Glutamate Receptors in the Medial Temporal Lobe Following Bilateral Vestibular Deafferentation

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Abstract

Dysfunction of the vestibular system can be caused by a number of conditions including Meniere's disease and aminoglycoside antibiotic induced damage. Bilateral vestibular damage results in symptoms such as vertigo, dysequilibrium and dysfunctional eye movements. Studies of vestibular loss have also documented decreases in the volume of the hippocampus, and electrophysiological changes such as alterations in place cell activity and theta rhythm. The hippocampus is an area of the brain associated with memory and learning, and vestibular damage can result in long-term cognitive problems, including spatial and non-spatial memory deficits. It is poorly understood what molecular changes occur in the hippocampus that lead to these alterations in function and structure. This study measured N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors levels in the entorhinal cortex (EC), perirhinal cortex, frontal cortex (FC) and the CA1, CA2/3 and dentate gyrus (DG) sub-regions of the rat hippocampus, at 24 hours, 72 hours, 1 week and 1 month following bilateral vestibular deafferentation (BVD) surgery. NMDA and AMPA receptor subunit levels were assessed because they are the two of the most important classes of ionotropic glutamate receptors in the hippocampus and are known to be involved in synaptic plasticity and memory. The protein kinase CaMKII and its active form, phosphorylated CaMKII, were also measured post-BVD, as it is a downstream signalling molecule that has a role in both NMDA and AMPA receptor activity. There was no significant difference between BVD and sham animals in the expression of individual receptor subunits in any of the brain regions at any time point following a one way analysis of variance. However, two multivariate statistical analysis techniques were used to analyse the relationship between variables within the treatment groups linear discriminant analysis detected a linear discriminant function based on 8 variables from these data that could distinguish between BVD and sham rats with 100% accuracy. These variables were: CaMKII at 24 hours in the DG, GluR2 at 1 week in the CA1, GluR4 at 1month in the EC, NR1 at 1 week in the CA1, NR1 at 1 week in the DG, NR1 at 1 month in the DG, NR1 at 1 month in the FC and NR2B at 1 month in the FC. Cluster analysis provided further information on the relationship between these 8 variables following BVD surgery. These results indicate that while the expression of individual glutamate receptor subunits did not change significantly when comparing BVD rats with controls that had undergone sham surgery, there was a change in the relationship between these variables following bilateral vestibular loss. Although BVD results in a number of electrophysiological and behavioural alterations, the

neurochemical changes appear to be more subtle and spread across a number of brain regions. Further studies need to be performed to assess changes in NMDAR and AMPAR affinity, efficacy and neuronal location in the medial temporal lobe of BVD animals.

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List of Abbreviations

ACh	acetylcholine
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate
ANOVA	analysis of variance
AP5	amino-5-phosphonovaleric acid
APV	aminophosphonovaleric acid
CA1	cornu ammonis area 1
CA2/3	cornu ammonis area 2 and 1
CaMKII	calcium/calmodulin-dependent kinase II
CNS	central nervous system
BVD	bilateral vestibular deafferentation
BVL	bilateral vestibular loss
DPMv	dorsal premotor cortex
EC	entorhinal cortex
EEG	electroencephalography
fEPSP	field excitatory postsynaptic potential
FC	frontal cortex
LDA	linear discriminant analysis
LTD	long term potentiation
LTP	long term depression
MK-801	(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine maleate
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
MVN	medial vestibular nucleus
NF-L	neurofilament-L
NMDA	N-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
PC	perirhinal cortex
PCR	polymerase chain reaction
PET	positron emission tomography
PKA	adenosine 3',5'-monophosphate (cAMP)-dependent protein kinase
PKC	protein kinase C
PKMζ	protein kinase M ζ
PSD	post-synaptic domain
tPA	tissue-type plasminogen activator
TEM	transmission electron microscopy
UVD	unilateral vestibular deafferentation
UVL	unilateral vestibular lesions
VMWT	virtual Morris water maze
VNC	vestibular nucleus complex
VOR	vestibulo-ocular reflex
VSR	vestibulo-spinal reflex

Chapter one

Introduction

1.1 The vestibular system

The vestibular system is a part of the labyrinth that lies in the otic capsule in the petrous portion of the temporal bone. It is a structure that detects gravity and self-motion, and uses this information to maintain balance and postural orientation. The sensory organ comprises five distinct end organs: the horizontal, anterior and posterior semicircular canals which are sensitive to angular acceleration, and the otolith organs, the utricle and saccule, which are sensitive to linear acceleration. The utricle is orientated horizontally, whereas the saccule is orientated primarily vertically (Glover 2004).

The semicircular canals are three ring-like structures, approximately perpendicular to each other, that detect angular acceleration. At the base of each semicircular canal is a swelling called the ampulla where the mechanoreceptors or hair cells are located. The canals are filled with endolymphatic fluid which bathes the hair cells. If a movement produces angular acceleration, inertia causes the endolymphtic fluid to lag behind in the canal which deflects the hair cells. The hair cells are orientated in one direction within each semi-circular canal, so that they are only deflected in the plane of that canal (Glover 2004).

The vestibular sensory-motor pathways comprise the sensory organs, sensory afferents and the vestibular nuclei in the brainstem, which project to interneurons and motor neurons in the spinal cord and brainstem. The hair cells are innervated by the eighth cranial nerve or vestibulocochlear nerve, which transmits vestibular information from the labyrinths to the vestibular nucleus complex (VNC) in the brainstem and the cerebellum (Carleton and Carpenter 1984). The VNC is made up of the lateral, medial, superior and inferior vestibular nuclei. The neurons in these sub-nuclei receive input from different regions of the labyrinth, with multiple inputs sometimes converging on the same neurons (Smith 1997). Vestibular information from the contralateral VNC is integrated with information from the ipsilateral VNC, in addition to visual and proprioceptive sensory input.

Vestibular information is vital for a number of reasons, including the generation of reflexive eye movements, controlled by the vestibulo-ocular reflexes (VORs) (Aw et al. 1996), and the vestibulo-spinal reflexes (VSRs) which are required for the correction of posture. The VORs and VSRs use short-latency, reflex arcs to rapidly correct for head movement (Goldberg 2000). The vestibular receptor cells in the vestibular organs detect acceleration and this signal

is converted so that it is in phase with head velocity (Shinoda and Yoshida 1974). The signal is then transmitted to specific subregions of the VNC by the vestibular nerve, where it is integrated into position signals. The motor neurons that innervate the eye muscles use the signal to generate VOR eye movements that are "equal and opposite to head movements" (Goldberg 2000, Smith 1997). The generation of this VOR takes approximately 7-10 ms (Aw et al. 1996). The medial and lateral vestibular nuclei also project to the spinal motor neurons at the cervical and thoracic levels which helps to generate the VSRs (Xerri et al. 1988).

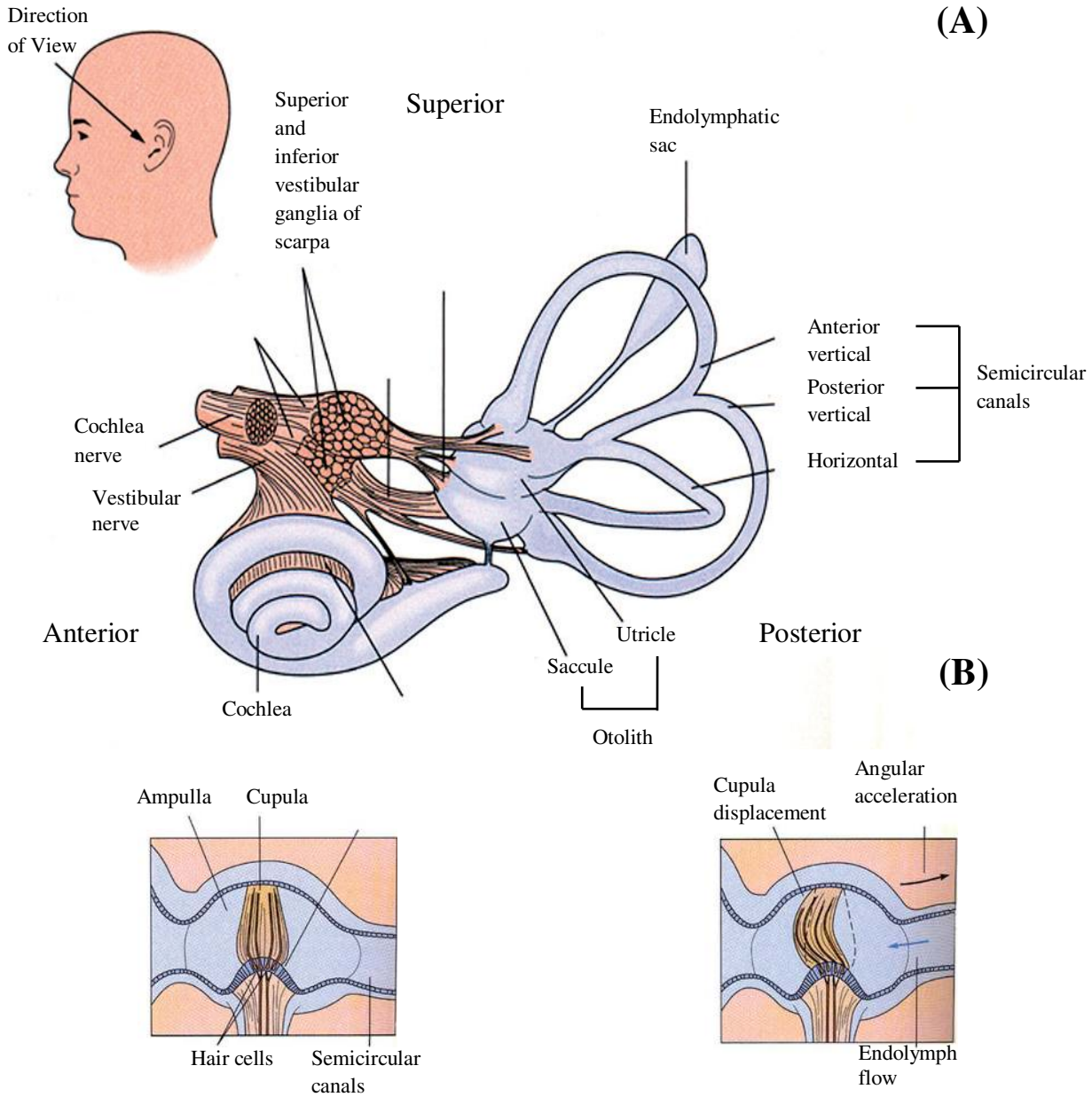


Figure 1.1: A simplified diagram of the vestibular system. *(A) The semicircular canals, otolith organs and the associated vestibular and cochlear ganglia and nerves. (B) Structure of the ampulla in the semicircular canal and a diagram of angular acceleration induced deflection of the cupula. Adapted from Glover JC. 2004. Encyclopedia of Neuroscience: 127-132, with permission from Elsevier.*

1.2 Vestibular dysfunction

Damage to the vestibular inner ear can occur as a result of Meniere's disease (Paparella and Mancini 1985), bacterial and viral infections such as vestibular neuritis and labyrinthitis (Davis 1993, Rasmussen et al. 1991), chemical toxicity resulting from aminoglycoside antibiotics (Brummett et al. 1972), tumours on the VIIIth nerve (Troost 1980), temporal bone trauma (Cannon and Jahrsdoerfer 1983) and a number of other causes. Vestibular dysfunction increases significantly with age, where the majority of individuals over 70 years of age report dizziness and postural imbalance (Agrawal et al. 2009). Patients with vestibular dysfunction who report dizziness have a much greater risk of falling, and balance-related falls account for more than one-half of accidental deaths in the elderly (Agrawal et al. 2009).

The loss of VORs and VSRs results in attention difficulties in humans (Yardley et al. 1998), decreased reaction time (Redfern et al. 2004), and symptoms such as oscillopsia (Shumway-Cook et al. 1997). Vestibular-lesioned patients have also been shown to perform worse on mental arithmetic tasks when compared to controls (Yardley et al. 2002). Patients with vestibular disorders present with severe spatial (Brandt et al. 2005, Hüfner et al. 2007, Schautzer et al. 2003) and non-spatial memory deficits (Grimm et al. 1989).

Mood disorders such as anxiety and depression (Godemann et al. 2004, Nagaratnam et al. 2004), agoraphobia (Jacob et al. 1996) and panic disorders (Godemann et al. 2006, Szirmai et al. 2005) have also frequently been associated with vestibular dysfunction in patients (Furman and Jacob 2001, Furman et al. 2006, Jacob and Furman 2001). However, this relationship is complex as patients with anxiety and depression have an increased rate of dizziness and vestibular disorders (Furman et al. 2006, Staab and Ruckenstein 2003). These mood disorders along with the behavioural symptoms explain why people with vestibular disorders often withdraw socially (Yardley et al. 1998, Zheng et al. 2008).

1.2.1 Vestibular damage and compensation

Unilateral and bilateral vestibular damage results in distinct behavioural syndromes. Some of these ocular and postural symptoms abate over time as a result of widespread compensation. However, not all symptoms undergo compensation and the patient or animal will continue to

suffer from deficits in their VORs and VSRs (Curthoys and Halmagyi 1995, Smith and Curthoys 1989).

1.2.1.1 Unilateral vestibular damage

Unilateral vestibular deafferentation (UVD) results in an imbalance in spontaneous resting activity between the contralateral and ipsilateral VNCs (Curthoys 2000). This imbalance results in the static symptoms of UVD, which are defined as symptoms that "are present without externally imposed vestibular stimulation" (Curthoys 2000). Static symptoms of UVD include spontaneous nystagmus defined as quick phase eye movements to the intact side, postural imbalances such as a marked head tilt towards the lesioned side, and ataxia or staggering gait (Curthoys and Halmagyi 1995, Dieringer 1995, Smith and Curthoys 1989). These static symptoms start to abate days or even hours following unilateral vestibular loss and this recovery is called vestibular compensation.

Dynamic vestibular responses are those that occur in response to passive vestibular stimuli (Smith and Curthoys 1989). The visual symptoms occur because dynamic responses such as the VOR and optokinetic reflexes are vital for stabilizing the visual gaze during head movements and require intact vestibular signals to function correctly (Aw et al. 1996). Such responses do not recover after deafferentation, resulting in abnormal sensory feedback (Curthoys and Halmagyi 1995, Dieringer 1995, Smith and Curthoys 1989).

The mechanisms behind vestibular compensation are poorly understood. While vestibular compensation is occurring, there is a corresponding return of resting activity to neurons in the VNC ipsilateral to the lesioned side (Ris and Godaux 1998, Ris et al. 1995). However, the electrical activity is below normal and there is no recovery in the ipsilateral vestibular nerve or vestibular receptor hair cells; therefore central nervous system (CNS) plasticity is responsible for this process (Curthoys and Halmagyi 1995).

1.2.1.2 Bilateral vestibular damage

Bilateral vestibular deafferentation (BVD) and UVD result in different behavioural symptoms. The UVD syndrome occurs as a result of an imbalance in vestibular output, which causes nystagmus, and a postural tilt towards the lesioned side. However, BVD results in a

greater loss of the vestibular reflexes, and there is no imbalance between the two vestibular nuclei in the brainstem (Smith and Curthoys 1989). BVD symptoms also undergo compensation, but to a lesser extent than UVD symptoms (Smith and Curthoys 1989).

The VORs and VSRs take longer to compensate following BVD and the symptom recovery varies (Deliagina et al. 1997), while some deficits never recover (Basile et al. 1999, Gilchrist et al. 1998). Loss of the VSRs results in symptoms such as gait ataxia and loss of the righting reflex, with compensation for these symptoms occurring gradually as vestibular nuclei resting activity recovers (Ris and Godaux 1998). There is no recovery in VOR function as the VNC neurons never recover their sensitivity to head movements. Compensation for deficits in these reflexes requires that the individual substitute alternative eye and postural movements, for instance saccadic or very rapid eye movements, which correct for the inappropriate axis of eye rotation (Halmagyi et al. 2003). Further compensation that stabilizes the gaze and head may take 6 months to a year to reduce symptoms like vertigo and oscillopsia, defined by Russell et al. (2003b) as the apparent movement of the visual world (Curthoys and Halmagyi 1995, Smith 1997, Smith and Curthoys 1989). However, symptoms such as hyperkinesis, defined as excessive locomotor activity (Goddard et al. 2008a), and horizontal head weaving (Basile et al. 1999), are permanent.

1.2.2 The vestibular system and spatial memory and navigation

In animal models and humans spatial navigation depends on the ability to learn and then remember how to get to a specific location. It is thought to be essential for higher cognitive processing (Zheng et al. 2006). Vestibular stimulation results in changes in spatial navigation (Matthews et al. 1989, Semenov and Bures 1989). Numerous papers highlight that peripheral vestibular lesions cause spatial memory deficits in both human patients and rats (Brandt et al. 2005, Cohen and Kimball 2002, Hüfner et al. 2007, Matthews et al. 1989, Péruch et al. 1999, Redfern et al. 2004, Russell et al. 2003a, Schautzer et al. 2003, Stackman and Herbert 2002b). These studies lend support to the idea that the vestibular system has a vital role in the spatial representations that are formed in the higher cognitive centres of the brain (McNaughton et al. 1991, Smith 1997, Taube et al. 1996). Spatial memory deficits are thought to be related to the vestibular system's failure to acquire information about self-movement (Zheng, et al., 2009).

Patients who had undergone bilateral neurectomy due to neurofibromatosis type 2 showed significant spatial learning and memory deficits when compared to healthy controls in the virtual Morris water task (VMWT) (Hamilton et al. 2002, Schautzer et al. 2003), a task that involves navigating to a hidden platform on a screen using visual cues. The task is used to measure hippocampal dependent learning and memory in humans. It is thought to require hippocampal circuitry without involving vestibular and proprioceptive signals (Hamilton et al. 2002).

Patients with bilateral vestibular loss (BVL) also showed significant spatial memory deficits in the VMWT when compared to healthy controls (Brandt et al. 2005). These BVL patients also showed an average atrophy of the hippocampus of 16.9% when compared to controls, as measured by magnetic resonance imaging (MRI) volumetry, which significantly correlated with the spatial memory loss of the BVL patients (Brandt et al. 2005). The patients were able to navigate to a cued location in the VMWT, which suggests that visual information is important for overcoming vestibular loss. This correlates with other studies demonstrating that people and animals with vestibular deficits perform better in spatial navigation tasks if there is a visible landmark present (Astur et al. 2002, Schautzer et al. 2003, Stackman and Herbert 2002b, Wallace et al. 2002).

In an animal model however, chemically-induced bilateral vestibular lesions resulted in impaired spatial learning and memory, but no impairment in non-spatial memory was observed in the object recognition task (Besnard et al. 2011, in press). However, BVD rats showed no hippocampal atrophy, in contrast to the human BVL patients (Balabhadrapatruni et al. 2011, unpublished data, Besnard et al. 2011, in press, Brandt et al. 2005). The deficits in spatial memory have been shown previously using rats that had undergone BVD (Russell et al. 2003a).

A potential confounding factor in behavioural studies testing spatial memory is that the deficits in the VOR and VSR following vestibular lesions are so severe that the animal cannot perform the tasks due to poor motor control and an inability to orient themselves. These symptoms are difficult to control for, particularly as all behavioural tasks involve some form of movement. The initial severe motion and postural deficits associated with the BVD syndrome could also potentially result in abnormal sensory feedback to the hippocampus. This is controlled for by assessing bilateral vestibular lesion induced deficits at longer time

points, as spatial memory impairments appear to be permanent (Baek et al. 2010, Brandt et al. 2005, Zheng et al. 2009) and some compensation for the lost vestibular reflexes has occurred (Curthoys 2000, Curthoys and Halmagyi 1995). BVD rats are also consistently shown to be hyperactive, exhibiting increased locomotor velocity, and distance travelled (Goddard et al. 2008a).

A study by Zheng et al. (2007) found that spatial memory deficits following bilateral vestibular lesions were likely to be permanent. BVD rats made significantly more errors than control rats in the spatial forced alternation T maze task at 3 weeks, 3 months and 5 months post-op. However, at 3 weeks post-surgery the BVD animal's percentage of correct choices was significantly lower than their percentage of correct choices at 5 months. This implies that while the BVD rats' hippocampal function and spatial memory is impaired over the long term, there is some improvement in performance as the animals adapt to deficits in their VORs and VSRs (Zheng et al. 2007).

Further evidence exists to support the hypothesis that spatial memory deficits associated with bilateral vestibular loss may be permanent. BVD and control rats performed the food foraging task 5 months post-surgery (Zheng et al. 2009). In light conditions the BVD animals exhibited significantly increased homing time, but used visual cues to find their way home. When the task was performed in darkness, the BVD rats exhibited significantly increased homing time, distance, errors and larger heading angles (Zheng et al. 2009). Similar results were observed in both light and dark conditions using rats with chemically-induced vestibular lesions (Wallace et al. 2002).

Dead reckoning is a type of spatial navigation that an animal uses to identify its current location and return to its starting position (McNaughton et al. 1991). Rats with bilateral vestibular loss were able to navigate home successfully in the light. However, in the absence of visual cues when dead reckoning is required, none of the rats were able to navigate home (Wallace et al. 2002, Zheng et al. 2009). The fact that two different methods were used and the same result was achieved confirms that it is the loss of vestibular function, and not the method used to produce the lesions, that resulted in deficits in the foraging task. When performing the food foraging task, the animal needs to continuously monitor and integrate self movement cues, in order to navigate an accurate path (Whishaw and Tomie 1997). Rats

without an intact vestibular system were not able to do this, as the animals relied on visual cues to navigate successfully.

1.2.2.1 Differences between unilateral and bilateral vestibular damage

BVL patients show atrophy of the hippocampus described in the previous section 1.2.2, whereas volumetry of the hippocampus of human patients with unilateral vestibular lesions (UVL) showed no difference in hippocampal volume in left or right sided UVL patients when compared to controls (Hüfner et al. 2007). Furthermore, unlike the BVL patients, UVL patients showed no significant impairment in spatial memory or navigation in the VMWT, 5-13 years following surgery. This suggests that vestibular input provided by one labyrinth is sufficient for the production and maintenance of spatial memory, and prevents the changes in the gross volume of the hippocampus observed in humans following BVL (Hüfner et al. 2007).

Conversely, in unilateral vestibular neuritis patients there was significant atrophy of the right superior temporal gyrus and atrophy of the left posterior hippocampus (zu Eulenburg et al. 2010). The patients also had increased gray matter in the medial vestibular nucleus (MVN), the right gracile nucleus, and increased white matter signal intensity in the pontine commissural vestibular fibers. This was measured 2.5 years after vestibular neuritis onset as assessed by voxel-based morphometry, which measures changes in gray and white matter using MRI (zu Eulenburg et al. 2010).

The different causative pathologies seen in these studies could account for the different results. Vestibular neuritis is thought to have a viral origin (Baloh et al. 1996) and is associated with failure of the vestibular nerve resulting in impaired transmission between the peripheral vestibular organs and the VNC (Baloh 2003). By comparison, the patients in the Hufner et al. (2007) study had undergone complete UVD surgery because of acoustic neuroma. It is still uncertain what effect the loss of unilateral vestibular input has on the function of the hippocampus in humans and animal models.

Differences between UVD and BVD have also been observed in rat models. Zheng et al. (2006) used the food foraging task to investigate changes in spatial memory at 3 and 6 months and compare bilaterally and unilaterally-lesioned animals against controls. The UVD rats

showed increased homing time, homing distance, number of errors and heading angles at 3 months in the dark, when correct self-movement cues were required. This indicates that UVD animals suffer spatial memory deficits. However, the animals showed no impairment at 6 months (Zheng et al. 2006). The improvement observed between 3 and 6 months is interesting as temporal bone histology confirmed that the vestibular labyrinth had not recovered. As one labyrinth was still intact it is possible that the animal had compensated for the loss of the other labyrinth (Zheng et al. 2006). It is possible that the compensation may occur in the hippocampus, due to plasticity-induced changes in the way vestibular information is processed (Zheng et al. 2006).

When BVD animals were tested in the food foraging task at 14 months post-surgery, no improvement was observed in homing behaviour, and the animals performed worse as they showed impairment in the light, when visual cues were available (Baek et al. 2010). This corresponds with the Brandt et al. (2005) study, who found spatial memory deficits in BVD patients 8-10 years after surgery. Together these studies suggest that in bilaterally lesioned animals and humans, navigational and spatial memory impairments may be permanent.

1.3 Hippocampus and spatial memory

In order to better understand the relationship between spatial memory and learning in the hippocampus, several experiments have studied human subjects using functional imaging while they navigated virtual environments. Positron emission tomography (PET) scanning showed strong activation of the right hippocampus while subjects were processing established spatial layouts (Maguire et al. 1997). Increased activation of the hippocampus was observed in subjects navigating out of a virtual maze using MRI scanning (Grön et al. 2000); increased hippocampal activation was also seen in subjects navigating a novel path or 'wayfinding' (Hartley et al. 2003). A more recent study used functional MRI scanning to show that hippocampal activation is strongest in the initial stages of navigational learning (Wolbers and Büchel 2005).

Numerous papers have shown that hippocampal lesions result in spatial memory deficits (Astur et al. 2002, Kessels et al. 2001, Maguire et al. 1996). Human unilateral hippocampal resection patients required spatial cues in order to navigate to a hidden escape platform in the VMWT. The deficits in spatial navigation occurred regardless of which side of the

hippocampus was lesioned (Astur et al. 2002). Significant spatial memory deficits were also observed in animals following medial septal nucleus lesions, which disrupted hippocampal theta activity (Winson 1978). These results suggest that the hippocampus is vital for spatial memory and learning (Maguire et al. 2000, McNaughton et al. 1996, O'Keefe and Nadel 1978, Whishaw et al. 2001, Wiener 1996).

The hippocampus and parahippocampal regions are both involved in navigation. MRI activity was observed in the parahippocampal gyri and cortical areas known to project to the parahippocampus in subjects navigating a virtual maze (Aguirre et al. 1996). Furthermore, place cell recordings in the medial temporal and frontal lobes of humans navigating in a virtual environment showed that the majority of cells that responded to the sight of specific locations were found in the hippocampus, whereas the cells that responded to views of landmarks were located in the parahippocampal regions (Ekstrom et al. 2003). The hippocampus and parahippocampal regions combine to make up the hippocampal formation (Anderson et al. 1998). The hippocampal formation is heavily interconnected with itself and the frontal cortex (FC); a simplified schematic of these connections is described in fig. 1.2. Any neurochemical or electrophysiological changes in one area will likely result in alterations in other areas.

The cellular changes behind the increased activation of the hippocampus following vestibular stimulation are unknown; however hippocampal neurogenesis may be responsible. Neuronal levels doubled in the rat dentate gyrus (DG) following training on an associative learning task (Gould et al. 1999). Adult neurogenesis may also be involved in the formation and consolidation of long-term hippocampal memories. Inhibition of neurogenesis in the rat hippocampus while the animals were training for a water maze task did not affect learning and performance of the task compared to controls at a 1 week time point, but at 2 and 4 weeks the rats' performance was significantly impaired (Snyder et al. 2005).

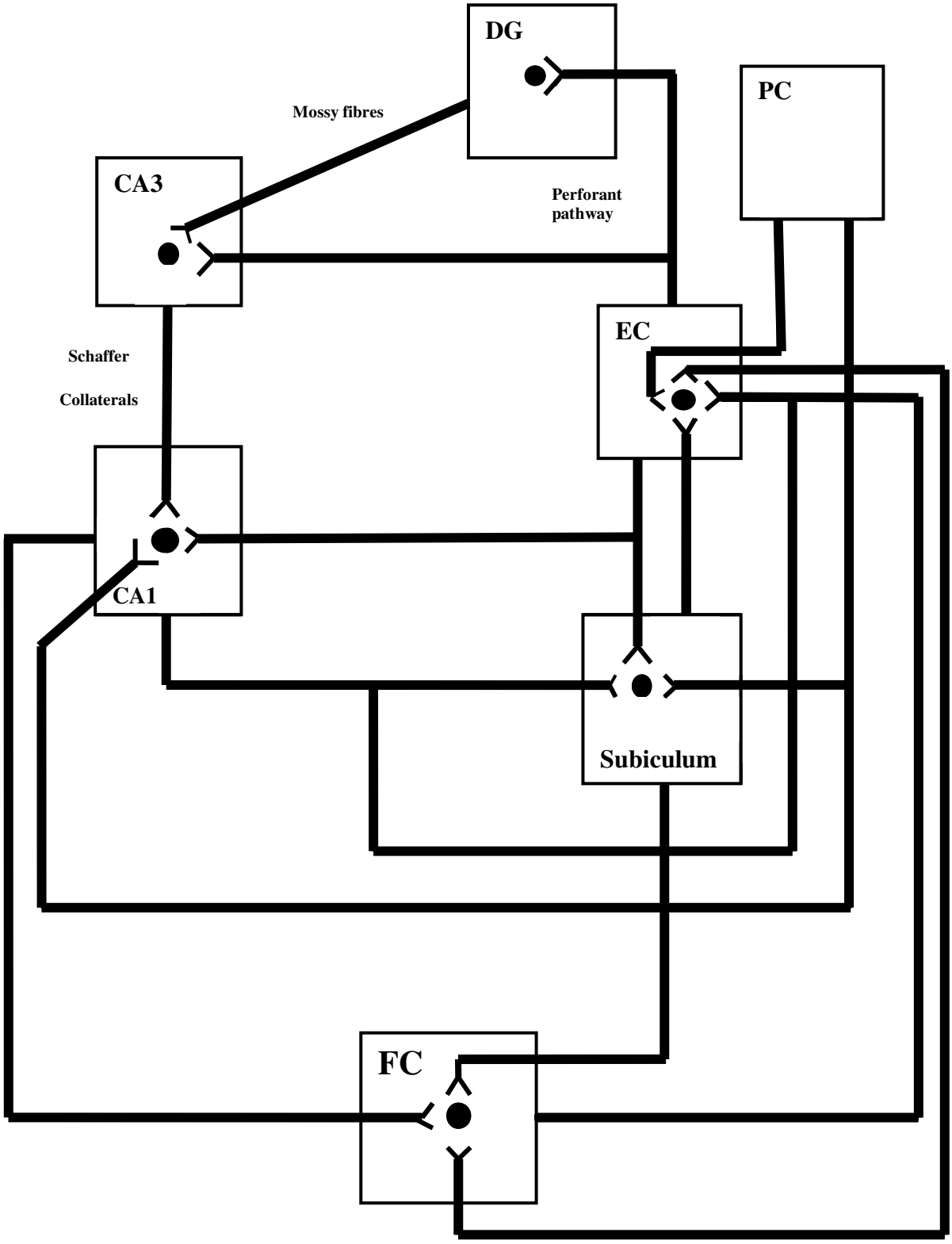


Figure 1.2: The hippocampal formation and connections to the Frontal Cortex. *Simplified schematic representation of the projections between the cornu ammonis area 1 (CA1), cornu ammonis area 3 (CA3), dentate gyrus (DG), Subiculum, entorhinal cortex (EC), perirhinal cortex (PC) and frontal cortex (FC). Includes synapses ( )and neural pathways ( ).Used to highlight the complicated interconnections between these various brain regions. (Andersen et al. 2007: 38, 71-72, 75-77, 92-93, 138).*

1.3.1 Memory and glutamate in the hippocampus

1.3.1.1 NMDA receptors

NMDA (N-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptors are the major classes of ionotropic receptors in central glutamatergic synapses (Hollmann and Heinemann 1994, Ottersen and Landsend 1997). NMDA receptors (NMDARs) are heteromeric ligand-gated ion channels composed of the subunits NR1, NR2A-D, NR3A-B (Kutsuwada et al. 1992, Monyer et al. 1992). The NR1 subunit is necessary for NMDAR function, as NMDARs contain two obligatory NR1 subunits (Dingledine et al. 1999, Petralia et al. 1994). Furthermore, expression of a functional NMDAR is thought to require co-expression of at least one NR1 and NR2 subunit (Dingledine et al. 1999). The form the NR2 subunit (NR2A-D) takes in a receptor has a significant effect on the receptors properties, such as channel conductance, sensitivity to Mg^{2+} and ligand affinity (Erreger et al. 2004, Ishii et al. 1993, Krupp et al. 1998, Kutsuwada et al. 1992, Monyer et al. 1994, Sucher et al. 1996, Williams et al. 1994).

In the adult rat brain, NR2A expression is widespread, while the majority of NR2B is in the forebrain (Ishii et al. 1993, Monyer et al. 1994). NR2A expression increases over time, whereas the majority of NR2B expression occurs during development. This causes a development-related shift in the functional properties of NMDARs (Hestrin 1992, Monyer et al. 1994, Sheng et al. 1994). NMDARs containing the subunits NR1/NR2B deactivate more slowly than receptors made up of NR1/NR2A (Vicini et al. 1998). Excitatory postsynaptic currents mediated by NMDARs containing NR1/NR2B also show slower decay times, than those containing NR1/NR2A (Flint et al. 1997).

1.3.1.2 AMPA receptors

AMPA receptors (AMPARs) mediate fast excitatory synaptic transmission in the CNS (Bliss and Collingridge 1993). AMPARs are heteromeric complexes made up of the four subunits GluR1-GluR4 (also referred to as GluRA1-GluRA4) (Hollmann and Heinemann 1994, Rosenmund et al. 1998). GluR1 and 2 are the most prominently expressed AMPAR subunits in the hippocampus, with lower levels of GluR3 and 4 (Craig et al. 1993, Geiger et al. 1995). In the hippocampus the receptor complexes are predominantly made up of GluR1/GluR2 or

GluR3/GluR2 (Wenthold et al. 1996). Different combinations of these AMPAR subunits change how the receptor responds to the agonists' kainate, AMPA and quisqualate (Egebjerg et al. 1991). The current-voltage relationship of the receptors also changes depending on subunit composition, and can be either linear or inwardly rectifying (Boulter et al. 1990, Egebjerg et al. 1991).

The AMPAR subunit GluR1 plays a central role in synaptic plasticity (Meng et al. 2003, Zamanillo et al. 1999). It is also required for NMDAR-dependent synaptic delivery of AMPARs (Hayashi et al. 2000, Passafaro et al. 2001, Shi et al. 1999). The majority of AMPARs in the CNS are heterodimers containing GluR2 (Greger et al. 2002, Wenthold et al. 1996). GluR2 is vital for AMPAR trafficking (Greger et al. 2002, Liu and Cull-Candy 2000, Rothman 1994) and internalization (Chung et al. 2000), so it is unsurprising that the majority of AMPARs in the CNS contain GluR2. Channels containing GluR2 have a linear current-voltage relationship, relatively low single-channel conductance and low permeability (Boulter et al. 1990, Geiger et al. 1995, Isaac et al. 2007), whereas homomeric channels formed from GluR1, GluR3, or GluR4 are inwardly rectifying and calcium permeable (Geiger et al. 1995).

GluR2 and GluR3 are thought to be important for activity-independent AMPAR movement and therefore more important for basal synaptic function (Lewis et al. 1989, Passafaro et al. 2001, Shi et al. 1999). It has been hypothesized that the subunit combination GluR2/3 is responsible for stabilising the AMPARs at the synaptic surface (Lee et al. 2002b, Meng et al. 2003, Noel et al. 1999, Osten et al. 2000). GluR4 is mainly expressed during early development and is thought to be important for developmental synaptic plasticity (Zhu et al. 2000).

1.3.1.3 Glutamate receptors and LTP

Long term potentiation (LTP) is a sustained increase in neurotransmission and synaptic efficacy induced by high frequency electrical stimulation. It is the primary model for learning and memory (Bliss and Lømo 1973, Bliss and Collingridge 1993, Nicoll and Malenka 1995). LTP is divided into two phases: the initiation or induction of synaptic potentiation and the maintenance of this potentiation over time. The induction phase of LTP is generally rapid and involves the release of glutamate and the activation of NMDARs, which are concentrated in

the cornu ammonis area 1 (CA1) and DG regions of the hippocampus (Collingridge et al. 1983). The longer lasting, maintenance phase of LTP is thought to involve changes in glutamate release and AMPAR expression and sensitivity (Collingridge et al. 1983).

NMDAR-dependent LTP was first observed in the hippocampus. Stimulation of NMDARs triggers postsynaptic membrane depolarization, resulting in the presynaptic release of glutamate. The voltage-dependent Mg^{2+} block of the NMDAR is released and Ca^{2+} enters postsynaptic dendritic spines. LTP is triggered by the increase in postsynaptic Ca^{2+} concentration, which activates intracellular signalling cascades of specific protein kinases, most importantly calcium/calmodulin-dependent protein kinase II (CaMKII). AMPAR trafficking is also altered, resulting in an increase in AMPARs at the postsynaptic membrane. This is the underlying mechanism behind the increase in synaptic strength during LTP (Nicoll and Malenka, 1999).

Following LTP, there are increased NMDAR and AMPAR subunit numbers in the hippocampus (Manahan-Vaughan et al. 2003, Williams et al. 2003). The addition of AMPARs results in increased synaptic transmission during LTP (Hayashi et al. 2000, Passafaro et al. 2001, Shi et al. 1999). A knockout mouse strain was developed with deletion of the *NMDAR1* gene limited to the CA1 pyramidal cells of the hippocampus. The resulting adult mice did not produce LTP or NMDAR–mediated synaptic currents in the CA1 synapses (Tsien et al. 1996).

Long term depression (LTD) is generated by prolonged low frequency stimulation; it occurs when the processes responsible for LTP are reversed (Linden and Connor 1995). The *de novo* induction of LTD requires activation of NMDARs containing NR2B, whereas LTP induction requires activation of NMDARs containing NR2A (Liu et al. 2004b, Massey et al. 2004). This result concurs with the finding that LTP is disrupted in mice lacking NR2A (Sakimura et al. 1995). However, this issue is complicated as it has also been found that mice over-expressing NR2B have enhanced LTP (Tang et al. 1999). Nevertheless, the NR2 composition of individual NMDARs clearly influences the induction of LTP and LTD.

Adult GluR1 knock-out mice could not generate LTP (Zamanillo et al. 1999). The AMPAR subunit GluR2 also has a role in the regulation of LTP. GluR2 knock-out mice showed enhanced LTP in the CA1 region of hippocampal slices but there were no changes in neuronal

excitability (Jia et al. 1996). This coincides with the fact that in regions of the brain where GluR2 is highly expressed, AMPARs show decreased Ca^{2+} permeability (Geiger et al. 1995), and regions with low or absent GluR2 expression exhibit high Ca^{2+} permeability (Koh et al. 1995).

NMDAR and AMPAR modulators affect various forms of memory in both animals and humans (Ingvar et al. 1997, Lynch et al. 1997, Puma et al. 1998, Pussinen and Sirviö 1999, Schwartz et al. 1996). The NMDAR agonist NMDA improved the consolidation of working memory in rats whereas the antagonist amino-5-phosphonovaleric acid (AP5) disrupted it (Puma et al. 1998). The NMDA antagonist aminophosphonovaleric acid (APV) also blocked the increase in GluR1 and GluR2 synaptic membrane expression in the hippocampus following LTP (Heynen et al. 2000). This is consistent with the finding that AMPAR binding is increased in the hippocampus following the induction of LTP (Maren et al. 1993).

