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Effects of Visceral Inputs on the Processing of Labyrinthine Signals by the Inferior and Caudal Medial Vestibular Nuclei: Ramifications for the Production of Motion Sickness

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Abstract

Neurons located in the caudal aspect of the vestibular nucleus complex have been shown to receive visceral inputs, and project to brainstem regions that participate in generating emesis, such as nucleus tractus solitarius and the “vomiting region” in the lateral tegmental field (LTF). Consequently, it has been hypothesized that neurons in the caudal vestibular nuclei participate in triggering motion sickness, and that visceral inputs to the vestibular nucleus complex can affect motion sickness susceptibility. To obtain supporting evidence for this hypothesis, we determined the effects of intragastric infusion of copper sulfate (CuSO₄) on responses of neurons in the inferior and caudal medial vestibular nuclei to rotations in vertical planes. CuSO₄ readily elicits nausea and emesis by activating gastrointestinal afferents. Infusion of CuSO₄ produced a >30% change in spontaneous firing rate of approximately one-third of neurons in the caudal aspect of the vestibular nucleus complex. These changes in firing rate developed over several minutes, presumably in tandem with the emetic response. The gains of responses to vertical vestibular stimulation of a larger fraction (approximately two-thirds) of caudal vestibular nucleus neurons were altered over 30% by administration of copper sulfate. The response gains of some units went up, and others went down, and there was no significant relationship with concurrent spontaneous firing rate change. These findings support the notion that the effects of visceral inputs on motion sickness susceptibility are mediated in part through the caudal vestibular nuclei. However, our previous studies showed that infusion of CuSO₄ produced larger changes in responses to vestibular stimulation of LTF neurons, as well as parabrachial nucleus neurons that are believed to participate in generating nausea. Thus, integrative effects of gastrointestinal inputs on the processing of labyrinthine inputs must occur at brain sites that participate in eliciting motion sickness in addition to the caudal vestibular nuclei. It seems likely that the occurrence of motion sickness requires converging inputs to brain areas that generate nausea and vomiting from a variety of regions that process vestibular signals.

Keywords

Vomiting; nausea; emesis; multisensory integration; copper sulfate; gastrointestinal receptors

Introduction

Motion sickness is a condition elicited by conflicting sensory inputs reflecting body position in space (Money 1970; Oman 1990; Yates 2009). Motion sickness does not occur in individuals with bilateral vestibular hypofunction, indicating that vestibular signals are critical for the production of this condition (Wang and Chinn 1956; Cheung et al. 1990). The hallmark symptoms of motion sickness include nausea and vomiting, which are also induced by other triggers, including ingested toxins (Borison and Wang 1953). The intragastric infusion of copper sulfate (CuSO_4) has often been employed to trigger nausea and vomiting in animal models. This agent readily produces emesis by activating gastrointestinal (GI) afferents sensitive to irritation of the stomach lining, presumably including chemoreceptors and pain receptors (Wang and Borison 1951a; Brizzee and Marshall 1960; Verbalis et al. 1987; Bountra et al. 1993; Endo et al. 1995b; Gardner et al. 1996; Gonsalves et al. 1996; Lang et al. 1999; Ariumi et al. 2000). Since CuSO_4 is not absorbed into the bloodstream in the stomach (Zimnicka et al. 2011), it can be delivered repeatedly during an experiment, and subsequently aspirated (Sugiyama et al. 2011; Moy et al. 2012; Suzuki et al. 2012).

In a series of studies, we examined the effects of intragastric delivery of CuSO_4 on the processing of vestibular signals by brainstem regions that participate in generating nausea and vomiting (Sugiyama et al. 2011; Moy et al. 2012; Suzuki et al. 2012). Amongst these areas were the dorsolateral reticular formation of the caudal medulla (Moy et al. 2012), often referred to as the lateral tegmental field (LTF) (Berman 1968), which was traditionally classified as the “vomiting center” because stimulation of the area evokes vomiting (Borison and Wang 1949; Fukuda and Koga 1991; Fukuda and Koga 1992), lesions of the area abolish the capacity for vomiting (Wang and Borison 1951b; Koga et al. 1998), and the delivery of emetic drugs triggers the expression of the intermediate-early gene *c-fos* by a large number of LTF neurons (Miller and Ruggiero 1994; Billig et al. 2001; Ito et al. 2003; Ito et al. 2005; Onishi et al. 2007). Another region examined was the parabrachial nucleus (Suzuki et al. 2012), which relays visceral signals to the hypothalamus and limbic system (Takeuchi et al. 1982; Cechetto et al. 1983; Fulwiler and Saper 1984; Cechetto and Calaresu 1985; Berkley and Scofield 1990), and has been hypothesized to play a critical role in generating nausea and related affective responses following the delivery of emetic stimuli (Balaban et al. 2011). A subset of both LTF (Moy et al. 2012) and parabrachial nucleus neurons (Suzuki et al. 2012) responded to whole-body rotations that activate labyrinthine receptors. The intragastric infusion of CuSO_4 substantially altered (>30%) the gains of these responses for approximately 50% of LTF neurons (Moy et al. 2012) and 75% of parabrachial nucleus neurons (Suzuki et al. 2012), with some responses being enhanced and others being attenuated. These findings suggested that the delivery of an emetic gastrointestinal stimulus could affect the processing of labyrinthine inputs by regions of the brainstem that generate the symptoms of motion sickness, thereby altering motion sickness susceptibility.

Another potential site where emetic gastrointestinal inputs could affect the processing of labyrinthine signals is the caudal aspect of the vestibular nucleus complex (inferior and caudal medial vestibular nuclei). Neurons in this region of the vestibular nuclei project to brainstem areas that participate in vomiting generation, including the LTF (Yates et al. 1995) and nucleus tractus solitarius (NTS) (Balaban and Beryozkin 1994; Yates et al. 1994; Ruggiero et al. 1996; Porter and Balaban 1997; Cai et al. 2007). Consequently, the caudal aspect of the vestibular nucleus complex likely plays a role in processing and transmitting vestibular signals to brainstem emetic centers during motion sickness. In addition, there is evidence caudal vestibular nucleus neurons receive visceral inputs, including those from the GI system. The injection of the retrograde transneuronal tracer pseudorabies virus into the caudal aspect of the vestibular nucleus complex resulted in the labeling of a large number of

NTS neurons following survival times adequate for the virus to pass across two synapses (Jian et al. 2005). In addition, electrical stimulation of vagal afferents altered the firing rate of approximately one-third of the neurons in the caudal vestibular nuclei (Jian et al. 2002). These observations raise the possibility that emetic gastrointestinal inputs could influence motion sickness susceptibility by affecting the processing of vestibular signals by neurons that receive direct inputs from the labyrinth.

