on vestibular circuits. Furthermore, this coordinated effect has been hypothesised as being the mediator of the activity produced by the structures implicated in the anxiety response, specifically the amygdala, PBN, infralimbic/insular cortex and frontal cortex (Balaban, 2002). An important implication of this serotonergic activity is the effect it has on the amygdala. When

there is an increase in serotonin release in the raphe nucleus-vestibular pathway, tolerance to aversion is increased which causes a decrease in stress related responses of the amygdala. Also, the increased serotonin leads to a decrease in conditioned anxiety responses but facilitates conditioned fear responses. The activity of the raphe nucleus-vestibular pathway also determines the activity that occurs in the vestibulo-parabrachial pathway (Balaban, 2002). Balaban (2002) found that the projections from the DRN that went to the vestibulo-parabrachial pathway influenced the way in which information was gathered in regards to response making, and, these projections influenced the sensitivity calibration of affective responses to aversive motion stimuli.

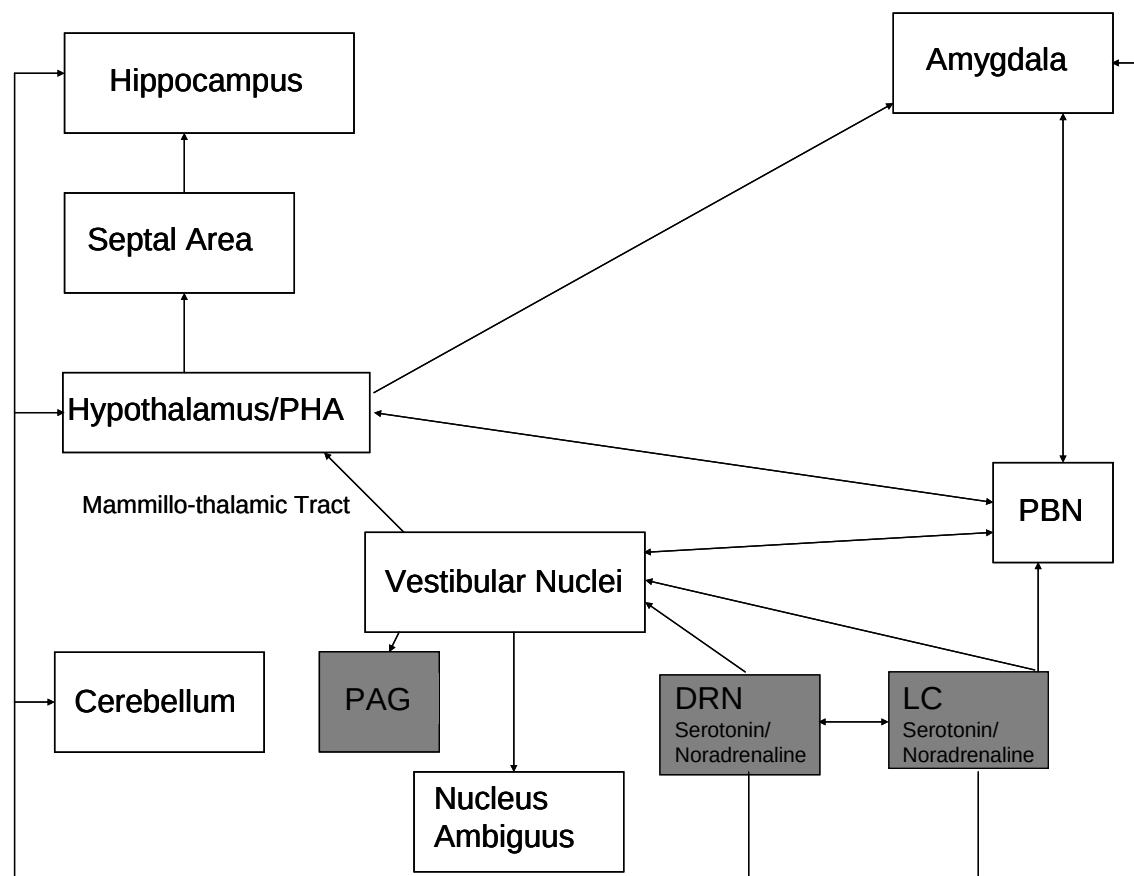


Figure 2.1. The vestibular network made up of the vestibulo-parabrachial (PBN), coeruleo-vestibular (LC) and raphe-vestibular pathways (DRN). Shaded boxes represent areas where neurons coming from the medial vestibular nucleus (MVN) were found using retrograde tracing. Information used in the diagram is from Matsuyama et al. (1996) and Balaban (2002, 2004).

2.2.4. The Head Direction Pathway

HD cells fire as a function of the animal's head direction in the horizontal plane (Biazoli Jr. et al., 2006). This activation is independent of the animal's behaviour or location in the surrounding environment (Biazoli Jr. et al., 2006). The cells are called HD cells because they only fire when the animal points its head in a direction that the cell specifically responds to (Taube, 1995). This direction has been described as the preferred firing direction of the cell. The integrity of the HD pathway is necessary for the animal to successfully navigate itself through its environment (Biazoli Jr. et al., 2006). The HD signal originates in the supragenual nucleus, an area of the subcortex which sends a dense projection to the dorsal tegmental nucleus (DTN; see Figure 2.2). Head angular velocity cells are located within the DTN. These velocity cells are involved in the generation of the HD signal (Biazoli Jr. et al., 2006). From the DTN, vestibular information is sent to the lateral mammillary nucleus. The HD signal ends up being integrated with spatial information in the hippocampus. This HD signal is relayed to the hippocampus via the anterior thalamic nuclei (ATN; Stackman & Taube, 1997). The projections that pass vestibular information from the vestibular nuclei to the ATN are a main pathway for the HD signal. If the pathway or areas along it are compromised, the HD signal can not get to the hippocampus via the ATN (Stackman & Taube, 1997). Using bilateral lesions to the lateral mammillary nucleus, Blair, Cho, and Sharp (1998) demonstrated that HD cells in the ATN lost their directional firing properties without input from the lateral mammillary nucleus, an area that is involved in the relay of vestibular information to the ATN.

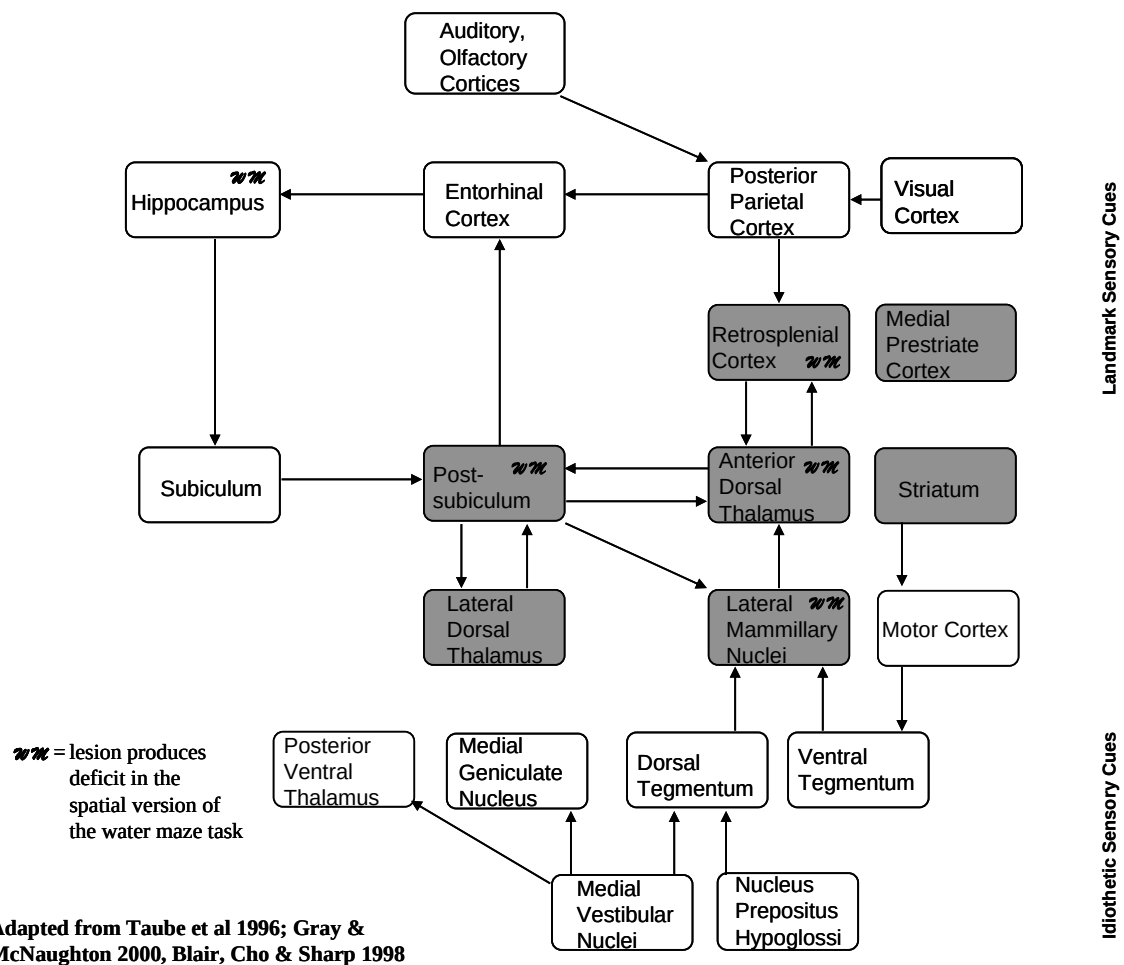


Figure 2.2. The main components of the head direction circuit. The shaded boxes are areas of the brain where head direction cells have been found. Information used in the diagram is from Taube et al. (1996), Gray and McNaughton (2000), and Blair et al. (1998).

2.2.5. Summary

The vestibular system provides inertial and head direction information that is sent to the hippocampus (Angelaki et al., 2009). This information is also processed by areas of the brain involved in the anxiety response. This relationship is evident through the serotonergic inputs to the vestibular system (Balaban, 2002). The head direction circuit has been hypothesised as the main way in which vestibular information reaches the hippocampus to be integrated with other spatial and emotional

information. When any component of the head direction pathway is compromised, including the vestibular system, place cells in the hippocampus demonstrate a decrease in firing specificity and begin to fire at random. This leads to deficits in spatial memory (Taube, 1995; Stackman & Taube, 1997).

3. Vestibular Lesion Effects

Vestibular lesions in animals are associated with deficient spatial memory and reduced anxiety-related behaviour. The effects of the damage caused by vestibular lesions are more severe when the lesions are bilateral rather than unilateral (Smith et al., 2005b). Navigation relies on theta rhythm in the hippocampus and following vestibular damage there is a reduction in theta power and frequency and a subsequent deficit in navigation (Stackman & Herbert, 2002). Structural and biochemical changes in the hippocampus have been implicated in this deficit (Goddard, Zheng, Darlington, & Smith, 2008).

In vestibular deficient humans, the hippocampus also suffers structural deficits which result in deficient spatial memory (Brandt et al., 2005). In contrast to the animal data, there appears to be an increase in agoraphobic tendencies amongst vestibular patients (Furman & Jacob, 1997). But this anxiety disorder and the level of distress associated with it is reported to be independent of the severity of the vestibular disorder. The research presented suggests that in humans, vestibular dysfunction may or may not lead to the development of an anxiety disorder, and that in groups of patients with anxiety disorders, abnormal vestibular functioning is a hallmark (Kalueff, et al., 2008).

3.1. Vestibular Lesion Effects in Animals

3.1.1. Immediate Effects of Vestibular Lesion Surgery

The vestibular system can be lesioned bilaterally (bilateral vestibular deafferentation, BVD) or unilaterally (unilateral vestibular deafferentation, UVD). These lesions result in immediate impairments. One immediate effect of vestibular

lesions in mammals is hyperactivity. Rats that received BVD displayed hyperactive behaviour with excessive movement, accompanied by alternating circling behaviour, gait ataxia and head dorsiflexion (Goddard et al., 2008).

Loss of Balance/Reflexes

Vestibular lesions also result in the immediate loss of two important reflexes- the vestibulo-ocular (VOR) and vestibulo-spinal reflexes (VSR). This is more severe in BVD animals compared to UVD (Smith et al., 2005b). This abolition of the two vestibular reflexes is known to cause severe oscillopsia (blurred vision) and ataxia. Damage to the vestibulo-ocular pathways leads to deficiencies associated with optokinetic reflexes, eye-head coordination and smooth pursuit eye movements. The damage done to the VSR is known to cause loss of balance and abnormal responses to sway and pitch stimuli (Goddard et al., 2008).

Other reflexes damaged by vestibular lesions include the air-righting reflex, the landing reflex and the contact- righting reflex. To demonstrate the air-righting reflex, subjects are held supine and dropped 40cm onto a soft surface. Animals with intact vestibular apparatus would right themselves immediately, but vestibular lesioned rats do not (Russell, Horii, Smith, Darlington, & Smith, 2003). To demonstrate the landing reflex, rats are lifted by the base of their tail, away from a horizontal surface, they then normally extend their forelimbs towards the horizontal surface but vestibular lesioned rats curl their bodies ventrally around towards their tail (Stackman & Herbert, 2002). The contact-righting reflex is demonstrated by placing a rat supine on a horizontal surface. While the rat is placed there, a clear plexiglass sheet is placed horizontally, in contact with the animal's feet. A non-vestibular rat rights itself immediately upon contact with the plexiglass sheet, vestibular rats do not (Russell et al., 2003).

Compensation

After vestibular damage, a process of adaptive plasticity occurs. This is commonly known as vestibular compensation. Following UVD, this compensation has been associated with the recovery of resting activity in the ipsilateral vestibular nucleus complex (VNC). To date, the actual mechanisms by which compensation occurs have not been found but may be partially reliant on the release of corticosterone during the early stages of recovery following UVD (Lindsay et al., 2005).

Ocular, motor and postural symptoms of UVD gradually subside within the first week following the lesion (Zheng, King, Darlington, & Smith, 2003). The static symptoms associated with vestibular lesions typically decrease in severity within three days of the lesion (Russell et al., 2003). The acute, reflexive symptoms take longer to compensate, with deficiencies still present up to two weeks after the lesion (Zheng et al., 2003). At the two week period, most vestibular-lesioned animals can walk through a maze in a similar way to non-vestibular animals. Although vestibular compensation reduces the severity of some symptoms, the process is never complete. Most animals will continue to exhibit deficiencies in both the VOR and VSR during head movements (Smith et al., 2005b).

3.1.2. Delayed Effects of Vestibular Lesions on Emotion

Behaviour

Vestibular-lesioned rats have been observed displaying behaviours in elevated T and plus mazes indicative of reduced anxiety relative to non-vestibular rats. This changes across the course of the compensation process. At three weeks post-operation, BVD rats show open arm entries that are not significantly different to those of the sham rats; however at three months post-operation, vestibular rats demonstrated

a large increase in the number of open arm entries when compared with shams (Zheng et al., 2008) suggesting reduced anxiety at three and five months post-operation when compared to controls. An experiment using a mutant strain of rat ($ci2^{-/-}$) with vestibular deficits demonstrated the same effect. The mutant rats spent more time investigating the open arm in an elevated plus maze when compared to the non-mutant controls (Lindemann, Gernert, Bennay, Koch, & Loscher, 2007, as cited in Zheng et al., 2008). The rats were exhibiting a lack of learned inhibitory avoidance caused by a vestibular lesion-induced deficit in the appraisal of potentially aversive or threatening stimuli. This deficit in appraisal led to interference in the expression of associative fear conditioning. It was also noted that, compared to sham rats, vestibular lesioned rats engaged in less social interactions with other rats and spent less time sniffing other rats. This is important because social interaction in rats is a measure of anxiety (Zheng et al., 2008).

When animals spontaneously alternate in an elevated T or plus maze, it is usually at levels greater than chance, which illustrates their willingness to explore the novel environment. Spontaneous alternation is dependent on low levels of anxiety. When the animal is very anxious, levels of alternation greatly decrease. Lesions of the vestibular system lead to a deficiency in alternation (Lalonde, 2002).

In the openfield test, vestibular lesioned rats again demonstrated behaviour that was indicative of reduced anxiety compared to sham rats (Goddard et al., 2008). When given a five minute trial in the openfield, BVD rats were observed travelling significantly farther than their sham counterparts (Goddard et al., 2008). Vestibular rats spent more time engaged in wall supported rearing and maintained the rearing position for longer periods of time compared to the shams. The vestibular rats also

spent more time in the middle and inner zones of the openfield showing a decreased fear of the open similar to diazepam treated rats (Goddard et al., 2008).

3.1.3. Possible Causes for Emotional Changes

Serotonin, Dopamine and Noradrenaline

Changes in biogenic amine levels following vestibular lesions play a role in the changes in attention and behaviours observed in vestibular lesioned animals (Goddard et al., 2008). In UVD rats, an increase in noradrenaline was found in the contralateral CA2 area of the hippocampus when compared to shams (Zheng, Smith, & Darlington, 1999). Vestibular damage is known to interfere with the transmission of dopamine and serotonin (Goddard et al., 2008). In BVD rats, the levels of the serotonin transporter (SERT), the dopamine transporter (DAT), tyrosine hydroxylase (TH), tryptophan hydroxylase (TryH) and dopamine β -hydroxylase (DH) in the medial temporal and frontal lobes were investigated (Goddard et al., 2008). SERT and DAT are high affinity transporter proteins whose role is to remove serotonin and dopamine from the synaptic cleft. TH and TryH are rate limiting enzymes which help with the metabolism of dopamine, noradrenaline (by TH) and serotonin (by TryH). The role of DH is to regulate the conversion of dopamine into noradrenaline. SERT expression was decreased in the frontal lobe and CA1, while TryH activity was decreased in the entorhinal cortex. A TH decrease was only observed in the frontal lobe (Goddard et al., 2008).

3.1.4. Effects of Vestibular Dysfunction on Spatial Memory

Spatial Memory Tasks

The cognitive deficits associated with vestibular damage appear to be long lasting, even permanent (Zheng et al., 2003). When rats receive vestibular lesions

they (1) lose their ability to return to a goal location after passive transport, (2) have reduced rates of spontaneous alternation, (3) have difficulty learning a radial arm maze task, and (4) have difficulty navigating without a visual landmark (Stackman & Herbert, 2002; Stackman, Clark, & Taube, 2002).

To test vestibular lesion effects on idiothetic navigation, sham and vestibular lesioned rats were trained to locate a water reward in one corner of a square enclosure (Stackman & Herbert, 2002). The goal location was fixed in relation to a cue card which was on one wall of the enclosure. The vestibular rats were able to learn the spatial task however their performance was critically dependent on the cue card being present. Two types of responding were considered correct during the trials in which the cue card was rotated, a cue response or a place response. When the cue card was rotated, the accuracy scores of both groups of rats were not affected but response type differed. Vestibular-lesioned rats made more cue responses whereas sham rats made more place responses. The predominance of cue responding in the vestibular rat group was indicative of a reliance on the cue card for navigation. Stackman and Herbert (2002) concluded that idiothetic navigation in rats is disrupted after a vestibular lesion.

Foraging behaviour is also deficient in BVD rats. In light and dark conditions, BVD and sham rats completed a foraging task (Wallace et al., 2002; as cited by Smith et al., 2005b). BVD rats found it very difficult to return home with food when the task was completed in darkness. The shams were able to successfully complete the task in both light and dark conditions. A correlation between foraging and the vestibular reflex deficit was reported - the more severe the reflex impairment, the worse the performance was on the foraging task. Vestibular information appeared to be necessary for place learning in rats (Wallace et al., 2002; as cited by Smith et al.,

2005b). To conclude, the discussed experiments have demonstrated a clear relationship between vestibular input and spatial performance. Stackman and Herbert (2002) have theorised that the vestibular signal is important for updating internal representations that allow for successful spatial behaviour. The vestibular lesion stops this updating of internal representations. The subsequent impairment means that vestibular lesioned rats are critically dependent on visual landmarks when navigating their environment (Stackman & Herbert, 2002).

