Abstract
The pulvinar nucleus of the tree shrew receives both topographic (specific) and nontopographic (diffuse) projections from superior colliculus (SC), which form distinct synaptic arrangements. We characterized the physiological properties of these synapses and describe two distinct types of excitatory postsynaptic potentials (EPSPs) that correlate with structural properties of the specific and diffuse terminals. Synapses formed by specific terminals were found to be significantly longer than those formed by diffuse terminals. Stimulation of these two terminal types elicited two types of EPSPs that differed in their latency and threshold amplitudes. In addition, in response to repetitive stimulation (0.5–20 Hz) one type of EPSP displayed frequency-dependent depression whereas the amplitudes of the second type of EPSP were not changed by repetitive stimulation of up to 20 Hz. To relate these features to vesicle release, we compared the synapsin content of terminals in the pulvinar nucleus and the dorsal lateral geniculate (dLGN) by combining immunohistochemical staining for synapsin I or II with staining for the type I or type 2 vesicular glutamate transporters (markers for corticothalamic and tectothalamic/retinogeniculate terminals, respectively). We found that retinogeniculate terminals do not contain either synapsin I or synapsin II, corticothalamic terminals in the dLGN and pulvinar contain synapsin I, but not synapsin II, whereas tectopulvinar terminals contain both synapsin I and synapsin II. Finally, both types of EPSPs showed a graded increase in amplitude with increasing stimulation intensity, suggesting convergence; this was confirmed using a combination of anterograde tract tracing and immunocytochemistry. We suggest that the convergent synaptic arrangements, as well as the unique synapsin content of tectopulvinar terminals, allow them to relay a dynamic range of visual signals from the SC.

Introduction
Three main types of glutamatergic terminals have been identified in the visual dorsal thalamus of rodents, carnivores, primates, and tree shrews. All contain round synaptic vesicles, but can be distinguished based on their size as small (RS), medium (RM) and large (RL) profiles. RS profiles originate from cortical layer VI [1,2,3,4,5,6], RM profiles originate from the superior colliculus [7,8,9,10] and RL profiles are contributed by primary sensory inputs, cortical layer V, or thalamocortical axon collaterals [1,2,7,11,12,13,14,15,16,17,18,19].

RL profiles, such as those originating from the retina, have been described as “drivers” because the receptive field properties of postsynaptic neurons are dependent on activation of these inputs. In contrast, corticothalamic RS profiles have been described as “modulators” because their activation changes the overall responsiveness of postsynaptic neurons, but does not dramatically change receptive field properties [20]. The role of tectothalamic RM profiles is less clear, but they appear to form a third functional class of terminals [21,22]; it has been suggested that the collective activities of multiple convergent RM inputs are critical for the formation of the receptive field properties of postsynaptic neurons. Comparisons of the excitatory postsynaptic potentials (EPSPs) elicited by stimulation of RS or RL profiles have revealed distinct forms of short-term synaptic plasticity, which are thought to reflect differences in the probability of glutamate release from each terminal type [23]. Each RL profile makes multiple synaptic contacts [7,14,16,17,24], and their stimulation elicits large amplitude EPSPs that depress when the stimulation is repeated at high frequency [25,26,27,28]. In contrast, RS profiles make single synaptic contacts [16,17,24] and their stimulation elicits smaller amplitude EPSPs that facilitate with high frequency stimulation [23,25,28,29,30]. Recently, these features have been linked to the distribution of synapsin I and synapsin II, proteins that tether a reserve pool of synaptic vesicles [31,32,33,34,35]. These proteins are found in corticogeniculate (RS) terminals but not retinogeniculate (RL) terminals, and in synapsin I/II knockout mice the short-term plasticity of corticogeniculate EPSPs is altered, while retinogeniculate EPSPs are unchanged [36].

