Research paper

Age differences in the purr call distinguished by units in the adult guinea pig primary auditory cortex

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ABSTRACT

Many communication calls contain information about the physical characteristics of the calling animal. During maturation of the guinea pig purr call the pitch becomes lower as the fundamental frequency progressively decreases from 476 to 261 Hz on average. Neurons in the primary auditory cortex (AI) often respond strongly to the purr and we postulated that some of them are capable of distinguishing between purr calls of different pitch. Consequently four pitch-shifted versions of a single call were used as stimuli. Many units in AI (79/182) responded to the purr call either with an onset response or with multiple bursts of firing that were time-locked to the phrases of the call. All had a characteristic frequency ≤5 kHz. Both types of unit altered their firing rate in response to pitch-shifted versions of the call. Of the responsive units, 41% (32/79) had a firing rate locked to the stimulus envelope that was at least 50% higher for one version of the call than any other. Some (14/32) had a preference that could be predicted from their frequency response area while others (18/32) were not predictable. We conclude that about 18% of stimulus-driven cells at the low-frequency end of AI are very sensitive to age-related changes in the purr call.

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1. Introduction

Species-specific communication calls provide ecologically relevant stimuli for investigating how the brain processes complex sounds. Some communication calls convey important information about the reproductive status, emotional state, age and or size of the animal producing the call (Clutton-Brock and Albon, 1979; Fitch and Hauser, 1995; Siemers et al., 2005; Charlton et al., 2009). This is also true of human speech: there is an almost linear relationship between age/weight and fundamental frequency (F0) of speech in adolescent boys between the ages of 12 and 15 years (Hollien et al., 1994). The F0 is related to glottal pulse rate. F0 and vocal tract length both have an effect on speech that is used by listeners to judge speaker size, sex and age (Smith and Patterson, 2005). Furthermore an increase in voice pitch has been shown in adult women just before they ovulate (Bryant and Haselton, 2009).

As a young animal increases in age and size, the vocal folds of the larynx become longer; this is associated with a lower voice pitch and a reduction in the F0 of harmonic calls (Charlton et al., 2009). The

first section of this study investigated to what extent there are age or size dependent differences within a guinea pig communication call. We focused on the short purr call because it is a stereotyped call and is the one most reliably produced by both infant and adult animals (Berryman, 1976). This call has been termed the drrr call by Berryman (1976) or an alarm rumble by Rood (1972) and Arvola (1974). The short purr call is an alerting call that catches the attention of surrounding guinea pigs and often elicits a corresponding call from all the animals in the group (Berryman, 1976). We show that the F0 of the short purr call decreases significantly as an animal increases in age. With an understanding of how calls naturally vary with age, acoustic features of a single exemplar call can be manipulated so that the new versions appear to come from humans or animals of different ages (Smith and Patterson, 2005).

The main aim of this study was to investigate whether single neurons in AI were sensitive to the age-related changes in the pitch of the short purr call. Pitch-sensitive neurons have been shown to occur at the low-frequency border of AI and the rostral field of marmoset monkeys (Bendor and Wang, 2005) but there has not been any evidence of a homologous area in the guinea pig. There is also evidence of one or more pitch sensitive areas in the human belt cortex (Hall and Plack, 2009 and see review by Bizley and Walker, 2010) but not so far in any non-primate species. We targeted the low-frequency region of AI because cells there have been shown to be better at locking to the waveform envelope of the purr call than

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cells in other cortical areas (Wallace et al., 2005a,b). Preference for individual versions of a call is more than just a measure of whether a unit simply does, or does not respond. A unit responding with a simple, onset response to one sound may not carry as much information as the same unit responding with a long-lasting, multi-peaked response to another sound (Wang et al., 1995). It has been proposed that a unit will respond with a sustained response, rather than an onset response, to its preferred stimulus (Wang et al., 2005). Romanski et al. (2004) categorised the specificity of ventrolateral prefrontal cortex units for communication calls by measuring the differences in their overall firing rate for a battery of different calls. From this they calculated the 'call preference index' of a unit. Gourévitch and Eggermont (2007) proposed that in cat auditory cortex the differences between natural and adapted calls were coded by differences in the overall firing rate of a unit, or the type of temporal response given, or by the overall synchrony of responses. The importance of temporal information in coding the characteristics of a neural response to a vocalization were also emphasised by other authors (Wang et al., 1995; Šuta et al., 2007; Huetz et al., 2009). This study investigates whether these methods of coding may also be used by cortical units to code for differences between pitch-shifted versions of the same communication call that mimic the age-related changes.

