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Auditory laterality in a nocturnal, fossorial marsupial (*Lasiorhinus latifrons*) in response to bilateral stimuli

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Behavioural lateralisation is evident across most animal taxa, although few marsupial and no fossorial species have been studied. Twelve wombats (*Lasiorhinus latifrons*) were bilaterally presented with eight sounds from different contexts (threat, neutral, food) to test for auditory laterality. Head turns were recorded prior to and immediately following sound presentation. Behaviour was recorded for 150 seconds after presentation. Although sound differentiation was evident by the amount of exploration, vigilance and grooming performed after different sound types, this did not result in different patterns of head turn direction. Similarly, left-right proportions of head turns, walking events and food approaches in the post-sound period were comparable across sound types. A comparison of head turns performed before and after sound showed a significant change in turn direction ($\chi^2_1 = 10.65$, $P = 0.001$) from a left preference during the pre-sound period (mean 58% left head turns, CI 49-66%) to a right preference in the post-sound (mean 43% left head turns, CI 40-45%). This provides evidence of a right auditory bias in response to the presentation of the sound. This study therefore demonstrates that laterality is evident in southern hairy-nosed wombats in response to a sound stimulus, although side biases were not altered by sounds of varying context.

Keywords

Wombat; Behaviour; Laterality; Auditory; Marsupial

Lateralised behaviour is evident in many animal species, including humans (Vallortigara & Rogers, 2005; Vallortigara, 2006; Corballis, 2007; MacNeilage, Rogers, Vallortigara, 2009), and is apparent in asymmetries of left-right cerebrum use, which manifests as a side bias (Rogers, 2000). Its expression can be affected by task function, emotion and perception, social structure, age or gender (de Latude, Demange, Bec, 2009; Pfannkuche, Bouma, Groothuis, 2009). Laterality assists cognitive processing by reducing reaction time (Rogers, 2000) and may enhance simultaneous performance of tasks that are controlled by opposite hemispheres (e.g. feeding and vigilance) (Rogers, Zucca, Vallortigara, 2004; Ghirlanda, Frasnelli, Vallortigara, 2009). At the population level, laterality may aid social communication or predator avoidance strategies (Vallortigara, Chiandetti, Sovrano, 2010).

Hemispheres appear to control different emotions and therefore the expression of laterality has the potential to indicate the animal's perception of a stimulus (Rodriguez, Gomez, Afonso, 1992; Hauser, 1993; Phillips, Llewellyn, Claudia, 2003; de Latude et al., 2009). A review of lateralization by Rogers (2010) suggests that dominant use of the right hemisphere may indicate an animal that is stressed or has a negative cognitive bias. The orienting asymmetry paradigm is a non-invasive measure of auditory laterality, using head turns in response to sounds of varied context (Hauser & Andersson, 1994; Teufel, Ghazanfar, Fischer, 2010). In dogs (*Canis familiaris*) and Rhesus macaques (*Macaca mulatta*) this test indicated that vocalisations from conspecifics that had been disturbed by a stranger knocking at the door or isolated in a room were processed with the right ear / left hemisphere (Hauser & Andersson, 1994; Siniscalchi, Quaranta, Rogers, 2008). These stimuli did not represent an immediate and severe threat to the dogs. By contrast, the left ear / right hemisphere was dominant in dogs for thunderstorm sounds and in Rhesus macaques for heterospecific vocalizations"

Few marsupial species have been tested for laterality despite cerebral differences from eutherian mammals (Lippolis. Westman, McAllan, Rogers, 2005). Similar to birds, marsupials lack a corpus callosum, which bridges the hemispheres and may play a role in lateralisation (Wiltschko, Traudt, Güntürkün, Prior, Wiltschko, 2002; Josse, Mohamed, Kherif, Price, 2008), although they do possess an anterior commissure, which interconnects the auditory fields of the hemispheres (Heath & Jones, 1971; Aitken, 1995). Unlike marsupials, lateralisation in birds has been extensively studied and the patterns of hemispheric use are similar to mammals (Rogers, 2008). The wombat presents a useful marsupial model for such studies due to its laterally placed eyes (Sanderson & Pearson, 1981), largely immobile ears, and its nocturnal, semi-fossorial behaviour. This study aimed to determine whether wombats are lateralised in their response to auditory stimuli, and whether this is influenced by sound type and context.

