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# Interlaminar differences of spike activation threshold in the auditory cortex of the rat

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**Abstract**

The neural circuits of the auditory cortex are a substrate for the dual purpose of representing and storing the auditory signal on one hand, and sending its relevant features to other cortical and subcortical areas on the other hand. The ability to process and transform the signal crucially depends on achievement of the neuronal spike threshold following spatiotemporal summation of the synaptic signals. We used patch-clamp recording in a thin slice preparation to compare neuronal responses to current injection of layer II/III and layer V neurons. We found that while the two classes of neurons do not differ in passive neuronal properties, layer II/III neurons possess a lower firing threshold relative to layer V neurons ($-44.8 \pm 2.4$ mV vs. $-34.3 \pm 4.0$ mV). We speculate that a lower spiking threshold in layer II/III neurons might favor local intracolumnar activation for representation and storage of the auditory information whereas a more positive spiking threshold for layer V neurons may prevent unnecessary cortical spread of a scarcely processed signal.


*Key words:* Cortical circuitry; Signal processing; Synaptic summation; Layer II/III; Layer V; AI; Patch-clamp

## 1. Introduction

The primary auditory cortex is the area of convergence for the auditory information transferred through the thalamic relay. Anatomical studies confirm, by and large (Smith and Populin, 2001), a similar interlaminar connectivity in auditory and other sensory cortices, where cells of a middle layer receive thalamic afferents and project to the supragranular layers II and III, which, in turn, send the processed signal to the local subgranular layer V and to non-local cortical and subcortical areas (for a review see Linden and Schreiner, 2003). Scant information is available on the mechanisms of intracortical processing, particularly whether or not the auditory signal is strongly reprocessed within a cortical column or unit, before eventually being transferred elsewhere. We have previously found that neurons of layer II/III are connected to each other with two types of synaptic connections distinguished by release probability and synaptic strength, which we called high-p and low-p (Atzori et al., 2001). In this study we measured the spike threshold of neurons within layer II/III and their local monosynaptic projection layer V, for determining the number of presynaptic neurons that must be simultaneously active in order to elicit downhill neuronal firing. We found no obvious interlaminar differences in the cell passive properties, whereas neurons in layer II/III displayed a much lower activation threshold with respect to layer V neurons.

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*Abbreviations:* $V_{thr}$, firing voltage threshold; $V_{max\,fir}$, voltage of maximum firing frequency; $f_{max}$, maximum firing frequency; $V_r$, resting potential; GABA, γ-aminobutyric acid

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## 2. Methods

### 2.1. Slice preparation

Wistar rats, 20–30 days old, were sacrificed after isoflurane anesthesia according to the National Institutes of Health guidelines, decapitated, and their brains sliced with a vibratome in a refrigerated solution (0–4°C) containing (mM) 130 NaCl, 3.5 KCl, 10 glucose, 24 $NaHCO_3$, 1.25 $NaH_2PO_4$, 1.5 $CaCl_2$ and 1.5 $MgCl_2$, saturated with a mixture of 95% $O_2$ and 5% $CO_2$ (ACSF). Coronal slices were incubated in ACSF at 32°C before being placed in the recording chamber. The recording area was selected using a Zeiss microscope with a 10× objective as previously described (Atzori et al., 2001). Layer II/III and V neurons with pyramidal cell morphology were visually identified with a 40× water immersion objective. Cells of layer II/III belonged to the high-density region lying approximately between 20% and 33% of the cortical span (0% = pial