2. Materials and methods

2.1. Recording vocalizations

Ten pigmented guinea pigs (*Cavia porcellus*) were used to study the development of the short purr call; seven male and three female. Vocalizations were recorded from between 1–3 animals at a time, placed in the centre of a sound attenuating room. When guinea pigs are isolated, young animals typically produce isolation calls, while the adults usually tend not to vocalize (Berryman, 1976). When the animals were frightened or stressed they would not produce a purr call and on any one day only a few of the animals would vocalize. Vocalizations were recorded using a single-diaphragm condenser microphone (Model, B-5 Behringer) and the signal was passed via a mixer (Eurorack UB802) and a sound blaster (Creative, SBO 490) to a lap-top computer and stored using Adobe Audition 1 software (stereo, 24 bit float, 48.8 kHz sample rate). Vocalizations were recorded from the animals at various postnatal ages between p9 and p100. Vocalizations were separated into four age groups for analysis: 1) young pups, below p10; 2) older pre-sexually mature pups of p20–p30; 3) adolescent

animals of p31–p55; 4) adult guinea pigs of p100. P30 was used as a boundary related to changes in sexual maturity because in our colony females have become pregnant as early as p28. Similarly in some strains of males the average age of first mounting occurs at about p30 while intromission/ejaculation occurs at p50–p55 (Harper, 1976). Animals vocalized in response to the experimenter clicking their tongue against the roof of their mouth (see Fig. 1a). Fundamental frequencies were measured from the spectra obtained by fast Fourier transformation of the call waveforms (Fig. 1b). The call duration and inter-pulse interval were also compared across ages.

2.1.1. Surgical preparation and cortical recording

Neural recordings were made in 9 pigmented guinea pigs (5 male and 4 virgin female) weighing 600–1007 g (aged p71–p133 days), all of which were also being used for another study. Neurophysiological recordings were undertaken within a sound attenuated chamber on a floating table. Anaesthesia was induced with urethane (0.9 g/kg in a 20% solution, i.p.). To suppress bronchial secretions a single injection of 0.2 ml atropine sulphate (0.06 mg/kg s.c.) was administered. Supplementary analgesia was maintained using injections (i.m.) of between 0.2 and 0.3 ml Hypnorm (Fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml, Janssen). Once surgical anaesthesia was established, the trachea was cannulated and the animal was artificially respired with 100% oxygen using a Harvard small animal ventilator model 683. Any wax in the ear canals was removed before the animal was placed into a stereotaxic frame so that its tympanic membranes were clearly visible through hollow Perspex speculae.

To prevent pressure building up in the middle ear, polyethylene tubing was inserted into the auditory bullae. A small incision was made in the connective tissue above the foramen magnum to release the pressure of the cerebro-spinal fluid. A craniotomy was performed on the right side, above AI. The dura was removed and the cortex was covered with agar solution to avoid desiccation. The animal's respiratory rate, end tidal CO_2 and core body temperature were monitored and maintained within normal limits.

To maximize data collection we used multi-channel electrode arrays. Arrays were custom-manufactured by attaching 4–8 glass-insulated tungsten electrodes (Bullock et al., 1988) to a circuit board that attached directly to a headstage amplifier (Medusa, Tucker-Davis Technologies, Alachua, Florida). Extracellular potentials were amplified and filtered (300–3000 Hz). Responses were collected using Brainware (v7.43, Jan Schnupp, Oxford University) and exported into Matlab for further analysis. The recorded spikes were
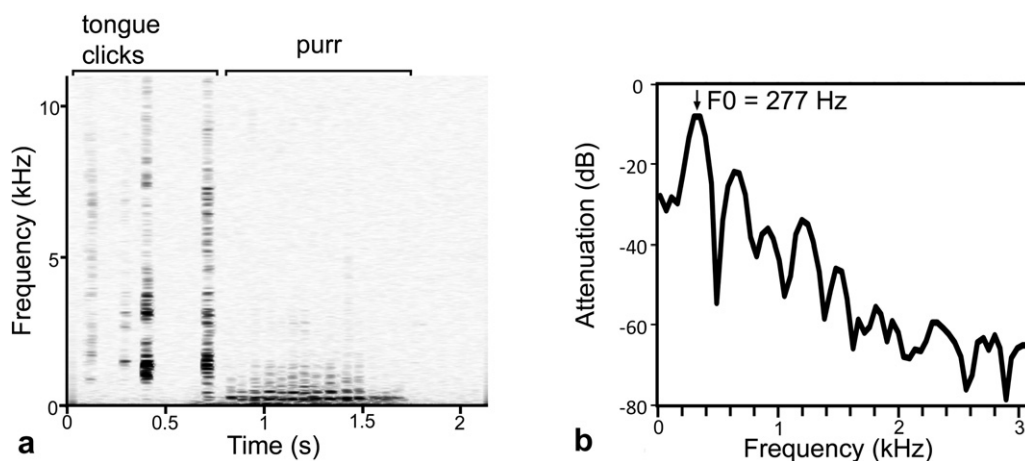

Fig. 1. (a) Spectrogram of four tongue clicks followed immediately by a short purr from an adult animal. (b) Fast Fourier transform of an adult short purr showing the fundamental frequency (F0) at 277 Hz.