Methods

This study used twelve adult southern hairy-nosed wombats (*Lasiorhinus latifrons*) maintained in four groups of one male with two females. Eleven of these were collected from the wild 5 - 8 years prior to this study and one was born at the facility in 2003. Each group was provided with a temperature-controlled burrow system, digging chamber, feeding house, log and an outside enclosure (76 m^2 - 249 m^2) with native grasses. Carrots, hay and macropod pellets were provided daily. The wombats were checked during routine cleaning and weighed fortnightly. Ethical clearance was obtained from the University of Queensland, Animal Ethics Committee (licence number: SAS/402/09).

Audio testing occurred in a vacant den (Figure 1) identical to those used by the wombats. A wooden and wire mesh feeding frame was placed centrally to control the wombat's position, and two speakers (Logitech, LS11, Dick Smith Electronics) were attached bilaterally (azimuth = 90° and 270°) to the den roof. Two infrared cameras (Sony IR Outdoor Night Vision CCIR 507L28) and surveillance software (Skyview Super-series, Skyview) were used to record behaviour at two frames per second in black and white. Wombats were habituated to the equipment to avoid confounding results from neophobia (Robins & Phillips, 2009).

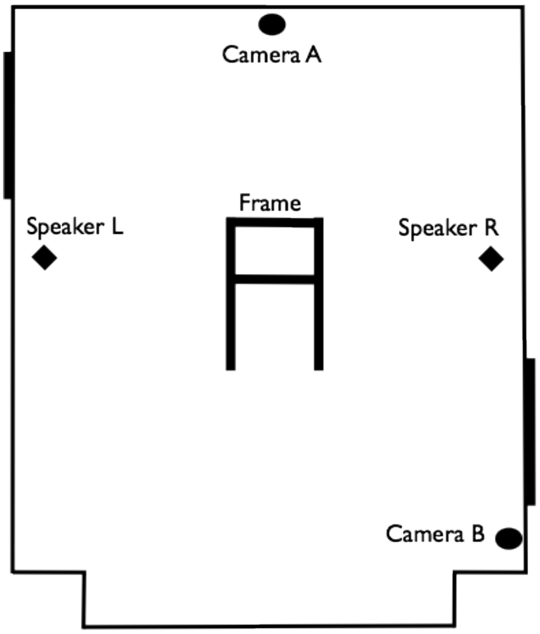

Figure 1: Two-dimensional representation of the test den.

Treatment sounds

Eight sound clips were used from three different contexts – threat, neutral and food-conditioned. Wombat hissing, and vocalisations from two predators (dog and dingo) were used as threatening sounds. Two sounds believed to be neutral (air-conditioning and aeroplane), were chosen because they were heard frequently at the test site without evoking behavioural responses from the wombats. Three novel and biologically irrelevant sounds (bells, opera and whistle) were positively conditioned to a food reward by offering a preferred treat immediately after presenting the sound. This was done six times per day for five days prior to testing.

All sound files were monophonic and five seconds in length, with a median volume of 62 - 67.5 dB SPL (Digital Sound Level Meter, Q1362, Dick Smith Electronics). The sound pressure level produced by each speaker was identical (mean difference in minimum level across 8 sounds was 0 dB SPL and maximum level was 0.5 dB SPL). Frequencies were analysed using Raven Pro (version 1.3) spectral analysis software (Figure 2). Spectrogram frequency outputs from the two speakers were compared and found to be less variable than output from the same speaker played two times (mean cross correlations for eight sounds was 0.958 for within speaker variation, compared with 0.971 for between speakers).