1.3.1.4 Glutamate receptors and spatial memory

The mechanisms that encode and maintain LTP are also thought to initiate and sustain spatial memory. The protein kinase M ζ (PKMζ) is required for the maintenance of LTP (Serrano et al. 2005). When PKMζ was introduced into hippocampal slices, the AMPAR response was strongly potentiated (Ling et al. 2006). When the PKMζ inhibitor ζ-pseudosubstrate inhibitory peptide was injected into the rat hippocampus; 1-day-old spatial memory was lost (Pastalkova et al. 2006).

Mice with deletion of the *NMDAR1* gene restricted to CA1 pyramidal cells of the hippocampus also showed spatial memory impairment in a water maze task (Tsien et al. 1996). This result suggests that NMDARs in CA1 synapses have an important role in the development of spatial memories. The mice did not present with any obvious behavioural abnormalities, as the mutation was targeted to the area of interest, unlike generic knockout mice where the mutation is global (Tsien et al. 1996).

Phosphorylation of GluR1 is also vital for LTD and LTP, and spatial memory retention. Knock-in mice with mutations in GluR1 phosphorylation sites showed deficits in LTP and LTD, and performed poorly in spatial learning tasks (Lee et al. 2003b). An animal model of GluR2 deficiency showed changes in the synaptic excitability of the hippocampus and

impairment in a spatial learning task (Shimshek et al. 2006). These findings suggest that NMDA and AMPAR subunit composition is as important for spatial memory and learning as it is for LTP.

The results of genetic studies are consistent with pharmacological ones. Delivery of the NMDAR antagonist APV to the rat hippocampus impaired spatial learning and this impairment correlated with LTP blockade (Davis et al. 1992). Rats' spatial working memory in an eight arm radial maze was not significantly impaired by administration of the NMDA antagonist (+)-10,11-dihydro-dibenzo [a, d] cycloheptene-5,10 imine (MK-801), except when tested in a novel environment (Caramanos and Shapiro 1994). This result suggests that NMDARs are important for spatial memory induction in a novel environment.

1.3.1.5 CaMKII and pCaMKII

Neuronal CaMKII is involved in gene expression, neurotransmitter synthesis, exocytosis, postsynaptic responses like LTP, and the control of cytoskeletal machinery (Schulman and Hanson 1993). CaMKII binds directly to the NMDAR subunits NR1 and NR2B (Leonard et al. 1999). NMDAR stimulation causes a large increase in intracellular calcium, which in turn increases the activation of CaMKII and leads to CaMKII autophosphorylation or pCaMKII (Giese et al. 1998). The AMPARs are then directly phosphorylated by CaMKII, which increases channel conductance (Barria et al. 1997a). The kinase also has a role in trafficking AMPARs to synapses, which results in enhanced synaptic transmission (Hayashi et al. 2000).

As CaMKII activity is so closely related to NMDAR and AMPAR activity, it is logical that CaMKII would have a role in LTP and many studies have confirmed this connection. LTP was disrupted in animals exposed to a selective CaMKII inhibitor, showing that CaMKII has a role in the induction of LTP (Malinow et al. 1989). In another study an increase in the postsynaptic expression of CaMKII in the CA1 region of the hippocampus was enough to enhance synaptic transmission and generate LTP (Pettit et al. 1994). One possible mechanism behind CaMKII-enhanced LTP is that CaMKII phosphorylates GluR1 at serine 831, and this has been shown to increase conductance through AMPARs and potentiate LTP (Barria et al. 1997a, Barria et al. 1997b, Derkach et al. 1999, Mammen et al. 1997, Roche et al. 1996).

There also appears to be a relationship between CaMKII activity and spatial memory and learning. Transgenic mice lacking CaMKII showed impaired spatial memory as the animals could not navigate to a specific location even though there were spatial cues available (Bach et al. 1995). However, another study using mice with mutated CaMKII found that the animals had significant spatial memory deficits, but could use cues to navigate (Silva et al. 1992). GluR1 is phosphorylated at serine 831 via CaMKII and serine 845 by adenosine 3′,5′-monophosphate (cAMP)-dependent protein kinase (PKA) (Barria et al. 1997b, Mammen et al. 1997, Roche et al. 1996). Spatial memory deficits were observed in knock-in mice with these serine residues mutated. The mutations meant that GluR1 could not be phosphorylated; however it is impossible to determine if this was due to loss of CaMKII or PKA activity or a combination of the two (Lee et al. 2003b). While the mechanisms are not clear it appears that CaMKII does have a role in spatial memory.

1.4 Vestibular-hippocampal connections

Current research suggests that there are four potential pathways connecting the vestibular system and hippocampus. Stimulation of the vestibular system produces a number of hippocampal responses. Hippocampal neuronal activity was altered in primates following vestibular stimulation (O'Mara et al. 1994). MRI imaging in human subjects showed that the hippocampus was activated following caloric stimulation, while eye movement was controlled for using a fixed visual target (Vitte et al. 1996). Most studies do not control for visual information when manipulating vestibular activity, nevertheless it is an important confounding factor. This is because the visual system is a major source of sensory information to the hippocampus (Tsanov and Manahan-Vaughan 2009) and it is disrupted by vestibular damage, due to symptoms such as oscillopsia (Chambers et al. 1985, Hamann et al. 2009).

Electrophysiological studies have also confirmed that the hippocampus has a role in processing vestibular information. Complex spiking cells or “place cells” (Vertes and Kocsis 1997) and non-complex spiking neurons or “theta cells” were recorded from in the CA1 region. Place cells and theta cells are both vital for accurate spatial navigation (Arleo and Gerstner 2000, Hasselmo et al. 2002). MVN stimulation increased the firing rates of complex spiking cells, while non-complex spiking cells showed a complex pattern of response, with some being activated, some inhibited and others not responding at all (Horii et al. 2004).

When areas adjacent to the MVN were stimulated there was no corresponding increase in firing of the CA1 neurons.

Unilateral stimulation of the vestibular system appears to result in a bilateral increase in hippocampal cell firing and evoked field potentials. This stimulation was low enough that VOR eye movements were not elicited (Cuthbert et al. 2000). Stimulation of one vestibular labyrinth also evoked theta activity in the hippocampus (Hicks et al. 2004). Interestingly all areas of the vestibular labyrinth were stimulated in these studies including the utricle and saccule and the semi-circular canals, suggesting that both angular and linear acceleration evoked vestibular signals are used by the hippocampus (Smith et al. 2005a). Furthermore, electrical stimulation of one vestibular nucleus has been shown to produce field potentials and evoke single unit activity in ipsilateral and contralateral CA1 neurons (Horii et al. 2004, Horii et al. 1994).

1.4.1 Potential pathways

There are several possible polysynaptic pathways connecting the brainstem vestibular nuclei and the hippocampus (Cuthbert et al. 2000, Horii et al. 2004, Smith 1997); these putative pathways are shown in fig 1.3. In the 'thalamo-cortical pathway', information from the VNC travels through the thalamus, parietal cortex, and then passes through the entorhinal and perirhinal cortices (EC and PC respectively) on the way to the hippocampus (McNaughton et al. 1994). The thalamus is also an important relay site for ascending vestibular projections (see Smith 1997 for review). Supporting this theory, a number of neurochemical changes were shown in the EC and PC following unilateral vestibular damage (Liu et al. 2004a).

In the 'head-direction pathway' vestibular information is transmitted to the prepositus hypoglossi via the MVN, which projects to the lateral mammillary nucleus via the dorsal tegmental nucleus (Liu et al. 1984). The lateral mammillary nucleus connects to the anterior dorsal region of the anterior thalamic nuclei, which projects to the hippocampus via the postsubiculum and EC, (reviewed by Taube et al. 1996). The name of the pathway originates from head direction cells in the lateral mammillary nuclei (Leonhard et al. 1996) and head direction cells in the anterodorsal thalamic nucleus, which were disrupted after bilateral inactivation of the peripheral vestibular system (Stackman and Taube 1997).

In the ‘theta generating pathway’ vestibular input from the VNC projects to the pedunculopontine tegmental nucleus, which in turn projects to the hippocampus via the supramammillary nucleus and the medial septum (Vertes and Kocsis 1997). There was an increase in the release of histamine from the medial septum when the round window of animals was electrically stimulated (Horii et al. 1994). Structures that generate theta rhythm are located in the pathway to the medial septum (Hayakawa et al. 1993, Kirk and McNaughton 1991, Semba et al. 1988, Vertes and Kocsis 1997) and theta activity is disrupted following BVD surgery (Russell et al. 2006).

Finally, there is a fourth possible pathway that has been put forward relatively recently; however there is less research available to substantiate this theory. In this pathway, known as the ‘vestibulo-cerebello-cortical pathway’, vestibular information is transmitted from the VNC to the cerebellar flocculus and paraflocculus, through to the posterior interpositus and dentate nucleus. These structures project to the thalamus via efferent fibres, then vestibular input is transmitted onto the temporal, parietal and frontal cortices which converge on the hippocampus (Fukushima 2003, Hüfner et al. 2007). It is important to note that whatever pathway vestibular information travels through, by the time it has left the VNC, it has been integrated with proprioceptive and subcortical visual information (Smith et al. 2009, Wilson and Jones 1979).

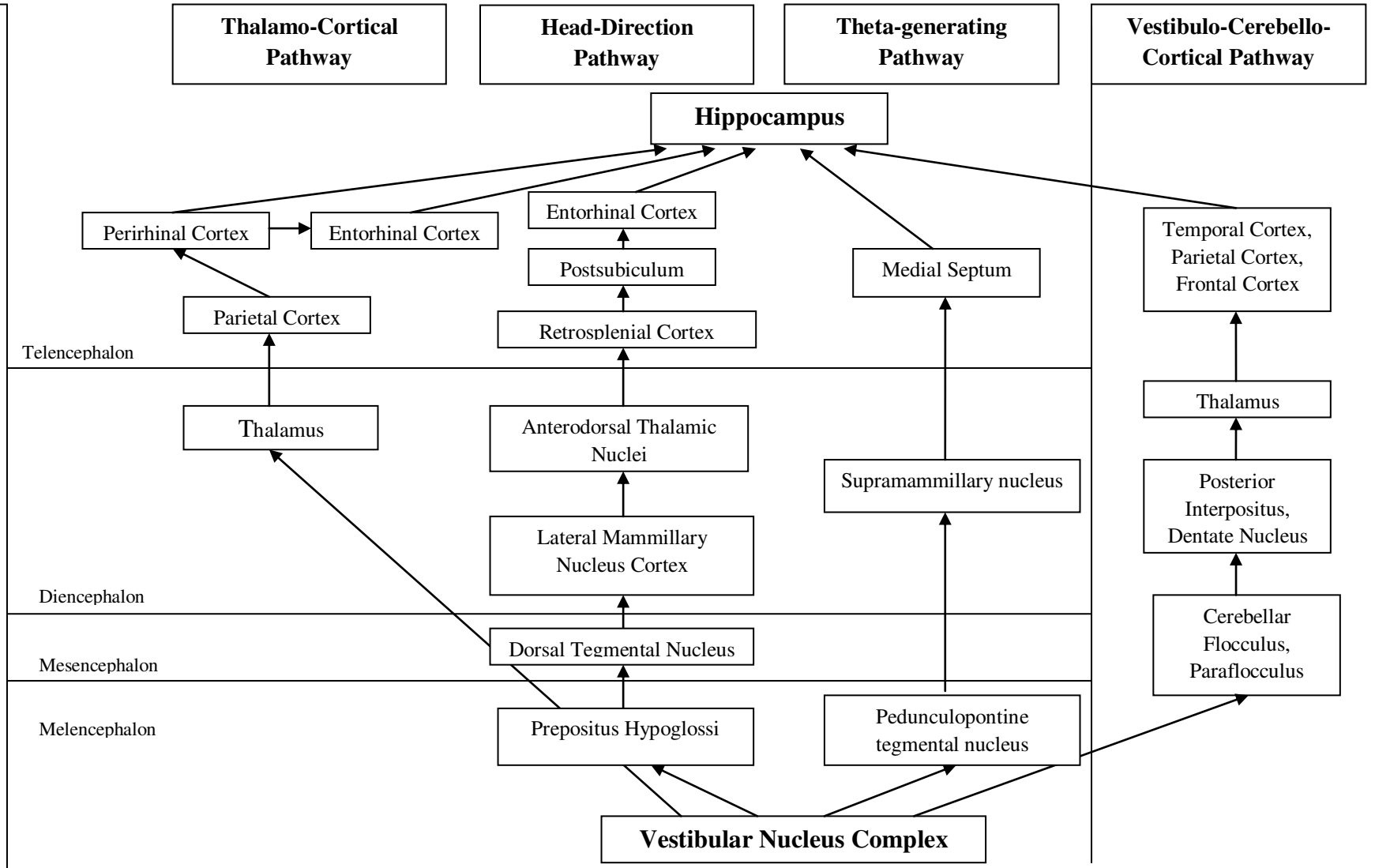


Figure 1.3: Putative vestibulo-hippocampal pathways. *Four putative pathways connecting the vestibular nucleus complex and the hippocampus, the possible vestibulo-hippocampal pathways include the Thalamo-Cortical, the Head-Direction, the Theta-Generating and the Vestibulo-Cerebello-Cortical pathways. The diagram is separated into the regions of the rat brain and only ascending pathways were depicted. Adapted slightly with permission from Hufner et al. (2007).*

1.4.2 The hippocampus, vestibular system and spatial navigation

The "path integration hypothesis" states that the hippocampus and other areas of the brain such as the neocortex use angular and linear velocity information from vestibular inputs to navigate through the spatial environment (Etienne and Jeffery 2004, McNaughton et al. 1991, McNaughton et al. 1996, Mittelstaedt and Mittelstaedt 1980, Taube et al. 1996). Spatial orientation and navigation are thought to require externally and internally generated cues (Berthoz et al. 1995). Internal or egocentric cues include information about self-movement, for instance vestibular, proprioceptive and motor efference signals (Horii et al. 2004), which are then integrated with visual information (McNaughton et al. 2006, McNaughton et al. 1996, Smith 1997, Taube et al. 1996). External or allothetic cues, require visual, auditory and olfactory sensory information (Wallace et al. 2002).

When an animal is navigating in the dark, in the absence of visual cues, self-motion cues are more important for the formation of intact place fields (Gothard et al. 2001). Vestibularly lesioned animals cannot navigate in the absence of visual cues (Russell et al. 2003b, Stackman and Herbert 2002b, Zheng et al. 2006), suggesting that vestibular self motion information is vital for the maintenance of hippocampal spatial firing activity (Russell et al. 2003b).

Self-movement information must be integrated with motor and sensory inputs in many cortical areas in order to correctly synchronize cognition and movement (Smith et al. 2009). This is indicated by the numerous areas of the brain receiving vestibular afferent input, including many parts of the limbic system and neocortex (Bottini et al. 1994, Dieterich et al. 2005, Eickhoff et al. 2006, Guldin et al. 1993), the FC (Bense et al. 2001, Dieterich et al. 2007), as well as areas in the temporal, occipital and insular lobes (Kahane et al. 2003). Vestibular lesion-induced changes in hippocampal function and anatomy are likely to be only a part of a complex cascade of neural changes throughout the brain (Karnath and Dieterich 2006, Smith et al. 2009).

1.5 Effects of vestibular damage on the hippocampus

While the effects of vestibular damage on learning and memory and emotional state have been well documented, the mechanisms behind these functional changes are poorly

understood. It is important to understand the connections between the vestibular system and the hippocampus, including the neurochemical and electrophysiological mechanisms behind these connections. Knowledge of these mechanisms will help us to understand how the hippocampus uses vestibular information, and what changes may occur in hippocampal function following vestibular damage.

1.5.1 Unilateral vestibular deafferentation

1.5.1.1 Neurochemical

Various neurochemical changes have been found in different sub-regions of the guinea pig hippocampus 10 hours post UVD. Glutamine levels were reduced in the DG of UVD animals when compared to sham animals, and taurine levels were significantly higher in the ipsilateral cornu ammonis area 2 and 3 (CA2/3) region of lesioned animals (Zheng et al. 1999b). UVD animals showed a significant increase in noradrenaline levels in the contralateral CA2/3 following UVD, compared to controls. The imbalance in ascending vestibular information following UVD may result in changes in the metabolism, release and synthesis of these neurochemicals (Zheng et al. 1999a).

In the rat hippocampus ten hours post-UVD there was a bilateral increase in nitric oxide synthase (Caramanos and Shapiro 1994) activity in the CA1 region. There was also a decrease in nitrite formation in the CA1 bilaterally and an increase in nitrite formation in the ipsilateral DG, the bilateral CA1 and ipsilateral DG (Zheng et al. 2000). Nitric oxide (NO) is synthesized by NOS (Caramanos and Shapiro 1994) from L-arginine (Vincent 1994). Therefore, this suggests that NO activity in the hippocampus is important in the initial stages of unilateral vestibular damage. This result is of significance because NO is thought to be important to the development of short-term memories in the hippocampus (Schuman and Madison, 1991). It also has a significant role in neuronal plasticity (Holscher 1997) and spatial memory (Böhme et al. 1993, Chapman et al. 1992, Liu et al. 2003a).

These earlier studies looked at neurochemical changes in the hippocampus while vestibular compensation was still occurring (Smith and Curthoys 1989). Any changes at the 10 hour time point are difficult to distinguish from the initial vestibular syndrome as the animals still suffer from the severe vestibular symptoms that follow lesion induction; however, by 2 weeks

the static vestibular symptoms of UVD have undergone partial compensation (King et al. 2002, Kitahara et al. 1995, Zheng et al. 2001a). Neurochemical changes after this point are most likely due to vestibular compensation induced plasticity in the hippocampus and/or are due to the chronic loss of vestibular information (Liu et al. 2003b).

The first study into neurochemical changes in the hippocampus after vestibular compensation had occurred showed a decrease in the expression of neuronal NOS (nNOS) in the ipsilateral DG 2 weeks after UVD surgery using Western blotting (Zheng et al. 2001a). However, some believe that NO is not necessary for the formation of LTP (Murphy and Bliss, 1999), therefore changes observed in NO production may have occurred as a result of changes in the normal synaptic transmission of the hippocampus (Zheng et al. 2001a). NO production is also linked to glutamatergic transmission as NMDAR activation causes increased intracellular Ca^{2+} levels, resulting in activation of nNOS and NO synthesis (Bredt and Snyder 1989, Zhang and Snyder 1995). This suggests there may be changes in glutamatergic transmission as well as NO activity following UVD.

NMDAR subunits NR1 and NR2A and the AMPA receptor subunit GluR2 were measured in sub-regions of the rat hippocampus post-UVD (Liu et al. 2003b). NR1 and NR2A were significantly decreased in the ipsilateral CA2/3 region 2 weeks after surgery; NR2A was also reduced in the contralateral CA2/3 region at 2 weeks when compared to shams, but to a lesser extent. There was also an increase in the NR2A subunit in the ipsilateral CA1 at 10 hours following UVD (Liu et al. 2003b).

Neurochemical changes have also been detected in brain areas that may be part of the connection between the VNC and hippocampus (Liu et al. 2004a). At 2 weeks post-UVD, nNOS expression in both the EC and PC was decreased on the contralateral side, while endothelial NOS (eNOS) expression was increased contralaterally in both. Furthermore, nNOS, eNOS, arginase I and arginase II expression were increased on the ipsilateral side in the EC. Arginase II increased bilaterally in the PC and arginase I increased on the contralateral and ipsilateral sides (respectively) in the PC. The protein levels were also measured at 10 and 50 hours, but no changes were found (Liu et al. 2004a). The two week post-UVD results support the ‘head-direction pathway’ as a possible connection between the VNC and hippocampus.

A confounding factor that could account for the neurochemical changes observed following UVD is stress. However, there were no changes in glucocorticoid receptor expression in the CA1, CA2/3 and DG regions of the rat hippocampus at 10 or 58 hours or 2 weeks post-UVD, except for a significant decrease in the ipsilateral CA1 2 weeks post-op. There were also no significant differences in serum corticosterone levels between UVD and sham animals at any of the time points (Lindsay et al. 2005). This result was complicated by the finding that salivary cortisol levels were significantly increased in guinea pigs following UVD surgery (Gliddon et al. 2003a). However, as blood cortisol can be inactivated during stress by binding to plasma proteins, salivary cortisol is considered a more accurate indicator of stress (Aardal and Holm 1995, Gliddon et al. 2003b). This result suggests that the neurochemical changes seen following UVD are unlikely to be due to increased stress in the animals that had undergone unilateral vestibular lesions.

1.5.1.2 Electrophysiological

Considering the neurochemical changes discussed above it was not surprising that there are corresponding alterations in hippocampal electrophysiology. Zheng et al. (2003) were the first to show that peripheral vestibular damage can produce long-term changes in hippocampal electrophysiological activity *in vitro*. In this study, the Schaffer collateral commissural pathway was electrically stimulated in hippocampal slices taken from UVD rats 5-6 months post-surgery and the field response was measured from the CA1. There was a reduction in population spike amplitude and field excitatory post synaptic potential (fEPSP) slope, and there was an increase in paired-pulse inhibition. Furthermore, the bilateral decrease in electrical excitability in the CA1 neurons was significantly larger at 5-6 months than at 4-6 weeks (Zheng et al. 2003). The changes in NMDAR subunit levels observed in the hippocampus previously (Liu et al. 2003b), indicate that changes in glutamatergic transmission may be responsible for this loss of neuronal electrical excitability. However, this was a much earlier time point and as no one has studied the changes in γ-aminobutyric acid (GABA) pathways following UVD, therefore it is impossible to know what plasticity underlies this loss in excitation.

1.5.2 Bilateral vestibular deafferentation

Bilateral vestibular lesions disrupt the resting discharge of the central vestibular system. This resting activity is regenerated in both vestibular nuclei, approximately a week after the lesion (Ris and Godaux 1998). However, there is still a permanent loss of vestibular information, which means these neurons never respond normally to head movements (Ris and Godaux 1998, Ris et al. 1997). This loss of vestibular information results in a number of anatomical, neurochemical and electrophysiological changes in the hippocampus.

1.5.2.1 Neurochemical

The decrease in hippocampal volume following bilateral vestibular damage (Brandt et al. 2005), and the behavioural changes shown in the human and animal studies mentioned previously, suggests that the hippocampus is undergoing neurochemical changes either as a result of the damage, or in order to compensate for the lesion.

Adaptations in neuronal plasticity and synaptic transmission could explain the anatomical changes observed in the hippocampus. The relative amounts of four proteins important for synaptic plasticity were measured in the hippocampus of BVD and sham rats using Western blotting. Synaptophysin is involved in neurotransmitter vesicle release (Fuentes-Santamaría et al. 2007) and SNAP-25 has a role in vesicle exocytosis (Delgado-Martinez et al. 2007). The synaptic protein drebrin is important for axonal and dendritic plasticity (Kobayashi et al. 2007) and neurofilament-L (NF-L) is a marker for cell death and axonal loss (Barry et al. 2007, Petzold et al. 2007). At 6 months post-surgery there was a significant increase in SNAP-25 in the DG compared to sham controls. Drebrin A was reduced in the EC and FC at the same time-point and drebrin E was significantly decreased in the EC. The protein NF-L was reduced in the FC of BVD rats when compared to shams. There were no changes in any of these proteins in the CA1 or CA2/3 regions at 6 months (Goddard et al. 2008c). The increased SNAP-25 in the DG is particularly interesting as it suggests a change in synaptic vesicle docking and fusion following neurotransmitter secretion in the hippocampus post-BVD (Goddard et al. 2008c). Furthermore, plasticity in the EC and FC and cell death and axonal loss in the FC may be reduced in BVD animals.

As discussed earlier, human patients with peripheral vestibular damage have a high incidence of anxiety disorders and depression (Furman and Jacob 2001, Godemann et al. 2004, Jacob and Furman 2001, Nagaratnam et al. 2004, Yardley et al. 1998). Depression is associated with changes in biogenic amine levels, such as serotonin, noradrenaline and dopamine, although this link is controversial (Castrén 2005, Maas 1975, Schildkraut and Kety 1967). Nevertheless, pharmacological interventions for patients suffering from these mood disorders, often alter the neurotransmitters noradrenaline, serotonin, and dopamine in the brain (Staab et al. 2002).

In BVD animals 6 months following surgery, tyrosine hydroxylase, the enzyme that metabolises noradrenaline, was significantly decreased in the FC compared to sham controls, and the protein that removes serotonin from the synaptic cleft, the serotonin transporter, was reduced in the FC and in the CA1 region. Tryptophan hydroxylase is the enzyme that metabolises serotonin; it was decreased in the EC and was significantly increased in the FC, CA2/3 and DG (Goddard et al. 2008b). Conversely, the decrease in the serotonin transporter and tryptophan hydroxylase would suggest an increase in serotonin in synaptic clefts. This result, along with the unchanged glucocorticoid levels observed in studies mentioned earlier, would suggest that the mood disorders and increased stress levels observed following vestibular damage are limited to humans.

Besnard et al. (2011, in press) induced bilateral vestibular lesions in rats using consecutive unilateral transtympanic injections of sodium arsanilate 3 weeks apart. This method is clinically relevant as Meniere's disease patients sometimes have one labyrinth lesioned because their symptoms are worse on that side and then have the contralateral labyrinth lesioned at a later time when symptoms become unbearable again (Strupp and Brandt 2009). This is known as Bechterew's phenomenon, which is the process of reversing post-unilateral labyrinthectomy spontaneous nystagmus by performing a labyrinthectomy on the contralateral side (Katsarkas and Galiana 1983). Autoradiography was used to measure changes in NMDARs 2 months after the initial injection, and showed an increase in hippocampal NMDAR density and a reduction in receptor affinity (Besnard et al. 2011, in press). It is possible that the receptors' affinity decreased to compensate for the increase in receptor expression.

1.5.2.2 Electrophysiological

In contrast to the Zheng et al. (2003) study which found that UVD surgery resulted in a bilateral reduction in electrical excitability at 5-6 months in the CA1 region, BVD surgery did not produce any detectable changes in LTP or basal synaptic transmission at 43 days or 7 months post-surgery in the CA1 or DG regions *in vivo* (Zheng et al. 2010). BVD surgery had no significant effect on either the induction or maintenance of LTP or baseline field potentials when compared to shams. There may be subtle changes occurring in LTP following BVD that are not detectable by field potential analysis (Zheng et al. 2010). Furthermore, any electrophysiological changes may have occurred in other areas of the hippocampus. However, it was suggested by the authors that the deficits in spatial memory observed following BVD may not be because of changes in LTP, but may be because of alterations in the encoding and consolidation of spatial memory. This process occurs via interactions between the hippocampus and other areas such as the limbic system and neocortex (Zheng et al. 2010).

1.5.2.2.1 Place cells

An animal's location in space is encoded by hippocampal place cells (O'Keefe and Dostrovsky 1971, O'Keefe and Nadel 1978). Each place cell fires when a subject is in a particular location in the environment or its "place field" (Muller et al. 1987). In order to navigate correctly, allothetic and egocentric cues are integrated so that place cells fire correctly and an accurate representation of space is formed (Etienne et al. 1996, McNaughton et al. 1996, Whishaw 1998). The vestibular system is a significant source of self-movement information and therefore is a vital egocentric cue (Berthoz et al. 1995, Matthews et al. 1989). This was confirmed by the finding that hippocampal place cells respond to vestibular information (Sharp et al. 1995).

Stackman et al. (2002a) showed that reversible inactivation of the vestibular system in rats disrupts location-specific firing of hippocampal place cells. Tetrodotoxin was injected bilaterally through the tympanic membrane of rats, creating a reversible vestibular lesion. The firing patterns of place cells in the CA1 region of the hippocampus were severely disrupted following injection and the hippocampal place fields also became unstable and their specificity was reduced. The place cells' spatial firing recovered, as the tetrodotoxin wore off and vestibular function returned to normal. These data support the hypothesis that the

vestibular system supplies self-movement information to the hippocampus and has an important role in hippocampal spatial representations. It could also explain why humans with vestibular dysfunction suffer from navigational and spatial memory deficits (Stackman et al. 2002a).

Russell et al. (2003b) showed that permanent vestibular inactivation also causes a loss of location-specific firing in hippocampal place cells. The hippocampal CA1 neurons of rats that had undergone either BVD or sham surgery were recorded extracellularly for 60 days after surgery. Complex spiking cells were also recorded and the neurons' place fields were examined. Location-specific firing was completely abolished in animals that had undergone bilateral labyrinthectomy, with the firing fields of the complex spiking neurons showing decreased spatial coherence. This study reinforces the finding that these lesions disrupt internal vestibular sensory inputs to the hippocampus, which interferes with the integration of self-movement signals with other egocentric cues in the formation of a coherent representation of space (Russell et al. 2003b).

1.5.2.2.2 Theta rhythm

During voluntary movement there is an electroencephalography (EEG) signal produced that oscillates at around 8 Hz. This signal is known as theta rhythm (Bland 1986, Bland and Colom 1993, Buzsáki 2002) and it is thought to have an important role in encoding spatial memory (Huxter et al. 2003, O'Keefe and Recce 1993). There is a correlation between theta frequency and movement velocity (Leung 1984), which suggests that theta rhythm has a role in the construction of correct representations of the spatial environment using egocentric and allocentric cues (McNaughton et al. 1996, Whishaw 1998).

Stimulation of the vestibular system has been shown to induce theta rhythm activity (Arnolds et al. 1984, Hicks et al. 2004) and increase the power of the theta frequency in the hippocampus in both light and dark conditions (Gavrilov et al. 1995). Correspondingly, theta rhythm in BVD animals has reduced power and frequency compared to sham animals (Russell et al. 2006). At least 60 days after surgery, hippocampal CA1 neurons of freely moving BVD animals fired with the same periodicity of around 8 Hz as sham animals, and the cell firing rate and movement velocity were positively correlated in both BVD and sham animals. Theta rhythm is known to be closely related to movement and the behavioural state of the animal

(Bland 1986). Research has shown that theta power and frequency generally increases with increasing movement (Terrazas et al. 2005). BVD animals are hyperkinetic (Goddard et al. 2008a), which is a possible confounder in theta studies. Therefore, the changes observed in theta in BVD animals could be due to the animals' hyperactive state. However, the lesioned animal's theta readings were less rhythmic even after the animals' velocity was controlled for by matching the lesioned animals to controls using speed of movement. These results indicate that while vestibular signals may have a role in theta rhythm, hippocampal neurons' velocity-related firing does not require intact vestibular input (Russell et al. 2006).

Russell et al. (2006) theorised that the coordination of neuronal firing in the hippocampus was disrupted by the vestibular lesion, so that while the neurons' intrinsic oscillations are maintained, synchronisation of the population of neurons is reduced, which affects EEG readings. This is interesting as there is a relationship between the pattern of place cell firing and theta frequency (O'Keefe and Recce 1993, Skaggs et al. 1996). This indicates that the vestibular lesion induced change in theta may be responsible for the loss of coherent place cell firing.

In contrast, Stackman et al. (2002a) reported no change in hippocampal theta after vestibular inactivation via transtympanic injections of the ototoxin sodium arsanilate. However, this method can produce an incomplete lesion due to poor penetration of the oval window (Jensen 1983), so that vestibular function is not completely eliminated (Saxon et al. 2001). By contrast, surgical lesions produce complete vestibular lesions (Campos-Torres et al. 2005). Stackman et al. (2002a) also used only one animal when assessing changes in hippocampal theta activity.

A recent study found that stimulation of the vestibular system during spatial navigation activated theta rhythm in the hippocampus. Rats were rotated around the vertical axis in light and dark conditions while EEG readings were taken from the CA1 region. Hippocampal theta rhythm was induced during rotation even though the rat was kept otherwise immobile. Passive rotation stimulates the vestibular system, and this has been shown to alter place fields thereby modifying the function of the hippocampus (Wiener et al. 1995). Rotation-induced theta rhythm was reduced in rats after bilateral chemical vestibular lesions (Tai et al. 2011, in press).

Medial septal cells act like 'pacemaker' cells for hippocampal theta rhythm (Petsche and Stumpf 1960, Petsche et al. 1962). Theta was attenuated in rats that had the cholinergic neurons in the medial septum lesioned and theta was also abolished by the muscarinic receptor antagonist atropine (Tai et al. 2011, in press). This result correlated with the finding that acetylcholine (ACh) is released from the hippocampus after electrical stimulation of the round window. This release of ACh was inhibited when the NMDAR antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) was injected into the ipsilateral vestibular nucleus, blocking second-order vestibular neurons (Horii et al. 1995). This result suggests that the cholinergic activity in the septohippocampal pathway may be important for vestibular induced changes in hippocampal spatial processing and that glutamate activity has a role in this process.

1.6 Conclusion

Vestibular damage-induced changes in hippocampal physiology may lead to long-term alterations in how the hippocampus processes information. Humans with vestibular inner ear damage experience cognitive problems that are thought to be related to a decrease in the size of the hippocampus, a part of the brain important for learning and memory. Despite the numerous changes observed in the rat hippocampus following BVD including alterations in electrical excitability (Zheng et al. 2003), theta rhythm (Russell et al. 2006) and place cell activity (Russell et al. 2003b, Stackman et al. 2002a), there are few neurochemical studies on the hippocampus in animal models that have undergone BVD surgery. The few studies that are available show subtle neurochemical changes across a number of brain regions (Goddard et al. 2008b, Goddard et al. 2008c).

1.7 Aims

The aim of this research project was to study the changes in glutamatergic receptors and signalling molecules involved in synaptic transmission in the hippocampus and other areas of the medial temporal lobe in an animal model after bilateral vestibular damage in order to help understand the subsequent alterations in hippocampal neurobiology and thus cognitive function.

The aim of this research is therefore to examine:

- Changes in NMDA and AMPA receptor subunits levels in the various subregions of the hippocampus, the entorhinal cortex, perirhinal cortex and frontal cortex, using western blotting, following bilateral vestibular lesions at various time points.
- Changes in CaMK-II and phosphorylated CaMKII protein levels in the various subregions of the hippocampus, using western blotting, following bilateral vestibular lesions at various time points.

Chapter two

Methods

2.2 Animals

Fifty six 9-12 week old male Wistar rats were randomly allocated to 24 hour (n=14), 72 hour (n=14), 1 week (n=14) or 1 month (n=14) time points. Subsequently the rats were randomly allocated to BVD (n =7) or sham surgery (n =7) conditions within each time point. Animals were maintained on a 12:12 h light:dark cycle at 22 ˚C and housed in individual cages.

2.3 Surgery

Bilateral vestibular deafferentiation surgery was performed on seven rats; the animals were anesthetized using 300 mg/kg fentanyl citrate (i.p.) and 300 mg/kg medetomidine hydrochloride (i.p.). The surgery was performed under microscopic control as detailed previously (Zheng et al. 2006). To summarise, a retro-auricular approach was used to expose the tympanic bulla and the tympanic membrane; once exposed the malleus and incus were removed. Next the stapedial artery was cauterized; and the horizontal and anterior semicircular canal ampullae were drilled open and their contents, including the saccule and utricle, were aspirated. Finally, the temporal bone was sealed using dental cement. After the surgical margins had been sutured, the postoperative analgesia carprofen (5 mg/kg, s.c.) was administered.

The remaining seven rats underwent sham surgery using the same anaesthesia. This involved exposing the temporal bone and removing the tympanic membrane without producing a vestibular lesion. Previous studies have confirmed, using temporal bone histology, that this BVD procedure produces a complete and permanent bilateral lesion of the vestibular labyrinth (Zheng et al. 2006).

It was felt that another control surgery using a sham group with no removal of the tympanic membrane was not needed, as a previous study has shown that damage to the auditory system does not alter place cell function in the hippocampus (see Smith et al. 2005 for a discussion). Removal of the tympanic membrane produced partial deafness in the control group as well as in the BVD group, therefore the neurochemical effects of BVD in comparison to the sham group were thought to reflect vestibular and not auditory damage.

All procedures were carried out in accordance with the regulations of the University of Otago Committee on Ethics in the Care and Use of Laboratory Animals. Following surgery, the animals recovered for 24 hours, 72 hours, 1 week or 1 month. It has been shown that by 1 month the animals have recovered from the severe, acute symptoms of BVD and some compensation has occurred, although the VORs and VSRs never return to normal following BVD. In the earlier time points the animals would have been suffering from the initial effects of the surgery (see Smith and Curthoys, 1989 for a review). The surgery is known to result in a number of behaviours that are now considered idiosyncratic of BVD, including gait ataxia, marked hyperactivity, head-dorsiflexion, head-weaving, and circling (Russell et al. 2003a, Zheng et al. 2006).

2.4 Tissue preparation

At 24 hours, 72 hours, 1 week and 1 month post-op., the animals were decapitated without anesthesia, and the hippocampal subregions (CA1, CA2/3 and the dentate gyrus (DG)), entorhinal cortex (EC), perirhinal cortex (PRC), and frontal cortex (FC) were dissected out using the methods described previously (Liu et al. 2003b, Zheng et al. 1999b, 1999c, Zheng et al. 2000), and stored in a -80 ˚C freezer until use.

2.4.1 Standard tissue preparation of 24 hour, 72 hour and 1 week tissue

The 24 hour, 72 hour and 1 week tissue underwent the standard tissue preparation for western blotting. Tissue buffer (containing Complete Proteinase Inhibitor, 50-mM Tris–HCl pH 7.6) was added to the samples on ice, then the tissue was homogenised using ultrasonification (Sonifier cell disrupter B-30, Branson Sonic Power Co.) and centrifuged at 12,000 g for 10 min at 4 °C.

The protein concentration in the supernatant was measured using the Bradford method. The supernatant and a Bio-Rad protein assay dye reagent concentrate were combined in a 96-well plate and analyzed using a spectramax microplate reader (Zheng et al. 2001a).

The protein concentrations in the samples were equalized, then the tissue homogenates were mixed with gel loading buffer (50 mM Tris-HCl, 10% SDS, 10% glycerol, 10% 2-mercaptoethanol, 2 mg/ml bromophenol blue) in a ratio of 1:1 and then boiled for 5 min.

2.4.2 Membrane preparation of 1 month tissue

The membranes were prepared according to the method described by Williams et al. (2003). The 1 month BVD and sham tissue was homogenised in 1.5 mL of 0.32 M sucrose using 10 strokes of a 1 mL hand-held glass teflon homogeniser on ice. The resulting homogenate was centrifuged for 10 min at 1000 x g (2820 RPM; using a JA-18.1 rotor). The resulting supernatant was collected and centrifuged at 20,000 x g (12630 RPM) for 20 min at 4^{o} C, and then the pellets were resuspended in ice-cold dH_2O, and centrifuged again for 20 min at 8,000 x g (7990 RPM). The supernatant fraction and buffy coat were collected and centrifuged for 10 min at 48,000 x g (19920 RPM; changing to a JA-20 rotor) at 4^{o} C; afterwards the pellets were resuspended in tris-HCl buffer (50 mM, pH 7.4), and centrifuged under the same conditions. The pellet was then frozen in dry ice for 2 minutes; this last step was repeated. Finally, the resulting membrane receptor preparations were stored at -80°C until the protein concentrations were estimated using a Bradford assay.

The protein concentrations in the samples were equalized to 2 or 1 mg/ml. Tissue homogenates were then mixed with gel loading buffer (50 mM Tris-HCl, 10% SDS, 10% glycerol, 10% 2- mercaptoethanol, 2 mg/ml bromophenol blue) in a ratio of 1:1 and then boiled for 5 min.

