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Research Paper

Suppression of the vestibular short-latency evoked potential by electrical stimulation of the central vestibular system

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ABSTRACT

In an attempt to view the effects of the efferent vestibular system (EVS) on peripheral dynamic vestibular function, we have monitored the Vestibular short-latency Evoked Potential (VsEP) evoked by pulses of bone conducted vibration during electrical stimulation of the EVS neurons near the floor of the fourth ventricle in the brainstem of anesthetized guinea pigs. Given the reported effects of EVS on primary afferent activity, we hypothesized that EVS stimulation would cause a slight reduction in the VsEP amplitude. Our results show a substantial (>50%) suppression of the VsEP, occurring immediately after a single EVS current pulse. The effect could not be blocked by cholinergic drugs which have been shown to block efferent-mediated vestibular effects. Shocks produced a short-latency P1-N1 response immediately after the electrical artifact which correlated closely to the VsEP suppression. Ultimately, we have identified that this suppression results from antidromic blockade of the afferent response (the VsEP). It would appear that this effect is unavoidable for EVS stimulation, as we found no other effects.

1. Introduction

The mammalian EVS has a bilateral and symmetric origin in the dorsal brainstem, at the floor of the fourth ventricle, lateral of the facial nerve genu (Goldberg and Fernandez, 1980; Strutz, 1982; Motts et al., 2008). It has an extensive and non-selective top-down projection to the periphery, where efferent fibers bifurcate and densely innervate type I calyx afferents, type II hair cells and also likely bouton afferents of all vestibular end-organs (Lysakowski and Goldberg., 1997, 2004; Holt et al., 2015).

EVS effects are mediated by cholinergic neurotransmission with both fast nicotinic (ionotropic) and slow muscarinic (metabotropic) kinetics (Luebke et al., 2005; Holt et al., 2017). Less understood pathways have also been implicated such as the Calcitonin Gene-Related Peptide (Wackym et al., 1991; Chi et al., 1999), Adenoside 5′-Triphosphate (Rennie and Ashmore, 1993; Rossi et al., 1994; Syeda and Lysakowski, 2001), Nitric Oxide (Lysakowski and Singer, 2000), Opioid peptides (Popper and Wackym, 2001), and GABA (Didier et al., 1990; Lopez et al., 1990). It is therefore likely the EVS has a range of functional time scales, with both rapid and gradual peripheral modulatory effects. Pharmacological modulation of the EVS in turtles using specific acetylcholine receptor (AChR) agonists and antagonists have demonstrated that fast calyx-dimorphic excitation is mediated by α4, α6 and β2 nicotinic AChR subunits, whereas fast bouton inhibition is attributed to the activation of α9/10 nicotinic AChRs on type II hair cells (Holt et al., 2006, 2015). Slow calyx-dimorphic excitation has also been implicated by M-current activation through muscarinic acetylcholine receptors (Holt et al., 2017). Knock-out mice for specific nAChR homomeric and heteromeric subtypes produced variations in VsEP threshold, latency and amplitude, suggesting a complex relationship between peripheral efferent cholinergic activation and primary dynamic afferent activity (Morley et al., 2017). However, despite ongoing work, the functional role of the EVS is currently unknown.

Plotnik et al. (2005) found large fluctuations in the background firing of irregular afferents which was shown to originate from the EVS. This was confirmed to be an artifact of the chinchillas

Abbreviations: AChR, Acetylcholine Receptor; BCV, Bone Conducted Vibration; CAP, Compound Action Potential; CM, Cochlear Microphonic; DMPP, 1,1-Dimethyl-4-phenylpiperazinium iodide; eCAP, electrically-evoked Compound Action Potential; ECG, Electrocardiography; ES, Electrical Stimulation; EVS, Efferent Vestibular System; I/O, Input/Output; VsEP, Vestibular short-latency Evoked Potential

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decerebrate state and lack of inhibitory feedback pathways, hence the explanation that the EVS may modulate peripheral afferent activity by a non-linear positive feedback mechanism (Plotnik et al., 2005). Experiments in the toadfish have demonstrated the EVS is activated during arousal, a behavior which precedes predatory movement in that species (Highstein and Baker, 1985), hence, the idea that the EVS may modify peripheral vestibular gain before or during specific activities. If the function of the EVS is somewhat specific to the behavioral requirements of an animal, it may explain the different vestibular efferent neuroanatomy and neurophysiology across species (Meredith, 1988).

It was originally hypothesized that the vestibular efferents send corollary discharges to the peripheral vestibular system to suppress afferent activity during active motions. However, whilst recent studies have shown that there is a strong corollary suppression of actively driven afferent throughput at the level of the vestibular nucleus (Cullen et al., 2011), this does not seem to occur in mammalian peripheral vestibular afferents (Cullen, 2012), unlike that of the amphibian species, Xenopus (Chagnaud et al., 2015). This suggests that the function of the mammalian vestibular efferents at the periphery is likely not involved with inhibitory silencing or distinguishing passive and active movements, but rather may be involved with slow adaptation and homeostasis. Recent research has demonstrated that mice lacking the α9-nAChR have impaired VOR adaptation (Hübner et al., 2015) and compensation (Hübner et al., 2017), further supporting this hypothesis.