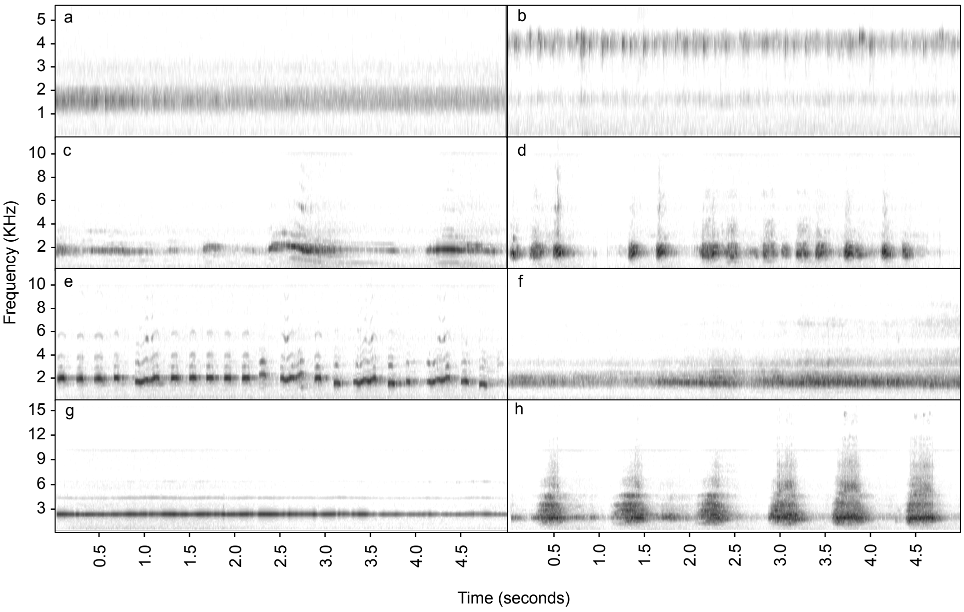

Figure 2: Spectrograms (Raven Pro, version 1.3) of eight test sounds: a) Air-conditioning, b) Bells, c) Dingo, d) Dog, e) Opera, f) Plane, g) Whistle, h) Wombat. Sounds a, b, g and h were recorded on-site using a digital audio recorder (Joybee 110, BenQ). Sounds c - f were sourced from soundboard.com.

Testing began at 0600 h, and was carried out in three blocks of 9, 9 and 6 days, with four wombats tested individually and in a random order each day. Testing lasted between 566 - 3612 seconds, and all wombats had an inter-test rest period of 3 days.

Behaviour was recorded over three periods: baseline (head turns prior to sound presentation), response (head turns within 30 seconds of sound presentation) and post-sound (all behaviour within 150 seconds of sound presentation). One trained observer scored all of the video data, and a second trained observer scored 5 % of the videos to check for inter-rater reliability (94.9 %). Behaviour was categorised into head turns (vigilance), feeding, locomotion, escape, exploration, resting and grooming. Food was placed into the bowl using a reaching tool (Nifty Nabber, Craftright) through a hole in the roof that also allowed discrete viewing of the den. The sound was presented once the animal was in the feeding frame with its head straight and of equal distance between the two speakers. Responses were considered invalid if the sound played when these criteria were not met. The procedure was repeated twice more using the same sound. Wombats that did not approach the bowl within three minutes (16 % occurrence) were lured to it using the reaching tool. On two occasions luring did not work within 20 min and the wombat was released back into its enclosure and re-tested at the end of the session.

Statistical analysis

Head turn in response to sound

The direction of responsive head turns was analysed for the effect of sound type using Chi-square tests of associations (Freq procedure, SAS®, version 8.2) for left and right turns, and no response. Head turn preferences for individuals and after each sound type were calculated using an Exact Binomial Test, using only responses in which a left or right choice had been made.

Behaviour post-sound

Post-sound behaviour (0 – 150 seconds) was categorised into feeding, locomotion, vigilance, escape, exploratory, resting and grooming. Data were not normally distributed by univariate analysis, therefore behavioural frequencies were analysed using a Genmod procedure with a Poisson distribution in SAS to determine the influence of sound type. Resting was too infrequent to include. If an overall effect of sound was indicated, post-hoc Chi-square tests were conducted to test for differences in behaviour between sounds. Three post-sound behaviours were directional: walking (clockwise/anti-clockwise), head turns (left/right) and food approach (bowl on left/right). These were analysed using the Genmod procedure in SAS with a binomial distribution to determine whether sound type influenced direction. Pre- and post-sound data were compared using the Genmod procedure with a binomial distribution in SAS.