Navigation

For an animal to successfully navigate its environment, representations involving the spatial relationship of the animal with its environment must be encoded and maintained regularly (Stackman & Taube, 1997). For this to occur successfully, the hippocampus must receive vestibular input. This input allows the hippocampus to fire in location-specific patterns (Stackman et al., 2002). Different types of spatial information influence spatial memory, in particular landmarks and internal cues. When familiar landmarks are unavailable, the animal uses any cues available. This change in cue availability usually leads to the animal relying on idiothetic navigation (Stackman & Herbert, 2002). Idiothetic navigation requires a continual monitoring of self motion signals. These self motion signals are then integrated and update the animal's internal representation of its environment. When the hippocampus does not receive vestibular input to update these representations, a decrease in successful navigation behaviour is seen (Stackman & Herbert, 2002).

HD Cells/Place Cells

The hippocampus receives angular velocity signals from the vestibular system. Stimulation of this influences the activity of hippocampal place cells and ATN HD cells (Stackman & Taube, 1997). A place cell is one that fires maximally when the animal is in or passing through a particular location, its “place field” (Stackman et al., 2002; Gray & McNaughton, 2000). Place cells and HD cells are interactive components and key contributors in a circuit which guides navigation. Place cell and HD cells still fire in the absence of external cues and will still fire even when the animal is blind or deaf.

The vestibular system provides important self-motion cues to both the HD cells and the place cells, which in turn provide spatial information to the hippocampus (Stackman et al., 2002). Place cell and HD cell firing are both influenced by landmark and idiothetic cues (Stackman & Herbert, 2002). Landmarks which are familiar to the animal exert stimulus control over both sets of cells. This can be illustrated when a familiar landmark is rotated. Place field location and preferred firing direction both shift an equal amount, which matches the shift of the landmark. When there is no landmark, the animal relies on idiothetic cues that update HD and place cells. When the vestibular system is lesioned, the spatial firing of the place cells and HD cells becomes non-specific, even if there is a familiar landmark present, suggesting that vestibular input may exert control over the spatial firing properties of the place and HD cells (Stackman & Herbert, 2002).

Spatial firing of hippocampal place neurons was measured before and after reversible inactivation (of the vestibular system) by tetrodotoxin injected into the inner ear. This inhibited the location-specific firing of the hippocampal place cells (Stackman et al., 2002). Inactivation of the vestibular system with sodium arsenite

also reduces HD cell activity in the anterior thalamic nucleus (ATN; Stackman & Taube, 1997) even when the animal is in a familiar environment with a landmark that has previously influenced the firing of the HD cells.

3.1.5. Possible Causes for the Spatial Memory Deficit

Changes in Firing Patterns and Biochemistry in the Hippocampus

Vestibular damage in animals causes changes in cell firing patterns within the hippocampus. Significant reductions in CA1 population spike amplitudes were observed in hippocampal slices from UVD rats (Zheng et al., 2003). This observation was specific to the slices from UVD rats as the slices from animals who received sham surgeries showed no difference in their population spike amplitudes. Changes in neuronal activity were also found in the CA2 region of the hippocampus. Field responses in CA1 were also different in UVD rats after stimulation of the Schaffer collateral commissural afferent pathway (Zheng et al., 2003).

Using western blotting techniques, changes in expression of the NR1 and NR2A subunits of the NMDA receptor in the hippocampus of UVD rats were measured (Liu, Zheng, King, Darlington, & Smith, 2003). The NR1 subunit is essential for NMDA receptor function, while the NR2A subunit potentiates NMDA receptor ion channels. Two weeks after UVD, there was a significant decrease in the expression of the NR1 subunit in the ipsilateral CA2/3 region of the UVD animals, compared to the sham animals. At two weeks post operation, the expression of NR2A was also reduced in the ipsilateral CA2/3 region of UVD rats (Liu et al., 2003).

UVD also causes a long lasting decrease in neuronal nitric oxide synthase (nNOS) expression in the dentate gyrus two weeks post operation (Liu et al., 2003). This did not occur at earlier time points, in other areas of the hippocampus or in the

sham animals. This decrease in nNOS expression may be indicative of a long lasting neural correlate of the damage to the hippocampus caused by UVD (Liu et al., 2003).

EEG/Theta

In BVD rats, even when movement is controlled for, theta is less rhythmic compared to theta recorded in shams (Russell, Horii, Smith, Darlington, & Bilkey, 2006). This is particularly noticed in the 6-9Hz and 10-12Hz ranges. Since movement had been controlled for, the change in theta rhythm is unlikely to be due to hyperkinetic behaviour. Further when rats were deprived of normal vestibular cues, and only given optic flow signals, theta rhythm in the hippocampus went through a large reduction in power. Vestibular signals seemed necessary for normal hippocampal function and so essential for successful navigation (Russell et al., 2006; Stackman & Herbert, 2002; Goddard et al., 2008).

3.1.6. Summary

A reduction in the rhythmicity and power of theta in the hippocampus was linked to the spatial and cognitive deficits associated with vestibular lesions (Russell et al., 2006). The spatial deficits observed in BVD rats were also suggested to be the result of a compromised head direction pathway; with the required inertial, movement and head direction information, some of which is provided by the vestibular system, failing to reach the hippocampus (Stackman & Taube, 1997). BVD rats exhibited reduced anxiety in T-maze and openfield tests, which was indicative of a deficit in the processing of aversive stimuli- a job that is done by the hippocampus (Zheng et al., 2008). This deficit could be due to the interference in the transmission of the biogenic amines which are necessary for affective responses (Goddard et al., 2008). The general conclusion was that bilateral vestibular lesions interfered with the

transmission of important spatial and affective information that the hippocampus needs for spatial memory and anxiety related responses.

3.3. Vestibular Lesion Effects in Humans

3.3.1. Types of Vestibular Disorders in Humans and Their

Symptoms

Common Causes of Damage

In humans, there are five commonly occurring vestibular disorders. First, about 20-30% of all cases of bilateral vestibular loss are caused by an auto-immunological inner ear disorder (Strupp & Brandt, 2006). Auto-antibodies attack the inner ear structures, which leads to bilateral loss of vestibular function. Second, unilateral acute or sub-acute vestibular failure is generally caused by acoustic neurinomas. These are sporadic tumours that begin in the Schwann cells that are located in the vestibular nerve (Hufner et al., 2007). Third, there is perilymph fistula syndrome. This syndrome is the result of a head trauma, chronic ear infection or acceleration forces that have torn the round window membrane in the inner ear resulting in unilateral vestibular damage which commonly manifests as benign paroxysmal positional vertigo (Smith et al., 2005a). Fourth there is paroxysmal, inadequate stimulation or inhibition of the peripheral vestibular system. This disorder is characterised by vertigo and oscillopsia (Strupp & Brandt, 2006). The fifth vestibular disorder is Ménière's disease which occurs when there is either too much endolymph produced or not enough absorbed. This causes the rupturing of the membrane that separates the endolymph from the perilymph space (Strupp & Brandt, 2006).

Symptoms of Damage

Bilateral loss of vestibular function is characterised by oscillopsia. This is a condition in which objects in the visual surround that are known to be stationary appear to be in motion. It is a result of the impaired vestibular system losing the vestibulo-ocular reflex and so being unable to generate compensatory eye movements (Ramos, 2006). This results in retinal slippage and so blurred vision during head movements, and accompanying instability of posture and gait (Strupp & Brandt, 2006).

Unilateral acute or sub-acute vestibular failure is characterised by a rotary vertigo, nausea, oscillopsia and unidirectional spontaneous nystagmus (spontaneous upward ocular drift due to the physiological inhibitory input, from the flocculus to the vestibular nuclei, being inhibited; Strupp & Brandt, 2006; Godemann, Linden, Neu, Heipp, & Dorr, 2004). The vertigo and nystagmus associated with the acute, unilateral loss of vestibular function usually subsides within a few weeks (Godemann et al., 2004).

Ménière's disease symptoms include chronic episodes of vertigo and tinnitus. If left untreated it can lead to hearing loss in the affected ear. In advanced cases of Ménière's disease a vestibular neurectomy is performed to alleviate the patient's vertigo episodes and to preserve hearing in the affected ear (Strupp & Brandt, 2006).

3.3.2. Vestibular Compensation in Humans

Following acute vestibular damage in humans, vestibular compensation usually occurs. Central compensatory mechanisms allow for a recovery of balance in approximately 70% of patients (Teggi, Caldirola, Fabiano, Recanati, & Bussi, 2009). Compensation in acute vestibular loss patients commonly results in the overweighting of visual and proprioceptive information used for balance control (Teggi et al., 2009).

However, most of the oculo-motor and postural symptoms associated with acute vestibular damage do not undergo compensation, and if they do, it is very often incomplete (Smith et al., 2005a). This lack of compensation or incomplete compensation is especially common with the vestibulo-ocular reflexes which never respond normally to high acceleration stimuli after vestibular damage (Smith et al., 2005a).

3.3.3. Changes Involving the Hippocampus after Vestibular Damage

Structural

Patients with bilateral vestibular trauma often have atrophy of the hippocampus (Brandt et al., 2005; Smith et al., 2005a). Using magnetic resonance imaging (MRI), a 17% decrease in hippocampal volume in bilateral vestibular patients has been reported compared to healthy controls (Brandt et al., 2005). Interestingly, the hippocampus was the only part of the brain to suffer this atrophy in vestibular patients and the degree of atrophy correlated with the size of impairment in their spatial memory (Smith et al., 2005a). In unilateral vestibular patients, the hippocampus did not suffer from atrophy. This was due to the one intact vestibular labyrinth providing sufficient information to maintain the overall function and so volume of the hippocampus (Hufner et al., 2007).

Spatial Learning and Memory Deficits

Following vestibular damage, patients have difficulty completing tasks involving path integration. Path integration tasks require patients to walk a specific path through their environment in various conditions, such as with their eyes open or shut (Smith et al., 2005a). This difficulty was especially noticed in patients with Ménière's disease, benign paroxysmal positional vertigo and chronic vestibulopathy.

Unilateral vestibular patients, who suffered from Ménière's disease, made significantly more turn errors during a path integration task (in their first post-operative week) when compared to controls (Smith et al., 2005a). In another study, bilateral vestibular patients could successfully complete goal directed linear locomotion, however when they had to negotiate corners along a triangular path, their accuracy decreased markedly (Brandt et al., 2005). These deficits in path integration are not due to motor dysfunction or vertigo, but are associated with deficits in learning and memory, specifically, spatial learning and memory (Smith et al., 2005a).

This idea that vestibular damage in humans leads to a deficit in spatial learning and memory is supported by maze performance and performance on other memory tests. During a hidden platform test, bilateral vestibular patients took longer to find the platform when compared to the control group (Brandt et al., 2005). Bilateral vestibular patients had matched memory performance with controls on standardised non-spatial tests, but exhibited a large deficit when they completed a virtual maze task. The deficits observed in bilateral vestibular patients were not seen in unilateral vestibular patients (Brandt et al., 2005).

Perceptual memory in patients with perilymph fistula syndrome was tested. The majority of the patients reported general memory loss as a result of their syndrome (Smith et al., 2005a). Although all participants had normal levels of intellectual function, most patients had an impaired performance on the digit symbol, block design (hippocampus sensitive) and picture arrangement tests. This diminished performance was also extended to auditory recall and paired association learning tests (hippocampus sensitive; Smith et al., 2005a).

3.3.4. Negative Physical Sensations Associated with Vestibular Dysfunction

Vertigo

Terms that are used to define vertigo, that is the result of behavioural, physical or psychiatric symptoms, include psychogenic vertigo (which has a primary psychiatric causality), visual vertigo and phobic postural vertigo (PPV; Staab, Ruckenstein, Solomon, & Shepard, 2002; Holmberg, Karlberg, Harlacher, & Magnusson, 2005). Psychogenic vertigo is often the result of panic disorder or hyperventilation, which causes the sensation of vertigo (Holmberg et al., 2005). Visual vertigo however arises from sensitivity to conflicting visual stimuli. This type of vertigo does not usually have co-morbid signs of anxiety. The symptoms associated with PPV include (1) dizziness and a disturbance of balance while standing or walking, despite having normal results on a clinical balance test, (2) fluctuating unsteadiness for seconds or minutes, or brief perceptions of illusory body perturbations, (3) a perceptual stimulus or a social situation can provoke an episode, with a tendency towards rapid conditioning, generalisation and avoidance behaviour, (4) anxiety and vegetative symptoms during or after vertigo, (5) obsessive compulsive type personality, labile affect or mild depression, and (6) onset frequently after a period of emotional stress, a serious illness or a vestibular disorder (Holmberg et al., 2005). Based on anxiety questionnaire responses, patients with PPV experience more anxiety and handicap than an unselected group of people suffering from balance disorders. In most vestibular patients, the perceived symptoms of dizziness and vertigo last longer than the actual acute phase of the vestibular trauma (Best, Tschann, Eckhardt-Henn, & Dieterich, 2009). This long lasting perception of dizziness and

vertigo then increases the likelihood of the patient suffering from disease specific handicap and reduced psychosocial functioning.

Dizziness

Dizziness is a symptom associated with hyperventilation and vestibular dysfunction. It has been estimated that 20-50% of all patients who complain of dizziness have psychiatric symptoms that can influence the course of the illness (Ramos, 2006). The symptom of dizziness has been divided into a number of categories, each defining its association with physical or psychiatric starting points. Dizziness has been categorised as (1) light headedness, feeling like you are about to faint, associated with hypotension, hypoglycaemia or anxiety, (2) the sensation of being off balance, this feeling is frequently associated with non-vestibular factors like abnormal somatosensory information or lower limb weakness, (3) vertigo (illusion of movement), usually episodic and linked to abnormalities of vestibular function, (4) oscillopsia (Ramos, 2006). Dizziness can be associated with a primary psychiatric disorder, such as generalised anxiety disorder or panic disorder. Dizziness can also be a prominent symptom that is not accompanied by a medical condition. An example of such dizziness is psychogenic vertigo. The third category encapsulates dizziness as being a symptom that occurs co-morbidly with something else, e.g. anxiety disorder and Ménière's disease (Ramos, 2006). In a group of patients suffering from chronic subjective dizziness, 17 out of the 20 had high levels of anxiety during their acute vestibular crises. Patients developed hypersensitivity to motion cues and exhibited a preoccupation with their dizziness even after they had recovered from the acute vestibular crises (Staab, 2006).

3.3.5. Anxiety Disorders Associated with Vestibular Dysfunction

Panic without Agoraphobia

Anxiety is said to involve a complex psychopathology that includes the following disorders: generalised anxiety, panic, phobias, post-traumatic stress disorder and obsessive compulsive disorder. Each one has been linked to vestibular or balance dysfunctions (Kaluëff et al., 2008). It was estimated that 30% of patients who suffered from vestibular disorders also experienced persistent panic and agoraphobic symptoms or generalised anxiety (Redfern, Furman, & Jacob, 2007). In a group of vestibular patients 14.9% suffered from panic disorder, which far exceeded the 12 month prevalence for the disorder when compared against the general population, which is approximately 2% (Stein, Asmundson, Ireland, & Walker, 1994). In another study, 75% of the patients suffering from panic disorder without agoraphobia exhibited a higher rate of vestibular abnormalities than healthy controls (Tecer, Tükel, Eradmer, & Sunay, 2004).

Panic with Agoraphobia

There is an increasing frequency post-lesion of agoraphobic tendencies in vestibular patients with situational and environmental fears including street neurosis, supermarket syndrome, space phobia and agoraphobia; with the most common fears being heights and boats. These situation-specific symptoms have not been observed in other neuro-otological conditions suggesting that the observed situational symptoms are specifically associated with vestibular damage (Furman & Jacob, 1997).

When vestibular and audiological tests are given to patients with anxiety or depressive disorders, including panic, abnormal vestibular functioning is a hallmark of patients with agoraphobia as opposed to the other disorders (Jacob, Furman,

Durrant, & Turner, 1996; Tecer et al., 2004; Perna et al., 2001; Furman & Jacob, 1997). The severity of the balance deficit correlates with the degree of agoraphobic avoidance and this could not be due to high anxiety levels during the tests as reported anxiety levels did not differ between the panic and the agoraphobia groups (Jacob et al., 1996).

Generalised Anxiety

A questionnaire study demonstrated that acute vestibular dysfunction patients experienced more anxiety, depression and subjective disability when compared to patients suffering from more acute, non-vestibular, neurological problems (Teggi et al., 2009). Adding to this, anxiety and depressive disorders are known to compound the feeling of dizziness (Teggi et al., 2009), with balance control being negatively correlated with state and trait anxiety (Kalueff et al., 2008). Vestibular patients have reported higher levels of psychological distress which is supported by their anxiety affect scores. Conversely, a fear of becoming dizzy and the total UCLA-DQ score were suggested as being accurate indicators of the possibility that vestibular subjective disability was conditioned by emotional distress (Monzani, Casolari, Guidetti, & Rigatelli, 2001). Another group of vestibular patients were tested posturographically one year after suffering from neuritis vestibularis (acute vestibular failure; Godemann et al., 2005). There was no apparent link between vertigo, anxiety and vestibular function. The majority of the patients demonstrated normal results in the posturography test (Godemann et al., 2005).

Space and Motion Discomfort

Space and motion discomfort (SMD) is defined by criteria in the DSM-IV, which include discomfort such as dizziness or imbalance or anxiety. This discomfort and anxiety is the result of situations that are defined by particular spatial or motion

characteristics. This discomfort then leads to avoidance behaviour so the patients do not experience the abnormal spatial and motion related sensations associated with SMD (Furman & Jacob, 1997; Holmberg et al., 2005). People suffering from vestibular disorders are highly prone to developing SMD because they are largely reliant on information from non-vestibular channels. This reliance means the patient becomes increasingly sensitive to misleading information from those non-vestibular channels (Redfern et al., 2007). Three sensory channels influence balance (vision, proprioception and vestibular). When there is sensory conflict, the postural control system usually fixes the integration process by up-weighting the input from the necessary sensory channel. Or, the postural control system down-weights the incorrect input from whichever sensory channel (Redfern et al., 2007). This increased sensitivity to conflicting stimuli increases the likelihood of vestibular patients with SMD developing avoidance behaviours in order to reduce their vestibular symptoms (Meli, Zimatore, Badaracco, De Angelis, & Tufarelli, 2007). In a group of panic-with-agoraphobia patients, a large number also suffered from SMD (Furman & Jacob, 1997). A questionnaire which measured SMD was a successful predictor of vestibular dysfunction in the agoraphobic patients.

3.3.6. Research Reporting No Link between Anxiety Disorders and Vestibular Dysfunction

A cross sectional study on vestibular patients one year after the onset of their disorder suggested that the level of emotional distress experienced did not influence the dizziness they experienced (Best et al., 2009). Levels of distress were significantly higher in patients who had suffered or were suffering from vestibular migraine, Ménière's disease, or primary psychiatric dizziness, but the distress was independent of the amount of the vestibular deficit. Interestingly, the patients who

had a known history of psychiatric disorders did experience increased levels of psychological strain and emotional distress. However, this increase in strain and distress was still independent of their vestibular diagnosis after the onset of the vestibular vertigo syndrome. Similarly, patients with chronic vestibular deficits reported instability, unsteadiness and fear of falling, but, they did not experience panic attacks or depressive symptoms, even when some patients exhibited an emotional influence on their perception of their symptoms (Meli et al., 2007). Kalueff et al. (2008) suggested that anxiety could lead to balance problems but balance problems did not lead to the development of an anxiety disorder.