2.5 Western blotting

Ten µl of each sample was loaded in each well on a 7.5% SDS-polyacrylamide mini-gel and pre-stained protein markers (10–250 kDa; Bio-Rad, Precision Plus: Dual colour) were used as molecular weight markers on each gel. The samples were electrophoresed with a 90 V variable current (Bio-Rad, PowerPack 3000) until protein flattened at the stacking/resolving interface, and 180 V thereafter. The proteins were transferred to polyvinylidene-difluoride (PVDF) membranes using a transblotting apparatus (2.5 L; Bio-Rad). The transfer was performed overnight in transfer buffer (25% methanol, 1.5% glycine and 0.3% Tris-base) at 10 V variable current overnight (Bio-Rad PowerPack 3000).

Non-specific IgG binding was blocked by incubation with 5% dried milk protein (Pams) and 0.1% bovine serum albumin (BSA) (Sigma) for 6-7 h at 4 C°. The membranes were then incubated with affinity-purified polyclonal goat antibodies raised against GluR1, GluR2, GluR3 and GluR 4, and affinity-purified polyclonal rabbit antibodies raised against NMDA ζ1 (NR1), NMDA ε1 (NR2A), and NMDA ε2 (NR2B) overnight at 4 °C; antibody details are in table 2.1. The secondary antibodies were anti-goat IgG linked to horseradish peroxidase and anti-rabbit IgG linked to horseradish peroxidise; details in table 2.1. Antibody concentrations were selected based on previous laboratory work (Liu et al. 2003b, Liu et al. 2004b). Detection was performed using the enhanced chemiluminescence (ECL) system (Amersham Biosciences, NZ). Hyperfilms (Amersham Biosciences, NZ) were analyzed by densitometry to determine the quantity of protein expressed in each group using a calibrated imaging densitometer (Bio-Rad) and a PowerPC Mac running OS 9.2 and Quantity One software.

Primary Antibody	**Dilution**	**Secondary Antibody**	**Dilution**
GluR1 (N-19), sc-7608 Santa Cruz	1:1000	donkey-anti-goat IgG-HRP, sc-2020 Sigma	1:5000
GluR2 (N-19), sc-7611 Santa Cruz	1:1000	donkey-anti-goat IgG-HRP, sc-2020 Sigma	1:5000
GluR3 (N-19), sc-7613 Santa Cruz	1:1000	donkey-anti-goat IgG-HRP, sc-2020 Sigma	1:5000
GluR4 (H-20), sc-31394 Santa Cruz	1:1000	donkey-anti-goat IgG-HRP, sc-2020 Sigma	1:5000
NR1 (H-54), sc-9058 Santa Cruz	1:1000	goat-anti-rabbit IgG-HRP, sc-2004 Sigma	1:1000
NR2A (H-50), sc-9056 Santa Cruz	1:1000	goat-anti-rabbit IgG-HRP, sc-2004 Sigma	1:1000
NR2B (H-300), sc-9057 Santa Cruz	1:1000	goat-anti-rabbit IgG-HRP, sc-2004 Sigma	1:1000
CaMKII (H-300), sc-13082 Santa Cruz	1:1000	goat-anti-rabbit IgG-HRP, sc-2004 Sigma	1:1000
p-CaMKII (Thr 286), sc-32289 Santa Cruz	1:1000	goat-anti-rabbit IgG-HRP, sc-2004 Sigma	1:1000
β-actin (I-19), sc-1616 Santa Cruz	1:5000	donkey-anti-goat IgG-HRP, sc-2020 Sigma	1:5000

Table 2.1: *Table containing the dilutions and companies of origin of the primary and secondary antibodies used for Western blotting.*

Results were expressed as volume of the band, i.e., optical density × area of the band. An antibody against β-actin (details in table 2.1), was used as a loading control to ensure that the same amount of protein was loaded in each lane, and the density of each target band was then expressed as a percentage of its corresponding loading control. It is possible that β-actin itself might change as a result of BVD; however it was decided that a loading control was necessary. Exploratory regression analyses already performed by our lab suggested that any changes in β-actin expression were unlikely to account for changes in the target protein expression (R^2=0.087) (Zheng et al. 2001b).

2.6 Statistics

The data were normalized to β-actin and then a 2-way analysis of variance (ANOVA), using surgery and time as factors, was used in Minitab 15 to analyze the data for each individual protein. In order to further investigate the data, and determine whether combinations of variables were changing rather than individual variables, we performed a series of multivariate statistical analyses. All of the data were standardised and expressed as z scores and then a stepwise linear discriminant analysis (LDA) was performed in SPSS 19, with Wilks' λ as the test statistic. Cross validation of the linear discriminant function was also carried out (Manly 2005). Finally, cluster analyses, using Ward's linkage and the correlation coefficient, were used to generate dendrograms so that relationships between the different variables could be inspected. The significance level was set at 0.005 for all comparisons.

Chapter three

Results

3.1 Protein and subunit expression in the hippocampus at 24 hours, 72 hours and 1 week post-BVD

Twenty four hours, seventy two hours and one week following surgery there was no significant difference in CaMKII, pCaMKII, GluR1, GluR2, GluR3, GluR4, NR1, NR2A or NR2B levels in the CA1, CA2/3 or DG sub-regions of the hippocampus of BVD rats compared to sham controls following a one-way ANOVA.

3.1.1 CaMKII expression levels in the hippocampus following BVD surgery

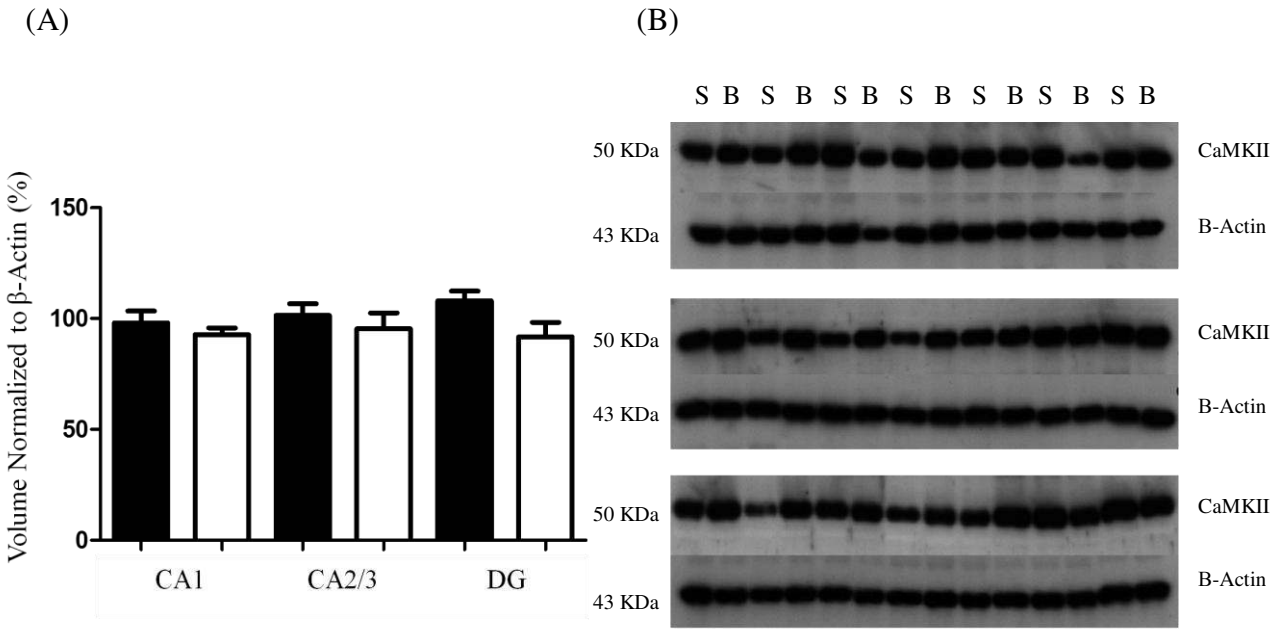


Figure 3.1: *A) Mean optical density for CaMKII expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of CaMKII expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of CaMKII is on the left on the immunoblot.*

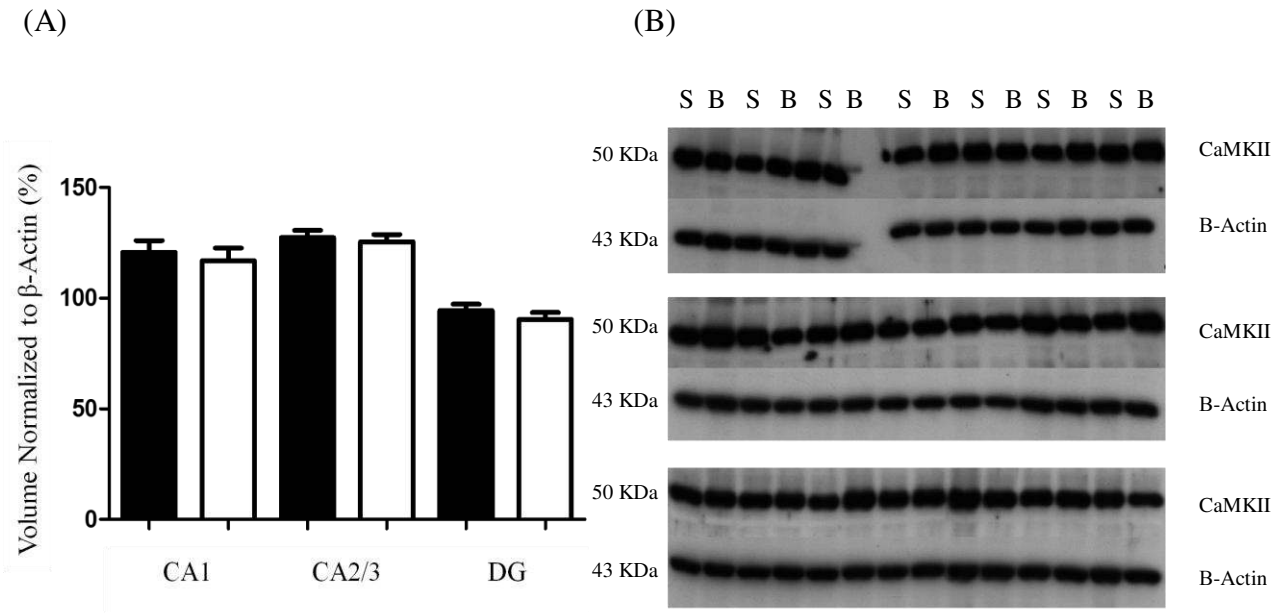


Figure 3.2: *A) Mean optical density for CaMKII expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of CaMKII expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of CaMKII is on the left on the immunoblot.*

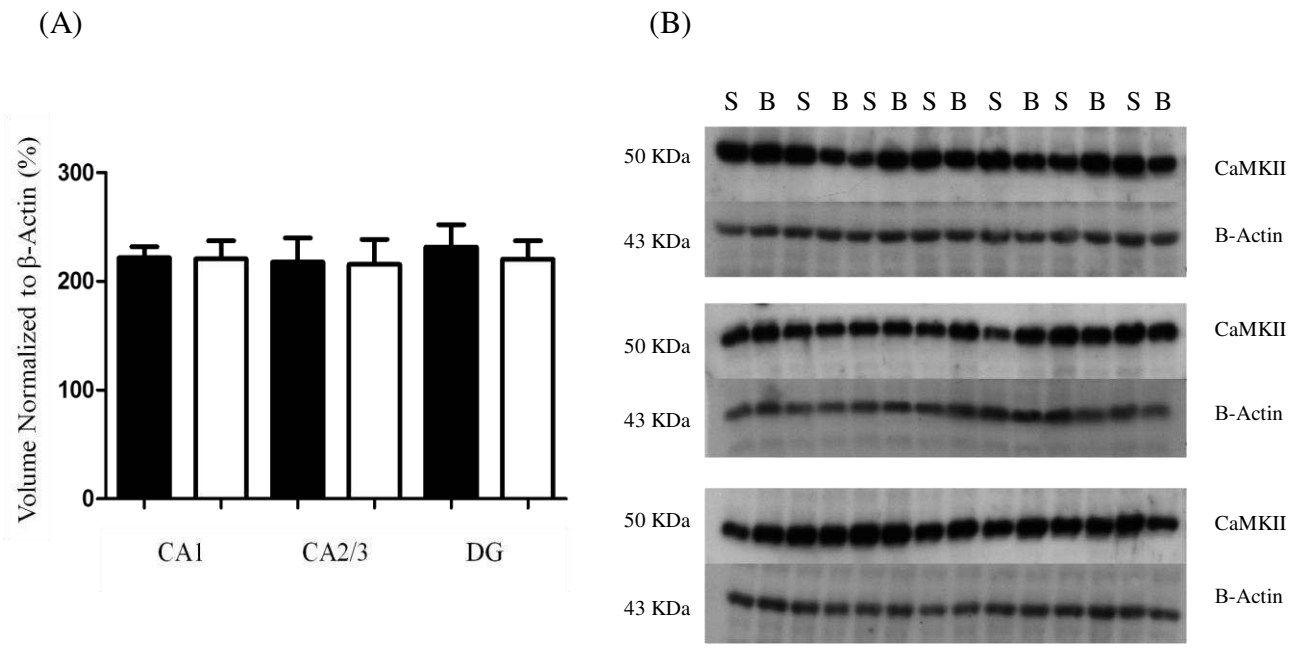


Figure 3.3: *A) Mean optical density for CaMKII expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of CaMKII expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of CaMKII is on the left on the immunoblot.*

3.1.2 pCaMKII expression levels in the hippocampus following BVD surgery

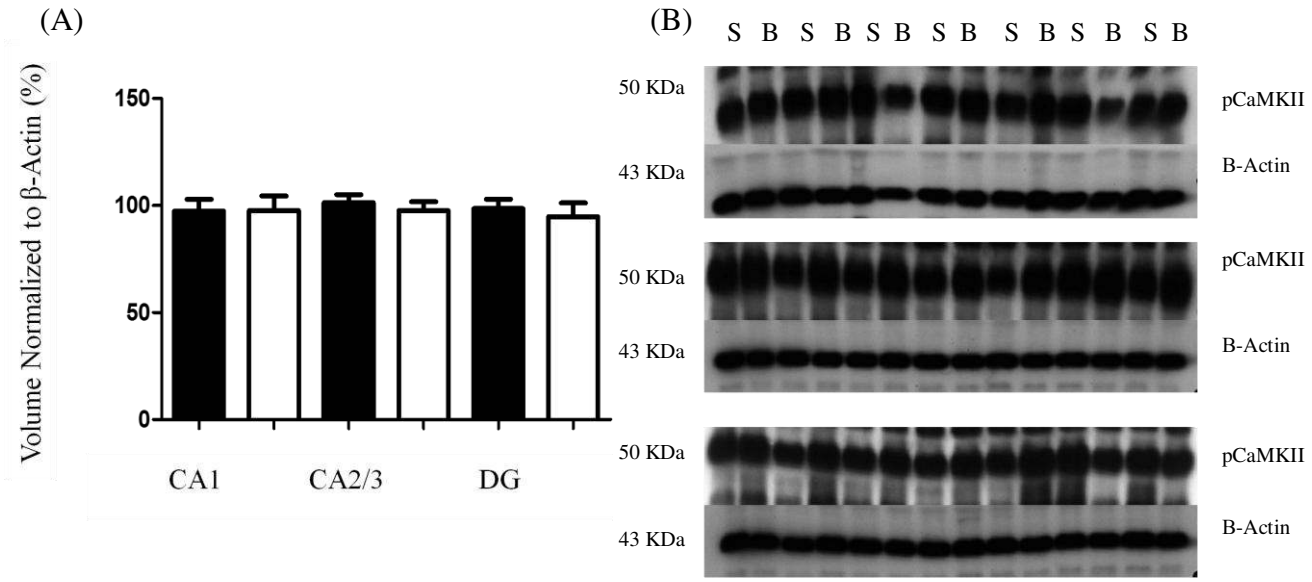


Figure 3.4: *A) Mean optical density for pCaMKII expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of pCaMKII expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of pCaMKII is on the left on the immunoblot.*

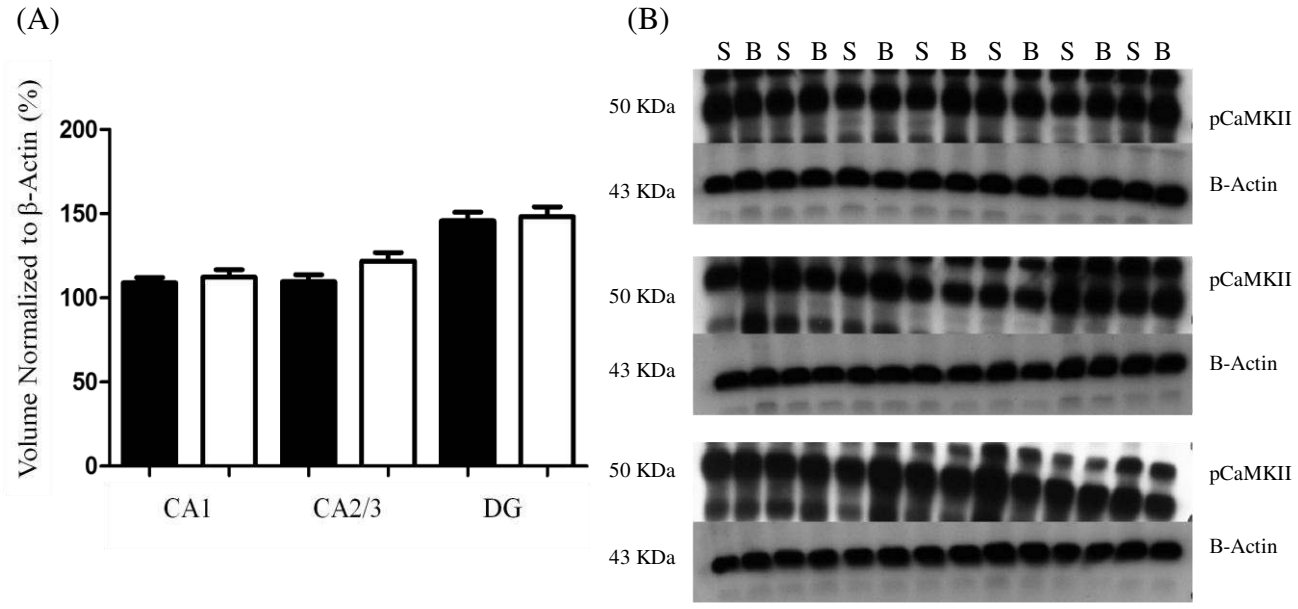


Figure 3.5: *A) Mean optical density for pCaMKII expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of pCaMKII expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of pCaMKII is on the left on the immunoblot.*

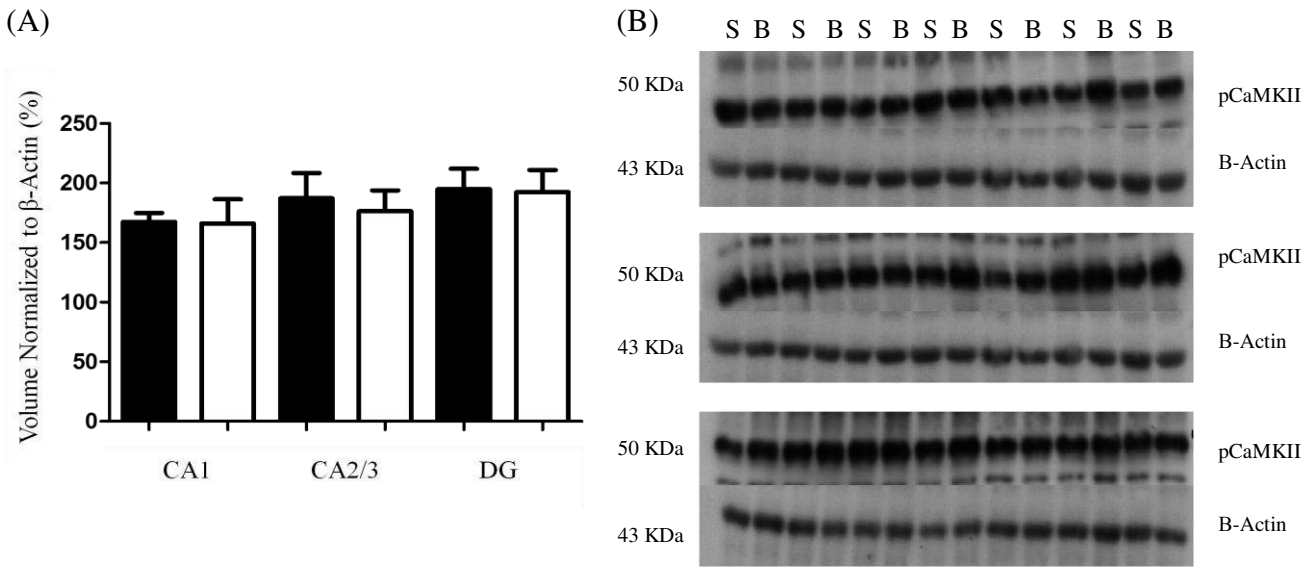


Figure 3.6: *A) Mean optical density for pCaMKII expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of pCaMKII expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of pCaMKII is on the left on the immunoblot.*

3.1.3 GluR1 expression levels in the hippocampus following BVD surgery

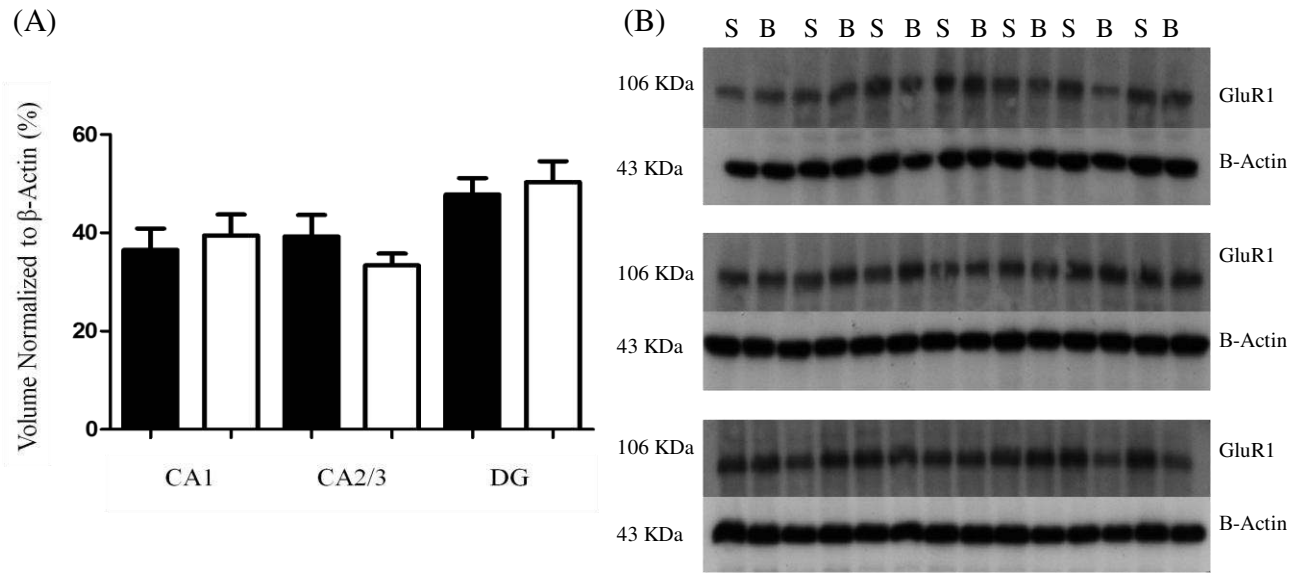


Figure 3.7: *A) Mean optical density for GluR1 expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR1 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR1 is on the left on the immunoblot.*

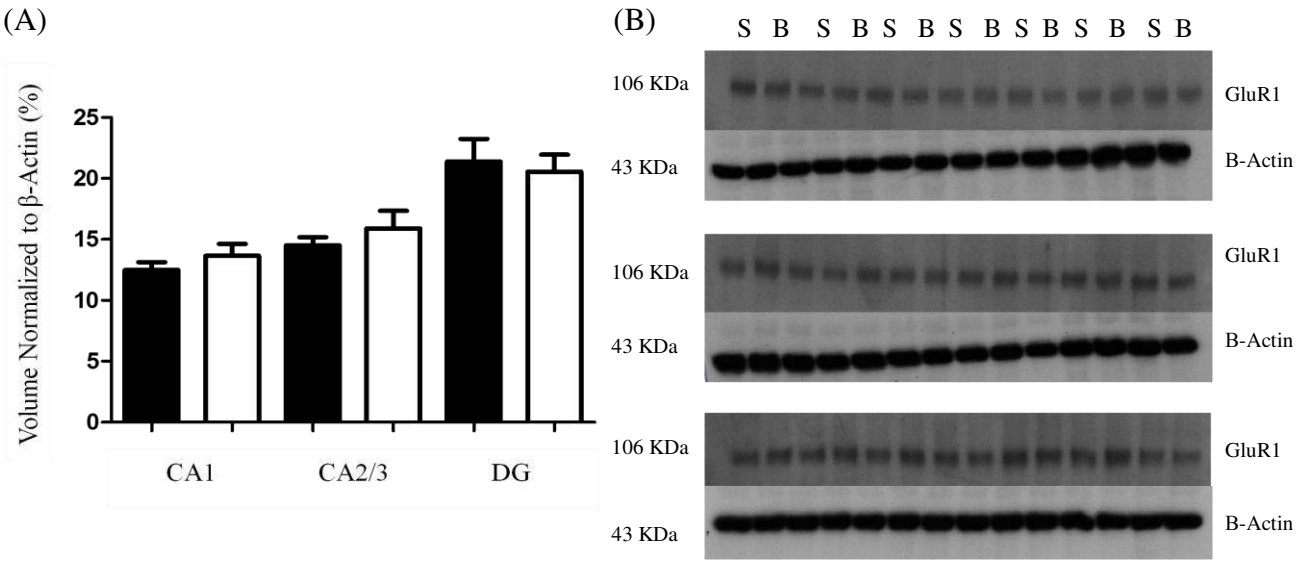


Figure 3.8: *A) Mean optical density for GluR1 expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR1 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR1 is on the left on the immunoblot.*

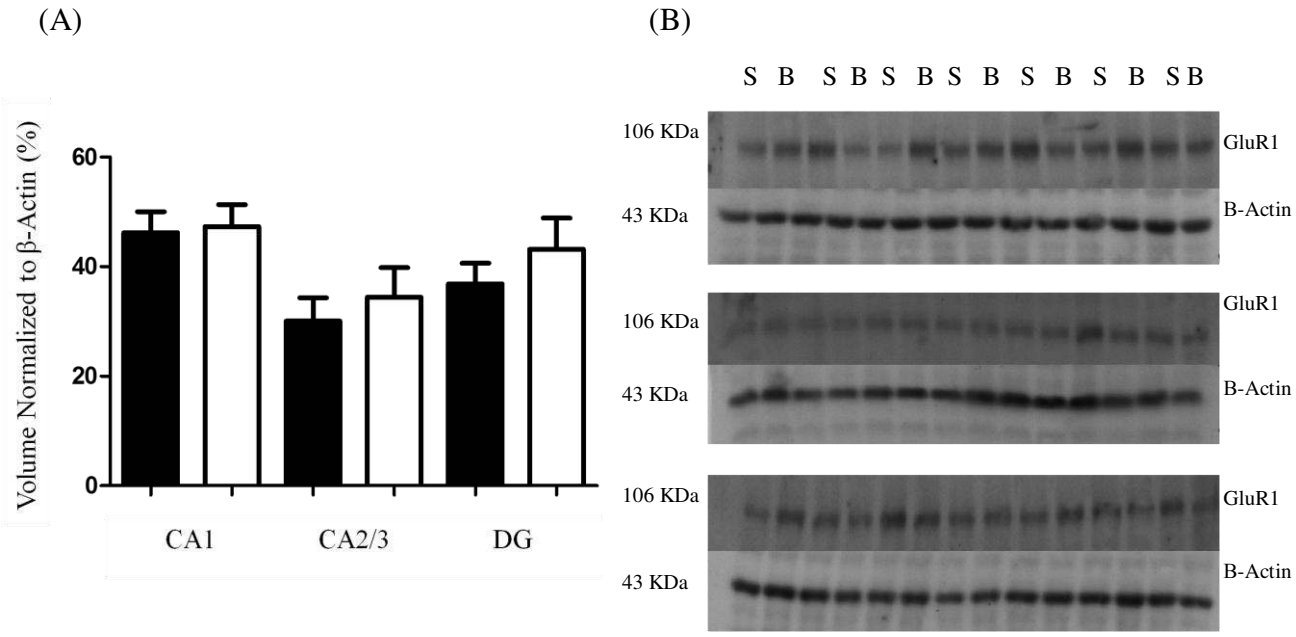


Figure 3.9: *A) Mean optical density for GluR1 expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR1 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR1 is on the left on the immunoblot.*

3.1.4 GluR2 expression levels in the hippocampus following BVD surgery

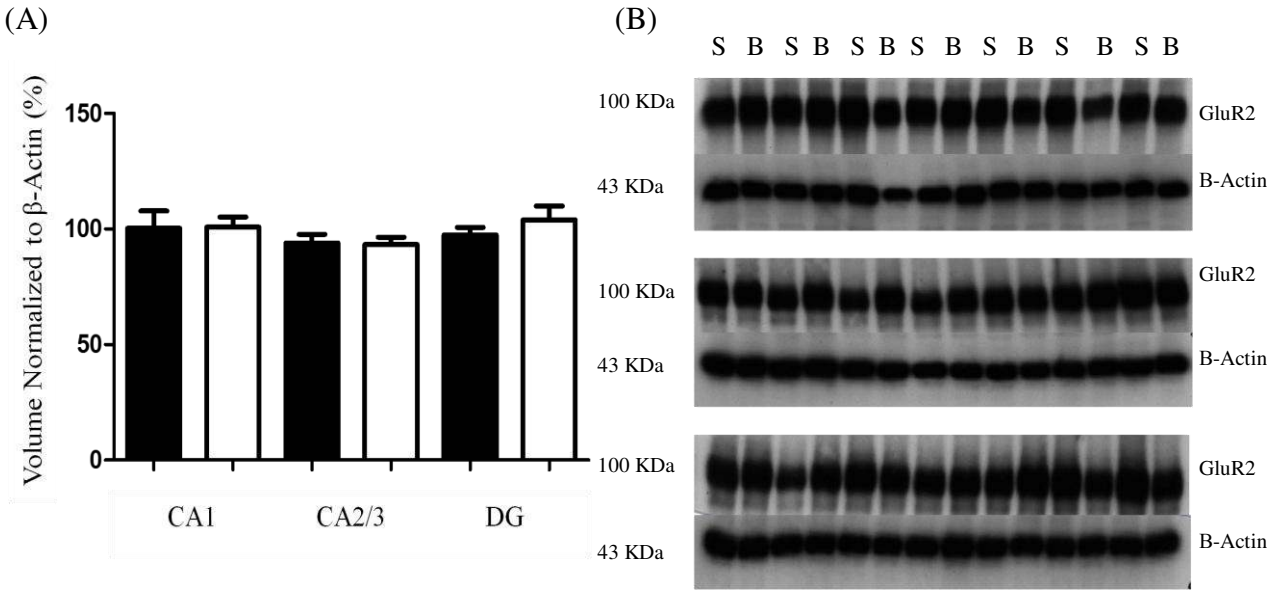


Figure 3.10: *A) Mean optical density for GluR2 expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR2 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR2 is on the left on the immunoblot.*

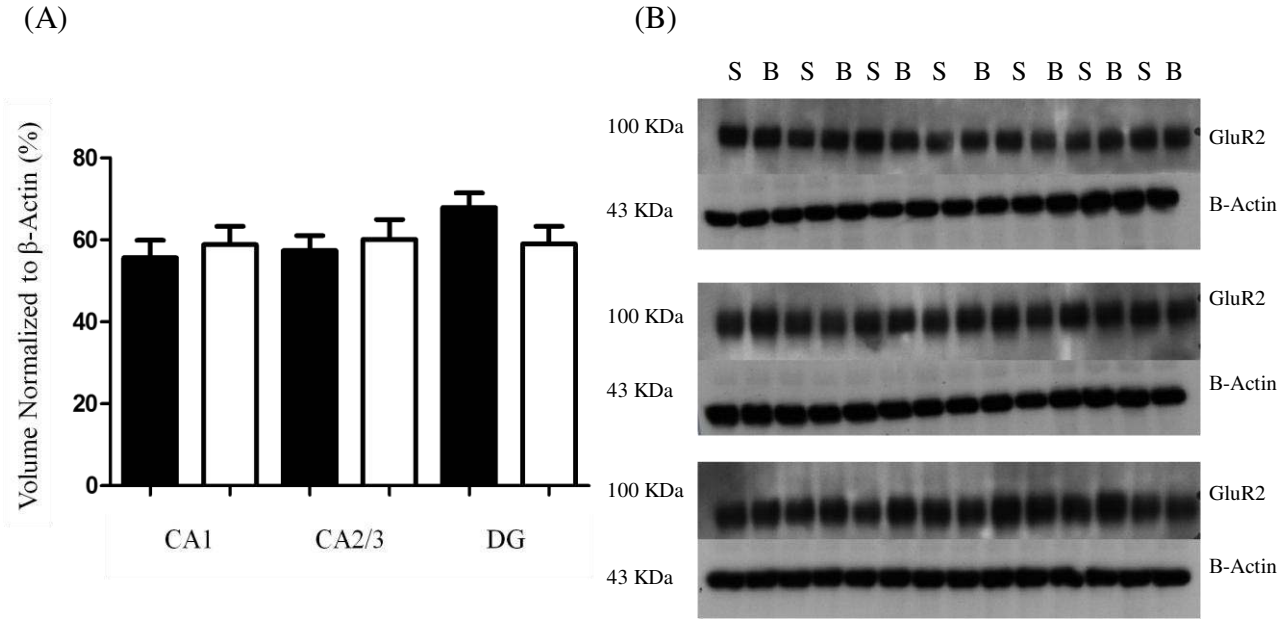


Figure 3.11: *A) Mean optical density for GluR2 expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR2 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR2 is on the left on the immunoblot.*

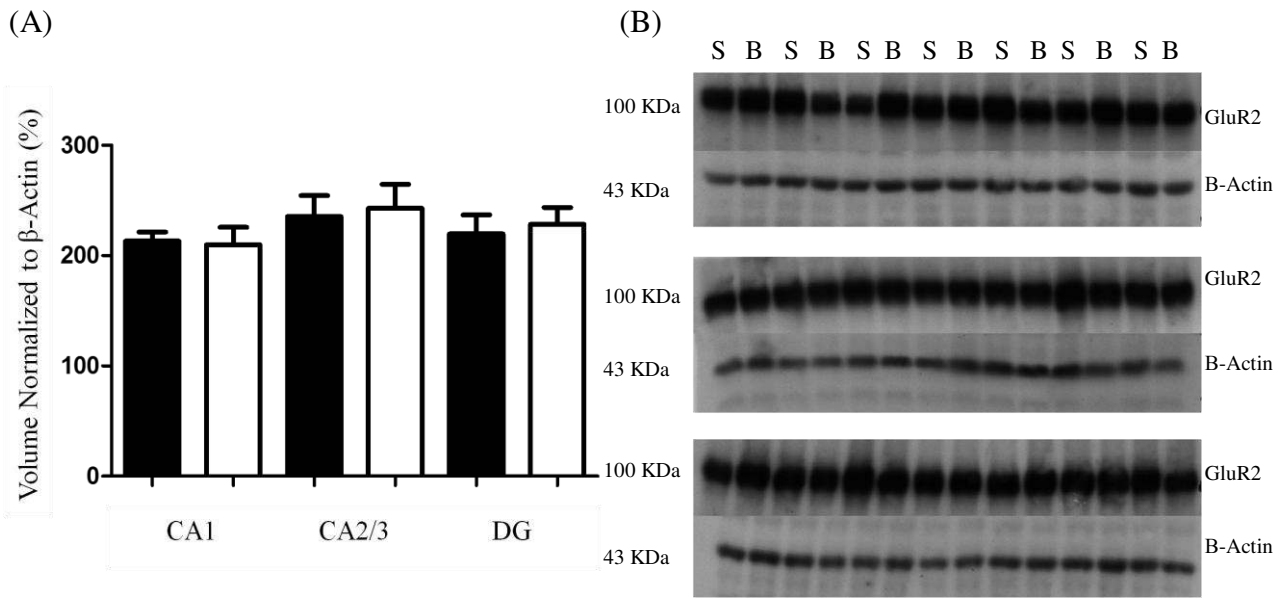


Figure 3.12: *A) Mean optical density for GluR2 expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR2 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR2 is on the left on the immunoblot.*

3.1.5 GluR3 expression levels in the hippocampus following BVD surgery

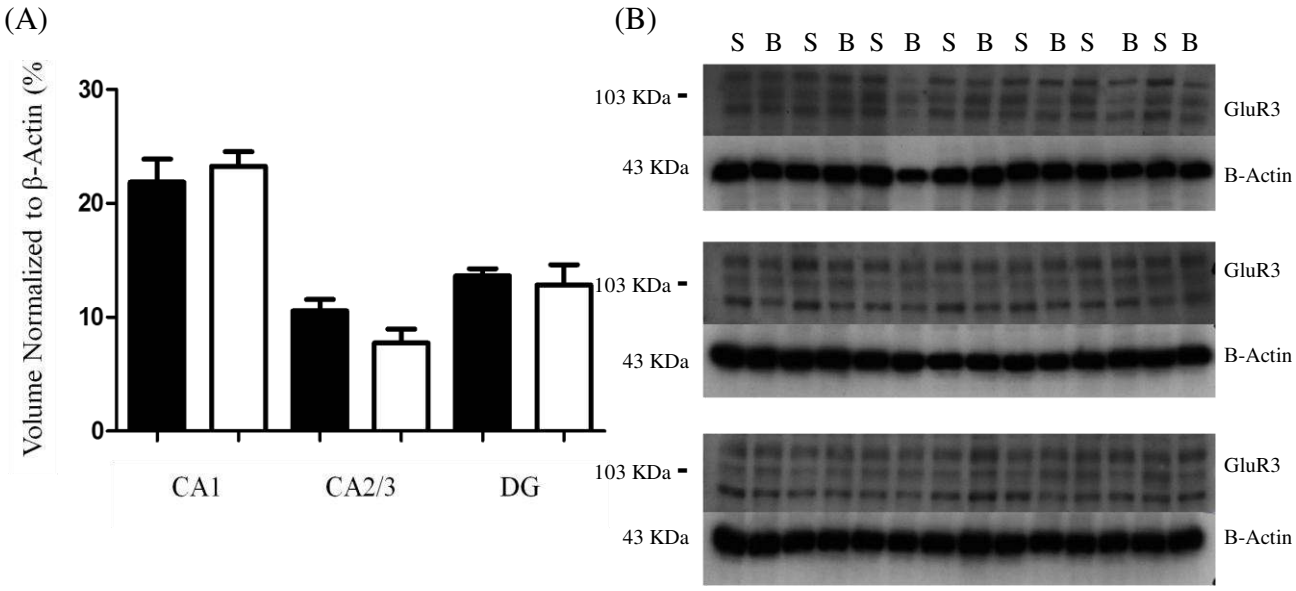


Figure 3.13: *A) Mean optical density for GluR3 expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR3 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR3 is on the left on the immunoblot.*