The goal of the present study was to ascertain the effects of intragastric infusion of CuSO_4 on the spontaneous activity and responses to whole-body rotations of neurons in the inferior and caudal medial vestibular nuclei. We tested the hypothesis that the delivery of CuSO_4 increases both the excitability (spontaneous activity) and the gain of responses to labyrinthine stimulation of neurons in the caudal aspect of the vestibular nucleus complex.

Methods and materials

Experiments were conducted on 12 laboratory bred (Liberty Research, Waverly, NY) cats of both sexes weighing 2.0–3.4 Kg. The University of Pittsburgh's Institutional Animal Care and Use Committee (IACUC) prospectively approved all procedures on animals, which were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, National Academies Press, Washington D.C., 2011). The experimental protocol used in these experiments has been validated and thoroughly described in previous manuscripts (Sugiyama et al. 2011; Moy et al. 2012; Suzuki et al. 2012), and thus will only be briefly described below.

Surgical procedures

Animals were anesthetized using isoflurane, tracheotomized, and a cannula was secured in the femoral vein for the delivery of drugs. Blood pressure was recorded using a Millar (Houston, TX) transducer introduced through the femoral artery into the abdominal aorta. Both carotid arteries were ligated, and an intragastric catheter was inserted through an esophagostomy to administer CuSO_4 . Animals were secured in a stereotaxic frame such that the head was pitched-down 30° to vertically align the anterior and posterior semicircular canals. The stereotaxic frame was mounted on a servo-controlled hydraulic tilt table (Neurokinetics, Pittsburgh, PA). A midcollicular decerebration was performed, the caudal aspect of the cerebellum was exposed, and 3–4 mm of the caudal cerebellum on one side was aspirated to expose landmarks on the surface of the brainstem. These landmarks were used to target the caudal aspect of the vestibular nucleus complex for recordings. In this process, the caudal vermis was ablated, although the fastigial nucleus was likely left intact.

Atropine sulfate and dexamethasone were injected every six hours to reduce airway secretions and brain swelling, respectively. Fluids were infused intravenously to replace loss, and if hypotension occurred phenylephrine was administered. Body temperature was measured rectally, and maintained at $37\text{--}38^\circ\text{C}$ using a DC-powered heating pad and heat lamp.

After all surgical procedures were completed, anesthesia was removed and animals were paralyzed using 0.1 mg/kg intravenous injections of vecuronium bromide every 20 min. Animals were artificially ventilated such that end tidal CO_2 was maintained near 4%. At the end of the experiment, animals were euthanized using Euthasol Euthanasia Solution.

Recording procedures

Extracellular recordings of unit activity were made from the inferior and medial vestibular nuclei (2–4 mm rostral to the obex) using 4–6 M Ω tungsten microelectrodes (FHC, Bowdoin, ME). Single unit activity was sampled at 25,000 Hz, and blood pressure and tilt

table position were sampled at 100 Hz, using a Cambridge Electronic Design (Cambridge, UK) 1401 data collection system and Spike2 version 6 software. We first determined the responses of units to 0.5 Hz wobble stimuli (Schor et al. 1984), fixed-amplitude tilts, the direction of which moves around the animal at a constant speed. Clockwise (CW) wobble stimuli were generated by driving the pitch axis of the tilt table with a sine wave, while simultaneously driving the roll axis with a cosine wave. During this stimulus, the animal's body (viewed from above) appeared to wobble, taking in succession nose-down, right ear-down, nose-up, and left ear-down positions. When the signal to the pitch axis of the tilt table was inverted, the stimulus direction rotated in the counterclockwise (CCW) direction. Responses to wobble stimuli were used to determine the response vector orientation, or the direction of tilt that produced the maximal modulation of a unit's firing rate (Schor et al. 1984). The response vector orientation was confirmed by comparing responses to tilts performed in the roll and pitch planes. Subsequently, fixed-plane tilts were delivered near the direction of the response vector orientation at frequencies ranging from 0.05–1 Hz to determine the response dynamics of the unit. The stimulus amplitudes were 2.5–5° at frequencies 0.2 Hz, and 5–10° below 0.2 Hz.

After a unit's responses to vertical vestibular stimulation were recorded, 83 mg of CuSO₄ dissolved in 10 ml of distilled water were injected into the stomach via the intragastric catheter. We recorded the effects of CuSO₄ injection on the unit's spontaneous firing rate for five min, as illustrated in Fig. 1. Afterwards, the vestibular stimulation protocol described above was repeated. At the conclusion of testing, the CuSO₄ solution was aspirated from the stomach, and six washes were performed, each using 10 ml of distilled water. The manipulation of the animal required for the washing process typically caused units to be lost, such that it was impossible to perform pre- and post-wash recordings from the same neuron.

Data analysis procedures

All data were subjected to offline spike sorting using Spike2 software to ensure that counts of action potentials generated by particular units were accurate. Neural activity recorded during whole-body rotations was binned (500 bins/cycle) and averaged over the sinusoidal stimulus period. Sine waves were fitted to responses with the use of a least-squares minimization technique (Schor et al. 1984). The response sinusoid was characterized by two parameters: phase shift from the stimulus sinusoid (subsequently referred to as phase) and amplitude relative to the stimulus sinusoid (subsequently referred to as gain). The signal-to-noise ratio for responses was also calculated (Schor et al. 1984). Responses were considered to be significant when the signal-to-noise ratio was > 0.5, only the first harmonic was prominent, and the responses were consistent from trial to trial.

Statistical analyses were performed using Prism 6 software (Graph-Pad Software, San Diego, CA). Pooled data are presented as means ± SE. Statistical significance was assumed if $P < 0.05$. Two variables were compared using a Mann-Whitney test, whereas multiple variables were compared using a nonparametric one-way ANOVA (Kruskal-Wallis test combined with Dunn's multiple comparisons test). A two-way ANOVA was used to determine the effect of CuSO₄ administration on two factors of a response. χ^2 tests were used to compare the number of neurons affected by a manipulation under two different conditions.

