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Evidence that spatial memory deficits following bilateral vestibular deafferentation in rats are probably permanent

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ABSTRACT

Previous studies of rats with bilateral vestibular deafferentation (BVD) have demonstrated spatial memory deficits, suggesting adverse effects on the hippocampus. However, the longest post-operative time interval that has been studied was approx. 5–7 months post-surgery. In this study, we investigated whether rats exhibited spatial memory deficits at 14 months following BVD and whether these deficits could be exacerbated by administration of cannabinoid (CB) drugs. Twenty-eight adult rats were divided into four groups: (1) sham surgery + vehicle; (2) sham surgery + the CB_1/CB_2 receptor agonist WIN55,212-2 ('WIN'); (3) BVD + vehicle; and (4) BVD + WIN. WIN (1.0 or 2.0 mg/kg/day) or vehicle, was administered (s.c.) on days 1–10 and 11–20 (respectively), 30 min before the rats performed in a foraging task. On day 21, the CB receptor inverse agonist, AM251 (3.0 mg/kg, s.c.), was administered before WIN or vehicle. To our surprise, BVD animals were impaired in using the visual cues during the probe test in light. In the dark trials, when visual cues were unavailable, BVD animals were unable to use self-movement cues in homing. However, WIN at 2 mg/kg, significantly improved BVD animals' homing time and number of errors in the dark through strategies other than the improvement in using self-movement cues. Furthermore, AM251 significantly improved heading angle in vehicle-treated animals and the first home choice in WIN-treated animals. These results suggest that at 14 months post-BVD, the animals are not only impaired in path integration, but also piloting and that the spatial memory deficits may be permanent. The involvement of the cannabinoid system is more complicated than expected.

1. Introduction

Bilateral lesions of the vestibular system have been reported to disrupt spatial memory in behavioural tasks ranging from the radial arm maze (e.g., Russell, Horii, Smith, Darlington, & Bilkey, 2003a; Stackman & Herbert, 2002) and spatial alternation tasks (e.g., Zheng, Goddard, Darlington, & Smith, 2007), to the foraging task (e.g., Wallace, Hines, Pellis, & Whishaw, 2002; Zheng, Darlington, & Smith, 2006; Zheng, Goddard, Darlington, & Smith, 2009b). Clinical studies in humans also indicate that bilateral loss of vestibular function is associated with significant deficits in spatial memory, as evidenced by poor performance on the virtual Morris water maze task (Brandt et al., 2005). Unlike studies conducted in rats, studies in humans have been conducted 5–10 years following the lesions, suggesting that the spatial memory deficits may be permanent (Brandt et al., 2005).

Over the last decade, many studies have shown that the vestibular system is connected to the hippocampus via long latency pathways (Cuthbert, Gilchrist, Hicks, MacDougall, & Curthoys, 2000; de Waele, Baudonnière, Lepecq, Tran Ba Huy, & Vidal, 2001; Horii, Russell, Smith, Darlington, & Bilkey, 2004; Vitte et al., 1996) and that bilateral lesions of the vestibular system result in the corruption of the response of hippocampal place cells (Russell, Horii, Smith, Darlington, & Bilkey, 2003b; Stackman, Clark, & Taube, 2002) and theta rhythm (Russell, Horii, Smith, Darlington, & Bilkey, 2006; but see Stackman et al., 2002 for contrary evidence). Such results suggest that the detrimental effects of bilateral vestibular deafferentation (BVD) on the hippocampus may be at least part of the explanation for the effects of BVD on spatial memory. On the other hand, basal synaptic transmission and the induction and maintenance of long-term potentiation (LTP) in the hippocampus appear to be similar to normal in rats with BVD (Zheng et al., 2010).

In the only MRI study to date of humans with BVD, the volume of the hippocampus was found to be approximately 17% smaller than in age- and sex-matched controls and this atrophy correlated with spatial memory deficits (Brandt et al., 2005). This observation, 5–10 years following the BVD, suggested that the hippocampus might undergo long-term changes that result in permanent spatial memory deficits. Whether or not the spatial memory deficits caused by BVD are permanent is a significant issue, because approximately 80% of patients with bilateral vestibulopathy do not recover significantly (Zingler et al., 2008) and ongoing memory

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problems may contribute to this phenomenon. To date, the longest post-operative time point that has been used to study the effects of BVD on spatial memory in the rat has been 5–7 months (Zheng et al., 2006, 2007, 2009b). In the current study we investigated whether such spatial memory deficits would persist at 14 months following BVD, when they could be considered permanent. By manipulating the available cues in the foraging task, potential changes in the animals' ability to use allocentric or egocentric cues were examined. Cannabinoid receptors have been demonstrated to contribute to some forms of plasticity in the hippocampus, such as depolarization-induced suppression of inhibition or excitation, long-term depression (e.g., Chiu & Castillo, 2008) and neurogenesis (e.g., Wolf et al., 2010); therefore it is possible that BVD affects endocannabinoid signalling in the hippocampus. Since cannabinoid receptor agonists are known to disrupt spatial memory (e.g. Robbe & Buzsáki, 2009; Suenaga & Ichitani, 2008; Wise, Thorpe, & Lichtman, 2009), we also investigated whether any spatial deficits apparent at 14 months post-op. could be exacerbated by administering the cannabinoid CB_1/CB_2 receptor agonist, WIN55,212-2 ('WIN'), or reduced, by administering the cannabinoid CB_1 receptor inverse agonist, AM251.