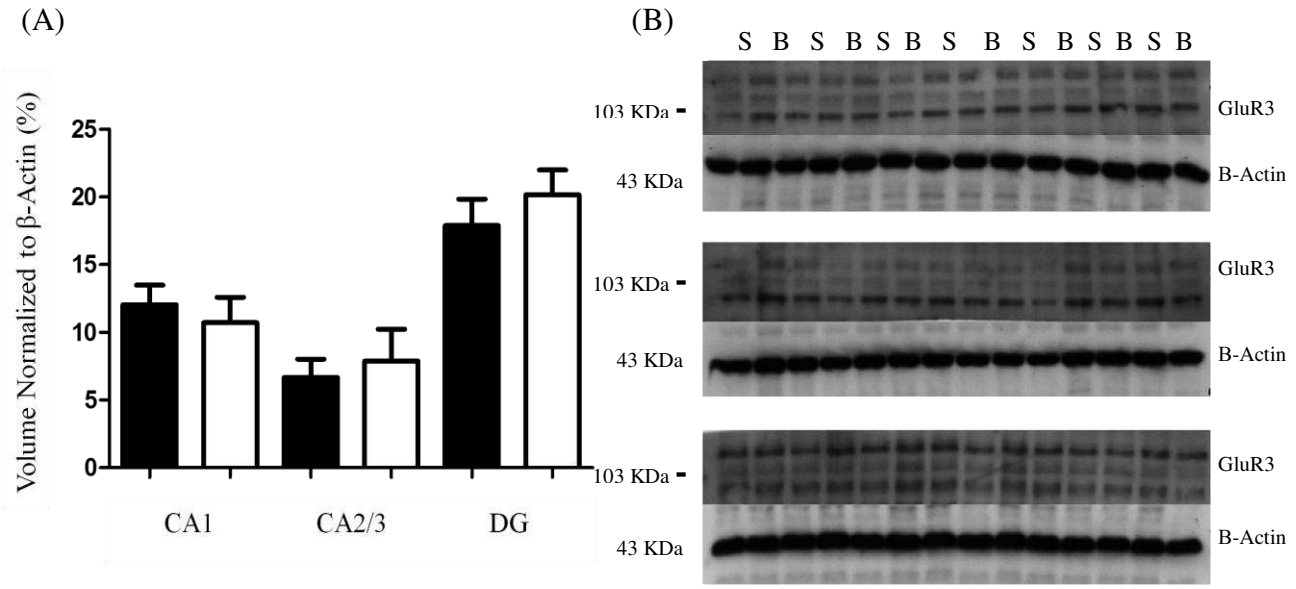


Figure 3.14: *A) Mean optical density for GluR3 expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR3 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR3 is on the left on the immunoblot.*

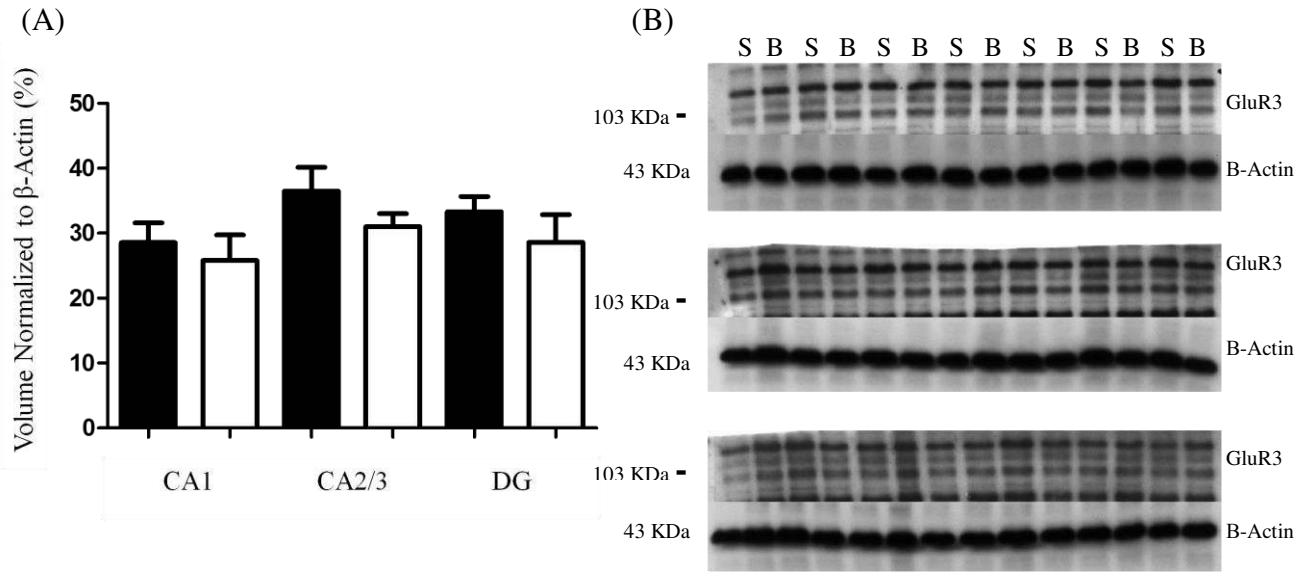


Figure 3.15: *A) Mean optical density for GluR3 expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR3 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR3 is on the left on the immunoblot.*

3.1.6 GluR4 expression levels in the hippocampus following BVD surgery

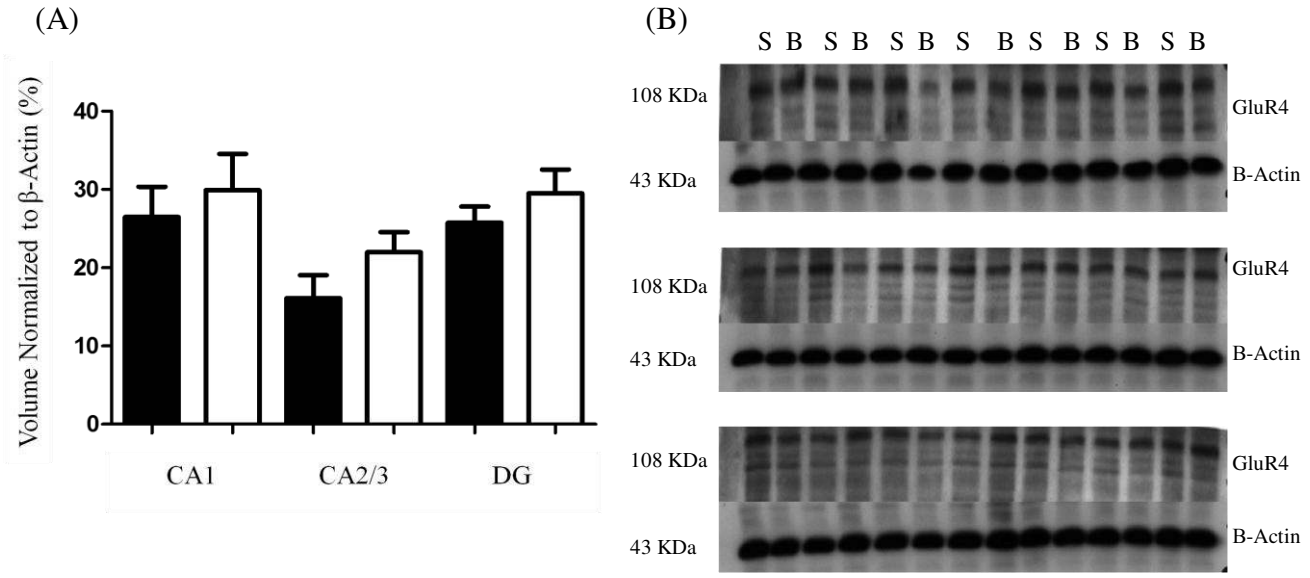


Figure 3.16: *A) Mean optical density for GluR4 expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR4 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR4 is on the left on the immunoblot.*

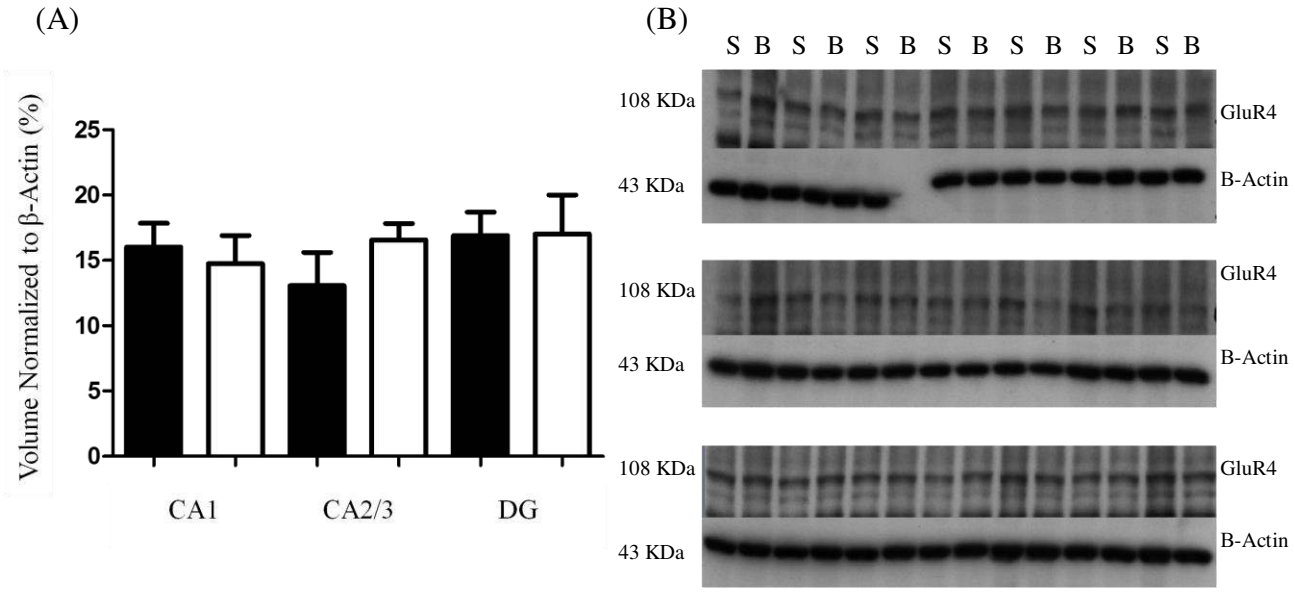


Figure 3.17: *A) Mean optical density for GluR4 expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR4 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR4 is on the left on the immunoblot.*

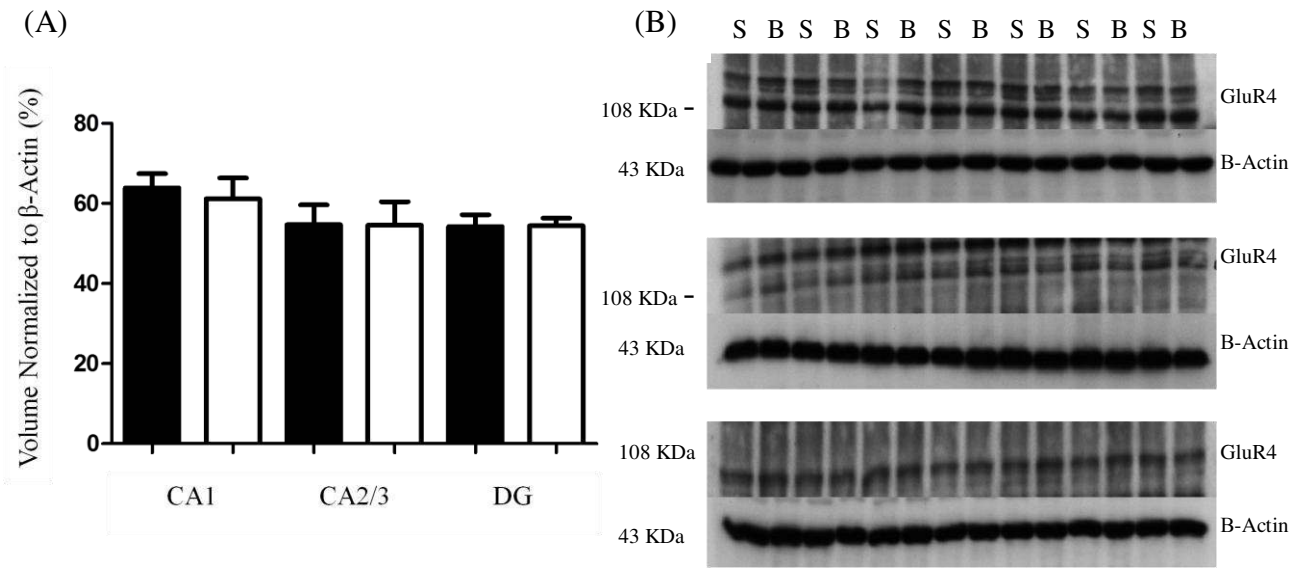


Figure 3.18: *A) Mean optical density for GluR4 expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR4 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR4 is on the left on the immunoblot.*

3.1.7 NR1 expression levels in the hippocampus following BVD surgery

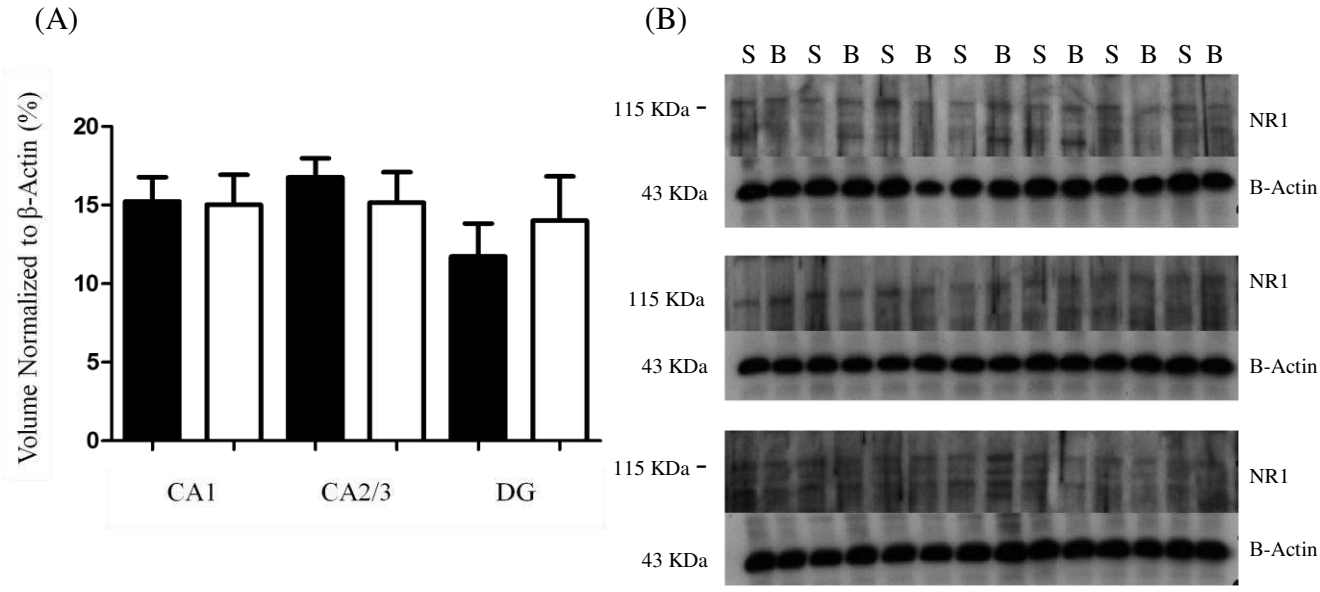


Figure 3.19: *A) Mean optical density for NR1 expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR1 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR1 is on the left on the immunoblot.*

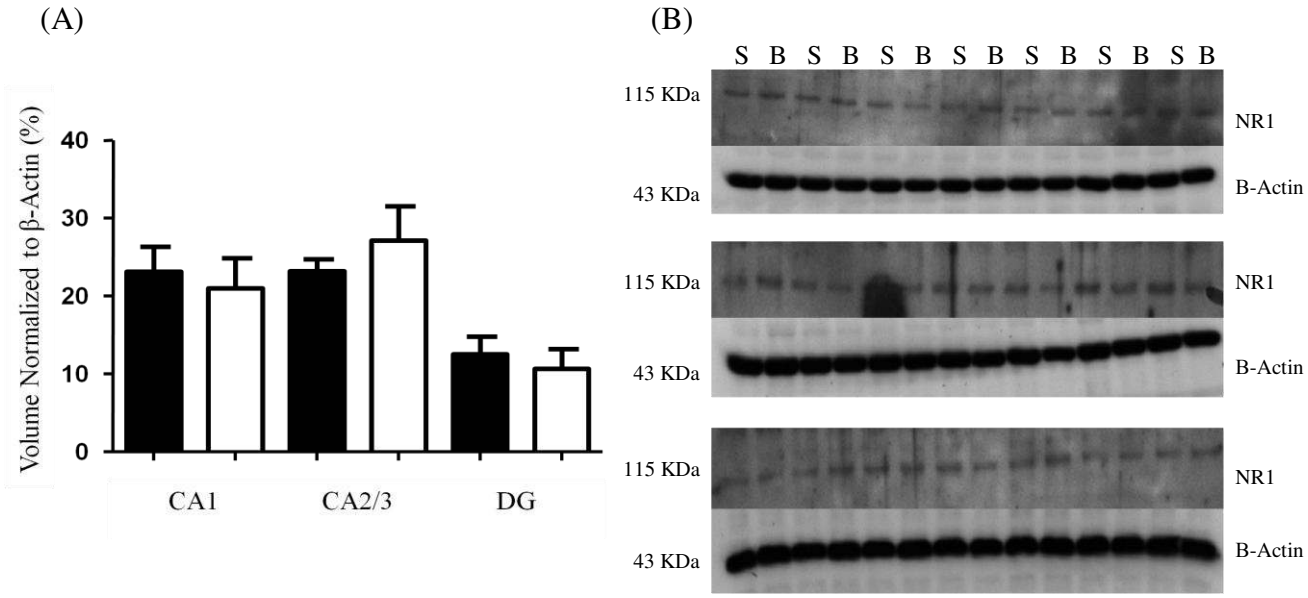


Figure 3.20: *A) Mean optical density for NR1 expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR1 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR1 is on the left on the immunoblot.*

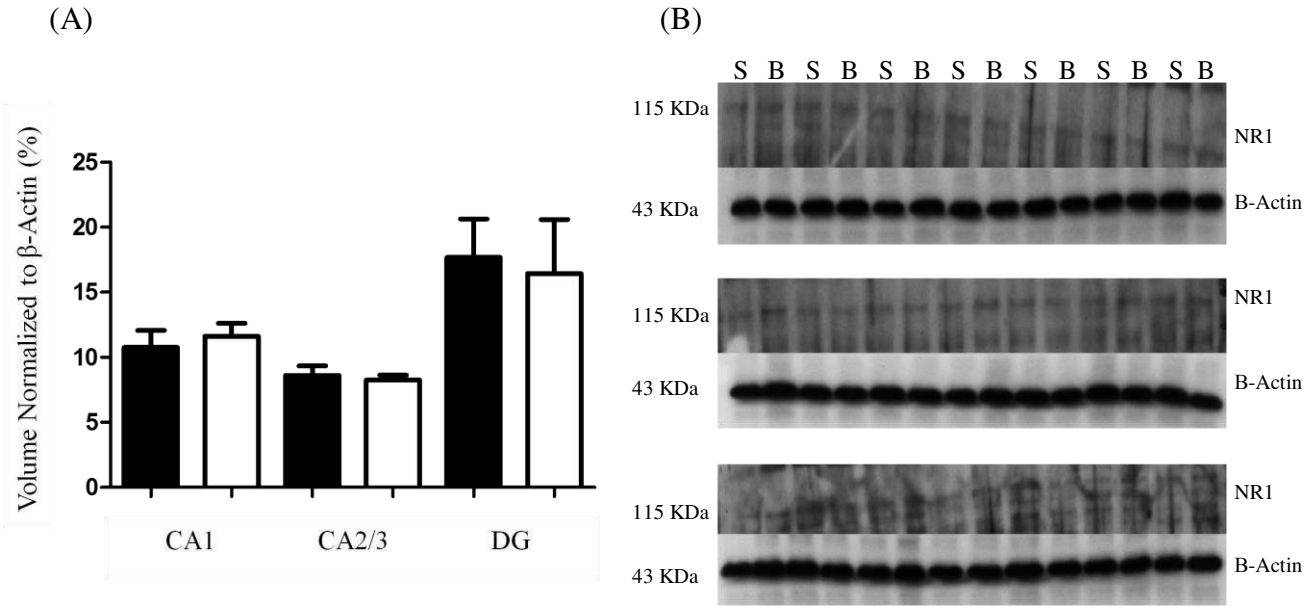


Figure 3.21: *A) Mean optical density for NR1 expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR1 expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR1 is on the left on the immunoblot.*

3.1.8 NR2A expression levels in the hippocampus following BVD surgery

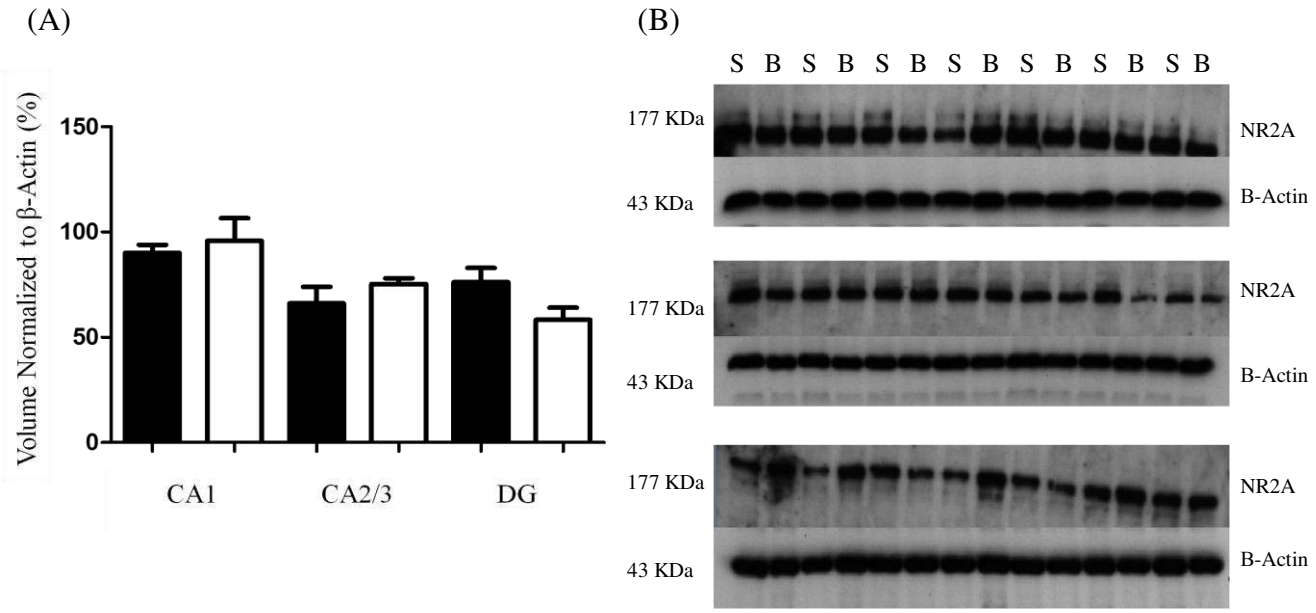


Figure 3.22: *A) Mean optical density for NR2A expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2A expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2A is on the left on the immunoblot.*

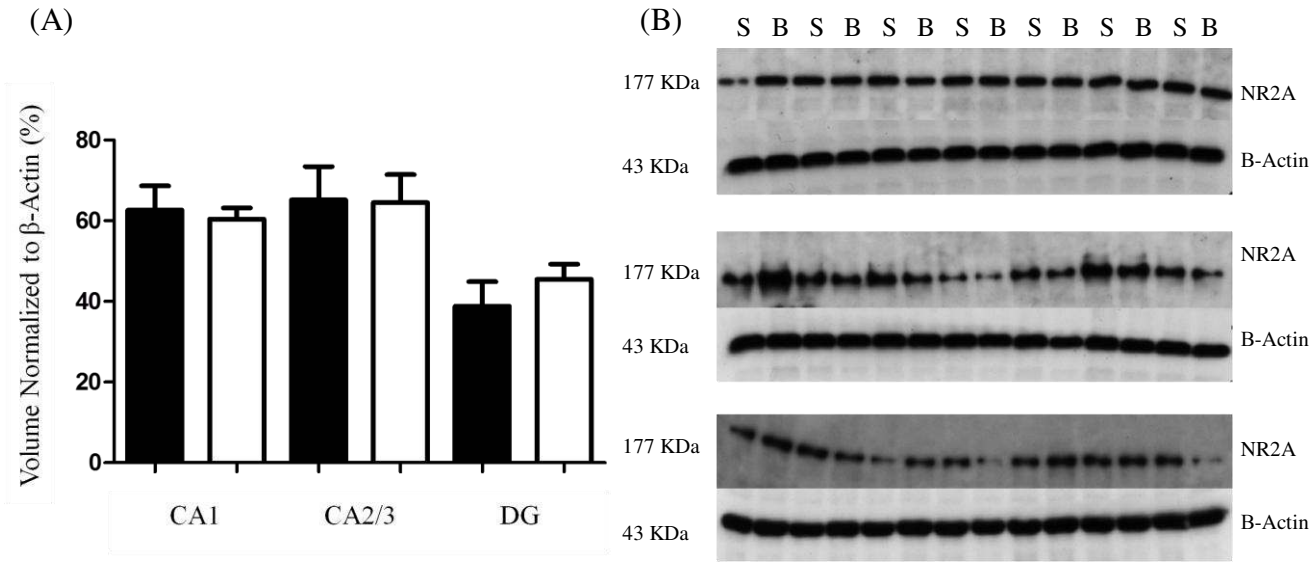


Figure 3.23: *A) Mean optical density for NR2A expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2A expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2A is on the left on the immunoblot.*

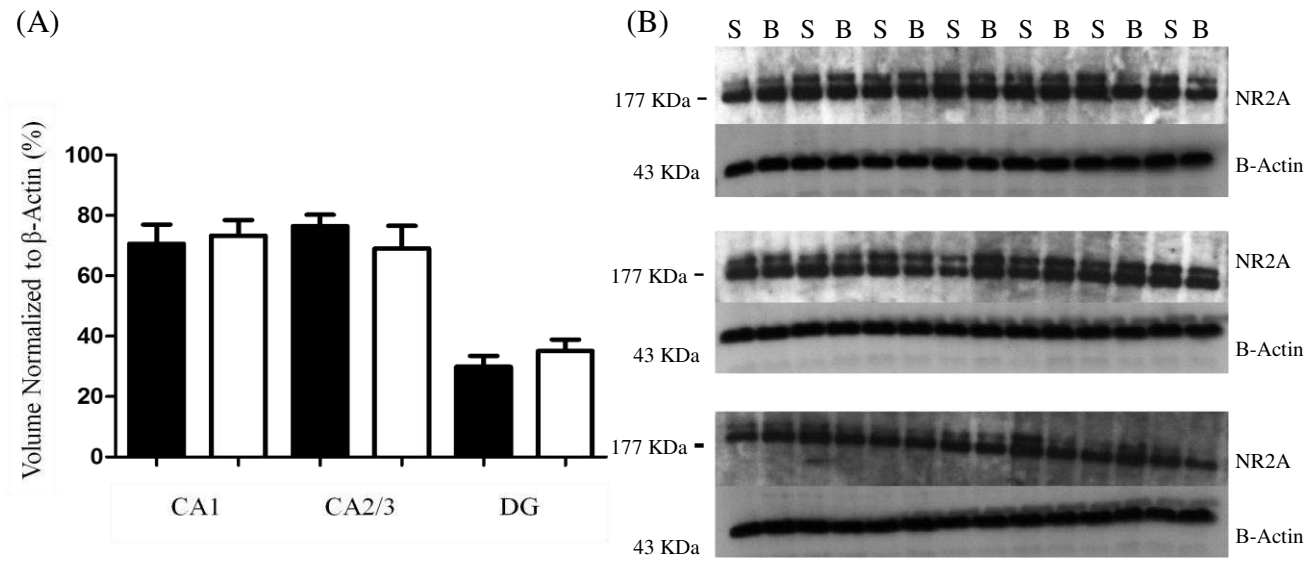


Figure 3.24: *A) Mean optical density for NR2A expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2A expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2A is on the left on the immunoblot.*

3.1.9 NR2B expression levels in the hippocampus following BVD surgery

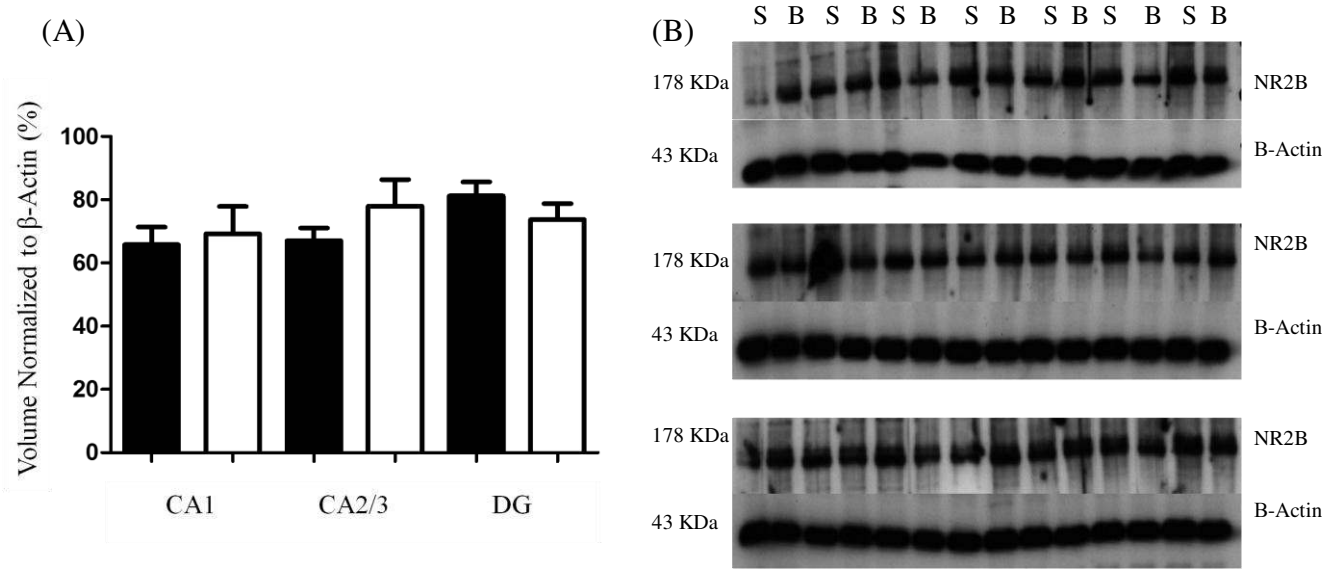


Figure 3.25: *A) Mean optical density for NR2B expression at 24 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2B expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 24 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2B is on the left on the immunoblot.*

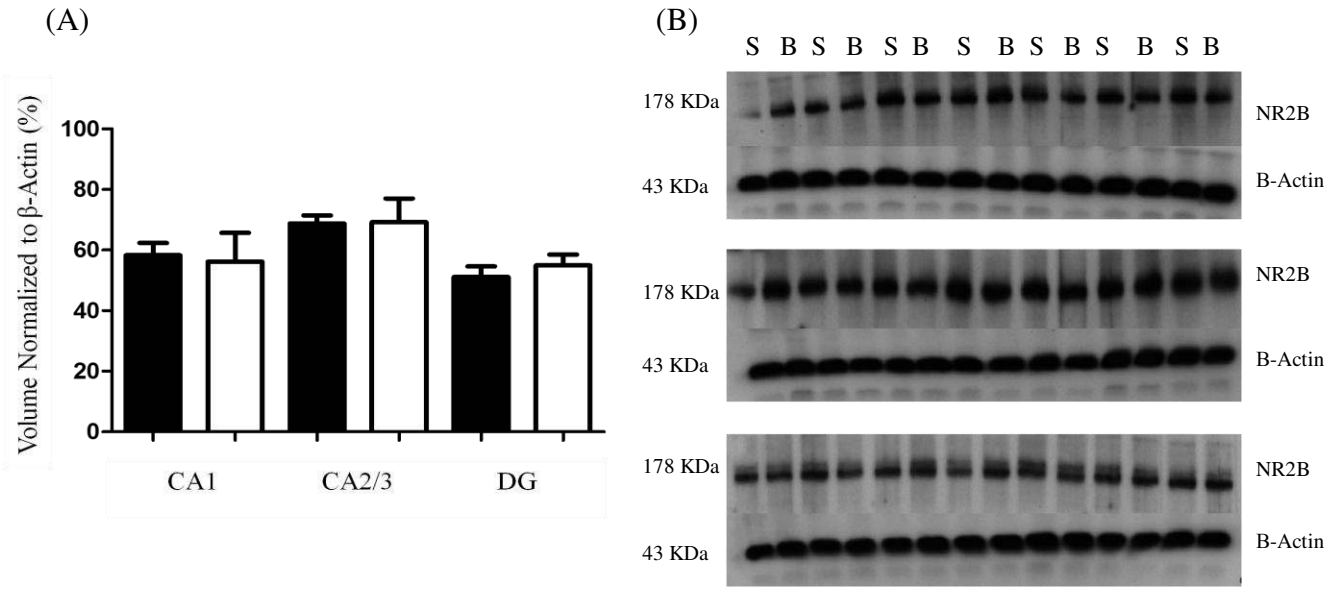


Figure 3.26: *A) Mean optical density for NR2B expression at 72 hours normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2B expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 72 hours respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2B is on the left on the immunoblot.*

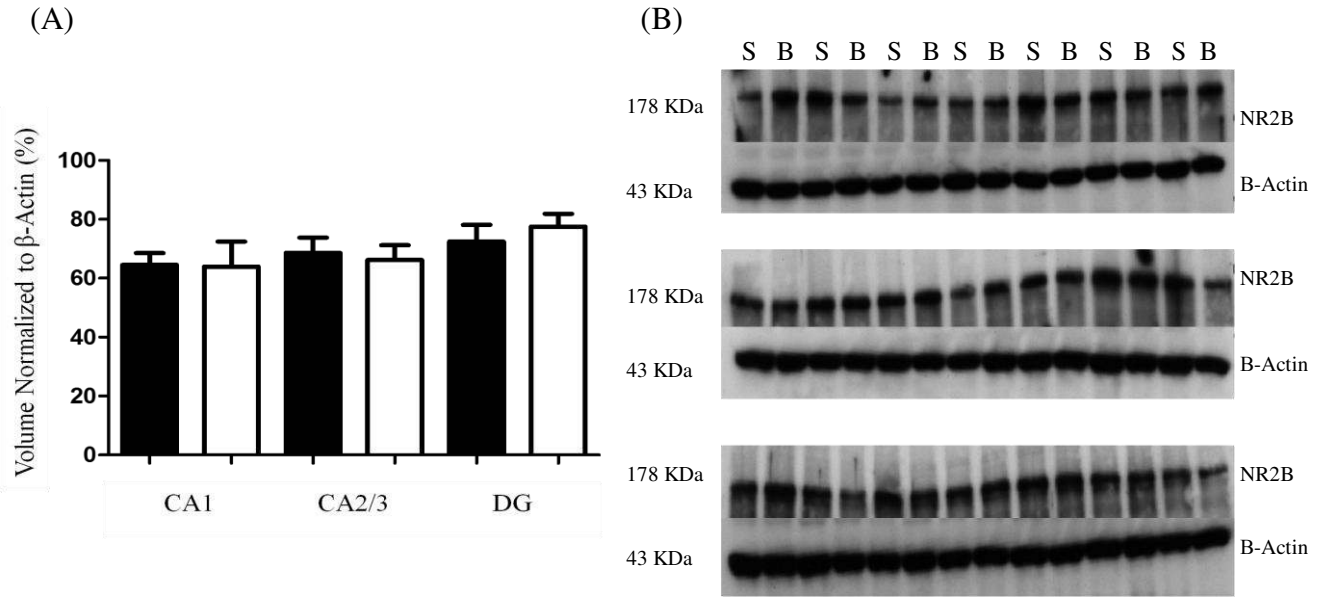


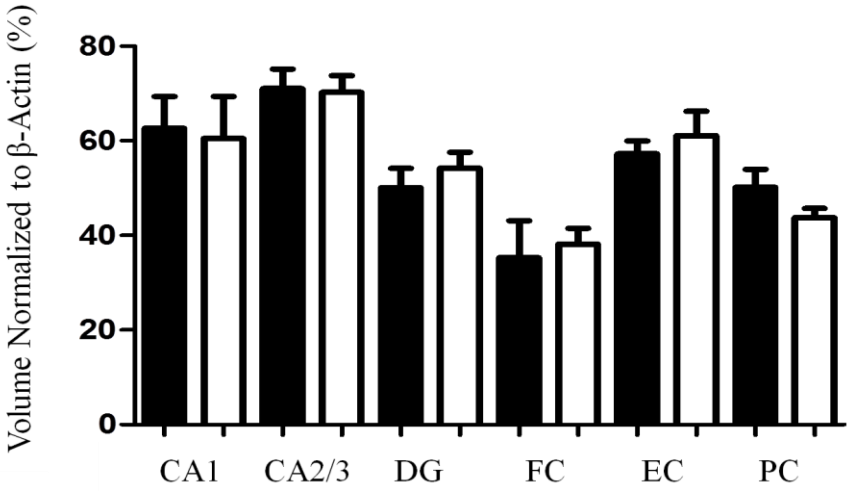
Figure 3.27: *A) Mean optical density for NR2B expression at 1 week normalized to β-actin in the CA1, CA2/3 and DG of the hippocampus post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2B expression in the CA1 (top), CA2/3 (middle) and DG (bottom) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2B is on the left on the immunoblot.*

3.2 Protein and subunit expression in the medial temporal lobe at 1 month post-BVD

One month following surgery there was no significant difference in GluR1, GluR2, GluR3, GluR4, NR1, NR2A or NR2B levels in the CA1, CA2/3 or DG, or the FC, EC or PC of BVD rats compared to sham controls following one-way ANOVA.

