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Vestibular nuclei characterized by calcium-binding protein immunoreactivity and tract tracing in *Gekko gekko*

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Abstract

Immunohistochemical techniques were used to describe the distribution of the calcium binding proteins calretinin, calbindin and parvalbumin as well as synaptic vesicle protein 2 in the vestibular nuclei of the Tokay gecko (*Gekko gekko*). In addition, tract tracing was used to investigate connections between the vestibular nerves and brainstem nuclei. Seven vestibular nuclei were recognized: the nuclei cerebellaris lateralis (Cerl), vestibularis dorsolateralis (Vedl), ventrolateralis (Vevl), ventromedialis (Vevm), tangentialis (Vetg), ovalis (VeO) and descendens (Veds). Vestibular fibers entered the brainstem with the ascending branch projecting to Vedl and Cerl, the lateral descending branch to Veds, and the medial descending branch to ipsilateral Vevl. Cerl lay most rostral, in the cerebellar peduncle. Vedl, located rostrally, was ventral to the cerebellar peduncle, and consisted of loosely arranged multipolar and monopolar cells. Vevl was found at the level of the vestibular nerve root and contained conspicuously large cells and medium-sized cells. Veds is a large nucleus, the most rostral portion of which is situated lateral and ventral to Vevl, and occupies much of the dorsal brainstem extending caudally through the medulla. VeO is a spherically shaped cell group lateral to the auditory nucleus magnocellularis and dorsal to the caudal part of Vevl. Vevm and Vetg were small in the present study. Except for VeO, all other vestibular nuclei appear directly comparable to counterparts in other reptiles and birds based on their location, cytoarchitecture, and connections, indicating these are conserved features of the vestibular system.

1. Introduction

Geckos possess remarkable motor capabilities compared to other reptilian taxa. For example, Tokay geckos (*Gekko gekko*) move freely on vertical surfaces and across ceilings (Aerts et al., 2000; Autumn and Peattie, 2002). In view of these behavioral abilities, it is reasonable to hypothesize that neural adaptations have arisen in this taxa compared to ground-moving lizards. A logical starting point for investigating the neural basis of such capabilities is the vestibular system, since previous studies have shown that the tree squirrel

possesses the longest striola and greatest number of cells in the saccular and utricular macule, as well as the longest cristae and greatest number of cells in the cristae ampullae in six rodent species when taking body mass into account (Desai et al., 2005a,b), thus implying that arboreal species adapted for moving or jumping may possess enlarged otolith organs and possibly other vestibular specializations Table 1.

Tokay gecko semicircular canals do not differ in length and diameter from snake and turtle canals, and the time constants of θ/Ω in the Steinhausen equation which describes the biophysics of fluid flow in the semicircular canals are of magnitudes similar to those reported in mammals (Ramprasad et al., 1986). Nevertheless, other vestibular specializations may exist, insofar as Tokay geckos are a freely roaming species where individuals exhibit rapid coordinated motor behaviors such as jumping on rocks and tree trunks, walking across the ceilings of buildings (Tang et al., 2001; Zaaf and Van Damme, 2001), and rapidly flipping themselves right-side-up by a tail swing if dropped accidentally from a height (Jusufi et al., 2008). Thus, it may be hypothesized that one or more brainstem vestibular nuclei are anatomically specialized in the Tokay gecko compared to other reptiles.

Six vestibular nuclei are generally recognized in birds and mammals. In mammals the vestibular complex is composed of four “classic” nuclear groups: the medial, descending, Deiters, superior nuclei, and “other” vestibular nuclei such as the parasolitary nucleus, Y-group and nucleus intercalatus (Barmack, 2003). Similarly, there are six homologous vestibular nuclei in chickens and pigeons: the medial, descending, lateroventral Deiters, laterodorsal Deiters, superior, and tangential nuclei (Wold, 1976; Schwarz and Schwarz, 1986; Dickman and Fang, 1996; Popratiloff and Peusner, 2007).

Turtles and lizards share four vestibular nuclei, including the dorsolateralis, descendens, ventrolateralis and ventromedialis nuclei. Lizards also possess the nucleus tangentialis, an additional vestibular nucleus that is not found in turtles (Foster and Hall, 1978; Bangma and Donkelaar, 1983; Barbas-Henry and Wouterlood, 1988). To date, the vestibular nuclear complex of reptiles has been visualized in Nissl stained material, which has provided information about the cytoarchitectonic features used for the description and identification of each nucleus. In the present study, we combined three methods, i.e. Nissl staining, neural tract tracing, and immunohistochemistry based on antibodies raised against calcium binding proteins, to delineate the vestibular nuclei of *G. gecko*. The use of calcium-binding protein immunohistochemistry is highly useful in studying the evolution of this system insofar as previous studies in birds and mammals have shown that these proteins are useful cross-phyletic markers (Baizer and Baker, 2005). Anterograde tracing methods were used to trace axonal projections from their source (the cell body or soma) to their point of termination (the synapse). The complementary technique is retrograde tracing, which is used to trace neural connections from their termination to their source (synapse to cell body). Tracers can be introduced into either living or fixed tissue, and are transported along nerve cell processes to reveal their origin and termination. Both anterograde and retrograde tracing techniques are based on axonal transport processes (Rajakumar et al., 1993).

2. Methods

2.1. Subjects

This study was based on data from 31 adult individuals of *G. gecko* of both sexes. Six animals were used for Nissl staining, 22 animals for immunohistochemistry including Western blots and histochemical staining of tissue. Three animals were used in tract tracing experiments. All animal care and anesthesia procedures followed those approved by the Institutional Animal Care and Use Committee of University of Maryland and the Chengdu Institute of Biology, Chinese Academy of Sciences. The geckos were anesthetized with a mixture of isoflurane and room air in a small chamber, followed by i.p. injections of euthasol at a dose of 7 mg/kg. Once the geckos were deeply anesthetized (no response to toe pinch, depressed respiration), they were perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer solution (PBS, pH 7.4) for 1 h.

2.2. Nissl staining and immunohistochemical procedures

The brains were postfixed in 4% paraformaldehyde overnight at 4 °C and cryoprotected in 30% sucrose in 0.1 M phosphate buffer. The brains were then embedded in tissue freezing medium (Jung, Heidelberg, Germany) and sectioned on a freezing microtome or cryostat at 40 µm thickness, with all sections collected in order in PBS. For Nissl staining, sections were mounted, counterstained with cresyl violet, dehydrated, and coverslipped.

