SPONTANEOUS ACTIVITY IN THE SENSORY SYSTEM

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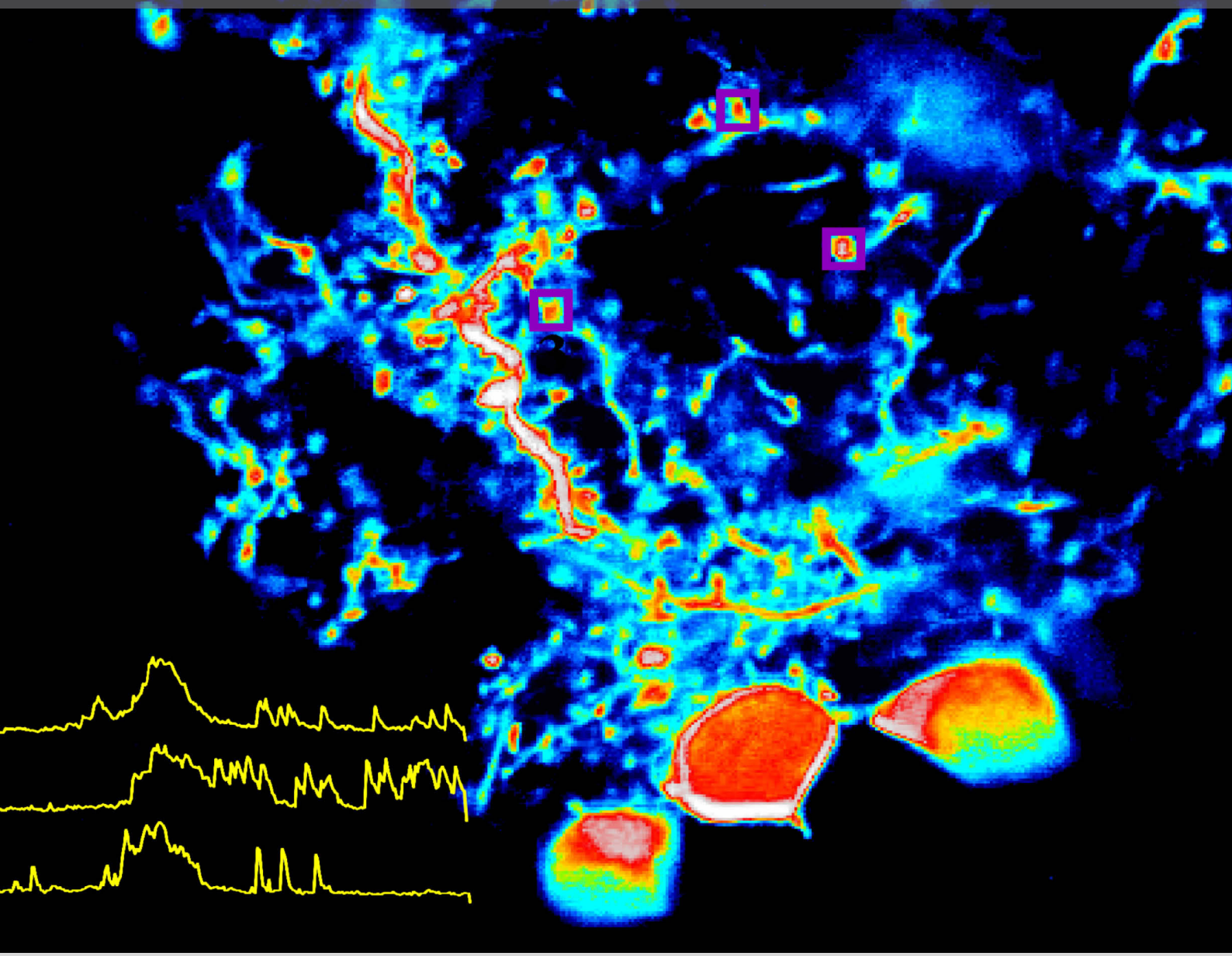

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SPONTANEOUS ACTIVITY IN THE SENSORY SYSTEM

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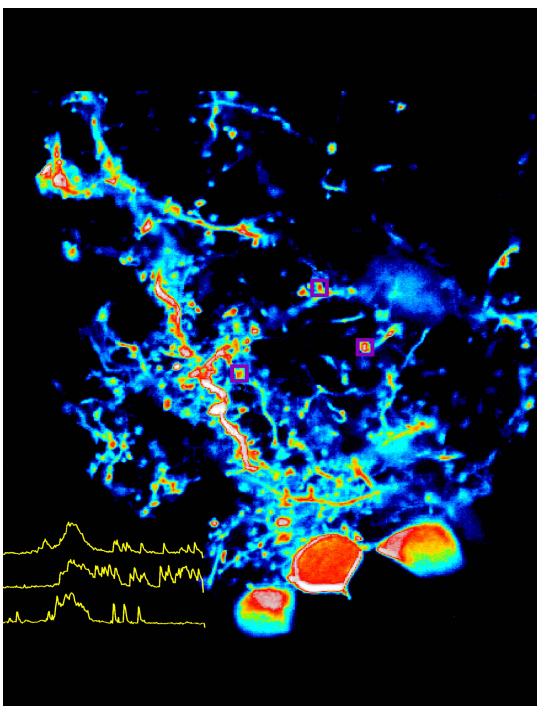

Neurons in the olfactory bulb of a Xenopus laevis tadpole were electroporated to express the calcium-indicator GCaMP7a. Hotter colors indicate a higher variance of fluorescence intensity of two-photon imaging. Both evoked and spontaneous calcium transients are plotted for three regions-of-interest in the dendrites. GCaMP7a was a gift of Drs. K. Kawakami and J. Nakai.
Image: Drs. Loïs Miraucourt, Valerie Higenell, and Edward Ruthazer.

Spontaneous activity in the nervous system is defined as neural activity that is not driven by an external stimulus and is considered a problem for sensory processing and computation. However, spontaneous activity is not completely random and often has unique spatiotemporal

patterns that instruct neural circuit development in the developing brain. Moreover, normal and aberrant patterns of spontaneous activity underlie behavioral states and diseased conditions in the adult brain. The recent technological development has shed light on these unique questions in spontaneous activity. This eBook provides both original and review articles in the propensity, mechanisms, and functions of spontaneous activity in the sensory system. Our goal is to define the state of knowledge in the field, the current challenges, and the future directions for research.