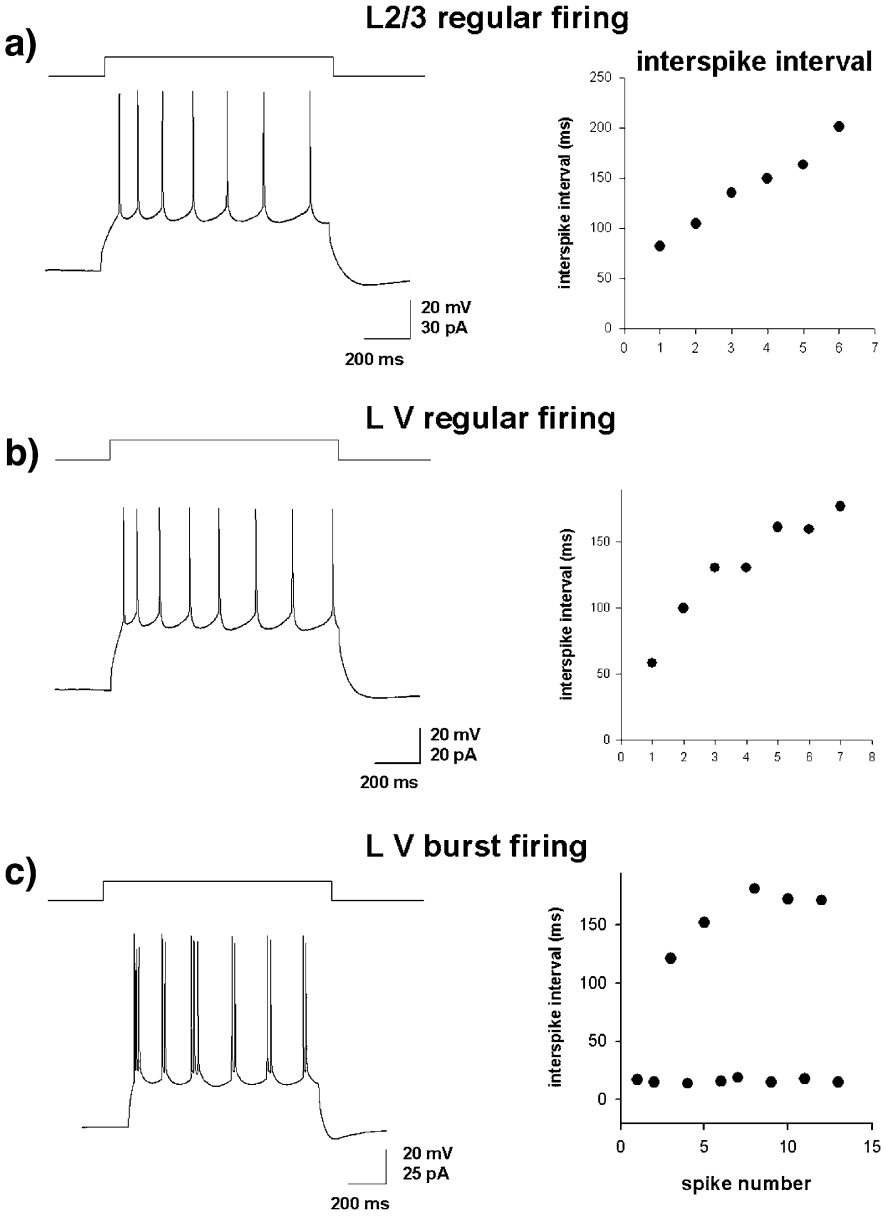


Fig. 1. Firing patterns in layer II/III and V. The firing patterns of neurons in layer II/III and V fell into three firing classes, regular-firing, bursting, and fast-spiking. The last one, associated with inhibitory cells, was not considered. Regular-firing neurons, displaying an increase of the interspike interval during the current pulse, were detected in both layer II/III and V (a,b). Bursting cells (c) were found in only 10% of layer V neurons (3/30) and were characterized by series of bursts with short interspike intervals followed by a longer delay (right).

Table 1

| | Layer II/III | *n* | Layer V | *n* |
|---|---|---|---|---|
| Input resistance (MΩ) | 266 ± 24 | 32 | 268 ± 27 | 30 |
| $\tau$ (ms) | 22 ± 2 | 32 | 21 ± 2 | 30 |
| $V_{resting}$ (mV) | −58.9 ± 1.9 | 33 | −60.4 ± 2.3 | 30 |
| Spike half width (ms) | 2.6 ± 0.2 | 32 | 2.5 ± 0.1 | 30 |
| Percent cells with pronounced afterhyperpolarization | 70% | 33 | 53% | 30 |
| Threshold (mV) | −44.8 ± 2.4 | 28 | −34.3 ± 4.0* | 26 |
| $V_{thr}$ (mV) | 15.1 ± 2.2 | 28 | 26.1 ± 3.1 | 26 |
| $V_{max\ firing}$ (mV) | −1.1 ± 8.8 | 28 | −2.2 ± 6.8 | 26 |
| $f_{max}$ (Hz) | 43.9 ± 3.8 | 28 | 36.0 ± 4.5 | 26 |
| Slope (Hz/mV) | 2.3 ± 0.5 | 28 | 2.6 ± 0.9 | 27 |

*$P < 0.05$.

surface). Layer V cells were selected in the lower-density large-cell-body-size region at approximately 60–75% from the cortical surface.