3.2.1 GluR1 expression levels in the medial temporal lobe following BVD surgery

(A)



(B)

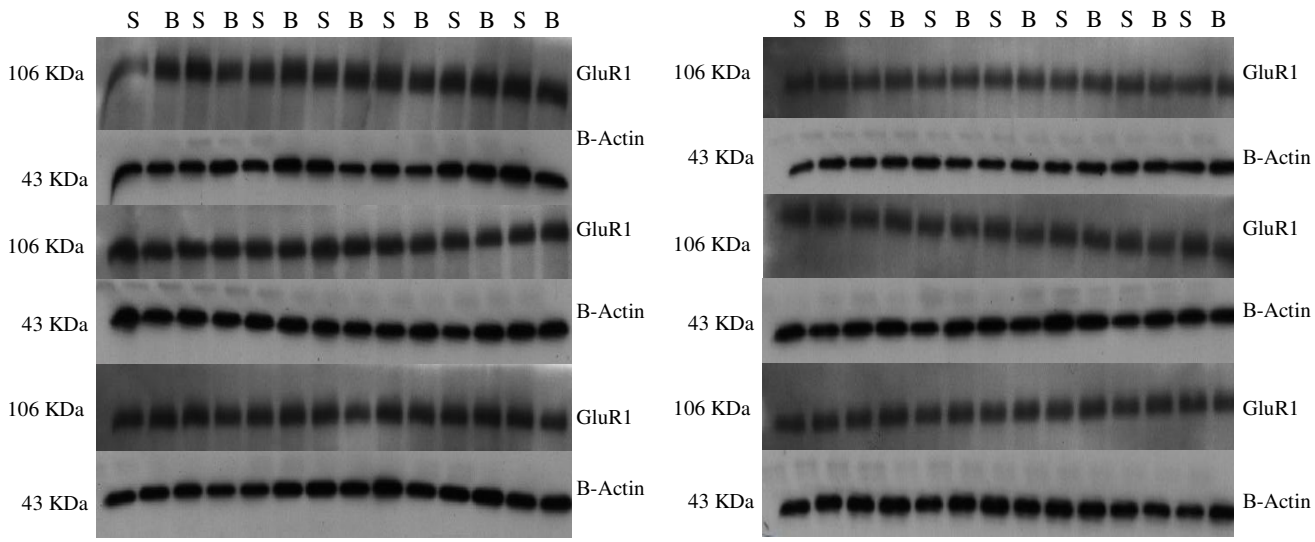
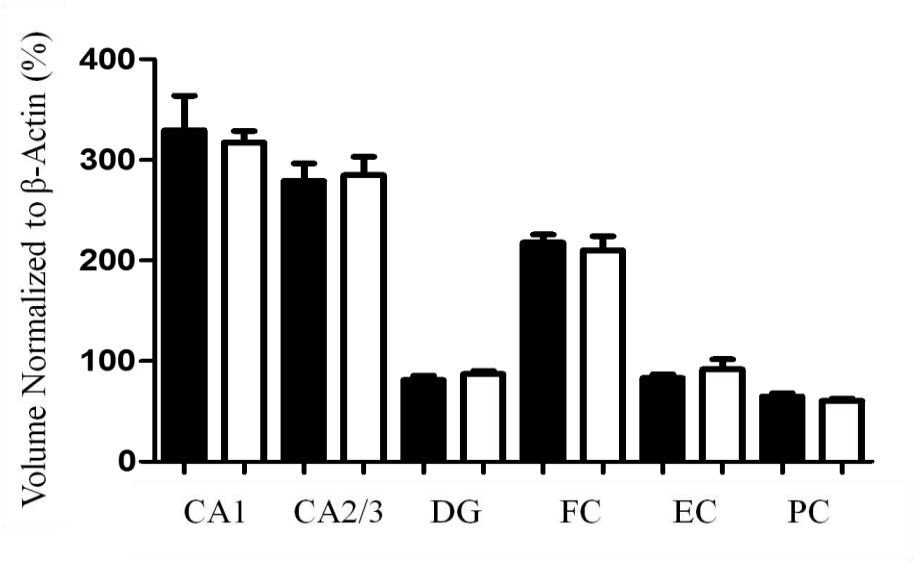


Figure 3.28: *A) Mean optical density for GluR1 expression at 1 month normalized to β-actin in the CA1, CA2/3, DG, FC, EC and PC post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR1 expression in the CA1 (top left), CA2/3 (middle left), DG (bottom left), FC (top right), EC (middle right) and PC (bottom right) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR1 is on the left on the immunoblot.*

3.2.2 GluR2 expression levels in the medial temporal lobe following BVD surgery

(A)



(B)

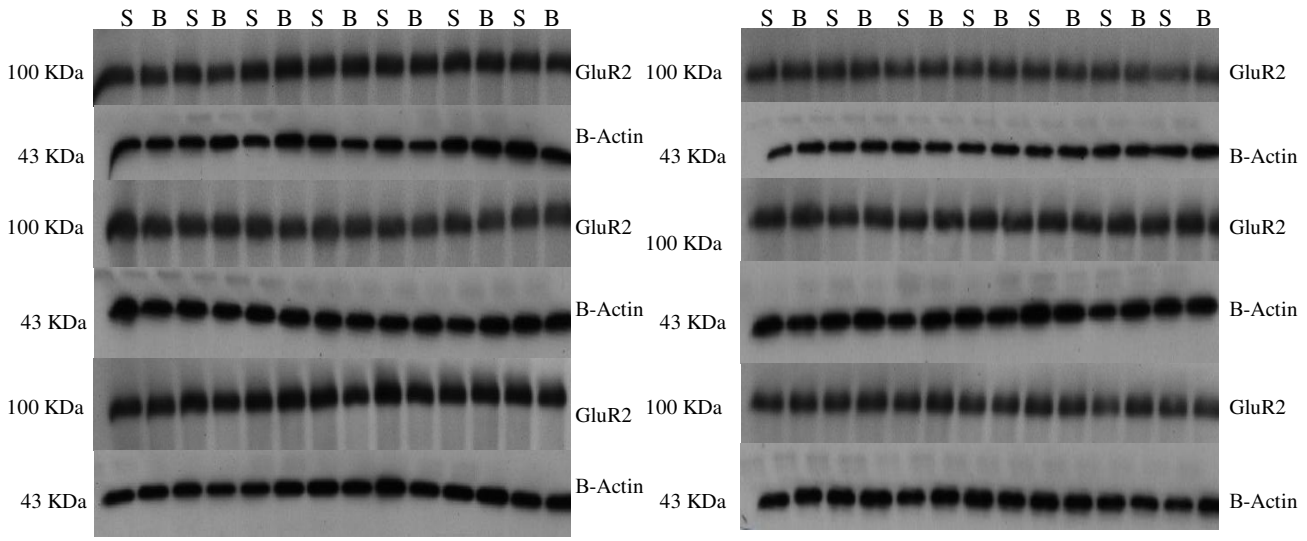


Figure 3.29: *A) Mean optical density for GluR2 expression at 1 month normalized to β-actin in the CA1, CA2/3, DG, FC, EC and PC post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR2 expression in the CA1 (top left), CA2/3 (middle left), DG (bottom left), FC (top right), EC (middle right) and PC (bottom right) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR1 is on the left on the immunoblot.*

3.2.3 GluR3 expression levels in the medial temporal lobe following BVD surgery

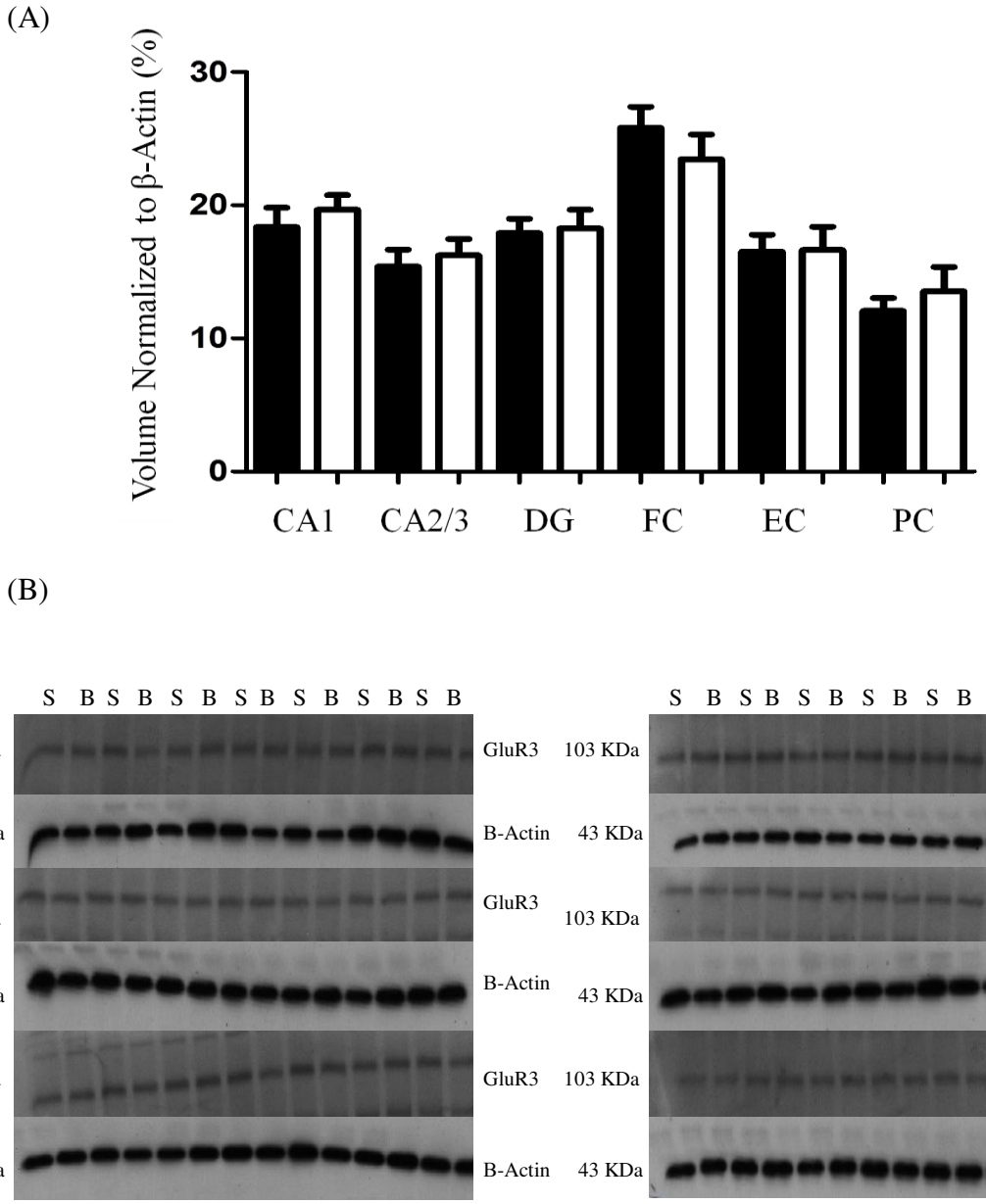


Figure 3.30: *A) Mean optical density for GluR3 expression at 1 month normalized to β-actin in the CA1, CA2/3, DG, FC, EC and PC post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR3 expression in the CA1 (top left), CA2/3 (middle left), DG (bottom left), FC (top right), EC (middle right) and PC (bottom right)of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR3 is on the left on the immunoblot.*

3.2.4 GluR4 expression levels in the medial temporal lobe following BVD surgery

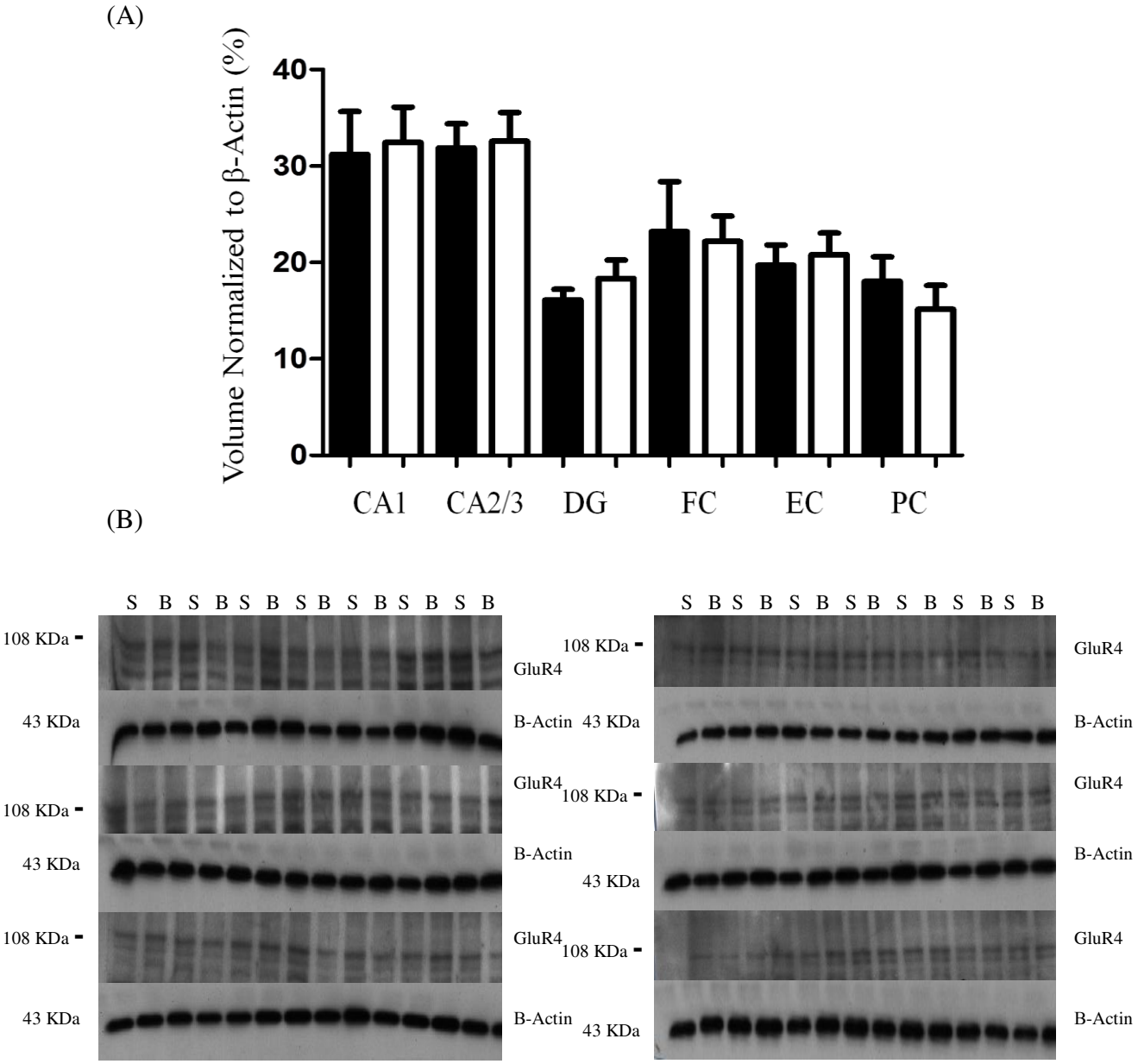


Figure 3.31: *A) Mean optical density for GluR4 expression at 1 month normalized to β-actin in the CA1, CA2/3, DG, FC, EC and PC post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of GluR4 expression in the CA1 (top left), CA2/3 (middle left), DG (bottom left), FC (top right), EC (middle right) and PC (bottom right) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of GluR4 is on the left on the immunoblot.*

3.2.5 NR1 expression levels in the medial temporal lobe following BVD surgery

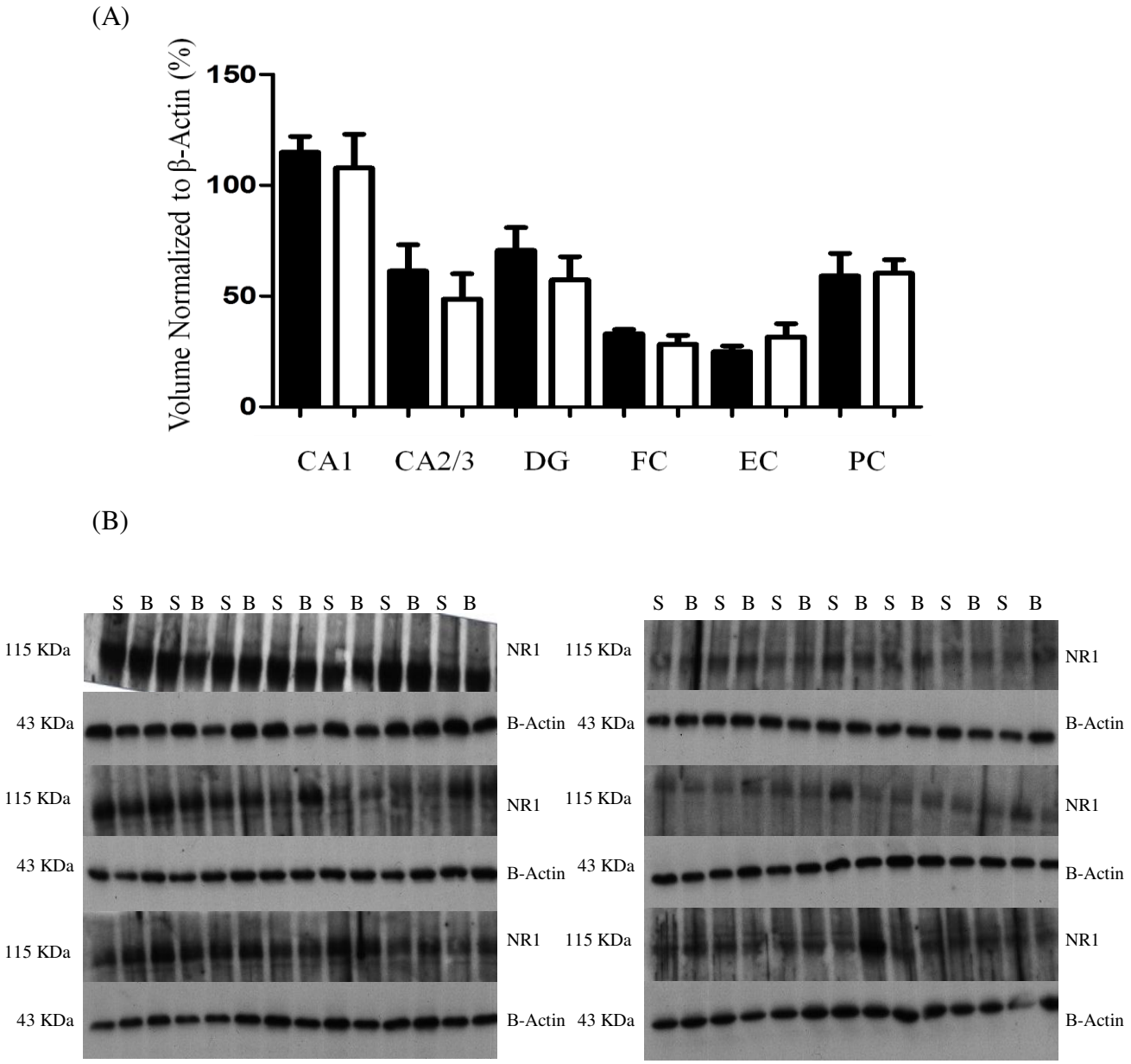


Figure 3.32: *A) Mean optical density for NR1 expression at 1 month normalized to β-actin in the CA1, CA2/3, DG, FC, EC and PC post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR1 expression in the CA1 (top left), CA2/3 (middle left), DG (bottom left), FC (top right), EC (middle right) and PC (bottom right) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR1 is on the left on the immunoblot.*

3.2.6 NR2A expression levels in the medial temporal lobe following BVD surgery

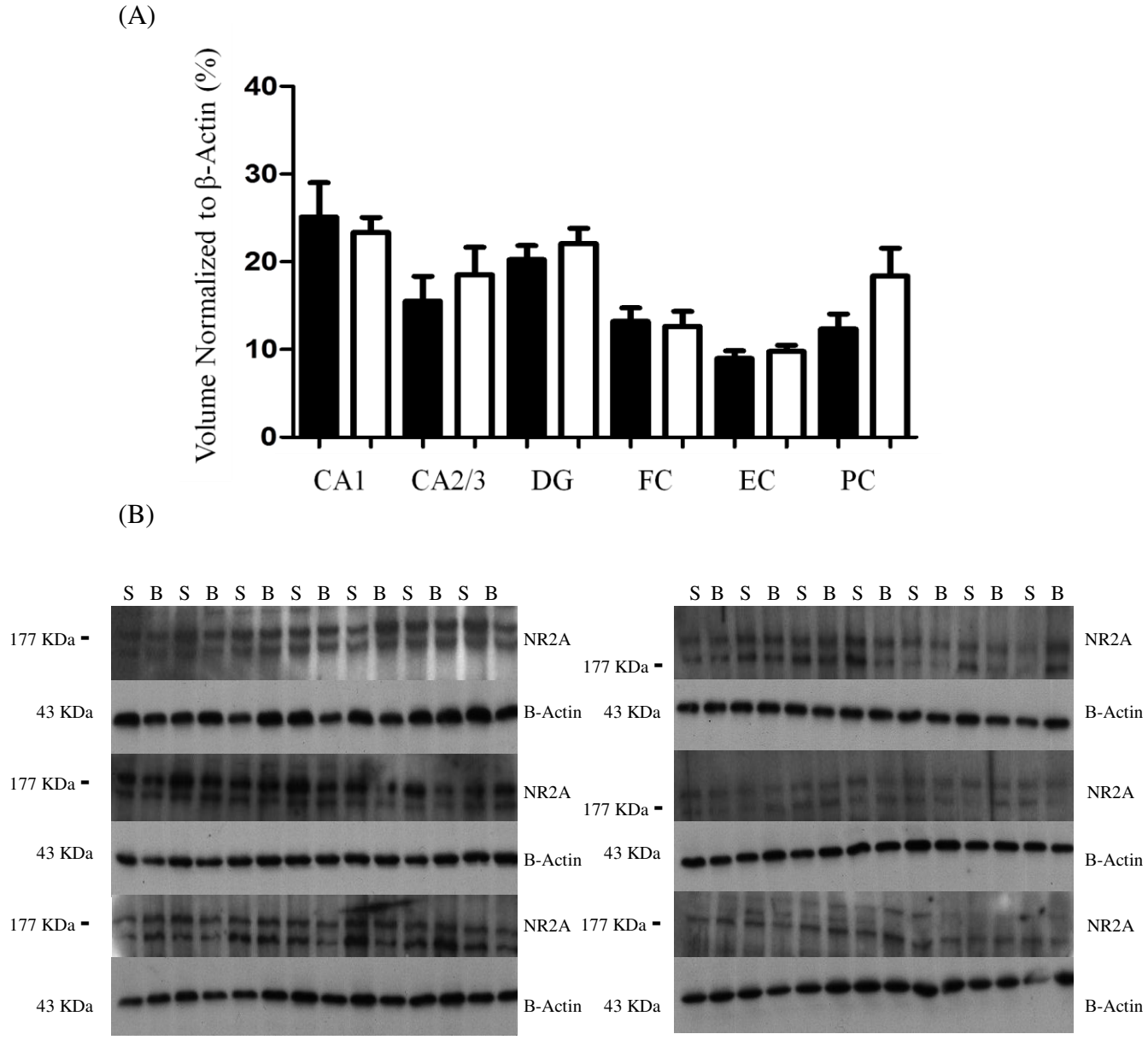


Figure 3.33: *A) Mean optical density for NR2A expression at 1 month normalized to β-actin in the CA1, CA2/3, DG, FC, EC and PC post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2A expression in the CA1 (top left), CA2/3 (middle left), DG (bottom left), FC (top right), EC (middle right) and PC (bottom right) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2A is on the left on the immunoblot.*

3.2.7 NR2B expression levels in the medial temporal lobe following BVD surgery

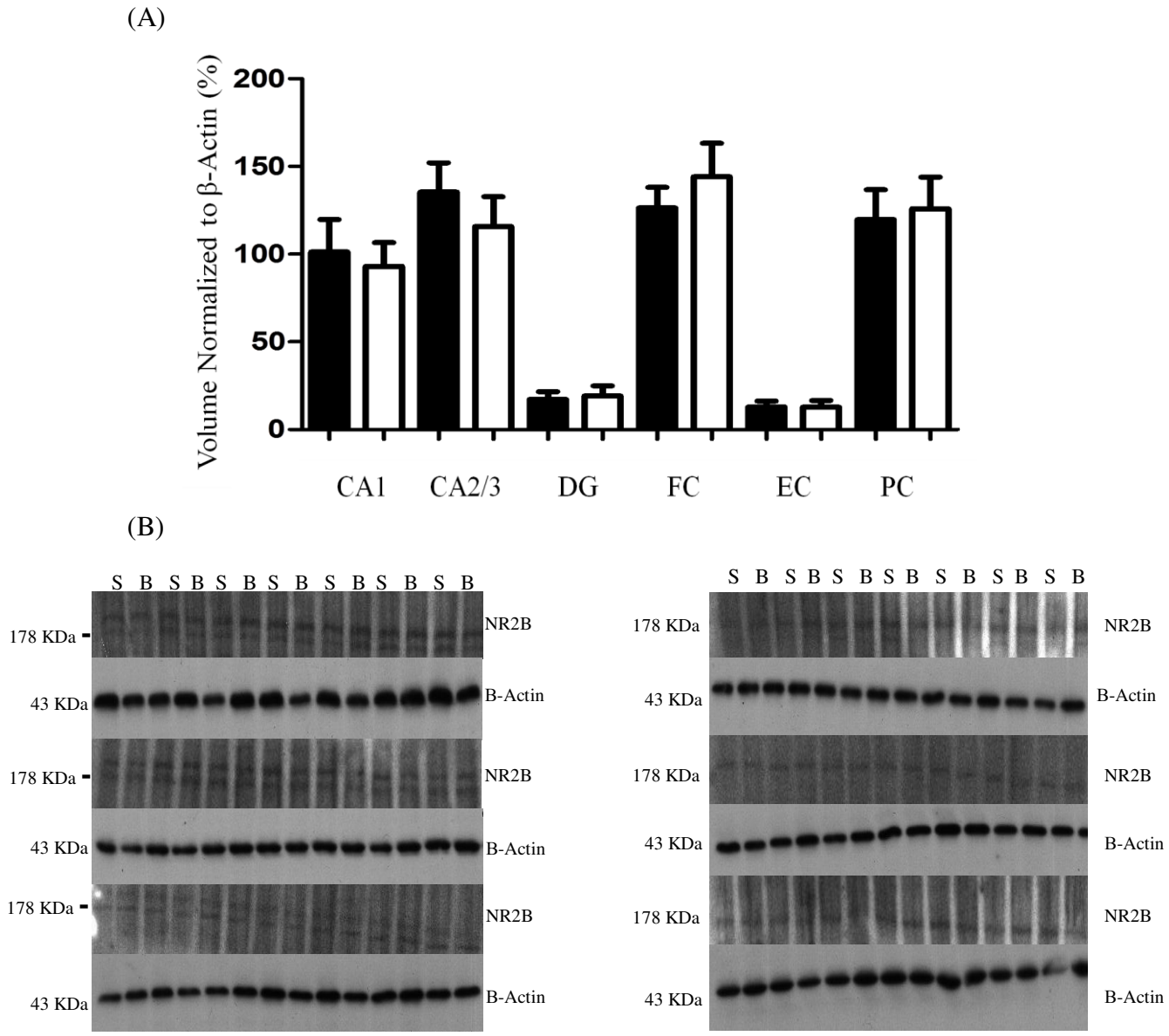


Figure 3.34: *A) Mean optical density for NR2B expression at 1 month normalized to β-actin in the CA1, CA2/3, DG, FC, EC and PC post-bilateral vestibular deafferentation (BVD) compared with sham surgery controls. Filled columns represent BVD animals, unfilled represents sham animals. Columns represent means, bars 1 S.E.M of the mean. B) Immunoblot of NR2B expression in the CA1 (top left), CA2/3 (middle left), DG (bottom left), FC (top right), EC (middle right) and PC (bottom right) of the hippocampus at 1 week respectively. B represents a BVD animal S represents a sham animal. The molecular weight in KDa of NR2B is on the left on the immunoblot.*

3.3 Linear Discriminant Analysis

In order to undertake multivariate analyses, to determine whether the relationship between different variables changed as a result of the BVD, the data were first converted to z scores by subtracting each optical density value in each group that had been normalised to β-actin, from its mean and dividing by the standard deviation of the group.

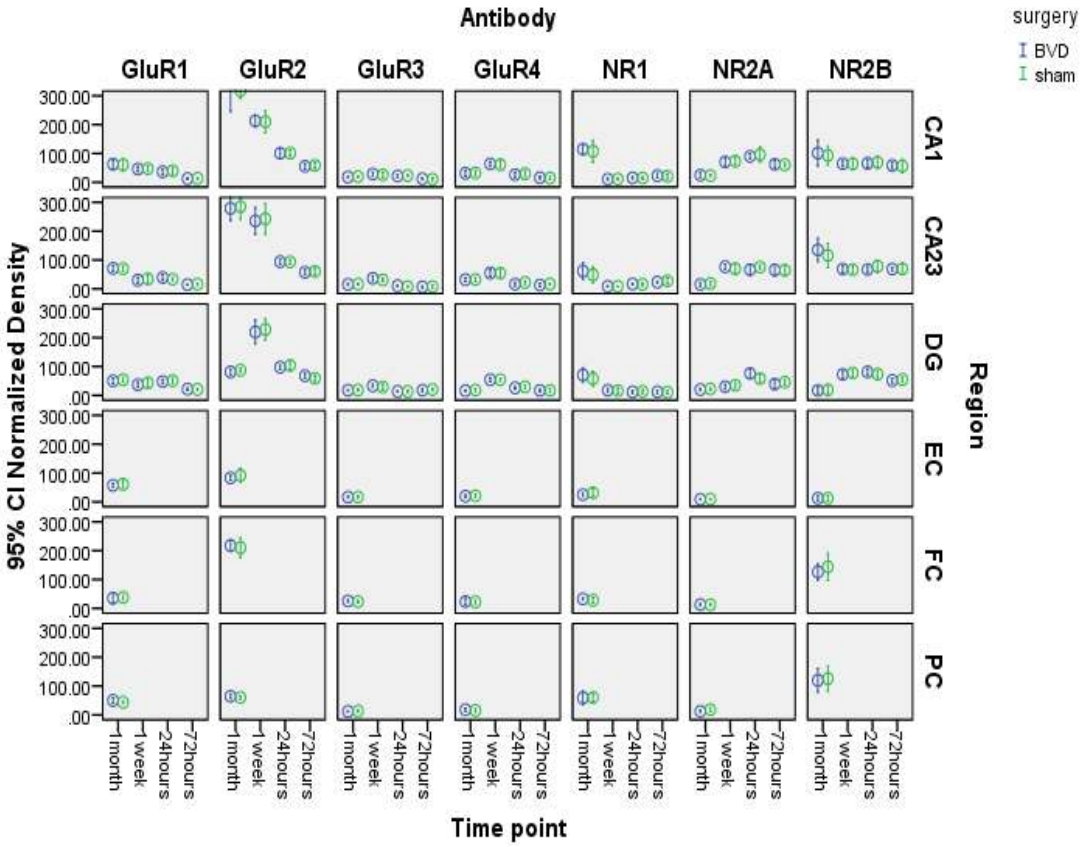


Figure 3.35: *This scatterplot matrix is showing the correlation of the mean normalized densities of the neurochemical variables at 24 hours, 72 hours, 1 week and 1 month in the medial temporal lobe.*

A stepwise LDA was performed to determine whether BVD animals could be separated from sham animals using a linear discriminant function using a subset of the variables. An LDA was identified that used only 8 variables: CaMKII at 24 hours in the DG, GluR2 at 1 week in the CA1, GluR4 at 1month in the EC, NR1 at 1 week in the CA1, NR1 at 1 week in the DG, NR1 at 1 month in the DG, NR1 at 1 month in the FC and NR2B at 1 month in the FC. Table 1 shows the standardised canonical discriminant function coefficients for this LDA. The LDA was significant ($P = 0.000$), according to Wilks' λ. The cross-validation, in which SPSS19 used the linear discriminant function to classify the animals blind to their surgical treatment group, indicated that the LDA discriminated between the BVD and sham control animals with 100% accuracy.

Standardized Canonical Discriminant Function Coefficients	
	Function
	1
Zscore (CaMKII 24 hours DG)	29.87
Zscore (GluR2 1 week CA1)	2.934
Zscore (GluR4 1 month EC)	-27.841
Zscore (NR1 1 week CA1)	3.295
Zscore (NR1 1 week DG)	15.035
Zscore (NR1 1 month DG)	5.584
Zscore (NR1 1 month FC)	26.613
Zscore (NR2B 1 month FC)	14.804

Table 3.1: *The standardised canonical discriminant function coefficients for the linear discriminant functions for the Western blotting data.*

3.4 Cluster Analysis

Since the LDA indicated that 8 variables could significantly discriminate between the BVD and sham animals with 100% accuracy, a Ward linkage cluster analysis was carried out on these variables for the BVD and sham groups. The results were consistent with the LDA in that the 8 variables clustered quite differently for the BVD and the sham animals. Cluster analysis of the BVD animals' data suggested that GluR2 at 1 week in the CA1 and NR1 at 1 month in the FC tended to vary together, whereas CaMKII at 24 hours in the DG, GluR4 at 1 month in the EC, NR1 at 1 month in the DG, NR1 at 1 week in the CA1, NR2B at 1 month in the FC and NR1 at 1 week in the DG tended to co-vary (fig. 3.36). The smallest cluster consisted of: CaMKII at 24 hours in the DG and GluR4 at 1 month in the EC; NR2B at 1 month in the FC and NR1 at 1 week in the DG; and GluR2 at 1 week in the CA1 and NR1 at 1 month in the FC (fig. 3.36).

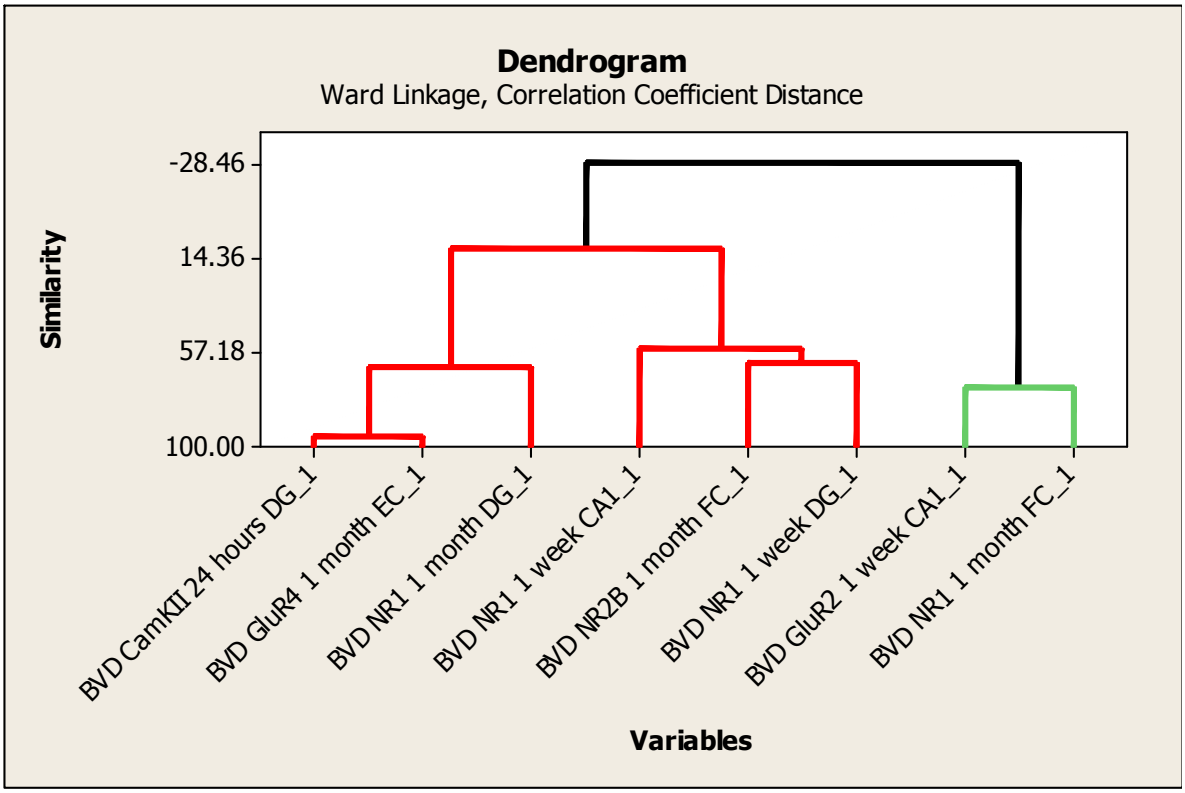


Figure 3.36: *Dendrogram showing the expression of the 8 stepwise linear discriminate analysis (LDA) neurochemical variables in the BVD rats.*

Cluster analysis of the sham animals' data suggested that CaMKII at 24 hours in the DG, NR2B at 1 month in the FC, GluR2 at 1 week in the CA1 and NR1 at 1month in the DG tended to vary together, whereas GluR4 at 1 month in the EC, NR1 at 1 month in the FC, NR1 at 1 week in the DG and NR1 at 1 week in the CA1 tended to co-vary (fig. 3.36). The smallest cluster consisted of: CaMKII at 24 hours in the DG and NR2B at 1 month in the FC; GluR2 at 1 week in the CA1and NR1 at 1 month in the DG; and NR1 at 1 month in the FC and NR1 at 1 week in the DG (fig. 3.36).

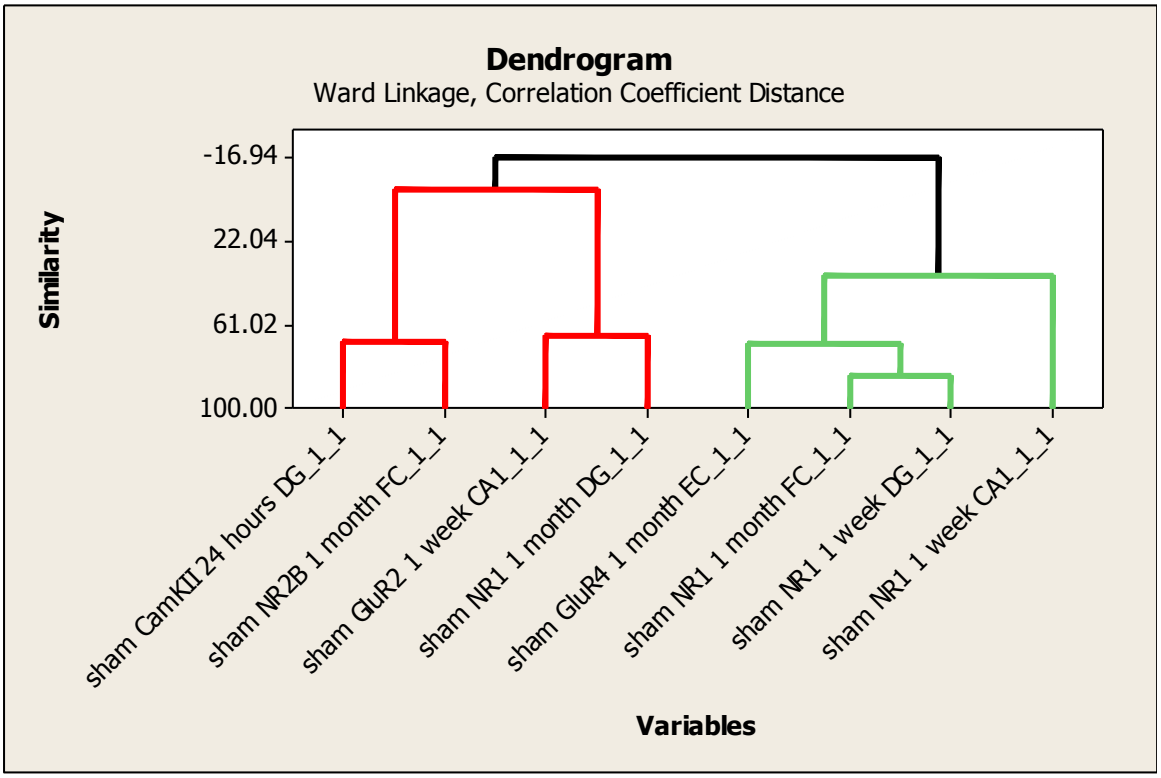


Figure 3.37: *Dendrogram showing the expression of the 8 stepwise linear discriminate analysis (LDA) neurochemical variables in the sham control animals.*

Chapter four

Discussion

The aim of this study was to determine if there were changes in NMDARs, AMPARs, CaMKII or phosphorylated CaMKII in various regions of the rat medial temporal lobe at 24 hours, 72 hours, 1 week and 4 weeks following BVD using western blotting. ANOVAs directly compared the individual subunit levels at different time points and in different brain regions following BVD and sham surgeries. When the expression of the individual variables was compared between treatment groups, there were no significant differences in the glutamate receptor subunits, CaMKII or activated CaMKII in any of the brain regions at any time point. However, LDA found eight variables from the data that could distinguish between BVD and sham rats with 100% accuracy.

