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Research paper

Identification of subdivisions in the medial geniculate body of the guinea pig

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Abstract

The accurate and reliable identification of subdivisions within the auditory thalamus is important for future studies of this nucleus. However, in the guinea pig, there has been no agreement on the number or nomenclature of subdivisions within the main nucleus of the auditory thalamus, the medial geniculate body (MGB). Thus, we assessed three staining methods in the guinea pig MGB and concluded that cytochrome oxidase (CYO) histochemistry provides a clear and reliable method for defining MGB subdivisions. By combining CYO with acetylcholinesterase staining and extensive physiological mapping we defined five separate divisions, all of which respond to auditory stimuli. Coronal sections stained for CYO revealed a moderate to darkly-stained oval core. This area (the ventral MGB) contained a high proportion (61%) of V-shaped tuning curves and a tonotopic organisation of characteristic frequencies. It was surrounded by four smaller areas that contained darkly stained somata but had a paler neuropil. These areas, the dorsolateral and suprageniculate (which together form the dorsal MGB), the medial MGB and the shell MGB, did not have any discernable tonotopic frequency gradient and contained a smaller proportion of V-shaped tuning curves. This suggests that CYO permits the identification of core and belt areas within the guinea pig MGB.

Keywords: Auditory thalamus; Cytochrome oxidase; Acetylcholinesterase; Frequency response area; Characteristic frequency

1. Introduction

In studying the forebrain auditory pathways it is important to be able to describe accurately the groups of neurones that make up the auditory thalamus, most of which are in the medial geniculate body (MGB). The MGB has been described as comprising a core division surrounded by several other divisions in a wide range of mammals (Jones, 1985), although the principal studies of this area have been made in the cat. On the basis of Golgi impregnation and Nissl staining, the cat MGB has been divided into ventral, dorsal and medial divisions, with the ventral and dorsal divisions each being further subdivided into at least three additional areas (Morest, 1964, 1965). However, Golgi impregnation is not compatible with physiological studies, while Nissl staining alone is sometimes insufficient for defining the precise location of borders between adjacent subdivisions. More recently a number of other histological techniques including; staining for acetylcholinesterase (AChE), nicotinamide adenine dinucleotide phosphate diaphorase, and immunohistochemistry for calcium binding proteins have been used

Abbreviations: AChE, acetylcholinesterase; APT, anterior pretectal nucleus; CF, characteristic frequency; CYO, cytochrome oxidase; D, dorsal; DL, dorsolateral subdivision of the MGB; L, Lateral; LGN, lateral geniculate nucleus; LP, lateral posterior nucleus; M, medial division of the MGB; MGB, medial geniculate body; PIN, posterior intralaminar nucleus; Po, posterior thalamic nucleus; R, rostral; S, shell division of the MGB; SC, superior colliculus; SG, suprageniculate nucleus; SNL, substantia nigra (lateral part); V, ventral division of the MGB

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in an attempt to delineate accurate borders between the MGB subdivisions (Cruikshank et al., 2001; de Venecia et al., 1995; Edeline et al., 1999; Hackett et al., 1998; Olucha-Bordonau et al., 2004). Attempts have been made to superimpose Morest's (1964, 1965) subdivisions onto the MGB in other species, e.g. mustached bat (Winer and Wenstrup, 1994), rat (Olucha-Bordonau et al., 2004), opossum (Winer et al., 1988), tree shrew (Oliver and Hall, 1978a,b) and human (Winer, 1984), however, these attempts have only been partially successful. Many authors have concluded that subdivisions homologous to those found in the cat cannot be identified in other species, e.g. guinea pig (Redies et al., 1989), rabbit (de Venecia et al., 1995; Tarlov and Moore, 1966) and monkey (Hackett et al., 1998).

The morphological differences between subdivisions are reflected in the physiological responses of the different subdivisions to acoustic stimuli, thus underlining the importance of assigning accurate subdivision borders. Neurones in the ventral MGB tend to be sharply tuned to tonal stimuli and have an orderly alignment of characteristic frequencies (Edeline et al., 1999; He, 2001, 2002; Redies and Brandner, 1991; Rodrigues-Dagaeff et al., 1989). Conversely, neurones in the dorsal MGB tend to have broad tuning curves and no tonotopic characteristic frequency arrangement (Calford, 1983; Calford et al., 1983; Edeline et al., 1999). Responses recorded from the medial MGB tend to encompass the full range of response properties encountered in the ventral and dorsal MGB, with a very wide range of tuning properties (Aitkin, 1973; Edeline et al., 1999; Rouiller et al., 1989). First spike latencies in response to acoustic clicks also varied between MGB subdivisions: the medial and ventral MGB contained the shortest latencies with the very shortest only found in the medial subdivision (Anderson et al., 2006; Rodrigues-Dagaeff et al., 1989; Rouiller et al., 1989). The dorsal and shell MGB had longer latencies than either medial or ventral subdivisions.

