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Physiological and anatomical investigation of the auditory brainstem in the Fat-tailed Dunnart (*Sminthopsis crassicaudata*)

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The fat-tailed Dunnart (*Sminthopsis crassicaudata*) is a small (10-20g) native marsupial endemic to the south west of Western Australia. Currently little is known about the auditory capabilities of the dunnart, and of marsupials in general. Consequently, this study sought to investigate several electrophysiological and anatomical properties of the dunnart auditory system. Auditory brainstem responses (ABR) were recorded to brief (5ms) tone pips at a range of frequencies (4-47.5 kHz) and intensities to determine auditory brainstem thresholds. The dunnart ABR displayed multiple distinct peaks at all test frequencies, similar to other mammalian species. ABR showed the dunnart is most sensitive to higher frequencies increasing up to 47.5 kHz. Morphological observations (Nissl stain) revealed that the auditory structures thought to contribute to the first peaks of the ABR were all distinguishable in the dunnart. Structures identified include the dorsal and ventral subdivisions of the cochlear nucleus, including a cochlear nerve root nucleus as well as several distinct nuclei in the superior olivary complex, such as the medial nucleus of the trapezoid body, lateral superior olive and medial superior olive. This study is the first to show functional and anatomical aspects of the lower part of the auditory system in the Fat-tailed Dunnart.

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Physiological and anatomical investigation of the auditory brainstem in the Fat-tailed Dunnart (*Sminthopsis crassicaudata)*

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Abstract

The fat-tailed Dunnart (*Sminthopsis crassicaudata*) is a small (10-20g) native marsupial endemic to the south west of Western Australia. Currently little is known about the auditory capabilities of the dunnart, and of marsupials in general. Consequently, this study sought to investigate several electrophysiological and anatomical properties of the dunnart auditory system. Auditory brainstem responses (ABR) were recorded to brief (5ms) tone pips at a range of frequencies (4-47.5 kHz) and intensities to determine auditory brainstem thresholds. The dunnart ABR displayed multiple distinct peaks at all test frequencies, similar to other mammalian species. ABR showed the dunnart is most sensitive to higher frequencies increasing up to 47.5 kHz. Morphological observations (Nissl stain) revealed that the auditory structures thought to contribute to the first peaks of the ABR were all distinguishable in the dunnart. Structures identified include the dorsal and ventral subdivisions of the cochlear nucleus, including a cochlear nerve root nucleus as well as several distinct nuclei in the superior olivary complex, such as the medial nucleus of the trapezoid body, lateral superior olive and medial superior olive. This study is the first to show functional and anatomical aspects of the lower part of the auditory system in the Fat-tailed Dunnart.

Keywords: cochlear nucleus, superior olivary nuclei, auditory brainstem response, hearing, marsupial

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Introduction

Marsupials evolved separately from eutherian mammals in the Cretaceous period and now form a highly diverse group with populations in the Americas and Australia (Luo, Yuan, Meng, & Ji, 2011; Nilsson et al., 2010). One marsupial, the fat-tailed dunnart (*Sminthopsis crassicaudata*), is a small (10-20g) insectivorous Australian marsupial (Frey, 1991; Morton, 1978a) that is named after its characteristic swollen tail that contains stored fat (Godfrey, 1968). The fat-tailed dunnart is a solitary animal with a widespread distribution across the southern and western parts of Australia inhabiting a variety of arid environments including open woodland, low scrublands, grasslands on clay or sand soils and farmlands (Morton, 1978a). Within these varied environments, the nocturnal dunnart hunts predominantly insects while itself being preyed upon by other predators such as snakes, feral cats and barn owls (Morton, 1978b)

Interestingly, the visual system in the fat-tailed dunnart has been shown to be different from most other marsupials as well as most eutherian mammals as they are trichromatic (Cowing, Arrese, Davies, Beazley, & Hunt, 2008; Ebeling, Natoli, & Hemmi, 2010). Being predominantly nocturnal (Levy, Dayan, Porter, & Kronfeld-Schor, 2019) the fat-tailed dunnart is likely to also heavily depend on its sense of hearing and its ability to localise sound as a means for prey detection, predator avoidance and species-specific communication (Osugi, Foster, Temple, & Poling, 2011). Previous work in a range of marsupial families such as northern quoll (Dasyurus hallucatus) (Aitkin, Nelson, & Shepherd, 1996), brush-tailed possums (Trichosurus vulpecula) (Signal, Foster, & Temple, 2001), and the tammar wallaby (Macropus eugenii) (Liu, 2003; Liu, Hill, & Mark, 2001) has shown that the overall structure of the auditory brainstem is largely consistent within eutherian mammals, enabling the distinction of several subnuclei in cochlear nuclei (CN), superior olivary complex (SOC) and inferior colliculus (Aitkin, 1998).