Immunohistochemical procedures started with free-floating sections pre-incubated for 1 h in a blocking solution of 10% normal goat serum diluted in 0.1M phosphate buffer solution (PBS, pH 7.4) containing 0.3% Triton X-100. Subsequently, sections were incubated for 2 days at 4 °C with antisera against 1) calretinin (CR, 7699/66; Swant, Belinzona, Switzerland) diluted 1:5000; 2) calbindin (CB, 300; Swant) diluted 1:3000; 3) parvalbumin (PV, PA-235; Sigma, St. Louis, MO) diluted 1:2000 and 4) synaptic vesicle protein 2 (SV2, Hybridoma Bank, University of Iowa) diluted 1:1000. After washes, sections were incubated for 1 h in biotinylated goat anti-rabbit (for CR), goat anti-mouse (for PV, CB, SV2) or mouse anti-goat (for CTX, see the tract tracing procedure below) secondary antisera, diluted 1:500 in PBS. Sections were then incubated in the avidin–biotin-peroxidase complex (Vector Laboratories, USA), followed by the SG reaction (Vector Laboratories, Burlingame, CA) which is a peroxidase substrate kit used to produce blue–gray reaction products. Sections were then mounted, airdried, dehydrated, and coverslipped. Some sections were additionally counterstained with neutral red.

Antibody specificity was determined using Western blot analyses of Tokay material (Yan et al., 2010) and histologically negative controls. Results showed single bands at estimated molecular weights of 29 kD for CR (Hack et al., 2000), 28 kD for CB (Ellis et al., 1991), 15 kD for PV (Celio, 1990; Lohmann and Friauf, 1996) and 18 kD for SV2 (Baumert et al., 1989). Three negative controls were designed to test labeling in the reactive condition in which the following changes: 1) primary antibody replaced with the blocking solution, 2) secondary antibody replaced with PBS or 3) avidin-biotin-peroxidase replaced with PBS. For tract tracing, 3 cases with robust tracer staining and transport were used in the analyses.

2.3. Tract tracing

For neurosurgery, geckos were maintained at about 25 °C using a heating blanket with feedback control. A dorsal surgical approach was used to expose the VIIIth nerve. If necessary, small paper probes were inserted between the brainstem and the internal wall of the skull, deflecting the medulla slightly and exposing the proximal part of the vestibulo-cochlear nerve (VIIIth). Following surgery, the head was held in a constant position by gluing a stainless-steel head to the prefrontal bone.

The retrograde tracer neurobiotin (NB, Molecular Probes, WA) diluted 1:200 and the anterograde tracer cholera toxin (CTX, List Biological Laboratories, CA) diluted 1:500 were injected into the VIIIth nerve with tracer-filled glass pipettes. Since Tokay brains are small, tracers were iontophoresed using alternating positive currents of 1–2.5 μ A for 10–20 min, i.e. 7 sec on, 7 s off. The craniotomy was sealed with a small gelfoam plug, followed by tissue glue (Vetbond, 3M) and the animal was unglued immediately from the stainless-steel head. Geckos recovered for 0.5–4 days in their home cages before they were euthanized and perfused as described above. Tract tracing with CTX was visualized by immunohistochemical procedures, using an antibody against CTX raised in goat. Similar steps were taken to visualize NB, beginning with incubation in the avidin–biotin-peroxidase complex (Vector Laboratories, USA, see above). In order to discriminate the boundary of each vestibular nucleus, the midbrain was injected with NB which retrogradely labeled somas in the vestibular nuclei of the brainstem. Fig. 1F shows the injection site within the nucleus oculomotorius which labeled exclusively vestibular cell bodies.

2.4. Data analyses

For both immunohistochemical and tract tracing materials, photomicrographs were captured with a digital camera (NIS-Elements F3.0) mounted on a Nikon Eclipse 55i microscope. Brightness and contrast were adjusted slightly using Image J 1.44e (NIH, USA) and Adobe Photoshop CS3 (Adobe Systems, Mountain View, CA).

Diameters of fibers in the VIIIth nerve and diameters and areas of somas in vestibular nuclei were measured with Image J 1.44e. Vestibular and auditory fibers were classified according to their location and relative size. The Shapiro–Wilk test was used to estimate the normality of the distribution of the values, and the Levene test was used to determine the homogeneity of variances. We conducted the square root transformation before any statistical analyses in case values were not normally distributed or if the variances were heterogeneous.

The diameters of VIIIth nerve rami labeled with the three antibodies were examined by analysis of variance (one-way ANOVA); comparisons of the diameters of the vestibular nerve (VN) and auditory nerve (AN) and between calcium binding protein immunopositive fibers and fibers labeled by tract tracing were executed by independent sample *t*-tests. The resulting values were expressed as mean $\pm$ SEM; differences with $p < 0.05$ were considered to be statistically significant, and differences with $p < 0.001$ were considered to be very statistically significant.

Ten Donkelaar's nomenclature proposed for vestibular nuclei in turtles (Bangma and Donkelaar, 1983) was adopted when identifying nuclei not previously considered vestibular

nuclei in geckos including Vetg and VeO although the latter was originally recognized and named by Foster and Hall (1978). As shown below, most reptilian vestibular nuclei correspond to avian vestibular nuclei (Tellegen et al., 2001), thus permitting homologies to be recognized between the two taxa.

3. Results

Each antibody tested with Western blots showed one band at the appropriate molecular weight for the corresponding protein (Yan et al., 2010) and each negative control in immunohistochemistry did not label any cells but only exhibited light background. Although more than ten animals were injected with different tracers, only three cases (two for NB and one for CTX) were used because they demonstrated robust staining at the injection site and transport. The vestibular brainstem of *G. gecko* contains the following targets of the vestibular nerve: 1) nucleus cerebellaris lateralis (Cerl); 2) nucleus vestibularis dorsolateralis (Vedl); 3) nucleus vestibularis ventrolateralis (Vevl); 4) nucleus vestibularis ventromedialis (Vevm); 5) nucleus vestibularis tangentialis (Vetg); 6) nucleus vestibularis descendens (Veds), and 7) nucleus vestibularis ovalis (VeO) (Foster and Hall, 1978).