3.3.7. Cognitive Behavioural Therapy and Vestibular Training for Negatively Perceived Symptoms Associated with Vestibular Dysfunction

Appraisal

Within a large proportion of patients who present with vestibular related symptoms like vertigo or dizziness, no definite pathology can be found and the patients also experience anxiety related symptoms (Holmberg et al., 2005). The handicap resulting from dizziness can be explained by the negative thoughts or cognitions related to the lack of control experienced during an attack of vertigo or other vestibular related events, rather than a neuro-otologic variable. These negative cognitions could also be related to somatic anxiety and the degree of autonomic arousal (Holmberg et al., 2005). The experience of vertigo has been described as being highly perceived due to it acquiring a threatening meaning over time (Godemann et al., 2005). This threat comes from the patient knowing that each time they have an attack of vertigo they will most likely experience an acute phase in

hospital and a temporary loss of control and independence. The physical symptoms experienced by people suffering from vestibular dysfunction may go on to act as unconditioned stimuli in an interoceptive conditioning paradigm (Meli et al., 2007).

Vestibular Training Cognitive Behavioural Therapy

Vestibular training has been found to reduce some of the symptoms associated with anxiety disorders. Likewise, cognitive behavioural therapy targeted at anxiety is known to improve performance on balance and postural tests (Kalueff et al., 2008). Vestibular patients suffering from dizziness were given rehabilitative therapy to treat their vestibular symptoms (Teggi et al., 2009). The role of anxiety disorders in the occurrence of balance compensation was examined. After the rehabilitation period, the rehabilitation group and the control group (no therapy) showed no difference on their posturography test but did show improvements in their total score on the dizziness handicap inventory. This improvement was also shown in the subscale scores of the dizziness handicap inventory in both groups. The rehabilitation group was the only group who showed improvements on the visual analogue for anxiety scale and dynamic index. The results from both groups indicate an increased dependence on visual information in vestibular patients. This dependence did decrease after rehabilitative therapy which suggests a critical condition of the balance system when it loses input from one of the three cues it relies on (Teggi et al., 2009). In another study, patients demonstrated an objective recovery of balance function two to three weeks after rehabilitation therapy (Meli et al., 2007). The patients reported a recovery of the subjective feeling of quality of life and a recovery of the handicap and disabilities associated with vestibular dysfunction.

3.3.8. Summary

A lack of vestibular input to the hippocampus in bilateral vestibular patients leads to atrophy of the hippocampus and a deficient spatial memory (Brandt et al., 2005; Smith et al., 2005a). This atrophy is not seen in unilateral vestibular patients but the deficient spatial memory and emotional deficits are still present (Brandt et al., 2005). Vestibular deficient patients have an increased reliance on other sensory inputs which increases their vulnerability to conflicting sensory inputs. This sensitivity to conflicting sensory inputs usually leads to vestibular patients developing SMD (Furman & Jacob, 1997). Vestibular patients also have a high rate of agoraphobia when compared to the general population (Jacob et al., 1996). This was suggested to be the result of patients trying to avoid experiencing the sensations of vertigo and oscillopsia that are hallmark symptoms of SMD and vestibular dysfunction (Meli et al., 2007). The general conclusion was that patients with vestibular damage were not likely to develop anxiety disorders, rather they were simply avoiding situations that would trigger their symptoms (Kalueff et al., 2008). This was further supported by the finding that SSRIs did not restore vestibular function in vestibular damaged patients, but they did reduce the level of anxiety experienced by highly anxious vestibular patients (Horii et al., 2007; Horii et al., 2004, as cited in Ramos, 2006; Simon et al., 2005).

4. The Hippocampus

The hippocampus is part of the limbic and behavioural inhibition systems. When the hippocampus is damaged, spatial memory and the processing of emotional stimuli are impaired (Derryberry & Tucker, 1992). The hippocampus relies on theta rhythm for the encoding of spatial information and the choosing of responses (Oddie & Bland, 1998). Therefore it has been suggested that there is a correlation between theta rhythm in the hippocampus and spatial memory and learning (Berry & Thompson, 1978). The hippocampus relies on inputs from brain areas such as the medial septum, dorsal striatum, vestibular nuclei and hypothalamus for theta rhythm, spatial information and motor programmes (Winson, 1978; Oddie & Bland, 1998; Gengler, Mallot, & Holscher, 2005). When any of this information is prevented from reaching the hippocampus, a deficit in spatial memory and sometimes in emotional behaviour is observed (Gengler et al., 2005).

4.1. What Does the Hippocampus Do?

4.1.1. Limbic System

The hippocampus is part of the brain's limbic system which has evolved to process emotional stimuli and spatial information (Derryberry & Tucker, 1992). This specialisation of function allows the hippocampus to selectively respond to competing relevant emotional and spatial stimuli (Derryberry & Tucker, 1992). The behavioural inhibition system (BIS) plays a role in this type of selective responding. The hippocampus and amygdala are both part of the BIS which allows the animal to respond efficiently to threatening or aversive stimuli. When such stimuli are encountered, the BIS inhibits ongoing motor activity. This inhibition then allows the

animal to focus its attention on the important threatening or aversive stimuli (Gray & McNaughton, 2000).

4.1.2. Spatial Memory

The hippocampus also contributes to spatial processing and learning (O'Keefe & Nadel, 1979). Damage to the hippocampus has been associated with a decrease in performance in spatial memory tasks such as the Morris water maze (Morris, Garrud, Rawlins, & O'Keefe, 1982). Both rats and humans (tested in a virtual, rather than a real water maze task) with hippocampal lesions, had increased latencies to reach the hidden platform and demonstrated a decreased recall of the location of the platform in probe trials (Brandt et al., 2005). Hippocampal impaired rats were tested on a radial arm maze place learning task (McDonald & White, 1995). Two adjacent arms (out of eight) were left open and contained food rewards. To solve this task, the animals needed to distinguish between the two open arms based on their location relative to distal cues around the room. Only the animals with a functioning hippocampus could solve this task which demonstrated that the hippocampus was necessary for place learning when the cues, indicating the locations that needed to be discriminated, were ambiguous. Resolution of this ambiguity required the unique processing properties of the hippocampus (McDonald & White, 1995).

4.2. Main Inputs/Outputs to/from the Hippocampus

For the hippocampus to process the spatial and emotional stimuli it receives, it relies on several other structures within the brain. The hippocampus receives information from the entorhinal cortex and the information leaves the hippocampus primarily via area CA3 and the subiculum (Gray & McNaughton, 2000).

Another input comes from the medial septum. The medial septum receives information from the midbrain reticular formation. The medial septum then sends non-specific, multimodal, sensory information to the hippocampus (Gray & McNaughton, 2000). This sensory information coming from the medial septum reaches the hippocampus via fibres that travel in the fimbria and fornix (Rawlins, Feldon, & Gray, 1979). This information indicates the presence of a goal and therefore the need for a behavioural response. Unless the hippocampus receives further information from the entorhinal cortex, the hippocampus responds in a way which causes the animal to produce exploratory behaviour (Gray & McNaughton, 2000). For the hippocampus to function properly, the medial septum has to be fully functional (Rawlins et al., 1979).

The hippocampus and dorsal striatum share a bidirectional connection (Gengler et al., 2005). Through its connection with other parts of the basal ganglia, the dorsal striatum is involved in the storage and retrieval of learned motor programmes, which are used by the hippocampus (Gengler et al., 2005). Interestingly, the vestibular system has outputs to the basal ganglia and thalamus and these play a role in the theta rhythm in these areas (Dieterich & Brandt, 2008; Angelaki et al., 2009).

4.3. Hippocampal Theta

4.3.1. Definition

Hippocampal theta rhythm has been described as being rhythmic slow wave activity that follows a sinusoidal pattern (James, McNaughton, Rawlins, Feldon, & Gray, 1977; Winson, 1974). It occurs at frequencies of 6 to 12Hz (Oddie & Bland,

1998) and a substantial amount of theta rhythm occurring in the hippocampus is cholinergically controlled (Feldon & Rawlins, 1978).

Theta activity is required for normal hippocampal function (Winson, 1978). It is common for hippocampal theta activity to occur during the presentation of naturally occurring sensory stimuli, electrical stimulation or particular types of movement (Winson, 1974). Due to this selective occurrence of theta rhythm, it has been described as being the arousal reaction of the hippocampus (Winson, 1974). Theta rhythm, within the hippocampus, plays an important part in sensorimotor integration, attention and motivation. This role is especially large in motor learning (Oddie & Bland, 1998).

4.3.2. Anatomy

Hippocampal theta rhythm relies on a number of brain areas in order for it to occur. The pedunculo pontine tegmental nucleus (PPT) provides cholinergic input to the hippocampus. This cholinergic input has been shown to influence hippocampal theta activity (Bland & Oddie, 2001). Cholinergic theta occurs when there is input, into the hippocampus, from the ascending cholinergic system as well as phasic inhibitory input. The ascending cholinergic system involves the PPT, superior colliculus, substantia nigra and the amygdala (Gray & McNaughton, 2000). As well as providing cholinergic input to the hippocampus, the PPT provides the main cholinergic input to the nucleus reticularis pontis oralis (RPO; Bland & Oddie, 2001). Projections from the PPT also reach the median raphe nucleus, medial thalamic nucleus, intralaminar nuclei, supramammillary nuclei (SuM) and the septum (Bland & Oddie, 2001).

When different levels of the brainstem reticular formation and the caudal diencephalon are stimulated, theta rhythm is produced in the hippocampus. The

synchronising effect these areas have on the field activity of the hippocampus is mediated via the medial septum and the synchronising effect originates in the RPO (Oddie & Bland, 1998). The neurons in the RPO project to the medial septum through SuM and the posterior hypothalamic nucleus (Oddie & Bland, 1998).

SuM can influence the frequency of theta via an input to the septum or via the direct connection it shares with the hippocampus (O'Keefe, 1993). The septo-hippocampal pathways also influence theta cell activity. Descending activation of these pathways is required for rhythmic cell firing in the medial mammillary bodies (Bland & Oddie, 2001).

The phasic information created by the theta cells in SuM, is sent to the medial septum. The medial septum acts as a cholinergic gate (Gray & McNaughton, 2000). Once information leaves the medial septum, it is sent to the posterior cingulate cortex, entorhinal cortex and the hippocampus (Bland & Oddie, 2001). Some of these projections leaving the medial septum are inhibitory, however the cholinergic projections are not. The outgoing projections inhibit the inhibitory interneurons (Gray & McNaughton, 2000). When particular stimuli are present, the medial septum fires and cholinergic theta occurs. This cholinergic theta originated in the reticular formation (Gray & McNaughton, 2000). The role of the medial septum has been suggested to be a pace maker of theta rhythm in the hippocampus. This role is also shared with the diagonal band of Broca (Wetzel, Ott, & Matthies, 1977; Bland & Oddie, 2001). The medial septum is thought to be the main external regulator of hippocampal theta rhythm and a controller of theta amplitude (Oddie & Bland, 1998).

Medial septal lesioned rats were tested in an elevated circular maze task (Winson, 1978). Rats were either lesioned to the point of completely theta, left with theta or were non-lesioned. Animals had to learn, using distal room cues, where on

the maze the water reward was located. One cup from eight contained the reward. Animals who received lesions, that abolished theta, could not perform the elevated circular maze task. Those animals that were lesioned, but still had intact theta rhythm, were able to successfully complete the maze task. Although the abolished theta lesions prevented the rats from locating the goal cup, when they did happen upon it by chance, they did recognise it as the goal location. Rats without theta rhythm could learn the spatial task however they used different means of learning, other than spatial cues (Winson, 1978).

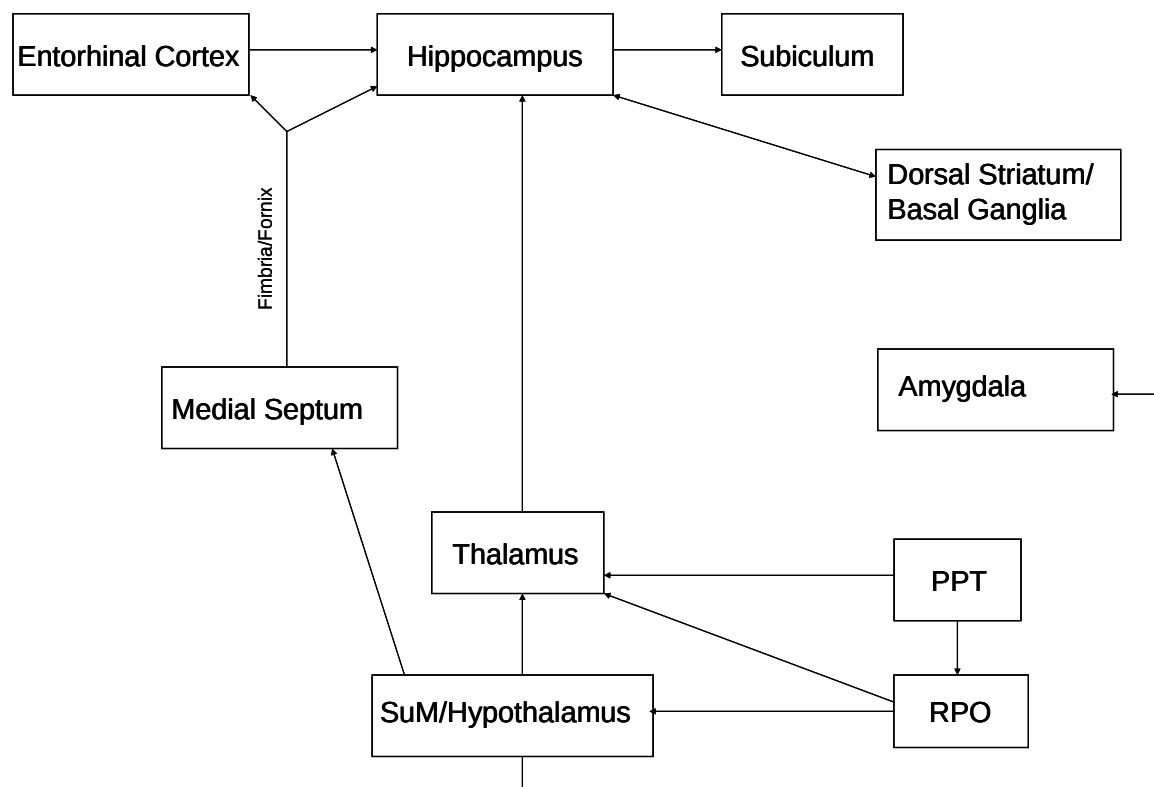


Figure 4.1. The hippocampal theta network. Information included in the diagram is from Winson (1978), Rawlins et al. (1979), O'Keefe (1993), Oddie and Bland (1998), Gray and McNaughton (2000) and, Gengler et al. (2005).

4.3.3. Movement

Cholinergic theta, or atropine sensitive theta occurs just before the animal begins a voluntary behaviour. Walking, turning, rearing, freezing, paradoxical sleep,

approach to and manipulation of food, approach to water and turning away after drinking, lying down, changing position or posture, climbing, struggling while being held and jumping to avoid a shock are classified as voluntary behaviours, and all show theta (Vanderwolf, 1969; Whishaw & Vanderwolf, 1973).

Cholinergic theta recorded before a voluntary motor behaviour is representative of the motor behaviour about to occur (Oddie & Bland, 1998). The amplitude and magnitude of theta activity recorded during a voluntary behaviour also appears to signal the magnitude of the behaviour. If the animal completed a large voluntary behaviour, such as a jump, the theta rhythm would also be large (Oddie & Bland, 1998). Theta rhythm is also correlated with the temporal occurrence of the voluntary behaviour. During the execution of a particular behaviour (e.g. jumping to avoid a shock), there is a steady increase in electroencephalogram (EEG) amplitude leading up to the jump, the largest amplitude just before the jump, and then a steady decrease in amplitude immediately after the jump has been completed (Vanderwolf, 1969). Large amplitude theta is also associated with novel movements. This can be observed during bar pressing. When the animal first experiences bar pressing, the EEG amplitude is large. As the novelty of the activity decreases, there is a decrease in EEG amplitude (Whishaw & Vanderwolf, 1973). Theta frequency is also correlated with movement velocity. As the velocity of a voluntary behaviour increases, there is an increase in the frequency of the recorded EEG (O'Keefe, 1993). Finally, the phase of firing of a place cell, relative to the phase of ongoing global theta, has been correlated with the animal's position in the environment (O'Keefe, 1993).

Oddie and Bland (1998) demonstrated the role of cholinergic theta and the role of the medial septum in hippocampal theta rhythm using a robber rat experiment. In

the experiment there were two groups of rats, the robbers and the victims. Victim rats were given a food item and the robber rats were not. The measured response was the victim's ability to successfully dodge the robber rat when he attempted to steal the food item. The decision to dodge relies on sensory integration and planning and this integration and planning relies on theta rhythm. Theta rhythm in the victim rats was manipulated with an infusion of atropine (cholinergic blockade) into the medial septum. Testing before the blockade of the medial septum revealed that victim rats were 96% accurate at dodging the robber rats. When the victim rat dodged the robber rat, there was an increase in the frequency of the theta rhythm. After the blockade of the medial septum, there was a reduction in cholinergic theta recorded from the victim rats. Also, there was a reduction in the victim rat's accuracy score. Fifty-nine percent of the time, the robber rats were able to steal the food from the victim rats (Oddie & Bland, 1998). The conclusion was that changing or blocking theta in the hippocampus causes a change in behaviour (Oddie & Bland, 1998).

Automatic behaviours do not show theta rhythm. Instead, they elicit large irregular EEG activity. Licking, chewing and grooming are examples of behaviours that have been described as being automatic (Vanderwolf, 1969). If an animal performs both a voluntary (theta producing) and an automatic (irregular activity producing) behaviour, theta will occur (Whishaw & Vanderwolf, 1973).

4.3.4. Learning

Hippocampal theta rhythm has been established as being a correlate of learning in animals. Time samples of hippocampal EEG taken before a training period can predict the learning rate of the animal. This relationship remains stable across days (Berry & Thompson, 1978). The relationship between theta and learning is also present in post-training retention performance tests. The amount of theta in the

post-training EEG has been positively correlated with retention performance in rats (Berry & Thompson, 1978). Changes in hippocampal frequency during training can predict the degree to which the animal learns the task. When there is a higher proportion of hippocampal EEG activity within the frequency range of theta subjects have decreased rates of conditioning (Berry & Thompson, 1978). The probability of reinforcement and theta activity are related, when observed in dogs. Gray and McNaughton (2000) theorised that this relationship could represent the number of different responses being considered by the subject. Hippocampal theta rhythm was observed in animals many hours after continued training, specifically repetition of the same motor act did not cause theta to disappear (Whishaw & Vanderwolf, 1969). Theta rhythm was still present during the later stages of running in rats that were forced to run for eight hours on a motor driven wheel.

4.3.5. Stimulation

Stimulation of areas connected to the hippocampus can enhance or disrupt hippocampal function. When the brainstem reticular formation or the caudal diencephalon was stimulated with trains of pulses at 100Hz, theta activity occurred in the hippocampus (Oddie & Bland, 1998). If the theta was produced at high frequencies during the reticular stimulation, movement was observed (James et al., 1977).

Stimulation of the hippocampus can disrupt the normal theta rhythm cycle (Bland & Vanderwolf, 1972). If stimulation was given to the hippocampus, of a rat, at a rate of 10-100c/sec, then spontaneous or specially trained movement patterns (e.g. bar pressing) were suppressed (Bland & Vanderwolf, 1972). Stimulation of the hippocampus also interferes with the execution of voluntary behaviours. If an animal received stimulation during a voluntary behaviour, the movement was halted. As

soon as stimulation was finished, the behaviour occurred (Bland & Vanderwolf, 1972).