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Table of Contents

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EDITORIAL
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Editorial: Spontaneous Activity in Sensory Systems

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Editorial on the Research Topic

Spontaneous Activity in Sensory Systems

Spontaneous activity in the nervous system, particularly in sensory systems, has become increasingly appreciated due to its important impact on the developing and mature brain. Although by its nature, spontaneous activity has been difficult to study experimentally, recent advances have shed light on its unique function in patterning the nervous system and information processing in normal and diseased conditions of the adult brain. In this special research topic for *Frontiers in Neural Circuits*, we bring together a collection of work from experts in the field that both summarizes past discoveries and introduces current advances to our understanding of spontaneous activity in both developing and mature sensory systems. Here, we provide a summary of their contributions.

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DEVELOPING SENSORY SYSTEMS

Among all of the sensory systems, spontaneous activity is perhaps first and most intensively investigated in the developing visual system, extending from local propagating activity in the retina to central visual pathways. In this regard, Kerschensteiner provides a review of the patterns, mechanisms, and functions of glutamatergic retinal waves, particularly their suitability for refining physiological properties in the visual thalamus (lateral geniculate nucleus: LGN) and the primary visual cortex (V1). The spatiotemporal properties of these retinal waves are further considered by Arroyo and Feller, who discuss the role of inter-eye competition and intraretinal correlated activity in the development of eye-specific segregation and retinotopy in the visual system.

The development of central visual structures is considered in more detail by a group of articles that focus on the projections from the retina to the optic tectum (the retinotectal projection) in tadpoles. In their article, Kutsarova et al. posit a set of rules underlying the development of the retinotectal pathway, which encompass a range of molecular, synaptic, physiological and homeostatic mechanisms. The tectothalamic pathway is further explored by Jang et al., who describe their computational network model of selective visual responses in the optic tectum to looming stimuli, which is able to predict simple behavioral responses in tadpoles. Finally, Pratt et al. examine the role of retinal waves and the development of the retinotectal projections in an evolutionary context, providing predictions for studies of retinal projections in mammalian systems to the superior colliculus (SC) and LGN.

Of course, spontaneous activity plays a key role in the development of all sensory systems. In their article, Leighton and Lohman provide an extensive review of the role of spontaneous activity in the wiring of the developing auditory, somatosensory and visual systems of rodents, and further integrate these with synaptic plasticity mechanisms. Furthermore, the development of higher order structures in each sensory system, particularly the cerebral cortex, is influenced by spontaneous neural activity. Luhman et al. discuss the role of spontaneous activity patterns in the development of neocortical networks and how these arise from changes in the biophysical properties of single neurons to the eventual involvement of complex network interactions. They suggest that early disturbance to these activity patterns may produce permanent neural alterations. Such early-life perturbations are examined by Shoykhet and Middleton, who describe alterations to spontaneous activity in the developing somatosensory and motor system of the rat thalamocortical system, following cardiac arrest-induced global brain hypoxia-ischemia, and they suggest that modulation of the abnormally induced spontaneous activity patterns could improve functions in cardiac arrest survivors.

MATURE SENSORY SYSTEMS

In mature sensory systems, spontaneous activity underlies both normal and diseased processing of information. In this context, the mature neocortex has been the focus of most investigations of the role of spontaneous activity in shaping and refining sensory information processing. In his review, Tan examines the diversity of such spontaneous activity across the sensory neocortex, highlighting both similarities and differences across cortical layers and areas, during fixation and slow oscillations, and the effects of attention, anesthesia and plasticity. Such activity patterns, may reveal facets of the diversity of functional properties across different regions of the neocortex. The role of behavioral state is further explored by McVea et al., who examine and compare the role of slow-wave activity in the adult brain with spindle-burst-related spontaneous activity in the developing brain. They note that both patterns of spontaneous activity have important, but likely independent, functional roles in developing and mature sensory systems.

The final set of articles examine the role of spontaneous activity in diseased sensory systems, in particular the auditory system. An important disorder here is tinnitus, which is the phantom perception of sound, largely thought to originate from excessive spontaneous activity throughout the auditory pathway. Chen et al. present evidence consistent with this view in their fMRI study of individuals with chronic tinnitus. Their study describes alterations in low-frequency fluctuations in several auditory and non-auditory neural centers of those with tinnitus. In an alternate view, Eggermont develops the notion that spontaneous activity is an information carrier, via firing rate and neural synchrony, throughout the auditory pathway, rather than a source of noise. In this context, he argues that increased spontaneous activity during tinnitus alters the activity of both auditory and non-auditory neural structures, which in turn results in the tinnitus percept.

CONCLUSION

As can be appreciated, the articles in this special topic on spontaneous activity in sensory systems cover a wide-range of advances in our understanding of its role in developing and mature nervous systems. However, these articles should also highlight avenues for future investigation, particularly as they pertain to the growing ability to manipulate neural activity to restore normal sensory function following aberrant development or in diseased states.

AUTHOR CONTRIBUTIONS

KI, ER, JM, and CL all contributed to the inception, solicitation, drafting and editing of this special topic and editorial.

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MINI REVIEW
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Glutamatergic Retinal Waves

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Spontaneous activity patterns propagate through many parts of the developing nervous system and shape the wiring of emerging circuits. Prior to vision, waves of activity originating in the retina propagate through the lateral geniculate nucleus (LGN) of the thalamus to primary visual cortex (V1). Retinal waves have been shown to instruct the wiring of ganglion cell axons in LGN and of thalamocortical axons in V1 via correlation-based plasticity rules. Across species, retinal waves mature in three stereotypic stages (I–III), in which distinct circuit mechanisms give rise to unique activity patterns that serve specific functions in visual system refinement. Here, I review insights into the patterns, mechanisms, and functions of stage III retinal waves, which rely on glutamatergic signaling. As glutamatergic waves spread across the retina, neighboring ganglion cells with opposite light responses (ON vs. OFF) are activated sequentially. Recent studies identified lateral excitatory networks in the inner retina that generate and propagate glutamatergic waves, and vertical inhibitory networks that desynchronize the activity of ON and OFF cells in the wavefront. Stage III wave activity patterns may help segregate axons of ON and OFF ganglion cells in the LGN, and could contribute to the emergence of orientation selectivity in V1.