3.1. Projections of the 8th nerve

The VIIIth nerve in the Tokay gecko is divided into an anteroventral root connected with the vestibular or intracranial ganglion (vestibular nerve, VN) and a posterodorsal root connected with the intraotic ganglion (auditory nerve, AN). The transverse sections from the entrance to the brainstem show that the rostral portion of the VN is located ventral to the caudal portion of the AN (Fig. 1A–C). After a small injection of NB into the VIIIth nerve, VN was visualized horizontally entering the brainstem beneath the AN, showing that the vestibular root divides into three branches: a dorsal ascending branch terminating within the Vedl area, a medial descending branch that projects to Vevl, and a lateral descending branch that projects to Veds (Fig. 1D). This connection pattern was confirmed by immunohistochemical labeling using antibodies against calbindin (Fig. 1E).

The fibers of the VN ramus were thicker than those of the AN, in immunoreactive material using antibodies against CR, PV and CB and in tracing material with NB and CTX injected into the VN (Fig. 1A–C). The average diameter of the VN fibers was $4.14 \pm 0.057 \mu\text{m}$, which was very significantly larger ($t = 26.840$, $p < 0.001$, $n = 60$) than that of the AN fibers ($2.23 \pm 0.043 \mu\text{m}$). There was no obvious difference in either the diameter of VN fibers ($F_{2,57} = 0.052$, $p > 0.05$, $n = 20$) or the diameter of AN fibers ($F_{2,57} = 1.101$, $p > 0.05$, $n = 20$) labeled with different antibodies. Nor was there any obvious difference ($t = 0.266$, $p > 0.05$, $n = 60$) in the VN diameter determined by immunohistochemical ($4.14 \pm 0.057 \mu\text{m}$) and tract tracing methods ($4.11 \pm 0.069 \mu\text{m}$).

3.2. Locations of vestibular nuclei

Nissl stained material revealed vestibular and auditory nuclei in the Tokay brainstem (Fig. 2A–D). Cerl is located ventral to the cerebellum (Fig. 2A) and rostradorsal to Vedl, which is located rostrally near the wall of the fourth ventricle (Figs. 2A and 3B). Vevl and Vevm are located immediately ventral to the cochlear nuclei (Figs. 2B, C, 3C). A small Vetg was

found lateral to Vevl and caudally; VeO is located lateral to the auditory nucleus magnocellularis (NM) (Figs. 2B, C, 3A), and adjacent to Veds, which appeared nearly circular in transverse sections (Figs. 2D and 3A).

3.2.1. Nucleus cerebellaris lateralis (Cerl)—In transverse sections, Cerl is located rostrally in the peduncle connecting the brainstem and cerebellum, with its long axis oriented dorsoventrally. The nucleus termed Cerm was located medial to Cerl, but does not receive input from VN. In contrast to Cerm, Cerl was labeled after injection of CTX to the VN (Fig. 4A). Neuronal density was low in Cerl with monopolar cells as the dominant type (Fig. 4B).

3.2.2. Nucleus vestibularis dorsolateralis (Vedl)—The rostral portion of Vedl was adjacent to Cerl. In horizontal sections, Vedl occupied most of the region lateral to the fourth ventricle and ended caudally at the level of the rostral pole of the nucleus angularis (NA) (Fig. 3B). Throughout its extent, Vedl was located adjacent to the nucleus vestibularis ventrolateralis (Vevl) and lateral to NA (Fig. 4C). Nissl staining revealed that the cells of Vedl were predominantly small to medium-sized, and stained darkly (Fig. 2A). Many somas in Vedl were stained using antibodies against CR and CB but not PV (Fig. 4C, D).

The terminals in Vedl were anterogradely labeled when CTX was injected into the VN (Fig. 4E). VN fibers were also labeled using antibodies raised against CB, with most labeled fibers found in the medioventral part of Vedl in transversal sections (Fig. 4D), consistent with our observation that CB positive fibers traverse this region in horizontal sections (Fig. 1E). In addition, NB injections in nucleus oculomotorius retrogradely label Vedl cells (Fig. 4F).

CR immuno-staining labeled somas, many varicose and coarse fibers as well as indications of axon terminals such as puncta in Vedl (Fig. 4C), while CB immuno-staining labeled mostly the cytoplasm of neurons (Fig. 4D). Cell sizes in Vedl in immunoreactive materials were consistent with those labeled by injection of NB to the nucleus oculomotorius in the midbrain (Fig. 4F), in which the average area of cells was $46.28 \pm 2.933 \mu\text{m}^2$ ($n = 21$). CR immunoreactive cell bodies were both monopolar and multipolar. Monopolar cells possessed generally spindle-like somata with one neurite at the pole, located mainly at dorsolateral part while multipolar ones were irregular in shape with prominently stained primary dendrites. These neurons were located primarily in the dorsolateral and ventromedial parts of the nucleus (Fig. 4C).

3.2.3. Nucleus vestibularis ventrolateralis (Vevl)—This nucleus forms a large part of the dorsomedial brainstem and receives a dense projection from the VN. Along the rostral–caudal axis, the main part of Vevl is situated caudal to Vedl, ventral to NA and NM and lateral to Vevm (Figs. 1C, 3B and 5A). The rostral portion of Vevl is separated from Vedl by NA dorsally (Fig. 3B) and not clearly separated ventrally. Moreover many cells in Vedl were labeled with antibody against CB, thus differing from cells of Vevl which exhibited no obviously labeling (Fig. 1E). The caudal portion of Vevl was located medial and rostral to the nucleus vestibularis descendens (Veds) (Fig. 3B). Terminals and somas were labeled in

Ve1 with injection of CTX into VN and NB into the nucleus oculomotorius, respectively (Fig. 5C, D).

Neurons in Ve1 had divergent shapes and were divided into three categories on the basis of size: medium, large and giant cells. Each category has evident discontinuity of cell sizes which were consistent between immunoreactive labeling and tract tracer labeling (Fig. 5D, E). The round or oval neurons of medium size had a mean area of $47.62 \pm 1.136 \mu\text{m}^2$ ($n = 60$, 3 cases). The area size of large neurons with bipolar shapes was $104.10 \pm 4.713 \mu\text{m}^2$ ($n = 30$, 3 cases) while the area size of giant cells with irregular shapes was $577.40 \pm 24.111 \mu\text{m}^2$ ($n = 30$, 3 cases). Most giant cells were found at ventromedial portion of Ve1 with a few located dorsolateral (Fig. 5E). Immunoreactivity with an antibody against SV2 revealed perisomatic synapses on the giant cells in addition to label on both somas and dendrites of middle and large cells (Fig. 5F).