When the medial septal region of rats is stimulated, at theta frequencies, but not higher frequencies, hippocampal theta rhythm occurs. In a septal stimulation experiment, thresholds for theta frequency in the hippocampus were tested (James et al., 1977). Subjects were given 5.9, 6.9, 7.7, 9.1 and 10Hz stimulation. Of all the subjects, 92% of the rats showed a minimum threshold at 7.7Hz. The behavioural significance, of the frequency of 7.7Hz, was that it was involved in the inhibition of ongoing behaviour, upon recognition of signals of punishment or non-reward (James et al., 1977).

4.4. Lesion/Pharmacological Block Effects on the Hippocampus

When the hippocampus is lesioned or inhibited with drugs, a loss of hippocampal theta rhythm occurs, as well as spatial memory deficits (Winson, 1978). Based on spatial memory tests on hippocampal lesioned animals, theta has been thought to play a key role in the integration of sensorimotor information (Bland, Seto, Sinclair, & Fraser, 1984).

Hippocampal lesioned rats were trained in a water maze task before and after receiving their lesion (O'Keefe, 1993). After receiving the lesion, the lesioned rats appeared to have a reduced capacity to perform the task. However, after a number of trials they did eventually relearn the task, but seemed to use different navigation strategies compared to the non-lesioned rats. O'Keefe (1993) also reported that when animals received lesions to both the hippocampus and the subiculum, the Morris water maze task was never learnt.

Another test used to illustrate the effects of hippocampal lesions on spatial memory is the Olton radial arm maze (O'Keefe, 1993). Working and reference memory were tested in ibotenic acid lesioned and non-lesioned rats. During the test, a subset of arms was consistently baited with a food reward on each trial, while the rest of the arms were always empty. A successful trial was where the animal entered every baited arm once (working memory) and avoided all of the non-baited arms (reference memory). The test had two versions which were spatial/place and cue. During the spatial/place version, the maze arms were specified by their location in the testing room. During the cue version, multi-cue inserts were placed in different sets of arms during each trial. It was these multi-cue inserts that provided the information needed to solve the task. The ibotenic acid lesioned animals were deficient on both the working and reference memory aspects of the spatial/place version of the task. In comparison, they were only mildly deficient on the cue version of the task. Pre-trained, lesioned rats were able to successfully complete the cue version of the task, but were unable to do so in the spatial/place version of the task. After a number of extra trials, the pre-trained, lesioned rats did eventually learn the spatial/place version of the task (O'Keefe, 1993).

Lesions of the hippocampus also have an effect on spontaneous alternation Lalonde (2002). When non-lesioned, control subjects were given this task, they often alternated at a rate greater than chance. In comparison, hippocampal lesioned rats alternated at a reduced rate (Lalonde, 2002). The literature reviewed by Lalonde (2002) indicated that the reduction in alternation seen in hippocampal lesioned rats could have been due to a delay in stimulus satiation during exposure to the maze arm. These same effects were observed in fornix and septal lesioned rats, both of which would have had disrupted hippocampal theta rhythm (Lalonde, 2002). When the

fornix of a rat is sectioned there is an abolition of theta and a disruption to regular hippocampal functioning. This effect is not observed when the fimbria is sectioned (Rawlins et al., 1979).

The hippocampus receives motor programme information from the dorsal striatum. This information is then integrated and synchronised with the spatial, sensory information in the hippocampus (Gengler et al., 2005). When theta activity was recorded simultaneously from both the hippocampus and the dorsal striatum, in rats during a T-maze task, it was synchronised. The relationship between the dorsal striatum and the hippocampus during the performance of a goal directed task was examined (Gengler et al., 2005). Rats were tested on two tasks and given different doses of sulpiride into the striatum (a D₂ antagonist that inactivates the dorsal striatum). The first task was a four-arm egocentric maze which tested dorsal striatum function. Animals were given their sulpiride treatment 15 minutes before their trial began. In the egocentric maze task, rats were started in the south arm and needed to go to the baited west arm. When subjects were given a dose of 5µl of 100mM of sulpiride, their number of errors increased. The second task tested the role of the dorsal striatum in hippocampal functioning, specifically its influence on hippocampal EEG. EEG recordings were collected while subjects were tested on a continuous alternation task, in a figure eight maze, and received different doses of sulpiride. During the task, subjects needed to go up the central stem and alternate between left and right turns at the upper junction. The food rewards were located at the end of each choice arm. Animals completed the continuous alternation task before, during and after sulpiride treatment. The same dose of sulpiride that caused the increase in errors in the egocentric maze task also caused a redistribution of theta power in the hippocampus during the continuous alternation task. The main theta activity of the

hippocampus was shifted from the 8-10Hz range, down to the 5-7Hz range (Gengler et al., 2005). The inactivation of the dorsal striatum inhibited the hippocampus from integrating and synchronising the motor inputs with its spatial sensory information. This inhibition stopped the hippocampus from being able to plan goal directed movements. This impaired integration of information was most likely the result of the redistribution of theta power because it caused an increase of theta power in the lower frequencies (Gengler et al., 2005).

4.4.1. Summary

The hippocampus plays an important role in the processing of emotional and spatial stimuli (Gray & McNaughton, 2000). When the hippocampus is damaged animals performance on hippocampal sensitive tests is indicative of a diminished spatial memory (Winson, 1978; O'Keefe, 1993). This is diminished spatial memory is attributed to a loss of theta rhythm in the hippocampus. Without theta rhythm, the hippocampus can not integrate incoming spatial information with the spatial memory and motor programmes already stored (Gengler et al., 2005). Lesioning or blocking the brain areas that provide spatial information or theta rhythm to the hippocampus causes deficits that are similar in effect to a hippocampal lesion (Rawlins et al., 1979; O'Keefe, 1993; Gengler et al., 2005). When the theta producing areas such as the medial septum are stimulated, the same frequency of theta can be recorded in the hippocampus (Oddie & Bland, 1998). This finding suggests that if the hippocampus is not receiving theta from an area of the brain that usually provides it, stimulation of a theta producing area can restore theta to the hippocampus.

5. General Conclusions of the Introduction leading to the Present Experiment

5.1. General Conclusions

A number of conclusions can be drawn from the literature presented. There appears to be evidence for cognitive and emotional deficits that are the result of vestibular lesions. These deficits appear to occur, at least in part, as a result of the damage to the hippocampus that is caused by the vestibular lesion. This is especially true for performance on spatial memory tasks such as the Morris water maze (in rats) and the virtual water maze and path integration tests (in humans). Emotional deficits are observed in both rats and humans – however the direction appears to be opposite in the two species. Rats demonstrate behaviour that suggests the vestibular lesion leads to a reduction in the anxiety experienced. This is opposite to humans, who demonstrate behaviour and report feelings that suggest the vestibular damage increases their anxiety. There is also evidence that vestibular damage in humans does not cause anxiety in and of itself. At this stage the general conclusion is that both effects do occur, in that anxiety produces vestibular-like symptoms (but not vestibular damage) and vestibular damage produces anxiety. However there are no data at present that suggest that anxiety produces vestibular lesions. The bi-directional interaction between vestibular dysfunction and anxiety is indicative of a positive feedback loop between the two. The difference in the effects the lesion has on emotional processing in the two species can be explained by humans being able to experience worry. The worry experienced by the vestibular damaged humans could exacerbate the effects of the lesion, in a negative feedback type cycle. The more the vestibular patients worry about experiencing for example an attack of vertigo, the

more anxious they become, which increases their sensitivity to the physical sensations of their symptoms which leads to them having an attack of vertigo. The most important conclusion taken from the reviewed research is that bilateral vestibular damage causes a deficit in hippocampal theta rhythm, at least in rats. Further, this interference with theta has been strongly associated with the discussed cognitive and emotional deficits observed in both rats and humans. This raises the possibility that restoring theta to the hippocampus may reverse the cognitive and emotional deficits caused by bilateral vestibular damage and so provide a potential therapeutic intervention in humans.

5.2. The Present Experiment

The aim of the present experiment was to test the effects of medial septal stimulation in bilaterally lesioned vestibular rats on tests of cognitive and emotional processing. It was hypothesised that stimulation of the medial septum would restore theta rhythm to the hippocampus and that this would then reverse the cognitive and emotional deficits caused by the bilateral vestibular lesion. Rats were given bilateral vestibular lesions or sham operations and tested in a spin test, openfield test, elevated T-maze test and forced alternation test. A range of tests were used in order to accurately measure both the emotional, cognitive and behavioural changes that occur as a result of bilateral vestibular lesions. The frequency of rearing, moving, non-movement, investigation and retreating; and EEG elicited by the subjects were measured and recorded.

6. Methods

6.1. Subjects

Subjects were 32 male, Sprague-Dawley rats. All subjects were experimentally naïve. The animals were obtained from the University of Otago Animal Breeding Station. Subjects were approximately two months old upon arrival at the Pharmacology Department laboratory. All subjects were handled for ten minutes every day to habituate them to being picked up. Subjects remained in the laboratory for at least 14 days before surgery. Surgery was performed when the rats weighed between 200-320g. Vestibular lesion surgery was performed first followed by electrode implantation surgery (see below for recovery times). Every subject was fitted with an electrode implant. Vestibular surgery was done in the Pharmacology department at the University of Otago. After vestibular surgery, the animals were kept in the Pharmacology department for the first seven days of the 14 day post operative special observation period. This was to reduce stress and increase the likelihood of a full recovery. On the seventh day rats were transported, via car, from the Pharmacology department to the Psychology department. For the transfer, subjects, in their cages, were placed in big canvas bags that completely covered the cages. Once at the Psychology department, subjects were placed in the colony room with a full water bottle and food hopper. Vestibular surgery required a recovery period of one month. This was to allow for vestibular compensation to occur. Electrode implantation surgery required a recovery period of two weeks.

Once at the Psychology Department, the animals were housed in a temperature controlled (21 +/- 2°C) colony room. Light was provided naturally via a large window and by overhead lighting between 5am and 5pm. Subjects were housed

individually in smaller, white plastic cages (21cm wide, 19cm high, 33cm long). These had stainless steel, detachable grill lids (21cm wide, 8cm high, 33cm long). The cages were lined with wood shavings and shredded paper. Water and food (Reliance Stock Food R49 pellets) were available ad libitum to all subjects, except when on food deprivation (see below). After vestibular lesion surgery, subjects were given raspberry jelly for one night. After each surgery subjects were given sugary mash for the first three to four days. This mash was made from the subjects' regular food pellets. Mash and water were placed in a double bowled, ceramic dish inside the cages, in addition to the usual supplies of food and water.

6.2. Surgery Apparatus and Procedure

6.2.1. Vestibular Lesion Surgery

All vestibular lesion surgery was carried out by Dr. Yiwen Zheng of the Pharmacology Department, University of Otago.

During the vestibular lesion surgery, each rat had either a bilateral vestibular deafferentation or a sham surgery performed. The sham surgery was the same as the bilateral lesion surgery, except that the temporal bone was exposed without producing a lesion of the vestibular apparatus.

Before the surgery, subjects were anaesthetised with 200mg/kg of Fentanyl citrate (injected intraperitoneally) and 500mg/kg of Medetomidine hydrochloride (injected intraperitoneally). Carprofen (5mg/kg) was routinely administered (subcutaneously) to each subject as a post-operative analgesic. Animals were also injected with Xylocaine (with 1:10000 adrenaline) around the wound margins.

The room was kept warm and subjects were kept under a heat lamp during the surgery. All surgical equipment was sterilised and disinfected. A full surgical gown, mask and gloves were worn at all times during the surgery.

Once the rat was anaesthetised, the hair on the top of the animal's head was shaved. The exposed skin was then disinfected. The subject was then placed in a custom made nose bar. The nose bar was used to keep the animal's head steady during the surgery. The tympanic bulla was exposed in each subject by using a retro-auricular approach. The tympanic bulla was located using an otolaryngological microscope. Following the removal of the tympanic membrane, the malleus and incus were also removed. The removal of these allowed the vestibule, above the ampullae of the horizontal and anterior semicircular canals, to be visualised. The stapedial artery was cauterized at two points. Following this, the horizontal and anterior semicircular canal ampullae were drilled open using a high speed dental drill with a fine burr. The contents of the canal ampullae, utricle and saccule were aspirated. The temporal bone was then sealed with dental cement. The procedure was then repeated on the opposite ear in each subject.

6.2.2. Electrode Implantation – Electrodes

All electrode construction was carried out by Dr. Phoebe Neo of the Psychology Department, University of Otago.

During the electrode implantation surgery, each rat had three arrays of electrodes implanted. One array was for stimulating and the other two were for recording. Each array of stimulating electrodes was made up of three strands of stainless steel wire (0.005mm bare, 0.008mm coated) twisted. The insulation was not present at the cut tips which were separated by 0.5mm by pulling the wires, which were then glued together. Each array of recording electrodes was made up of four

strands of stainless steel wire (0.003mm bare, 0.0055mm Teflon coated) and the tips separated by 0.5mm (for spread of 1.5mm overall) in the same manner as the stimulating electrode. Each strand of wire was then soldered to one pin of a square, 32 channel connector (SK-MGA6 / 32A-01, Ironwood Electronics). This was done using phosphoric acid flux. An earth wire made of uninsulated silver and two indifferent electrodes (made from insulated wire soldered to Amphenol gold pins) were also soldered to the gold pins of the connector.

6.2.3. Electrode Implantation – Surgery

All implantation surgery was carried out by Dr. Phoebe Suat-Hong Neo, Department of Psychology, University of Otago.

On the day of the surgery, subject's were weighed and placed in a black plastic box. This was covered with a towel to keep the rats calm. Ketamine (100mg/ml; 75mg/kg; 0.75ml/kg) and Domitor (1mg/ml; 0.5mg/kg; 0.5ml/kg) were prepared and injected subcutaneously into the subject. Once the subject was asleep, Atropine (0.065mg/ml; 0.05mg/kg; 0.77ml/kg; Sigma Chemical Company) was injected. This was to aid breathing by reducing mucus secretion while the animal was anaesthetised. The level of anaesthesia was assessed during the surgery via the foot pinch reflex test. Once the animal was anaesthetised, the top of the head was shaven. The shaved area was made larger than the incision area to allow for thorough disinfection. The shaved area was wiped with alcohol (70% ethanol) and infused subcutaneously with Lopaine and Marcaine solution. Lopaine (20mg/ml; 4mg/kg; 0.2ml/kg) and Marcaine (5mg/ml; 2mg/kg; 0.4ml/kg) were prepared together in a syringe and diluted with saline at a ratio of 1:3. Before the first incision was made, Tricin ointment (Jurox, Australia) was applied to the subject's eyes to protect them from drying out. A piece of gauze was placed over the subject's eyes to protect them from the disinfectant.

After the application of Tricin, the animal was given a 5ml subcutaneous injection of saline in each side of its body. This was to keep the animal hydrated during the surgery. At the end of the operation, the animal was given Antisedan (5mg/ml; 2.5mg/kg; 0.5ml/kg; subcutaneous), an antidote to Domitor. This usually produced fast recovery.

During the surgery, the rat was kept on a heat pad to help maintain a normal body temperature whilst under anaesthesia. All items that were going to be used or that were likely to be touched during the procedure were sterilised using Chlorhexidine. These items included the stereotaxic frame, lamp, drill bit and drill casing and the dental cement container. All surgical tools were sterilised in an autoclave before the surgery. All surgical tools were placed on a stainless steel tray previously sterilised with Chlorhexidine. Surgical gloves, a gown and a mask were worn at all times during the surgery. Gloves were dipped regularly in a 70% ethanol bath to maintain sterility throughout the operation.

The subject was then positioned in the stereotaxic frame. The rat's head was held in place by ear bars and an incisor bar. The skull was adjusted to be flat between bregma and lamda. A sterilised, plastic sheet was placed over the rat's head and incisions were made through the sheet. An incision down the midline of the scalp was made. Forceps were used to hold aside the muscle and skin. Connective tissue and blood were cleared away from the surface of the skull using a sterile swab. Six holes were drilled for stainless steel anchor screws and an earth screw and a dental cement dam was created around the electrode insertion area and connected with adjacent screws. Holes were drilled in appropriate sites and then electrodes were inserted. The stimulating electrode was aimed at the medial septum. The coordinates were (with skull flat, referenced to bregma) AP 1.0mm, L (to the right) 0.96mm, D 4.0mm, at an

Angle of 10 degrees. The recording electrode aimed at the hippocampus had the coordinates AP -3.8mm, L (to the left) -2.5mm, D 4.0mm (Angle 0 degrees). The other recording was aimed at the supramammillary bodies with the coordinates AP - 4.8mm, L 1.56mm, D 9.4mm, and Angle 10 degrees. Each inserted electrode was firmly cemented to a skull screw before the next one was inserted. Finally the gold-pin indifferent electrodes were held in place in contact with a dent drilled in the skull just behind lamda (taking care not to penetrate through to dura) and cemented in place. The earth wire was then positioned around the implant and finally wound around one of the screws. After the positioning of the earth wire, the electrode plug was cemented up, with its front facing the nose of the rat. The incision was sutured up and the saline drip was removed. Antisedan (5mg/ml; 2.5mg/kg; 0.5ml/kg) was administered. For post operation analgesia animals were given 0.05mg/kg of Temgesic twice a day for two days. Animal's were returned to the animal room and placed on a heating pad overnight. Mash (2Tb finely crushed pellets with water and sugar) and water were given to each subject after the operation in a two compartment ceramic cat food dish. During the post operation special observation period, rats were given subcutaneous injections of 5 to 10mls of saline if the skin fold test was indicative of dehydration. A recovery period of ten days followed before animals were tested.

6.3. Electrophysiological Testing Apparatus and Procedures

6.3.1. EEG Recording

EEG recordings were done via a Grass Model 15 Neurodata amplifier system connected to a CED Micro-1401 system with Spike2 software (Cambridge Electronic Design, Cambridge, England). A video web camera was linked to the Spike2

programme. This camera was attached to the ceiling, in the middle of the testing room. The camera recorded the rat's behaviour in synchrony with the EEG recordings. Once the animal was connected, the Spike2 EEG programme was set to start and EEG and video recording began.

6.3.2. EEG Connector

In all behavioural tests, subjects were connected to the EEG recorder. The animals had the 32 channel, gold, female connector, described above, on top of their heads. This connector has both a male and female side so can be plugged into another connector of the same type. The EEG recorder was connected to the female connector via a similar male 32 channel, gold, connector. This connector in turn was plugged into a similar one soldered into the cable. The intermediate connector was kept unplugged into the cable to protect the gold pins so that the cable did not have to be dismantled if the pins connecting to the rat were damaged. The soldered connector was wired to a source follower and a long, flexible cable. This cable was connected to a commutator that was fixed to the ceiling. This, in turn, was connected to the power supply and connecting box for the source follower, and the latter was connected to the Grass amplifier system.

6.3.3. Stimulation

The bilateral vestibular deafferentation (BVD) rats were stimulated with trains of bursts of four pulses (0.1ms pulse width), with a within burst frequency of 100Hz. Each burst was followed by a pause, followed by the next four pulse burst. The interval between the first pulse of one burst and the first pulse of the next burst was 128ms. As a result, the frequency of driven theta, resulting from the stimulation, was 7.81Hz. The 128ms interval was chosen to allow ms accuracy of timing using a

system clock that is polled only every 16ms. Stimulation was delivered via a purpose built, programmable, constant current stimulator. All parameters were controlled via a Visual Basic programme. This allowed adjustment on a burst by burst basis. The stimulation current was adjusted manually, on a continuous basis, to generally maintain stimulation above the threshold for driving theta and to ensure that evoked potentials, and consequent seizures did not occur. Pilot experiments suggested that when a single pulse, as opposed to a four pulse burst, was used, seizures were more likely. In comparison, no seizures were observed in any animal receiving the four-pulse burst stimulation. Stimulation was given for the duration of all behavioural tests, except the spin test (where elicited theta rather than behaviour was assessed). Stimulation was also given during the exposure trial of the elevated T maze experiment and during the pre-training trials of the forced alternation experiment.