4.1 Interpretation of results

There were no significant differences when individual subunit levels were compared between BVD and sham animals. However, the LDA and cluster analysis showed there was a change in the relationship between the subunits and proteins when comparing treatment groups. While overall NMDAR and AMPAR subunit levels were not significantly different, subunit composition of individual receptors may have altered following BVD surgery. Differences in NMDAR and AMPAR subunit composition in the hippocampus would confer distinct pharmacological and gating properties (Monyer et al. 1994) and could alter the intracellular signalling molecules coupled to AMPARs and NMDARs. This could have a number of anatomical and electrophysiological consequences. For example, during LTP induction there is an increase in GluR1 AMPAR homomers but in the maintenance phase these are replaced with AMPARs containing GluR2 (Plant et al. 2006). Therefore, overall AMPAR and NMDAR levels may not change in the hippocampus following BVD, but receptor composition may undergo subtle alterations resulting in deficits in spatial navigation and/or place cell activation.

It is possible that there were changes in the affinity and efficacy of the receptors, as opposed to overt changes in synaptic numbers. This could occur by altering glutamate receptor phosphorylation, associations with cytoskeletal proteins, enzymes and neuromodulators. Glutamate receptors are tightly controlled by phosphokinases (Roche et al. 1994, Soderling et al. 1994). Measuring the activity of phosphokinases such as PKA, and conventional/novel isoforms of PKCs (Sanes and Lichtman 1999) could provide information about changes in NMDAR and AMPAR affinity and efficacy. Mice lacking the regulatory phosphorylation

sites Ser 831 and Ser 845 from GluR1, show deficits in LTP, LTD and spatial learning tasks (Lee et al. 2003b). These sites are targeted by CaMKII and PKA. PKA, like CaMKII, alters AMPAR activity and (Greengard et al. 1991) modulates the generation of LTP (Blitzer et al. 1995). Subtle changes in mechanisms such as these could alter AMPAR function and therefore the receptor's role in synaptic transmission generally, and spatial memory specifically (Lisman et al. 2002, Malinow and Malenka 2002, Soderling and Derkach 2000).

AMPAR phosphorylation and dephosphorylation are vital for receptor trafficking and synaptic changes and therefore synaptic plasticity (Daw et al. 2000, Keller et al. 1992, Mammen et al. 1997, Matsuda et al. 1999, Xia et al. 2000). It has been hypothesised that AMPARs are trafficked to extrasynaptic sites through cyclic AMP signalling alone (Oh et al. 2006); this trafficking correlates with the phosphorylation of the Ser 845 site on GluR1 by PKA (Esteban et al. 2003). Mice lacking Ser845 and Ser831 show deficits in LTP and spatial memory (Lee et al. 2003b). This indicates that the activity-dependent trafficking of AMPARs via Ser845 phosphorylation may facilitate LTP and spatial memory. Subtle changes in AMPAR and NMDAR trafficking could account for the deficits in spatial memory and hippocampal atrophy post-BVD.

The identity of the NR2 subunit affects receptor trafficking and synaptic targeting (Cull-Candy and Leszkiewicz 2004), whereas the NR1 subunit is vital for NMDAR release from the endoplasmic reticulum (Fukaya et al. 2003). Kinases such as tyrosine kinase bind to scaffolding proteins like the membrane-associated guanylate kinase PSD-95. PSD-95 contains PDZ domains which bind to the NR2 subunits in NMDARs and stabilize the receptors in the plasma membrane (Sheng and Sala 2001, Wenthold et al. 2003). Changes in NR2 subunit location and function could also be caused by changes in the subunits C-termini's affinity for PSD-95 (Kornau et al. 1995, Niethammer et al. 1996).

Changes in receptor targeting and sensitivity may have occurred following BVD surgery. For example, Stocca and Vicini (1998) found that NMDAR-EPSCs in the visual cortex had lost sensitivity to NR2B-selective antagonists, however blockage of extra-synaptic receptors still occurred. This indicated that NR1/NR2B receptors were still present, but were no longer targeted to the synapses (Stocca and Vicini 1998). A similar change could have occurred following vestibular lesions resulting in electrophysiological adaptations but unchanged receptor numbers.

AMPARs are recycled between synapses and the cytosol, as a result receptors are either degraded in the cytosol or reinserted into synapses (Ehlers 2000, Passafaro et al. 2001). Following LTP induction AMPAR exocytosis is enhanced as the receptors are recycled through an endosomal pathway (Park et al. 2004). During synaptic potentiation AMPARs are stabilized on the membrane surface because the receptors are anchored to PSD proteins (Derkach et al. 2007). AMPAR internalization is mediated via PKC phosphorylation of GluR2 (Chung et al. 2003, Chung et al. 2000). Activity of these molecules can also alter the synthesis of AMPARs, and receptor abundance at synapses (Ju et al. 2004). Clearly AMPAR trafficking and localization has a large effect on synaptic activity. Following BVD changes in receptor composition and movement may occur without altering subunit levels.

4.1.1 Interpretation of Linear Discriminant Analysis data

There was a very strong correlation between eight variables: CaMKII at 24 hours in the DG, GluR2 at 1 week in the CA1, GluR4 at 1month in the EC, NR1 at 1 week in the CA1, NR1 at 1 week in the DG, NR1 at 1 month in the DG, NR1 at 1 month in the FC and NR2B at 1 month in the FC. This combination of variables could predict whether the rat had undergone BVD or sham surgery with 100% certainty. The result indicates there is a change in the relationship between the subunits and CaMKII in BVD animals when compared to controls, as opposed to differences in the levels of individual subunits. This is logical as the different subunits make up whole receptor complexes and CaMKII and pCaMKII are part of the downstream signalling mechanisms of these receptors. CaMKII is also involved in glutamate receptor activity and trafficking (Hayashi et al. 2000, Lisman et al. 2002). Slight changes in the distribution of one subunit could have a significant effect on the function, affinity and/or efficacy of that receptor. For instance, the NR2A subunit has a role in the efficacy of calcium channels associated with NMDARs, which affects synaptic excitability (Dingledine et al. 1999).

Four of the eight variables that distinguish BVD animals from sham include NR1 at 1 week or 1 month in the CA1, DG and FC. NR1 is the only NMDAR subunit required for the formation of a functional receptor (Cull-Candy et al. 2001). NR1-knockout mice do not survive past a day because NMDARs in the midbrain have a vital role in breathing (Forrest et al. 1994). CA1-region-specific NR1 knock-out mice develop normally but show significant spatial

learning deficits in the MWT (Tsien et al. 1996). This may be because the coordination of cell firing and place fields was reduced in the CA1 pyramidal cells of CA1-NR1-knock-out mice, indicating that the spatial representations in the mice were less coherent (McHugh et al. 1996). Therefore, changes in NR1 could have a role in the altered place cell activity observed in BVD animals (Russell et al. 2003b, Stackman et al. 2002a).

Two of the eight correlating LDA variables included NR1 in the DG. Mice with NR1 selectively knocked out of granule cells of the DG showed impairment in a spatial working memory task and severely impaired LTP in the medial and lateral perforant path inputs (Niewoehner et al. 2007). This subunit in this sub-region of the hippocampus may have a role in the spatial memory deficits observed in BVD patients and animals (Brandt et al. 2005, Russell et al. 2003a, Zheng et al. 2006). The significance of NR1 in differentiating between BVD and sham animals may be that there are changes in the ligands and modulators that control NR1 efficacy and affinity. For example, serine protease tissue-type plasminogen activator (tPA) increases NMDAR-mediated Ca^{2+} influx, by cleaving NR1 within its N-terminal domain (Fernández-Monreal et al. 2004). Preventing the interaction between NR1 and tPA has already been shown to disrupt the encoding of new spatial memories (Benchenane et al. 2007). This may be a mechanism behind altered NMDAR activity post-BVD surgery.

Two variables in the model of BVD included the NMDAR subunits NR1 and NR2B in the FC at 1 month. Lesions of the prefrontal cortex resulted in deficits in spatial working memory (Granon et al. 1994). Additionally, there are electrophysiological studies showing that the prefrontal cortex is stimulated during working memory tasks (Funahashi et al. 1997, Miller et al. 1996) and lesions of the prefrontal cortex resulted in deficits in spatial working memory (Granon et al. 1994). NMDARs have a role in spatial working memory in the FC, but the neurochemical mechanisms are complicated. The NMDR antagonist MK-801 reduced working memory performance because dopamine D_1 receptor activation was attenuated in the medial prefrontal cortex (mPFC) during the working memory task (Rios Valentim et al. 2009). Furthermore, CaMKII phosphorylation of NR1 was induced when a D_1 receptor agonist was infused into the mPFC (Mouri et al. 2007), so clearly there is a link between dopamine receptors and NMDARs in the FC. This is a potential target for future studies.

The dorsal hippocampus and the mPFC are thought to process short-term spatial memory in parallel, acting as a compensatory mechanism for each other (Lee and Kesner 2003a). This correlates with the finding that the frontal lobes were activated when the vestibular nerve was electrically stimulated (De Waele et al. 2001) and when the vestibular system was activated using galvanic (Bense et al. 2001) and caloric stimulation (Suzuki et al. 2001). The regions of the FC that respond to vestibular stimulation and/or receive projections from the vestibular nuclei (Akbarian et al. 1993, Guldin et al. 1993) are collectively named the dorsal premotor cortex (DPMv) (Akbarian et al. 1993, Fukushima et al. 2005). Correspondingly, the majority of neurons in the DPMv respond to head movement (Fukushima et al. 2005). The DPMv also contains the smooth pursuit region of the frontal eye fields which is thought to have a role in encoding gaze (Fukushima et al. 2004, Shinder and Taube 2010). Therefore, these regions may use vestibular sensory input to improve visual target pursuit during head movements (Fukushima et al. 2006) and to update self-motion feedback enabling the accurate coordination of motor responses (Roy and Cullen 2004, Shinder and Taube 2010, Sugiuchi et al. 2005). Bilateral loss of vestibular sensory information would disrupt the neural pathways in this region and that there would be corresponding neurochemical changes as a result of this loss.

One of the predictive variables included GluR4 in the EC at 1 month. The correlation of the eight variables shows that there are strong connections between the hippocampus, EC and FC and that these links are being affected following BVD. The lateral part of the EC projects to the dorsal hippocampus and the neurons in the medial parts of the EC connect to the ventral hippocampus (Witter et al. 2000). The EC provides important input to the CA1 region and entorhinal neurons may have a role in the encoding of novel information in the CA1 region (Brun et al. 2002, McNaughton et al. 1989, Nakazawa et al. 2004). The prefrontal cortex projects to the EC via a number of pathways (Van Hoesen et al. 1975). Fig. 4.1 highlights the complex connections between the hippocampal formation and the FC. Bilateral vestibular lesion-induced changes in hippocampal function and anatomy are likely to be part of a complex cascade of neural changes occurring throughout the brain, particularly in other regions of the medial temporal lobe and the frontal cortex (Karnath and Dieterich 2006, Smith et al. 2009).

One of the variables from the LDA model included GluR2 in the CA1 region. This is unsurprising as the majority of AMPARs in the CNS are heterodimers containing GluR2

(Greger et al. 2002, Wenthold et al. 1996) and GluR2 is vital for AMPAR trafficking (Greger et al. 2002, and Cull-Candy 2000, Rothman 1994) and internalization (Chung et al. 2000). GluR2 knock-out mice show significantly altered LTP in the CA1 region (Jia et al. 1996). GluR2 mutant mice show significantly less precise and more unstable place fields, and deficits in a spatial learning task (Yan et al. 2002).

NMDAR and AMPAR expression, distribution and function are controlled by alterations in the assembly of different subunits into various receptor complexes via post-translational modifications such as phosphorylation. This was investigated in this study by measuring both CaMKII and its active form, phosphorylated CaMKII, which has an important role in modulating NMDAR and AMPAR activity (Hayashi et al. 2000, Shen and Meyer 1999). One of the variables from the LDA model included CaMKII in the DG. AMPAR activity is potentiated by CaMKII (Tan et al. 1994), and consequently changes in CaMKII activity may have altered GluR4 and GluR2 activity in BVD animals.

The majority of the variables from the eight variables in the LDA were at either 1 week or 1 month. This indicates that the change in the relationship between the variables did not occur because of the initial BVD-induced damage. It also suggests that the subtle changes in NMDAR and AMPAR caused by BVD in the hippocampus, EC and FC were not due to vestibular compensation.

4.1.2 Interpretation of cluster analysis

The NR1 subunits tended to cluster together in both the BVD and sham animals. As NR1 expression is required for the formation of functional NMDARs (Dingledine et al. 1999, Petralia et al. 1994), this result suggests that the formation of functional NMDARs was tightly regulated in both groups. NR1 and GluR2 also co-varied in both BVD and sham groups. The majority of AMPAR heterodimers in the CNS contain GluR2 (Greger et al. 2002, Wenthold et al. 1996) and its presence in a receptor changes channel activity, for example, lowering channel conductance and Ca^{2+} permeability (Boulter et al. 1990, Geiger et al. 1995, Isaac et al. 2007). Therefore, the activity of AMPARs appears to be coordinated with the formation of NMDARs in both BVD and shams. However, GluR2 at 1 week in the CA1 clustered with NR1 at 1 month in the FC in BVD animals, and clustered with NR1 at 1 month in the DG in sham animals. Perhaps the hippocampal connections to the frontal cortex result in a number of

changes to both regions following BVD. It is unclear whether this is due to the bilateral loss of vestibular input affecting both regions, or the changes to one brain region producing secondary consequences in the other.

The clustering of the remaining variables varied between the BVD and sham rats. CaMKII at 24 hours in the DG co-varied with GluR4 at 1month in the EC in BVD animals, but clustered with NR2B at 1 month in the FC in sham animals. This result indicates that the phosphorylation of glutamate receptors is different in BVD animals, with an emphasis on AMPAR phosphorylation. The EC connects to the DG as shown in fig. 4.1, via the perforant pathway. The perforant pathway is a vital projection from the EC which connects with the hippocampal formation (Anderson et al. 1998). Therefore, any alterations in AMPAR and CaMKII expression in one of these regions would likely affect neurochemical activity in connecting regions of the cortex.

NR1 at 1 week in the DG clustered with NR2B at 1 month in the FC in BVD rats. The majority of NR2B is expressed in the forebrain (Ishii et al. 1993, Monyer et al. 1994). This result suggests that NMDAR expression in the FC is linked to NMDAR expression in the DG and therefore, in the hippocampus. The presence of NR2B in an NMDAR changes the properties of the receptor, as these receptors deactivated more slowly (Vicini et al. 1998) and show slower EPSC decay times (Flint et al. 1997). This connection is potentially important for the electrophysiological changes observed following BVD (Stackman et al. 2002a, Russell et al. 2003b, Russell et al. 2006).

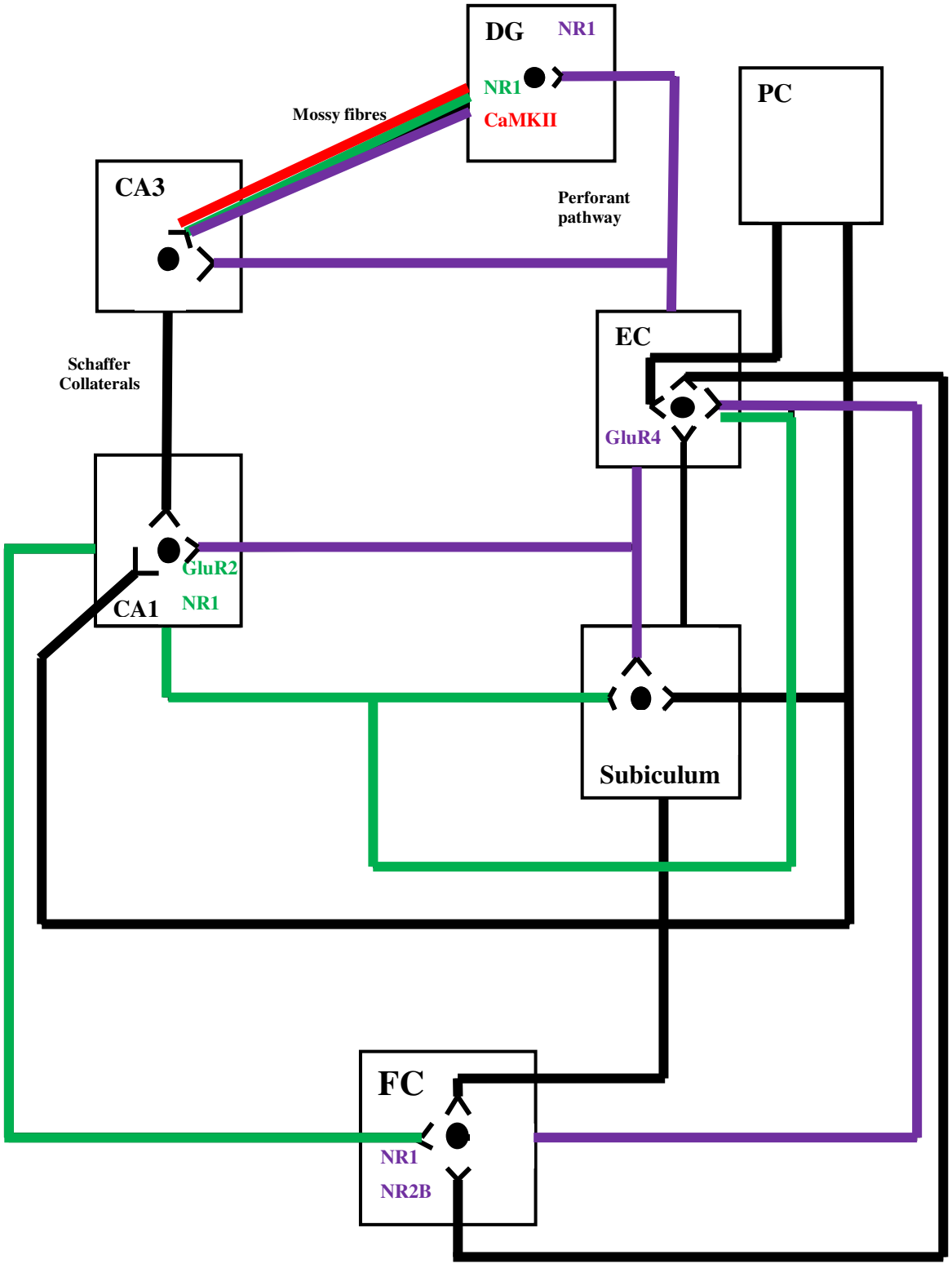


Figure 4.1: The hippocampal formation and connections to the Frontal Cortex to LDA data. *Simplified schematic representation of the projections between the CA1, CA3, DG, Subiculum, EC, PC and FC. Includes synapses ( )and neural pathways ( ).Used to highlight the complicated interconnections between these various brain regions. (Andersen et al. 2007: 38, 71-72, 75-77, 92-93, 138).The eight variables from the stepwise linear discriminate analysis added to the figure. Colour coded based on time point: red is 24 hours; green is 1 week; and purple is 1 month.*

4.2 Comparison to previous literature

To the authors knowledge there are no previous studies investigating NMDAR, AMPAR and CaMKII levels in the hippocampus following BVD surgery. The most relevant studies are those that investigate NMDAR and AMPAR subunits in the rat hippocampus following UVD surgery, and NMDAR levels following the induction of chemically induced consecutive unilateral vestibular lesions.

4.2.1 Other vestibular lesion studies

As referred to in the Introduction, NMDAR and AMPAR subunit changes were found in the hippocampus of rats post-UVD (Liu et al. 2003b). UVD results in loss of one vestibular labyrinth and therefore results in an imbalance in spontaneous firing of the bilateral VNCs (Smith and Curthoys 1989). However, following BVD the input to both labyrinths is lost and the initial spontaneous firing of both VNCs is disrupted (Curthoys 2000). There is no imbalance in the sensory information that is transmitted to the hippocampus and other cortical regions, therefore these surgeries result in different behavioural paradigms. The animals perform differently on behavioural tasks (Baek et al. 2010, Zheng et al. 2006) because of the differences in input to the hippocampus. Unilateral vestibular loss also results in different electrophysiological (Zheng et al. 2003, Zheng et al. 2010) and anatomical (Brandt et al. 2005, Hüfner et al. 2007) changes in the human and rat brain, when compared to bilateral vestibular loss.

There was a significant increase in the distribution and decrease in the affinity of NMDARs in the hippocampus following sequential bilateral vestibular damage (Besnard et al. 2011, in press). The method used in this study involved inducing a unilateral lesion, which creates an imbalance in vestibular sensory input. Then the animals were left for 3 weeks while they underwent compensation. Some spontaneous firing of the VNC on the lesioned side was restored following compensation. Subsequently the other labyrinth was lesioned, inducing an imbalance in the spontaneous firing activity of the ipsilateral and contralateral VNCs for a second time. The loss of vestibular function is confounded by the continuing compensation of the side that was lesioned first. The effect of inducing a unilateral vestibular lesion and then inducing a second one after the first has undergone compensation is known as ‘Bechterew’s phenomenon’ (Katsarkas and Galiana 1983).

Besnard et al. (2011, in press) found no significant changes in behaviour when these animals performed the object recognition task, whereas BVD animals showed decreased exploration of a novel object at 3 and 6 months (Zheng et al. 2004). However, Besnard et al. (2011, in press), like Zheng et al. (2009) and Baek et al. (2010), found that animals with bilateral vestibular loss had severe spatial memory deficits. During BVD both labyrinths are lesioned in the same procedure so that initially there is no spontaneous firing of either VNC, and consequently no imbalance in firing (Curthoys 2000). The initial symptoms of UVD caused by an imbalance in firing of the VNCs, for example vertigo, nystagmus and postural asymmetries such as roll head tilt and yaw head tilt (Curthoys and Halmagyi 1995), are thought to be more severe clinically than BVD symptoms. Therefore, while this procedure and BVD both induce bilateral vestibular lesions, the symptoms and behaviours induced are different. Consequently any neurochemical and/or electrophysiological changes that occur as a result of these procedures could be different.

Besnard et al. (2011, in press) used intratympanic injections of the chemical sodium arsanilate to induce vestibular lesions. This method is often used to induce vestibular lesions in animal models as it is less complicated than UVD or BVD surgery (Stackman et al. 2002a, Wallace et al. 2002). Histological study of the temporal bone is often used in studies that involve damaging the vestibular labyrinth, to ensure that a complete lesion of the vestibular labyrinth has occurred (Zheng et al. 2006). Chemicals injected through the tympanic membrane often produce an incomplete lesion due to poor penetration of the round window and inconsistent circulation through the vestibular and auditory labyrinth (Jensen 1983), as a result vestibular function is not completely eliminated (Saxon et al. 2001). A complete lesion is always induced following BVD surgery and consequently the procedure produces a consistent behavioural syndrome. When chemical ablation is used vestibular function cannot be quantified using behavioural tests alone. The only way to guarantee that each animal has completely lost vestibular function is to use scleral search coils to record eye movements while applying high acceleration, unpredictable head movements to test the VOR (Aw et al. 1996, Gilchrist et al. 1998). For a number of reasons, including expense and equipment availability, this technique is rarely used.

Permanent and complete destruction of the vestibular labyrinths results in degeneration of the vestibular nerve fibres in the vestibular nuclei (Smith and Curthoys 1989) and distinct

neurochemical changes in the VNC (Campos-Torres et al. 2005). This damage is likely to have effects in other areas of the CNS, for example glial cell induced changes or compensatory processes with downstream effects. These changes may not occur in animals who have received incomplete and/or transient lesions (Russell et al. 2003b). If any vestibular labyrinth function is intact following chemical ablation, disrupted sensory information could be relayed to the cortex. This could also alter the compensatory processes of these animals and the corresponding behavioural alterations and the neurochemical and electophysiological changes occurring in the brain. For example, the circling behaviour characteristic of BVD animals does not occur following this procedure (Hunt et al. 1987). Therefore, any cortical alterations following chemically induced vestibular lesions are unlikely to be comparable to those produced by BVD.

4.2.2 Long term potentiation following bilateral vestibular deafferentation

The disruption of spatial memory is severe and consistent for both humans and animals with vestibular loss (Astur et al. 2002, Russell et al. 2003a). LTP is considered to be the synaptic mechanism behind spatial memory (Pastalkova et al. 2006). Loss of NMDAR function in the hippocampus results in deficits in LTP and spatial memory (Davis et al. 1992, Sakimura et al. 1995, Tsien et al. 1996). However, there were no significant electrophysiological changes in BVD rats (Zheng et al. 2010). BVD animals perform poorly in tasks that require spatial navigation, particularly if the task does not have learned visual cues (Stackman and Herbert 2002b). It is possible that the maintenance of spatial memory is intact even though the encoding of novel spatial memories is disrupted. Future research could measure LTP when BVD rats are introduced into a new environment or while BVD rats are performing a task that required spatial navigation. BVD animals have shown severe deficits in the food foraging task in the absence of visual cues, as accurate performance requires vestibular input (Etienne et al. 1996, Stackman and Herbert 2002b, Zheng et al. 2009b). Therefore, electrophysiological recordings could be taken from the lesioned animals while they perform this task in the dark.

Electrophysiological recordings were taken in the DG and CA1 regions of BVD animals (Zheng et al. 2010). LTP may have been affected in other regions of the hippocampus such as the CA3 region. The CA3 sub-region has a role in the formation of spatial memories, the association of a stimulus with a spatial location and the retrieval of spatial memories (Gilbert and Brushfield 2009). A mouse strain with the NR1 gene knocked out of the CA3 region

showed deficits in the acquisition of novel spatial information (Nakazawa et al. 2003). NMDAR function may be more important for the acquisition of spatial memory in the CA3 region, than the CA1 or DG. The NMDAR antagonist AP5 was injected bilaterally into either the CA1, DG or CA3 region of the hippocampus in rats that had been trained in the radial eight-arm maze. The animals that received the drug in the CA3 region were significantly impaired when performing the task in a new environment when compared to animals with lesions of the CA1 or DG sub-regions (Lee and Kesner 2002a). Potentially, the animals could not use known spatial cues to navigate and did not possess functioning spatial learning so could not acquire the task. LTP and the processes behind spatial memory may be impaired in the CA3 region of BVD rats.

4.3 Critical evaluation of my research

4.3.1 Bilateral vestibular damage in human patients compared to the animal model

Neurochemical changes in BVD animals may not be comparable to neurochemical changes that occur in humans following bilateral vestibular loss. The atrophy of the hippocampus in BVL humans was observed at 5-10 years post-lesion (Brandt et al. 2005). However, the recent measurement of neuronal numbers in the whole hippocampus and within the sub-regions of the hippocampus of BVD rats 17 months post-surgery showed that there was no significant difference between BVD rats and shams (Balabhadrapatruni et al. 2011, unpublished data). Besnard et al. (2011, in press) also reported no change in hippocampal volume following bilateral vestibular loss in rats.

These results suggest that the hippocampal atrophy that occurs following BVL in humans does not occur in BVD rats. Anxiety disorders are common in human patients with vestibular loss (Yardley et al. 1998, Zheng et al. 2008), but BVD rats do not appear to be anxious (Zheng et al. 2008). Complete BVD is also rare in human patients (Zheng et al. 2008), however researchers are still attempting to quantify and understand the changes that occur following vestibular loss in humans. So while BVD is not the best model of human disease it is an effective technique for measuring what complete loss of the vestibular sensory system does to the CNS.

4.3.2 Sham control rats

The sham rats were anaesthetized and their tympanic membrane was removed as a necessary control. Following temporal bone surgery NOS levels were significantly lower in the ipsilateral guinea pig hippocampus of UVD and sham animals when compared to controls that had only been anaesthetised (Zheng et al. 1999c). This study confirmed that the act of opening the temporal bone without damaging the vestibular labyrinth results in neurochemical changes in the hippocampus. Therefore, sham animals that have undergone surgery are vital in BVD studies as anaesthetic controls are not sufficient.

4.3.3 Tissue preparation

The standard western blotting tissue preparation technique was used on the tissue from the BVD and sham animals that had been sacrificed 24 hours, 72 hours and 1 week after surgery. The membrane preparation technique was used on the 1 month tissue thereby, isolating crude synaptic membranes (Broutman and Baudry 2001). Interestingly, there were no obvious differences between the blots following analysis and the density readings were at similar levels. CaMKII and pCaMKII levels were not measured at the 1 month time point because the membrane preparation technique was used and CaMKII is not membrane bound as it is an intracellular signalling protein (Miller and Kennedy 1986).

The theory behind using the membrane preparation is that the functional AMPARs and NMDARs are largely located in the membrane (Craig et al. 1993, Monyer et al. 1994). NMDARs are believed to be tightly associated with the synaptic cytostructure and firmly bound to the postsynaptic complex (Allison et al. 1998). However, while almost all NR2B subunits appear to be expressed in the plasma membrane, conversely less than half of the total NR1 subunits are found in the membrane. This suggests that there is a large intracellular pool of NR1 subunits (Hall and Soderling 1997). In addition, Shi et al. (1999) found that non-synaptic AMPARs greatly outnumber synaptic AMPARs (Shi et al. 1999). Therefore, there may not be an overt change in receptor activity, but there may be changes in the available pool of receptors. A more specific research technique such as transmission electron microscopy (TEM) would show the cellular localization of receptors (Williams and Carter 2009).

The tissue preparation was performed on the left cortex of the BVD and control animals. It is possible that greater or more complex changes would have been seen in the sub-regions of the right hippocampus. There were no significant deficits observed in UVD patients with left vestibular failure when their spatial memory was assessed using the VMWT. Conversely, there was a slight decrease in the performance of UVD patients with right vestibular loss (Hüfner et al. 2007). This is probably because the right hippocampus is primarily involved in spatial memory and navigation in humans (Grön et al. 2000, Hartley et al. 2003). For example, the right hippocampus of human subjects was activated more strongly than the left during navigation (Ghaem et al. 1997, Maguire et al. 1997). However, it seems unlikely that the use of the right or left brain is a significant issue when studying BVD, as past neurochemical and electrophysiological studies have shown no laterality following bilateral vestibular lesions (Goddard et al. 2008c, Russell et al. 2003b, Zheng et al. 2010). This issue could be clarified in future immunohistochemical studies which could measure neurochemical changes in glutamatergic receptors in the right hippocampus.

Whole sub-regions of the hippocampus were used in this study. However, the ventral and rostral hippocampus may show different alterations following BVD. Genetic markers showed distinct spatial expression domains between the dorsal and ventral hippocampus, confirming that there is a functional separation in the hippocampus at a molecular level (Dong et al. 2009). Lesions to the dorsal/posterior hippocampus resulted in impaired spatial memory in a MWT and the level of impairment correlated with the size of the lesion. Conversely, when the ventral/anterior hippocampus was lesioned there was no impairment in spatial learning (Moser et al. 1993). Pothuizen et al. (2004) found the same result using a radial arm maze task where lesions of the dorsal hippocampus disrupted working and reference spatial memory, whereas animals with ventral lesions and control animals were not significantly different (Pothuizen et al. 2004).

While animals with lesions of the ventral hippocampus do not exhibit spatial memory deficits (Moser et al. 1993, Pothuizen et al. 2004), they do present with changes in the regulation of motor output and anxiety, as the animals showed increased movement and the lesion appeared to have an anxiolytic effect (Bannerman et al. 2002, Bannerman et al. 2003, Trivedi and Coover 2004). This coincides with the finding that BVD rats are hyperactive (Goddard et al. 2008a) and that they spent more time in the open arms of the elevated plus maze when compared to shams, which suggests BVD has an anxiolytic effect (Zheng et al. 2008).

Alterations in physiology in the ventral and rostral hippocampus may be responsible for different symptoms following BVD.

The results from these studies are consistent with the knowledge that the dorsal hippocampus receives the majority of the visuo-spatial information from sensory modalities (Moser and Moser 1998); whereas the ventral hippocampus projects to the prefrontal cortex (Goldman-Rakic et al. 1984) and is closely connected to the hypothalamus and amygdala (Jacobson and Sapolsky 1991, Petrovich et al. 2001, Pitkänen et al. 2000, Swanson and Cowan 1977). Perhaps the differences in anxiety between humans with vestibular deficits and BVD rats are caused by different anatomical connections between the hippocampus and other brain regions in animals and humans. Another possibility is that the behavioural deficits induced by vestibular damage in humans, result in additional stress, anxiety and depression due to societal pressures that rats do not experience (Yardley 1994, Zheng et al. 2008). The additional pressures caused by being unable to live a normal life may overcome any anxiolytic effects from the ventral hippocampus.

4.3.4 Western Blotting

The power of the study was estimated to be 0.9 based on previous studies using an n of 7 per treatment group and therefore the result was unlikely to be insignificant because of insufficient sample size. It is also possible that changes in NMDAR and AMPAR subunit levels were below the resolution of Western blotting. Unfortunately there is no literature available that quantifies the resolution of western blotting when compared to other detection methods.

Western blotting cannot differentiate between protein expression in neurons and/or glial cells. AMPARs are highly expressed on glial cells (Seifert and Steinhäuser 2001) and in microglia (Noda et al. 2000). Astroglia expressing AMPARs have been found in almost all brain regions including the hippocampus (Gallo and Ghiani 2000, Matthias et al. 2003, Seifert and Steinhäuser 2001). NMDARs have also been detected in several types of cortical astrocytes (Conti et al. 1999, Lalo et al. 2006). It is possible that the loss of vestibular input induced by BVD could result in changes in glial cell expression in the hippocampus, particularly in the earlier time points, potentially masking changes in neuronal expression.

The molecular weight of human NR2A is 170 kDa, whereas the molecular weight in rats is 165 kDa (Laurie et al. 1997). Mouse, rat and human NR2A subunits are highly homologous and contain the same number of amino acids (Bourdellès et al. 1994, Kutsuwada et al. 1992, Monyer et al. 1992). The differences in human and rat NR2A may be due to different protein processing. Following deglycosylation, the molecular weight of NR2A in both rats and humans decreases by 10 kDa (Laurie et al. 1997). The changes may be due to differences in phosphorylation at the PSD (Kornau et al. 1995). These alterations could result in variations in glutamate receptor function and location between species.

There was no internal control used in the individual membranes making comparisons between different Western blots difficult. This was overcome by normalizing the data to β-actin and then converting the data to z-scores, so that the optical densities in the different blots were normalised to their own means and standard deviations.

CaMKII and pCaMKII had non-specific binding patterns at the same molecular weights of around 25, 40 and 60 kDa. The specific and non-specific densities were much stronger in the pCaMKII blots, indicating there is more of the activated form of CaMKII in the hippocampus. However, the tissue preparation for the pCaMKII antibody may have not been optimal for gauging the activity of this antibody. In another study the brains were snap frozen in situ with liquid nitrogen so that the phosphorylation status of CaMKII was preserved (Atkins et al. 2006). Phosphatase inhibitors should also be used in tissue preparation when measuring the phophorylated form of a protein (Atkins et al. 2006, Martínez-Turrillas et al. 2007).

Many of the studies that use Western blotting to measure AMPAR and NMDAR subunit levels crop their blots, or do not discuss non-specific antibody binding. This makes a literature review difficult. However, the same non-specific binding was found in another CaMKII and pCaMKII Western blot study (Pollak et al. 2005). The NMDAR subunits in particular consistently showed non-specific binding. Glycosylation of a protein can result in changes in molecular weight and therefore different bands of specific binding. For example, there are at least six possible N-linked glycosylation sites at the N-terminus of the NR2A and NR2B subunits (Wang et al. 1995). Antibody quality can also be extremely variable even between antibody batches from the same company (Wortley et al. 2009). This could be overcome by employing a technique that does not require antibodies so that antibody specificity is not an

issue, for example non-immunohistochemical techniques such as in situ hybridisation that measures mRNA.

4.3.5 Confounding factors of study

NMDAR and AMPAR levels were studied in the hippocampus of BVD rats at 24 and 72 hours and 1 week. In the initial 48-72 hours following vestibular damage, symptoms include severe postural instability and oscillopsia, due to the loss of VORs and VSRs (Smith and Curthoys 1989). The animals also suffer from abnormal visual and proprioceptive feedback (Curthoys and Halmagyi 1995). Permanent damage to the vestibular labyrinths causes the vestibular nerve fibres in the vestibular nuclei to degenerate (Smith and Curthoys 1989). This loss could also result in glial cell-induced changes in other areas of the CNS. Soon after the initial damage to the vestibular system, compensation for these deficits starts to occur (Curthoys 2000). The severe initial symptoms, abnormal sensory feedback, and vestibular compensation are all confounders when assessing the earlier time points of BVD. The hippocampus is also compensating for the loss of the vestibular system and abnormal sensory feedback. The hippocampus may adapt by altering the amount it uses other sensory inputs (McEwen 1999, Zamanillo et al. 1999).

Another potential confounding factor in this study was the loss of auditory input to the hippocampus. A retroauricular approach is used when performing surgical labyrinthectomy (Zheng et al. 1999b, Zheng et al. 1999c, Zheng et al. 2001b). This involves removing the malleus and incus, which transmits auditory information to the stapes. It is difficult if not impossible to damage the vestibular system in rats and guinea pigs without harming the cochlea and auditory middle ear. However, it has been observed that hippocampal place cells function normally in deaf rats (Hill and Best 1981) and it was shown that auditory cues alone are not sufficient information for spatial navigation (Rossier et al. 2000). The sham animals' act as a partial control for loss of auditory information, as removal of the tympanic membrane disrupts sound to the malleus, incus and stapes. However, this only partially stops sound being transmitted to the cochlea. Nevertheless, these control animals consistently perform better in tasks than vestibular lesioned animals (Zheng et al. 2006, Zheng et al. 2007, Zheng et al. 2008).

The VOR is disrupted in BVD animals which disrupts the animals' vision and results in symptoms such as oscillopsia (Curthoys 2000). The visual system is a major source of sensory information to the hippocampus (Tsanov and Manahan-Vaughan 2009). Hippocampal place cells were disrupted when the visual cortex was lesioned (Paz-Villagràn et al. 2002) and light deprivation results in altered LTP in the CA1 region (Talaei et al. 2010). The glial fibrillary acidic protein is an important astrocyte cytoskeleton protein that was reduced in the hippocampus following light deprivation and astrocyte shape was also altered (Corvetti et al. 2006). The vestibular induced changes in vision may result in abnormal visual sensory feedback to the hippocampus, thereby confounding the damage induced by BVD. However, oscillopsia undergoes compensation (Curthoys 2000) and blind and sighted rats can form and maintain place fields (Save et al. 1998).

It is impossible to isolate vestibular information being transmitted to the hippocampus from other inputs. Vestibular information is integrated with visual, proprioceptive and motor efference signals in the VNC (Horii et al. 2004), and projected to the limbic system and neocortex. Lesions of the vestibular system will change the way this information is integrated resulting in a corresponding change in the signal that is transmitted to the various regions of the brain (Smith 1997).