3.2.4. Nucleus vestibular tangentialis (Vetg)—This nucleus was embedded in the vestibular nerve root, in a narrow region between the edge of the brainstem and Ve1 (Figs. 2B and 6B). The obvious differences between Vetg and Ve1 were that neurons in Vetg were lightly labeled after an injection of tracer to the nucleus oculomotorius compared with Ve1. Vetg contained mainly small to medium sized cell bodies (Fig. 6A). In addition, some terminals were observed in the nucleus after CTX injection to VN (Fig. 6B).

3.2.5. Nucleus vestibular ventromedialis (Vevm)—Vevm formed a triangular profile ventral to NM and caudal NA, and ventromedial to Ve1 (Fig. 2C). As seen in former studies (Donkelaar et al., 1983; Barbas-Henry and Lohman, 1988), labels were found in this nucleus after injection of CTX tracers to VN (Fig. 6D). Neurons in Vevm were relatively homogeneous in size and shape, as visualized with NB injection to the midbrain (Fig. 6C).

3.2.6. Nucleus vestibular ovalis (VeO)—VeO was an almost spherically shaped nucleus based on profiles observed in sections in both the horizontal and transverse planes and located lateral to NM (Figs. 2C and 3A). Somas and neuropils were heavily labeled with PV antibody and only slightly with CR or CB antibody (Fig. 7A–C). An injection of NB to the nucleus oculomotorius that labeled exclusively vestibular nuclei in the brainstem labeled some neurons in VeO which was composed of a fairly uniform population of medium-sized neurons (Fig. 7A, D).

3.2.7. Nucleus vestibularis descendens (Veds)—Nissl and CB immunoreaction labeling revealed that the rostral Veds was located ventrally within the vestibular complex, merging with the ventral Ve1 and Vevm at the level of NM (Figs. 2C and 3B). The boundaries between Veds, Ve1 and Vevm were readily visualized (Fig. 3C). At the central level, Veds was an oval nucleus occupying much of the dorsal portion of the brainstem in transverse section (Figs. 2D and 8A), while at the caudal levels, Veds occupied the dorsal-most portion of the brainstem (Fig. 8B). The nucleus was labeled after injections of CTX to VN (Fig. 8C) and NB to the nucleus oculomotorius in the midbrain (Fig. 8D), respectively. The somas exhibited uniform size and similar shape (Fig. 8D).

4. Discussion

The distributions of the calcium-binding protein immunoreactivity for calbindin, calretinin and parvalbumin and the synaptic vesicle protein SV2 present a firm basis for recognizing the similarities and differences in the organization of the vestibular complex in *Gekko* compared to other taxa (Baizer and Baker, 2005). Tract tracing provides additional information about the projections and connections of the vestibular nuclei in the brainstem.

4.1. Calcium-binding proteins in the vestibular system

Previous studies have emphasized the utility of calcium-binding proteins as cross-phyletic markers for the vestibular system (for mRNA, Rogers, 1987, 1989; for proteins; Kevetter, 1996; Baizer and Baker, 2005). In the mammalian vestibular system, calbindin is found mainly in fibers and axon terminals of the vestibular nerve and Purkinje cells (Celio, 1990; Baurle and Grusser-Cornehls, 1994). Calretinin immunoreactive fibers contribute to pathways involved in eye movement modulation but not generation (Baizer and Baker, 2005). Although the physiological functions of these proteins in the vestibular system are currently unknown, they are useful markers for anatomical mapping. For example, lesions of the vestibular nerve largely eliminate expression of calretinin in the vestibular nucleus complex and will also lower levels of calbindin. Cerebellar lesions greatly reduce calbindin labeling with no change in calretinin expression (Kevetter and Leonard, 1997).

While several studies have used calcium binding protein expression patterns as cross-phyletic markers to study brainstem auditory nuclei in both reptiles and birds (Takahashi et al., 1987; Braun, 1990; Hack et al., 2000; Belekova et al., 2004, 2008; Popratiloff and Peusner, 2007; Yan et al., 2010), only two studies have mapped the distributions of calretinin and calbindin in chick vestibular systems. Using *in situ* hybridization and immunohistochemistry (Rogers, 1987, 1989), these studies showed that different antibodies selectively labeled specific populations of vestibular neurons and terminals. Many large cells in the superior vestibular nucleus and Deiter's nucleus expressed calretinin, and were contacted or enclosed by calbindin-positive fibers from the vestibular nerve (Popratiloff and Peusner, 2007; Belekova et al., 2008). A large population of neurons in the descending nucleus was found to be calretinin- and calbindin-negative while many of the large neurons were calretinin-positive (Rogers, 1989).

In geckos, calcium-binding proteins exhibited specific distribution patterns in both auditory (Yan et al., 2010) and vestibular nuclei. First, all three antibodies against calcium-binding proteins labeled vestibular nerve fibers. Second, calretinin-ir labeled neurons in Vdl and central Vds. Third, parvalbumin-ir and calbindin-ir labeled terminals in Vevl, while labeled neurons were located in the ventral Vevl with giant calbindin-ir labeled neurons mainly in ventromedial Vevl.

4.2. Vestibular nerve fibers

Calbindin, calretinin, and parvalbumin have been localized to neurons in the vestibular ganglion (Sans et al., 1986; Dememes et al., 1993; Raymond et al., 1993; present study). The diameters of vestibular nerve fibers are significantly greater than those of auditory nerve

fibers in all vertebrates studied to date (Kevetter, 1996; Baizer and Baker, 2005). Wide intervals between neighboring labeled vestibular fibers suggests a sparse density of fibers containing calcium-binding proteins. The existence of large-diameter vestibular nerve fibers is consistent with the idea that these axons propagate with high velocity and fidelity (Hursh, 1939; Cullheim and Ulfhake, 1979). Moreover, SV2-ir synapses in the Tokay gecko anchored directly on the large and giant cells in Vevl, suggesting a rapid and reliable information processing. These characteristics of the vestibular system may reflect sensorimotor adaptations previously identified in this species such as the ability to rapidly flip upright when dropped (Jusufi et al., 2008).