The purpose of this study was to seek a reliable histological method of identifying subdivisions of the guinea pig MGB and confirm its usefulness by comparing frequency response properties of the histological subdivisions. Cytochrome oxidase (CYO) is a mitochondrial marker which indicates the levels of energy utilisation and is correlated with the average level of functional activity in a particular part of the brain (Wong-Riley, 1979). There has apparently not been any comprehensive attempt to link CYO levels to different divisions of the auditory thalamus before, however, preliminary work in a variety of species has indicated that it should be a useful marker (Anderson et al., 2006 (guinea pig); Gonzalez-Lima and Cada, 1994 (mouse); Gonzalez-Lima and Jones, 1994 (gerbil); Hackett et al., 1998 (monkey)). Here, we compared CYO distribution to an alternative marker (AChE) which has previously been used to define subdivisions of the rat MGB (Olucha-Bordonau et al., 2004).

2. Materials and methods

2.1. Stimulation and recording

Twenty-eight adult pigmented guinea pigs (12 females, 16 males, mean weight 0.473 kg ± 0.161 kg SD) with clean middle ears were anaesthetised by an intraperitoneal injection of urethane (0.9–1.3 g/kg in a 20% solution, Sigma). Anaesthesia was supplemented when necessary by intramuscular injections of 0.2 ml Hypnorm (Fentanyl citrate 0.315 mg/ml, fluanisone 10 mg/ml, Janssen) to maintain areflexia. Atropine sulphate (0.06 mg/kg s.c., Phoenix Pharma) was administered sub-cutaneously to suppress bronchial secretions. All animals were tracheotomised and mounted in a stereotaxic frame with hollow ear bars. The bulla on each side was vented with a polyethylene tube (22 cm long, 0.5 mm diameter), and the connective tissue above the foramen magnum was opened to release the pressure of the cerebro-spinal fluid and increase stability for recording. A craniotomy 5 mm in diameter was centred on 4.5 mm lateral to midline and 5.0 mm anterior to ear-bar zero, consistent with the coordinates of Rapisarda and Bacchelli (1977), enabling vertical access to the MGB. The brain was covered by 1.5% agar in 0.9% saline to prevent desiccation and to aid stability. The animal's core temperature was maintained at 38 °C by a heating blanket controlled by a rectal thermistor. End-tidal CO_2 was monitored and kept within normal physiological limits by artificially respiring with oxygen. The electrocardiogram was monitored via a pair of electrodes inserted into the skin on either side of the animal's thorax.

Experiments were carried out in a sound-attenuated booth. Stimuli were delivered diotically via a closed-field system (modified Radioshack 40–1377 tweeters; M. Ravicz, Eaton Peabody Laboratory, Boston, MA, USA) coupled to damped 4 mm diameter probe tubes, which fitted into the hollow ear bars. A probe-tube microphone (Brüel and Kjaer 4134 with a calibrated 1 mm probe tube) was used to calibrate the sound system close to the tympanic membrane. The sound system response was flat to within ±10 dB from 100 to 35,000 Hz. All stimuli were generated by an array processor (TDT AP2; Tucker-Davis Technologies, Alachua, FL, USA), which was housed in a personal computer. The stimuli were output via a digital-to-analogue converter and waveform reconstruction filter at rates of at least 100 kHz (TDT System II). The maximum output level of the system was approximately 100 dB SPL. Binaural frequency response plots were obtained using pure tones (2 ms rise/fall time, 50 ms duration, repetition rate 2 bursts/s) of varying frequencies and intensities, presented in a pseudo-random order. Frequency response areas covered most of the behavioural audiogram of the guinea pig (0.05–27 kHz), with three steps per octave. Intensity ranged from 10 dB SPL to 100 dB SPL in steps no greater than 7 dB. The characteristic frequency (CF) of a neurone was defined