6.4. Behavioural Testing Apparatus and Procedures

6.4.1. Testing Room

The testing room, where all the behavioural tests were carried out, was approximately 4m by 5m, with a ceiling height of 6.5m. During all the tests, all the lights in the room were turned on. There was a ceiling light, in the centre of the room, and 6 wall lamps around the room. In the centre of the room, where the testing apparatus were placed, the light measured 45 lux. The door to the testing room was to the South East. To the South West, was the computer used for recording. The North West corner of the room had another computer. The testing room had no windows. The door to the testing room was closed at all times during behavioural tests.

6.4.2. Spin Test

Subjects were placed in a tapered dustbin which was mounted on a rotating base. The original bin used was a tapered white, plastic dustbin. It had a diameter of 42cm at the top and 35cm at the bottom and was 49cm high. The white bin was mounted on a piece of fibre board (32cm in diameter), which was attached to a rotating metal base (25.3cm wide; 9cm high). The dustbin was filled to a depth of approximately 3cm with wood shavings.

Due to an engineering fault, a second, replacement bin had to be used. This bin was a tapered, red metal dustbin measuring 23cm in diameter at the bottom, 30.5cm in diameter at the top and was 29cm high. The bin was mounted on a piece of fibre board (15cm in diameter). The fibre board was fixed to a rotating metal base (25.5cm in diameter; 9.5cm high). The dustbin was filled to a depth of 3cm with wood shavings.

Subjects were connected to the EEG recorder and placed in the spin bin. Recording began just before subjects were placed in the bin. The subjects were left to explore the bin and become habituated to the new surroundings. Habituation to the bin was judged as a cessation in movement. Once movement had ceased, the spin trials were carried out. The bin was spun, by the experimenter, with a rotation speed of one rotation per 1.5 seconds, with five rotations in total. Spin start and spin stop were achieved with a single hand movement and so had high rates of acceleration with free spinning for approximately seven seconds of rotation. Spin trials occurred at 2 minute intervals. These trials were alternated between clockwise and anticlockwise spin directions. Subjects completed six trials and were then returned to their home cages.

6.4.3. Open Field Test

The open field was a rigid, black, plastic drum with a diameter of 75cm and a height of 55cm. Around the top was a 2cm lip facing inwards. The drum was empty and set in the middle of the testing room. Before each trial began, the open field was wiped with an alcohol solution (20% ethanol). All the lights in the testing room were on during the open field trials. This included the main, central ceiling light and all wall mounted lamps.

Each subject was connected to the EEG recorder and placed in the open field, as close as possible to the outer wall. Each subject completed a 15 minute trial once a day, for three consecutive days. Rearing, grooming, freezing and the number of fecal boli were all counted and recorded. At the end of the 15 minutes, recording was stopped and the subjects were returned to their home cages.

6.4.4. Elevated T-Maze Test

The elevated T-maze was built to match that of Zangrossi and Graeff (1997). The maze had four arms of the same dimensions (50 x 12cm). The maze was 50cm above the floor. Two of the arms were enclosed by 40cm high opaque walls (closed arms). These walls were made from medium density fibreboard and were painted black. One of the closed arms was blocked off using a block of wood which was 40cm high and 12cm wide. During the trials with the non-vestibular rats, the open arms were surrounded by a 1cm high clear, plexiglass rim. This was to reduce the likelihood of subjects falling off the T-maze. During the trials with the vestibular lesioned and sham lesioned rats, and as a result of pilot testing, the open arms were surrounded by 14.5cm high, 6mm thick, plexiglass walls. This was to prevent lesioned animals from falling from the maze because of their impaired balance and

increased motility. The elevated T-maze was placed in the middle of the testing room. All lights in the testing room were on during the trials. Before each subject was tested, the maze was wiped down with an alcohol solution (20% ethanol).

Exposure Trial: The T-maze was wiped down with 20% alcohol solution before each trial. A block was placed in the centre junction point of the maze to keep the animal in the open arm. Subjects were placed in the maze, at the extreme end of the open arm. If the animal fell, or jumped, they were placed back on the arm at the point where they had begun the trial. The exposure trial went for 30 minutes. At the end of the 30 minutes, subjects were placed back in their home cages. The exposure trial was completed once and on a day separate from the other T-maze trials.

Avoidance and Escape Trials: Subjects were connected to the EEG recorder and placed in a plastic holding box. This box was located at the end of one of the closed arms. The box was the same as the subjects' home cages. Recording began when the rat had been placed in the box. After two minutes, the rat was placed in the closed end of the closed arm of the T-maze. The rat was cupped with two hands and brought into the arm of the maze via the open end, rather than putting the animal straight down at the closed end. During this, the rat was facing the centre of the maze. The time taken for the rat to leave the closed arm was recorded (baseline latency). Leaving the closed arm was defined as the animal having all four paws over the white lines on the maze that represent the end of an arm. The subject was given a maximum of 300 seconds to complete the trial. If the rat had not responded after this time, the trial was terminated and the latency was recorded as 300 seconds. After the subject moved to another arm, it was removed from the maze and placed back in the holding box for 30 seconds. The same procedure was continued for two more consecutive trials (avoidance 1 and 2). On the fourth trial, the subject was placed at the end of the

open arm used for exposure training. The time taken to leave this arm was recorded. Time taken to leave the arm was defined as the rat's four paws crossing the white line at the end of the arm. The subject did not have to enter a new arm. As with the avoidance trials, the animal was given a maximum of 300 seconds to leave the open arm. Once the subject crossed the line, it was placed back in the holding box for 30 seconds. This sequence was continued for two more trials (escape 2 and 3). At the end of the third escape trial, the cable was unplugged from the rat and the animal was placed back in its home cage.

6.4.5. Forced Alternation Test

For this test, the subjects were food deprived. Food deprivation began ten days before the first exposure trial. On day one of food deprivation the food was taken off the subjects at 5pm. On day two the food was returned to the animals at 9am and then taken away at 5pm. On day three, the food was returned at 12pm and taken away at 5pm. On day four, the food was returned at 3pm and taken away at 5pm. On day five, the food was returned to the subjects at 4pm and then removed at 5pm. The day five feeding schedule was then maintained throughout the duration of the testing period. If, during this time, subjects lost more than 80% of their free-feeding body weight, they were given three extra pellets after the feeding time. These extra pellets were only given until the animals weight was back above the 80% requirement.

The forced alternation test used was the same T-maze as for the elevated T-maze test. The maze was placed in the middle of the testing room. The open arms of the maze were pointed to the East and West walls of the testing room. All lights in the testing room were on during the trials. The maze was kept in the same place for all trials. All subjects to be tested on the maze for the given day were placed along

the wall, in their home cages. Subjects were arranged in the order they were to be tested. Before subjects were placed on the maze, it was wiped down with alcohol solution (20% ethanol).

Exposure Trials: Subjects were given three exposure trials. These trials were over three consecutive days. The trials had no fixed duration time. After the maze was wiped down with the alcohol solution, coco pops were placed in all the arms of the maze. Approximately 15 coco pops were in each arm. The coco pops were arranged in a line, leading the rat from the start of the arm, to the end of the arm. At the end of the open arms there were food wells. These contained approximately three coco pops. Stimulation and recording was not done on these exposure trials. The rat was placed in the south arm (a closed arm) of the maze and left to wander the maze and eat the coco pops. A trial was complete once the animal had visited all the arms and eaten all the coco pops.

Forced Alternation Trials – General Method: During trials, EEG recording and stimulation was done. Stimulated rats were placed in the maze once theta driving occurred. Recording and stimulation was done for the duration of the trial.

The closed arms of the maze were the North and South arms and were where the trials started. During a trial, one of the closed arms was always blocked off. The open arms of the maze were the East and West arms and were where the food rewards were located. The food rewards were in food wells, at the end of each open arm. Each food well contained approximately three coco pops. At the beginning of a trial, a closed arm and an open arm would always be blocked off.

Rats began a trial by being placed in the closed end of one of the closed arms (North or South). The subject was held at the closed end of the arm, by a hand held barricade, for approximately ten seconds. This holding period was to encourage the

animal to take notice of where it was in relation to the spatial cues around it. After the ten second holding period, the barricade was removed and the rat was left to go down to the end of the one available open arm (East or West). When the rat had entered the open arm, the wooden block, closing off entry to the opposite open arm, was placed in the centre of the maze. This held the animal in the visited open arm. After the animal had eaten the food reward, it was placed in a closed arm again (North or South). After the ten second holding period, the wooden block in the centre of the maze was removed and the animal was free to choose one of the two available open arms. Once the animal had chosen, the wooden block was placed back in the middle of the maze and the subject's choice (East or West) was recorded. After 15 seconds the subject was removed from the maze, disconnected from the EEG recorder and placed back in their home cage. At the end of each day, each subject's percentage accuracy score was calculated. This was: the number of trials correct (x) / the total number of trials (six).

Pretraining Trials: These trials were run as per the general method above.

There were six trials, per subject, per day, for six days. The same start position was used for the two starts in each trial. Fifty percent of the trials had the North arm as the start arm and 50% of the trials had the South.

Learning Trials: These trials were run as per the general method above.

There were six trials, per subject, per day, for four days. Fifty percent of the trials had different start positions for the two starts in each trial, e.g. start in the North arm, visit open arm, start again in the South arm, visit one of the open arms. Fifty percent of the trials had the same start position for the two starts in each trial (as per the learning trials). Eighty-five percent accuracy, over two consecutive days was the criterion used to decide if the animals had learnt the task.

Correction Trials: These trials were run for the first group of rats because the majority of the group appeared to have learnt the wrong rule. This was apparent from their consistent percentage accuracy score of less than 50% during the learning trials. There were six trials, per subject, per day, for five days. To maintain the trial sequence, the subjects from the first group that had learnt the correct rule were also included.

These trials were run as per the general method above, with a number of differences. The open arms (East and West) were the start arms. This was done to try and provide the subject with a greater chance of seeing the spatial cues around the testing room. The closed arms (North and South) were where the food rewards were located.

The trial schedule was the same as that during the testing trials. However, if a subject got a trial wrong, it was given that same trial, each time it was that subjects turn in the testing sequence, until it chose the correct arm. Once the animal chose the correct arm, on it's next turn, it was given the actual trial that was next in the schedule e.g. if an animal started on Trial 1 and chose the incorrect arm, and was given Trial 1 again and chose the correct arm, it would then complete Trial 3 that was in the counterbalanced testing schedule. The last 4 days of the corrections trials were used in the analysis so they could be compared to the 4 days of the learning trials completed by subjects who did not need the correction trials.

6.5. Histology

After they had completed testing in the forced alternation T-maze test, rats were deeply anaesthetised (sodium pentobarbital) and perfused transcardially, with saline, followed by 10% formalin-saline. The brains were removed and kept overnight in 10% formalin-distilled water. They were then kept in 30% sucrose-

formalin for seven days. Brains were frozen and sliced, using a freezing microtome. Coronal sections (90 μ) were placed onto glass slides and stained with thionin. Slides were viewed under magnification and the positions of the recording and stimulating electrodes were reconstructed according to the atlas of Paxinos and Watson (1998).

6.6. Data Analysis

During testing, all EEG was recorded simultaneously with a video of the animal. Behaviours that occurred during each test were marked at a later time.

During the spin test, the beginning and ending of spin and movement were marked in the video. Spin Start was defined as the beginning of the spinning of the wastebin. Spin End was defined as the ending of the spinning. Move Start was defined as the animal starting to move. Movement was not marked if it occurred during a spin. Move End was defined as the animal remaining still, this was normally just before the wastebin was about to be spun.

During the open field test, move, rear and still behaviours were marked in the video. Translational movement was defined as the animal moving from one spot in the open field to another. Rearing was defined as the animal lifting its front two paws off the ground and either balancing on its hind legs or leaning on the side of the open field. Non-translational movement or still was defined as the animal staying in one place and staying relatively still. Grooming was included in the non-translational movement category because the animal remained in one place.

Move, rear and non-move in the elevated T-maze were as per the definitions given above. Two additional behaviours were marked. Investigation behaviour was defined as the rat keeping its hind legs in one spot and stretching forward with its two front legs. Retreating was defined as the animal keeping its two hind legs in one place and moving the front half of its body backwards, usually into a hunched position.

When these behaviours had been marked in the EEG video, files were exported from Spike2 into a custom made analysis program. Each file then underwent a Fast Fourier Transform with an Overlapping Hanning analysis. The sample rate was at 128Hz and the window contained 128 values. This allowed for one second of EEG. Each tail end of the Overlapping Hanning contained 32 values, with 64 in the middle with the tails having high attenuation by the window and the centre portion little attenuation. The tail of each Overlapping Hanning window, overlapped with the next window in the sequence. Therefore, each transform derived the bulk of its power from 0.5 seconds of EEG that was consecutive with the 0.5s central portion of the subsequent window. The use of the overlapping Hanning effectively doubles the frequency resolution of the power spectrum without losing temporal resolution.

7. Behavioural and EEG Results

7.1. Behavioural Results

7.1.1. Openfield Rearing

Vestibular lesions reduced rearing, and this effect was not reversed by theta stimulation (Figure 7.1; treatment, $F(2,15) = 30.22$, $p < 0.001$). The sham group reared more often, across all days, than the non-stimulated and stimulated vestibular groups. This difference between the shams and the two vestibular groups remained stable across the three days of openfield tests (Figure 7.1; days x treatment group, $F(4, 53) = 0.57$, $p = 0.688$).

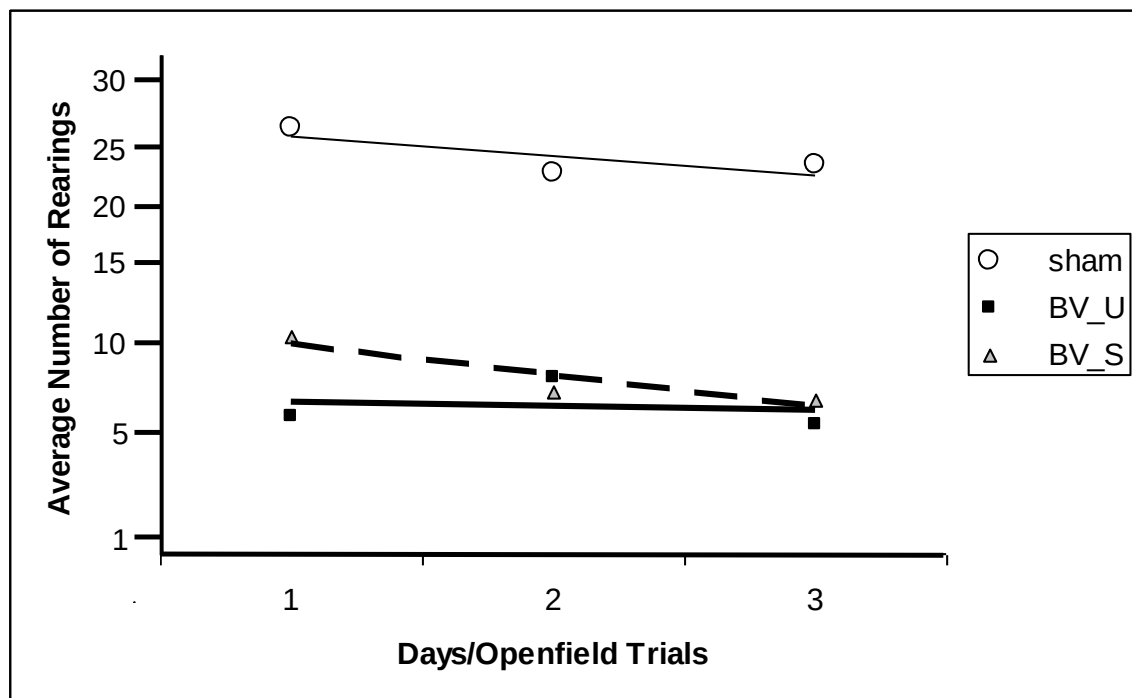


Figure 7.1. Average number of times subjects from each group reared in the openfield. The non-linear Y-axis is the result of LOG transformation of the data. BV_U subjects are those which had a vestibular lesion but were not stimulated. BV_S subjects are those which had a vestibular lesion and were stimulated.

7.1.2. Elevated T-Maze Latencies – Avoidance

As seen in Figure 7.2, the sham group began the avoidance trials with smaller latencies than the vestibular-lesioned groups. As the trials progressed, the latencies seemed to increase. In comparison, both of the vestibular lesioned groups began the trials with larger latencies, compared to the sham group, and over the trials, both vestibular groups' latencies seemed to decrease. The slopes of the lines in each of the three groups appeared to differ. The sham group latencies increased, where the two vestibular groups had decreasing latencies, with the stimulated vestibular group decreasing more than the non-stimulated vestibular group (trials x treatment, $\ln x$ dev, $F(2, 28) = 3.75$, $p = 0.036$). The overall mean values for the groups (as indicated by the centre of the regression lines) appeared to differ, with the non-stimulated vestibular group having larger latencies in each trial. However, this apparent difference was not statistically significant (treatment, $F(2, 14) = 0.94$, $p = 0.412$).

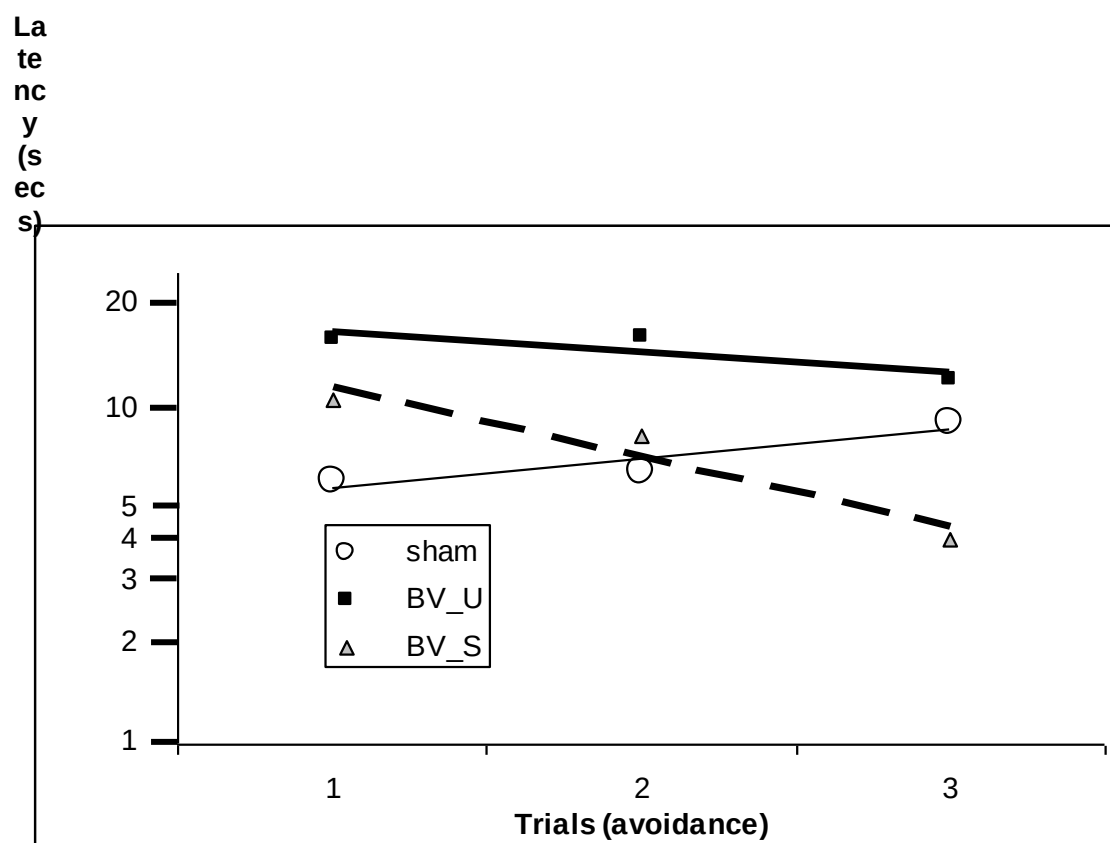


Figure 7.2. Latency (secs) to leave the closed arm during the avoidance trials. The non-linear Y-axis is the result of LOG transformation of the data. BV_U subjects are those which had a vestibular lesion but were not stimulated. BV_S subjects are those which had a vestibular lesion and were stimulated.