However, the relative size of the subcortical structures in the auditory system is known to be highly varied both in eutherian mammals and marsupials (Glendenning & Masterton, 1998). For example, the CN represents about 13% of the whole auditory system in the swamp wallaby, but approximately 37% in the pocket gopher. In addition, there exists a large degree of heterogeneity in the anatomical architecture of the CN and principal nuclei of the SOC (Glendenning &

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Masterton, 1998). For example, in some of the Muridae such as rat, mouse and gerbil (López, Merchán, Bajo, & Saldaña, 1993)as well as in some marsupials (Willard, 1993) the auditory nerve contains a small group of large neurons, the so-called cochlear nerve root neurons, whereas this does not appear to be the case in for instance cat or guinea pig. In the SOC, the lateral superior olive (LSO) forms a S-shaped segment in many species such as guinea pig, cat and gerbil (Grothe & Park, 2000) but has been described as a triangle shape in marsupials (Aitkin 1996).

With regard to functional studies, the auditory brainstem response (ABR) has been shown to reveal the typical waveforms (i.e. waves I-V present) between 1-90kHz with lowest thresholds between 12-16kHz in the short-tailed opossum (Monodelphus domestica) (Reimer, 1996b). Click-evoked ABRs obtained from tammar wallaby also showed typical peaks and the appearance of the peaks during development correlated with the development of the known anatomical substrates of the ABR waves (Liu, 2003; Liu, Hill, & Mark, 2001).

With the exception of a few references to the striped-faced dunnart (Sminthopsis macroura) by Aitkin (1998) very little is known about the anatomy and physiology of the dunnart auditory system. In view of the fact that the fat-tailed dunnart has specific adaptations in its visual system, this paper explored functional and anatomical aspects of its auditory system to investigate whether this sensory system also has distinct features compared to other marsupials. For this purpose, we combined electrophysiological (ABR) and anatomical (Nissl staining) investigations of the auditory brainstem in the dunnart. For the latter we focussed on cochlear nucleus and the main nuclei in the SOC, known to be involved in sound localization.

Materials and Methods

Animals

Eight fat-tailed dunnarts (*Sminthopsis crassicaudata*) aged between 12 and 18months (12-18g weight) of either sex were used for this study. Precise age was not known but was estimated based on arrival in the animal facilities, weight and time of experimentation. The animals were separately housed in enriched cages containing running discs, rocks and a covered nest. Food

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(Science Diet Sensitive Stomach Cat Food supplemented with live crickets and mealworms) and water were supplied ad libitum. The vivariums were maintained at 22^{o} C with a 12-hour Day night cycle. All procedures conformed to NIH guidelines on the use of animals for experimentation (USA) and were approved by the University of Western Australia's Animal Ethic Committee (RA/3/100/1123).

Auditory Brainstem Response Measurements

The fat-tailed dunnarts were anaesthetised via intraperitoneal injection with Ketamine (75mg/kg) and Medetomidine (1mg/kg). Animals were maintained at near physiological temperature (38°C) using both a heating pad and an ambient room heater for the entirety of the auditory brainstem response (ABR) recording (60-90 minutes per animal). ABRs were measured as previously described (Yates, Robertson, Martin-Iverson, & Rodger, 2014). In brief, ABRs were recorded in a sound attenuated room and sound stimuli were generated by custom made Neurosound software (M. Lloyd Cambridge) via a RME DIGI 9636 sound card (96 kHz sampling rate). Average ABRs (n=400 stimuli) were evoked using pure tone bursts (5ms duration, 1ms rise-fall-time, rate 10/s), delivered to the animal using a plastic cone attached to a reverse driven ¼ inch condenser microphone (Bruel and Kjaer type 4134). The acoustic coupler was placed using a surgical microscope to touch the lower edge of the left tragus and was directed towards external auditory meatus. During the course of the experiments, we observed no movement of the animal or auditory coupler.

ABR responses were recorded via an insulated silver-wire electrode inserted subdermally at the vertex. A reference electrode was placed above the left mastoid at the base of the pinna and a ground electrode was inserted into the tail. Differential recordings were made using an AC coupled amplifier (DAM50, World Precision Instruments) with a gain of 1000x and band pass filtering at (300-3000Hz). Average ABR responses were sampled by Powerlab/4ST (AD Instruments) and stored for offline analyses.

ABR thresholds were determined at 4, 8, 16, 24, 32 and 47.5 kHz. In view of the sampling rate of our sound card 47.5 kHz was the maximum frequency tested. Each sound stimulus was presented first at 10dB attenuation followed by sound intensities decreasing in 10dB increments

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until after the disappearance of overt ABR peaks (I and V) in the recording. Upon disappearance of the ABR, the sound intensity was increased in 5dB steps until the visual reappearance of the peaks in the waveform. Sound stimuli were converted into sound pressure (SPL, re 20µPa) levels using a Bruel and Kjaer pistonphone (94dB SPL at 1000Hz). ABR traces were analyzed using AxoGraph X V1.5.0 (J. Clements, Australia) and thresholds were determined by visual inspection. ABR threshold was estimated as the lowest intensity where peaks I and V could still be identified. The threshold estimation procedure employed here, was undertaken by 3 different observers and yielded consistent estimates.