Results

The direction of responsive head turns was not influenced by sound type ($\chi^2_{14} = 14.24$, $P = 0.43$), although one sound (air-conditioning) was significantly skewed to the right (Figure 3). Only one individual had a significant directional preference (Figure 3). The direction of post-sound walking, head turning or food approach was similar between sounds (Table 1). Head turn direction significantly changed ($\chi^2_1 = 10.65$, $P = 0.001$) from a left preference [proportion left (with 95% CI) = 0.58 (0.49 – 0.66)] in the baseline period to a right preference in the post-sound period [proportion left (with 95% CI) = 0.43 (0.40 – 0.45)]. This change was not affected by the type of sound presented ($\chi^2_7 = 6.77$, $P = 0.45$) (Table 2).

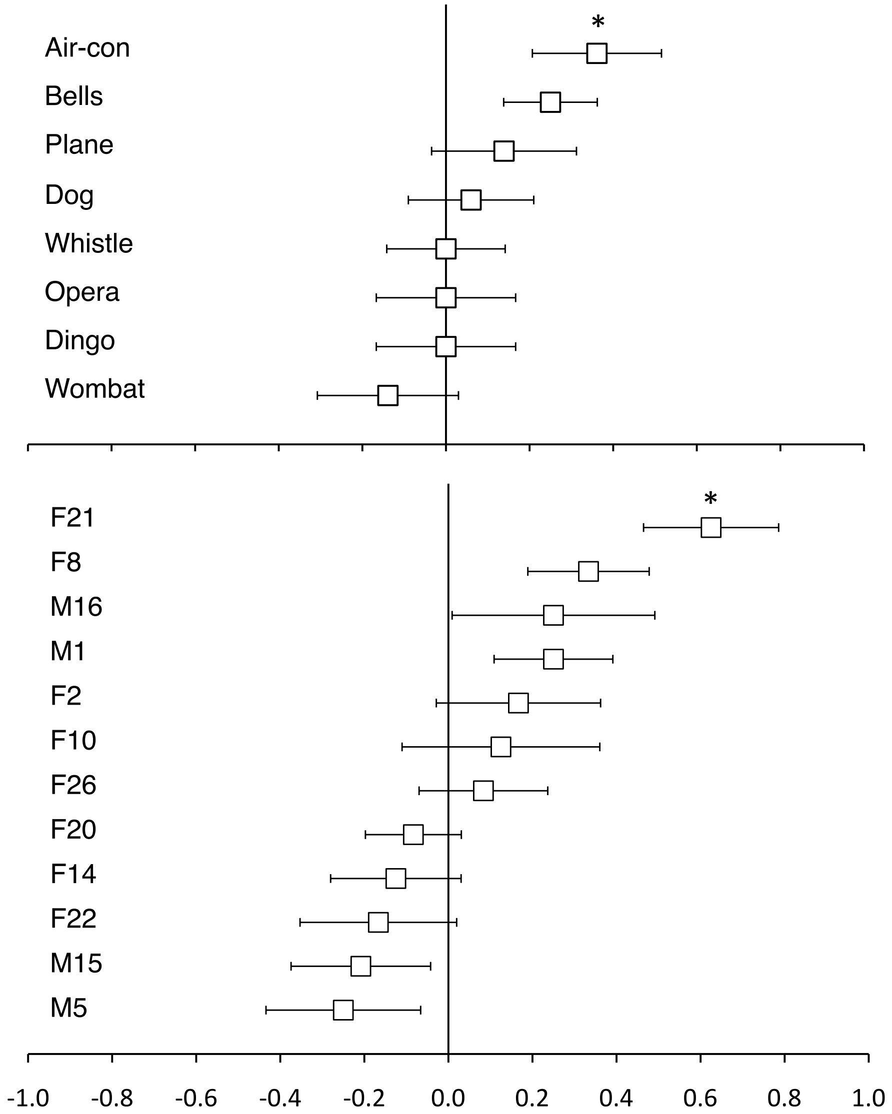

*Figure 3. Mean head turn preferences (± SE) for individual wombats and sounds. Negative and positive means indicate a left and right preference respectively. Preferences were calculated by attributing a left response with -1, right response with +1 and no response with 0 then calculating means for each individual or sound. * indicates a significant ($p < 0.05$) preference using an Exact Binomial Test.*

Table 1. Back-transformed proportions (with 95% CI) of left or anti-clockwise behaviour after 8 sound types, and the overall sound effect on direction (χ^2 statistic, degrees of freedom, probability value).