Histological procedures

Near the end of the recording sessions, lesions were made at defined coordinates by passing a 200- μ A negative current through the recording electrode for 60 sec. After euthanasia, the brainstem was removed and fixed in 10% formaldehyde solution. The brainstem was cut transversely at 100- μ m thickness using a freezing microtome, mounted serially on slides,

stained using thionine, and coverslipped. Recording sites were reconstructed on drawings of sections with reference to the locations of electrolytic lesions, the relative positions of electrode tracks, and microelectrode depths.

Results

We characterized the effects of intragastric infusion of CuSO_4 on the spontaneous activity and responses to rotations in vertical planes of 47 vestibular nucleus neurons. A median of three units was successfully tested per animal, and the data from all animals were combined for analyses. Fig. 1 and Fig. 2A illustrate the effects of CuSO_4 on the firing rate of two units. Although modest changes in activity of units were often noted within a few sec, pronounced and prolonged alterations in firing rates were not observed until 1–2 min following delivery of CuSO_4 . Such changes in neuronal activity were not correlated with perturbations in blood pressure (see Fig. 2A). For each unit, the interspike intervals during the 60 sec period before and five min period following the infusion of CuSO_4 were compared using a Mann-Whitney test. In every case when the mean firing rate changed >30% after CuSO_4 was administered, the interspike intervals before and after the delivery of the chemical were significantly different ($p < 0.05$). Consequently, we assumed that the induction of a 30% change in a unit's spontaneous firing rate by CuSO_4 indicated that the chemical affected its excitability.

The spontaneous firing rates of 15 of the 47 tested vestibular nucleus units (32%) were altered $\geq 30\%$ by intragastric infusion of CuSO_4 , as indicated in Fig. 3A. The activity of seven of these units decreased, whereas the firing rate of eight units increased. Since some units were excited by the chemical, and others were inhibited, the median change in firing rate for the population of vestibular nucleus neurons sampled was ~ 0 . Prior to administration of CuSO_4 , we established that the baseline firing rate of the neuron was stable, and there was no trend of increasing or decreasing firing rate. The coefficient of variation in firing rate for the baseline period was relatively low (median of 0.75, with just 12% of units having a coefficient of variation > 1).

We also examined the effects of CuSO_4 delivery on the responses of neurons in the caudal aspect of the vestibular nucleus complex to whole-body rotations. These responses were similar to those described in previous publications focused on the caudal vestibular nuclei of decerebrate cats (Endo et al. 1995a; Jian et al. 2002), and thus will not be described in detail in this manuscript. In brief, most (>80%) of the neurons had response vector orientations closer to the roll plane than to the pitch plane. In addition, the dynamic properties of responses to tilts of over half of the neurons were similar to those of otolith afferents: the response gains increased only modestly as stimulus frequency was advanced, and the response phases were near stimulus position or lagged position slightly (Fernandez and Goldberg 1976; Anderson et al. 1978). However, some neurons had response dynamics like semicircular canal afferents: the response phases were near stimulus velocity, and the response gains increased sharply as stimulus frequency was advanced (Fernandez and Goldberg 1971; Anderson et al. 1978). Consequently, the average Bode plots for caudal vestibular nucleus neurons reflected a summation of the response properties of the two types of vestibular afferents, such that the response gain increased modestly at higher stimulus frequencies, while the response phases were midway between stimulus position and stimulus velocity (Fig. 4B).

In previous studies considering responses to vestibular stimulation of neurons in brainstem areas that generate nausea and vomiting, we ascertained whether units exhibited spatiotemporal convergence (STC) behavior (Baker et al. 1984; Schor et al. 1984; Schor and Angelaki 1992). This response pattern has been attributed to the convergence of inputs with different spatial and temporal properties (for instance, the convergence of inputs from

semicircular canal afferents activated by roll rotations and otolith organ afferents activated by pitch rotations). When wobble stimuli are used, neurons with STC behavior respond to one direction of rotation (CW or CCW), but not the other (Schor and Angelaki 1992). Just one of the 47 vestibular nucleus neurons exhibited pronounced STC behavior, such that a response vector orientation could not be determined. Thus, as reported in previous studies (Endo et al. 1995a; Jian et al. 2002), this response pattern does not appear to be common for neurons in the caudal aspect of the vestibular nucleus complex.

Following administration of CuSO_4 , the gains of responses to vertical plane rotations was altered $>30\%$ for 30 of the 47 neurons (64%); the responses increased in magnitude for 12 cells, and were attenuated for 18 units. Fig. 2B illustrates responses of a caudal vestibular nucleus neuron to wobble rotations whose gain decreased $\sim 65\%$ following the infusion of CuSO_4 . Fig. 4A shows responses to roll tilts that were attenuated after CuSO_4 was administered. The effects of CuSO_4 on the responses of all of the neurons to wobble rotations are indicated in Fig. 3B. For this analysis, we averaged the gains of responses to CW and CCW wobble rotations, and then compared the averages for trials before and after CuSO_4 was provided. For the entire population of vestibular nucleus neurons examined, the gain of responses to wobble stimuli decreased by a median value of 18% following the administration of CuSO_4 . Filled symbols show data for neurons whose spontaneous firing rate changed $>30\%$ following the delivery of CuSO_4 . The response gain of responses to wobble stimuli was altered for 12/15 neurons (80%) whose spontaneous firing rate changed over 30% following injection of CuSO_4 into the stomach. In contrast, the gains of responses to wobble stimuli were affected for 18/32 (56%) of the neurons whose firing rates remained relatively stable after CuSO_4 . However, a χ^2 test failed to confirm that infusion of CuSO_4 affected the gains of responses to vestibular stimulation of a higher fraction of neurons whose spontaneous activity was also altered by the chemical ($p=0.11$). When a unit's spontaneous firing rate decreased significantly following administration of CuSO_4 , the gain of its responses to vestibular stimulation tended to increase (4/7 cells). In contrast, when a unit's spontaneous firing rate increased significantly after CuSO_4 was provided, the gain of its responses to vestibular stimulation tended to decrease (7/8 cells). Hence, a parallel change in both a unit's excitability (firing rate) and gain of responses to vestibular stimulation, as illustrated in Fig. 2, was relatively uncommon. The average change in magnitude of responses to vestibular stimuli (average of absolute values of changes in gains of responses to wobble stimuli) was $52 \pm 7\%$ for neurons whose firing rates were altered by CuSO_4 infusion, and $70 \pm 23\%$ for neurons whose firing rates remained stable. The changes in response gains for the two populations of units were not significantly different ($p=0.25$). In addition, we compared the effects of CuSO_4 infusion on the magnitudes of responses to 0.5 Hz wobble and single-plane stimuli. The averages were $64 \pm 15\%$ and $54 \pm 17\%$, respectively, and were not significantly different ($p=0.73$).