Previous studies on pigeons showed that the superior, dorsal lateral, ventral lateral, medial, and descending vestibular nuclei received afferent projections from the semicircular canals and otoliths (Schwarz and Schwarz, 1986; Dickman and Fang, 1996). Semicircular canals project approximately to medial portions of all five nuclei, and otoliths to lateral portions, with some overlap (Dickman and Fang, 1996). The pattern of vestibular terminals in pigeons differed from that in chicks in that the vestibular nuclei received terminals from both semicircular canals and otoliths with no obvious subdivisions (Popratiloff and Peusner, 2007).

In geckos, the primary afferent vestibular fibers projected to the ipsilateral vestibular nuclei Cerl, Vedl, Vevl, Vetg, Vevm and Veds but not to nucleus VeO. These observations are partially supported by studies on the monitor lizard in which injection of VN labeled Vedl, Vevl, Vevm, Vetg, Veds and rc/cc (Barbas-Henry and Wouterlood, 1988) and Iguana in which Vedl, Vevl, Vevm, Vetg and VeO were recognized by VN lesion technology (Foster and Hall, 1978). Similarly, VeO were not labeled in other reptiles with injection of tracers to VN (Donkelaar et al., 1983).

4.3. Organization of brainstem vestibular nuclei

The nuclear divisions revealed in the expression of calcium binding proteins in the vestibular nuclei of *G. gecko* proposed in this study largely agree with previous descriptions in other reptiles (Foster and Hall, 1978; Schwab, 1979; Bangma and Donkelaar, 1983; Donkelaar et al., 1983; Barbas-Henry and Wouterlood, 1988) in which five distinct vestibular nuclei are recognized. The nucleus VeO has only been described in *Iguana iguana* (Foster and Hall, 1978) and *G. gecko* (present study). Nucleus vestibular tangentialis has been recognized consistently in birds including chickens and pigeons (Wold, 1976; Schwarz and Schwarz, 1986), and in some lizards but not in turtles and snakes (Bangma and Donkelaar, 1983; Donkelaar et al., 1983; Barbas-Henry and Wouterlood, 1988). In the present study, a putative homolog to the nucleus tangentialis was found lateral to Vevl. The conflicting reports on the existence of nuclei Vetg and VeO in the literature may reflect variation in its development or that the criteria for recognizing the cell groups are somewhat obscure (Foster and Hall, 1978; Donkelaar et al., 1983; Barbas-Henry and Wouterlood, 1988).

As our results in the Tokay demonstrated, Vedl in red-eared turtles is located immediately ventral to the cerebellar peduncle and consists of loosely-arranged multipolar and spindle-shaped neurons (Barbas-Henry and Wouterlood, 1988). Many neurons in Vedl in turtles and

in the superior vestibular nucleus in chicks were labeled after injection of HRP into the contralateral vestibular nuclear complex (Wold, 1979a; Bangma and Donkelaar, 1983). We hypothesize that reptilian V_{edl} is homologous to the superior vestibular nucleus of birds and the dorsal nucleus in frog (Straka et al., 2003) based on its similar location, commissural connections and loosely arranged neurons (Wold, 1976).

In *G. gecko*, V_{evl} contains mostly medium to large or giant cells, similar to the V_{evl} nucleus of the monitor lizard. It is reasonable to hypothesize that the reptilian V_{evl} is homologous to the nucleus Deiters (dorsalis and ventralis) in birds since both the lizard and avian nuclei lack commissural connections and instead project to the ipsilateral spinal cord (Wold, 1979a,b; Bangma and Donkelaar, 1983; Donkelaar et al., 1983; Tellegen et al., 2001). In dolphins, nucleus Deiters appears to have a close functional relationship with the cerebellum and its relatively large size seems to correspond to the physical conditions of aquatic life and locomotion in three dimensions (Kern et al., 2009). Accordingly, the profile of V_{evl} was larger in Tokay than that found in other reptiles, which might correlate to the gecko's special pattern of locomotion.

Tokay V_{evm} appears to be homologous to the medial vestibular nucleus in birds. The reptilian V_{evm} receives projections from the contralateral midbrain oculomotor centers, projects to the contralateral spinal cord and brainstem and has commissural connections in the lizard, turtle and snake (Wold, 1978; Bangma and Donkelaar, 1983; Donkelaar et al., 1983; present study). These characteristics are most similar to the medial vestibular nucleus in chicks (Wold, 1979a). Interestingly, based on its position in the avian brainstem and cytoarchitecture, the avian medial vestibular nucleus is similar to the medial vestibular nucleus of mammals (Brodal and Pompeiano, 1957; Sadjadpour and Brodal, 1968).

Although V_{etg} is evident in birds, there is little evidence for it in reptiles. The V_{etg} nucleus is difficult to delimit in mallards, pigeons and lizards (Donkelaar et al., 1987; Dickman and Fang, 1996; Tellegen et al., 2001) due to its small size and concealment by VN fibers. In chicks, the vestibular fibers with the largest diameters were found to form large axosomatic endings on the V_{etg} principal cells (Peusner, 1980, 1981, 1984), whereas the smaller vestibular fibers formed synaptic terminals primarily on the dendrites (Cox and Peusner, 1990; Dickman and Fang, 1996). In reptiles, no study to date shows anatomically how primary vestibular nerves project to V_{etg}, which is only identified by location and the staining pattern of its neurons. V_{etg} in birds receives VN projection from both semicircular canals and otoliths (Dickman and Fang, 1996; Popratiloff and Peusner, 2007). Although we were able to discriminate nucleus V_{etg} in geckos, its function and connections in reptiles remain unknown.