Sound type	Head movement left	Approach food left	Walking anti-clockwise
Air-con.	0.40 (0.32 – 0.47)	0.55 (0.28 – 0.79)	0.65 (0.56 – 0.72)
Bells	0.40 (0.33 – 0.48)	0.52 (0.27 – 0.76)	0.60 (0.51 – 0.67)
Dingo	0.46 (0.39 – 0.53)	0.66 (0.43 – 0.83)	0.57 (0.49 – 0.64)
Dog	0.41 (0.34 – 0.49)	0.61 (0.35 – 0.82)	0.66 (0.58 – 0.73)
Opera	0.45 (0.38 – 0.52)	0.46 (0.22 – 0.73)	0.61 (0.52 – 0.69)
Plane	0.39 (0.32 – 0.46)	0.49 (0.25 – 0.73)	0.66 (0.58 – 0.73)
Whistle	0.44 (0.37 – 0.52)	0.39 (0.18 – 0.65)	0.71 (0.63 – 0.78)
Wombat	0.47 (0.40 – 0.54)	0.64 (0.41 – 0.82)	0.68 (0.61 – 0.75)
Sound effect	$\chi^2_7 = 5.37, p = 0.62$	$\chi^2_7 = 3.81, p = 0.80$	$\chi^2_7 = 10.61, p = 0.16$

Table 2. Back-transformed proportions (with 95% CI) of left head turns after 8 sound types.

Sound type	Proportion left pre-sound	Proportion left post-sound
Air-con.	0.47 (0.27 – 0.69)	0.40 (0.32 – 0.47)
Bells	0.72 (0.43 – 0.89)	0.41 (0.33 – 0.49)
Dingo	0.55 (0.31 – 0.78)	0.46 (0.39 – 0.53)
Dog	0.37 (0.19 – 0.61)	0.41 (0.34 – 0.49)
Opera	0.60 (0.46 – 0.73)	0.45 (0.38 – 0.52)
Plane	0.74 (0.44 – 0.92)	0.38 (0.32 – 0.46)
Whistle	0.51 (0.33 – 0.68)	0.44 (0.37 – 0.52)
Wombat	0.60 (0.35 – 0.81)	0.47 (0.40 – 0.81)

Sound type significantly affected the expression of three behaviours in the post-sound period: exploration ($\chi^2_7 = 16.8$, $p = 0.02$), vigilance ($\chi^2_7 = 26.2$, $p = 0.0005$) and grooming behaviour ($\chi^2_7 = 24.1$, $p = 0.001$) (Figure 4). Vigilance behaviour was exhibited most frequently after the plane and wombat sounds and least frequently for bells and whistles. Exploratory behaviour occurred most commonly after hearing bells, while the whistle elicited the most grooming and the wombat the least.