2. Materials and methods

2.1. Animals

Thirty-two adult male Wistar rats weighing 250–300 g at the time of surgery were used. All animals received either the sham or BVD surgery 14 months prior to the behavioural testing. However, four animals were lost due to aging before starting the behavioural testing. The animals were randomly divided into four treatment groups: (1) sham with vehicle control ($n = 8$); (2) sham with WIN ($n = 7$); (3) BVD with vehicle control ($n = 6$); and (4) BVD with WIN ($n = 7$). The animals were food deprived to 85% of their normal feeding weight before and during the behavioural testing. The animals' body weight was measured and recorded every day. Large oat and wheat honey cereal loops were used as food pellets during behavioural testing. Rats normally do not consume large food pellets where they find them, but rather carry the pellets back to the home cage (Whishaw, Coles, & Bellerive, 1995). Furthermore, rats display different food-handling behaviour toward different sizes of food pellet (Whishaw, Oddie, McNamara, Harris, & Perry, 1990). The size of the food pellet used in the current study has been used previously in the foraging task in our laboratory (Zheng et al., 2006, 2009b). After each day of testing, the animals were given supplementary normal laboratory rodent food in their home cage in order to maintain body weight. The rats were sprayed with black paint (Donaghys Industries Ltd., Christchurch, New Zealand) on the neck so that their movement could be tracked and analysed using the custom-made software.

2.2. BVD surgery

A complete bilateral surgical vestibular deafferentation was performed using an otolaryngological microscope and the methods described in detail elsewhere (e.g., Zheng, Kerr, Darlington, & Smith, 2003; Zheng et al., 2006; Zheng et al., 2007, 2009a, 2009b, 2010; Zheng, Goddard, Darlington, & Smith, 2008). Briefly, animals were anaesthetised with ketamine hydrochloride (760 μg/kg, s.c.), medetomidine hydrochloride (300 μg/kg, s.c.) and atropine sulfate (80 μg/kg, s.c.). The tympanic bulla was exposed using a retroauricular approach and the tympanic membrane, malleus and incus were removed. The stapedial artery was cauterised and the horizontal and anterior semicircular canal ampullae drilled open. The contents of the canal ampullae and the utricle and saccule were aspirated and the temporal bone sealed with dental cement. Carprofen (5 mg/kg, s.c.) was used for post-operative analgesia. Our histological studies have shown that this procedure results in complete destruction of the vestibular sensory epithelia (Zheng et al., 2006). Sham surgery consisted of exposing the temporal bone and removing the tympanic membrane without producing a vestibular lesion. Although some sound is still transmitted to the inner ear, the removal of the tympanic membrane served as a partial auditory control.

2.3. Drugs

The cannabinoid CB_1/CB_2 receptor agonist, WIN55,212-2, and the cannabinoid CB_1 receptor inverse agonist, AM251, were both purchased from Tocris Bioscience (Bristol, UK). WIN was dissolved in dimethylsulfoxide (DMSO, Merck) and diluted in saline (0.9% sodium chloride), at a final concentration of 1:1 (DMSO:Saline). AM251 was dissolved in a 75% DMSO solution in saline. The drugs were administered s.c. at doses of 1 or 2 mg/kg for WIN and 3 mg/kg for AM251. The doses used were determined based on a previous study (Baek, Zheng, Darlington, & Smith, 2009). The vehicle solution was prepared in a similar manner, except that the drug was omitted.

2.4. Foraging task apparatus

The apparatus was similar to the ones used previously by ourselves and other researchers (Maaswinkel, Jarrard, & Whishaw, 1999; Wallace et al., 2002; Whishaw & Tomie, 1997; Zheng et al., 2006, 2009b). The apparatus consisted of a 140 cm diameter circular wooden white table that was elevated 105 cm above the floor. Eight 10 cm diameter holes, centered 10.5 cm from the table's edge, were located at equal distances around the perimeter of the table. The table was mounted on a central bearing such that it could be rotated between animals. A home cage was placed beneath one of the holes from which a rat could climb onto the table to search for food. A metal sheet was inserted at the bottom of every other hole to prevent accidental falls by BVD rats; however, the eight holes still looked the same to the rats on the table. A 5.5 cm high transparent perspex edging was also fitted around the table to prevent falls from the edge. Twenty-three food cups (4 cm in diameter and 1 cm in height) were attached to the table. The apparatus was located in a test room in which many visual cues were on the wall. A disc (195 cm in diameter) was mounted on the ceiling, with a centre bearing, 130 cm above the table. An opaque curtain was hung on this disc so that the table could be enclosed from all visible light during dark conditions. The room lights were turned off for both light and dark conditions. In the light conditions, the apparatus was illuminated by three lights, which were positioned on the disc, 120° apart from each other. In the dark conditions, the disc lights were turned off and an infrared light source was located inside the curtain. An infrared camera was placed above the centre of the table to record the movements of the animals under both light and dark conditions. A speaker playing white noise was also mounted above the centre of the table.

2.5. Foraging task procedure

2.5.1. Pre-training

The rats were trained to reliably retrieve four food pellets per 10 min session. During pre-training, the room lights were turned off, the ceiling frame lights were on and the curtain surrounding the table was drawn back. All of the food cups were baited for the first few days, but the number of baited cups was gradually decreased until only one food cup was baited per trial. A trial was defined as an exit from the home cage and a return to the cage