7.1.3. Elevated T-Maze Latencies – Escape

Figure 7.3 shows that, during the escape trials, all groups began with similar latencies. As the trials progressed, both the sham and the non-stimulated vestibular groups appeared to increase their latencies slightly compared to the stimulated group. However, these apparent differences in trend were not reliable (trials x treatment x lin x dev, $F(2, 28) = 0.28$, $p = 0.757$). The centre points of each of the lines in the groups were quite close together, suggesting that there was no effect of any treatment on latency (treatment, $F(2, 28) = 0.36$, $p = 0.703$).

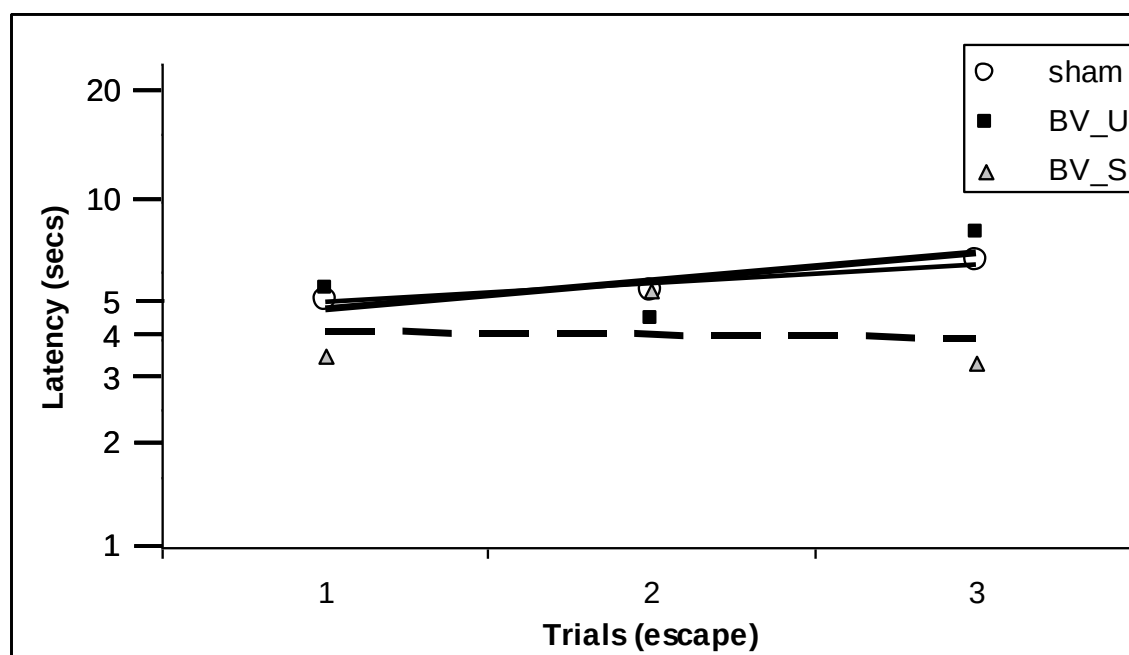


Figure 7.3. Latency (secs) to leave the open arm during the escape trials. The non-linear Y-axis is the result of LOG transformation of the data. BV_U subjects are those which had a vestibular lesion but were not stimulated. BV_S subjects are those which had a vestibular lesion and were stimulated.

7.1.4. Forced Alternation

During the forced alternation test, both the non-stimulated and stimulated vestibular groups appeared to consistently perform at an accuracy level of 50%. In contrast, the sham group consistently performed above 80% accuracy. This difference in accuracy between the shams and both the vestibular groups was reliable (Figure 7.4; treatment, $F(2, 15) = 5.51$, $p = 0.016$). The vestibular lesion appeared to lead to a deficit in learning the forced alternation task, and the stimulation did not appear to reverse this deficit with the stimulated group showing, if anything, poorer performance.

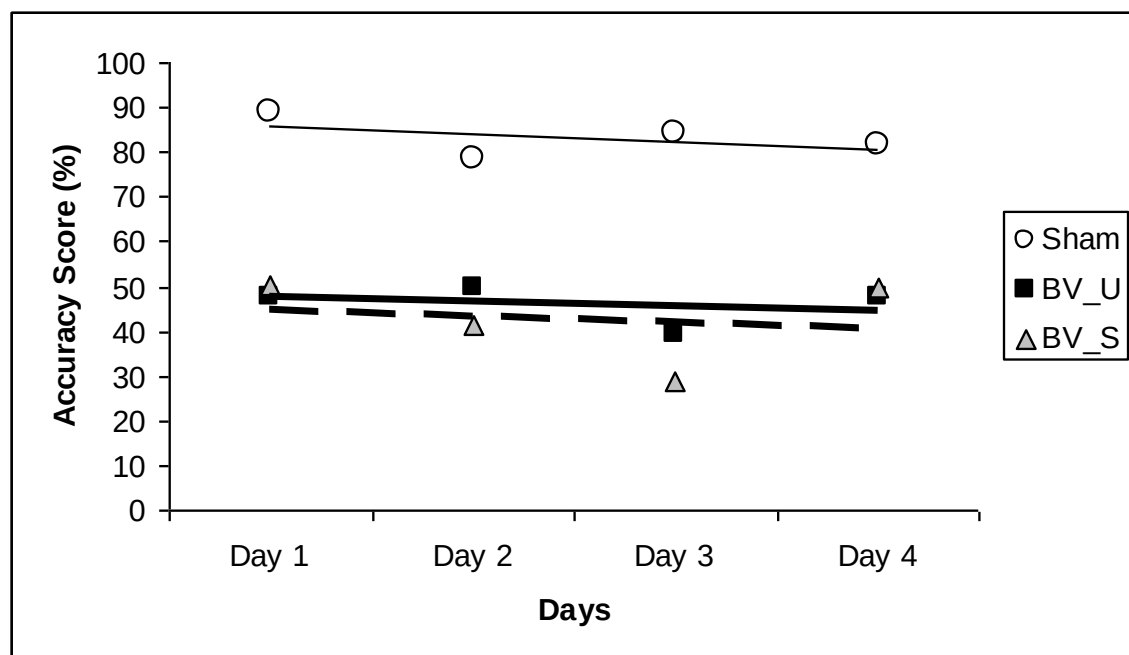


Figure 7.4. The percentage accuracy scores for each group, across the 4 days of the forced alternation test. BV_U subjects are those which had a vestibular lesion but were not stimulated. BV_S subjects are those which had a vestibular lesion and were stimulated.

7.2. EEG Results

7.2.1. Spin Test Data

Thirteen rats, who had received only electrode implantation, and neither sham nor real vestibular lesions, were tested in the spin apparatus. The difference in EEG activity, during spin and move trials, and across subcortical and hippocampal channels, was measured. For data to be included, each subject's EEG had to have defined peaks in the power spectrum, in the theta range, for both subcortical and hippocampal channels. Five rats met these criteria. In each case the hippocampal channel and the subcortical channel with the greatest theta power were selected for further analysis.

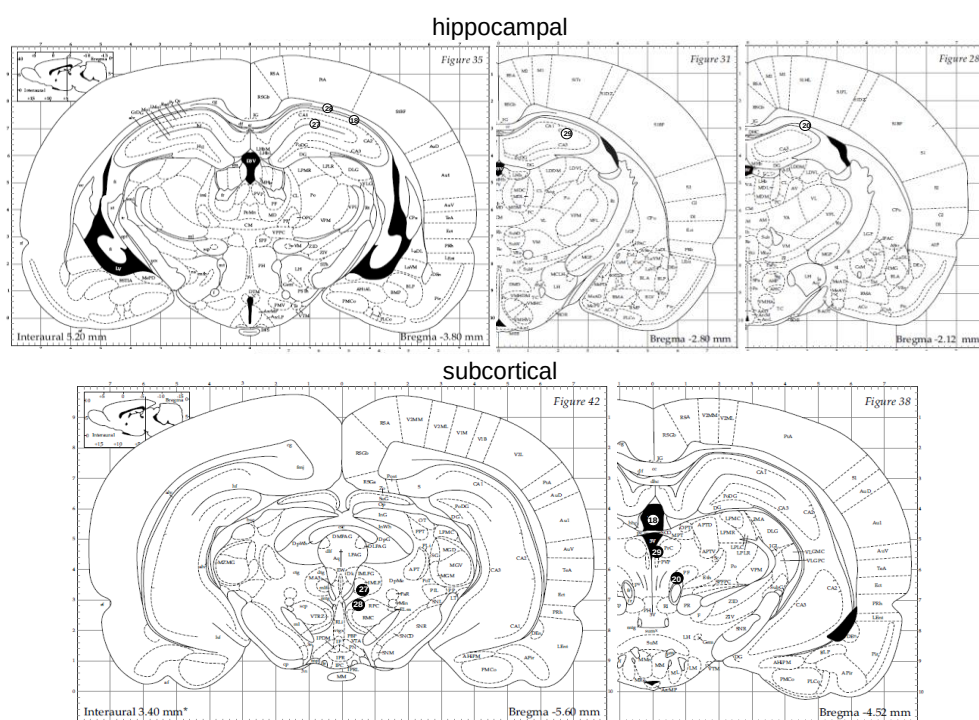


Figure 7.5. Histology results for the spin test. Histology is from the five rats used for the spin test. Slices were prepared by Dr. Phoebe Neo.

Hippocampal Channels: During both move and spin, the hippocampal channels showed a peak of activity at 7Hz (Figure 7.6B). At this frequency, move

appeared to produce slightly more power than spin. In the higher frequency range (9-13Hz), spin appeared to produce more power.

Subcortical Channels: As seen in the hippocampal channels, during both move and spin, the subcortical channels produced a peak of EEG activity at 7Hz (Figure 7.6A). At this frequency, move had more power and a more defined peak of activity. At 9Hz, move and spin seemed to have the same amount of EEG power.

Hippocampus-Subcortical Differences: During movement and spinning, the hippocampal channels in each rat, produced greater EEG power in the theta band than the subcortical channel, with all theta power values in the 4-9Hz range above 1.5 log units for the hippocampal channels and below 1.5 log units for the subcortical channels (Figure 7.6A, B). This difference in the amount of theta power produced by the different channels was significant (channel, $F(1, 24) = 183.38$, $p < 0.001$). The power curves for the different channels showed different variations in relation to frequency (channel x frequency, $F(9, 7128) = 14.02$, $p < 0.001$).

Move Spin Difference: (see Panel D of Figure 7.6) A difference in the shapes of the curves produced by spinning and moving was observed (spin x frequency, $F(9, 7128) = 6.13$, $p < 0.001$; quad x dev, $F(1, 7128) = 22.25$, $p < 0.001$; cub x dev, $F(1, 7128) = 8.01$, $p = 0.005$; quart x dev, $F(1, 7128) = 9.60$, $p = 0.002$). In the 7-10Hz range, the move spin difference appeared to be larger in the subcortical channels, compared to the hippocampal channels. This changed in the lower and higher end frequencies (4-6Hz and 10-13Hz), where the difference between move and spin appeared to be larger in the hippocampal channels. However, these apparent differences between move and spin were not significantly different between the channels (channel x frequency x spin, $F(9, 7128) = 0.34$, $p = 0.960$).

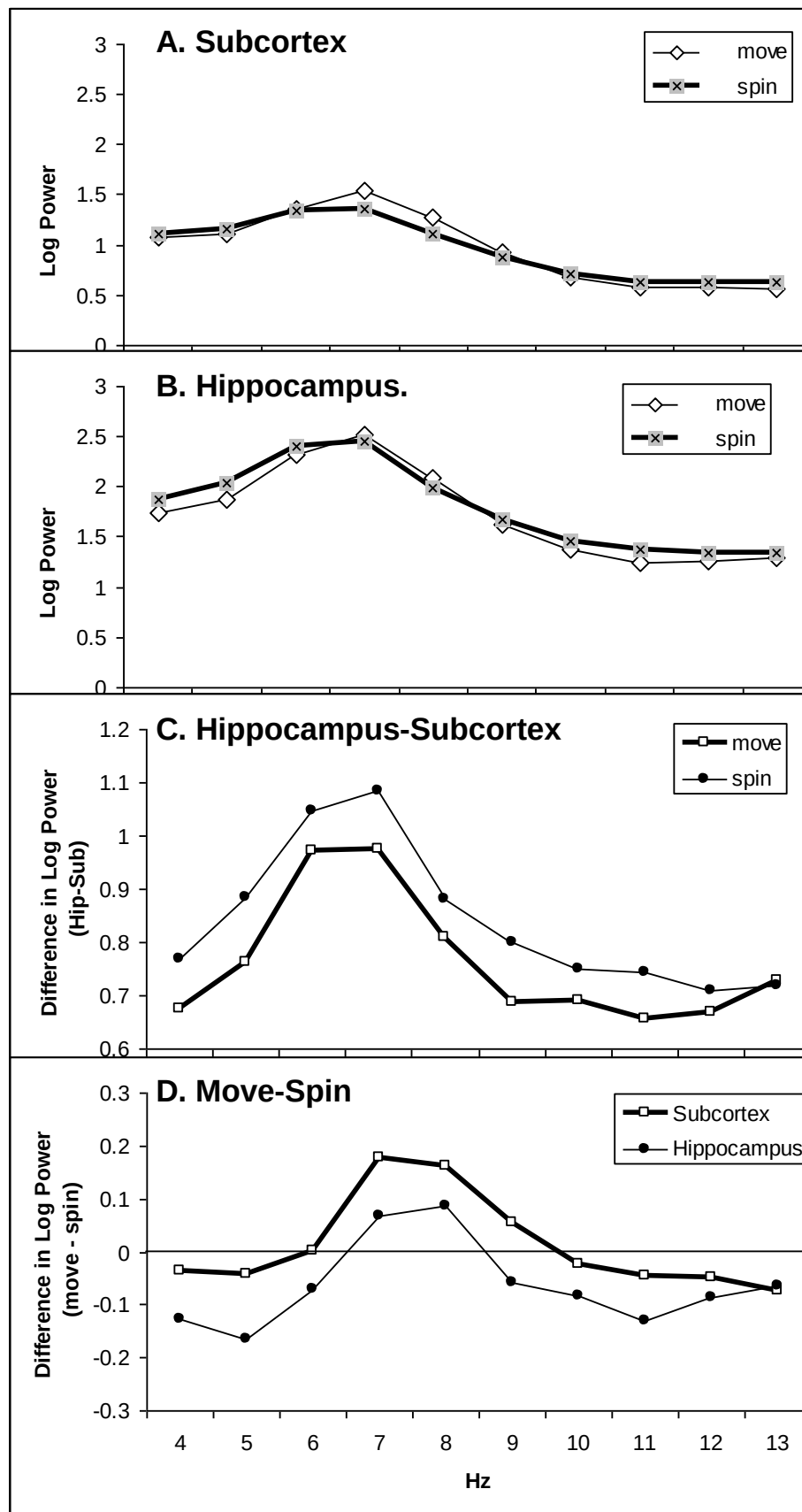


Figure 7.6. Panel A: EEG activity in subcortical channels during move and spin motions. Panel B: EEG activity in hippocampal channels during move and spin motions. Panel C: Difference

between move and spin motions in subcortical and hippocampal channels. Panel D: Move spin difference.

7.2.3. Openfield

All three of the experiment groups showed very small but reliable differences in EEG power while emitting the measured behaviours (refer to page 74 for definitions) in the openfield (Figure 7.7; behaviour x frequency x treatment, $F(40, 25220) = 1.93$, $p = <0.001$).

Move: During movement in the openfield, the stimulated vestibular group showed a peak of EEG activity at 8Hz consistent with the imposed frequency of stimulation (Figure 7.7A). This was at a higher frequency than the sham and non-stimulated vestibular group, both of which had a peak of activity at 7Hz. The stimulated vestibular group had larger EEG power at all frequencies when compared to the sham and non-stimulated vestibular groups. This difference in power appeared to be greatest at 8Hz. The sham and non-stimulated vestibular groups appeared to have very similar power in the 7-14Hz range. The stimulated vestibular group had a more defined curve than the other two groups. The sham group had a slightly more defined peak compared with the non-stimulated vestibular peak.

Rear: When the subjects reared in the openfield, the stimulated vestibular group had a peak of EEG activity at 8Hz (Figure 7.7). This peak of activity was at a higher frequency and had more power than the peak of the sham and non-stimulated vestibular groups. The peak for the sham and non-stimulated vestibular group occurred at 7Hz. This peak appeared to be more defined than the peak which occurred during movement. The stimulated vestibular group had more power at all frequencies than the sham and non-stimulated vestibular groups.

Non-movement: When there was an absence of translational movement in the openfield, the stimulated vestibular group had the greatest EEG power across all the frequencies, when compared to the sham and non-stimulated vestibular groups. The stimulated vestibular group had a peak of EEG activity at 8Hz (Figure 7.7). This was at a higher frequency and of greater power than the peak of the other two groups. The sham and non-stimulated vestibular groups had a peak of activity at 7Hz. This peak was not as defined during stillness as it was during rearing. It also had slightly less power than the peak that occurred during rearing. The difference in EEG power between the stimulated vestibular group and the other two groups was greater during stillness than it was during rearing. The sham and non-stimulated vestibular groups had very similar EEG power from 8-14Hz.

Overall: To summarise, the sham and non-stimulated vestibular groups had peaks of activity at 7Hz during all the behaviours. These groups consistently had less power at all frequencies than the stimulated vestibular group. The sham and non-stimulated vestibular group had very similar EEG activity in the absence of translational movement. The sham group had a more defined peak of activity than the non-stimulated vestibular group, but had similar power. This trend was present in all three behaviours. The stimulated vestibular group had peaks of activity at 8Hz, across all behaviours, consistent with the generation of rhythmic activity by the 7.81Hz stimulation. It appeared the stimulated vestibular group had the most EEG power during movement. Between 7-11Hz, there appeared to be a slight difference in power across the three behaviours, in the stimulated vestibular group. This trend is different to the more uniform activity seen in the sham and non-stimulated vestibular groups EEG across the behaviours.