In mammals, the descending vestibular nucleus is recognized on the basis of position in the brainstem and by the existence of numerous longitudinally running fibers. A similar nucleus exists in birds; however, it is not known if the avian descending vestibular nucleus is characterized by both efferent and afferent connections with the cerebellum, as is the case in mammals (Brodal, 1972; Walberg, 1972). Both the turtles' and chicks' nucleus descendens project to the contralateral spinal cord while mallards' descendens project mainly to the ipsilateral cord (Wold, 1979a, b; Bangma and Donkelaar, 1983; Tellegen et al., 2001). It is

reasonable to hypothesize that the nucleus descendens is an evolutionarily conserved structure in the amniote vestibular system. It is notable that in the Tokay gecko, Veds is extremely large and occupies much of the lateral brainstem. In contrast, Veds occupies a far smaller portion of the brainstem in turtles (Bangma and Donkelaar, 1983). Since cells in Veds are uniform in size and similar in shape in tokays and distributed evenly in the nucleus, the large size of the nucleus indicates that more cells are available for locomotor control. In view of these connections with the spinal cord, we hypothesize that the large size of Veds in geckos may correlate with the enhanced climbing and locomotor ability of this species.

VeO was first identified as a vestibular nucleus in a lesion study of the VIIIth nerve in iguana lizards (Foster and Hall, 1978), which might be duplicated with tract tracing technology in monitor lizards (Barbas-Henry and Wouterlood, 1988). Significantly, injection of NB to the midbrain vestibular nucleus labeled neurons in VeO, suggesting its ascending projection. Our previous study showed that injection of anatomical tracers into the auditory nerve does not result in labeled terminals and/or neurons in VeO (Christensen-Dalsgaard et al., 2011). The homologs of VeO have not been described in birds, and its functions remain unknown in lizards.

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References

- Aerts P, Van Damme R, Vanhooydonck B, Zaaf A, Herrel A. Lizard locomotion: how morphology meets ecology. *Neth. J. Zool.* 2000; 50:261–277.
- Autumn K, Peattie AM. Mechanisms of adhesion in geckos. *Integr. Comp. Biol.* 2002; 42:1081–1090. [PubMed: 21680391]
- Baizer JS, Baker JF. Immunoreactivity for calcium-binding proteins defines subregions of the vestibular nuclear complex of the cat. *Exp. Brain Res.* 2005; 164:78–91. [PubMed: 15662522]
- Bangma G, Donkelaar HJT. Some afferent and efferent connections of the vestibular nuclear complex in the red-eared turtle *Pseudemys scripta elegans*. *J. Comp. Neurol.* 1983; 220:453–464. [PubMed: 6643738]
- Barbas-Henry HA, Lohman AHM. Primary projections and efferent cells of the VIIIth cranial nerve in the monitor lizard, *Varanus exanthematicus*. *J. Comp. Neurol.* 1988; 277:234–249. [PubMed: 2466058]
- Barbas-Henry HA, Wouterlood FG. Synaptic connections between primary trigeminal afferents and accessory abducens motoneurons in the monitor lizard, *Varanus exanthematicus*. *J. Comp. Neurol.* 1988; 267:387–397. [PubMed: 3343407]
- Barmack NH. Central vestibular system: vestibular nuclei and posterior cerebellum. *Brain Res. Bull.* 2003; 60:511–541. [PubMed: 12787870]
- Baumert M, Maycox P, Navone F, De Camilli P, Jahn R. Synaptobrevin: an integral membrane protein of 18,000 daltons present in small synaptic vesicles of rat brain. *EMBO J.* 1989; 8:379–384. [PubMed: 2498078]
- Baurle J, Grusser-Cornehls U. Calbindin D-28k in the lateral vestibular nucleus of mutant mice as a tool to reveal Purkinje cell plasticity. *Neurosci. Lett.* 1994; 167:85–88. [PubMed: 8177535]

- Belekhova M, Kenigfest N, Karamian O, Vesselkin N. Distribution of calcium-binding proteins in the central and peripheral regions of the turtle mesencephalic center torus semicircularis. *Dokl Biol. Sci.* 2004; 399:451–454. [PubMed: 15717605]
- Belekhova M, Chudinova T, Kenigfest N, Krasnoshchekova E. Distribution of metabolic activity (cytochrome oxidase) and immunoreactivity to calcium-binding proteins in the turtle brainstem auditory nuclei. *J. Evol. Biochem. Physiol.* 2008; 44:354–364.
- Braun K. Calcium-binding proteins in avian and mammalian central nervous system: localization, development and possible functions. *Prog. Histochem. Cytochem.* 1990; 21:1–64. [PubMed: 2198609]
- Brodal A. Some features in the anatomical organization of the vestibular nuclear complex in the cat. *Prog. Brain Res.* 1972; 37:31–53. [PubMed: 4642045]
- Brodal A, Pompeiano O. The vestibular nuclei in the cat. *J. Anat.* 1957; 91:438–454. [PubMed: 13475144]
- Celio M. Calbindin D-28k and parvalbumin in the rat nervous system. *Neuroscience.* 1990; 35:375–475. [PubMed: 2199841]
- Christensen-Dalsgaard J, Tang Y, Carr CE. Binaural processing by the gecko auditory periphery. *J. Neurophysiol.* 2011; 105:1992–2004. [PubMed: 21325679]
- Cox RG, Peusner KD. Horseradish peroxidase labeling of the central pathways in the medulla of the ampullary nerves in the chicken, *Gallus gallus*. *J. Comp. Neurol.* 1990; 297:564–581. [PubMed: 1696590]
- Cullheim S, Ulfhake B. Relations between cell body size, axon diameter and axon conduction velocity of triceps surae α -motoneurons during the postnatal development in the cat. *J. Comp. Neurol.* 1979; 188:679–686. [PubMed: 521509]
- Dememes D, Eybalin M, Renard N. Cellular distribution of parvalbumin immunoreactivity in the peripheral vestibular system of three rodents. *Cell. Tissue Res.* 1993; 274:487–492. [PubMed: 8293446]
- Desai SS, Zeh C, Lysakowski A. Comparative morphology of rodent vestibular periphery. I. Saccular and utricular maculae. *J. Neurophysiol.* 2005a; 93:251–266. [PubMed: 15240767]
- Desai SS, Ali H, Lysakowski A. Comparative morphology of rodent vestibular periphery. II. Cristae ampullares. *J. Neurophysiol.* 2005b; 93:267–280. [PubMed: 15240768]
- Dickman JD, Fang Q. Differential central projections of vestibular afferents in pigeons. *J. Comp. Neurol.* 1996; 367:110–131. [PubMed: 8867286]
- Donkelaar H, Bangma G, Boer-van Huizen R. Reticulospinal and vestibulo-lospinal pathways in the snake *Python regius*. *Anat. Embryol.* 1983; 168:277–289. [PubMed: 6606996]
- Donkelaar, HJ.; Bangma, GC.; Barbas-Henry, HA.; de Boer-van Huizen, R.; Walters, JG. The brain stem in a lizard, *Varanus exanthematicus*. In: Beck, F.; Hild, W.; Kriz, W.; Ortman, R.; Pauly, JE.; Schiebler, TH., editors. *Advances in Anatomy, Embryology and Cell Biology*. Berlin: Springer-Verlag; 1987. p. 1-168.
- Ellis J, Richards D, Rogers J. Calretinin and calbindin in the retina of the developing chick. *Cell. Tissue Res.* 1991; 264:197–208. [PubMed: 1878940]
- Foster RE, Hall WC. The organization of central auditory pathways in a reptile, *Iguana iguana*. *J. Comp. Neurol.* 1978; 178:783–831. [PubMed: 632382]
- Hack NJ, Wride MC, Charters KM, Kater SB, Parks TN. Developmental changes in the subcellular localization of calretinin. *J. Neurosci.* 2000; 20:1–5. [PubMed: 10627575]
- Hursh JB. Conduction velocity and diameter of nerve fibers. *Am. J. Physiol.* 1939; 127:131–139.
- Jusufi A, Goldman DI, Revzen S. Active tails enhance arboreal acrobatics in geckos. *Proc. Natl. Acad. Sci. U. S. A.* 2008; 105:4215–4219. [PubMed: 18347344]
- Kern A, Seidel K, Oelschläger H. The central vestibular complex in dolphins and humans: functional implications of Deiters' nucleus. *Brain Behav. Evol.* 2009; 73:102–110. [PubMed: 19390175]
- Kevetter GA. Pattern of selected calcium-binding proteins in the vestibular nuclear complex of two rodent species. *J. Comp. Neurol.* 1996; 365:575–584. [PubMed: 8742303]
- Kevetter GA, Leonard RB. Use of calcium-binding proteins to map inputs in vestibular nuclei of the gerbil. *J. Comp. Neurol.* 1997; 386:317–327. [PubMed: 9295155]