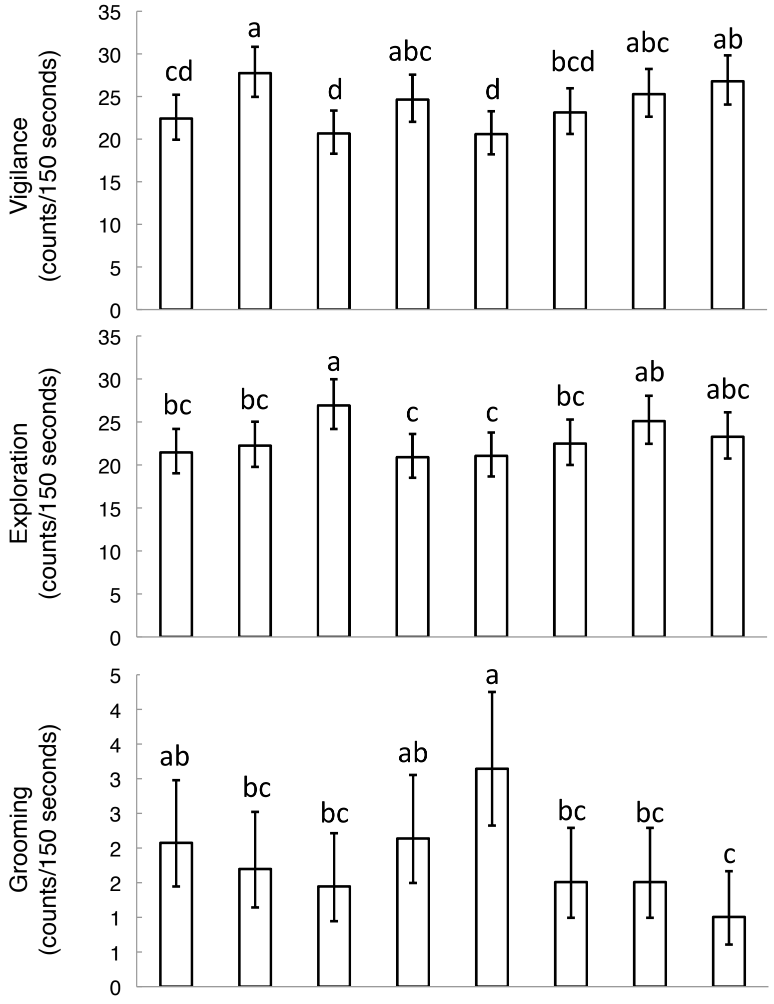

Figure 4. Mean counts (± 95% CI) of vigilance, exploration and grooming after the presentation of eight sound types. Means with different letters are significantly different ($p < 0.05$).

Discussion

It is evident that the wombats could distinguish between different sounds, as the amount of vigilance, exploration and grooming was significantly affected by sound type. Vigilance was performed most frequently after plane and wombat sounds and least after whistles and bells. Wombat hissing represents a threat, while the whistle and bells were conditioned to food and therefore these results are mostly unsurprising. The plane sound, however, was believed to be neutral due to its frequency in the test vicinity and usual lack of response by the wombats. The higher amount of vigilance shown after this sound suggests that threat perception was influenced by context (Thorson, Morgan, Brown, Norman, 1998), and habituated sounds encouraged alertness when they were presented in a novel setting. Grooming was infrequent after all agonistic sounds (wombat, dog, dingo), which is probably because threatened animals direct less energy towards maintenance behaviour such as scratching (Hirsch, 2002; Stojan-Dolar & Heymann, 2010). Differences in exploration and grooming were apparent between food-conditioned sounds, indicating that conditioned responses may be affected by sound type. Despite sound differentiation, wombats did not demonstrate lateralised behavioural responses to sounds of varying context. No side preferences were evident for responsive head turns or post-sound directional behaviour, a result that concurs with Fischer et al.'s (2009) orienting study in humans.

Exposure to sound of any type significantly changed the direction of head turns from a left bias in the baseline period to a right bias after sound presentation. This may be due to a right auditory bias, as seen in humans (Tallus, Hugdahl, Alho, Medvedev, Hämäläinen, 2007; Devlin, Raley, Tunbridge, Lanary, Floyer-Lea, et al. 2003), which becomes strengthened after sound presentation due to increased focus on that side (Tallus et al., 2007). Alternatively the change may result from habituation to the test situation with the wombats displaying vigilance (left side) on first entering the den, and then switching to the right as they become familiar with the environment. This would concur with previous studies that show most species (60 – 95%) exhibit a right hemisphere / left side preference for vigilance (Vallortigara & Rogers, 2005), while familiar objects are usually processed by the left hemisphere / right side (Robins & Phillips, 2009). Speaker differences may also have influenced the change in head turn, as speaker positions were not randomised. However, this is unlikely as turn direction changed significantly while measured differences between speakers were minimal, and discrepancies would need to be consistently in the same direction to have caused this change.

This study concludes that the southern hairy-nosed wombats exhibited lateralised behaviour in response to sound presentation, although the expression was unaffected by different types of auditory stimuli. Further studies using this species are recommended to clarify the functional drivers of this hemispheric specialisation in marsupials.

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