Keywords: retina, development, spontaneous activity, visual system, synaptic refinement, asynchronicity

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Retinal waves have been observed in many species including primates (Warland et al., 2006). In evolution, waves seem to have emerged as a source of patterned activity in species that refine ganglion cell projections while visually deprived inside a shell or womb (Demas et al., 2012). Consistent with this idea, waves have been found in all amniotes tested, but not in amphibians, which use vision to find food and avoid predators as soon as ganglion cell axons reach their targets (Holt and Harris, 1983; Demas et al., 2012). In this review, I will focus on recent data obtained in mice. An excellent review of earlier work in ferrets, chickens, and turtles can be found here (Wong, 1999).

PATTERNS OF GLUTAMATERGIC RETINAL WAVES

Glutamatergic waves pervade the mouse retina from postnatal day 10–14 (stage III, P10–P14). They are preceded by cholinergic waves (stage II, P1–P10) and localized bursts of activity mediated by gap-junctional coupling among nearby ganglion cells (stage I, embryonic day 17–P1; Bansal et al., 2000; Demas et al., 2003; Blankenship and Feller, 2010; Maccione et al., 2014). The beginning of stage III waves and the end of stage II waves appear to be mechanistically linked; and cholinergic waves persist when glutamatergic waves are disrupted (Blankenship et al., 2009; Xu et al., 2016); and precocious glutamatergic waves are observed when cholinergic waves are disrupted (Bansal et al., 2000; Xu et al., 2016). Glutamatergic waves

subside around the time of eye opening (P14) as light-evoked signals begin to drive retinal activity. The disassembly of glutamatergic waves requires normal signaling between photoreceptors and bipolar cells, but occurs independent of visual experience (Demas et al., 2003, 2006).

In waves of all stages, bursts of ganglion cell activity spread across the retina (Meister et al., 1991). Based on large-scale multielectrode array recordings and calcium imaging *in vitro*, glutamatergic waves are estimated to spread laterally at 150–200 μm/s (Blankenship et al., 2009; Maccione et al., 2014). Compared to stage II waves (0.5–1 mm^2), individual stage III waves encompass smaller areas of the retina (∼0.2 mm^2; Maccione et al., 2014). In each glutamatergic wave, ganglion cells fire 2–5 bursts of action potentials lasting ∼0.6 s per burst; and consecutive waves are separated by ∼60 s of silence (Demas et al., 2003; Kerschensteiner and Wong, 2008; Blankenship et al., 2009; Maccione et al., 2014). A unique feature of glutamatergic waves is the asynchronous recruitment of ganglion cells that respond to light increments (ON) and decrements (OFF), respectively. In each wavefront, neighboring ON and OFF ganglion cells fire in sequence: ON before OFF (Kerschensteiner and Wong, 2008; Akrouh and Kerschensteiner, 2013). Cross-correlations of ON and OFF ganglion cell spike trains peak at 0.8–1 s, indicating that opposite sign cells fire temporally adjacent non-overlapping bursts, whereas same sign pairs (i.e., ON—ON and OFF—OFF) fire synchronously with cross-correlations peaking at 0 s (Kerschensteiner and Wong, 2008; Akrouh and Kerschensteiner, 2013).

In addition to spreading within the retina, waves propagate forward through the visual system. *In vivo* imaging in awake mice revealed that stage II waves dominate activity in superior colliculus (SC) and lateral geniculate nucleus (LGN), two major targets of ganglion cell axons (Ackman et al., 2012), and in primary visual cortex (V1), the primary target of LGN axons (Hanganu et al., 2006; Ackman et al., 2012). In addition to a preliminary report on forward propagation of stage III waves *in vivo* (Gribizis et al., 2015), one study found that a subset of spontaneous activity patterns in developing V1 (P10–P14) were suppressed by enucleation (Siegel et al., 2012). This suggests that glutamatergic retinal waves are relayed through the early visual system up to V1.

MECHANISMS OF GLUTAMATERGIC RETINAL WAVES

Early in the exploration of waves, stage III ganglion cell activity was shown to rely on glutamatergic input (Wong et al., 2000); subsequently, bipolar cells were identified as the source of this input (Blankenship et al., 2009). However, the circuits that activate bipolar cells (i.e., wave initiation), spread activity laterally (i.e., wave propagation), and desynchronize the firing of neighboring ON and OFF ganglion cells (i.e., wave patterning), until recently, remained obscure.

The mouse retina has 13 bipolar cell types, which can be grouped into functional classes based on whether their dendrites preferentially contact rods (1 type) or cones (12 types). Cone bipolar cells can further be divided into those that respond to light increments (ON, 7 types) and those that respond to light decrements (OFF, 5 types; Euler et al., 2014). All bipolar cells release glutamate, which they package into vesicles via VGluT1 (Johnson et al., 2003); and stage III waves are abolished in VGluT1 knockout (*VG1 KO*) mice (Blankenship et al., 2009). A recent study found that ON cone bipolar, OFF cone bipolar, and rod bipolar cells contribute differently to glutamatergic waves (Akrouh and Kerschensteiner, 2013). I will use the responses of these functional bipolar cell classes to organize the following discussion of the circuit mechanisms that generate, propagate, and pattern stage III waves (**Figure 1**).

In each glutamatergic wave, ON cone bipolar cells depolarize, causing a transient increase in intracellular calcium and eliciting glutamate release onto ON ganglion cells, which as a result fire bursts of action potentials (Akrouh and Kerschensteiner, 2013; Firl et al., 2013). Electrophysiological "sniffer patch" recordings, and imaging studies using soluble and cell-surface-bound fluorescent reporters have shown that glutamate escapes from synaptic clefts and spreads extracellularly during stage III waves (Blankenship et al., 2009; Firl et al., 2013; Rosa et al., 2015). This excites a subset of developing ON cone bipolar cells that express ionotropic glutamate receptors (iGluRs) on their axons (Akrouh and Kerschensteiner, 2013). The remaining ON cone bipolar cells lack such receptors and are instead excited via gap junctions with nearby iGluR-expressing ON cone bipolar cells and/or amacrine cells (Akrouh and Kerschensteiner, 2013). Together, these two excitatory mechanisms, coordinated by extracellular glutamate, support the lateral propagation of glutamatergic waves (**Figure 1**, *left panel*). Pharmacological blockade of either iGluRs or gap junctions is sufficient to block stage III activity in ganglion cells and bipolar cells (Akrouh and Kerschensteiner, 2013). Interestingly, glutamatergic waves persist when the connexin subunits that make up bipolar cell gap junctions are deleted in the germline (Blankenship et al., 2011), a manipulation that may trigger compensatory changes in iGluR expression. Spontaneous network activity can either be initiated by pacemaker activity of individual neurons or by amplification of coincident membrane potential fluctuations in recurrent networks (Kerschensteiner, 2013). The observation that ON cone bipolar cell depolarizations are abolished when networks are pharmacologically uncoupled, argues against them functioning as pacemakers (Akrouh and Kerschensteiner, 2013). Similarly, AII amacrine cells, which are gap-junctionally coupled to ON cone bipolar cells (Lin et al., 2005; Marc et al., 2014), and which at maturity can generate rhythmic bursting (Cembrowski et al., 2012), do not function as pacemakers during glutamatergic waves (Firl et al., 2015). Together these findings suggest that the lateral excitatory networks that propagate glutamatergic retinal waves also initiate them by amplifying coincident fluctuations in the membrane potential of nearby ON cone bipolar and/or amacrine cells.