Although CuSO_4 administration altered the gains of responses of vestibular nucleus neurons to vertical vestibular stimulation, the spatial and temporal properties of the responses were relatively unchanged. Fig. 3C shows that the intragastric delivery of CuSO_4 had little effect on the response vector orientations of vestibular nucleus neurons. The median change in response vector orientation was just 6° , and only three neurons had alterations in response vector orientation $> 45^\circ$. Fig. 4B shows that CuSO_4 administration did not appreciably change the mean response dynamics for the population of vestibular nucleus neurons sampled in this study. A two-way ANOVA (factors were presence of CuSO_4 and stimulus frequency) indicated that administration of the chemical did not contribute significantly to response variation ($p=0.61$ for gain; $p=0.85$ for phase). Although CuSO_4 altered response gains to single-plane tilts for individual neurons, some gains were enhanced and others diminished, such that the response gains for the population remained relatively constant.

The locations of the neurons whose activity was sampled before and after infusion of CuSO₄ are shown in Fig. 5. Most of the recordings occurred in the inferior vestibular nucleus. Different colors are used to indicate whether the spontaneous activity increased or decreased (< 30% change), or remained constant following the administration of CuSO₄. Different symbols are used to indicate whether the response gain increased or decreased (>30% change), or remained constant after the intragastric infusion of the chemical. There was no indication that neurons with distinct responses to CuSO₄ were confined to a particular region of the caudal vestibular nuclei.

Discussion

The main finding of this study is that the spontaneous activity and responses to whole-body rotations of a subset of neurons in the caudal aspect of the vestibular nucleus complex are affected by the intragastric infusion of CuSO₄. This chemical elicits emesis by irritating the stomach lining, thereby activating a subset of GI receptors sensitive to such irritation (Wang and Borison 1951a; Brizzee and Marshall 1960; Verbalis et al. 1987; Bountra et al. 1993; Endo et al. 1995b; Gardner et al. 1996; Gonsalves et al. 1996; Lang et al. 1999; Ariumi et al. 2000). Thus, these results show that inputs from the GI system can affect the processing of labyrinthine signals by the portion of the vestibular nuclei that makes connections with regions of the caudal medulla that participate in autonomic regulation, and presumably is responsible for triggering motion sickness (Balaban and Beryozkin 1994; Yates et al. 1994; Yates et al. 1995; Ruggiero et al. 1996; Porter and Balaban 1997; Cai et al. 2007).

The spontaneous firing rate of approximately one-third of neurons in the caudal portion of the vestibular nuclei was altered by the intragastric infusion of CuSO₄. The activity of approximately the same percentage of neurons was affected by electrical stimulation of vagal afferents (Jian et al. 2002). Although it was impossible to determine precise onset latencies for the changes in excitability, the firing rates following CuSO₄ delivery did not plateau at new levels until 1–2 min following the injection of the chemical (see Figs. 1–2). In contrast, changes in the firing rate of NTS neurons, which receive vagal afferent inputs, occurred within a few sec after CuSO₄ was administered (Sugiyama et al. 2011). The long latency of the effects of CuSO₄ on the firing rate of vestibular nucleus neurons was similar to that for LTF (Moy et al. 2012) and parabrachial nucleus neurons (Suzuki et al. 2012). Since these areas are respectively involved in the generation of emesis (Borison and Wang 1949; Wang and Borison 1951b; Fukuda and Koga 1991; Fukuda and Koga 1992; Koga et al. 1998) and nausea (Balaban et al. 2011), it was concluded that changes in firing rate of LTF and parabrachial nucleus neurons following CuSO₄ infusion were related to the production of the emetic response (Moy et al. 2012; Suzuki et al. 2012). Thus, although neurons in the caudal vestibular nuclei receive disynaptic inputs from NTS (Jian et al. 2005), these connections may not be responsible for the changes in activity noted after CuSO₄. Instead, the changes in excitability may occur as the emetic response is triggered within the brainstem.

The gains of responses of approximately two-thirds of the neurons in the caudal aspect of the vestibular nucleus complex were altered over 30% following the delivery of CuSO₄. The median absolute change in response gain was approximately 40%, and was similar for neurons whose spontaneous firing rates were and were not influenced by CuSO₄ injection. Neurons whose firing rate remained stable after CuSO₄ was injected either presumably did not receive inputs from brainstem areas powerfully activated by the chemical, or received a combination of excitatory and inhibitory signals from these areas that were negating. Moreover, the changes in firing rate of a unit and its responses to vestibular stimulation usually did not occur in parallel (e.g., units with an increase in firing rate following CuSO₄ typically had diminished responses to vestibular stimulation). As discussed above, since the

caudal aspect of the vestibular nucleus complex receives relatively direct inputs from brainstem regions such as NTS that process visceral inputs (Jian et al. 2005), we hypothesized that these connections mediate the effects of CuSO₄ administration on the activity of vestibular nucleus neurons. In other words, we presumed that CuSO₄ would affect motion sickness sensitivity by altering the excitability of vestibular nucleus neurons that receive direct inputs from the labyrinth. However, this hypothesis is not consistent with our observations that changes in the gain of responses to vestibular stimulation typically either occurred without a change in a unit's spontaneous firing rate, or were opposite to the change in excitability indicated by the alteration in firing rate (i.e., the response gain increased despite a decrease in the unit's firing rate). These observations raise the alternate possibility that GI signals affect the processing of vestibular signals by neurons located outside the vestibular nuclei, but which make connections with vestibular nucleus neurons and affect their responses to whole-body rotations.