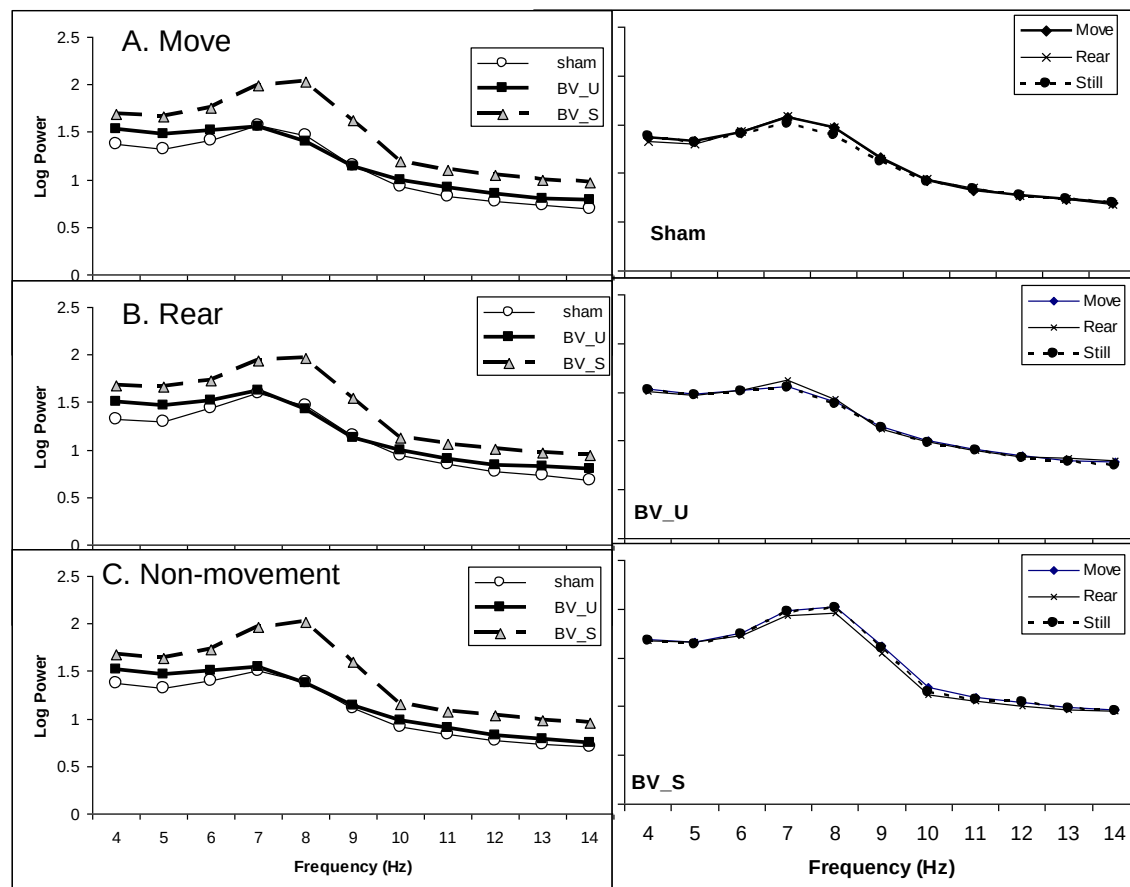


Figure 7.7. EEG power during behaviours in the openfield test. The Y-axes have been LOG transformed. Panel A: Move. Panel B: Rear. Panel C: Non-movement. BV_U subjects are those which had a vestibular lesion but were not stimulated. BV_S subjects are those which had a vestibular lesion and were stimulated.

7.2.4. Elevated T-Maze

Overall, the sham group appeared to have less overall EEG power, compared to the stimulated and non-stimulated vestibular groups, across all the behaviours. However, the form of the curves suggests that this is a function of greater background low frequency noise-like activity, rather than a greater power peak in the theta range. In Figure 7.8, therefore, the data are presented in both the form of the original transform and with an assumed exponential noise function subtracted. The stimulated vestibular group had the largest amount of EEG power, compared to the sham and non-stimulated group, during rearing and non-movement. The non-stimulated

vestibular group had the largest amount of EEG power, compared to the sham and stimulated vestibular group, when subjects were retreating. Both vestibular groups had very similar EEG activity during investigation and moving. Figure 7.8 indicated a difference in EEG, across behaviour and groups (behaviour x frequency x treatment group, $F(80, 9940) = 13.30$, $p = <0.001$). This is explored further in post hoc analysis below.

Investigation Behaviour: When subjects were investigating in the elevated T-maze, both the non-stimulated and stimulated vestibular groups had more EEG power than the sham group, and this was maintained across all the frequencies (Figure 7.8A). Both the sham and the non-stimulated vestibular group had a defined peak at 7Hz. The stimulated vestibular group had a defined peak at 8Hz and had greater power, compared to the other two groups, at this frequency. All groups showed a decrease in EEG power from 9Hz onwards.

Moving Behaviour: During movement in the elevated T-maze, the sham group had less EEG power, across all frequencies, than both the vestibular groups (Figure 7.8B). At 7Hz, the sham group had a defined peak and an increase in EEG power. The non-stimulated vestibular group also had an increase in EEG power at 7Hz. The stimulated vestibular group had the greatest EEG power at a higher frequency of 8Hz. After 8Hz, all groups show a loss of EEG power. A post hoc analysis found these differences to be statistically significant (frequency x treatment group, $F(20, 3140) = 5.43$, $p = <0.001$).

Rearing Behaviour: During rearing in the elevated T-maze, there was an apparent group difference in EEG power, with the sham group having less power, across all the frequencies, than the other two groups (Figure 7.8C). The stimulated vestibular group had a defined peak at 7Hz and had the greatest amount of EEG

power, compared to the two other groups. Both the sham and non-stimulated groups had defined peaks at 7Hz. After 8Hz, all groups showed a gradual decrease in EEG power.

Retreating Behaviour: When animals were retreating, the non-stimulated vestibular group had more EEG power than the sham and stimulated vestibular group, across all the frequencies (Figure 7.8D). The sham group had a marked decrease in EEG power over the 6-8Hz range. The stimulated vestibular group did not show a loss of EEG power until 9Hz, while the non-stimulated vestibular group had a loss of EEG power at a lower frequency of 7Hz. The sham and non-stimulated vestibular groups had a peak of EEG activity at 6Hz, however, the sham group had greater EEG power at this frequency. The stimulated vestibular group showed a peak of activity at 8Hz and this was of similar power shown by the sham group.

Non-movement: When there was a lack of translational movement (still or remaining in one place while grooming) in the elevated T-maze, EEG activity was different in each group (Figure 7.8E). Overall, the sham group had the least EEG power, while the stimulated vestibular group had the greatest EEG power. The sham and non-stimulated groups had a defined peak at 7Hz, with the sham group having greater EEG power at this frequency. The stimulated vestibular group had a defined peak at 8Hz, with slightly less power than that shown by the sham group during their peak of activity. From 8Hz, the sham group showed a steady decay of EEG power throughout the higher frequencies. The stimulated vestibular group maintained a larger amount of EEG power across the higher frequencies when compared to the non-stimulated and sham groups.

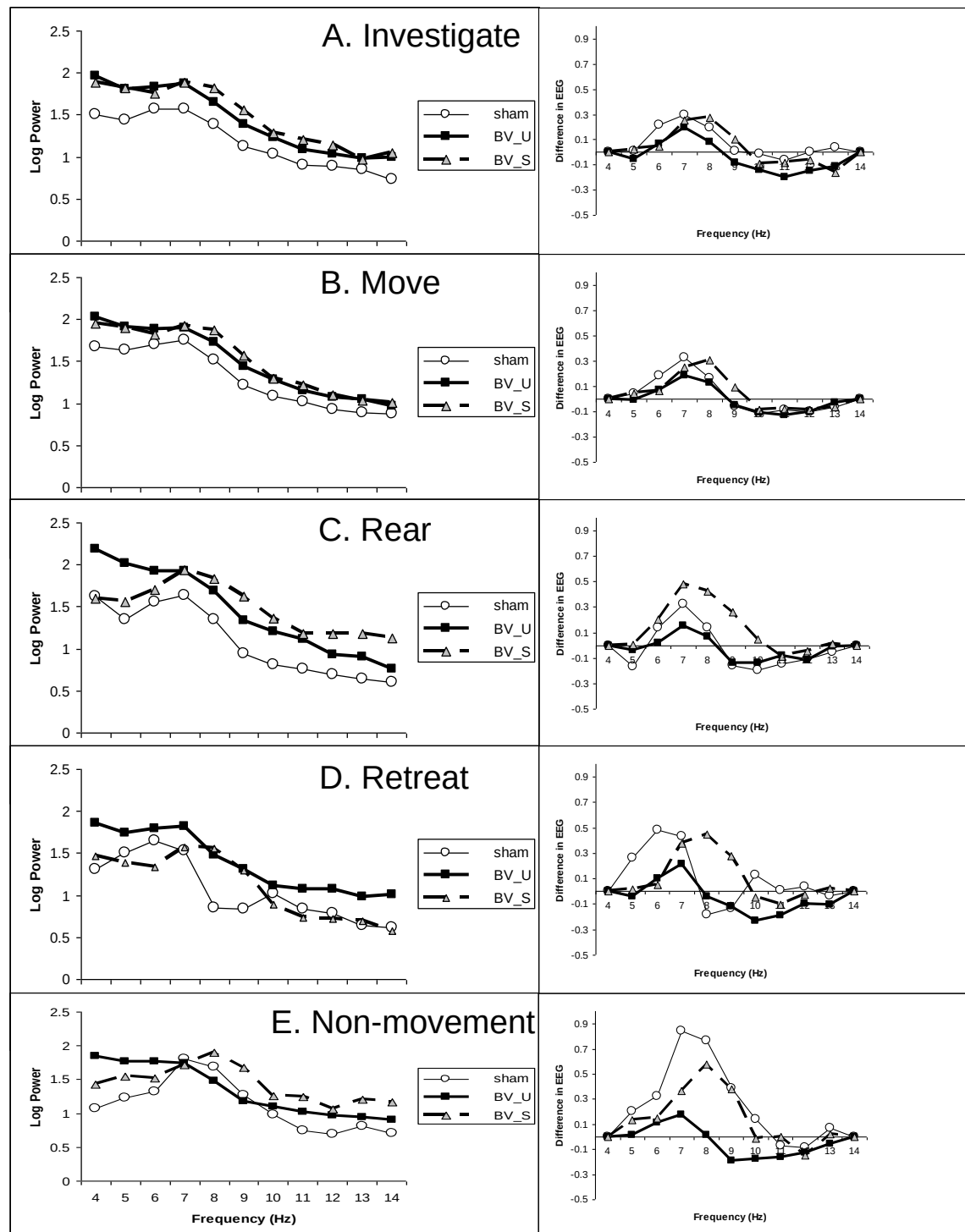


Figure 7.8. EEG power during behaviours in the elevated T-maze. Panels on the right are detrended versions of their matching panel on the left. The Y-axis of all the graphs are LOG transforms of the original data. Panel A: Investigate. Panel B: Move. Panel C: Rear. Panel D: Retreat. Panel E: Non-movement. BV_U subjects are those which had a vestibular lesion but were not stimulated. BV_S subjects are those which had a vestibular lesion and were stimulated.

A further comparison of the differences in the EEG power curves focussing on differences between the measured behaviours was completed for each group.

Sham Group: After the subtraction of background noise, the power curve of sham group appeared to be larger and more defined when there was an absence of movement (Figure 7.9A). Retreating caused a defined peak in EEG activity at 6Hz, which was a lower frequency of peak activity than what was observed during the other behaviours. All other behaviours had a peak of activity at 7Hz. Overall, the sham group showed marked differences in EEG activity during different behaviours.

Non-stimulated Vestibular Group: The non-stimulated vestibular group showed very similar EEG activity, across all frequencies and across all behaviours (Figure 7.9B). There was a peak of EEG activity at 7Hz, during all the behaviours, and the power at this frequency was very similar in each of the behaviours.

Stimulated Vestibular Group: EEG power was at its greatest during non-movement and rearing behaviours (Figure 7.9C). This was similar to the sham group, who showed an increase in EEG power during an absence of movement. During rearing, there was a peak of activity at 7Hz. All other behaviours caused a peak in EEG activity at 8Hz. The stimulated vestibular group showed less of a difference in EEG activity across behaviours when compared to the sham group. However, the stimulated vestibular group had more of a difference in EEG activity across behaviours, when compared to the non-stimulated vestibular group.

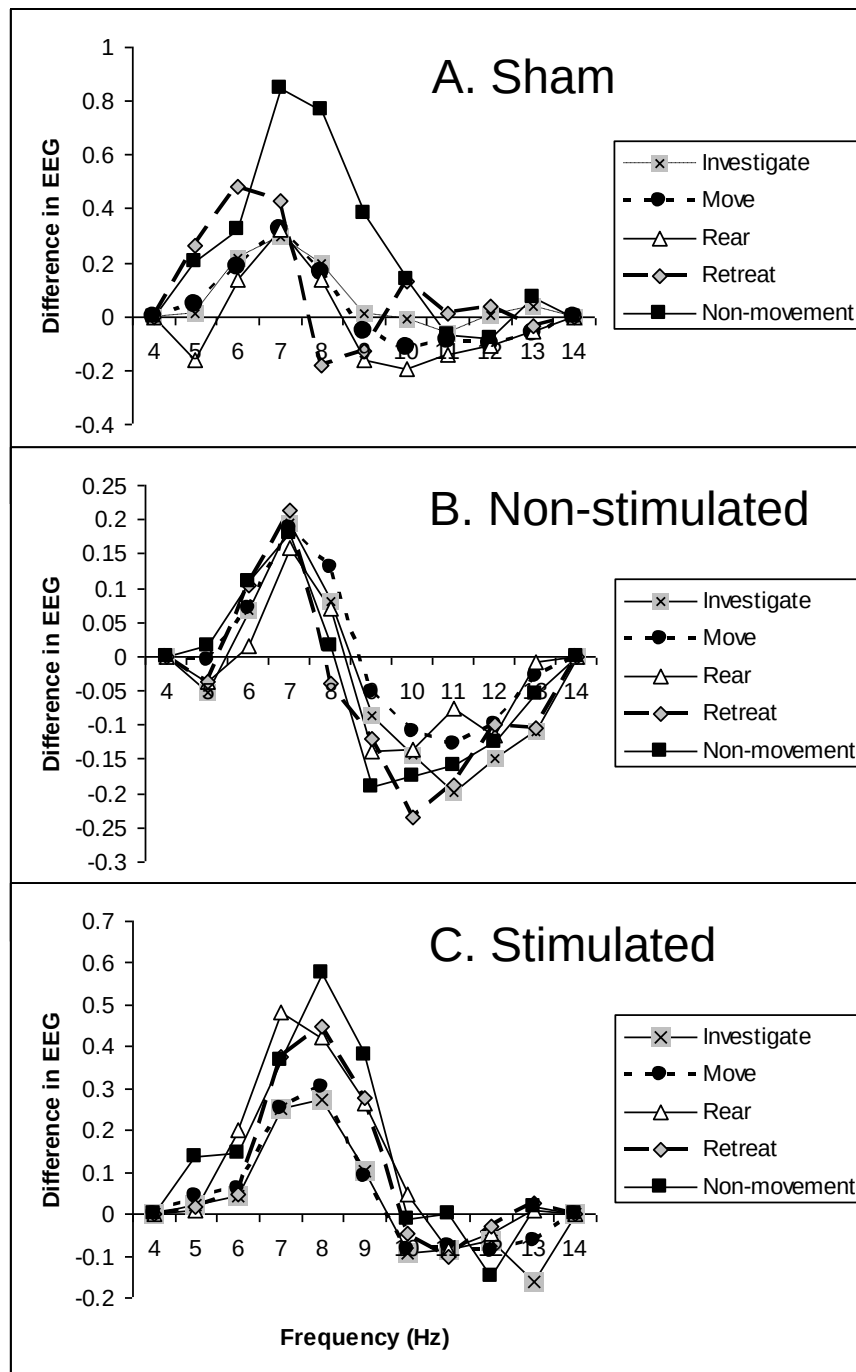


Figure 7.9. Group EEG activity during the measured behaviours in the elevated T-maze. Y-axis scales are not uniform.

8. Discussion

8.1. Behavioural Results

The behavioural data demonstrated that stimulation given to BVD rats did not reverse the cognitive or emotional deficits associated with bilateral vestibular lesions. In the openfield test, vestibular lesioned rats reared less compared to shams and stimulation did not reverse this. In the elevated T-maze test, the lesion appeared, if anything, to reduce anxiety and stimulation seemed to increase this anxiolytic effect. The shams displayed the opposite effect, taking longer on each trial to leave the closed arm, a sign of anxiety. During the escape trials in the elevated T-maze (a test of fear) there were no reliable differences in the latencies of the groups. The forced alternation test showed that stimulation did not reverse the cognitive deficit associated with bilateral vestibular lesions. Both the stimulated and non-stimulated vestibular groups consistently performed with an accuracy of 50%. This differed to the shams who demonstrated they had learnt the task, with an accuracy score of approximately 80% on all the trials. In general, the behavioural results indicated that the emotional effects of the bilateral lesions were not large in contrast to the cognitive effects.

8.2. EEG Results

The EEG data showed that the hippocampus produced more EEG power than the subcortical areas during both movement and spinning. The type of motion (movement or spinning) did not change the amount of EEG power being produced in the hippocampus. This suggested that the hippocampus reliably responds to all types of theta-producing motion stimuli and that spinning activated the same general theta pathways that are activated during movement. The EEG recorded from the non-stimulated BVD rats in the openfield test did not show a clear theta deficit, with the

non-stimulated rats having slightly more absolute EEG power at 7Hz compared to the sham groups EEG. If the openfield graphs had been de-trended this power difference would have been marginally reversed. Interestingly, when the de-trended elevated T-maze graphs are considered, the non-stimulated BVD group did appear to have an obvious theta deficit. This de-trended theta deficit was present in all of the behaviours measured during the elevated T-maze test. The stimulation produced a peak of activity at 8Hz during all of the behaviours with a power that reversed any reduction in theta there may have been as a result of the lesion. This was slightly higher frequency than the sham group which had a peak of activity at 7Hz.

8.3. Behavioural Effects of the Lesions

In the present study the BVD rats spent less time compared to shams engaged in rearing behaviour. The lesioned animals also appeared to be less anxious than the shams in the elevated T-maze test, with the lesioned group having a decreased latency to leave the closed arm during the avoidance trials. Based on these findings, it was concluded that the vestibular lesions caused anxiolytic behaviour in the present experiment. Vestibular rats in the present experiment also demonstrated a loss of spatial memory, with the vestibular groups unable to learn the forced alternation task.

Rats' behaviour in the present study's openfield test differed to the behaviour of vestibular lesioned rats in the Goddard et al. (2008) study. Vestibular lesioned rats in that experiment reared more often and spent more time in the rearing position compared to shams. It is possible that because a low stress version of the openfield test was used in the present study the difference in rearing can be attributed to the difference in perceived risk (which is influenced by the level of stress being experienced), rather than the effect of the bilateral lesion on the animals behaviour. Rearing is a behaviour that is indicative of risk assessment and is sensitive to the

effects of anxiolytic drugs (Gray & McNaughton, 2000). Gray and McNaughton (2000) described the relationship between rearing and perceived risk as being an inverted U-shape. When there is either little potential threat or a high level of potential threat the frequency of rearing is low. If the perceived risk is intermediate then the frequency of rearing is at its greatest. When animals are given anxiolytic drugs rearing frequency changes as a function of the level of perceived threat. Specifically, the anxiolytic moves the frequency of the behaviour towards the level associated with lower perceived threat. Anxiolytic-treated animals in a low stress version of the openfield test have decreased levels of rearing, whereas anxiolytic treated animals in the high stress version of the openfield have increased levels of rearing.

An anxiolytic action of BVD has also been reported in other studies. Zheng et al. (2008) found that at three months after BVD surgery, the rats had an increase in the number of open arm entries in the elevated plus maze. Although in the present study latency to leave the closed arm and enter the open arm was measured in the elevated T-maze, instead of number of arm entries, both are measures of anxiety. Zheng et al. (2008) believed that the vestibular lesion caused a deficit in learned inhibitory avoidance which led to a deficit in the appraisal of potentially threatening stimuli. Further, Goddard et al. (2008) reported that vestibular damage interferes with the transmission of serotonin and dopamine, two biogenic amines that have a large role in anxiety related behaviours. So, as far as the discussed literature goes, the emotional and cognitive deficits found in the rats in the present experiment match those found in previous studies.