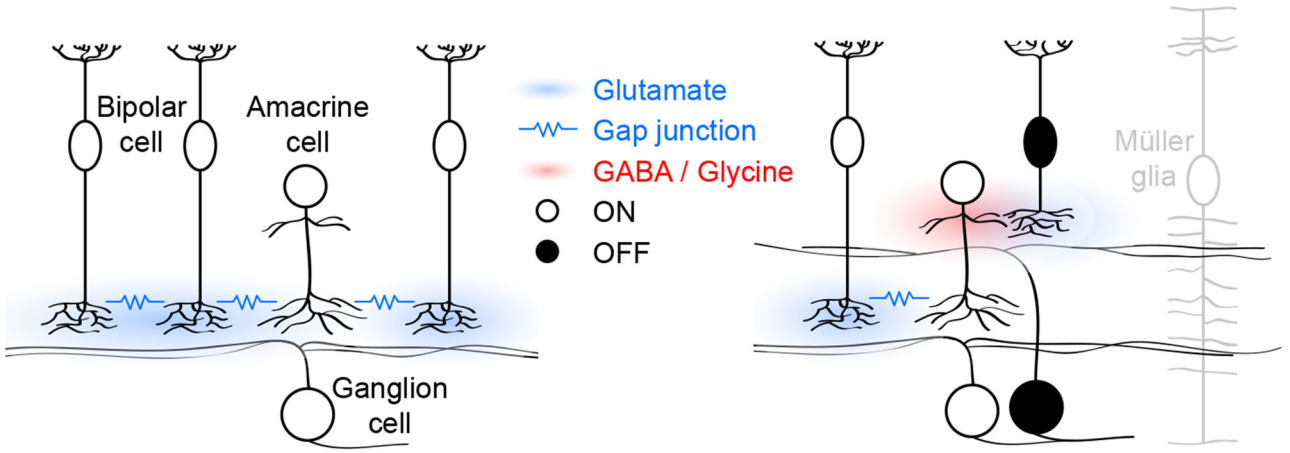

FIGURE 1 | Schematic of glutamatergic wave circuits. The *left panel* illustrates the lateral excitatory (*blue*) network of ON cone bipolar and amacrine cells, which generates and propagates stage III waves. Activity spreads by lateral glutamatergic transmission and gap junctions among neighboring cells. The *right panel* illustrates the vertical inhibitory (*red*) pathway through which ON cone bipolar cells hyperpolarize OFF cone bipolar cells and delay excitatory input to and spiking off OFF ganglion cells. Müller glia limit the spread of glutamate and maintain temporal separation of ON and OFF activity.

Imaging and electrophysiological studies both indicate that rod bipolar cells, which do not provide direct input to ganglion cells, are not reliably recruited into stage III waves (Akrouh and Kerschensteiner, 2013; Firl et al., 2013). This is likely because they are not integrated into the gap-junctional network of ON cone bipolar cells (Akrouh and Kerschensteiner, 2013). In addition, rather than iGluRs, rod bipolar cells express the excitatory amino acid transporter 5 (EAAT5) on their axon terminals, a glutamate uptake transporter with a large chloride conductance (Veruki et al., 2006; Wersinger et al., 2006; Ichinose and Lukasiewicz, 2012; Akrouh and Kerschensteiner, 2013).

As ON cone bipolar cells depolarize during stage III waves, OFF cone bipolar cells hyperpolarize (Akrouh and Kerschensteiner, 2013). This hyperpolarization results from dominant inhibitory synaptic inputs to the axon terminals of OFF cone bipolar cells by a combination of GABAergic and glycinergic amacrine cells with neurites that stratify diffusely in the inner plexiform layer (Akrouh and Kerschensteiner, 2013). Diffuse amacrine cells are depolarized by glutamatergic input from ON cone bipolar cells and form a vertical inhibitory pathway through which ON cone bipolar cells hyperpolarize OFF cone bipolar cells (**Figure 1**, *right panel*). Simultaneous recordings of bipolar cell voltage and excitatory input to ganglion cells, indicate that OFF cone bipolar cells release glutamate as their voltage returns to baseline from the wave-associated hyperpolarization (Akrouh and Kerschensteiner, 2013). Bipolar cell axons release glutamate at ribbon synapses, which enable them to adjust release continuously to gradual changes in voltage (Matthews and Fuchs, 2010). Why does the return to sustained baseline voltage elicit transient glutamate release from OFF cone bipolar cells? One possible explanation is that the readily releasable pool of vesicles in OFF cone bipolar cells is depleted between waves and replenished during the wave-associated hyperpolarization, causing a subsequent transient increase in release. This remains to be experimentally tested, but similar rapid changes in vesicle pool occupancy have been observed at mature bipolar cell synapses (Mennerick and Matthews, 1996; Burrone and Lagnado, 2000; Singer and Diamond, 2003, 2006) where they contribute to adaptive computations (Manookin and Demb, 2006; Dunn and Rieke, 2008; Jarsky et al., 2011; Oesch and Diamond, 2011). As waves propagate through lateral excitatory networks, ON cone bipolar cells thus engage vertical inhibitory pathways that hyperpolarize OFF cone bipolar cells. The synchronous opposite sign responses of ON and OFF cone bipolar cells are translated into a time-locked sequence of glutamate release and spiking of ON and OFF ganglion cells in the wavefront.

