Functional Architecture and Spike Timing Properties of Corticofugal Projections From Rat Ventral Temporal Cortex

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INTRODUCTION

The temporal association and parahippocampal cortex (TeA) is a poly-sensory structure that plays a critical role in high-order sensory recognition and control of top down attention. According to current neurophysiological model of attention control (Desimone and Duncan 1995; Fuster 1997; Gilbert and Sigman 2007), sensory experience and awareness can be represented as network-driven persistent neuronal firing in the form of working memory or template of attention (Desimone and Duncan 1995; Fuster 1997; Gilbert and Sigman 2007). When this internally generated template activity is propagated to downstream cortical and subcortical structures, it leads to an enhancement of the salience of the attended environmental stimulus, thereby creating a selection bias in sensorimotor processing (Desimone and Duncan 1995; Fuster 1997; Gilbert and Sigman 2007; Komura et al. 2001; Miyashita 2004; Naya et al. 2001).

Although the cortical projection system is a critical element for the TeA network, the functional organization of specific feedback pathways remain poorly defined. In particular, there is insufficient evidence on whether and how individual projection cells can form a large axonal network that requires multiple target pathways transcending the boundaries of different sensorimotor modalities. It is also unknown how fast spike conduction and synchronized activation can be maintained in such a network. Large-scale axonal network with diffuse projections not only raises uncertainty in terms of the fidelity of signal conduction but also the issue of spike timing. Recent studies have shown that spike timing can encode neural information in the cortex on a scale of a few milliseconds (deCharms and Merzenich 1996; Steinmetz et al. 2000) or less (Shmiel et al. 2005). The timing of presynaptic spike volleys and their relative temporal order to a postsynaptic spike can also exert strong influence on neuronal plasticity and development (Hauser et al. 2001; Markram et al. 1997). Hence, large temporal jitters or delays in axonal conduction may disrupt these timing-based mechanisms of information processing.

Anatomical tracing studies in different species have thus far shown that deep layer neurons in motor, sensory, and temporal association cortex project to multiple cortical and subcortical regions (Arnault and Roger 1990; Bourassa and Deschenes 1995; Cruikshank et al. 2002; Deschenes et al. 1994, 1998; Shi and Cassell 1997; Vaudano et al. 1991; Winer et al. 2001). These studies, however, do not necessarily provide data on the prevalence of the uncovered projection pathways. That is, are they derived from highly branched axons from single neurons or primarily formed by many individual cells with fewer axonal branches? Moreover it is not feasible to demonstrate the relative spike timing properties of the cerebral cortical networks based on pathway tracing. To address these issues, we developed a large horizontal slice preparation where multiple electrodes can be placed in different brain regions for antidromic activation of axonal pathways. This slice preparation was previously used to demonstrate that layer V neurons in the rat TeA can maintain a myelinated and nonmyelinated collateral pathway in the posterior thalamus (Chomiak and Hu 2007).

In this study, the architecture, branching pattern and conduction velocity from individual layer V axons were evaluated. The results show that most, if not all, layer V neurons in this brain region can form multiple axonal pathways that project to many brain structures, both proximal and remote, and a striking isochronicity of spike conduction was found in this brain region.

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Because layer V neurons convey top-down cortical modulatory signals to many other sensorimotor structures (Berespolova et al. 2006; Guillery and Sherman 2002; Jones 2001; Ma and Suga 2001; Winkowski and Knudsen 2006), it was hypothesized that single layer V neurons may utilize multiple axonal collaterals and an icoschonic conduction mechanism to synchronize its spike delivery to multiple targets located at different distances. Preliminary results have been reported previously in abstract form (Chomiak and Hu 2006).