One potential site of influences of GI inputs on the processing of labyrinthine inputs is the cerebellum. Several physiological studies have shown that Purkinje cells in the posterior cerebellar vermis respond to noxious visceral stimuli (Saab and Willis 2001) or electrical stimulation of vagal afferents (Okahara and Nisimaru 1991; Tong et al. 1993). In addition, the posterior cerebellar vermis receives direct inputs from brainstem regions that process visceral inputs, including NTS (Somana and Walberg 1979), area postrema (Shapiro and Miselis 1985), and the dorsal motor nucleus of the vagus (Zheng et al. 1982). The regions of the cerebellar vermis that process visceral afferent signals also receive labyrinthine inputs (Precht et al. 1977), and project to the caudal aspect of the vestibular nucleus complex (Angaut and Brodal 1967; Precht et al. 1976; Walberg and Dietrichs 1988). Furthermore, the posterior cerebellar vermis projects to the fastigial nucleus (Ruggiero et al. 1977), which likewise receives afferents from the vestibular nuclei (Ruggiero et al. 1977) and brainstem areas that process GI inputs, including the dorsal motor nucleus of the vagus (Zheng et al. 1982) and the area postrema (Shapiro and Miselis 1985). Like the posterior vermis, the fastigial nucleus modulates the responses to whole-body rotations of neurons in the caudal aspect of the vestibular nucleus complex (Carleton and Carpenter 1983; Andrezik et al. 1984).

These previous findings support the notion that an extensive cerebellar circuitry integrates GI and labyrinthine inputs, and affects the activity of neurons in the caudal aspect of the vestibular nucleus complex. Although electrical polarization of the labyrinths can still evoke symptoms of motion sickness in decerebrate cats following ablation of the caudalmost cerebellar vermis (Miller and Wilson 1983), it is unknown whether motion sickness can occur following a complete cerebellectomy. In fact, a number of lines of evidence suggest that the cerebellum participates in the generation of motion sickness (Wang and Chinn 1956; Cohen et al. 2003). Consequently, the alterations in the gain of responses of vestibular nucleus neurons following infusion of CuSO₄ could have been a consequence of cerebellar modulation of their activity. Further research will be needed to ascertain the role of the cerebellum in modifying signal transduction in the vestibular nuclei following emetic stimuli.

Since the caudal aspect of the vestibular nucleus complex projects to regions of the brainstem that are believed to be paramount in generating nausea and vomiting (Balaban and Beryozkin 1994; Yates et al. 1994; Yates et al. 1995; Balaban 1996; Ruggiero et al. 1996; Porter and Balaban 1997; Cai et al. 2007), it is tempting to speculate that the effects of GI inputs on motion sickness susceptibility (Wang et al. 1958) are a reflection of integration of visceral and labyrinthine inputs by vestibular nucleus neurons. However, the magnitude of the effects of CuSO₄ administration on the gain of responses of vestibular nucleus neurons to whole-body rotations was less than that for LTF (Moy et al. 2012) or parabrachial nucleus

neurons (Suzuki et al. 2012). The average absolute value of response gains following the infusion of CuSO₄ was 64±15% for vestibular nucleus neurons, as opposed to 106 ± 43% for LTF neurons (Moy et al. 2012) and 119 ± 28% for parabrachial nucleus neurons (Suzuki et al. 2012). In addition, the responses of parabrachial nucleus and LTF neurons to whole-body rotations was much more complex than those observed for vestibular nucleus neurons. For example, many LTF and parabrachial nucleus neurons exhibited pronounced STC behavior, such that the neurons responded to one direction of wobble stimulation, but not the other (Moy et al. 2012; Suzuki et al. 2012). Thus, it seems likely that the integrative responses of LTF and parabrachial nucleus neurons to GI and vestibular inputs reflect the convergence of inputs from a variety of sources, one of which is the vestibular nuclei.

In conclusion, the present results show that an emetic GI stimulus affects the processing of labyrinthine signals by neurons in the caudal aspect of the vestibular nucleus complex. These findings are consistent with the premise that this region participates in generating motion sickness, and contributes to eliciting vomiting. However, a number of questions remain. The responses of some vestibular nucleus neurons to whole-body rotations were enhanced by the delivery of CuSO₄, whereas others were diminished. Additional studies will be needed to determine if neurons whose responses to vestibular stimulation were affected differently by CuSO₄ have distinct projections or physiological roles. Further research is also needed to determine if direct projections from the caudal vestibular nuclei to areas of the brainstem that coordinate emesis, such as the LTF, play a predominant role in triggering motion sickness, or if this condition is principally elicited through projections to LTF neurons from regions such as the caudal cerebellar vermis and fastigial nucleus. It is also feasible that the occurrence of motion sickness requires converging inputs to brain areas that generate nausea and vomiting from a variety of regions that process vestibular signals. Studies in conscious animals will be needed to address these important questions.

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References

- Anderson JH, Blanks RHI, Precht W. Response characteristics of semicircular canal and otolith systems in the cat. I. Dynamic responses of primary vestibular fibers. *Exp Brain Res.* 1978; 32:491–507. [PubMed: 28960]
- Andreuzik JA, Dormer KJ, Foreman RD, Person RJ. Fastigial nucleus projections to the brain stem in beagles: pathways for autonomic regulation. *Neurosci.* 1984; 11:497–507.
- Angaut P, Brodal A. The projection of the “vestibulocerebellum” onto the vestibular nuclei in the cat. *Arch Ital Biol.* 1967; 105:441–479. [PubMed: 5585723]
- Ariumi H, Saito R, Nago S, Hyakusoku M, Takano Y, Kamiya H. The role of tachykinin NK-1 receptors in the area postrema of ferrets in emesis. *Neurosci Lett.* 2000; 286:123–126. [PubMed: 10825652]
- Baker J, Goldberg J, Hermann G, Peterson B. Spatial and temporal response properties of secondary neurons that receive convergent input in vestibular nuclei of alert cats. *Brain Res.* 1984; 294:138–143. [PubMed: 6697230]
- Balaban CD. Vestibular nucleus projections to the parabrachial nucleus in rabbits: implications for vestibular influences on the autonomic nervous system. *Exp Brain Res.* 1996; 108:367–381. [PubMed: 8801117]
- Balaban CD, Beryozkin G. Vestibular nucleus projections to nucleus tractus solitarius and the dorsal motor nucleus of the vagus nerve: potential substrates for vestibulo-autonomic interactions. *Exp Brain Res.* 1994; 98:200–212. [PubMed: 8050507]