In the inner plexiform layer of the retina, ON and OFF circuits are separated vertically. For glutamate release from ON and OFF cone bipolar cells to stimulate ON and OFF ganglion cells sequentially, the vertical spread of glutamate needs to be restricted (**Figure 1**, *right panel*). DL-threo-beta-benzyloxyaspartate (TBOA), an antagonist of EAATs, synchronizes excitatory input to ON and OFF ganglion cells, indicating that EAAT-mediated uptake limits the spread of glutamate and is critical for patterning stage III waves (Akrouh and Kerschensteiner, 2013). Müller glia express EAAT1 and at maturity are the primary agent of glutamate uptake in the retina (Pow and Crook, 1996; Pow et al., 2000). Electrophysiological recordings from Müller glia showed that they depolarize during stage III waves (Akrouh and Kerschensteiner, 2013) possibly due to electrogenic glutamate uptake via EAAT1 (Owe et al., 2006). Moreover, a recent study identified calcium transients in Müller glia processes in the inner plexiform layer during stage III waves (Rosa et al., 2015). The function of these signals, which are mediated by iGluRs on Müller glia (Rosa et al., 2015), remains to be determined.

FUNCTIONS OF GLUTAMATERGIC RETINAL WAVES

In this section, I will discuss how glutamate release from bipolar cells during stage III waves regulates circuit development in the retina, and how the patterns of ganglion cell activity, which propagate forward through the visual system, may shape wiring in retinorecipient structures (e.g., LGN and SC) and in V1.

As outlined in the previous section, ON and OFF cone bipolar cells release glutamate with each stage III wave. The effects of glutamate release from bipolar cells on circuit development in the retina have been analyzed in several recent studies. Glutamate release from ON cone bipolar cells was suppressed by transgenic expression of tetanus toxin (i.e., *TeNT* mice). In *TeNT* mice, ON cone bipolar and ON ganglion cells are connected by fewer synapses at maturity (Kerschensteiner et al., 2009). Live imaging showed that reduced connectivity is a result of lower rates of synapse formation, whereas synapse elimination is unaffected in *TeNT* mice (Kerschensteiner et al., 2009). Effects of release suppression are pathway specific; and excitatory synapses form normally on OFF ganglion cells and OFF dendrites of ON-OFF ganglion cells (Kerschensteiner et al., 2009). Furthermore, in ON ganglion cells that receive convergent input from multiple ON cone bipolar cell types, connectivity is reduced in a cell-type-specific manner in *TeNT* mice (Morgan et al., 2011). In a mouse model in which glutamate release is enhanced, rates of synapse formation are elevated with similar cell type specificity (Soto et al., 2012). When expression of tetanus toxin is limited to a sparse subset of ON cone bipolar cells, synapse formation on ON ganglion cell dendrites is reduced locally without competition among neighboring axons (Johnson and Kerschensteiner, 2014; Okawa et al., 2014). In addition to effects of glutamate release from bipolar cells on synapse formation between their axons and ganglion cells dendrites, retrograde plasticity was observed, in which axonal glutamate release affects the ability of bipolar cell dendrites to recruit input from photoreceptors (Johnson and Kerschensteiner, 2014). Whereas no differences in ganglion cell dendrite structure were observed in *TeNT* mice (Kerschensteiner et al., 2009), in CD3ζ knockout mice, which generate fewer stage III waves, the number of filopodia on ganglion cell dendrites was increased (Xu et al., 2010). This effect is phenocopied by intraocular injections of glutamate receptor blockers (Xu et al., 2010).

In LGN and SC, eye-specific segregation and retinotopic refinement of ganglion cell axons emerge during stage II waves (Kerschensteiner, 2013; Ackman and Crair, 2014). Waves promote this organization via burst-time-dependent plasticity as they synchronize activity of nearby ganglion cells in the same eye more than of ganglion cells further apart in the same eye or in different eyes (Grubb et al., 2003; McLaughlin et al., 2003; Butts et al., 2007; Shah and Crair, 2008; Xu et al., 2011; Ackman et al., 2012; Zhang et al., 2012; Burbridge et al., 2014). Stage III waves are critical for the maintenance of these wiring patterns. In no b-wave mice, in which stage III waves are replaced by high-frequency bursting, ganglion cell axons from the two eyes desegregate (Demas et al., 2006). Similarly, synchronous optogenetic stimulation of ganglion cells in both eyes during the period of stage III waves, disrupts eye-specific segregation and retinotopy (Zhang et al., 2012). Finally, in mice with disrupted stage II waves, stage III waves are able to support eye-specific segregation and retinotopic refinement, but this organization is lost when both stage II and III waves are abolished (Xu et al., 2016).

In addition to maintaining eye-specific and retinotopic organization, the patterns of glutamatergic waves may promote ON/OFF segregation in LGN (Kerschensteiner and Wong, 2008; Kerschensteiner, 2013). Given burst-time-dependent plasticity rules (Butts et al., 2007), the asynchronous recruitment of neighboring ON and OFF ganglion cells during stage III waves is expected to separate converging ON and OFF axons in retinotopically refined projections to LGN (Kerschensteiner and Wong, 2008). Although this remains to be directly tested, the following circumstantial evidence supports a role for glutamatergic waves in ON/OFF segregation in mouse LGN. First, in ferrets, pharmacologic blockade of stage III waves prevents ON/OFF segregation (Hahm et al., 1991; Cramer and Sur, 1997); and pharmacologic enhancement of stage III waves accelerates receptive field maturation (Davis et al., 2015). Second, artificial neuronal networks with plasticity rules observed in subcortical visual circuits (Butts et al., 2007; Shah and Crair, 2008) undergo reliable ON/OFF segregation in response to recorded retinal activity patterns (Gjorgjieva et al., 2009). Third, mice with precocious stage III waves exhibit excessive ON/OFF segregation: SC neurons, which normally respond to both ON and OFF stimuli, become purely ON or OFF responsive (Chandrasekaran et al., 2005); ON and OFF neurons in LGN, which normally intermingle, form clusters (Grubb et al., 2003). An interesting question in this context is why the same sequence of ganglion cell activity promotes ON/OFF segregation in LGN, but not in SC. The answer may lie in the different timing of critical periods of refinement in these circuits. In SC, synaptic remodeling appears complete by P7 (Chandrasekaran et al., 2005), before the onset of stage III waves, whereas in LGN, most early synaptic remodeling occurs between P11 and P14 (Hooks and Chen, 2006).