Unlike RS and RL profiles, the amplitudes of EPSPs elicited by stimulation of tectothalamic terminals (RM profiles) remain relatively stable at stimulation frequencies of 1–10 Hz [21,22]. To investigate potential mechanisms responsible for the stability of RM EPSPs, we correlated morphological features of tectopulvinar terminals with the postsynaptic responses elicited by their stimulation in tissue obtained from tree shrews (Tupaia belangeri). In this species, tectopulvinar terminals are classified as RM profiles, but they exhibit two different types of synaptic
arrangements, referred to as "diffuse" and "specific" [9,37]. The specific projections are topographically arranged and distributed throughout both the dorsal (Pd) and central (Pc) pulvinar nuclei. The diffuse projections are nontopographic projections that only innervate the Pd. Our recent tract tracing studies indicate that the Pd and Pc project to the temporal cortex and striatum, and the Pd additionally projects to the amygdala [6,39]. Thus, we have suggested that the specific projections relay topographic visual information from the SC to the cortex and striatum to aid in guiding precise movements, while the diffuse projection relays nontopographic visual information from the SC to the amygdala to alert the animal to potentially dangerous visual images.

In the current study, we characterized the length of synaptic contacts made by diffuse and specific tectopulvinar terminals, the synapsin content of RS, RM and RL profiles in the pulvinar nucleus and dorsal lateral geniculate nucleus (dLGn), and the properties of tectopulvinar EPSPs. We conclude that the short-term synaptic plasticity of RM profiles is distinct from that of RS and from that of RL profiles because each terminal type contains a unique distribution of synapsins. Furthermore, we suggest that the diffuse and specific tectopulvinar projections display subtle differences in short-term plasticity due to differences in glutamate release probability.

Materials and Methods

A total of 16 tree shrews (Tupaia belangeri); 7 adults (more than 3 months old) and 9 juveniles (3 weeks old), were used for these experiments. Twelve tree shrews were used for in vitro physiology experiments, 1 tree shrew received injections of biotinylated dextran amine (BDA) in the SC, and tissue from 3 tree shrews was used for immunocytochemistry. In addition, we analyzed material from 2 tree shrews used in a previous study [9]. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Louisville (Animal Welfare Assurance number A358601).

Slice preparation

Thalamic slices (400 µm) were prepared from 9 juvenile and 3 adult male and female tree shrews using procedures previously described [22]. Briefly, the animals were deeply anesthetized with carbon dioxide and decapitated, the brain was excised and a block of tissue containing the thalamus was removed and placed in an ice-cold oxygenated solution of modified artificial cerebrospinal fluid (ACSF) containing (in mM) 206 sucrose, 2.5 KCl, 1 CaCl2, 1 MgSO4, 1 MgCl2, 1.25 NaH2PO4, 26 NaHCO3 and 10 glucose at a pH of 7.4 and equilibrated with 95% O2/5% CO2, adjusted to 7.2 with KOH (osmolarity 290–295 mOsm). Biocytin (0.5%) was added to allow morphological reconstruction of the recorded neurons. Current-clamp recording were made with an Axoclamp 2B amplifier (Axon Instruments, Foster City, CA); the bridge was continually monitored and adjusted as needed. Data were digitized and stored on an IBM-compatible computer for offline analyses. Only recordings showing a stable resting membrane potential more negative than −50 mV and over shooting action potentials were included in this study (n = 46). To stimulate the tectothalamic fibers a multipolar stimulation electrode (matrix microelectrode; FHG, Bowell, ME) was placed in the superficial layers of the superior colliculus (SC). Stimulating electrodes were at least 2 mm away from the recording electrode. Once a stable whole-cell recording was obtained, paired-pulse or train stimulation was produced by using any two adjacent electrodes (115 µm apart) in the arrays until the best response was achieved.

Histochemistry

A subset of neurons was filled with 0.5% biocytin by diffusion from the pipette during recording (20 recovered). At the end of each recording, slices were fixed at 4°C overnight in 4% paraformaldehyde and rinsed several times in 0.1 M phosphate buffer (PB). Slices were then incubated in 10% methanol in PB with 3% hydrogen peroxide to react with the endogenous peroxidase activity of red blood cells. After several rinses in PB, slices were incubated overnight at 4°C under agitation in a 1% solution of avidin and biotinylated-horseradish peroxidase (ABC Kit Standard, Vector Laboratories) prepared in 0.3% Triton X-100. The slices were subsequently rinsed, reacted with nickel-intensified 3,3'-diaminobenzidine (DAB) for 5 min, and washed in PB. After rinses in phosphate buffer, slices were mounted onto slides and reconstructed with a Neurolucida system (Micro Bright Field Inc., USA). In some cases, biocytin-filled neurons were revealed by incubating slices in a 1:100 dilution of streptavidin conjugated to Alexa Fluor 546 (Molecular Probes, Eugene, OR) and confocal images of the cell were obtained using an Olympus Fluoview laser scanning microscope (BX61W1).