- Balaban CD, Jacob RG, Furman JM. Neurologic bases for comorbidity of balance disorders, anxiety disorders and migraine: neurotherapeutic implications. *Expert Rev Neurotherapeutics*. 2011; 11:379–394.
- Berkley KJ, Scofield SL. Relays from the spinal cord and solitary nucleus through the parabrachial nucleus to the forebrain in the cat. *Brain Res*. 1990; 529:333–338. [PubMed: 1704286]
- Berman, AI. *The Brain Stem of the Cat*. University of Wisconsin Press; Madison: 1968.
- Billig I, Yates BJ, Rinaman L. Plasma hormone levels and central c-Fos expression in ferrets after systemic administration of cholecystokinin. *Am J Physiol Regul Integr Comp Physiol*. 2001; 281:R1243–1255. [PubMed: 11557633]
- Borison HL, Wang SC. Functional localization of central coordinating mechanism for emesis in cat. *J Neurophysiol*. 1949; 12:305–313. [PubMed: 18137708]
- Borison HL, Wang SC. Physiology and pharmacology of vomiting. *Pharmacol Rev*. 1953; 5:193–230. [PubMed: 13064033]
- Bountra C, Bunce K, Dale T, Gardner C, Jordan C, Twissell D, Ward P. Anti-emetic profile of a non-peptide neurokinin NK1 receptor antagonist, CP-99,994, in ferrets. *Eur J Pharmacol*. 1993; 249:R3–4. [PubMed: 7506663]
- Brizzee KR, Marshall KR. Developmental studies on emetic response to tartar emetic and copper sulfate in the cat. *Proc Soc Exp Biol Med*. 1960; 103:839–842. [PubMed: 13804527]
- Cai YL, Ma WL, Li M, Guo JS, Li YQ, Wang LG, Wang WZ. Glutamatergic vestibular neurons express Fos after vestibular stimulation and project to the NTS and the PBN in rats. *Neurosci Lett*. 2007; 417:132–137. [PubMed: 17412503]
- Carleton SC, Carpenter MB. Afferent and efferent connections of the medial, inferior and lateral vestibular nuclei in the cat and monkey. *Brain Res*. 1983; 278:29–51. [PubMed: 6315158]
- Cechetto DF, Calaresu FR. Central pathways relaying cardiovascular afferent information to amygdala. *Am J Physiol*. 1985; 248:R38–45. [PubMed: 3970185]
- Cechetto DF, Ciriello J, Calaresu FR. Afferent connections to cardiovascular sites in the amygdala: a horseradish peroxidase study in the cat. *J Auton Nerv Syst*. 1983; 8:97–110. [PubMed: 6619513]
- Cheung BS, Money KE, Jacobs I. Motion sickness susceptibility and aerobic fitness: a longitudinal study. *Aviation Space Environ Med*. 1990; 61:201–204.
- Cohen B, Dai M, Raphan T. The critical role of velocity storage in production of motion sickness. *Ann NY Acad Sci*. 2003; 1004:359–376. [PubMed: 14662476]
- Endo K, Thomson DB, Wilson VJ, Yamaguchi T, Yates BJ. Vertical vestibular input to and projections from the caudal parts of the vestibular nuclei of the decerebrate cat. *J Neurophysiol*. 1995a; 74:428–436. [PubMed: 7472343]
- Endo T, Nemoto M, Minami M, Yoshioka M, Saito H, Parvez SH. Changes in the afferent abdominal vagal nerve activity induced by cisplatin and copper sulfate in the ferret. *Biog Amine*. 1995b; 11:399–407.
- Fernandez C, Goldberg JM. Physiology of peripheral neurons innervating semicircular canals of the squirrel monkey. II. Response to sinusoidal stimulation and dynamics of peripheral vestibular system. *J Neurophysiol*. 1971; 34:661–675. [PubMed: 5000363]
- Fernandez C, Goldberg JM. Physiology of peripheral neurons innervating otolith organs of the squirrel monkey. III. Response dynamics. *J Neurophysiol*. 1976; 39:996–1008. [PubMed: 824414]
- Fukuda H, Koga T. The Botzinger complex as the pattern generator for retching and vomiting in the dog. *Neurosci Res*. 1991; 12:471–485. [PubMed: 1665553]
- Fukuda H, Koga T. Non-respiratory neurons in the Botzinger complex exhibiting appropriate firing patterns to generate the emetic act in dogs. *Neurosci Res*. 1992; 14:180–194. [PubMed: 1331922]
- Fulwiler CE, Saper CB. Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. *Brain Res Rev*. 1984; 7:229–259.
- Gardner CJ, Armour DR, Beattie DT, Gale JD, Hawcock AB, Kilpatrick GJ, Twissell DJ, Ward P. GR205171: a novel antagonist with high affinity for the tachykinin NK1 receptor, and potent broad-spectrum anti-emetic activity. *Regul Pept*. 1996; 65:45–53. [PubMed: 8876035]
- Gonsalves S, Watson J, Ashton C. Broad spectrum antiemetic effects of CP-122,721, a tachykinin NK1 receptor antagonist, in ferrets. *Eur J Pharmacol*. 1996; 305:181–185. [PubMed: 8813551]