If stage III waves are faithfully transmitted to V1, they could contribute to the formation of orientation selective receptive fields. Orientation selectivity in V1 arises in part from the convergence of axons of ON and OFF LGN neurons with spatially offset receptive fields (Hubel and Wiesel, 1962; Lien and Scanziani, 2013; Niell, 2013). Spontaneous activity patterns that synchronize the activity of ON and OFF neurons at appropriate distances could help set up this organization (Miller, 1994). The centers of the ON and OFF portions of orientation-selective V1 receptive fields are ∼5° apart (Lien and Scanziani, 2013), equivalent to ∼170 μm in retinal space (Remtulla and Hallett, 1985; Schmucker and Schaeffel, 2004). Given propagation speeds of 150–200 μm/s (Blankenship et al., 2009; Maccione et al., 2014) and a temporal delay of 0.8–1 s between the firing of neighboring of ON and OFF ganglion cells (Kerschensteiner and Wong, 2008;

Akrouh and Kerschensteiner, 2013), it is possible the stage III wave synchronize the activity of opposite sign LGN neurons at appropriate distances to drive their convergence in V1. If such spatially offset synchronization exists in glutamatergic waves and whether it contributes to orientation selectivity in V1 remains to be experimentally tested.

SUMMARY

Recent studies have described in detail the patterns of glutamatergic waves in the retina and have identified the circuit mechanisms that generate, propagate and shape this activity. Activity patterns observed in the retina appear well-suited to promote ON/OFF segregation in LGN and orientation selectivity in V1. However, forward propagation of stage III waves through the visual system remains to be clearly demonstrated; and *in vivo* manipulations that alter patterns of glutamatergic waves without affecting activity levels are needed to test their role in circuit organization in LGN and V1.

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DK wrote the manuscript.

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frontiers in Neural Circuits

MINI REVIEW
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Spatiotemporal Features of Retinal Waves Instruct the Wiring of the Visual Circuitry

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Coordinated spontaneous activity is present in different sensory systems during early stages of development. This activity is thought to play a critical role in the development of sensory representations before the maturation of sensory experience. In the visual system, the mechanisms by which spatiotemporal properties of retinal spontaneous activity, called retinal waves, drive developmental events has been well studied. Recent advancements in pharmacological, genetic, and optogenetic manipulations have provided further understanding of the contribution of specific spatiotemporal properties of retinal waves to eye-specific segregation and retinotopic refinement of retinofugal projections. Here we review some of the recent progress in understanding the role of retinal waves in the early stages of visual system development, prior to the maturation of vision.

Keywords: activity-dependent development, ferret, mouse, retinotopy, retinal waves, eye-specific segregation

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INTRODUCTION

The cellular and molecular mechanisms underlying the spatiotemporal properties ofs retinal waves are well understood (Blankenship and Feller, 2010; Kerschensteiner, 2013). There are three developmental stages of waves mediated by different forms of neurotransmission; stage 1 waves are mediated by a combination of gap junctions and cholinergic circuits (Bansal et al., 2000), stage 2 waves are cholinergic (Feller et al., 1996), and stage 3 waves are glutamatergic (Wong et al., 2000). All three stages generate waves with different spatiotemporal properties (Maccione et al., 2014), as determined *in vitro*. Waves are characterized by their speed, frequency, covered area (size), within-burst spiking frequency, intra-retina distance-dependent correlations, and inter-eye correlations. More recently it has been established that the spatiotemporal properties of stage 2 retinal waves are preserved *in vivo* and are synaptically propagated to central visual targets (Colonnese and Khazipov, 2010; Ackman et al., 2012).

It is widely accepted that retinal waves are critical for the refinement of visual maps in retinofugal targets. Retinal projections to the superior colliculus (SC) and the dorsal lateral geniculate nucleus (dLGN) are retinotopically organized and segregate into eye-specific regions in a manner dependent on retinal activity (**Figures 1**, **2A**; Wong, 1999; Bansal et al., 2000; Huberman et al., 2008). A classic debate in this field has been whether retinal waves