Histological preparation

Dunnarts were terminally anaesthetised with 0.2ml Euthal (pentobarbitone sodium 170mg/mL, phenytoin sodium 25mg/mL). Animals were then perfused with saline (0.9%) followed by paraformaldehyde (4% in 0.1M phosphate buffered saline, PBS). Regions of brainstem containing auditory nuclei were removed and cryoprotected (30% sucrose in 0.1M PBS for 24h) and sectioned at 30µm using a cryostat (Leica CM1900).

For cresyl violet staining, horizontal sections were washed with PBS for four minutes and then dehydrated in graded ethanol solutions (70% - 95%, one minute). Slides were heated in a microwave for 2 minutes in a 500mL solution of 95% ethanol and 5% Glacial acetic acid (Sigma), followed by rehydration in descending ethanol solutions (95% to 70%, 20 seconds each) and washed in PBS for one minute. Sections were then placed in warmed Cresyl Violet solution (0.5% Cresyl Violet) for eight minutes. After staining, sections were rapidly exchanged through ascending ethanol solutions (70%-95%, 15 seconds each) and differentiated at room temperature in 95% ethanol and 5% acetic acid for 5 minutes. Finally, slides were washed with three 100% ethanol and cleared in xylene. Slides were cover-slipped with DePeX (ProSciTech) mounting media and dried overnight prior to microscopy.

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Microscopy and analysis

Images of Cresyl Violet stained sections were captured using an Olympus DP70 camera and DP Controller (Olympus Corporation, image size 4080x3072pixels). High-power micrographs were captured using a Nikon DS-U2/L2 camera with NIS-Elements (Nikon AR 3.0, image size 2560x1920pixels). Using standard anatomical markers such as neuronal shape, neuronal density, and somatic alignment, the auditory nuclei (CN and SOC) were identified in the dunnart. Nuclei were observed under low power to determine the area and extent of the nucleus. Images for publication underwent minor adjustments in brightness and contrast.

Results

Auditory Brainstem Response

A typical ABR was observed in the fat-tailed dunnart (figure 1). At moderate to high sound intensities, the ABR showed five distinct peaks within the first 6ms after onset of the tone stimuli. ABRs were evoked at all frequencies tested in this study (between 4 and 47.5 kHz). ABR threshold was estimated as the lowest intensity where peak I and V could still be identified (typical example at 47.5kHz shown in figure 2a). Average thresholds (n= 6-8) depicted as audiograms (figure 2b) reveal the fat-tailed dunnart ABR is more sensitive (lower thresholds) with increasing frequency. Currently however, it cannot be established whether 47.5 kHz is the most sensitive frequency or if ABR thresholds decline rapidly at higher frequencies.

In agreement with the known characteristics of ABR responses (Reimer, 1996), peak I amplitudes increased with increasing sound intensity (figure 2c). Similarly, increasing sound intensities resulted in a shortening of ABR latencies (data for 4, 24 and 47.5kHz shown in figure 2d).

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Histological analysis

The cochlear nerve root and cochlear nuclei

Similar to other known marsupial species such as the brush-tailed possum and quoll, the cochlear nuclei (CN) reside medial to the restiform body (rb in figure 3a-c) (Aitkin, Byers, & Nelson, 1986; Aitkin & Kenyon, 1981). The ventral cochlear nucleus (VCN) as a whole is clearly identifiable in the dunnart (figure 3c,h) with round small closely packed cells of the anteroventral cochlear nucleus (AVCN) in rostral levels to the dorsal cochlear nucleus (DCN). A more sparsely populated posteroventral cochlear nucleus (PVCN) containing larger nuclei was observed in more caudally located sections (figure 3c,d,e,f).

On gross appearance, the DCN in the dunnart was a large trigonal nucleus that did not appear as densely packed with neurons as the mouse DCN (Godfrey et al., 2016). Throughout its extent, the prominent tri-laminar DCN could clearly be subdivided into a superficial (I in figure 3f), granule cell layer (II in figure 3f) and deep polymorphic layers (III in figure 3f). The DCN was bounded laterally by the small cell cap layer (scc, figure 3d, f).

The dunnart brainstem also shows a clearly defined cochlear nerve root nucleus (CNR) (figure 3e and g), consisting of large neurons clustered within the passing nerve fascicles. The CNR nucleus is similar in appearance not only to other marsupials such as brush-tailed possum (Aitkin, 1996) but also to rodents such as the rat (Merchan, Collia, Lopez, & Saldana, 1988).

The superior olivary complex nuclei

The nuclei of the superior olivary complex (SOC) in the dunnart closely resembled their anatomical correlates found in eutherian mammals. Of the three principal SOC nuclei lateral superior olive (LSO), medial superior olive (MSO), and the medial nucleus of the trapezoid body (MNTB), the most prominent and distinguishable nucleus in the dunnart was the MNTB (figure 4a,b). The MNTB occupied a familiar position within the brainstem and the cells of the MNTB were not densely packed presumably due to their location within the passing trapezoid body projection (see figure 4 b). A small MSO (typically observed within one to two histological sections) was observed as a linear cluster of pleiomorphic cells aligned along a dorsal-ventral

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axis (figure 4a-c). The gross appearance of the MSO (single linear nucleus) has been shown previously in arboreal marsupials (Aitkin, 1996).