- Ito H, Nishibayashi M, Kawabata K, Maeda S, Seki M, Ebukuro S. Induction of Fos protein in neurons in the medulla oblongata after motion- and X-irradiation-induced emesis in musk shrews (*Suncus murinus*). *Auton Neurosci*. 2003; 107:1–8. [PubMed: 12927221]
- Ito H, Nishibayashi M, Maeda S, Seki M, Ebukuro S. Emetic responses and neural activity in young musk shrews during the breast-feeding/weaning period: comparison between the high and low emetic response strains using a shaking stimulus. *Exp Anim*. 2005; 54:301–307. [PubMed: 16093643]
- Jian BJ, Acernese AW, Lorenzo J, Card JP, Yates BJ. Afferent pathways to the region of the vestibular nuclei that participates in cardiovascular and respiratory control. *Brain Res*. 2005; 1044:241–250. [PubMed: 15885222]
- Jian BJ, Shintani T, Emanuel BA, Yates BJ. Convergence of limb, visceral, and vertical semicircular canal or otolith inputs onto vestibular nucleus neurons. *Exp Brain Res*. 2002; 144:247–257. [PubMed: 12012162]
- Koga T, Qu R, Fukuda H. The central pattern generator for vomiting may exist in the reticular area dorsomedial to the retrofacial nucleus in dogs. *Exp Brain Res*. 1998; 118:139–147. [PubMed: 9547082]
- Lang IM, Sarna SK, Shaker R. Gastrointestinal motor and myoelectric correlates of motion sickness. *Am J Physiol*. 1999; 277:G642–G652. [PubMed: 10484390]
- Miller AD, Ruggiero DA. Emetic reflex are revealed by expression of the Immediate-Early gene C-Fos in the cat. *J Neurosci*. 1994; 14:871–888. [PubMed: 8301366]
- Miller AD, Wilson VJ. Vestibular-induced vomiting after vestibulocerebellar lesions. *Brain Behav Evol*. 1983; 23:26–31. [PubMed: 6606480]
- Money KE. Motion sickness. *Physiol Rev*. 1970; 50:1–39. [PubMed: 4904269]
- Moy JD, Miller DJ, Catanzaro MF, Boyle BM, Ogburn SW, Cotter LA, Yates BJ, McCall AA. Responses of Neurons in the Caudal Medullary Lateral Tegmental Field to Visceral Inputs and Vestibular Stimulation in Vertical Planes. *Am J Physiol Regul Integr Comp Physiol*. 2012; 303:R929–R940. [PubMed: 22955058]
- Okahara K, Nisimaru N. Climbing fiber responses evoked in lobule VII of the posterior cerebellum from a vagal nerve in rabbits. *Neurosci Res*. 1991; 12:232–239. [PubMed: 1660985]
- Oman CM. Motion sickness: a synthesis and evaluation of the sensory conflict theory. *Can J Physiol Pharmacol*. 1990; 68:294–303. [PubMed: 2178753]
- Onishi T, Mori T, Yanagihara M, Furukawa N, Fukuda H. Similarities of the neuronal circuit for the induction of fictive vomiting between ferrets and dogs. *Auton Neurosci*. 2007; 136:20–30. [PubMed: 17478125]
- Porter JD, Balaban CD. Connections between the vestibular nuclei and brain stem regions that mediate autonomic function in the rat. *J Vestib Res*. 1997; 7:63–76. [PubMed: 9057160]
- Precht W, Volkind R, Blanks RH. Functional organization of the vestibular input to the anterior and posterior cerebellar vermis of cat. *Exp Brain Res*. 1977; 27:143–160. [PubMed: 65291]
- Precht W, Volkind R, Maeda M, Giretti ML. The effects of stimulating the cerebellar nodulus in the cat on the responses of vestibular neurons. *Neurosci*. 1976; 1:301–312.
- Ruggiero D, Batton RR 3rd, Jayaraman A, Carpenter MB. Brain stem afferents to the fastigial nucleus in the cat demonstrated by transport of horseradish peroxidase. *J Comp Neurol*. 1977; 172:189–209. [PubMed: 65366]
- Ruggiero DA, Mtui EP, Otake K, Anwar M. Vestibular afferents to the dorsal vagal complex: Substrate for vestibular-autonomic interactions in the rat. *Brain Res*. 1996; 743:294–302. [PubMed: 9017258]
- Saab CY, Willis WD. Nociceptive visceral stimulation modulates the activity of cerebellar Purkinje cells. *Exp Brain Res*. 2001; 140:122–126. [PubMed: 11500804]
- Schor RH, Angelaki DE. The algebra of neural response vectors. *Ann NY Acad Sci*. 1992; 656:190–204. [PubMed: 1599143]
- Schor RH, Miller AD, Tomko DL. Responses to head tilt in cat central vestibular neurons. I. Direction of maximum sensitivity. *J Neurophysiol*. 1984; 51:136–146. [PubMed: 6319622]
- Shapiro RE, Miselis RR. The central neural connections of the area postrema of the rat. *J Comp Neurol*. 1985; 234:344–364. [PubMed: 3988989]

- Somana R, Walberg F. Cerebellar afferents from the nucleus of the solitary tract. *Neurosci Lett*. 1979; 11:41–47. [PubMed: 431884]
- Sugiyama Y, Suzuki T, Destefino VJ, Yates BJ. Integrative responses of neurons in nucleus tractus solitarius to visceral afferent stimulation and vestibular stimulation in vertical planes. *Am J Physiol Regul Integr Comp Physiol*. 2011; 301:R1380–1390. [PubMed: 21832211]
- Suzuki T, Sugiyama Y, Yates BJ. Integrative responses of neurons in parabrachial nuclei to a nauseogenic gastrointestinal stimulus and vestibular stimulation in vertical planes. *Am J Physiol Regul Integr Comp Physiol*. 2012; 302:R965–975. [PubMed: 22277934]
- Takeuchi Y, McLean JH, Hopkins DA. Reciprocal connections between the amygdala and parabrachial nuclei: ultrastructural demonstration by degeneration and axonal transport of horseradish peroxidase in the cat. *Brain Res*. 1982; 239:583–588. [PubMed: 7093703]
- Tong G, Robertson LT, Brons J. Climbing fiber representation of the renal afferent nerve in the vermal cortex of the cat cerebellum. *Brain Res*. 1993; 601:65–75. [PubMed: 8431787]
- Verbalis JG, Richardson DW, Stricker EM. Vasopressin release in response to nausea-producing agents and cholecystokinin in monkeys. *Am J Physiol*. 1987; 252:R749–753. [PubMed: 3032008]
- Walberg F, Dietrichs E. The interconnection between the vestibular nuclei and the nodulus: a study of reciprocity. *Brain Res*. 1988; 449:47–53. [PubMed: 2456133]
- Wang SC, Borison HL. Copper sulphate emesis; a study of afferent pathways from the gastrointestinal tract. *Am J Physiol*. 1951a; 164:520–526. [PubMed: 14810961]
- Wang SC, Borison HL. The vomiting center; its destruction by radon implantation in dog medulla oblongata. *Am J Physiol*. 1951b; 166:712–717. [PubMed: 14885461]
- Wang SC, Chinn HI. Experimental motion sickness in dogs; importance of labyrinth and vestibular cerebellum. *Am J Physiol*. 1956; 185:617–623. [PubMed: 13340001]
- Wang SC, Renzi AA, Chinn HI. Mechanism of emesis following x-irradiation. *Am J Physiol*. 1958; 193:335–339. [PubMed: 13533550]
- Yates BJ. Motion Sickness. In: Binder, MD.; Hirokawa, N.; Windhorst, U., editors. *Encyclopedia of Neuroscience*. Springer; Heidelberg: 2009. p. 2410-2413.
- Yates BJ, Balaban CD, Miller AD, Endo K, Yamaguchi Y. Vestibular inputs to the lateral tegmental field of the cat: potential role in autonomic control. *Brain Res*. 1995; 689:197–206. [PubMed: 7583323]
- Yates BJ, Grelot L, Kerman IA, Balaban CD, Jakus J, Miller AD. Organization of vestibular inputs to nucleus tractus solitarius and adjacent structures in cat brain stem. *Am J Physiol*. 1994; 267:R974–983. [PubMed: 7524372]
- Zheng ZH, Dietrichs E, Walberg F. Cerebellar afferent fibres from the dorsal motor vagal nucleus in the cat. *Neurosci Lett*. 1982; 32:113–118. [PubMed: 7145232]
- Zimnicka AM, Ivy K, Kaplan JH. Acquisition of dietary copper: a role for anion transporters in intestinal apical copper uptake. *Am J Physiol Cell Physiol*. 2011; 300:C588–599. [PubMed: 21191107]