Tracer injections

As described in detail in our previous study [9], single tracer injections in the SC label dense topographic clusters of terminals ("specific" terminals); the medial SC projects to the Pd, while the central and lateral regions of the SC project to the Pc. In contrast, the “diffuse” terminals are nontopographic; injections in any part of the SC label sparsely-distributed terminals in the Pd [37]. Therefore, when BDA is injected into the medial SC, tracer-labeled diffuse and specific terminals overlap within the Pd. However, when BDA is injected into the central or lateral SC, diffuse terminals are labeled in the Pd, while specific terminals are labeled in the Pc, allowing analysis of these segregated populations. Therefore we placed injections into the central and/or lateral regions of the SC to enable us to examine the isolated "diffuse" and “specific” projections in the Pd and Pc respectively.

Tree shrews that received BDA (3,000 MW; Molecular Probes, Eugene, OR) injections were initially anesthetized with intramuscular injections of ketamine (100 mg/kg) and xylazine (6.7 mg/kg). Additional supplements of ketamine and xylazine were administered approximately every 45 minutes to maintain deep anesthesia through completion of the tracer injections. Prior to injection, the tree shrews were placed in a stereotaxic apparatus and prepared for sterile surgery. A small area of the skull overlying the superior colliculus was removed and the dura reflected, a glass pipette containing either BDA (5% in saline, tip diameter 3 µm) was lowered vertically and the tracer was ejected iontophortically.

Tectopulvinar Synapses and Synaptic Potentials
(2 µA positive current for 15–30 minutes) into the central and/or lateral SC. After a 7-day survival period, the tree shrews were given an overdose of ketamine (600 mg/kg) and xylazine (130 mg/kg) and were perfused through the heart with Tyrode solution, followed by a fixative solution of 4% paraformaldehyde.

The BDA was revealed by incubating sections in a 1:100 dilution of avidin and biotinylated horseshadish peroxidase (ABC; Vector Laboratories, Burlingame, CA) in phosphate-buffered saline (0.01 M PB with 0.9% NaCl, pH 7.4; PBS) with 1% normal goat serum (NGS) overnight at 4°C. The sections were subsequently rinsed, reacted with nickel-intensified 3,3′-diaminobenzidine (DAB) for 5 minutes, and washed in PB. For confocal microscopy, the BDA was revealed by incubating sections in a 1:100 dilution of streptavidin conjugated to Alexa Fluor 546 (Molecular Probes, Eugene, OR).

Immunohistochemistry

Three adult tree shrews were given an overdose of sodium pentobarbital (tree shrew) were perfused through the heart with Tyrode solution, followed by a fixative solution of 4% paraformaldehyde in 0.1 M PB. The brain was removed from the skull, sectioned into 50-µm-thick slices using a vibratome (Leica VT1000S, Leica Microsystems, Bannockburn, IL) and collected in a solution of 0.1 M PB. The sections were incubated at 4°C overnight with one of the following antibodies: guinea pig monoclonal anti-vGLUT1 or anti-vGLUT2 (1:5000, Chemicon Temecula, CA; catalogue #’s AB5905 and AB2251), rabbit polyclonal anti-synapsin I (1:1000, Millipore, Billerica, MA; catalogue #AB1543P) or rabbit monoclonal anti-synapsin II (1:500, Abcam, Cambridge, MA; catalogue #AB76494). The following day the sections were rinsed in PB and incubated for 1 hour in an anti-guinea pig antibody conjugated to Alexafluor-488 (1:100, Invitrogen/Molecular Probes, Carlsbad, CA; catalogue #A11073) or anti-rabbit antibody conjugated to Alexafluor-546 (1:100, Invitrogen/Molecular Probes, Carlsbad, CA; catalogue # A11010). The sections were subsequently rinsed in PB and mounted on slides for confocal microscopic examination.