The lateral superior olive (LSO) of the dunnart was not as well defined as found in similarly sized eutherian species (Ollo & Schwartz, 1979) (figure 4c). Despite this, the LSO was observed as a round nucleus located near the latero-ventral surface of the brainstem in transverse sections often containing the MNTB. Densely stained elongated cells occupied the periphery of the nucleus whereas lightly stained bipolar nuclei were found to occupy more central locations.

Discussion

Here we characterise some of the anatomical and electrophysiological features of the ascending auditory pathway in the fat-tailed dunnart. With the exception of Aitkin (1998), there has been very little characterisation of the dunnart auditory system, therefore we sought to establish normative values of the fat-tailed dunnart auditory system. In addition to identifying common auditory nuclei, we found that the anesthetised fat-tailed dunnart auditory system is remarkably sensitive to high frequency stimuli.

The ABR represents the average response to repetitive sound stimuli of neuronal populations in the auditory pathway. Waveform analysis of the ABR revealed 5 definite peaks (Reimer, 1996) with short latency, corresponding to the action-potential volleys from the auditory nerve through to inferior colliculus (Liu et al., 2001). In the current study, not only were we still able to evoke ABR responses to high frequency stimuli (47.5kHz), but ABR thresholds improved at high frequencies. These ABR findings are puzzling and present a contrast to the only previously published data from a dunnart species (*Sminthopsis macroaura*), which displayed a frequency range of 1-40kHz and a minimum, or best threshold at 10kHz (Aitkin, 1998). However, this study was limited by low animal numbers (n=2) and lack of detail in the methodology, making it unclear whether 40 kHz was the highest frequency attempted.

Nonetheless, high frequency sensitivity is quite common in small non-echolocating mammals such as the leaf-eared mouse and spiny mouse (Heffner, Koay, & Heffner, 2001). In fact, upon closer inspection of cochlear and ABR audiograms taken from several rodent species including

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the mouse (*Mus musculus*), a second local minimum is present (20-30dB SPL) at around 50kHz (Ehret, 1976; Heffner et al., 2001), and similarly, secondary local minima are also found in echo-locating mammals (~15dB SPL at >45kHz) (Koay, Heffner, & Heffner, 1998).

With the exception of the cat (*Felis catus*), animals with smaller head sizes have small functional interaural distances and tend to have higher audible frequencies (Heffner et al., 2001; Koay et al., 1998). In agreement with this, another marsupial, the northern quoll (*Dasyurus hallucatus*) which is larger than the dunnart (adults 400g, 5cm snout-ear), is most sensitive at 10kHz (10dB SPL) with rapid loss of sensitivities at 40kHz (50-80dB SPL) (Aitkin, Nelson, & Shepherd, 1994; Oakwood, 2002). Similarly, the Brazilian short-tailed opossum (*Monodelphis domestica*) also a marsupial larger than the fat-tailed dunnart (rat-size) shows best thresholds between 8 and 12 kHz (20 dB SPL) and an upper audible frequency limit of 60kHz (Reimer, 1995). Therefore, given its small size (12-18g), the high frequency sensitivity observed in the fat-tailed dunnart may be in line with its size, but conflicts with the limited data from the stripe faced dunnart (Aitkin, 1998), which is of similar size. Therefore, we cannot exclude the possibility that this audiogram of the fat-tailed dunnart represents a specific adaptation to its auditory environment, in line with the specific adaptation found in its visual system (Cowing et al., 2008; Ebeling et al., 2010). The reasons for such specialised adaptations within its sensory system remain unclear. As discussed in Ebeling et al. it may represent specific adaptations to the visual and auditory ecology or, alternatively, adaptations in early ancestors (Ebeling et al., 2010).

The anatomy of the auditory brainstem in the fat-tailed dunnart reveals a similar pattern of auditory nuclei as reported previously across a range of marsupials (Aitkin, 1998). The CNR is present in many small marsupials including the yellow-bellied glider (*Petaurus australis*), Northern quoll (Aitkin et al., 1986) but also in muridae (López et al., 1993; Merchan et al., 1988) While neurons in the CNR nucleus are considered as an extension of the ventral cochlear nucleus (Osen, Lopez, Slyngstad, Ottersen, & Storm-Mathisen, 1991), it projects to motor components of the pontine reticular and facial nuclei (Lopez, Saldana, Nodal, Merchan, & Warr, 1999). Although few in number, neurons in the CNR nucleus in the rat respond to sound and thus likely represent an initial auditory nucleus (Sinex, Lopez, & Warr, 2001). Given its early position within the auditory pathway, sensitivity to sound, and efferent projections to the pontine motor

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nuclei, the CNR nucleus is thought to play a role in sensorimotor control of acoustic startle responses (Lee, Lopez, Meloni, & Davis, 1996).