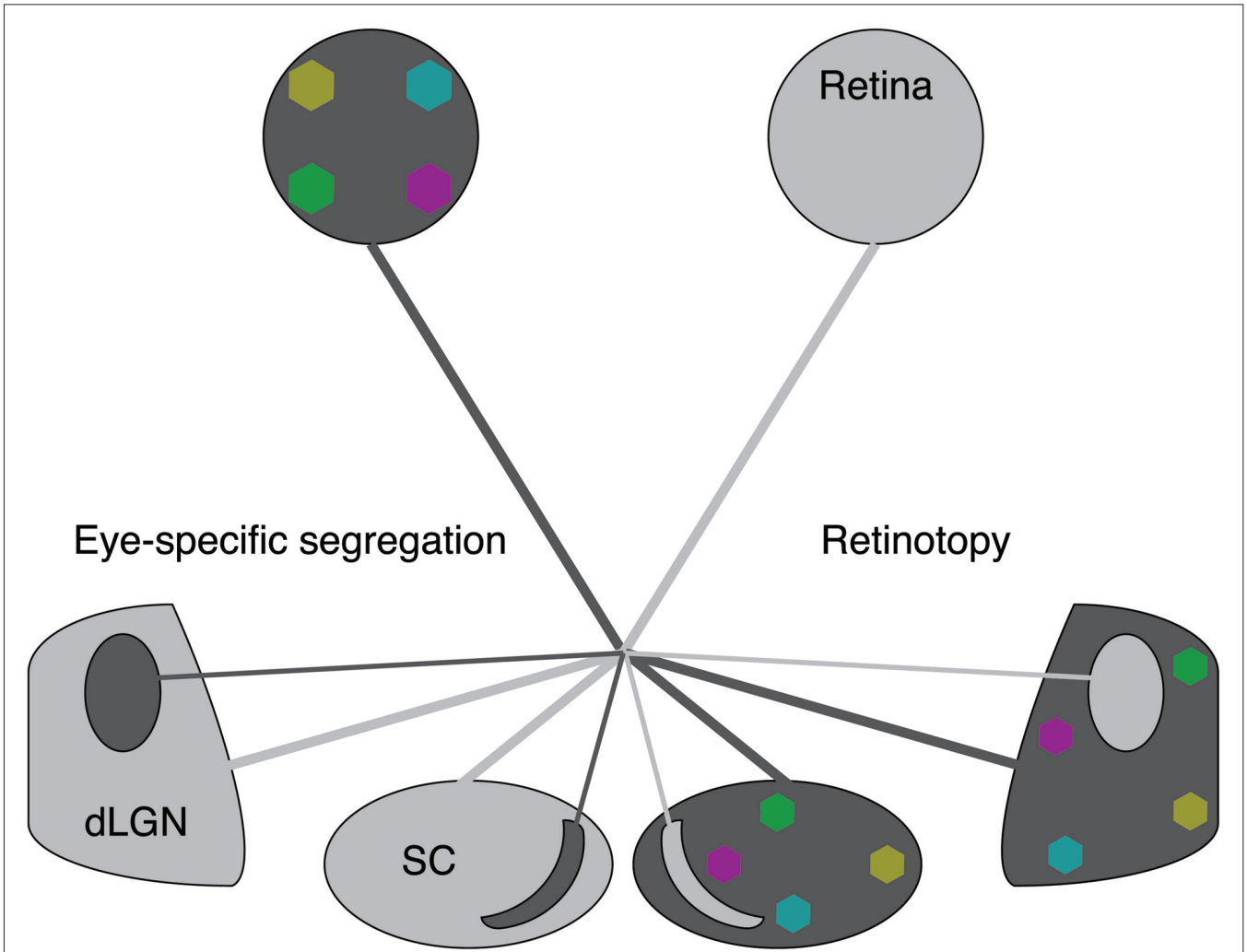

FIGURE 1 | Spontaneous retinal waves mediate eye-specific segregation and retinotopic refinement of retinofugal projections. The axons of retinal ganglion cells (RGCs) target the dorsal lateral geniculate nucleus (dLGN) of the thalamus and the superior colliculus (SC). RGC projections from opposite eyes are segregated into ipsilateral and contralateral regions (black/gray oppositions). RGC projections from each retina are retinotopically organized (colored regions). Note that for simplicity we have depicted retinotopic maps only for one eye. Retinotopic maps and eye-specific segregations form for both eyes.

are permissive or instructive for driving these developmental processes (Crair, 1999; Chalupa, 2009; Feller, 2009; Maccione et al., 2014). If waves are permissive, it implies that a minimum level of activity is required for some other instructive process to drive segregation, such as gene regulation. If waves are instructive, it implies that information about the target map is contained within the pattern of action potential firing during retinal waves. However, manipulations that alter the pattern of retinal waves also change their overall firing levels, making it difficult to differentiate between instructive and permissive roles. Here we review recent efforts that have used novel manipulations, in both mouse and ferret, to restrict perturbations of specific spatiotemporal properties of waves and thus advance the understanding of their contribution to the development of the visual circuitry.

INTER-EYE COMPETITION INSTRUCTS EYE-SPECIFIC SEGREGATION

Inter-eye competition generated by spontaneous retinal waves was hypothesized to be instructive for eye-specific segregation of retinofugal projections (Butts et al., 2007). During retinal waves, individual retinal ganglion cells (RGCs) fire short bursts of action potentials that propagate to neighboring RGCs within a well defined region and are separated by 1–2 min of silence—hence the firing within retinotopically identified regions of the retina have stronger intra-retinal correlation than inter-retinal correlations. One of the unexpected findings of *in vivo* calcium imaging used to record activity of RGC terminals projecting to the SC was that activity of the two eyes is more correlated than