Electrical stimulation of the efferent vestibular system has been used to study efferent-mediated effects on the peripheral vestibular afferents (Goldberg and Fernandez, 1980; Brichta and Goldberg, 2000), and experiments in mammals (Marlinski et al., 2004), toadfish (Boyle and Highstein, 1990; Boyle et al., 1991), frogs (Rossi et al., 1980; Bernard et al., 1985) and turtles (Brichta and Peterson, 1994; Holt et al., 2006) demonstrate the EVS plays an active role in modulating peripheral vestibular afferent activity. Specifically, in mammals, high-frequency shocks to the floor of the fourth ventricle result in both fast (10–100 ms) and slow (5–20 s) increases in spontaneous irregular afferent firing, and slow yet smaller regular primary afferent excitation (Goldberg and Fernandez, 1980; Goldberg, 2000). Results are not homogenous across species, and show mixed excitatory and inhibitory spontaneous afferent activity in frogs (Sugai et al., 1992) and turtles (Brichta and Goldberg, 1996) depending on neuroepithelial location (Holt et al., 2006). Moreover, electrical stimulation in the EVS region may potentially evoke antidromic stimulation of the vestibular afferents (Goldberg and Fernandez, 1980), which terminate in close proximity to, or indeed within the EVS nucleus (Ohgaki et al., 1988). Such antidromic effects may potentially confound any efferent-mediated effects. Presently, it is thought such antidromic affects occur secondary to efferent-mediated effects.

That high frequency electrical stimulation of the EVS is needed to modify afferent activity suggests the vestibular efferents may act as a high-pass filter, attenuating tonic low-frequency stimuli whilst amplifying phasic high-frequency information, which may function to rapidly modify peripheral vestibular gain during large dynamic stimuli (Holt et al., 2011).

Despite continued research, the functional role of the peripheral EVS remains elusive. We have attempted to further study the function of the EVS by electrically stimulating the efferent vestibular cell bodies at the floor of the fourth ventricle, whilst monitoring the VsEP, evoked by Bone-Conducted Vibration (BCV) in guinea pigs. This is analogous to the research undertaken in auditory physiology, with medial-olivocochlear (MOC) stimulation, whilst monitoring the acoustic Compound Action Potential (CAP) (Galambos, 1956; Gifford and Guinan, 1987). Since the VsEP is a measure of irregular jerk-sensitive afferent activity (Jones et al., 2011; Chihara et al., 2013), which primarily innervate the calyx/dimorphic units at the striola (Curthoys et al., 2006, 2016), we are solely interested in the efferent driven mechanisms of these irregular units. Based on the few studies available, and the known characteristics of efferent-mediated calyx/dimorphic excitation, we hypothesized EVS stimulation will result in VsEP suppression. This is supported by Goldberg and Fernandez (1980), who found that pairing fast efferent-mediated excitation with rotation, resulted in a modest reduction in the rotational gain of irregular vestibular afferents. Recordings in the turtle showed that canal duct indentation paired with an efferent-mediatedfast response resulted in a gain decrease (Holt, 2008), and Boyle and Highstein (1990) noticed an inhibition of the dynamically driven vestibular response during EVS stimulation. These results can be explained by a parallel-conductance model, whereby fast efferent responses cause large conductance increases at the efferent-afferent synapse, resulting in a decreased conductance of the main afferent terminal through electrical shunting (Holt et al., 2011). In this study, suppression of the VsEP by electrical stimulation of the central vestibular system at the floor of the fourth ventricle does not appear to rely on the activation of EVS neurons.

2. Materials and methods

2.1. Animal preparation & surgery

Experiments were performed on 15 adult tri-colored guinea pigs (*Cavia porcellus*), of either sex weighing between 200 and 600 g. All experimental procedures were approved by The University of Sydney Animal Ethics Committee (protocol #829). Animals received pre-anaesthetic intraperitoneal injections of Atropine Sulphate (0.6 mg/ml; Apex Laboratories, NSW, Australia), and were thereafter anaesthetized using either Ketamine (Ketamil, Ilium − 100 mg/kg) and Xylazine (Xylazil-20, Ilium − 4 mg/kg) (n = 6) or Isoflurane (IsoFlo - 2–4%) (n = 9). Animals anesthetized using Isoflurane received 0.05 ml intraperitoneal pre-anaesthetic injections of the analgesic Temgesic (Buprenorphreine Hydrochloride, 324 μg/ml; Reckitt Benckiser, Auckland, NZ). In experiments using Ketamine and Xylazine anesthesia, for the first 4 h animals received hourly injections of Ketamine and Xylazine at half the initial dose, delivered intramuscularly. Thereafter, animals received top up injections of only Ketamine (50 mg/kg) every 45 min. In both anaesthetic regimes, once sedated and lacking a foot-withdrawal reflex, animals were transferred to the surgical table to be tracheotomized and artificially ventilated with oxygen. Heart rate and blood oxygen saturation were continuously monitored throughout the experiment, and body temperature was maintained using a blanket and infrared heating pad (Kent Scientific, CT, USA).

The animal's head was mounted between custom-made ear bars, housing a canalphone speaker (ATH-IM70, Audio-Technica, Tokyo, Japan). A BCV vibration/modal shaker device was attached to the ipsilateral earbar in a lateral-medial orientation via a 5 cm metal rod, with an attached 3-axis accelerometer (bandpass: 0.02–6 kHz; TE Connectivity, Ch-8200, Switzerland) (Fig. 1). In the dorsal position, a small incision was made behind the pinna, removing musculature and exposing the dorsolateral bulla, so that a small window of bone could be removed (~2 mm^2), providing a clear view of the round window and facial nerve canal.

A Teflon-coated Ag/AgCl wire with the tip exposed was used as a non-inverting electrode, and was either inserted onto the round window niche, or 3 mm into the facial nerve canal. A bare Ag/AgCl wire inserted into the neck musculature served as the inverting electrode. The animal was grounded via a low-resistance Ag/AgCl electrode placed in the nape of the neck.

For most experiments where the focus was on VsEP recordings,