The auditory cochlear nuclei in the dunnart were similar in location to other marsupial species studied such as the brush-tailed possum (*Trichosurus vulpecula*) (Aitkin & Kenyon, 1981), multiple glider species (Aitkin, 1996), northern quoll (Aitkin et al., 1986). Also in agreement with other marsupials, the fat-tailed dunnart's trilaminar DCN was larger than the VCN (Aitkin, 1996, 1998). Despite widespread variation across mammalian species (Glendenning & Masterton, 1998; Illing, Kraus, & Michler, 2000), the organisation of the SOC was again largely consistent with previous reports. In common laboratory rodents, the three main SOC (LSO, MSO and MNTB) are known targets of the cochlear nuclei and it is likely that a similar connectivity exists in marsupials (Aitkin et al., 1986; Bazwinsky-Wutschke, Hartig, Kretzschmar, & Rubsamen, 2016). The presence of a MSO is not surprising as it is known to persist in almost all mammalian species analysed including the mouse (Fischl et al., 2016; Ollo & Schwartz, 1979). The MSO is involved in detecting interaural timing differences related to sound localization of lower frequencies (Grothe & Sanes, 1994). Therefore, it is likely that the functional role of the MSO in these small animals with high frequency sensitivity is relatively limited (Grothe & Pecka, 2014) and hence its small size in the fat-tailed dunnart is as expected. The LSO and MNTB, involved in detection of higher frequencies based on interaural level differences (Caird & Klinke, 1983; Grothe & Koch, 2011) were both present in the fat-tailed dunnart in line with its high frequency sensitivity. The relative size of the MNTB is known to vary between species, its relative size being about 5% of the subcortical auditory system in kangaroo rat and less than 1% in humans (Glendenning & Masterton, 1998). In addition, a study by Hilbig et al comparing different primates, showed a marked reduction in MNTB size from macaque to human (Hilbig, Beil, Hilbig, Call, & Bidmon, 2009). The MNTB in the fat-tailed dunnart was clearly distinguishable with large neurons comparable to the anatomy in rat (Reuss, Disque-Kaiser, De Liz, Ruffer, & Riemann, 1999). The LSO is often described as an S-shaped or horseshoe shaped nucleus in many species such as guinea pig, cat and gerbil (Grothe & Park, 2000). However, a distinct shape could not be observed in our histological material, rather the LSO boundary remained diffuse, in line with the description of Aitkin (1996) in some arboreal marsupials (Aitkin, 1996).

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While the presence of CN and SOC in the dunnart suggests an ability to process incoming auditory information particularly in terms of sound localisation, further investigations into the synaptic morphology, neurochemistry and electrophysiology would further help to refine our understanding of the roles these nuclei play within the dunnart and their environment.

Conclusions

Here we show that the fat-tailed dunnart is an animal species that displays a remarkable high frequency sensitivity. In addition, the auditory brainstem nuclei reveal a large and well developed CN as well as a MNTB. These nuclei are important in early binaural auditory processing and sound localisation, and their presence in the dunnart suggests similar processing capabilities. In addition to extending the ABR audiograms to higher frequencies, it would be of immediate interest to determine how the hearing sensitivities correspond to species specific communication as well as predator / prey detection and avoidance. (Aitkin et al., 1994). In light of recent reports on the role of the DCN in the analysis of vocalisations (Roberts & Portfors, 2015), it would be of interest to determine if the DCN performs a similar role in the marsupial.

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Figure legends

Figure 1. Characteristic ABR recording from the fat-tailed dunnart (tone burst indicated with black bar below the graph, 47.5 kHz, 5 ms duration, 52 dB SPL). Grey line represents the background noise from the recording equipment. Main peaks of ABR indicated by roman numerals and accompanied by abbreviated corresponding auditory nuclei. AN – auditory nerve, CV- cochlear nuclei, SOC – superior olivary nuclei, LL – lateral lemniscus, IC- inferior colliculus.

Figure 2. ABR thresholds in fat-tailed dunnart. (a): ABR recordings at 6 different intensities (42, 32, 22, 12, 7 and 2 dB SPL indicated right of waveforms) in response to a 47.5 kHz tone burst. Black bar indicates duration of tone burst. (b): Audiogram showing ABR thresholds at different frequencies. Individual animal thresholds are shown in grey with numbers in brackets above each point indicate number of animals per data point. Due to several animals with the same thresholds, the number of individual response points, may not appear to correspond with the number of animal in parenthesis (c): Input-output function of the peak I amplitude at 4, 24 and 47.5 kHz. (d) Input-output function of the latency of peak I at 4, 24 and 47.5 kHz. Each data point shows mean ± SEM. N.B. in panel (c) and (d) some of the points at very low sound intensity are the values derived from 1 or 2 animals.