Ultrastructural Analysis

Electron micrographs of diffuse and specific tectopulvinar terminals labeled by the anterograde transport of BDA injected into the SC, which were collected in a previous study [9], were re-analyzed. As described above, following BDA injections in the central and/or lateral SC, “diffuse” terminals were labeled in the Pd and “specific” terminals were labeled in the Pc. Resin-embedded sections were first examined with a light microscope to select “specific” terminals in the Pc and “diffuse” terminals in the Pd for electron microscopic analysis. Selected areas of the Pc or Pd were mounted on blocks, ultrathin sections (70–80 nm, silver-gray interference color) were cut using a diamond knife, and every fifth section was collected on Formvar-coated nickel slot grids. One in four of the collected sections was subsequently stained for the presence of gamma amino butyric acid (GABA) using gold particles, and examined using an electron microscope. Within each examined section, all labeled terminals involved in a synapse were photographed. The section spacing ensured that no single terminal was examined more than once. In the current study, we used ImageJ software (Rasband, W.S., U. S. National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2009) to measure the length of each synaptic contact, and the area of each presynaptic BDA-labeled terminal, within the previously collected images.

Statistical Analysis

Student t-tests were used to test for statistical significance. Quantitative data are expressed as means ± SD. The significance level was set at p<0.05 for all statistical comparisons.

Figure 1. Convergence of tectopulvinar terminals. Injections of biotinylated dextran amine (BDA) in the SC labels tectopulvinar axons that form widespread (“diffuse”) axons and boutons as well as more discrete clustered (“specific”) boutons. Representative images of BDA-labeled “diffuse” (A) and “specific” (B) axons in 50 µm thick sections are illustrated using transmitted light, a 40x objective, and Nomarski optics. C–G) Confocal images (single 0.1 µm scan with a 100x objective) illustrate tectopulvinar terminals labeled with BDA (purple) and tectopulvinar terminals labeled with antibodies against the type 2 vesicular glutamate transporter (vGLUT2, green). Terminals labeled with both BDA and vGLUT2 appear white. Clusters of vGLUT2-stained terminals contain at most 1 bouton contributed by “diffuse” axons (F), while “specific” axons contributed several boutons to each cluster (C–E, G) Scale in B = 10 µm and applies to A. Scale in D = 10 µm and applies to C, F and G. Scale in E = 2 µm.

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Results

Tectopulvinar clusters and convergence

As previously reported [9], injections of biotinylated dextran amine (BDA) in the SC label tectopulvinar axons that terminate in nontopographically organized widespread boutons (“diffuse” projections; Figure 1A) as well as topographically organized clusters of boutons (“specific” projections; Figure 1B). By comparing BDA-labeled tectopulvinar axons to synapses labeled by the vGLUT2 antibody (a marker for tectothalamic terminals; [9,10]), we previously concluded that both the diffuse and specific projections are convergent [9]. We confirmed this by staining tissue that contained BDA-labeled tectopulvinar axons with the vGLUT2 antibody. As illustrated in Figure 1C–G, for both types of tectopulvinar axons, the BDA-labeled terminals made up a small proportion of the total terminals within a vGLUT2-stained cluster. However, vGLUT2-stained clusters contained at most 1 bouton contributed by “diffuse” axons (Figure 1F), whereas “specific” axons contributed several boutons to each cluster (Figure 1C–E, G). This suggests the “diffuse” pathway exhibits a greater degree of convergence onto individual dendrites than does the “specific” pathway.

Specific synapses are longer than diffuse synapses

We re-analyzed electron micrographs collected in our previous ultrastructural study of tectopulvinar terminals labeled by the anterograde transport of BDA injections into the SC [9] to compare the synapse length of “specific” terminals (Figure 2A, n = 47) imaged within Pc tissue and “diffuse” terminals (Figure 2B, n = 49) imaged in Pd tissue. As illustrated in Figure 2C, the distribution of the synapse lengths overlapped, but as a population, specific synapses (0.25±0.07 μm) were found to be significantly longer than diffuse synapses (0.22±0.07 μm, P<0.05). We found no significant difference in the size of the specific and diffuse terminals measured at the site of synaptic contacts (specific 0.46±0.21 μm2; diffuse 0.47±0.21 μm2). In this single section analysis most terminals made single synaptic contacts (46 in Pc, 47 in Pd), but a few made more than one contact (1 in Pc, 2 in Pd).