### 2.2. Electrophysiology

Current-clamp recording was performed with a MultiClamp 700A amplifier (Axon) at room temperature with 3–6 MΩ electrodes, filtered at 2 kHz with a Butterworth filter, sampled at 10 kHz and stored in a PC with a Digidata 1320 A/D converter (Axon). The pipette solution contained (mM) 105 KMeS, 1 $MgCl_2$, 10 HEPES, 4 glutathione, 1.5 $ATPMg_2$, 0.2 EGTA, 20 phosphocreatine, 0.3 $GTPNa_2$. The pH was adjusted to 7.2–7.4 and the osmolarity was 265–275 mOsm. Cellular firing patterns were evoked by injecting square pulses of current at increasing amplitudes. Recordings whose resting potential were more positive than −50 mV or whose action potential was smaller than 50 mV were discarded.

## 3. Results

We classified cortical neurons according to their response to current injection, into regular-spiking, bursting, or fast-spiking (Connors and Gutnick, 1990). Since this last feature is indicative of γ-aminobutyric acidergic inhibitory cells we discarded all fast-spiking cells from the current analysis. All of the 33 recorded neurons in

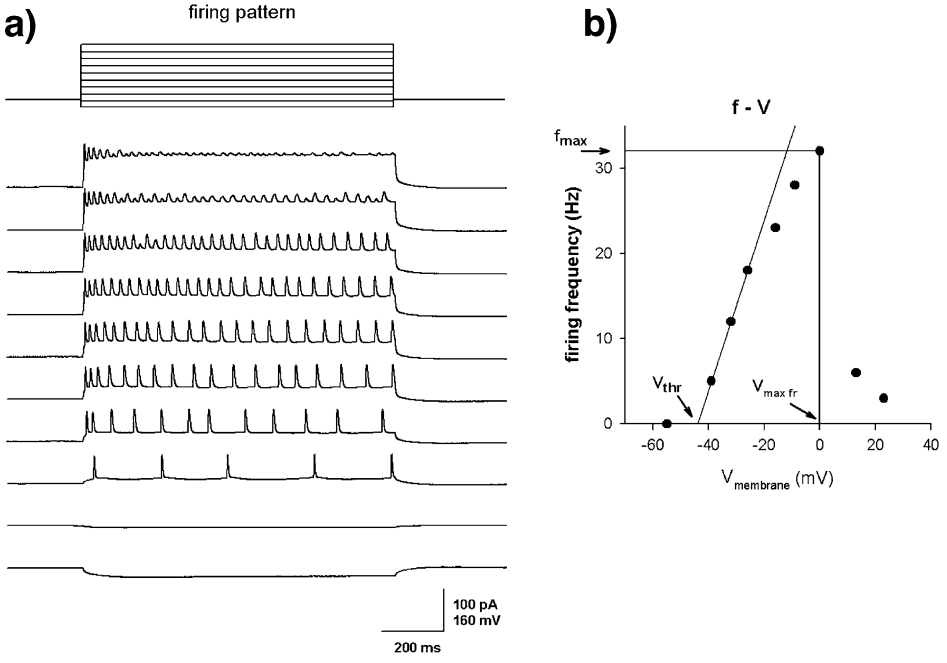


Fig. 2. Computation of $f_{max}$, $V_{max\ fir}$, $V_{thr}$ and slope. The number of spikes was obtained counting the peaks larger than half the initial spike amplitude from a series of current clamp traces at increasing current values (a). The voltage is calculated before the beginning of the second spike. (b) $f_{max}$ and $V_{max\ fir}$ are taken directly from the experimental data. $V_{thr}$ and the slope of the voltage–frequency data were derived from the straight line that best fit the linear part of the curve.