Figure 3. Overview of the fat-tailed dunnart auditory brainstem. Nissl staining reveals prominent auditory nerve root nucleus and cochlear nuclei. Images are organised caudal to rostral. The dorsal cochlear nucleus resides medio-dorsal to the restiform body in the caudal regions (shown in a, with high power image in b). (c and d): More rostrally the ventral cochlear nucleus shows prominently as well. (d), (e), and (f): further rostral the trilaminar arrangement of the dorsal cochlear nucleus is clearly visible (f) as well as the cochlear nerve root nucleus (g). At more rostral level (h) the ventral cochlear nucleus shows a separation between posteroventral and anteroventral cochlear nucleus. Scale bars are 500µm in a, c, e, h and 200µm in b,d and f,g. Distance between panel a and c: 240µm, between c and e 90µm, and between e and h 210µm, Abbreviations: cnr – cochlear nerve root, cb – cerebellum, dcn – dorsal cochlear nucleus, fn –

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facial nucleus, rb – restiform body, avcn – anteroventral cochlear nucleus, pvcn- posteroventral cochlear nucleus.

Figure 4. The superior olivary complex (SOC) nuclei in the fat-tailed dunnart. The three main nuclei evident include the medial nucleus of the trapezoid body (MNTB) (a and b) residing within the fibres of the trapezoid body (tb marked in a). Located laterally to the MNTB is the linear medial superior olive (MSO) (a, b with outline in c). The lateral superior olive (LSO) (outline in c) can be seen lateral to the MSO. The boundary of the LSO shown in panel c is tentative and derive from alignment of neuronal somata across. Micrographs are taken at 2x (a) and 10x (b). Scale bars denote 1mm in a and 200µm in b, c. Abbreviations: lso – lateral superior olive, mntb – medial nucleus of the trapezoid body, mso – medial superior olive, tb – trapezoid body.

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Figure 1

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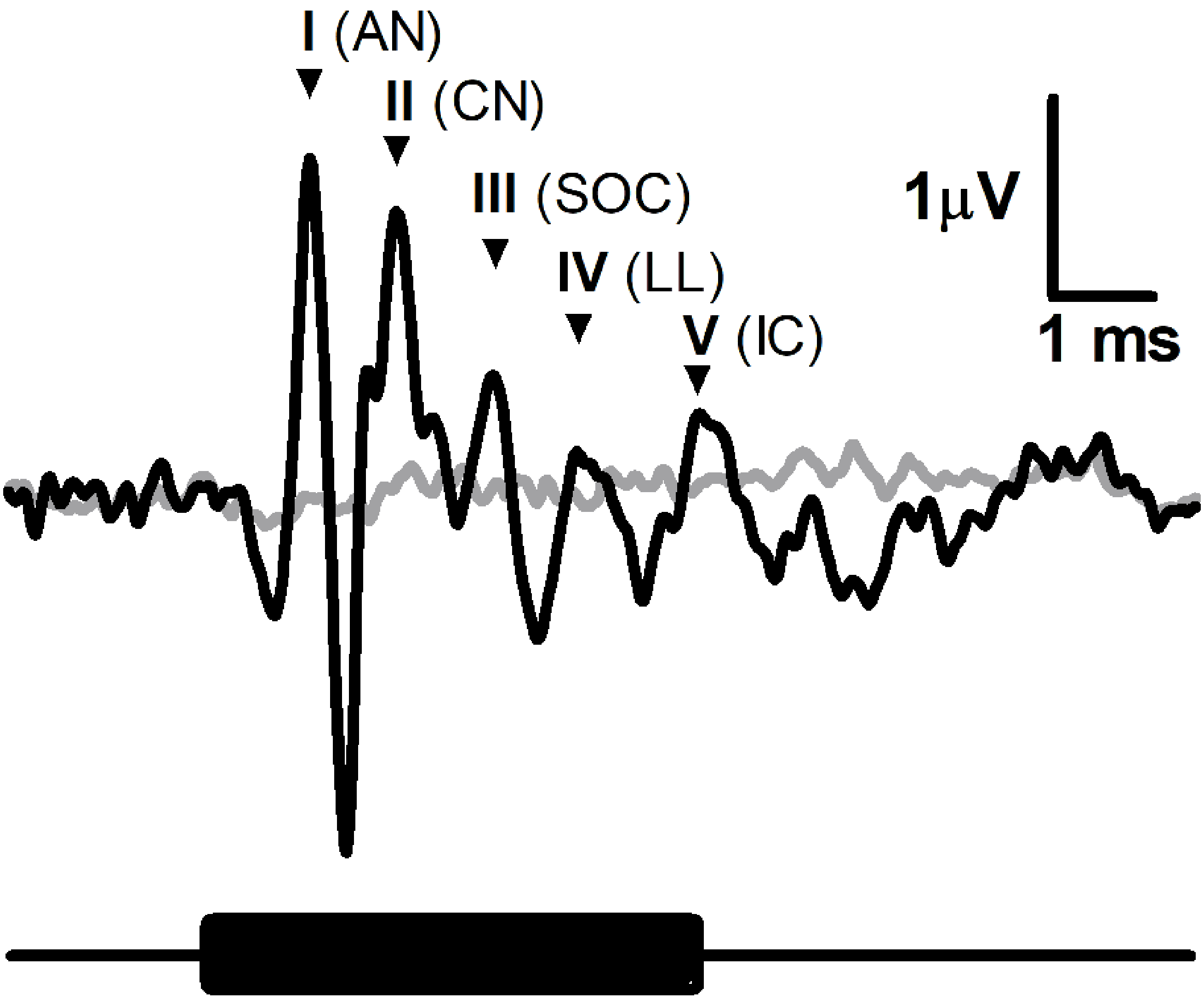