**METHODS**

In vivo electrophysiology

All experimental protocols were approved by the University of Calgary Animal Care Committee. Male Long Evans or Sprague Dawley rats (~300 g) were initially anesthetized with sodium pentobarbital (Somnotol) and later maintained at stable anesthetic level via intravenous drip or intraperitoneal infusion of 85% ketamine and 15%/xylazine. Lidocaine hydrochloride (1%, Sigma) was injected subcutaneously in the scalp and lidocaine gel (Xylocaine) was applied to the skin prior to surgical incisions. After a craniotomy, bipolar electrodes for local field potential (LFP) recording were lowered to the following locations relative to bregma according to the rat atlas (Paxinos and Watson 1986): TeA, anterior/posterior −7.0, medial/lateral 6.0, dorsal/ventral 4.5; caudate putamen (CPU), −1.4, 4.0, 4.0; medial geniculate dorsal division/lateral posterior thalamic nuclei (PT), −5.6, 3.4, 4.0; basolateral amygdala: −0.28, 5.0, 8.0; Par, −4.5, 4.0, 2.0. Single or trains of monophasic pulse (150 μA, 100–250 μs) stimulation were applied via a bipolar stimulating electrode to the white matter immediately beneath the TeA. LFPs were amplified, digitized at 4–5 kHz (National Instruments NIDAQ 6300E) and captured on a laptop running Igor software (Wavemetrics). Onset latency data were obtained using concentric and side-by-side bipolar electrodes, consistent with previous reports (Bagshaw and Evans 1976; McIntyre et al. 2004; Nowak and Bullier 1996), the estimated current spreading edge in our preparation is generally <500 μm. Compared with fast-conducting fibers, slow-conducting fibers typically required higher stimulation intensities (Chomiak and Hu 2007). Antidromic latencies or conduction times were determined by measuring the time interval between the end of the stimulation pulse and the onset (foot) of the antidromic evoked action potential (Lipski 1981) (see Fig. 3). Similar latency data were obtained using concentric and side-by-side bipolar electrodes, consistent with previous reports (Bagshaw and Evans 1976).

Histology

For fiber tract tracing, rats were anesthetized with isoflurane (4% induction, 2–2.5% maintenance), and a Hamilton syringe containing 80% biotin dextran amine (BDA, Molecular Probes) was lowered stereotaxically to the temporal lobe associational cortex. After 5 min of settling time, injection proceeded manually at a rate of 200 nl every 3 min to a total volume of 1 μl BDA. Six minutes was allowed prior to syringle removal to ensure proper diffusion of the tracer. The animals were allowed to recover for 24–48 h before transcardially perfused and fixed at 4°C for over 24 h.

For pathway length measurement, horizontal slices (100 μm) were cut in the same way as those used for electrophysiology. The sections were treated with a 0.3% H₂O₂ solution in methanol for 20 min to block the remaining endogenous peroxidase activity. They were incubated in avidin–biotin complex solution (1:100; ABC Elite kit, Vector Laboratories) overnight at room temperature. After three washes for 10 min each in 1× phosphate-buffered solution (PBS), the sections were reacted with a 3,3′-diaminobenzidine solution, rinsed with distilled water, and covered with a coverslip. Same staining reactions were also performed as controls on uninjected side of the brain sections (n = 4) and in rats received only vehicle injections (n = 3) or with missed target injections (n = 3). There was no endogenous BDA background staining or false positive deposit of BDA in these specimens. Clearly identifiable BDA-positive axons from both gray matter and white matter where measured using the imaging software provided by Northern Eclipse (Empix Imaging).