- Lohmann C, Friauf E. Distribution of the calcium-binding proteins parvalbumin and calretinin in the auditory brainstem of adult and developing rats. *J. Comp. Neurol.* 1996; 367:90–109. [PubMed: 8867285]
- Peusner K. Developmental changes of synaptic endings in the nucleus vestibularis tangentialis of the chicken: degeneration of spoon endings. *Dev. Neurosci.* 1980; 3:266–276. [PubMed: 7460798]
- Peusner K. An ultrastructural study of the development of synaptic endings in the nucleus vestibularis tangentialis of the chick embryo. *Neuroscience.* 1981; 6:2335–2350. [PubMed: 7329550]
- Peusner KD. The development of synapses and “spoon” synaptic terminal space in the tangential vestibular nucleus: a quantitative electron microscope study. *J. Comp. Neurol.* 1984; 230:372–385. [PubMed: 6520239]
- Popratiloff A, Peusner KD. Otolith fibers and terminals in chick vestibular nuclei. *J. Comp. Neurol.* 2007; 502:19–37. [PubMed: 17335047]
- Rajakumar N, Elisevich K, Flumerfelt B. Biotinylated dextran: a versatile anterograde and retrograde neuronal tracer. *Brain Res.* 1993; 607:47–53. [PubMed: 7683244]
- Ramprasad F, Landolt J, Money K, Laufer J. Comparative morphometric study of the vestibular system of the vertebrata: Reptilia, Aves, Amphibia, and Pisces. *Acta Otolaryngol. Suppl.* 1986; 427:1–42. [PubMed: 3485883]
- Raymond J, Dechesne CJ, Desmadryl G, Dememes D. Different calcium-binding proteins identify subpopulations of vestibular ganglion neurons in the rat. *Acta Otolaryngol.* 1993; 113:114–118.
- Rogers JH. Calretinin: a gene for a novel calcium-binding protein expressed principally in neurons. *J. Cell. Biol.* 1987; 105:1343–1353. [PubMed: 3654755]
- Rogers JH. Two calcium-binding proteins mark many chick sensory neurons. *Neuroscience.* 1989; 31:697–709. [PubMed: 2594198]
- Sadjadpour K, Brodal A. The vestibular nuclei in man. A morphological study in the light of experimental findings in the cat. *J. Hirnforsch.* 1968; 10:299–323. [PubMed: 5732949]
- Sans A, Etchecopar B, Brehier A. Immunocytochemical detection of vitamin D-dependent calcium-binding protein (CaBP-28K) in vestibular sensory hair cells and vestibular ganglion neurones of the cat. *Brain Res.* 1986; 364:190–194. [PubMed: 3512032]
- Schwab ME. Variation in the rhombencephalon. *Biol. Reptilia.* 1979; 10:201–246.
- Schwarz DWF, Schwarz IE. Projection of afferents from individual vestibular sense organs to the vestibular nuclei in the pigeon. *Acta Otolaryngol.* 1986; 102:463–473. [PubMed: 3788546]
- Straka H, Holler S, Goto F, Kolb FP, Gilland E. Differential spatial organization of otolith signals in frog vestibular nuclei. *J. Neurophysiol.* 2003; 90:3501–3512. [PubMed: 12853438]
- Takahashi TT, Carr CE, Brecha N, Konishi M. Calcium binding protein-like immunoreactivity labels the terminal field of nucleus laminaris of the barn owl. *J. Neurosci.* 1987; 7:1843–1856. [PubMed: 2439666]
- Tang YZ, Zhuang LZ, Wang ZW, McEachran J. Advertisement calls and their relation to reproductive cycles in *Gekko gecko* (Reptilia, Lacertilia). *Copeia.* 2001; 2001:248–253.
- Tellegen AJ, Arends JJA, Dubbeldam JL. The vestibular nuclei and vestibuloreticular connections in the mallard (*Anas platyrhynchos* L.). *Brain Behav. Evol.* 2001; 58:205–217. [PubMed: 11964497]
- Walberg F. Descending and reticular relations to the vestibular nuclei: anatomy. *Prog. Brain Res.* 1972; 37:585–588. [PubMed: 4642055]
- Wold JE. The vestibular nuclei in the domestic hen (*Gallus domesticus*). I. Normal anatomy. *Anat. Embryol.* 1976; 149:29–46. [PubMed: 1267187]
- Wold JE. The vestibular nuclei in the domestic hen (*Gallus domesticus*). III. Ascending projections to the mesencephalic eye motor nuclei. *J. Comp. Neurol.* 1978; 179:393–405. [PubMed: 641224]
- Wold JE. The vestibular nuclei in the domestic hen (*Gallus domesticus*). VII. Afferents from the spinal cord. *Arch. Ital Biol.* 1979a; 117:30–44. [PubMed: 554568]
- Wold JE. The vestibular nuclei in the domestic hen: V. Commissural connections. *Exp. Brain Res.* 1979b; 34:217–232. [PubMed: 421748]
- Yan K, Tang YZ, Carr CE. Calcium-binding protein immunoreactivity characterizes the auditory system of *Gekko gecko*. *J. Comp. Neurol.* 2010; 518:3409–3426. [PubMed: 20589907]