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Figure 2

ABR thresholds in fat-tailed dunnart.

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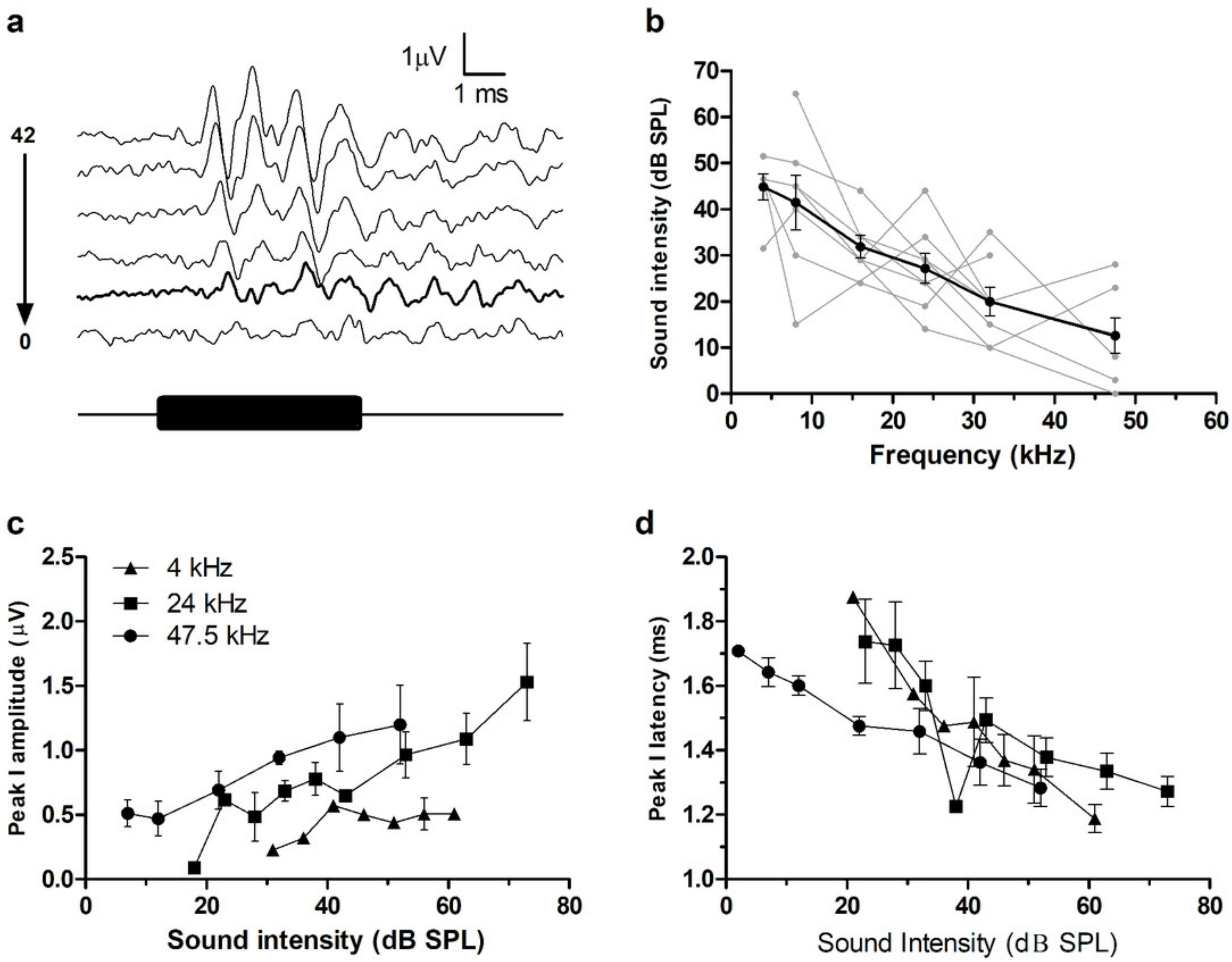

a
1μV
1 ms
42
0
b
Sound intensity (dB SPL)
Frequency (kHz)
c
4 kHz
24 kHz
47.5 kHz
Peak I amplitude (μV)
Sound intensity (dB SPL)
d
Peak I latency (ms)
Sound Intensity (dB SPL)