Tectopulvinar terminals contain both synapsin I and synapsin II

Because the expression of synapsins has been related to short-term plasticity [36], we examined the synapsin content of RS, RM and RL profiles in the dorsal lateral geniculate nucleus (dLGN, Figure 2. “Specific” tectopulvinar synapses are longer than “diffuse” tectopulvinar synapses. Electron micrographs illustrate examples of specific tectopulvinar terminals in the Pc (A) and diffuse tectopulvinar terminals in the Pd (B) labeled by the anterograde transport of biotinylated dextran amine (BDA) from the superior colliculus. The synapse length (arrows) of each terminal type was measured. The distribution of synapse lengths is plotted (C). As a population, the length of specific synapses was found to be significantly longer than the length of diffuse synapses (P<0.05). Scale bar = 0.5 μm. doi:10.1371/journal.pone.0023781.g002
and pulvinar nucleus (Figure 4) of the tree shrew by combining immunohistochemical labeling for synapsin I or synapsin II with immunohistochemical labeling for vGLUT2 (a marker for both tectothalamic and retinogeniculate terminals; [9,10,18]) or vGLUT1 (a marker for corticothalamic terminals; [39,40,41]). As illustrated in Figure 3, we found that retinogeniculate terminals (RL profiles labeled with the vGLUT2 antibody) do not contain either synapsin I or synapsin II (as was previously reported in the mouse; [36]). We also found that corticothalamic terminals in the dLGN and pulvinar nucleus (RS profiles labeled with the vGLUT1 antibody) contain synapsin I but not synapsin II (Figures 3–4). Finally, we observed that tectopulvinar terminals (RM profiles labeled with the vGLUT2 antibody) contained both synapsin I and synapsin II (Figure 4). We found a similar distribution of synapsins in vGLUT1- and vGLUT2-labeled terminals in the rat dLGN and lateral posterior nucleus (unpublished data).

Because tectopulvinar terminals form dense clusters, and individual terminals are difficult to distinguish at the light microscopic level, we used ImageJ software to quantify the labeling patterns by calculating the number of white pixels (double labeled areas) as a percentage of the total area labeled with the vGLUT antibodies (white + green pixels). For each image, we only analyzed areas that included both synapsin and vGLUT labeling to control for differences in antibody penetration. Three images of each antibody combination in the dLGN, Pd and Pc were analyzed.

This analysis confirmed our qualitative assessment. There was very little overlap of vGLUT2 in the dLGN (retinogeniculate terminals) with either synapsin I (1.62 ± 0.40%) or synapsin II (1.99 ± 0.43%). For vGLUT1 staining in the dLGN (corticothalamic terminals) there was significant double-labeling with synapsin I (33.74 ± 6.12%) but not with synapsin II (3.85 ± 0.84%). This was also the case for vGLUT1 staining in the Pd and Pc (corticopulvinar terminals; Pd vGLUT1 29.36 ± 1.54% overlap with synapsin I and 3.83 ± 1.29% overlap with synapsin II; Pc vGLUT1 38.32 ± 5.51% overlap with synapsin I and 3.24 ± 1.29% overlap with synapsin II). In contrast, for vGLUT2 staining in the Pd and Pc (tectopulvinar terminals) there was significant double-labeling with both synapsins (Pd vGLUT2 57.86 ± 2.45% overlap with synapsin I and 48.79 ± 1.31% overlap with synapsin II; Pc vGLUT2 34.84 ± 5.49% overlap with synapsin I and 47.83 ± 4.25% overlap with synapsin II).