Table 2

| | Quantal amplitude | | Number of presynaptic cells for threshold | |
|---|---|---|---|---|
| | pA | mV | Layer II/III | Layer V |
| High-p connections | 6.9 | 1.84 | 8 | 14 |
| Low-p connections | 1.1 | 0.29 | 52 | 90 |

layer II/III and 27/30 layer V neurons were regular-firing (Fig. 1a,b). Three of the 30 neurons recorded in layer V displayed bursts for particular values of injected current (Fig. 1c) and were discarded from further calculations.

In 32 layer II/III and 27 layer V cells we measured input resistance, membrane potential and membrane time constant ($\tau$) by delivering a single negative current pulse. The spike width was measured at 50% amplitude. No significant differences between layers were detected (Table 1). Since the values of the spike threshold obtained by a single current pulse injection are dependent on the size of the current pulse, we decided to measure the activation threshold by analyzing the whole extent of the voltage–frequency curve for every given neuron.

On 28 layer II/III and 26 layer V neurons we delivered 1-s current pulses of increasing amplitude (range of maximum injected current: 80–300 pA) in order to measure a series of parameters: the activation threshold ($V_{thr}$), the voltage of maximum firing ($V_{max\ fir}$), the maximum firing frequency ($f_{max}$) and the frequency–voltage slope (slope, see Fig. 2). The frequency was calculated by dividing the number of spikes whose amplitude was larger than half of the first spike by the duration of the pulse. To avoid ambiguities due to a fast rise time during the first spike of the train, the voltage was measured right before the beginning of the second spike. $V_{max\ fir}$ and $f_{max}$ were measured directly from the maximum values recorded. The values of $V_{thr}$ and of the slope were derived by fitting a straight line with a minimum square algorithm from the linear part of the frequency–voltage curve, corresponding usually to the first three to five points of the curve. The activation threshold $V_{thr}$ and the slope were calculated accordingly. No differences were detected in the parameters measured except for the voltage activation threshold $V_{thr}$, which was

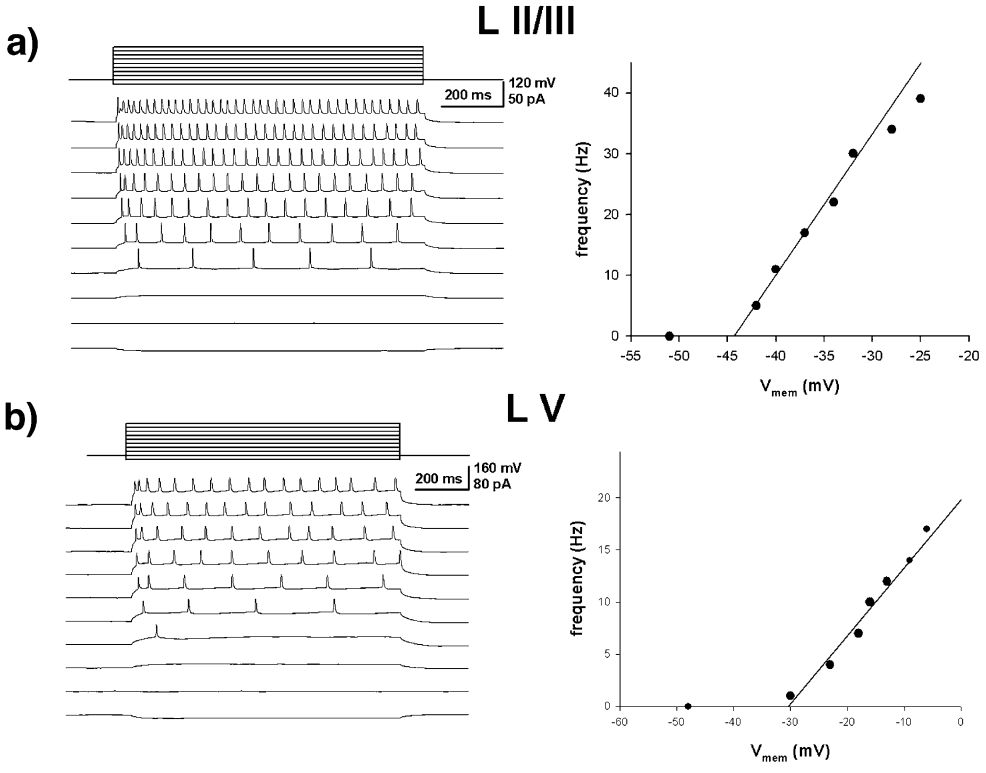


Fig. 3. Layer II/III and layer V have different activation thresholds. Representative example of traces recorded from a layer II/III neuron (a) and the corresponding voltage–frequency curve. $V_{thr} \approx -44$ mV. Representative traces (c) and voltage–frequency curve for a layer V neuron. $V_{thr} \approx -32$ mV.

more negative for layer II/III neurons than for layer V neurons ($V_{thr}$(LII/III) = −44.8 ± 2.4 vs. $V_{thr}$(LV) = −34.3 ± 4.0, see Table 1 and Fig. 3).