**Pathway tracking and length measurement**

Our experimental design required that the full length of long axonal efferents from TeA can be measured along multiple trajectories and terminal sites in the same slices that were used for electrophysiological latency measurement. We found, under this circumstance, slicing the brains with pre-BDA injection gave the best results. In contrast,
we were unable to uncover the axonal branches of a layer V cell to any significant length via intracellular dye injection (unpublished observations). The BDA-labeled pathways were first manually traced and drawn to first generate a silhouette of BDA-labeled fibers (see Fig. 2 for example). This was done by employing two-dimensional color enhancing of brown BDA products followed by template subtracting (Empix Imaging). The silhouette was then overlaid to the photograph of live slice preparation to help localize the pathway trajectory and stimulation electrode locations (Fig. 1B). Our tracing data revealed that the axonal trajectories and target sites similar to that described in the literature (Cruikshank et al. 2002; Meltzer and Ryugo 2006; Vaudano et al. 1991). To localize the gray matter structures in the slice, we used the atlas by Paxinos and Watson and by Kruger et al. (Kruger et al. 1995; Paxinos and Watson 1986), the latter of which provides clear demarcations between gray and white matters based on myelin staining. To more accurately measure fiber length with curvature, the pathway images were first overlaid onto the brain slice image (Fig. 1Inset, the pathway images were first overlaid onto the brain slice image). Using curvature measuring tool (Empix Imaging), the absolute pixel length, including its angular proportions, for each pathway branch was calculated between the stimulation and recording sites and converted into millimeters. As previously noticed, long contralateral branches (CLBs) unlike other pathways exhibited poor tracer retention (Parent and Parent 2006) and cannot be kept with full length in the in vitro horizontal slice. However, CLBs have been shown to almost exclusively course through the external capsule (EC) and corpus callosum (Hackett et al. 1999; Parent and Parent 2006; Vaudano et al. 1991). We therefore took the shortest route connecting the EC, callosum and then the contralateral EC (stimulation site) as the CLB pathway length (Fig. 1B; ⋯).

Statistical tests

Data are expressed as means ± SD if they pass the D’Agostino and Pearson normality test. Otherwise they are expressed as geometric mean ± 95% confidence interval (CI). P values were either determined from the test statistic derived from a two-sampled t-test (parametric), Kruskal-Wallis (nonparametric ANOVA) test followed by Dunn’s multiple-comparison test (nonparametric).

RESULTS

In vivo tract tracing and orthrodromic stimulation

Our first goal was to obtain a slice preparation where the length of some of the axonal pathways from TeA can be anatomically traced and their time delays measured, preferably in the same cell and with minimal confounding factors. To ensure the tracer injection was targeted at the TeA area, we first conducted experiments in vivo to see if orthodromic stimulation in this brain region can evoke synaptic responses in multiple subcortical sites. We stimulated the TeA and recorded orthodromically evoked local field potentials (LFPs). In anesthetized rats, single electrical stimulus in TeA area elicited LFPs in the CPU, parietal cortex (Par), limbic region, PT (Fig. 1A), indicating these brain sites indeed contain axonal terminals from TeA. The postsynaptic nature of the recorded LFPs was indicated by their long duration (typically lasted from 10 to 20 ms), much longer than action potentials and their sensitivity to stimulation frequency (potentiation or depression; data not shown). Furthermore, we found that the average LFP latency of all ipsilateral sites were rapid and synchronized (mean: 3.1 ± 0.26 ms and comparable among almost all ipsilateral sites, coefficient of variance = 8%, P > 0.05; n = 6). However, in spite of corticofugal axons forming axonal plexuses of terminals in the external cortex of the inferior colliculus (ECIC) (Arnault and Roger 1990), the evoked LFPs in this region were small and variable. This is likely

![Fig. 1](http://jn.physiology.org/)