In vitro recording of tectopulvinar synaptic potentials

In 400 μm thick parasagittal sections, whole cell recordings were obtained in the pulvinar nucleus and the rostral SC was stimulated to evoke EPSPs. The pulvinar EPSPs evoked by SC stimulation can be identified as tectopulvinar EPSPs because the cortical input to the dorsal (Pd) and central (Pc) pulvinar...
subdivisions originates exclusively from layer VI [6] while cortical input to the SC originates exclusively from layer V [42]. Therefore SC stimulation cannot activate corticopulvinar inputs. In addition, the pretectum does not project to the Pd or Pc [9], so current spread to this region does not activate any pulvinar inputs. Finally, cholinergic inputs from the parabigeminal nucleus that travel through the superficial SC and optic tract to the dLGN [43] do not innervate the pulvinar nucleus [9].

Figure 5A illustrates the approximate location of the recording pipette in the pulvinar nucleus and the location of the 8 electrode stimulation array in the SC (spanning the superficial layers, stratum griseum superficiale and stratum opticum). The array spanned a distance of 1 mm and stimulation could be produced between any two electrodes in the array; the anode and cathode positions were varied to obtain the best response. With this configuration, tectopulvinar EPSPs could be elicited with a high rate of success (28 of 46 cells; 10 of 29 cells in juvenile tissue, and 10 of 17 cells in adult tissue) in the caudal pulvinar, which corresponds to the Pd (as described by Lyon et al., 2003[44]). In sections stained for vGLUT2, the Pd is identified as a region with densely distributed large clusters of terminals (Figure 5C) while the Pc contains smaller clusters of terminals that are more sparsely distributed (Figure 5D). We were unable to elicit tectopulvinar responses from more rostral regions of the pulvinar nucleus; in the parasagittal slice preparation, tectal axons traveling to the rostral pulvinar were most likely severed before they reached their synaptic targets.

As illustrated in Figure 5B, in response to the injection of depolarizing or hyperpolarizing current steps, all recorded neurons fired with either tonic action potentials or low threshold calcium spikes respectively, firing properties exhibited by thalamic relay cells [45]. Biocytin was included in the recording pipette and all recovered cells (n = 20) displayed morphologies consistent with their identification as relay cells (Figure 5E, F).

Tectopulvinar EPSPs can be divided into two groups based on latency, and amplitude

As illustrated in Figure 6, tectopulvinar EPSPs fell into two groups based on differences in their average latencies, as well as their threshold and peak amplitudes, suggesting that two different types of axon arbors were activated. EPSPs with the shortest latencies (2.11 ± 0.10 ms, n = 17; 10 in juvenile tissue and 7 in adult tissue) exhibited the largest threshold EPSP amplitudes (4.82 ± 0.57 mV, n = 17; Figure 6B) and EPSPs with longer latencies (3.20 ± 0.15 ms, n = 8; 5 in juvenile tissue and 3 in adult tissue) exhibited smaller threshold amplitudes (1.58 ± 0.27 mV, n = 8; Figure 6C). These parameters were found to be significantly different (latencies p<0.05; threshold amplitudes p<0.05), supporting the division of the tectopulvinar EPSPs into two groups. In
addition, the rise time (10-90% of maximum amplitude) of short latency, large amplitude EPSPs (2.90 ± 0.25 ms, n = 17) was significantly faster than that of the slow latency, small amplitude EPSPs (3.82 ± 0.27 ms, n = 8, p < 0.05).

The two types of tectopulvinar EPSPs display distinct short term plasticity

The short-term plasticity of these two types of EPSPs was tested with trains of stimuli varying in frequency from 1 Hz to 10 Hz (Figure 7), as well as paired-pulse stimuli with interstimulus intervals of 0.1 s to 2 s (Figure 8). For each type of EPSP we used stimulation currents sufficient to evoke EPSPs at 50% of maximum. The short latency/large amplitude EPSPs were depressed by high frequency stimulation (frequencies > 1 Hz, n = 10). With train stimuli, relative to the first EPSP of the train, the second EPSP decreased by 16.67 ± 2.35% at 2.5 Hz, 23.29 ± 3.88% at 5 Hz, and 29.42 ± 3.42% at 10 Hz (Figure 7A and B). Similar values were observed using paired-pulse stimuli (a 9.19 ± 4.99% reduction in the amplitude of the second pulse relative to the first pulse at interstimulus intervals of 0.6 s; 17.17 ± 7.51% at 0.2 s and 37.18 ± 8.27% at 0.1 s; Fig. 8A and C, n = 5). In contrast, for the slower latency/smaller amplitude EPSPs, there was no correlation between mean amplitudes and stimulation frequencies or paired-pulse interstimulus intervals (Fig. 7C and D, n = 6; Fig. 8B and D, n = 4).