Figure 1.

Changes in spontaneous firing rate of a vestibular nucleus neuron produced by the intragastric infusion of copper sulfate ($CuSO_4$). **A:** a raster plot of neuronal activity before and following $CuSO_4$ delivery. Each tick mark indicates the occurrence of an action potential. Shaded areas indicate periods of neuronal activity that are expanded in panels **C–E**. **B:** tracing of blood pressure recorded simultaneously with unit activity. **C–E:** 10 msec periods of unit activity recorded before (**C**), immediately after (**D**), and approximately 150 sec following (**E**) the infusion of $CuSO_4$. Unit activity was sampled at 25,000 Hz. The spontaneous firing rate of the unit decreased after $CuSO_4$ was provided.

Figure 2.

A: average unit activity (*top*) and blood pressure (*bottom*) during 20-sec intervals before (*gray bars*) and after (*white bars*) the administration of CuSO₄. Error bars indicate one SEM, and asterisks indicate intervals when unit activity and blood pressure were significantly and >30% different from those during the first 20 sec of recording (*leftmost bar in each histogram*). **B:** averaged responses to clockwise (CW) and counterclockwise (CCW) wobble stimuli (0.5 Hz, 5° amplitude) recorded before and after the delivery of CuSO₄. Each panel shows unit activity, with a superimposed sine wave fit to the response. Responses to the following number of wobble rotations were averaged to generate each trace: pre-CuSO₄ CW, 10; pre-CuSO₄ CCW, 11; post-CuSO₄ CW, 72; post-CuSO₄ CCW, 43. Following the administration of CuSO₄, the average response gain to wobble rotations decreased from 7.6 spikes/sec/° to 2.8 spikes/sec/°. Abbreviations: CED, contralateral ear down roll; IED, ipsilateral ear down roll; ND, nose-down pitch; NU, nose-up pitch.

Figure 3.

Effects of intragastric CuSO_4 on the spontaneous activity (**A**), gain of responses to wobble stimuli (**B**), and response vector orientation (**C**) of vestibular nucleus neurons. For **A** and **B**, the values plotted indicate: (pre-lesion value – post-lesion value)/pre-lesion value $\times 100\%$. Each symbol indicates values for a particular neuron; filled symbols denote data for the subset of units whose spontaneous activity changed $>30\%$ after CuSO_4 was injected, with red symbols indicating the subset of units whose firing rate decreased. Horizontal lines indicate median values. Responses to wobble stimuli were so attenuated after CuSO_4 in four cases that the signal-to-noise ratio no longer met our criteria for significance. Data for such cases were used to calculate the change in response gain (**B**), but not the change in response vector orientation (**C**). Hence, fewer data values are plotted in (**C**) than (**A**) or (**B**).

Figure 4.

Effects of intragastric CuSO_4 on the responses of vestibular nucleus neurons to fixed-plane tilts delivered near the plane of the response vector orientation. **A:** averaged responses of one neuron to roll tilts delivered at 0.1 Hz (5°), 0.5 Hz (5°) and 1 Hz (2.5°). Dashed red lines indicate table position. Following the administration of CuSO_4 , the response gain decreased 30% at 0.1 Hz, 45% at 0.5 Hz, and 32% at 1.0 Hz. However, response phases were similar before and after the injection of CuSO_4 . **B:** average Bode plots for all neurons whose responses to rotations in a single vertical plane were recorded before and after CuSO_4 infusion. Response gains and phases were plotted with respect to stimulus position. Error bars indicate one SEM. Abbreviations: *CED*, contralateral ear down roll; *IED*, ipsilateral ear down roll.

Figure 5.

Locations of neurons tested for the effects of CuSO_4 on responses to whole-body tilts. Unit locations are plotted on standard transverse sections through the caudal medulla, which were generated through reference to Berman's atlas (Berman 1968). Numbers below each section designate distance in mm rostral to the obex. Red symbols indicate units whose spontaneous firing rate decreased >30% following CuSO_4 , green symbols indicate units whose spontaneous firing rate increased >30%, and blue symbols indicate units whose spontaneous firing rate remained stable. Filled symbols indicate cells whose response gains to rotations increased >30% after CuSO_4 , open symbols indicate cells whose response gains decreased >30%, and "X"s indicate units whose response gains remained stable. Abbreviations: *T2*, hypoglossal nucleus; *CN*, cochlear nucleus; *EC*, external cuneate; *IO*, inferior olivary nucleus; *PH*, prepositus hypoglossi; *IVN*, inferior vestibular nucleus; *MVN*, medial vestibular nucleus; *RB*, restiform body; *S*, solitary nucleus; *SA*, stria acoustica; *SNV*, spinal trigeminal nucleus; *STV*, spinal trigeminal tract.