It is important to consider whether the changes in spatial firing and theta in BVD animals occur as a direct result of loss of vestibular input to the hippocampus, or due to vestibular-induced changes in another cortical area affecting the hippocampus. It is also possible that alterations occurred as a secondary consequence of other sensory systems becoming dysfunctional because of the vestibular lesion. For example, the visual field needs vestibular information to remain stable (Curthoys and Halmagyi 1995). Adaptations may be occurring in multiple regions of the CNS in order to compensate for this change in normal sensory function.

Movement has a large effect on the hippocampus (Neeper et al. 1996, Smith et al. 2009). The characteristic hyperactivity of BVD rats results in increased velocity (Goddard et al. 2008a). Rats are bipedal, allowing for greater postural stability, particularly when moving. Humans with vestibular deficits have shown greater stability while moving if their velocity is higher (Brandt et al. 1999). Humans with permanent vestibular loss often become agoraphobic (Jacob et al. 1996), and this may be due to the increased anxiety associated with vestibular

loss. Vestibular loss also results in staggering gait and postural instability. These symptoms can have social ramifications for humans, as patients are often accused of public drunkenness (Yardley 1994). As a result human patients frequently become less active.

Humans are generally older when vestibular loss occurs (Agrawal et al. 2009), whereas the rats were relatively young when the BVD surgeries were performed. Synaptic plasticity of the hippocampus alters with age (Detoledo-Morrell et al. 1988). Potentially, the rats were better able to adapt following the vestibular lesions and may have shown a greater degree of plasticity. To adjust for this the surgeries could be performed in older rats to improve the correlation between humans with bilateral vestibular disorders and BVD animals. The disadvantage of this approach is that the older animals may not cope as well with the surgery, which would probably result in a greater loss of animals, and very long time points could not be used.

Chronic stress is a confounding factor as in vestibular lesion studies as it could induce neurochemical changes in the hippocampus. Patients with neuro-otologic deficits have shown short term increases in cortisol that were significantly higher than patients without neuro-otologic disorders (Horii et al. 2007). However, animal studies have shown that the increased blood corticosterone levels (Lindsay et al. 2005, Russell et al. 2006), and salivary cortisol levels (Gliddon et al. 2003a) decrease quickly following surgery and are normal within 2 weeks. It is known that patients with Meniere's disease suffer from long term increases in salivary and serum cortisol (Van Cruijsen et al. 2005); however this disease is relapsing and remitting in nature (da Costa et al. 2002), unlike BVD, which is chronic. The increased stress observed in humans with vestibular loss does not appear to occur in BVD animals.

4.4 Further research

4.4.1 Time points

The latest time-point of this study was 1 month and the atrophy of the hippocampus in BVL humans was observed at 5-10 years post-lesion (Brandt et al. 2005). The molecular changes in the hippocampus that caused this atrophy to occur may not have happened by 1 month. Therefore, NMDAR and AMPAR levels following BVD should be measured at a later time

point. Potential time points could include 14 months, as severe spatial memory deficits were exhibited in rats at 14 months in the light and dark in the foraging task (Baek et al. 2010).

4.4.2 Western blotting protocol

There were no negative controls used, in the future a specific antibody blocking peptide could be included in the Western blotting protocol, this would confirm the specific band reactivity of the antibodies (Ong et al. 2002).

4.4.3 Brain regions

While western blotting was performed on the FC, EC and PC at the 1 month time point, due to time limitations, receptor subunit and CaMKII levels were not measured in the earlier time points. This research should be performed in future studies, particularly as subunits from the EC and FC at 1 month were 3 of the 8 variables that could successfully distinguish between BVD and sham animals and the FC obviously has an important role in plasticity following bilateral vestibular lesions (Goddard et al. 2008b, Goddard et al. 2008c). Without further research on the effects of BVD throughout the CNS, it is difficult to distinguish cause and effect.

As shown in figure 1.2, the subiculum is an important relay point in the hippocampal formation that has projections to the frontal cortex (Andersen et al. 2007). The subiculum is also a brain region in the head-direction pathway that connects the vestibular system to the hippocampus (Taube et al. 1996). Due to these connections the subiculum could undergo changes in NMDAR, AMPAR and CaMKII expression in following BVD and is therefore a potential region for assessing changes post-surgery.

4.4.4 Alternative techniques

Western blotting was a good starting point, but other techniques may provide more information about glutamatergic receptors. The alterations in NMDARs and AMPARs are clearly subtle and require more sensitive techniques to determine subunit levels and changes in the ratio, efficacy and affinity of these receptors. Immunohistochemistry is a high

resolution form of antigen staining that could distinguish on which neuronal cells and fibres the NMDARs and AMPARs are localized (Franklin and Martin 1981, Tsou et al. 1997). It is possible that behavioural and electrophysiological changes induced by BVD surgery were due to alterations in glutamate receptor affinity and efficacy as opposed to overt changes in subunit levels. Autoradiography measures changes in receptor affinity and it is therefore a more sensitive method of measuring neurochemical changes than Western blotting. There is also TEM which has very high spatial and analytical resolution and would show the number, localization and ratio of synaptic NMDARs and AMPARs (Williams and Carter 2009). Pharmacological studies using subunit-selective agonists and antagonists could be used to gauge changes in NMDAR and AMPAR function following BVD.

Genetic studies would also provide a lot of information about NMDARs, AMPARs and CaMKII following BVD. NMDAR subtypes can be targeted within a cell in a 'synapse-selective manner'. When the NR2A subunit gene was selectively disrupted there was a reduction in NMDAR-EPSCs and LTP at apical dendrites in CA3 pyramidal neurons (Ito et al. 1997). Disruption of the NR2B subunit gene decreased NMDAR-EPSCs and LTP at the synapses of basal dendrites (Ito et al. 1997). Genetic targeting has a large effect on NMDAR-mediated LTP and synaptic plasticity. Polymerase chain reaction (PCR) is a fast and powerful technique that amplifies DNA (Mullis et al. 1992) and quantitative reverse transcriptase polymerase chain reaction measures mRNA (Freeman et al. 1999). GluR2 undergoes RNA editing where the glutamine codon for residue 607 is replaced with the arginine codon. This edited version of the subunit controls the AMPAR properties (Swanson et al. 1997). Techniques such as these could be used to compare the genetic alterations of BVD animals with sham animals.

One deficit in the available neurochemical, electrophysiological and behavioural literature is the lack of direct comparison between UVD and BVD animals, as there are marked differences in UVD and BVD postural and ocular motor symptoms (Curthoys 2000). There are also no studies using animals that have undergone chemical and surgical vestibular lesions which would enable comparison between the two. It would be interesting to compare the differences and similarities between these vestibular lesion techniques in the same studies.

A possible alternative to deafferentation surgery is to use aminoglycoside antibiotics to lesion the vestibular system. Aminoglycoside antibiotics have varying vestibulo- and cochleo-

toxicities (Kotecha and Richardson 1994). However, there is still some slight cochlea hair cell damage produced by aminoglycoside antibiotics like gentamicin that preferentially target the vestibular system (Forge and Schacht 2000) and rats are also far more susceptible to aminoglycoside-induced renal damage than humans. Alternately, guinea pigs could be used as an animal model of bilateral vestibular damage as their metabolism of aminoglycoside antibiotics is similar to humans (Brummett and Fox 1982). Delivery of the drug would be difficult due to the issues with intratympanic injection mentioned previously. Microstimulation of the vestibular labyrinth is a more reliable and specific method for inducing transient changes in vestibular input, where vestibular receptors are activated or inactivated using an anode and cathode (Cuthbert et al. 2000). Another advantage of this technique is that the auditory system is not affected (Smith et al. 2005b).

4.4.5 Other NMDA receptor subunits

Due to the time constraints of this project some NMDAR and AMPAR subunits were not quantified following BVD. It was important to measure potential changes in the expression of NR2A and NR2B subunits as they are abundant in the cortex and hippocampus. Alternately, NR2C is located primarily in the cerebellum and NR2D is located in sub-cortical areas, such as the midbrain and brainstem (Ishii et al. 1993). However, NR2C is important developmentally in both synaptic and extra-synaptic NMDARs (Akazawa et al. 1994). Although there is little evidence for the expression of NR1/NR2D-containing receptors at central synapses (Cull-Candy et al. 2001) and NR2D expression appears to be largely extra-synaptic (Momiyama et al. 1996), extra-synaptic NMDARs containing NR2D were recruited to synapses in the DG when NMDAR dependent-LTP was induced (Harney et al. 2008).

The NMDAR subunits NR3A and NR3B were also not researched in this study. Currently there is little pharmacological data on the NR3A receptor subunit, but it is thought to have high expression in astrocytes and oligodendrocytes (Paoletti and Neyton 2007). Nonetheless, incorporating an NR3 subunit into an NMDAR decreases Ca^{2+} permeability, Mg^{2+} block and single-channel conductance (Sasaki et al. 2002). NR3 may have a role in BVD-induced synaptic plasticity in the hippocampus, but it was outside the scope of this project.

4.5 Conclusion

The loss of vestibular input results in more subtle changes in the hippocampus, when compared to the imbalance created by UVD. However, the recovery of spatial memory impairments following UVD and the permanent spatial memory deficits observed following BVD would suggest that the compensation for this loss is less effective. Even though BVD results in a number of electrophysiological changes such as alterations in theta rhythm and place cell activity, the neurochemical changes appear to be subtle and spread across a number of brain regions. Further studies need to be performed to gauge changes in NMDAR and AMPAR affinity, efficacy and neuronal location in the hippocampus, entorhinal cortex, perirhinal cortex and frontal cortex of BVD animals.

Chapter five

References

Aardal E, Holm A. 1995. Cortisol in saliva-reference ranges and relation to cortisol in serum. European Journal of Clinical Chemistry and Clinical Biochemistry 33: 43.

Agrawal Y, Carey J, Della Santina C, Schubert M, Minor L. 2009. Disorders of balance and vestibular function in US adults. Archive of Internal Medicine 169: 938-944.

Aguirre G, Detre J, Alsop D, D'Esposito M. 1996. The parahippocampus subserves topographical learning in man. Cerebral Cortex 6: 823-829.

Akazawa C, Shigemoto R, Bessho Y, Nakanishi S, Mizuno N. 1994. Differential expression of five N-methyl-D-aspartate receptor subunit mRNAs in the cerebellum of developing and adult rats. The Journal of Comparative Neurology 347: 150-160.

Akbarian S, Grüsser O, Guldin W. 1993. Corticofugal projections to the vestibular nuclei in squirrel monkeys: further evidence of multiple cortical vestibular fields. The Journal of Comparative Neurology 332: 89-104.

Allison D, Gelfand V, Spector I, Craig A. 1998. Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. The Journal of Neuroscience 18: 2423-2436.

Andersen P, Morris R, Amaral D, Bliss T, O'Keefe J. 2007. The Hippocampus Book. Oxford.

Anderson T, Moulton A, Sansom A, Kerr D, Laverty R, Darlington C, Smith P. 1998. Evidence for reduced nitric oxide synthase (NOS) activity in the ipsilateral medial vestibular nucleus and bilateral prepositus hypoglossi following unilateral vestibular deafferentation in the guinea pig. Brain Research 787: 311-314.

Arleo A, Gerstner W. 2000. Spatial cognition and neuro-mimetic navigation: a model of hippocampal place cell activity. Biological Cybernetics 83: 287-299.

Arnolds D, Lopes da Silva F, Boeijinga P, Kamp A, Aitink W. 1984. Hippocampal EEG and motor activity in the cat: the role of eye movements and body acceleration. Behavioural Brain Research 12: 121-135.

Astur R, Taylor L, Mamelak A, Philpott L, Sutherland R. 2002. Humans with hippocampus damage display severe spatial memory impairments in a virtual Morris water task. Behavioural Brain Research 132: 77-84.

Atkins C, Chen S, Alonso O, Dietrich W, Hu B. 2006. Activation of calcium/calmodulin-dependent protein kinases after traumatic brain injury. Journal of Cerebral Blood Flow & Metabolism 26: 1507-1518.

Aw S, Halmagyi G, Haslwanter T, Curthoys I, Yavor R, Todd M. 1996. Three-dimensional vector analysis of the human vestibuloocular reflex in response to high-acceleration head rotations. II. Responses in subjects with unilateral vestibular loss and selective semicircular canal occlusion. Journal of Neurophysiology 76: 4021-4030.

Bach M, Hawkins R, Osman M, Kandel E, Mayford M. 1995. Impairment of spatial but not contextual memory in CaMKII mutant mice with a selective loss of hippocampal LTP in the range of the theta frequency. Cell 81: 905-915.

Baek J, Zheng Y, Darlington C, Smith P. 2010. Evidence that spatial memory deficits following bilateral vestibular deafferentation in rats are probably permanent. Neurobiology of Learning and Memory 94: 402-413.

Balabhadrapatruni S, Zheng Y, Baek J, Darlington C, Napper R, Strupp M, Brandt T, Smith P. 2011, unpublished data.

Baloh R. 2003. Vestibular neuritis. New England Journal of Medicine 348: 1027-1032.

Baloh R, Lopez I, Ishiyama A, Wackym P, Honrubia V. 1996. Vestibular neuritis: clinical-pathologic correlation. Otolaryngology-Head and Neck Surgery 114: 586-592.

Bannerman D, Deacon R, Offen S, Friswell J, Grubb M, Rawlins J. 2002. Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. Behavioral Neuroscience 116: 884-901.

Bannerman D, Grubb M, Deacon R, Yee B, Feldon J, Rawlins J. 2003. Ventral hippocampal lesions affect anxiety but not spatial learning. Behavioural Brain Research 139: 197-213.

Barria A, Derkach V, Griffith L, Muller D, Soderling T. 1997a. Regulatory phosphorylation of AMPA-type glutamate receptors by CaMKII during long-term potentiation. Science 276: 2042-2045.

Barria A, Derkach V, Soderling T. 1997b. Identification of the Ca2+/Calmodulin-dependent protein kinase II regulatory phosphorylation site in the α-Amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. Journal of Biological Chemistry 272: 32727-32730.

Barry D, Millecamps S, Julien J, Garcia M. 2007. New movements in neurofilament transport, turnover and disease. Experimental Cell Research 313: 2110-2120.

Basile A, Brichta A, Harris B, Morse D, Coling D, Skolnick P. 1999. Dizocilpine attenuates streptomycin-induced vestibulotoxicity in rats. Neuroscience Letters 265: 71-74.

Benchenane K, et al. 2007. Anti-NR1 N-terminal-domain vaccination unmasks the crucial action of tPA on NMDA-receptor-mediated toxicity and spatial memory. Journal of Cell Science 120: 578-585.

Bense S, Stephan T, Yousry T, Brandt T, Dieterich M. 2001. Multisensory cortical signal increases and decreases during vestibular galvanic stimulation (fMRI). Journal of Neurophysiology 85: 886-899.

Berthoz A, Israël I, Georges-François P, Grasso R, Tsuzuku T. 1995. Spatial memory of body linear displacement: what is being stored? Science 269: 95-98.

Besnard S, Machado M, Vignaux G, Boulouard M, Coquerel A, Bouet V, Freret T, Denise P, Lelong Boulouard V. 2011, in press. Influence of vestibular input on spatial and nonspatial memory and on hippocampal NMDA receptors. Hippocampus: 1-13.

Bland B. 1986. The physiology and pharmacology of hippocampal formation theta rhythms. Progress in Neurobiology 26: 1-54.

Bland B, Colom L. 1993. Extrinsic and intrinsic properties underlying oscillation and synchrony in limbic cortex. Progress in Neurobiology 41: 157-208.

Bliss T, Lømo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. The Journal of Physiology 232: 331-356.

Bliss T, Collingridge G. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361: 31-39.

Blitzer R, Wong T, Nouranifar R, Iyengar R, Landau E. 1995. Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. Neuron 15: 1403-1414.

Böhme G, Bon C, Lemaire M, Reibaud M, Piot O, Stutzmann J, Doble A, Blanchard J. 1993. Altered synaptic plasticity and memory formation in nitric oxide synthase inhibitor-treated rats. Proceedings of the National Academy of Sciences of the United States of America 90: 9191-9194.

Bottini G, Sterzi R, Paulesu E, Vallar G, Cappa S, Erminio F, Passingham R, Frith C, Frackowiak R. 1994. Identification of the central vestibular projections in man: a positron emission tomography activation study. Experimental Brain Research 99: 164-169.

Boulter J, Hollmann M, O'Shea-Greenfield A, Hartley M, Deneris E, Maron C, Heinemann S. 1990. Molecular cloning and functional expression of glutamate receptor subunit genes. Science 249: 1033-1037.

Bourdellès B, Wafford K, Kemp J, Marshall G, Bain C, Wilcox A, Sikela J, Whiting P. 1994. Cloning, functional coexpression, and pharmacological characterisation of human cDNAs encoding NMDA receptor NR1 and NR2A subunits. Journal of Neurochemistry 62: 2091-2098.

Brandt T, Strupp M, Benson J. 1999. You are better off running than walking with acute vestibulopathy. The Lancet 354: 746.

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.

Bredt D, Snyder S. 1989. Nitric oxide mediates glutamate-linked enhancement of cGMP levels in the cerebellum. Proceedings of the National Academy of Sciences of the United States of America 86: 9030-9033.

Broutman G, Baudry M. 2001. Involvement of the secretory pathway for AMPA receptors in NMDA-induced potentiation in hippocampus. The Journal of Neuroscience 21: 27-34.

Brummett R, Fox K. 1982. Studies of aminoglycoside ototoxicity in animal models. New York: Marcel Dekker.

Brummett R, Himes D, Saine B, Vernon J. 1972. A comparative study of the ototoxicity of tobramycin and gentamicin. Archives of Otolaryngology- Head and Neck Surgery 96: 505-512.

Brun V, Otnæss M, Molden S, Steffenach H, Witter M, Moser M, Moser E. 2002. Place cells and place recognition maintained by direct entorhinal-hippocampal circuitry. Science 296: 2243-2246.

Buzsáki G. 2002. Theta oscillations in the hippocampus. Neuron 33: 325-340.

Campos-Torres A, Touret M, Vidal P, Barnum S, De Waele C. 2005. The differential response of astrocytes within the vestibular and cochlear nuclei following unilateral labyrinthectomy or vestibular afferent activity blockade by transtympanic tetrodotoxin injection in the rat. Neuroscience 130: 853-865.

Cannon C, Jahrsdoerfer R. 1983. Temporal bone fractures: review of 90 cases. Archives of Otolaryngology-Head and Neck Surgery 109: 285-288.

Caramanos Z, Shapiro M. 1994. Spatial memory and N-Methyl-D-Aspartate receptor antagonists APV and MK-801: memory impairments depend on familiarity with the environment, drug dose, and training duration. Behavioural Neuroscience 108: 30-43.

Carleton S, Carpenter M. 1984. Distribution of primary vestibular fibers in the brainstem and cerebellum of the monkey. Brain Research 294: 281-298.

Castrén E. 2005. Is mood chemistry? Nature Reviews Neuroscience 6: 241-246.

Chambers B, Mai M, Barber H. 1985. Bilateral vestibular loss, oscillopsia, and the cervico-ocular reflex. Otolaryngology-Head and Neck Surgery 93: 403-407.

Chapman P, Atkins C, Allen M, Haley J, Steinmetz J. 1992. Inhibition of nitric oxide synthesis impairs two different forms of learning. NeuroReport 3: 567-570.

Chung H, Steinberg J, Huganir R, Linden D. 2003. Requirement of AMPA receptor GluR2 phosphorylation for cerebellar long-term depression. Science 300: 1751-1755.

Chung H, Xia J, Scannevin R, Zhang X, Huganir R. 2000. Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. The Journal of Neuroscience 20: 7258-7267.

Cohen H, Kimball K. 2002. Improvements in path integration after vestibular rehabilitation. Journal of Vestibular Research 12: 47-51.

Collingridge G, Kehl S, McLennan H. 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. The Journal of Physiology 334: 33-46.

Conti F, Barbaresi P, Melone M, Ducati A. 1999. Neuronal and glial localization of NR1 and NR2A/B subunits of the NMDA receptor in the human cerebral cortex. Cerebral Cortex 9: 110-120.

Corvetti L, Aztiria E, Domenici L. 2006. Reduction of GFAP induced by long dark rearing is not restricted to visual cortex. Brain Research 1067: 146-153.

Craig A, Blackstone C, Huganir R, Banker G. 1993. The distribution of glutamate receptors in cultured rat hippocampal neurons: postsynaptic clustering of AMPA selective subunits. Neuron 10: 1055-1068.

Cull-Candy S, Brickley S, Farrant M. 2001. NMDA receptor subunits: diversity, development and disease. Current Opinion in Neurobiology 11: 327-335.

Cull-Candy S, Leszkiewicz D. 2004. Role of distinct NMDA receptor subtypes at central synapses. Science's STKE: Signal Transduction Knowledge Environment 2004: 1-9.

Curthoys I. 2000. Vestibular compensation and substitution. Current Opinion in Neurology 13: 27-30.

Curthoys I, Halmagyi G. 1995. Vestibular compensation: a review of the oculomotor, neural, and clinical consequences of unilateral vestibular loss. Journal of Vestibular Research: Equilibrium and Orientation 5: 67-107.

Cuthbert P, Gilchrist D, Hicks S, MacDougall H, Curthoys I. 2000. Electrophysiological evidence for vestibular activation of the guinea pig hippocampus. NeuroReport 11: 1443-1447.

da Costa S, De Sousa L, Piza M. 2002. Meniere's disease: overview, epidemiology, and natural history. Otolaryngologic Clinics of North America 35: 455-495.

Davis L. 1993. Viruses and vestibular neuritis: review of human and animal studies. Acta Oto-Laryngologica 113: 70-73.

Davis S, Butcher S, Morris R. 1992. The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. The Journal of Neuroscience 12: 21-34.

Daw M, Chittajallu R, Bortolotto Z, Dev K, Duprat F, Henley J, Collingridge G, Isaac J. 2000. PDZ proteins interacting with C-terminal GluR2/3 are involved in a PKC-dependent regulation of AMPA receptors at hippocampal synapses. Neuron 28: 873-886.

De Waele C, Baudonniere P, Lepecq J, Tran Ba Huy P, Vidal P. 2001. Vestibular projections in the human cortex. Experimental Brain Research 141: 541-551.

Delgado-Martinez I, Nehring R, Sørensen J. 2007. Differential abilities of SNAP-25 homologs to support neuronal function. The Journal of Neuroscience 27: 9380-9391.

Deliagina T, Popova L, Grant G. 1997. The role of tonic vestibular input for postural control in rats. Archives Italiennes de Biologie 135: 239-262.

Derkach V, Barria A, Soderling T. 1999. Ca2+/calmodulin-kinase II enhances channel conductance of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. Proceedings of the National Academy of Sciences of the United States of America 96: 3269-3274.

Derkach V, Oh M, Guire E, Soderling T. 2007. Regulatory mechanisms of AMPA receptors in synaptic plasticity. Nature Reviews Neuroscience 8: 101-113.

Detoledo-Morrell L, Geinisman Y, Morrell F. 1988. Age-dependent alterations in hippocampal synaptic plasticity: relation to memory disorders. Neurobiology of Aging 9: 581-590.

Dieringer N. 1995. 'Vestibular compensation: neural plasticity and its relations to functional recovery after labyrinthine lesions in frogs and other vertebrates. Progress in Neurobiology 46: 97-129.

Dieterich M, Bartenstein P, Spiegel S, Bense S, Schwaiger M, Brandt T. 2005. Thalamic infarctions cause side-specific suppression of vestibular cortex activations. Brain 128: 2052-2067.

Dieterich M, Bauermann T, Best C, Stoeter P, Schlindwein P. 2007. Evidence for cortical visual substitution of chronic bilateral vestibular failure (an fMRI study). Brain 130: 2108-2116.

Dingledine R, Borges K, Bowie D, Traynelis S. 1999. The glutamate receptor ion channels. Pharmacological Reviews 51: 7-62.

Dong H, Swanson L, Chen L, Fanselow M, Toga A. 2009. Genomic–anatomic evidence for distinct functional domains in hippocampal field CA1. Proceedings of the National Academy of Sciences 106: 11794-11799.

Egebjerg J, Bettler B, Hermans-Borgmeyer I, Heinemann S. 1991. Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA. Nature 351: 745-748.

Ehlers M. 2000. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. Neuron 28: 511-525.

Eickhoff S, Lotze M, Wietek B, Amunts K, Enck P, Zilles K. 2006. Segregation of visceral and somatosensory afferents: an fMRI and cytoarchitectonic mapping study. Neuroimage 31: 1004-1014.

Ekstrom A, Kahana M, Caplan J, Fields T, Isham E, Newman E, Fried I. 2003. Cellular networks underlying human spatial navigation. Nature 425: 184-188.

Erreger K, Chen P, Wyllie D, Traynelis S. 2004. Glutamate receptor gating. Critical Reviews in Neurobiology 16: 187-224.

Esteban J, Shi S, Wilson C, Nuriya M, Huganir R, Malinow R. 2003. PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. Nature Neuroscience 6: 136-143.

Etienne A, Jeffery K. 2004. Path integration in mammals. Hippocampus 14: 180-192.

Etienne A, Maurer R, Séguinot V. 1996. Path integration in mammals and its interaction with visual landmarks. Journal of Experimental Biology 199: 201-209.

Fernández-Monreal M, López-Atalaya J, Benchenane K, Cacquevel M, Dulin F, Le Caer J, Rossier J, Jarrige A, MacKenzie E, Colloc'h N. 2004. Arginine 260 of the amino-terminal domain of NR1 subunit is critical for tissue-type plasminogen activator-mediated

enhancement of N-methyl-D-aspartate receptor signaling. Journal of Biological Chemistry 279: 50850-50856.

Flint A, Maisch U, Weishaupt J, Kriegstein A, Monyer H. 1997. NR2A subunit expression shortens NMDA receptor synaptic currents in developing neocortex. The Journal of Neuroscience 17: 2469-2476.

Forge A, Schacht J. 2000. Aminoglycoside antibiotics. Audiology and Neurotology 5: 3-22.

Forrest D, Yuzaki M, Soares H, Ng L, Luk D, Sheng M, Stewart C, Morgan J, Connor J, Curran T. 1994. Targeted disruption of NMDA receptor 1 gene abolishes NMDA response and results in neonatal death. Neuron 13: 325-338.

Franklin R, Martin M. 1981. Pre-embedding immunohistochemistry as an approach to high resolution antigen localization at the light microscopic level. Histochemistry and Cell Biology 72: 173-190.

Freeman W, Walker S, Vrana K. 1999. Quantitative RT-PCR: pitfalls and potential. Biotechniques 26: 112-125.

Fuentes-Santamaría V, Alvarado J, Henkel C, Brunso-Bechtold J. 2007. Cochlear ablation in adult ferrets results in changes in insulin-like growth factor-1 and synaptophysin immunostaining in the cochlear nucleus. Neuroscience 148: 1033-1047.

Fukaya M, Kato A, Lovett C, Tonegawa S, Watanabe M. 2003. Retention of NMDA receptor NR2 subunits in the lumen of endoplasmic reticulum in targeted NR1 knockout mice. Proceedings of the National Academy of Sciences of the United States of America 100: 4855-4860.

Fukushima K. 2003. Roles of the cerebellum in pursuit-vestibular interactions. The Cerebellum 2: 223-232.

Fukushima K, Akao T, Kurkin S, Fukushima J. 2005. Role of vestibular signals in the caudal part of the frontal eye fields in pursuit eye movements in three dimensional space. Annals of the New York Academy of Sciences 1039: 272-282.

Fukushima J, Akao T, Kurkin S, Kaneko C, Fukushima K. 2006. The vestibular-related frontal cortex and its role in smooth-pursuit eye movements and vestibular-pursuit interactions. Journal of Vestibular Research 16: 1-22.

Fukushima J, Akao T, Takeichi N, Kurkin S, Kaneko C, Fukushima K. 2004. Pursuit-related neurons in the supplementary eye fields: discharge during pursuit and passive whole body rotation. Journal of Neurophysiology 91: 2809-2825.

Funahashi S, Inoue M, Kubota K. 1997. Delay-period activity in the primate prefrontal cortex encoding multiple spatial positions and their order of presentation. Behavioural Brain Research 84: 203-223.

Furman J, Jacob R. 2001. A clinical taxonomy of dizziness and anxiety in the otoneurological setting. Journal of Anxiety Disorders 15: 9-26.

Furman J, Redfern M, Jacob R. 2006. Vestibulo-ocular function in anxiety disorders. Journal of Vestibular Research 16: 209-215.

Gallo V, Ghiani C. 2000. Glutamate receptors in glia: new cells, new inputs and new functions. Trends in Pharmacological Sciences 21: 252-258.

Gavrilov V, Wiener S, Berthoz A. 1995. Enhanced hippocampal theta EEG during whole body rotations in awake restrained rats. Neuroscience Letters 197: 239-241.

Geiger J, Melcher T, Koh D, Sakmann B, Seeburg P, Jonas P, Monyer H. 1995. Relative abundance of subunit mRNAs determines gating and Ca2+ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron 15: 193-204.

Ghaem O, Mellet E, Crivello F, Tzourio N, Mazoyer B, Berthoz A, Denis M. 1997. Mental navigation along memorized routes activates the hippocampus, precuneus, and insula. NeuroReport 8: 739-744.

Giese K, Fedorov N, Filipkowski R, Silva A. 1998. Autophosphorylation at Thr286 of the calcium-calmodulin kinase II in LTP and learning. Science 279: 870-873.

Gilbert P, Brushfield A. 2009. The role of the CA3 hippocampal subregion in spatial memory: a process oriented behavioral assessment. Progress in Neuro-Psychopharmacology and Biological Psychiatry 33: 774-781.

Gilchrist D, Curthoys I, Cartwright A, Burgess A, Topple A, Halmagyi M. 1998. High acceleration impulsive rotations reveal severe long-term deficits of the horizontal vestibulo-ocular reflex in the guinea pig. Experimental Brain Research 123: 242-254.

Gliddon C, Darlington C, Smith P. 2003a. Activation of the hypothalamic-pituitary-adrenal axis following vestibular deafferentation in pigmented guinea pig. Brain Research 964: 306-310.

Gliddon C, Smith P, Darlington C. 2003b. Interaction between the hypothalamic-pituitary-adrenal axis and behavioural compensation following unilateral vestibular deafferentation. Acta Oto-Laryngologica 123: 1013-1021.

Glover J. 2004. Vestibular System. Encyclopedia of Neuroscience: 127-132.

Goddard M, Zheng Y, Darlington C, Smith P. 2008a. Locomotor and exploratory behavior in the rat following bilateral vestibular deafferentation. Behavioral Neuroscience 122: 448-459.

Goddard M, Zheng Y, Darlington C, Smith P. 2008b. Monoamine transporter and enzyme expression in the medial temporal lobe and frontal cortex following chronic bilateral vestibular loss. Neuroscience Letters 437: 107-110.

Goddard M, Zheng Y, Darlington C, Smith P. 2008c. Synaptic protein expression in the medial temporal lobe and frontal cortex following chronic bilateral vestibular loss. Hippocampus 18: 440-444.

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, Schabowska A, Naetebusch B, Heinz A, Strohle A. 2006. The impact of cognitions on the development of panic and somatoform disorders: a prospective study in patients with vestibular neuritis. Psychological Medicine 36: 99-108.

Goldberg J. 2000. Afferent diversity and the organization of central vestibular pathways. Experimental Brain Research 130: 277-297.

Goldman-Rakic P, Selemon L, Schwartz M. 1984. Dual pathways connecting the dorsolateral prefrontal cortex with the hippocampal formation and parahippocampal cortex in the rhesus monkey. Neuroscience 12: 719-743.

Gothard K, Hoffman K, Battaglia F, McNaughton B. 2001. Dentate gyrus and CA1 ensemble activity during spatial reference frame shifts in the presence and absence of visual input. The Journal of Neuroscience 21: 7284-7292.

Gould E, Beylin A, Tanapat P, Reeves A, Shors T. 1999. Learning enhances adult neurogenesis in the hippocampal formation. Nature Neuroscience 2: 260-265.

Granon S, Vidal C, Thinus-Blanc C, Changeux J, Poucet B. 1994. Working memory, response selection, and effortful processing in rats with medial prefrontal lesions. Behavioral Neuroscience 108: 883-891.

Greengard P, Jen J, Nairn A, Stevens C. 1991. Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. Science 253: 1135-1138.

Greger I, Khatri L, Ziff E. 2002. RNA editing at arg607 controls AMPA receptor exit from the endoplasmic reticulum. Neuron 34: 759-772.

Grimm R, Hemenway W, Lebray P, Black F. 1989. The perilymph fistula syndrome defined in mild head trauma. Acta Oto-Laryngologica 108: 1-40.

Grön G, Wunderlich A, Spitzer M, Tomczak R, Riepe M. 2000. Brain activation during human navigation: gender-different neural networks as substrate of performance. Nature Neuroscience 3: 404-408.

Guldin W, Mirring S, Grüsser O. 1993. Connections from the neocortex to the vestibular brain stem nuclei in the common marmoset. NeuroReport 5: 113-116.

Hall R, Soderling T. 1997. Differential surface expression and phosphorylation of the N-methyl-D-aspartate receptor subunits NR1 and NR2 in cultured hippocampal neurons. Journal of Biological Chemistry 272: 4135-4140.

Halmagyi G, Black R, Thurtell M, Curthoys I. 2003. The human horizontal vestibulo ocular reflex in response to active and passive head impulses after unilateral vestibular deafferentation. Annals of the New York Academy of Sciences 1004: 325-336.

Hamann K, Weiss U, Ruile A. 2009. Effects of acute vestibular lesions on visual orientation and spatial memory, shown for the visual straight ahead. Annals of the New York Academy of Sciences 1164: 305-308.

Hamilton D, Driscoll I, Sutherland R. 2002. Human place learning in a virtual Morris water task: some important constraints on the flexibility of place navigation. Behavioural Brain Research 129: 159-170.

Harney S, Jane D, Anwyl R. 2008. Extrasynaptic NR2D-containing NMDARs are recruited to the synapse during LTP of NMDAR-EPSCs. The Journal of Neuroscience 28: 11685-11694.

Hartley T, Maguire E, Spiers H, Burgess N. 2003. The well-worn route and the path less travelled: distinct neural bases of route following and wayfinding in humans. Neuron 37: 877-888.

Hasselmo M, Hay J, Ilyn M, Gorchetchnikov A. 2002. Neuromodulation, theta rhythm and rat spatial navigation. Neural Networks 15: 689-707.

Hayakawa T, Ito H, Zyo K. 1993. Neuroanatomical study of afferent projections to the supramammillary nucleus of the rat. Anatomy and Embryology 188: 139-148.

Hayashi Y, Shi S, Esteban J, Piccini A, Poncer J, Malinow R. 2000. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluR1 and PDZ domain interaction. Science 287: 2262-2267.

Hestrin S. 1992. Developmental regulation of NMDA receptor-mediated synaptic currents at a central synapse. Nature 357: 686-689.

Heynen A, Quinlan E, Bae D, Bear M. 2000. Bidirectional, activity-dependent regulation of glutamate receptors in the adult hippocampus in vivo. Neuron 28: 527-536.

Hicks S, Gilchrist D, Cuthbert P, Curthoys I. 2004. Hippocampal field responses to direct electrical stimulation of the vestibular system in awake or anesthetised guinea pigs. Journal of Vestibular Research 15: 1-9.

Hill A, Best P. 1981. Effects of deafness and blindness on the spatial correlates of hippocampal unit activity in the rat. Experimental Neurology 74: 204-217.

Hollmann M, Heinemann S. 1994a. Cloned glutamate receptors. Annual Review of Neuroscience 17: 31-108.

Holscher C. 1997. Nitric oxide, the enigmatic neuronal messenger: its role in synaptic plasticity. Trends in Neurosciences 20: 298-303.

Horii A, Russell N, Smith P, Darlington C, Bilkey D. 2004. Vestibular influences on CA1 neurons in the rat hippocampus: an electrophysiological study in vivo. Experimental Brain Research 155: 245-250.

Horii A, Takeda N, Mochizuki T, Okakura-Mochizuki K, Yamamoto Y, Yamatodani A. 1994. Effects of vestibular stimulation on acetylcholine release from rat hippocampus: an in vivo microdialysis study. Journal of Neurophysiology 72: 605-611.

Horii A, Uno A, Kitahara T, Mitani K, Masumura C, Kizawa K, Kubo T. 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, et al. 2007. Spatial memory and hippocampal volume in humans with unilateral vestibular deafferentation. Hippocampus 17: 471-485.

Hunt M, Miller S, Nielson H, Horn K. 1987. Intratympanic injection of sodium arsanilate (atoxyl) solution results in postural changes consistent with changes described for labyrinthectomized rats. Behavioral Neuroscience 101: 427-428.

Huxter J, Burgess N, O'Keefe J. 2003. Independent rate and temporal coding in hippocampal pyramidal cells. Nature 425: 828-832.

Ingvar M, Ambros-Ingerson J, Davis M, Granger R, Kessler M, Rogers G, Schehr R, Lynch G. 1997. Enhancement by an ampakine of memory encoding in humans. Experimental Neurology 146: 553-559.

Isaac J, Ashby M, McBain C. 2007. The role of the GluR2 subunit in AMPA receptor function and synaptic plasticity. Neuron 54: 859-871.

Ishii T, Moriyoshi K, Sugihara H, Sakurada K, Kadotani H, Yokoi M, Akazawa C, Shigemoto R, Mizuno N, Masu M. 1993. Molecular characterization of the family of the N-methyl-D-aspartate receptor subunits. Journal of Biological Chemistry 268: 2836-2843.

Ito I, Futai K, Katagiri H, Watanabe M, Sakimura K, Mishina M, Sugiyama H. 1997. Synapse-selective impairment of NMDA receptor functions in mice lacking NMDA receptor epsilon 1 or epsilon 2 subunit. The Journal of Physiology 500: 401-408.

Jacob R, Furman J. 2001. Psychiatric consequences of vestibular dysfunction. Current Opinion in Neurology 14: 41-46.

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

Jacobson L, Sapolsky R. 1991. The role of the hippocampus in feedback regulation of the hypothalamic-pituitary-adrenocortical axis. Endocrine reviews 12: 118-134.

Jensen D. 1983. Survival of function in the deafferentated vestibular nerve. Brain Research 273: 175-178.

Jia Z, Agopyan N, Miu P, Xiong Z, Henderson J, Gerlai R, Taverna F, Velumian A, MacDonald J, Carlen P. 1996. Enhanced LTP in mice deficient in the AMPA receptor GluR2. Neuron 17: 945-956.

Ju W, Morishita W, Tsui J, Gaietta G, Deerinck T, Adams S, Garner C, Tsien R, Ellisman M, Malenka R. 2004. Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. Nature Neuroscience 7: 244-253.

Kahane P, Hoffmann D, Minotti L, Berthoz A. 2003. Reappraisal of the human vestibular cortex by cortical electrical stimulation study. Annals of Neurology 54: 615-624.

Karnath H, Dieterich M. 2006. Spatial neglect—a vestibular disorder? Brain 129: 293-305.
Katsarkas A, Galiana H. 1983. Bechterew's phenomenon in humans: A new explanation. Acta Oto-Laryngologica 96: 95-100.

Katsarkas A, Galiana H. 1983. Bechterew's phenomenon in humans: A new explanation. Acta Oto-Laryngologica 96: 95-100

Keller B, Hollmann M, Heinemann S, Konnerth A. 1992. Calcium influx through subunits GluR1/GluR3 of kainate/AMPA receptor channels is regulated by cAMP dependent protein kinase. The EMBO Journal 11: 891-896.