The two types of tectopulvinar EPSP display different degrees of convergence

All recorded tectopulvinar EPSP amplitudes (n = 28) increased as the stimulation intensity was increased, suggesting that each postsynaptic cell receives input from multiple convergent tectal axons. However, the degree of convergence was found to be greatest for the slower/smaller/nondepressing EPSPs. The peak amplitudes of these EPSPs increased up to 3.34 fold above threshold amplitude values, while peak amplitudes of the faster/larger/depressing EPSPs increased to 2.11 fold above threshold values. As illustrated in Figure 6 (B and C), the rate of amplitude increase as a function of stimulation current was greater for nondepressing EPSPs compared to the depressing EPSPs.

“Mixed” EPSPs

For the majority of tectopulvinar EPSPs (25 of 28) the latencies did not change as the stimulation intensity was increased (Figure 6B, C). However, in some cases (n = 3) EPSPs appeared to contain a mixture of the two EPSP types described above. The amplitude of these “mixed” EPSPs initially increased gradually as the stimulation current was increased, but then exhibited a sudden large increase in amplitude, suggesting that the threshold was reached to recruit a different type of axon. Supporting this conclusion, there was a corresponding decrease in EPSP latency with the jump in EPSP amplitude (Fig. 9, A and B). Furthermore,
Figure 6. Two types of EPSPs. A) With increasing stimulation intensity, tecto-pulvinar EPSPs in the Pd show a graded increase in amplitude. B) Average first type EPSP amplitudes and latencies as a function of stimulation intensity (n = 17), graph show a graded increase in peak amplitude correlate to the increase in stimulation current but the latency of the EPSP is not relative to stimulation current. C) Average second type EPSP amplitudes and latencies as a function of stimulation intensity (n = 8), second type EPSP show a graded increase in peak amplitude and no change in latency with increasing stimulation intensity, but the threshold amplitude was smaller and latency was longer (p<0.05).

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for “mixed” EPSPs the amplitudes were unaffected by stimulus frequency when stimuli were delivered with threshold current levels, but frequency-dependent depression was observed when the stimuli were delivered with current levels that elicited the larger amplitude EPSPs.

**Discussion**

**Diffuse and specific EPSPs**

Based on differences in their convergence patterns, we suggest that the slower latency, smaller amplitude EPSPs represent activation of diffuse tectopulvinar projections, while the faster, larger amplitude EPSPs represent activation of specific tectopulvinar projections. This suggestion is also based on comparisons to our previous study of EPSPs elicited by stimulation of tectothalamic axons in the rat. Tectothalamic projections in the rodents are nontopographic [46] and EPSP amplitudes remain stable at stimulation frequencies of up to 20 Hz [22]. The lack of short-term frequency-dependent plasticity in the rat tectothalamic pathway compares to the slower latency, smaller amplitude tectopulvinar EPSPs of the tree shrew. In contrast, the faster, larger amplitude tectopulvinar EPSPs of the tree shrew exhibited a small frequency-dependent depression (29.42% at 10Hz), a feature which was not observed in our studies of the rat tectothalamic EPSPs. This difference does not appear to be a function of age because we detected these two types of tectopulvinar EPSPs in both juvenile and adult tree shrew tissue.

At threshold, specific EPSPs may be larger than diffuse EPSPs because stimulation of a single specific axon activates clusters of terminals that innervate the same dendrite [9], while stimulation of a single diffuse axon activates unitary inputs. In addition, relative to diffuse terminals, the glutamate release is likely to be higher for specific terminals because their synaptic zones are significantly larger. This conclusion is tentative since we measured the length of specific synapses in the Pc, while we recorded EPSPs in the Pd. However, assuming specific synapses are similar in the Pc and Pd, their longer length relative to diffuse synapses would help to explain why we observed frequency-dependent depression in one group of EPSPs; the pool of synaptic vesicles ready for release in specific tectopulvinar terminals may not be fully replenished during short interstimulus intervals. Diffuse tectopulvinar EPSPs may exhibit relatively stable due to a lower glutamate release probability. Since we detected no difference in the overall size of specific and diffuse terminals, a lower probability of neurotransmitter release would imply less vesicle depletion.
Synapsin distribution varies in glutamatergic terminals of the thalamus