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Nissl staining reveals prominent auditory nerve root nucleus and cochlear nuclei. Images are organised caudal to rostral. The dorsal cochlear nucleus resides medio-dorsal to the restiform body in the caudal regions (shown in a, with high power image in b). (c and d): More rostrally the ventral cochlear nucleus shows prominently as well. (d), (e), and (f): further rostral the trilaminar arrangement of the dorsal cochlear nucleus is clearly visible (f) as well as the cochlear nerve root nucleus (g). At more rostral level (h) the ventral cochlear nucleus shows a separation between posteroventral and anteroventral cochlear nucleus. Scale bars are 500µm in a, c, e, h and 200µm in b,d and f,g. Distance between panel a and c: 240µm, between c and e 90µm, and between e and h 210µm, Abbreviations: cnr – cochlear nerve root, cb – cerebellum, dcn – dorsal cochlear nucleus, fn – facial nucleus, rb – restiform body, avcn – anteroventral cochlear nucleus, pvcn- posteroventral cochlear nucleus.

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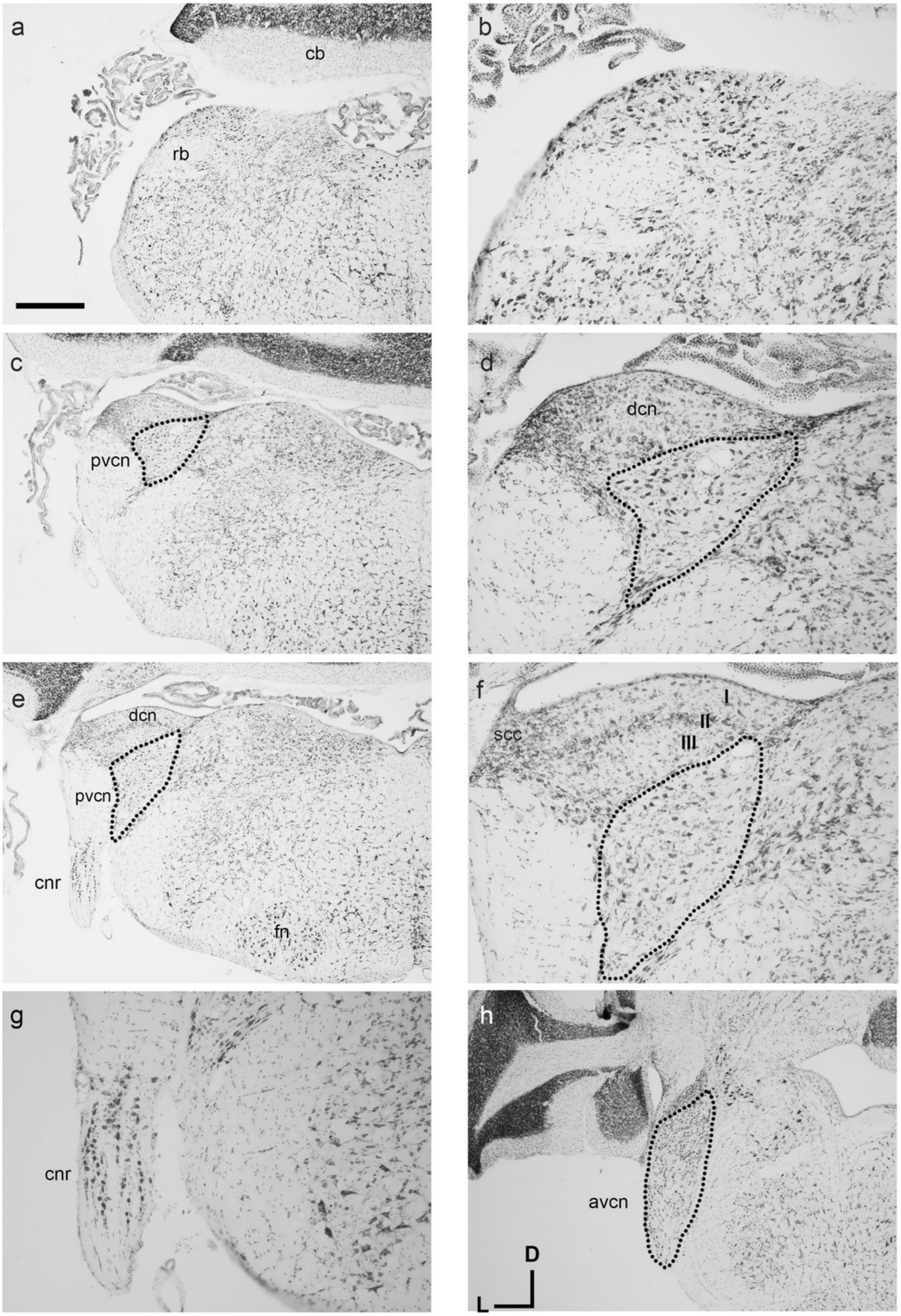
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Figure 4

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