Kessels R, de Haan E, Kappelle L, Postma A. 2001. Varieties of human spatial memory: a meta-analysis on the effects of hippocampal lesions. Brain Research Reviews 35: 295-303.

King J, Zheng Y, Liu P, Darlington C, Smith P. 2002. NMDA and AMPA receptor subunit protein expression in the rat vestibular nucleus following unilateral labyrinthectomy. Neuroreport 13: 1541-1545.

Kirk I, McNaughton N. 1991. Supramammillary cell firing and hippocampal rhythmical slow activity. NeuroReport 2: 723-725.

Kitahara T, Takeda N, Saika T, Kubo T, Kiyama H. 1995. Effects of MK801 on Fos expression in the rat brainstem after unilateral labyrinthectomy. Brain Research 700: 182-190.

Kobayashi C, Aoki C, Kojima N, Yamazaki H, Shirao T. 2007. Drebrin a content correlates with spine head size in the adult mouse cerebral cortex. The Journal of Comparative Neurology 503: 618-626.

Koh D, Geiger J, Jonas P, Sakmann B. 1995. Ca2+ permeable AMPA and NMDA receptor channels in basket cells of rat hippocampal dentate gyrus. The Journal of Physiology 485: 383-402.

Kornau H, Schenker L, Kennedy M, Seeburg P. 1995. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science 269: 1737-1740.

Kotecha B, Richardson G. 1994. Ototoxicity in vitro: Effects of neomycin, gentamicin, dihydrostreptomycin, amikacin, spectinomycin, neamine, spermine and poly-L-lysine. Hearing research 73: 173-184.

Krupp J, Vissel B, Heinemann S, Westbrook G. 1998. N-terminal domains in the NR2 subunit control desensitization of NMDA receptors. Neuron 20: 317-327.

Kutsuwada T, Kashiwabuchi N, Mori H, Sakimura K, Kushiya E, Araki K, Meguro H, Masaki H, Kumanishi T, Arakawa M. 1992. Molecular diversity of the NMDA receptor channel. Nature 358: 36-41.

Lalo U, Pankratov Y, Kirchhoff F, North R, Verkhratsky A. 2006. NMDA receptors mediate neuron-to-glia signaling in mouse cortical astrocytes. The Journal of Neuroscience 26: 2673-2683.

Laurie D, Bartke I, Schoepfer R, Naujoks K, Seeburg P. 1997. Regional, developmental and interspecies expression of the four NMDAR2 subunits, examined using monoclonal antibodies. Molecular Brain Research 51: 23-32.

Lee I, Kesner R. 2002a. Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. Nature Neuroscience 5: 162-168.

Lee S, Liu L, Wang Y, Sheng M. 2002b. Clathrin adaptor AP2 and NSF interact with overlapping sites of GluR2 and play distinct roles in AMPA receptor trafficking and hippocampal LTD. Neuron 36: 661-674.

Lee S, Liu L, Wang Y, Sheng M. 2003a. Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. The Journal of neuroscience 23: 1517-1523.

Lee H, Takamiya K, Han J, Man H, Kim C, Rumbaugh G, Yu S, Ding L, He C, Petralia R. 2003b. Phosphorylation of the AMPA receptor GluR1 subunit is required for synaptic plasticity and retention of spatial memory. Cell 112: 631-643.

Leonard A, Lim I, Hemsworth D, Horne M, Hell J. 1999. Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-D-aspartate receptor. Proceedings of the National Academy of Sciences of the United States of America 96: 3239-3244.

Leonhard C, Stackman R, Taube J. 1996. Head direction cells recorded from the lateral mammillary nuclei in rats. Social for Neuroscience 22: 1873.

Leung L. 1984. Model of gradual phase shift of theta rhythm in the rat. Journal of Neurophysiology 52: 1051-1065.

Lewis M, Phelan K, Shinnick-Gallagher P, Gallagher J. 1989. Primary afferent excitatory transmission recorded intracellularly in vitro from rat medial vestibular neurons. Synapse 3: 149-153.

Linden D, Connor J. 1995. Long-term synaptic depression. Annual Review of Neuroscience 18: 319-357.

Lindsay L, Liu P, Gliddon C, Zheng Y, Smith P, Darlington C. 2005. Cytostolic glucocortioid receptor expression in the rat vestibular nucleus and hippocampus following unilateral vestibular deafferentation. Experimental Brain Research 162: 309-314.

Ling D, Benardo L, Sacktor T. 2006. Protein kinase Mζ enhances excitatory synaptic transmission by increasing the number of active postsynaptic AMPA receptors. Hippocampus 16: 443-452.

Lisman J, Schulman H, Cline H. 2002. The molecular basis of CaMKII function in synaptic and behavioural memory. Nature Reviews Neuroscience 3: 175-190.

Liu R, Chang L, Wickern G. 1984. The dorsal tegmental nucleus: an axoplasmic transport study. Brain Research 310: 123-132.

Liu S, Cull-Candy S. 2000. Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. Nature 405: 454-458.

Liu P, Smith P, Appleton I, Darlington C, Bilkey D. 2003a. Regional variations and age-related changes in nitric oxide synthase and arginase in the sub-regions of the hippocampus. Neuroscience 119: 679-687.

Liu P, Zheng Y, King J, Darlington C, Smith P. 2003b. Long-term change in hippocampal N-methyl-D-asparate receptor subunits following unilateral vestibular damage in rat. Neuroscience 117: 965-970.

Liu P, Gliddon C, Lindsay L, Darlington C, Smith P. 2004a. Nitric oxide synthase and arginase expression changes in the rat perirhinal and entorhinal cortices following unilateral vestibular damage: a link to deficits in object recognition? Journal of Vestibular Research 14: 411-417.

Liu L, Wong T, Pozza M, Lingenhoehl K, Wang Y, Sheng M, Auberson Y, Wang Y. 2004b. Role of NMDA receptor subtypes in governing the direction of hippocampal synaptic plasticity. Science 304: 1021-1024.

Lynch G, Granger R, Ambros-Ingerson J, Davis C, Kessler M, Schehr R. 1997. Evidence that a positive modulator of AMPA-type glutamate receptors improves delayed recall in aged humans. Experimental Neurology 145: 89-92.

Maas J. 1975. Biogenic amines and depression: biochemical and pharmacological separation of two types of depression. Archives of General Psychiatry 32: 1357-1361.

Maguire E, Burke T, Phillips J, Staunton H. 1996. Topographical disorientation following unilateral temporal lobe lesions in humans. Neuropsychologia 34: 993-1001.

Maguire E, Frackowiak R, Frith C. 1997. Recalling routes around London: activation of the right hippocampus in taxi drivers. The Journal of Neuroscience 17: 7103-7110.

Maguire E, Gadian D, Johnsrude I, Good C, Ashburner J, Frackowiak R, Frith C. 2000. Navigation-related structural change in the hippocampi of taxi drivers. Proceedings of the National Academy of Sciences of the United States of America 97: 4398-4403.

Malinow R, Malenka R. 2002. AMPA receptor trafficking and synaptic plasticity. Annual Review of Neuroscience 25: 103-126.

Malinow R, Schulman H, Tsien R. 1989. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science 245: 862-866.

Mammen A, Kameyama K, Roche K, Huganir R. 1997. Phosphorylation of the α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. Journal of Biological Chemistry 272: 32528-32533.

Manahan-Vaughan D, et al. 2003. An increased expression of the mGlu5 receptor protein following LTP induction at the perforant path-dentate gyrus synapse in freely moving rats. Neuropharmacology 44: 17-25.

Manly BFJ. 2005. Multivariate statistical methods: a primer (third ed.). London: Chapman & Hall/CRC.

Maren S, Tocco G, Standley S, Baudry M, Thompson R. 1993. Postsynaptic factors in the expression of long-term potentiation (LTP): increased glutamate receptor binding following LTP induction in vivo. Proceedings of the National Academy of Sciences 90: 9654-9658.

Martínez-Turrillas R, Del Río J, Frechilla D. 2007. Neuronal proteins involved in synaptic targeting of AMPA receptors in rat hippocampus by antidepressant drugs. Biochemical and Biophysical Research Communications 353: 750-755.

Massey P, Johnson B, Moult P, Auberson Y, Brown M, Molnar E, Collingridge G, Bashir Z. 2004. Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. The Journal of Neuroscience 24: 7821-7828.

Matsuda S, Mikawa S, Hirai H. 1999. Phosphorylation of serine 880 in GluR2 by protein kinase C prevents its C terminus from binding with glutamate receptor interacting protein. Journal of Neurochemistry 73: 1765-1768.

Matthews B, Ryu J, Bockaneck C. 1989. Vestibular contribution to spatial orientation. Acta Oto-Laryngologica 108: 149-154.

Matthias K, Kirchhoff F, Seifert G, Hüttmann K, Matyash M, Kettenmann H, Steinhäuser C. 2003. Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. The Journal of Neuroscience 23: 1750-1758.

McEwen B. 1999. Stress and hippocampal plasticity. Annual Review of Neuroscience 22: 105-122.

McHugh T, Blum K, Tsien J, Tonegawa S, Wilson M. 1996. Impaired hippocampal representation of space in CA1-specific NMDAR1 knockout mice. Cell 87: 1339-1349.

McNaughton B, et al. 1996. Deciphering the hippocampal polyglot: the hippocampus as a path integration system. Journal of Experimental Biology 199: 173-185.

McNaughton B, Barnes C, Meltzer J, Sutherland R. 1989. Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge. Experimental Brain Research 76: 485-496.

McNaughton B, Battaglia F, Jensen O, Moser E, Moser M. 2006. Path integration and the neural basis of the 'cognitive map'. Nature Reviews Neuroscience 7: 663-678.

McNaughton B, Chen L, Markus E. 1991. “Dead Reckoning,” landmark learning, and the sense of direction: a neurophysiological and computational hypothesis. Journal of Cognitive Neuroscience 3: 190-202.

McNaughton B, Mizumori S, Barnes C, Leonard B, Marquis M, Green E. 1994. Cortical representation of motion during unrestrained spatial navigation in the rat. Cerebral Cortex 4: 27-39.

Meng Y, Zhang Y, Jia Z. 2003. Synaptic transmission and plasticity in the absence of AMPA glutamate receptor GluR2 and GluR3. Neuron 39: 163-176.

Miller E, Erickson C, Desimone R. 1996. Neural mechanisms of visual working memory in prefrontal cortex of the macaque. The Journal of Neuroscience 16: 5154-5167.

Miller S, Kennedy M. 1986. Regulation of brain type II Ca2+/calmodulin-dependent protein kinase by autophosphorylation: a Ca2+-triggered molecular switch. Cell 44: 861-870.

Mittelstaedt M, Mittelstaedt H. 1980. Homing by path integration in a mammal. Naturwissenschaften 67: 566-567.

Momiyama A, Feldmeyer D, Cull-Candy S. 1996. Identification of a native low-conductance NMDA channel with reduced sensitivity to Mg2+ in rat central neurones. The Journal of Physiology 494: 479-492.

Monyer H, Burnashev N, Laurie D, Sakmann B, Seeburg P. 1994. Developmental and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron 12: 529-540.

Monyer H, Sprengel R, Schoepfer R, Herb A, Higuchi M, Lomeli H, Burnashev N, Sakmann B, Seeburg P. 1992. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science 256: 1217-1221.

Moser M, Moser E. 1998. Functional differentiation in the hippocampus. Hippocampus 8: 608-619.

Moser E, Moser M, Andersen P. 1993. Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. The Journal of Neuroscience 13: 3916-3925.

Mouri A, Noda Y, Noda A, Nakamura T, Tokura T, Yura Y, Nitta A, Furukawa H, Nabeshima T. 2007. Involvement of a dysfunctional dopamine-D1/N-methyl-D-aspartate-NR1 and Ca2+/calmodulin-dependent protein kinase II pathway in the impairment of latent learning in a model of schizophrenia induced by phencyclidine. Molecular Pharmacology 71: 1598-1609.

Muller R, Kubie J, Ranck JJ. 1987. Spatial firing patterns of hippocampal complex-spike cells in a fixed environment. Journal of Neuroscience 7: 1935-1950.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. 1992. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symposia on Quantitative Biology 52: 263-273.

Nagaratnam N, Ip J, Bou-Haidar P. 2004. The vestibular dysfunction and anxiety disorder interface: a descriptive study with special reference to the elderly. Archives of Gerontology and Geriatrics 40: 253-264.

Nakazawa K, McHugh T, Wilson M, Tonegawa S. 2004. NMDA receptors, place cells and hippocampal spatial memory. Nature Reviews Neuroscience 5: 361-372.

Nakazawa K, Sun L, Quirk M, Rondi-Reig L, Wilson M, Tonegawa S. 2003. Hippocampal CA3 NMDA receptors are crucial for memory acquisition of one-time experience. Neuron 38: 305-315.

Neeper S, Gómez-Pinilla F, Choi J, Cotman C. 1996. Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain. Brain Research 726: 49-56.

Nicoll R, Malenka R. 1995. Contrasting properties of two forms of long-term potentiation in the hippocampus. Nature 377: 115-118.

Niethammer M, Kim E, Sheng M. 1996. Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. The Journal of Neuroscience 16: 2157-2163.

Niewoehner B, Single F, Hvalby Ø, Jensen V, Meyer zum Alten Borgloh S, Seeburg P, Rawlins J, Sprengel R, Bannerman D. 2007. Impaired spatial working memory but spared spatial reference memory following functional loss of NMDA receptors in the dentate gyrus. European Journal of Neuroscience 25: 837-846.

Noda M, Nakanishi H, Nabekura J, Akaike N. 2000. AMPA–kainate subtypes of glutamate receptor in rat cerebral microglia. The Journal of Neuroscience 20: 251-258.

Noel J, Ralph G, Pickard L, Williams J, Molnar E, Uney J, Collingridge G, Henley J. 1999. Surface expression of AMPA receptors in hippocampal neurons is regulated by an NSF-dependent mechanism. Neuron 23: 365-376.

O'Keefe J, Dostrovsky J. 1971. The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely-moving rat. Brain Research 34: 171-175.

O'Keefe J, Nadel L. 1978. The hippocampus as a cognitive map. Oxford: Clarendon Press

O'Keefe J, Recce M. 1993. Phase relationship between hippocampal place units and the EEG theta rhythm. Hippocampus 3: 317-330.

O'Mara S, Rolls E, Berthoz A, Kesner R. 1994. Neurons responding to whole-body motion in the primate hippocampus. The Journal of Neuroscience 14: 6511-6523.

Oh M, Derkach V, Guire E, Soderling T. 2006. Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. Journal of Biological Chemistry 281: 752-758.

Ong HL, Chen J, Chataway T, Brereton H, Zhang L, Downs T, Tsiokas L, Barritt G. 2002. Specific detection of the endogenous transient receptor potential (TRP)-1 protein in liver and airway smooth muscle cells using immunoprecipitation and Western-blot analysis. Biochemical Journal 364: 641-648.

Osten P, Khatri L, Perez J, Köhr G, Giese G, Daly C, Schulz T, Wensky A, Lee L, Ziff E. 2000. Mutagenesis reveals a role for ABP/GRIP binding to GluR2 in synaptic surface accumulation of the AMPA receptor. Neuron 27: 313-325.

Ottersen OP, Landsend AS. 1997. Organization of glutamate receptors at the synapse. Eur. J. Neurosci. 9: 2219-2224.

Paoletti P, Neyton J. 2007. NMDA receptor subunits: function and pharmacology. Current Opinion in Pharmacology 7: 39-47.

Paparella M, Mancini F. 1985. Vestibular Meniere's disease. Otolaryngology-head and neck surgery 93: 148-151.

Park M, Penick E, Edwards J, Kauer J, Ehlers M. 2004. Recycling endosomes supply AMPA receptors for LTP. Science 305: 1972-1975.

Passafaro M, Piëch V, Sheng M. 2001. Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. Nature Neuroscience 4: 917-926.

Pastalkova E, Serrano P, Pinkhasova D, Wallace E, Fenton A, Sacktor T. 2006. Storage of spatial information by the maintenance mechanism of LTP. Science 313: 1141-1144.

Paz-Villagràn V, Lenck-Santini P, Save E, Poucet B. 2002. Properties of place cell firing after damage to the visual cortex. European Journal of Neuroscience 16: 771-776.

Péruch P, Borel L, Gaunet F, Thinus-Blanc G, Magnan J, Lacour M. 1999. Spatial performance of unilateral vestibular defective patients in nonvisual versus visual navigation. Journal of Vestibular Research 9: 37-47.

Petralia R, Yokotani N, Wenthold R. 1994. Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. The Journal of Neuroscience 14: 667-696.

Petrovich G, Canteras N, Swanson L. 2001. Combinatorial amygdalar inputs to hippocampal domains and hypothalamic behavior systems. Brain Research Reviews 38: 247-289.

Petsche H, Stumpf C. 1960. Topographic and toposcopic study of origin and spread of the regular synchronized arousal pattern in the rabbit. Electroencephalography and Clinical Neurophysiology 12: 589-600.

Petsche H, Stumpf C, Gogolak G. 1962. The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus I. The control of hippocampus arousal activity by the septum cells. Electroencephalography and Clinical Neurophysiology 14: 202-211.

Pettit D, Perlman S, Malinow R. 1994. Potentiated transmission and prevention of further LTP by increased CaMKII activity in postsynaptic hippocampal slice neurons. Science 266: 1881-1885.

Petzold A, Keir G, Warren J, Fox N, Rossor M. 2007. A systematic review and meta-analysis of CSF neurofilament protein levels as biomarkers in dementia. Neurodegenerative Diseases 4: 185-194.

Pitkänen A, Pikkarainen M, Nurminen N, Ylinen A. 2000. Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex, and postrhinal cortex in rat: A review. Annals of the New York Academy of Sciences 911: 369-391.

Plant K, Pelkey K, Bortolotto Z, Morita D, Terashima A, McBain C, Collingridge G, Isaac J. 2006. Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. Nature Neuroscience 9: 602-604.

Pollak D, Herkner K, Hoeger H, Lubec G. 2005. Behavioral testing upregulates pCaMKII, BDNF, PSD-95 and egr-1 in hippocampus of FVB/N mice. Behavioural Brain Research 163: 128-135.

Pothuizen H, Zhang W, Jongen Rêlo A, Feldon J, Yee B. 2004. Dissociation of function between the dorsal and the ventral hippocampus in spatial learning abilities of the rat: a within subject, within task comparison of reference and working spatial memory. European Journal of Neuroscience 19: 705-712.

Puma C, Baudoin C, Bizot J-C. 1998. Effects of intraseptal infusions of N-methyl-D-aspartate receptor ligands on memory in an object recognition task in rats. Neurosci. Lett 244: 97-100.

Pussinen R, Sirviö J. 1999. Effects of D-cycloserine, a positive modulator of N-methyl-D-aspartate receptors, and ST 587, a putative alpha-1 adrenergic agonist, individually and in combination, on the non-delayed and delayed foraging behaviour of rats assessed in the radial arm maze. J. Psychopharmacol. 13: 171-179.

Rasmussen N, Johnsen N, Bohr V. 1991. Otologic sequelae after pneumococcal meningitis: a survey of 164 consecutive cases with a follow up of 94 survivors. The Laryngoscope 101: 876-882.

Redfern M, Talkowski M, Jennings J, Furman J. 2004. Cognitive influences in postural control of patients with unilateral vestibular loss. Gait & Posture 19: 105-114.

Rios Valentim S, Gontijo A, Peres M, de Melo Rodrigues L, Nakamura-Palacios E. 2009. D1 dopamine and NMDA receptors interactions in the medial prefrontal cortex: modulation of spatial working memory in rats. Behavioural Brain Research 204: 124-128.

Ris L, Capron B, de Waele C, Vidal P, Godaux E. 1997. Dissociations between behavioural recovery and restoration of vestibular activity in the unilabyrinthectomized guinea-pig. The Journal of Physiology 500: 509-522.

Ris L, de Waele C, Serafin M, Vidal P, Godaux E. 1995. Neuronal activity in the ipsilateral vestibular nucleus following unilateral labyrinthectomy in the alert guinea pig. Journal of Neurophysiology 74: 2087-2099.

Ris L, Godaux E. 1998. Neuronal activity in the vestibular nuclei after contralateral or bilateral labyrinthectomy in the alert guinea pig. Journal of Neurophysiology 80: 2352-2367.

Roche K, O'Brien R, Mammen A, Bernhardt J, Huganir R. 1996. Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16: 1179-1188.

Roche K, Tingley W, Huganir R. 1994. Glutamate receptor phosphorylation and synaptic plasticity. Current Opinion in Neurobiology 4: 383-388.

Rosenmund C, Stern-Bach Y, Stevens C. 1998. The tetrameric structure of a glutamate receptor channel. Science 280: 1596-1599.

Rossier J, Haeberli C, Schenk F. 2000. Auditory cues support place navigation in rats when associated with a visual cue. Behavioural Brain Research 117: 209-214.

Rothman J. 1994. Mechanisms of intracellular protein transport. Nature 372: 55-63.

Roy J, Cullen K. 2004. Dissociating self-generated from passively applied head motion: neural mechanisms in the vestibular nuclei. The Journal of Neuroscience 24: 2102-2111.

Russell N, Horii A, Smith P, Darlington C, Bilkey D. 2003a. Bilateral peripheral vestibular lesions produce long-term changes in spatial learning in the rat. Journal of Vestibular Research 13: 9-16.

Russell N, Horii A, Smith P, Darlington C, Bilkey. 2003b. 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. Journal of Neurophysiology 96: 4-14.

Sakimura K, Kutsuwada T, Itot I, Manabel T, Takayama C, Shinichi Aizawa Y, Suglyamat H, Mishina M. 1995. Reduced hippocampal LTP and spatial learning in mice lacking NM DA receptor ε1 subunit. Nature 373: 151-155.

Sanes J, Lichtman J. 1999. Can molecules explain long-term potentiation? Nature Neuroscience 2: 597-604.

Sasaki Y, Rothe T, Premkumar L, Das S, Cui J, Talantova M, Wong H, Gong X, Chan S, Zhang D. 2002. Characterization and comparison of the NR3A subunit of the NMDA receptor in recombinant systems and primary cortical neurons. Journal of Neurophysiology 87: 2052-2063.

Save E, Cressant A, Thinus-Blanc C, Poucet B. 1998. Spatial firing of hippocampal place cells in blind rats. The Journal of Neuroscience 18: 1818-1826.

Saxon D, Anderson J, Beitz A. 2001. Transtympanic tetrodotoxin alters the VOR and Fos labelling in the vestibular complex. NeuroReport 12: 3051-3055.

Schautzer F, Hamilton D, Kalla R, Strupp M. 2003. Spatial memory deficits in patients with chronic bilateral vestibular failure. Annals of the New York Academy of Sciences 1004: 316-324.

Schuman E, Madison D. 1991. A requirement for the intracellular messenger nitric oxide in long-term potentiation. Science 254: 1503-1506.

Schildkraut J, Kety S. 1967. Biogenic amines and emotion. Science 156: 21-30.

Schulman H, Hanson P. 1993. Multifunctional Ca2+/calmodulin-dependent protein kinase. Neurochemical Research 18: 65-77.

Schwartz BL, Hashtroudi S, Herting RL, Schwartz P, Deutsch SI. 1996. d-Cycloserine enhances implicit memory in Alzheimer patients. Neurology 46: 420-424.

Seifert G, Steinhäuser C. 2001. Ionotropic glutamate receptors in astrocytes. Progress in Brain Research 132: 287-299.

Semba K, Reiner P, McGeer E, Fibiger H. 1988. Brainstem afferents to the magnocellular basal forebrain studied by axonal transport, immunohistochemistry, and electrophysiology in the rat. The Journal of Comparative Neurology 267: 433-453.

Semenov L, Bures J. 1989. Vestibular stimulation disrupts acquisition of place navigation in the Morris water tank task. Behavioral and Neural Biology 51: 346-363.

Serrano P, Yao Y, Sacktor T. 2005. Persistent phosphorylation by protein kinase Mζ maintains late-phase long-term potentiation. The Journal of Neuroscience 25: 1979-1984.

Sharp P, Blair H, Etkin D, Tzanetos D. 1995. Influences of vestibular and visual motion information on the spatial firing patterns of hippocampal place cells. The Journal of Neuroscience 15: 173-189.

Shen K, Meyer T. 1999. Dynamic control of CaMKII translocation and localization in hippocampal neurons by NMDA receptor stimulation. Science 284: 162-167.

Sheng M, Cummings J, Roldan L, Jan Y, Jan L. 1994. Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. Nature 368: 144-147.

Sheng M, Sala C. 2001. PDZ domains and the organization of supramolecular complexes. Annual Review of Neuroscience 24: 1-29.

Shi S, Hayashi Y, Petralia R, Zaman S, Wenthold R, Svoboda K, Malinow R. 1999. Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. Science 284: 1811-1816.

Shimshek D, Jensen V, Celikel T, Geng Y, Schupp B, Bus T, Mack V, Marx V, Hvalby;, Seeburg P. 2006. Forebrain-specific glutamate receptor B deletion impairs spatial memory but not hippocampal field long-term potentiation. The Journal of Neuroscience 26: 8428-8440.

Shinder M, Taube J. 2010. Differentiating ascending vestibular pathways to the cortex involved in spatial cognition. Journal of Vestibular Research 20: 3-23.

Shinoda Y, Yoshida K. 1974. Dynamic characteristics of responses to horizontal head angular acceleration in vestibuloocular pathway in the cat. Journal of Neurophysiology 37: 653-673.

Shumway-Cook A, Woollacott M, Kerns K, Baldwin M. 1997. The effects of two types of cognitive tasks on postural stability in older adults with and without a history of falls. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 52: 232-240.

Silva A, Paylor R, Wehner J, Tonegawa S. 1992. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257: 206-211.

Skaggs W, McNaughton B, Wilson M, Barnes C. 1996. Theta phase precession in hippocampal neuronal populations and the compression of temporal sequences. Hippocampus 6: 149-172.

Smith P. 1997. Vestibular-hippocampal interactions. Hippocampus 7: 465-471.

Smith P, Curthoys I. 1989. Mechanisms of recovery following unilateral labyrinthectomy: a review. Brain Research Reviews 14: 155-180.

Smith P, Darlington C, Zheng Y. 2009. Move it or lose it-Is stimulation of the vestibular system necessary for normal spatial memory? Hippocampus 20: 36-43.

Smith P, Horii A, Russell N, Bilkey D, Zheng Y, Liu P, Kerr D, Darlington C. 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. 2005. Does vestibular damage cause cognitive dysfunction in humans? Journal of Vestibular Research 15: 1-10.

Snyder J, Hong N, McDonald R, Wojtowicz J. 2005. A role for adult neurogenesis in spatial long-term memory. Neuroscience 130: 843-852.

Soderling T, Derkach V. 2000. Postsynaptic protein phosphorylation and LTP. Trends in Neurosciences 23: 75-80.

Soderling T, Tan S, McGlade McCulloh E, Yamamoto H, Fukunaga K. 1994. Excitatory interaction between glutamate receptors and protein kinases. Journal of Neurobiology 25: 304-311.

Staab J, Ruckenstein M. 2003. Which comes first? Psychogenic dizziness versus otogenic anxiety. The Laryngoscope 113: 1714-1718.

Staab J, Ruckenstein M, Solomon D, Shepard N. 2002. Serotonin reuptake inhibitors for dizziness with psychiatric symptoms. Archives of Otolaryngology, Head & Neck Surgery 128: 554-560.

Stackman R, Clark A, Taube J. 2002a. Hippocampal spatial representations require vestibular input. Hippocampus 12: 291-303.

Stackman R, Herbert A. 2002b. 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.

Stocca G, Vicini S. 1998. Increased contribution of NR2A subunit to synaptic NMDA receptors in developing rat cortical neurons. The Journal of Physiology 507: 13-24.

Strupp M, Brandt T. 2009. Vestibular neuritis. Seminars in Neurology 29: 509-519.

Sucher N, Awobuluyi M, Choi Y, Lipton S. 1996. NMDA receptors: from genes to channels. Trends in Pharmacological Sciences 17: 348-355.

Sugiuchi Y, Izawa Y, Ebata S, Shinoda Y. 2005. Vestibular cortical area in the periarcuate cortex. Annals of the New York Academy of Sciences 1039: 111-123.

Suzuki M, Kitano H, Ito R, Kitanishi T, Yazawa Y, Ogawa T, Shiino A, Kitajima K. 2001. Cortical and subcortical vestibular response to caloric stimulation detected by functional magnetic resonance imaging. Cognitive Brain Research 12: 441-449.

Swanson L, Cowan W. 1977. An autoradiographic study of the organization of the efferet connections of the hippocampal formation in the rat. The Journal of Comparative Neurology 172: 49-84.

Swanson G, Kamboj S, Cull-Candy S. 1997. Single-channel properties of recombinant AMPA receptors depend on RNA editing, splice variation, and subunit composition. The Journal of Neuroscience 17: 58-69.

Szirmai A, Kisely M, Nagy G, Nedeczky Z, Szabados E, Tóth A. 2005. Panic disorder in otoneurological experience. International Tinnitus Journal 11: 77-80.

Tai S, Ma J, Ossenkopp K, Leung L. 2011, in press. Activation of immobility related hippocampal theta by cholinergic septohippocampal neurons during vestibular stimulation. Hippocampus: 1-12.

Talaei S, Sheibani V, Salami M. 2010. Light deprivation improves melatonin related suppression of hippocampal plasticity. Hippocampus 20: 447-455.

Tan S, Wenthold R, Soderling T. 1994. Phosphorylation of AMPA-type glutamate receptors by calcium/calmodulin-dependent protein kinase II and protein kinase C in cultured hippocampal neurons. The Journal of Neuroscience 14: 1123-1129.

Tang Y, Shimizu E, Dube G, Rampon C, Kerchner G, Zhuo M, Liu G, Tsien J. 1999. Genetic enhancement of learning and memory in mice. Nature 401: 63-69.

Taube J, Goodridge J, Golob E, Dudchenko P, Stackman R. 1996. Processing the head direction cell signal: a review and commentary. Brain Research Bulletin 40: 477-484.

Terrazas A, Krause M, Lipa P, Gothard K, Barnes C, McNaughton B. 2005. Self-motion and the hippocampal spatial metric. The Journal of Neuroscience 25: 8085-8096.

Trivedi M, Coover G. 2004. Lesions of the ventral hippocampus, but not the dorsal hippocampus, impair conditioned fear expression and inhibitory avoidance on the elevated T-maze. Neurobiology of Learning and Memory 81: 172-184.

Troost B. 1980. Dizziness and vertigo in vertebrobasilar disease. Part II. Central causes and vertebrobasilar disease. Stroke 11: 413-415.

Tsanov M, Manahan-Vaughan D. 2009. Visual cortex plasticity evokes excitatory alterations in the hippocampus. Frontiers in Integrative Neuroscience 3: 1-13.

Tsien J, Huerta P, Tonegawa S. 1996. The essential role of hippocampal CA1 NMDA receptor dependent synaptic plasticity in spatial memory. Cell 87: 1327-1338.

Tsou K, Brown S, Sañudo-Peña M, Mackie K, Walker J. 1997. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83: 393-411.

Van Cruijsen N, Dullaart R, Wit H, Albers F. 2005. Analysis of cortisol and other stress-related hormones in patients with Ménière's disease. Otology & Neurotology 26: 1214-1219.

Van Hoesen G, Pandya D, Butters N. 1975. Some connections of the entorhinal (area 28) and perirhinal (area 35) cortices of the rhesus monkey. II. Frontal lobe afferents. Brain Research 95: 25-38.

Vertes R, Kocsis B. 1997. Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. Neuroscience 81: 893-926.

Vicini S, Wang J, Li J, Zhu W, Wang Y, Luo J, Wolfe B, Grayson D. 1998. Functional and pharmacological differences between recombinant N-Methyl-D-Aspartate receptors. Journal of Neurophysiology 79: 555-566.

Vincent S. 1994. Nitric oxide: A radical neurotransmitter in the central nervous system. Progress in Neurobiology 42: 129-160.

Vitte E, Derosier C, Caritu Y, Berthoz A, Hasboun D, Soulié D. 1996. Activation of the hippocampal formation by vestibular stimulation: a functional magnetic resonance imaging study. Experimental Brain Research 112: 523-526.

Wallace D, Hines D, Pellis S, Whishaw I. 2002. Vestibular information is required for dead reckoning in the rat. The Journal of Neuroscience 22: 10009-10017.

Wang Y, Bosy T, Yasuda R, Grayson D, Vicini S, Pizzorusso T, Wolfe B. 1995. Characterization of NMDA receptor subunit specific antibodies: distribution of NR2A and NR2B receptor subunits in rat brain and ontogenic profile in the cerebellum. Journal of Neurochemistry 65: 176-183.

Wenthold R, Petralia R, Blahos J, Niedzielski A. 1996. Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. The Journal of Neuroscience 16: 1982-1989.

Wenthold R, Prybylowski K, Standley S, Sans N, Petralia R. 2003. Trafficking of NMDA receptors Annual Review of Pharmacology and Toxicology 43: 335-358.

Whishaw I. 1998. Place learning in hippocampal rats and the path integration hypothesis. Neuroscience & Biobehavioral Reviews 22: 209-220.

Whishaw I, Hines D, Wallace D. 2001. Dead reckoning (path integration) requires the hippocampal formation: evidence from spontaneous exploration and spatial learning tasks in light (allothetic) and dark (idiothetic) tests. Behavioural Brain Research 127: 49-69.

Whishaw I, Tomie J. 1997. Piloting and dead reckoning dissociated by fimbria-fornix lesions in a rat food carrying task. Behavioural Brain Research 89: 87-97.

Wiener S. 1996. Spatial, behavioral and sensory correlates of hippocampal CA1 complex spike cell activity: implications for information processing functions. Progress in Neurobiology 49: 335-361.

Wiener S, Korshunov V, Garcia R, Berthoz A. 1995. Inertial, substratal and landmark cue control of hippocampal CA1 place cell activity. European Journal of Neuroscience 7: 2206-2219.

Williams D, Carter C. 2009. The Transmission Electron Microscope. Transmission Electron Microscopy: 3-22.

Williams JM, Guevremont D, Kennard JTT, Mason-Parker SE, Tate WP, Abraham WC. 2003. Long-term regulation of N-methyl-D-aspartate receptor subunits and associated synaptic proteins following hippocampal synaptic plasticity. Neuroscience 118: 1003-1013.

Williams K, Zappia A, Pritchett D, Shen Y, Molinoff P. 1994. Sensitivity of the N-methyl-D-aspartate receptor to polyamines is controlled by NR2 subunits. Molecular pharmacology 45: 803-809.

Wilson V, Jones G. 1979. Mammalian vestibular physiology. New York: Plenum Publishing Corporation.

Winson J. 1978. Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. Science 201: 160-163.

Witter M, Wouterlood F, Naber P, Van Haeften T. 2000. Anatomical organization of the parahippocampal-hippocampal network. Annals of the New York Academy of Sciences 911: 1-24.

Wolbers T, Büchel C. 2005. Dissociable retrosplenial and hippocampal contributions to successful formation of survey representations. The Journal of Neuroscience 25: 3333-3340.

Wortley A, Mckinley K, Whittle R, Calvert A, Shaw O, Fernando R, Pearse A, Hopkins K, Clark B. 2009. Investigations into the lack of consensus in the reporting of HLA antibody specificities in the UK. Journal of Clinical Pathology 62: 270-274.

Xerri C, Borel L, Barthelemy J, Lacour M. 1988. Synergistic interactions and functional working range of the visual and vestibular systems in postural control: neuronal correlates. Progress in Brain Research 76: 193-203.

Xia J, Chung H, Wihler C, Huganir R, Linden D. 2000. Cerebellar long-term depression requires PKC-regulated interactions between GluR2/3 and PDZ domain-containing proteins. Neuron 28: 499-510.

Yan J, Zhang Y, Jia Z, Taverna F, McDonald R, Muller R, Roder J. 2002. Place-cell impairment in glutamate receptor 2 mutant mice. The Journal of Neuroscience 22: 1-5.

Yardley L. 1994. Contribution of symptoms and beliefs to handicap in people with vertigo: a longitudinal study. British Journal of Clinical Psychology 33: 101-113.

Yardley L, Burgneay J, Nazareth I, Luxon L. 1998. Neuro-otological and psychiatric abnormalities in a community sample of people with dizziness: a blind, controlled investigation. Journal of Neurology, Neurosurgery & Psychiatry 65: 679-684.

Yardley L, Papo D, Bronstein A, Gresty M, Gardner M, Lavie N, Luxon L. 2002. Attentional demands of continuously monitoring orientation using vestibular information. Neuropsychologia 40: 373-383.

Zamanillo D, Sprengel R, Hvalby Ø, Jensen V, Burnashev N, Rozov A, Kaiser K, Köster H, Borchardt T, Worley P. 1999. Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. Science 284: 1805-1811.

Zhang J, Snyder S. 1995. Nitric oxide in the nervous system. Annual Review of Pharmacology and Toxicology 35: 213-233.

Zheng Y, Appleton I, Smith P, Darlington C. 2000. Subregional variation in the effects of unilateral vestibular deafferentation on nitric oxide synthase activity and nitrite formation in the guinea pig hippocampus. Neuroscience Research Communications 27: 109-116.

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

Zheng Y, Smith P, Darlington C. 1999b. Subregional analysis of amino acid levels in the guinea pig hippocampus following unilateral vestibular deafferentation. Journal of Vestibular Research: Equilibrium & Orientation 9: 335-345.

Zheng Y, Smith P, Darlington C. 1999c. Temporal bone surgery causes reduced nitric oxide synthase activity in the ipsilateral guinea pig hippocampus. Neuroscience Letters 259: 130-132.

Zheng Y, Darlington C, Smith P. 2004. Bilateral labyrinthectomy causes long-term deficit in object recognition in rat. Neuroreport 15: 1913-1916.

Zheng Y, Darlington C, Smith P. 2006. Impairment and recovery on a food foraging task following unilateral vestibular deafferentation in rats. Hippocampus 16: 368-378.

Zheng Y, Kerr D, 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, Goddard M, Darlington C, Smith P. 2007. Bilateral vestibular deafferentation impairs performance in a spatial forced alternation task in rats. Hippocampus 17: 253-256.

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, Goddard M, Darlington C, Smith P. 2009. Long-term deficits on a foraging task after bilateral vestibular deafferentation in rats. Hippocampus 19: 480-486.

Zheng Y, Horii A, Appleton I, Darlington C, Smith P. 2001a. Damage to the vestibular inner ear causes long-term changes in neuronal nitric oxide synthase expression in the rat hippocampus. Neuroscience 105: 1-5.

Zheng Y, Horii A, Smith P, Darlington C. 2001b. Differences in NOS protein expression and activity in the rat vestibular nucleus following unilateral labyrinthectomy. Molecular Brain Research 88: 166-170.

Zheng Y, Mason-Parker S, Logan B, Darlington C, Smith P, Abraham W. 2010. Hippocampal synaptic transmission and LTP in vivo are intact following bilateral vestibular deafferentation in the rat. Hippocampus 20: 461-468.

Zhu J, Esteban J, Hayashi Y, Malinow R. 2000. Synaptic potentiation during early development: delivery of GluR4-containing AMPA receptors by spontaneous activity. Nature Neuroscience 3: 1098-1106.

zu Eulenburg P, Stoeter P, Dieterich M. 2010. Voxel-based morphometry depicts central compensation after vestibular neuritis. Annals of Neurology 68: 241-249.