**Fig. 1.** Characterization of functional temporal association and parahippocampal cortex (TeA) efferent pathways in vivo and in vitro. A: in vivo recordings of local field potentials (LFPs) in cortical and subcortical targets evoked by ipsilateral TeA stimulation (shown as individual traces). LFPs show short-latency (<4 ms) responses in the caudate putamen (CPU), parietal cortex (Par), amygdala (Amyg), and posterior thalamic nuclei (PT). The data were compiled from 6 rats for each recording site. Inset scale bars: vertical, 70 μV; horizontal, 5 ms. B: brain slice used for in vitro pathway tracing and electrophysiology. Axonal pathway trajectories (orange) are overlaid onto the horizontal preparation. Blue dots indicate stimulation sites. Inset (bottom): a typical layer V neuron with an approaching patch electrode. Inset (left): higher-magnification images of biotin dextran amine (BDA)-positive axons traveling within the external capsule located beneath the TeA (indicated by the red arrow). ECIC, external cortex of the inferior colliculus; HF, hippocampal formation; Ic, internal capsule; EC, external capsule; CC, corpus callosum; and CLB, contralateral branch.
due to terminal field dispersion and/or its susceptibility to anesthesia. It should be noted that the waveforms of LFPs recorded in vivo may be subject to the influences of anesthetics. Moreover, their latencies include synaptic and membrane ion channel delays at postsynaptic sites, in addition to axonal delays (Hu 1995; Marsalek et al. 1997). Therefore although LFPs may reveal the sites of axonal endings, the absolute latency values of LFPs cannot be equalized to presynaptic axonal conduction time.

In a separate set of experiments, we injected a small amount of BDA into the same site as we applied electrical stimulation. Brains from these rats were cut into horizontal slices (115 μm) with a 30° angle (similar to that used for electrophysiology) to keep all the branches of TeA pathways as complete as possible (see Methods). In these slices, BDA-labeled fibers could be followed from TeA to CPU, PT, tectum, and ECIC (Figs. 1B and 2). The CLBs, although they could be activated in the slices by stimulating the contralateral external capsule, were not labeled by the BDA. This likely reflects the poor ability for tracer retention of callosal fibers (Hackett et al. 1999; Parent and Parent 2006).

Antidromic activation of layer V neurons in vitro

Whole cell recordings were made from layer V pyramidal neurons maintained in in vitro horizontal preparation (Fig. 1B). The majority of layer V neurons can be classified as bursting type although some neurons, especially in younger rat group, regular spiking, and initially did not seem to fire action potentials in response to current injections or antidromic stimulation (unpublished observations). Single electrical stimulus applied at threshold intensities to each of the four stimulation sites all evoked a single fast action potential (AP). The waveform and the latency of a typical AP are shown in Fig. 3 (left). As previously noted (Chomiak and Hu 2007), the stimulus threshold for evoking an antidromic AP from different stimulation sites can differ significantly, which is in part related to the local branch pattern and level of tissue myelination (e.g., between posterior thalamus and brain stem). During repetitive antidromic stimuli, antidromic responses exhibited small (<0.1 ms) temporal jitters in antidromic response. The antidromic nature of the evoked axonal responses were further confirmed by the presence of collision tests by pairing an antidromic AP with either an

FIG. 2. Pathway tracing and geometrical measurements of TeA efferent axons. Middle (A): montage of TeA descending pathways prelabeled by BDA. Light microscope images were taken (×10) from a horizontal slice that was used for electrophysiology. Injection of BDA (brownish deposit at the TeA site) was made 48 h prior to tissue processing as described in Methods. BDA containing fibers appear in the external (EC) and internal capsules as well as in the subcortical white matters. Prominent fibers with terminal like morphology are also seen in PT, CPU, ECIC, and tectum. Some amplified (×20) images are shown as insets for EC and ECIC sites. The red arrow indicates the location where descending fibers entering the PT and tectum are split from those to CPU. Left (B): TeA descending pathways were manually traced and laid over to the computer-generated silhouette as shown in gray (Northern Eclipse). Right (C): images of BDA-labeled pyramidal cells at the TeA injection site. Bottom graph: the presence of dense plexuses of labeled thin fibers or swellings (also see enlarged images in A) in the gray matter, which is taken as the indication of terminal fields. It is noteworthy that subcortical projections to nonthalamic regions arise primarily from layer V neurons. Abbreviations are given in text and Fig. 1.
orthodromic AP or an antidromic AP initiated from another collateral and by the absence of synaptic potentials during antidromic stimulation at different membrane potentials (Chomiak and Hu 2007; Lipski 1981).