A limitation with the present study is that the increased height of the open arm walls in the elevated T-maze could have reduced the aversive effects of the open arms

to some extent. However, this effect would be the same for all of the groups tested in the vestibular experiment, including sham controls.

8.4. *Theta Effects*

The EEG data are consistent with a theta deficit in BVD rats. This means that changes in theta could account for the bulk of the behavioural changes observed in the subjects of the present experiment. This theta deficit was not as clear in the elevated T-maze data before de-trending. The general form of these curves suggested that there was background noise occurring in the vestibular rats but not the sham rats. One possible explanation of this is the large amount of forward and backward head wagging in which vestibular rats engage. The apparent replication of reduced theta in BVD rats was only possible when this background noise was eliminated after the data was de-trended and so must be treated as tentative. A limitation of using de-trended data is that by de-trending you are removing background noise and other artefacts. If the de-trending is too extensive, actual data values may be removed. This then raises the question of how accurate the de-trended representation is.

Previous research has reported that bilateral vestibular lesions cause a loss of theta rhythmicity in the hippocampus (Russell et al., 2006). This finding was supported by the present experiment's results which showed that the lesions did cause a loss of theta in the hippocampus. A loss of theta rhythm in the hippocampus has also been associated with a loss of spatial memory and processing (Stackman & Herbert, 2002; Stackman et al., 2002).

8.5. *Stimulation Effects*

The EEG recordings in the present study indicated that stimulation of the medial septum in vestibular lesioned rats created theta rhythm in the hippocampus.

The conclusion that stimulation did produce theta driving (as determined by oscilloscope observation during testing) in the hippocampus of BVD rats was supported by the finding that in each test, the stimulated rats had the greatest EEG power and peaks of activity at 8Hz, the frequency band of the imposed 7.81Hz stimulation. These 8Hz peaks of activity were higher frequency than the peaks of activity seen in the non-stimulated and sham rats.

Theta restoration is usually associated with restoration of cognitive functioning. A deficit in spatial memory was recorded in the BVD rats in the present study but restoring theta power and rhythmicity did not repair this deficit. This was observed when the stimulated vestibular rats could not learn the forced alternation task. The stimulation did not reverse the emotional deficits caused by the lesion either. In the elevated T-maze test, stimulated BVD rats in the present study produced behaviour in the avoidance trials that was indicative of reduced anxiety. Combining the present study's finding with the previous literature, it could be possible that the stimulation in this study produced a decreased level of anxiety which added to the moderate anxiolytic effect of the lesion.

A limitation of this study is that the frequency of stimulation could have been incorrect. The control rats in the spin test consistently produced hippocampal theta rhythm during both move and spin at 7Hz. The sham rats in the openfield and elevated T-maze tests also produced theta rhythm at 7Hz. This is a lower frequency than that produced by the stimulation which was 8Hz, and this was consistent across all tests. The power of the theta curve was also at a higher level in the stimulated rats and stayed at a higher power across all the frequencies compared to EEG from the sham and non-stimulated rats. While previous results could suggest that large amounts of high frequency theta produce optimum performance, it is possible that the

restoration of theta must be at the specific frequency required by a specific situation to be effective. That is both higher than normal and lower than normal theta frequency are detrimental.

8.6. Implications of Rat Research When Compared to Human Research

The spatial deficits observed in the vestibular lesioned rats have been found in humans suffering from vestibular damage. Vestibular damaged humans can not successfully complete path integration tasks or virtual water maze tasks (Smith et al., 2005a; Brandt et al., 2005), which is the same for BVD rats. Changes in emotionality after vestibular damage are also observed in humans. At this stage the majority of the literature suggests that vestibular damage in rats is anxiolytic (Goddard et al., 2008) while vestibular damage in humans is anxiety provoking, with some exceptions (Staab, 2006). It is only on the emotional changes caused by vestibular damage where the human and rat literature begin to diverge, possibly because the emotional effects are more labile than the spatial effects.

Most vestibular patients suffer from vertigo and oscillopsia (Ramos, 2006). These symptoms then cause the patient to become overly sensitive to the accompanying sensations and display agoraphobic behaviour and behaviour that is indicative of an anxiety disorder (Staab, 2006). However, these behaviours can be remedied when the patient undergoes vestibular rehabilitation therapy or cognitive behavioural therapy (Meli et al., 2009). So, the conclusion is that vestibular damage in humans does not cause anxiety, it is the negative interpretation of the symptoms that leads to the anxiety related behaviour rather than the development of an organic anxiety disorder (Godemann et al., 2005).

Simply restoring theta to the hippocampus after vestibular lesions is not enough to repair the cognitive and emotional deficits caused by the lesions. Secondly, the stimulation used in this study is not a viable treatment option for people suffering from a vestibular disorder. This is because the stimulation did not restore spatial memory function or alleviate the emotional deficits. If the stimulation did not work in an animal model it is unlikely it would work in humans. It must also be noted that because humans have conscious awareness and are capable of forethought, a whole extra dimension is added to the emotional effects of their vestibular damage.

8.7. Future Research

In future it would be beneficial to provide a definitive answer to how best reverse the cognitive and emotional deficits caused by vestibular lesions, since it is apparent that restoring theta is not enough. It would also be worthwhile to examine the effects of vestibular lesions in humans, while isolating the effects of our unique ability of conscious thought and worry about future events. The role of negative thought in the severity of the vestibular symptoms is where the human literature begins to produce different results to those reported in the rat literature.

8.8. General Summary

Bilateral vestibular lesions in the rat produce cognitive, specifically spatial memory deficits, and moderate emotional deficits. Stimulation of the medial septum normally restores theta to the hippocampus after it has been blocked. In this experiment the stimulation was successful in generating theta but not enough to repair the cognitive and emotional deficits. Rat and human vestibular literature appears to differ in the effects of vestibular damage on the emotional processes of the subject. This difference is somewhat supported by the present study and can be explained by

humans being overly sensitive to the negative physical sensations associated with vestibular damage and being able to think about it before it occurs. As a treatment for humans, the specific stimulation protocol used in this study is not suitable (although a different protocol might be) and further research is needed to find an appropriate treatment.

References

- Angelaki, D., & Cullen, K. (2008). Vestibular system: The many facets of a multimodal sense. *Annu. Rev. Neurosci.*, 31, 125-150.
- Angelaki, D., Klier, E., & Snyder, L. (2009). A vestibular sensation: Probabilistic approaches to spatial perception. *Neuron*, 64, 448-461.
- Balaban, C. (2002). Neural substrates linking balance control and anxiety. *Physiology & Behavior*, 77, 469-475.
- Balaban, C. (2004). Projections from the parabrachial nucleus to the vestibular nuclei: Potential substrates for autonomic and limbic influences on vestibular responses. *Brain Research*, 996, 126-137.
- Bland, B., & Oddie, S. (2001). Theta band oscillation and synchrony in the hippocampal formation and associated structures: The case for its role in sensorimotor integration. *Behavioural Brain Research*, 127, 119-136.
- Barmack, N. (2003). Central vestibular system: Vestibular nuclei and posterior cerebellum. *Brain Research Bulletin*, 60, 511-541.
- Berry, S., & Thompson, R. (1978). Prediction of learning rate from the hippocampal electroencephalogram. *Science*, 200, 1298-1300.
- Best, C., Tschan, R., Eckhardt-Henn, A., & Dieterich, M. (2009). Who is at risk for ongoing dizziness and psychological strain after a vestibular disorder? *Neuroscience*, 164, 1579-1587.
- Biazoli Jr, C., Goto, M., Campos, A., & Canteras, N. (2006). The supragenual nucleus: A putative relay station for ascending vestibular signals to head direction cells. *Brain Research*, 1094, 138-148.

- Blair, H., Cho, J., & Sharp, P. (1998). Role of the lateral mammillary nucleus in the rat head direction circuit: A combined single unit recording and lesion study. *Neuron*, 21, 1387-1397.
- Bland, B., & Oddie, S. (2001). Theta band oscillation and synchrony in the hippocampal formation and associated structures: The case for its role in sensorimotor integration. *Behavioral Brain Research*, 127, 119-136.
- Bland, B., Seto, M., Sinclair, B., & Fraser, S. (1984). The pharmacology of hippocampal theta cells: Evidence that the sensory processing correlate is cholinergic. *Brain Research*, 299, 121-131.
- Bland, B., & Vanderwolf, C. (1972). Electrical stimulation of the hippocampal formation: Behavioral and bioelectrical effects. *Brain Research*, 43, 89-106.
- Brandt, T., Schautzer, F., Hamilton, D., Brüning, R., Markowitsch, H., Kalla, R., Darlington, C., Smith, P., & Strupp, M. (2005). Vestibular loss causes hippocampal atrophy and impaired spatial memory in humans. *Brain*, 128, 2732-2741.
- Derryberry, D., & Tucker, D. (1992). Neural mechanisms of emotion. *Journal of Consulting and Clinical Psychology*, 60, 329-338.
- Dieterich, M., & Brandt, T. (2008). Functional brain imaging of peripheral and central vestibular disorders. *Brain*, 131, 2538-2552.
- Feldon, J., & Rawlins, J. (1978). The anatomical basis of hippocampal theta rhythm. *Journal of Physiology*, 284, 81.
- Fukushima, K. (1997). Corticovestibular interactions: Anatomy, electrophysiology, and functional considerations. *Exp. Brain. Res.*, 117, 1-16.
- Furman, J., & Jacob, R. (1997). Psychiatric dizziness. *Neurology*, 48, 1161-1166.

- Gengler, S., Mallot, H., & Holscher, C. (2005). Inactivation of the rat dorsal striatum impairs performance in spatial tasks and alters hippocampal theta in the freely moving rat. *Behavioural Brain Research*, 164, 73-82.
- Goddard, M., Zheng, Y., Darlington, C., & Smith, P. (2008). Locomotor and exploratory behaviour in the rat following bilateral vestibular deafferentation. *Behavioral Neuroscience*, 122, 448-459.
- Godemann, F., Linden, M., Neu, P., Heipp, E., & Dörr, P. (2004). A prospective study on the course of anxiety after vestibular neuronitis. *Journal of Psychosomatic Research*, 56, 351-354.
- Godemann, F., Siefert, K., Hantschke-Brüggemann, M., Neu, P., Seidl, R., & Ströhle, A. (2005). What accounts for vertigo one year after neuritis vestibularis-anxiety or a dysfunctional vestibular organ? *Journal of Psychiatric Research*, 39, 529-534.
- Gray, J., & McNaughton, N. (2000). *The neuropsychology of anxiety: an enquiry into the functions of the septo-hippocampal system* (2nd edition, revised). New York: Oxford University Press.
- Holmberg, J., Karlberg, M., Harlacher, U., & Magnusson, M. (2005). Experience of handicap and anxiety in phobic postural vertigo. *Acta Oto-Laryngologica*, 125, 270-275.
- Horii, A., Uno, A., Kitahara, T., Mitani, K., Masumura, C., Kizawa, et al. (2007). Effects of fluvoxamine on anxiety, depression, and subjective handicaps of chronic dizziness patients with or without neuro-otologic diseases. *Journal of Vestibular Research*, 17, 1-8.

Hüfner, K., Hamilton, D., Kalla, R., Stephan, T., Glasauer, S., Ma, J., et al. (2007).

Spatial memory and hippocampal volume in humans with unilateral vestibular deafferentation. *Hippocampus*, 17, 471-485.

Jacob, R., Furman, J., Durrant, J., & Turner, S. (1996). Panic, agoraphobia, and vestibular dysfunction. *The American Journal of Psychiatry*, 153, 503-512.

James, D., McNaughton, N., Rawlins, J., Feldon, J., & Gray, J. (1977). Septal driving of hippocampal theta rhythm as a function of frequency in the free-moving male rat. *Neuroscience*, 2, 1007-1012.

Kalueff, A., Ishikawa, K., & Griffith, A. (2008). Anxiety and otovestibular disorders: Linking behavioral phenotypes in men and mice. *Behavioural Brain Research*, 186, 1-11.

Lalonde, R. (2002). The neurobiological basis of spontaneous alternation. *Neuroscience and Biobehavioral Reviews*, 26, 91-104.

Lindsay, L., Liu, P., Gliddon, C., Zheng, Y., Smith, P., & Darlington, C. (2005).

Cytosolic glucocorticoid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. *Exp. Brain. Res.*, 162, 309-314.

Liu, P., Zheng, Y., King, J., Darlington, C., & Smith, P. (2003). Long-term changes in hippocampal N-methyl-D-aspartate receptor subunits following unilateral vestibular damage in rat. *Neuroscience*, 117, 965-970.

Matsuyama, T., Kayahara, T., Nomura, J., & Nakano, K. (1996). Direct projections from the medial vestibular nucleus to the posterior hypothalamic area in the monkey (*Macaca fuscata*). *Neuroscience Letters*, 219, 199-202.

McDonald, R., & White, N. (1995). Hippocampal and nonhippocampal contributions to place learning in rats. *Behavioral Neuroscience*, 109, 579-593.

- Meli, A., Zimatore, G., Badaracco, C., De Angelis, E., & Tufarelli, D. (2007). Effects of vestibular rehabilitation therapy on emotional aspects in chronic vestibular patients. *Journal of Psychosomatic Research*, 63, 185-190.
- Monzani, D., Casolari, L., Guidetti, G., & Rigatelli, M. (2001). Psychological distress and disability in patients with vertigo. *Journal of Psychosomatic Research*, 50, 319-323.
- Morris, R. G. M., Garrud, P., Rawlins, I. N. P., & O'Keefe, I. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, 297, 681-683.
- Oddie, S., & Bland, B. (1998). Hippocampal formation theta activity and movement selection. *Neuroscience and Biobehavioral Reviews*, 22, 221-231.
- O'Keefe, J. (1993). Hippocampus, theta, and spatial memory. *Current Opinion in Neurobiology*, 3, 917-924.
- O'Keefe, J. & Nadel, L. (1979). Precis of O'Keefe and Nadel's The hippocampus as a cognitive map. *The Behavioral and Brain Sciences*, 2, 487-583.
- Perna, G., Dario, A., Caldirola, D., Stefania, B., Cesarani, A., & Bellodi, L. (2001). Panic disorder: The role of the balance system. *Journal of Psychiatric Research*, 35, 279-286.
- Ramos, R. (2006). Antidepressants and dizziness. *Journal of Psychopharmacology*, 20, 708-713.
- Rawlins, J., Feldon, J., & Gray, J. (1979). Septo-hippocampal connections and the hippocampal theta rhythm. *Experimental Brain Research*, 37, 49-63.
- Redfern, M., Furman, J., & Jacob, R. (2007). Visually induced postural sway in anxiety disorders. *Journal of Anxiety Disorders*, 21, 704-716.

- Russell, N., Horii, A., Smith, P., Darlington, C., & Bilkey, D. (2003). Long-term effects of permanent vestibular lesions on hippocampal spatial firing. *The Journal of Neuroscience*, 23, 6490-6498.
- Russell, N., Horii, A., Smith, P., Darlington, C., & Bilkey, D. (2006). Lesions of the vestibular system disrupt hippocampal theta rhythm in the rat. *J. Neurophysiol.*, 96, 4-14.
- Smith, P. (1997). Vestibular-hippocampal interactions. *Hippocampus*, 7, 465-471.
- Smith, P., Horii, A., Russell, N., Bilkey, D., Zheng, Y., Liu, P., et al. (2005a). The effects of vestibular lesions on hippocampal function in rats. *Progress in Neurobiology*, 75, 391-405.
- Smith, P., Zheng, Y., Horii, A., & Darlington, C. (2005b). Does vestibular damage cause cognitive dysfunction in humans? *Journal of Vestibular Research*, 15, 1-9.
- Snyder, L. (1999). This way up: Illusions and internal models in the vestibular system. *Nature Neuroscience*, 2, 396-398.
- Staab, J. (2006). Chronic dizziness: the interface between psychiatry and neuro-otology. *Current Opinion in Neurology*, 19, 41-48.
- Staab, J., Ruckenstein, M., Solomon, D., & Shepard, N. (2002). Serotonin reuptake inhibitors for dizziness with psychiatric symptoms. *Arch. Otolaryngol. Head. Neck. Surg.*, 128, 554-560.
- Stackman, R., Clark, A., & Taube, J. (2002). Hippocampal spatial representations require vestibular input. *Hippocampus*, 12, 291-303.
- Stackman, R., & Herbert, A. (2002). Rats with lesions of the vestibular system require a visual landmark for spatial navigation. *Behavioural Brain Research*, 128, 27-40.

- Stackman, R., & Taube, J. (1997). Firing properties of head direction cells in the rat anterior thalamic nucleus: Dependence on vestibular input. *The Journal of Neuroscience*, 17, 4349-4358.
- Stein, M., Asmundson, G., Ireland, D., & Walker, J. (1994). Panic disorder in patients attending a clinic for vestibular disorders. *The American Journal of Psychiatry*, 151, 1697-1700.
- Strupp, M., & Brandt, T. (2006). Pharmacological advances in the treatment of neuro-otological and eye movement disorders. *Curr. Opin. Neurol.*, 19, 33-40.
- Taube, J. (1995). Head direction cells recorded in the anterior thalamic nuclei of freely moving rats. *The Journal of Neuroscience*, 15, 70-86.
- Tecer, A., Tükel, R., Erdamar, B., & Sunay, T. (2004). Audiovestibular functioning in patients with panic disorder. *Journal of Psychosomatic Research*, 57, 177-182.
- Teggi, R., Caldirola, D., Fabiano, B., Recanati, P., & Bussi, M. (2009). Rehabilitation after acute vestibular disorders. *The Journal of Laryngology & Otology*, 123, 397-402.
- Vanderwolf, C. (1969). Hippocampal electrical activity and voluntary movement in the rat. *Electroencephalography and Clinical Neurophysiology*, 26, 407-418.
- de Waele, C., Baudonniere, P., Lepecq, J., Tran Ba Huy, P., & Vidal, P. (2001). Vestibular projections in the human cortex. *Exp. Brain. Res.*, 141, 541-551.
- Wetzel, W., Ott, T., & Matthies, H. (1977). Hippocampal rhythmic slow activity ("theta") and behavior elicited by medial septal stimulation in rats. *Behavioral Biology*, 19, 534-542.
- Whishaw, I., & Vanderwolf, C. (1973). Hippocampal EEG behavior: Changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous

and learned movement patterns in rats and cats. *Behavioral Biology*, 8, 461-484.

Winston, J. (1974). Patterns of hippocampal theta rhythm in the freely moving rat.

Electroencephalography and Clinical Neurophysiology, 36, 291-301.

Winston, J. (1978). Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. *Science*, 201, 160-163.

Zangrossi, H., & Graeff, F. (1997). Behavioral validation of the elevated T-maze, a new animal model of anxiety. *Brain Research Bulletin*, 44, 1-5.

Zheng, Y., Goddard, M., Darlington, C., & Smith, P. (2008). Effects of bilateral vestibular deafferentation on anxiety-related behaviours in Wistar rats. *Behavioural Brain Research*, 193, 55-62.

Zheng, Y., Kerr, S., Darlington, C., & Smith, P. (2003). Unilateral inner ear damage results in lasting changes in hippocampal CA1 field potentials in vitro. *Hippocampus*, 13, 873-878.

Zheng, Y., Smith, P., & Darlington, C. (1999). Noradrenaline and serotonin levels in the guinea pig hippocampus following unilateral vestibular deafferentation. *Brain Research*, 836, 199-202.