Excitatory neurons in layer II/III are interconnected by two networks of synapses (Atzori et al., 2001), one with a high release probability and relatively high quantal amplitude, and another one with opposite characteristics, which we called high-p and low-p, respectively. On the basis of the mean quantal amplitude and of the activation threshold ($V_{thr}-V_r$), we estimated the number of local cells necessary for eliciting an action potential in layer II/III (Table 2). Since layer II/III neurons project to layer V (Wallace et al., 1991; Winer, 1984; Linden and Schreiner, 2003), we repeated a similar calculation for determining the number of presynaptic cells needed for activation of layer V neurons assuming connections similar to layer II/III local connections. Assuming the existence of similar connections between layer II/III and layer V, the results showed that the simultaneous activation of approximately eight high-p or 50 low-p presynaptic neurons is needed for eliciting a postsynaptic action potential within layer II/III, while almost twice as many presynaptic layer II/III neurons need to be active in order to elicit a layer V spike (Table 2).

## 4. Discussion

In spite of a large morphological variety of neuronal types in the auditory cortex (Winer, 1984, 1985; Winer and Prieto, 2001), layer II/III and V neurons display a limited number of firing patterns referred to as regular-firing, fast-firing and bursts already described previously (McCormick et al., 1985; Connors and Gutnick, 1990). In the present analysis we only considered regular-firing neurons, corresponding presumably to excitatory neurons. The main finding of our study is that neurons in layer II/III have a lower activation threshold with respect to layer V neurons. Using the voltage activation thresholds (Table 1) and the quantal amplitude determined in our previous work (Table 2), we calculated that approximately eight high-p or 50 low-p presynaptic neurons (or any combination) is sufficient for evoking a postsynaptic spike in a neuron in layer II/III. An error due to the underestimation of the whole-brain postsynaptic response with respect to the brain slice is partly corrected by the overestimation of the input resistance, which is measured at the resting potential and not dynamically, and should not substantially compromise the validity of the estimate. Assuming the existence of similar connections between layer II/III and layer V (Thomson and Bannister, 1998), almost twice as many layer II/III presynaptic cells are required to elicit a postsynaptic spike in one layer V neuron. This information suggests a specific role of excitatory neurons in the different cortical layers. From anatomical and physiologic studies it is known that cortical layers II and III receive cortico-cortical projections from layer IV, in addition to intralaminar and direct thalamic input (Beierlein et al., 2002; Shen et al., 1999). Furthermore, it is known that layer V receives afferent fibers from supragranular layers II and III, and it is proposed to represent the major cortical output (Linden and Schreiner, 2003; Winer and Prieto, 2001). In the cat, layers II and III are characterized by a high cell density associated with relatively small somata (Winer, 1984, 1985), whereas layer V displays a lower cell density and larger cell body size (Winer and Prieto, 2001). Layer V also possesses an extended inhibitory network (Foeller et al., 2001) modulating and limiting its output, which is less significant in layer II/III. The lower activation threshold of layer II/III neurons with respect to layer V neurons would suggest that while auditory information can easily circulate and overflow in and from layer II/III, the activation of information processing in layer V requires a more robust presynaptic activation and/or higher presynaptic synchrony. One consequence of this is that the storage of the auditory information would be easier in synapses within layer II/III rather than in synapses from layer II/III to layer V. Furthermore, a high density of excitatory cells and a lower activation threshold would indicate layer II/III as the appropriate substrate for the immediate representation of auditory stimuli utilizing local excitatory networks shaped by previous synaptic plasticity. On the contrary, the presence of strong local inhibitory mechanisms and a higher spike activation threshold in layer V cells could indicate that layer V acts as a barrier for the spread of the signal, allowing only a limited amount of information selected by the local circuitry to cross the boundaries of a local functional unit (Sugimoto et al., 1997). A recent in vivo study highlighted a major difference between layer II and layer III pyramidal neurons, expressed as a prominent inhibition following the onset depolarization in response to 50-ms tones in layer III but not in layer II pyramidal neurons (Ojima and Murakami, 2002). The differential thalamocortical innervation of layer II and III, together with their hyperexcitability, a consequence of the low activation threshold, could contribute to explain the presence of an effective synaptic inhibition in layer III but not in layer II. Synaptic inhibition could indeed prevent layer III pyramidal cells from saturating their response to thalamic input, while leaving layer II available to uninhibited signal processing, one synapse downstream on the auditory signal pathway.