Population latency and conduction velocity

We constructed the spike latency histograms for each axonal pathway obtained from 61 neurons (Fig. 3, right). At the population level, the mean onset latency of antidromic spikes of all the ipsilateral pathways clustered around 0.57 ± 0.37 ms (Fig. 3B), demonstrating an identical synchronized timing pattern relationship seen in vivo (Fig. 1A). In contrast, the average latency from all responding fibers in the contralateral projection was significantly longer than all ipsilateral pathways (1.2 ± 0.12 ms; n = 34; P < 0.001; see Table 1). Although as a population, latency variation among ipsilateral pathways appears to be somewhat variable, intrabranch comparison in single cells showed an opposite trend (see following text).

Next we calculated the mean conduction velocities of individual axonal pathways (Table 1). The axonal length data were obtained by measuring the distance of each fiber projection from TeA to the stimulation sites (Fig. 1B). These pathways are prelabeled with an anterograde tracer deposit and cut in same way as the slices used for electrophysiology (see METHODS). As shown in Table 1, the conduction velocities of individual pathways are evidently heterogeneous.

Spike conduction among different branches from the same cell

If axonal geometrical properties reflect a self-adaptation process of individual fibers, how does this regulation mani-

FIG. 3. Population latency distribution of TeA axonal pathways. Left: an example action potential evoked by antidromic stimulation of axonal terminal field regions. The axonal conduction time is defined here as the time interval (t) between the stimulation artifact and the onset of the initial segment spike (IS-SD) or the “foot” of the AP. Right: population histograms of axonal spike latency distributions for each of the four TeA efferent pathways. Scale bars: horizontal 0.5 ms, vertical 20 mV.
TABLE 1. Summary of individual efferent pathways properties

<table>
<thead>
<tr>
<th>Site</th>
<th>Spike latency, ms</th>
<th>distance, mm</th>
<th>V_{abs} mm/ms</th>
<th>df</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>0.52 ± 0.06</td>
<td>5.8 ± 0.25</td>
<td>11.2 ± 1.2</td>
<td>(56)</td>
</tr>
<tr>
<td>ECIC</td>
<td>0.41 ± 0.05</td>
<td>9.9 ± 0.25</td>
<td>24.1 ± 2.6</td>
<td>(60)</td>
</tr>
<tr>
<td>CPU</td>
<td>0.53 ± 0.11</td>
<td>4.1 ± 0.25</td>
<td>7.7 ± 1.3</td>
<td>(53)</td>
</tr>
<tr>
<td>CLB</td>
<td>1.20 ± 0.12</td>
<td>19.5 ± 0.25</td>
<td>16.3 ± 1.7</td>
<td>(33)</td>
</tr>
</tbody>
</table>

Mean spike latency of individual pathways. The fiber length was determined for each projection using the method mentioned in METHODS. Experimentally determined conduction velocity (V_{abs}) was obtained by dividing the general pathway distance by the axonal spike latency. df, degrees of freedom. See text for details. Note: all ipsilateral sites are significantly shorter than the contralateral site (P < 0.001). PT, posterior thalamic nuclei; ECIC, external cortex of the inferior colliculus; CPU, caudate putamen; CLB, contralateral branch.

fested among individual axonal branches in the same cell? In other words, are there relative timing patterns between individual collaterals? To address this question, we evaluated the relative timing pattern differences between axonal branches in individual neurons. As shown in Fig. 4, two distinct timing patterns emerged. First, the three ipsilateral pathways exhibit a very small intrabranch latency shifts among themselves (0.24 ± 0.21 ms; n = 20). Second, and in contrast, the latency difference between the three ipsilateral branches and the CLB of the same cell is disproportionately large (0.78 ± 0.31 ms; n = 10), with the CLB delays being shifted by approximately equal to threefold (P < 0.001; Fig. 4). Note, however, that this value underestimates the true intrabranch latency shift because the entire length of the contralateral branch cannot be maintained in the preparation. Nevertheless, the timing pattern of the ipsi- and contralateral pathways showed a systematic asymmetry. The conduction delays of ipsilateral pathways tend to be minimized and tuned to isochrony, whereas the contralateral fibers tend to be significantly slower and lag the ipsilateral spike conduction, suggesting a co-existence of different axonal timing strategies at a level of single cells.