Zaaf A, Van Damme R. Limb proportions in climbing and ground-dwelling geckos (Lepidosauria, Gekkonidae): a phylogenetically informed analysis. *Zoomorphology*. 2001; 121:45–53.

Fig. 1. Photomicrographs illustrating labeled vestibular nerve fibers (A–E) and an NB tracer injection site in the midbrain (F). (A–C) vestibular nerves were labeled with antisera against, in order, CR, PV and CB. (D) Horizontal section showing vestibular nerve fibers labeled by a microinjection of NB into the 8th nerve. (E) Horizontal section through the brainstem at a level 500 μm ventral to the brainstem surface showing neurons and terminals labeled with antisera against CB. (F) The injected site in the midbrain centered within the oculomotor nucleus. Scale bar = 200 μm in A–C; scale bar = 100 μm in D; scale bar = 150 μm in E;

scale bar = 300 μm in F. Note: except for Figs. 1D, 1E and 3, all sections are coronal orientation.

Fig. 2.

Locations and shapes of all vestibular nuclei stained with Nissl method in the Tokay brainstem. (A) Rostrally, the vestibular nuclei include Vedl, Cerl and Cerm. (B) The rostromedial brainstem contains the auditory nucleus NA and the vestibular nuclei Vetg, Vevl and Vevm. (C) The caudomedial brainstem includes the auditory nucleus NM and the vestibular nuclei VeO, Vetg, Vevl, Vevm and Veds. (D) Caudally, most of the area was occupied by Veds. Scale bar = 200 μ m.

Fig. 3. Fibers and nuclei of the brainstem vestibular and auditory systems at different levels using antisera against CB. (A) The horizontal photomicrograph captured around 200 μm beneath top surface. (B) The horizontal photomicrograph around 400 μm beneath top surface. (C) The horizontal photomicrograph around 1300 μm from top surface. Scale bar = 150 μm.

Fig. 4. Position, shape and cytoarchitecture of Cerl and Vedl. (A) Cerl labeled after injection of CTX to the VN branch. (B) Monopolar cells labeled by NB injection to the nucleus oculomotorius. (C) Section labeled using antisera against CR and (D) against CB. (E) Vedl was labeled by injection of CTX to VN. (F) Neurons of Vedl are labeled by injection of NB to the midbrain. Scale bar = 50 μm in A, E; scale bar = 25 μm in B; scale bar = 200 μm in C, D; scale bar = 10 μm in F.

Fig. 5. Position, shape and cytoarchitecture of Vevl. (A) Vevl labeled with antisera against PV. (B) Vestibular afferent fibers terminated in rostral and caudal levels of Vevl. (C) Vevl received projections of medial descending branch of VN labeled by injection of CTX to the nerve. (D) Cell shapes and sizes in Vevl, with injection of NB to the nucleus oculomotorius. (E) Vevl cells were labeled with antisera against CB and (F) against SV2. The black arrowhead indicates synapses distributing perisomatically while the white arrowhead shows some dendritical synapses in (F). Scale bar = 200 μ m in A, E; scale bar = 50 μ m in B-D, F.

Fig. 6. Labeled terminals and neurons of Vetg and Vevm. (A) NB injection to the midbrain labeled neurons in Vetg. (B) CTX injection to VN labeled terminals in Vetg. (C) Vevm cells were labeled after injection of NB to the oculomotorius nucleus. (D) Vevm was labeled after injection of CTX tracers to VN. Scale bar = 25 μ m in A; scale bar = 50 μ m in B–D.

Fig. 7.
Position, shape and cytoarchitecture of VeO. (A–C) sections labeled with antisera against CR, PV and CB, respectively. (D) Injection of NB to the midbrain illustrates the cytoarchitecture of VeO. Scale bar = 200 μm in A–C, scale bar = 10 μm in D.

Fig. 8. Brainstem sections displaying features of Veds. (A) Nucleus labeled with antisera against CR, (B) in a posterior position than (A). (C) The nucleus was labeled with an injection of CTX to VN and (D) NB to the vestibular midbrain. Scale bar = 100 μ m in A, B; scale bar = 50 μ m in C and 10 μ m in D.

Table 1

List of abbreviations used in the text and figures.

AN	Auditory nerve
CB	Calbindin
cereb	Cerebellum
Cerl	Nucleus cerebellaris lateralis
Cerm	Nucleus cerebellaris medialis
CR	Calretinin
CR	Calretinin
CTX	Cholera toxin
ir	Immunoreaction
mlf	Medialis longitudinalis fasciculus
NA	Nucleus angularis
NB	Neurobiotin
NL	Nucleus laminaris
NM	Nucleus magnocellularis
nV	Nervus trigeminus
nVIII	Nervus octavus
Pb	Nucleus parabrachialis
PBS	Phosphate buffer solution
Phy	Perihypoglossal nuclear complex
PV	Parvalbumin
SOC	Superior Olivary Complex
SV2	Synaptic vesicle protein 2
TS	Torus semicircularis
Vedl	Nucleus vestibularis dorsolateralis
Veds	Nucleus vestibularis descendens
VeO	Nucleus vestibularis ovalis
Vetg	Nucleus vestibularis tangentialis
VeS	Nucleus vestibularis superior
Vevl	Nucleus vestibularis ventrolateralis
Vevm	Nucleus vestibularis ventromedialis
VN	Vestibular nerve