We speculate that layer II/III and V could carry out complementary roles in the local cortical circuitry, in which layer II/III would be the preferred substrate for

local stimulus representation and storage whereas layer V would be the feature extracting filter for non-local signal processing. Alternatively, layer II/III cells could swiftly extract auditory features by virtue of their low activation threshold, by using an experience-modified circuitry, while the higher activation threshold in layer V could prevent an unnecessary leakage of incompletely processed auditory information to the areas downstream in the auditory pathway. Further studies would be necessary to assess possible threshold differences between layer II and layer III, which were not separated in our study.

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## References

Atzori, M., Lei, S., Evans, D.I., Kanold, P.O., Phillips-Tansey, E., McIntyre, O., McBain, C.J., 2001. Differential synaptic processing separates stationary from transient inputs to the auditory cortex. Nat. Neurosci. 4, 1230–1237.

Beierlein, M., Fall, C.P., Rinzel, J., Yuste, R., 2002. Thalamocortical bursts trigger recurrent activity in neocortical networks: layer 4 as a frequency-dependent gate. J. Neurosci. 22, 9885–9894.

Connors, B.W., Gutnick, M.J., 1990. Intrinsic firing patterns of diverse neocortical neurons. Trends Neurosci. 13, 99–104.

Foeller, E., Vater, M., Kossl, M., 2001. Laminar analysis of inhibition in the gerbil primary auditory cortex. J. Assoc. Res. Otolaryngol. 2, 279–296.

Linden, J.F., Schreiner, C.E., 2003. Columnar transformations in auditory cortex? A comparison to visual and somatosensory cortices. Cereb. Cortex 13, 83–89.

McCormick, D.A., Connors, B.W., Lighthall, J.W., Prince, D.A., 1985. Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J. Neurophysiol. 54, 782–806.

Ojima, H., Murakami, K., 2002. Intracellular characterization of suppressive responses in supragranular pyramidal neurons of cat primary auditory cortex in vivo. Cereb. Cortex 12, 1079–1091.

Shen, J.X., Xu, Z.M., Yao, Y.D., 1999. Evidence for columnar organization in the auditory cortex of the mouse. Hear. Res. 137, 174–177.

Smith, P.H., Populin, L.C., 2001. Fundamental differences between the thalamocortical recipient layers of the cat auditory and visual cortices. J. Comp. Neurol. 436, 508–519.

Sugimoto, S., Sakurada, M., Horikawa, J., Taniguchi, I., 1997. The columnar and layer-specific response properties of neurons in the primary auditory cortex of Mongolian gerbils. Hear. Res. 112, 175–185.

Thomson, A.M., Bannister, A.P., 1998. Postsynaptic pyramidal target selection by descending layer III pyramidal axons: dual intracellular recordings and biocytin filling in slices of rat neocortex. Neuroscience 84, 669–683.

Wallace, M.N., Kitzes, L.M., Jones, E.G., 1991. Intrinsic inter- and intralaminar connections and their relationship to the tonotopic map in cat primary auditory cortex. Exp. Brain Res. 86, 527–544.

Winer, J.A., 1984. The pyramidal neurons in layer III of cat primary auditory cortex (AI). J. Comp. Neurol. 229, 476–496.

Winer, J.A., 1985. Structure of layer II in cat primary auditory cortex (AI). J. Comp. Neurol. 238, 10–37.

Winer, J.A., Prieto, J.J., 2001. Layer V in cat primary auditory cortex (AI): cellular architecture and identification of projection neurons. J. Comp. Neurol. 434, 379–412.