Velocity-length relationship

We constructed the velocity-length relationship for layer V axons. This method was used previously to evaluate the degree of synchrony in spike conduction time among parallel axonal projections in olivocerebellar pathways (Sugihara et al. 1993). We plotted the observed conduction velocities (V_{obs}) of individual branches against their pathway lengths (D) as shown in Table 1. It has been demonstrated that for a network to achieve isochronous spike conduction (ICT), a linear relationship between velocity (V_{abs}) and pathway length D must exist (Sugihara et al. 1993). This relationship is expressed as V_{obs} = k·D; where k (ms⁻¹) is a rate constant and equivalent to the reciprocal of ICT (i.e., k = 1/ICT). In Fig. 5, the experimental velocity and distance data are plotted for all the fiber pathways. We found a quasi-linear relationship in velocity-distance plots for experimental data with a best-fit k value of 2.2 ± 0.2 ms⁻¹. Strikingly, the contralateral projection did not fit into this linear plot (Fig. 5; “contralateral”).

DISCUSSION

In the present study, we examined timing properties of the axonal network formed by cortical layer V neurons. We first found that a single layer V neuron can issue multiple axonal collaterals of different length. This reticular-like axonal network, however, exhibits robust isochronous spike conduction. Moreover, the ICT appears to be asymmetrical and is strongly biased toward ipsilateral projections.

Methodological considerations

STIMULATION SITES. The placement of our stimulation electrodes was targeted at brain regions that have been previously shown to host rich axonal terminal labelings when anterograde transport tracers were deposited in the TeA area (Arnault and Roger 1990; Shi and Cassell 1997; Vaudano et al. 1991; Winer et al. 2001). This labeling pattern has also been reconfirmed in our own tracing experiments. Although we cannot be certain of the exact site of spike initiation during stimulation (which is relevant to all stimulation studies), it is important to keep in mind that the errors associated with this are exceedingly small relative to the total path-length (i.e., a few hundred micrometers vs. several millimeters) and fall within the 95% confidence interval range of the experimental data (Table 1).

STIMULATION CURRENT SPREAD. Our antidromic collision tests showed that individual axonal pathways can be selectively

![FIG. 4. Intra-pathway delays among individual branches from single TeA neurons. Average intra-pathway latency difference among all ipsilateral branches and the contralateral branch (i.e., the difference between the contralateral site with at least one (and ≤3) of the ipsilateral sites for an individual cell). The phase or latency shifts between contra- and ipsilateral branches are significantly different. See text for details. Scale bars: horizontal, 2 ms; vertical, 25 mV. **P < 0.001.](http://jn.physiology.org/)

![FIG. 5. Velocity-length relationship of layer V efferent axons. The velocity (V_{abs})-distance plot is based on the data in Table 1. The isochronous spike conduction ICT is represented by the inverse slope (1/k) of the line. The velocity distance demonstrates a linear relationship (R^2 = 0.94) and can be described as V_{obs} = k·D, where the slope factor k equals 1/ICT (ms⁻¹). The best fit value for k is 2.2 ± 0.2.](http://jn.physiology.org/)

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activated in slices. This result cannot be due to current spread. The individual stimulation sites in our slices were separated by distances >1 mm. We estimated that with a threshold current intensity <110 μA, the edge of spreading current in our preparation does not seem to exceed 500 μm. This is consistent with that reported in previous studies (Bagshaw and Evans 1976; McIntyre et al. 2004; Nowak and Bullier 1996). Furthermore, we have also shown that slow conducting fibers require higher stimulation intensities than fast conducting fibers, also confirming that different types of axonal collaterals can be activated independently in our preparation (Chomiak and Hu 2007).