Synapsins have been associated with the tethering of synaptic vesicles to cytoskeletal elements [47,48,49,50,51,52,53,54] and are thought to regulate the equilibrium of a reserve pool of synaptic vesicles and a population of vesicles that are docked for ready release [55,56]. It has been hypothesized that synapsins are necessary to sustain the release of neurotransmitter at high rates of synaptic transmission [30,51,52]. We found that, within the visual thalamus, tectothalamic terminals are the only glutamatergic terminals that contain both synapsin I and synapsin II. Retinogeniculate terminals contained neither synapsin, and corticothalamic terminals contained synapsin I, but not synapsin II. These distributions provide further support for the division of thalamic glutamatergic terminals into 3 functional classes, and are presumably related to the distinct synaptic properties of these terminal types. Retinogeniculate EPSPs exhibit strong frequency-dependent depression [25,26,27], corticothalamic EPSPs exhibit strong frequency-dependent facilitation [25,29,30], while tectothalamic EPSP amplitudes remain relatively stable during high frequency stimulation [22]. Even the tectopulvinar EPSPs that showed a small decrease in amplitude with high stimulation frequencies (29.42% decrease at 10Hz) stimulation are more stable than retinogeniculate EPSPs which can depress by as much as 60% at 10Hz [25].

Recent studies have suggested that synapsin IIa is a key regulator of the reserve pool of synaptic vesicles. Gitler et al. [35]...
found that synapsin IIa was the only synapsin isoform that could increase the reserve pool of synaptic vesicles and slow synaptic depression in neurons obtained from synapsin I/II/III triple knockout mice. The synapsin II content of tectopulvinar terminals may make them particularly resistant to fatigue and able to follow high-frequency stimulation. Synapsin I in tectothalamic terminals may additionally regulate plasticity. In the dLGN of synapsin I knockout mice, the short-term frequency-dependent facilitation of corticogeniculate EPSPs was increased, while long-term posttetanic potentiation was decreased [36]. This change presumably occurred because fewer vesicles were tethered to retain a reserve pool. If this is correct, we would predict that tectothalamic EPSPs in synapsin I/II knockout mice would have larger initial amplitudes compared to controls, but would depress with high frequency stimulation. However, direct comparison of corticothalamic and tectothalamic EPSP characteristics attributable to synapsin content is complicated by the fact that other proteins are likely involved in the frequency-dependent facilitation of corticothalamic EPSPs. For example, growth associated protein 43 is distributed specifically in RS profiles [57] and may mediate an activity-dependent enhancement of neurotransmitter release [58,59].

Functional implications

Tree shrew tectopulvinar neurons are located in the lower stratum griseum superficiale (SGS) of the SC and have been termed wide-field vertical cells due to their widespread, obliquely-oriented dendrites that extend throughout the retinorecipient SGS [9,60]. Neurons in the lower SGS of the tree shrew have large receptive fields, exhibit brisk responses to movement and are direction selective [61]. Firing rates of up to 250 Hz have been recorded in the tree shrew SGS. Moreover, many cells exhibit sustained activity as the leading or trailing edge of a stimulus moves across their receptive field [61]. Therefore, tree shrew tectopulvinar terminals must be uniquely equipped to sustain transmission from the SC to pulvinar neurons. We suggest that the convergent synaptic arrangements, as well as the synapsin content of tectopulvinar terminals, allows these terminals to relay a dynamic range of visual signals from the SC to the pulvinar. These features may be particularly important for the reliable transfer of visual signals to initiate and guide the appropriate actions in response to the movements of predator or prey.

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Author Contributions

Conceived and designed the experiments: HW SPM HMP MEB. Performed the experiments: HW SPM. Analyzed the data: HW MEB. Contributed reagents/materials/analysis tools: HMP MEB. Wrote the paper: HW MEB.

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