**ANTIDROMIC LATENCY MEASUREMENTS.** The latency and conduction time were determined based on the time interval between the end of the stimulation pulse and the onset (foot) of the antidromically evoked action potential (Lipski 1981) (see Fig. 3). This is because antidromic spike initiation in the axon follows the termination or peak of a stimulus pulse, which in turn reflects the brief time needed to charge the membrane of an axon (Davies 1968; McIntyre and Grill 1999). Indeed even in our preparation, we found that shortening the pulse duration of an activating stimulus (e.g., from 150 to 10 μs for a given intensity) led to an all-or-none activation failure, consistent with the fact that axonal excitation occurs near the terminating edge of the stimulus pulse (data not shown). Using the end of the pulse for latency determination can also help to improve the comparability of population data where different stimulus parameters are used for activating different axons (Ranck 1975). Furthermore, because the pulse durations we used here are similar to the apparent utilization time of axons, the latencies measured at the end of stimulus pulse is, in theory, more closely matched to the actual conduction time (Jankowska and Roberts 1972).

**Descending reticular network**

TeA in rat comprises the areas corresponding to the polysensory temporal cortex present in monkey and human, including part of the dorsal aspect of parahippocampal area 36 (Burwell and Amaral 1998; Palomero-Gallagher and Zilles 2004). Previous axonal tracing studies in rats show that the TeA projects to multiple cortical and subcortical regions (Arnault and Roger 1990; Shi and Cassell 1997; Vaudano et al. 1991; Winer et al. 2001). These include the ipsilateral conjoint regions of the dorsal medial geniculate body and lateral posterior nucleus (Burwell and Amaral 1998; Shi and Cassell 1997), caudal portion of the CPU (Vaudano et al. 1991), the ECIC (Arnault and Roger 1990), the superior colliculus (Arnault and Roger 1990; Vaudano et al. 1991), brain stem nuclei (Arnault and Roger 1990; Vaudano et al. 1991), the amygdala and peri-/entorhinal cortices (Burwell and Amaral 1998; Shi and Cassell 1997), parietal and occipital cortices (Burwell and Amaral 1998; Shi and Cassell 1997), prefrontal and orbital cortices (Shi and Cassell 1997; Vaudano et al. 1991). TeA also sends projections to the contralateral TeA region (Vaudano et al. 1991). However, it remains uncertain that efferent projections to these high-order brain structures are mainly derived from the same neuron or formed predom- inately by many disparate cell groups without significant axonal branching.

In this study, using electrophysiological methods, we provide strong evidence that the long distance axonal projections from layer V neurons are mainly derived from collaterals arising from the same cell. Although previous studies have already alluded this structural organization in sensory and motor cortical neurons (Bourassa and Deschenes 1995; Deschenes et al. 1998; O’Leary and Koester 1993), to our knowledge, this is the first unambiguous demonstration that most, if not all, layer V neurons form divergent axonal pathways to multiple subcortical regions. The cortical neurons we recorded from also frequently showed antidromic spikes to ipsilateral intra-cortical stimulation (unpublished observations). However, due to shorter axonal length, the latency of these intra-cortical branches cannot be determined as precisely as other pathways. Therefore from a structural point of view, the axonal architecture of layer V axons may form a “reticular” network through which a cortically generated signal can be distributed effectively via presumably a small group of projection neurons, transcending the boundaries of many sensorimotor modalities. Interestingly, this type of descending network has already been proposed for the association and adjacent parahippocampal cortex as an important network structure for facilitating sensory recognition and memory recall (Damasio 1989). The highly branched innervation pattern may allow time-locked co-activation of the same geographically separate sites in early sensory pathways that were engaged during the initial experience (Damasio 1989; Fuster 1997; Gilbert and Sigman 2007).

**Conduction velocity and isochronous spike conduction**

Although layer V projections are considered fast-conducting fibers, the antidromic latency data obtained in vitro slice can be approximately equal to fivefold faster than that reported in vivo (Beloozerova et al. 2003; Swadlow and Weyand 1981). Several technical factors may account for this difference, notably the absence of anesthetics, interference of spontaneous activity (Swadlow 1998), difference in intracellular versus extracellular measurements of spike waveform latency (Lipski 1981), and the requirement of membrane depolarization for observing antidromic spikes (Chomiak and Hu 2007). Nevertheless, it is important to note that fast-conducting cortical antidromic responses, with comparable latencies, have also been observed in vivo (Sirota et al. 2005; Swadlow 1991).

In a number of central neural pathways such as olivocerebellar (Lang and Rosenbluth 2003; Sugihara et al. 1993), thalamocorti- cal (Salami et al. 2003), and amygdalocortical projections (Pelle- tier and Pare 2002), the axonal delays appear rather uniform and isochronous. In our study, the k value (2.2 ms⁻¹) appears to be slightly different from that found in olivocerebellar and thalamocortical fiber systems (Salami et al. 2003; Sugihara et al. 1993). This difference may be due to the fact that we used pure axonal conduction velocity to calculate ICT without implicating synaptic delays as was done in other studies. This may yield a shorter but more accurate conduction time for axon collaterals and, as a result, allow us to separate out composite conduction delays (i.e., synaptic and axonal). In support of this conjecture, we found that the average in vivo latencies of orthodromically evoked LFPs in ipsilateral pathways, although isochronous, were longer than antidromic spike latencies measured in vitro. Finally, the diameters of the axons in layer V network appear larger (up to ~3 μm; Supplementary Fig. S1) than those of the thalamocortical system (see Salami et al. 2003).
Possible mechanism of ICT

Despite a number of axonal networks that are known to exhibit ICT or strong tendencies toward synchronous spike conduction, it is not intuitively apparent how this is achieved. The conduction time of an axon is largely determined by its geometrical properties, e.g., the diameter, length, and degree of myelination (Lang and Rosenbluth 2003; Waxman and Bennett 1972; Waxman and Swadlow 1977). To account for our experimental data, we derived a simple model (see supplementary material) based on Rushton’s quantitative theory of axon diameter and speed of conduction for myelinated and nonmyelinated fibers (Rushton 1951). With the use of experimentally determined structural data (Supplementary Figs. S1 and S2), we found that the appearance of ICT in this layer V network can be readily accounted for by regional myelination and axonal geometry adaptation among ipsilateral branches (Supplementary Fig. S3). Interestingly, in our model, the conduction time of CLBs from layer V neurons does not fit the isochronous spike timing pattern of the ipsilateral pathways despite having a conduction velocity comparable to the fastest ipsilateral branches (Fig. 5 and Table 1). We interpret this result as that the longer path-length of CLBs (>10 mm) incurs an extra time delay (Supplementary Fig. S3). These results are consistent with the suggestion that both regional myelination and axonal geometry play an important role in the occurrence of ICT (Salami et al. 2003).

Functional implications

Corticofugal projection network plays an important role in top-down attention modulation and facilitation of sensory cue responses in the basal ganglia, tectum, and posterior thalamus (Komura et al. 2001; Ma and Suga 2001; Mooney et al. 2004; Winkowski and Knudsen 2006). By having the long axonal fibers consolidated to a relatively few projection neurons, layer V network may significantly enhance its functionality. For example, the spike timing control can be executed faster and more precisely in this way, thereby facilitating orientating response to behaviorally significant events that often requires speedy sensorimotor coordination across different modalities. Fast spike conduction in the ipsilateral layer V network may also help dispatch cortical attention signals to multiple brain regions simultaneously. This may allow an internal frame of when, what, and where, to be rapidly reconstituted across different sensorimotor modalities that are physically separate in space but become closely connected in time.

GRANTS

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