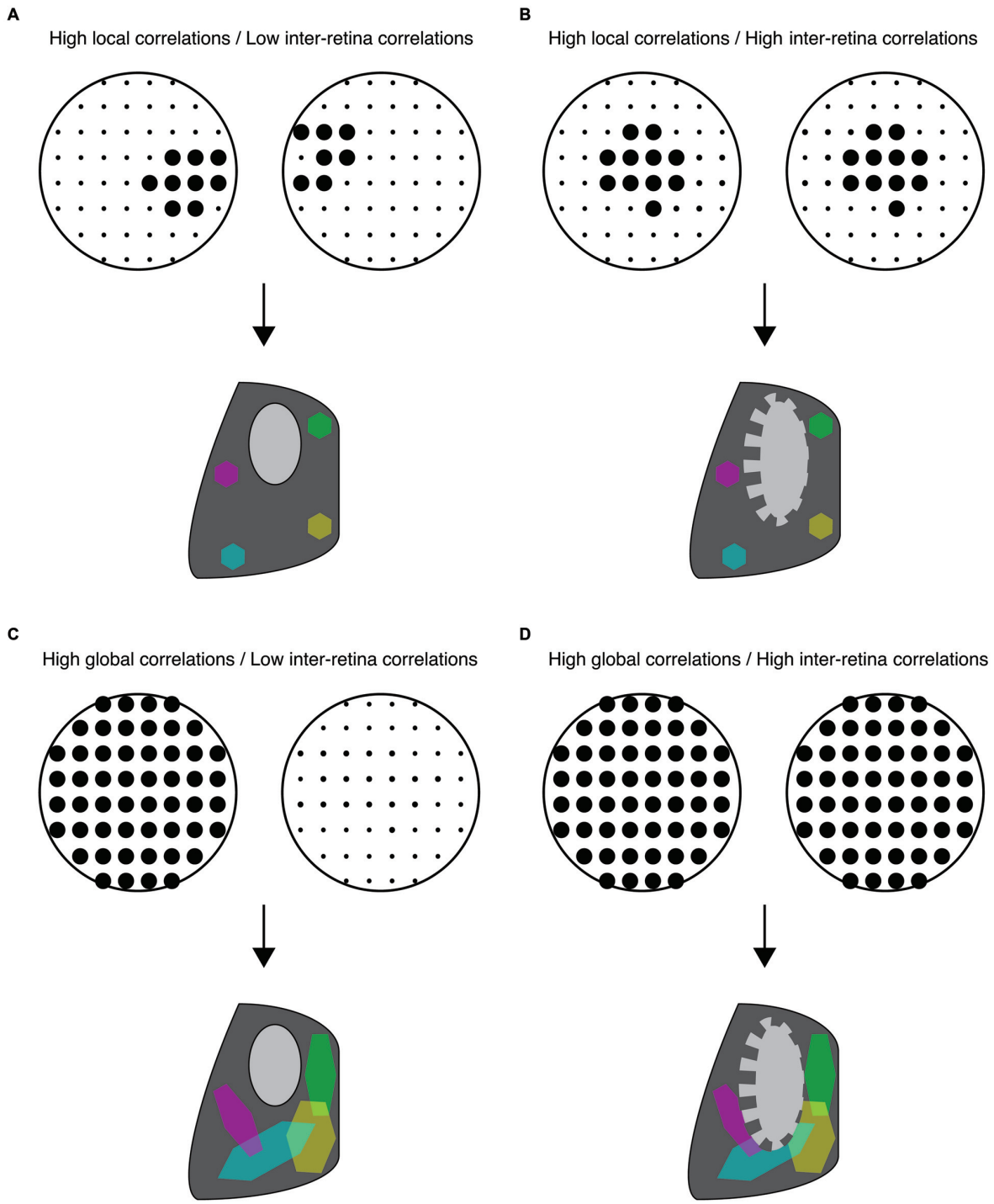

FIGURE 2 | Distinct spatiotemporal patterns of retinal waves instruct different features of retinofugal projections in mice. (A–D) Top: schematic of retinal wave firing patterns. Dots are representative of RGCs with larger dots indicating elevated firing rates during a single retinal wave. Bottom: schematic of retinogeniculate wiring using same code as **Figure 1**—colored hexagons represent retinotopy while gray/black regions represent eye-specific segregation. **(A)** Under normal conditions, RGCs exhibit high local correlations while activity of the two retinas is minimally correlated. This pattern of activity supports both eye-specific segregation and retinotopic maps in both the dLGN and SC. **(B)** High correlations between retinal waves of opposite retinas induced by optogenetic stimulation is detrimental to eye-specific segregation while retinotopic maps are unaffected (Zhang et al., 2011). **(C)** Disruption of local correlations either by an increase in uncorrelated firing or by abnormally elevated correlations between distant RGCs (global correlations), such as that observed in β2KO and Retβ2-cKO mice, is detrimental to retinotopic map formation. Local correlations are sufficient for normal retinotopic maps in Retβ2-cKO, Rxβ2-cKO and β2(TG) (Xu et al., 2011, 2015; Burbridge et al., 2014). **(D)** High global correlations paired with high inter-retina correlations such as that observed in the β2KO mouse, is detrimental to both eye-specific segregation and retinotopic maps (Xu et al., 2011; Burbridge et al., 2014).

would be expected by chance (Ackman et al., 2012). However, this correlation is weak and most waves occur independently.

The *β2KO* mouse is a classic model for studying the role of retinal waves in visual system development. This mouse model lacks the β2 subunit of the nicotinic acetylcholine receptor (β2-nAChR), which is required for cholinergic waves. This mouse has significantly impaired eye-specific segregation and retinotopic refinement of retinal projects to the dLGN of the thalamus and the SC. Unfortunately, there are several conflicting descriptions of β2KO retinal activity *in vit*ro, with different spatiotemporal properties reported based on recording conditions such as temperature and media used (McLaughlin et al., 2003; Sun et al., 2008; Kirkby and Feller, 2013). In contrast, sensitivity to temperature is not observed in waves of wild type (WT) mice (Stafford et al., 2009). Recent *in vivo* imaging of RGC axon terminals in the SC of *β2KO* revealed waves to be infrequent but dramatically larger and faster than waves in WT mice, and were described by the authors as "flashes" (Burbridge et al., 2014). Importantly, depolarizations induced by RGCs in *β2KO* mice were not sufficient to entrain postsynaptic SC neurons, which exhibited an activity pattern different from that of RGC axons. Hence *β2KO* have strong long-range intra-retinal correlations but weak and infrequent activity. Moreover, when flashes occur, they are likely to be correlated between the two eyes, indicating stronger inter-retinal correlations than those observed in WT (Burbridge et al., 2014).

Though these studies are consistent with the hypothesis that a lack of correlated activity between opposing retinas is important for eye-specific segregation (**Figure 2B**), they do not rule out the possibility that impaired map refinement in *β2KO* mice is due to an overall reduction in retinal activity. Recent studies have separated out the contributions of an overall decrease in retinal activity, the increase in inter-retinal correlations, and the disruption of the slowly propagating waves that drive local intra-retinal correlations. First, the frequency of waves is increased in the *β2KO* via intraocular injection of CP-cAMP (Burbridge et al., 2014), a manipulation known to increase the frequency of retinal waves *in vitro* (Stellwagen et al., 1999; Stellwagen and Shatz, 2002). Increasing wave frequency and thus overall wave activity improves eye-specific segregation in *β2KO*. Second, both correlated and anticorrelated inter-retinal activity were induced using optogenetic manipulations (Zhang et al., 2011). In these studies, correlated stimulation of the two retinas induced impaired eye-specific segregation while anticorrelated stimulation permitted eye-specific segregation and even partially rescued it in *β2KO* mice. These results were complemented by studies based on a transgenic mouse in which β2-nAChR subunits are knocked out exclusively from the retina starting early in development (*Rx-β2cKO* mice). In these mice local intra-retinal correlation properties of neighboring RGCs were largely maintained, but RGCs fired at low levels. This decrease in activity resulted in impaired eye specific segregation (Xu et al., 2015). Together, these studies strongly implicate that large-scale asynchronous activity between opposite eyes instructs eye-specific segregation while the local intra-retinal correlations induced by slowly propagating activity do not (**Figures 2B,C**).

Ferrets provide an interesting comparison to mice for assessing whether retinal waves are instructive for eye-specific segregation. Like mice, significant disruption of stage 2 cholinergic retinal waves disrupts eye-specific segregation of retinogeniculate afferents (Huberman et al., 2008). In contrast to mice, ipsilateral and contralateral retinal afferents of ferrets project to distinct cellular laminae in the dLGN, which may indicate differences in the mechanisms underlying visual map refinement between the two species. Using an immunotoxin to ablate starburst amacrine cells, a key component for generating Stage 2 retinal waves, Speer et al. (2014) were able to disrupt both local and global features of retinal waves while maintaining eye-specific segregation, similar to a previous study (Huberman et al., 2003). In contrast to mice, they found that large scale firing and the overall level of activity were not instructive, instead, the amount of uncorrelated firing exhibited by RGCs during the inter-wave periods was detrimental to eye-specific segregation.

A new insight into how waves influence eye-specific segregation in ferrets was achieved using an enucleated ferret model (Failor et al., 2015). Monocular enucleation results in expanded ipsilateral projections in the dLGN. Pharmacologically blocking waves in the remaining eye reduces this expansion and causes abnormal fragmentation of the ipsilateral laminae. To a lesser degree, blocking waves also induces fragmentation of contralateral projections in monocularly enucleated animals. This study suggests that intra-retinal activity itself plays a role in the development of eye-specific segregation that is independent of inter-eye competition. Furthermore, single-unit spike recordings from dLGN cells showed that blocking waves in monocularly enucleated animals causes abnormal enlargement and misalignment of receptive fields, thereby matching functional connectivity of the retino-geniculate projections with the observed anatomical phenotypes.

Together, these studies indicate that in ferrets intra-retinal correlations may be more critical for targeting axons to the correct layers and may also influence eye-specific segregation, in contrast with the observations in mice (**Figures 2B,D**).

LOCAL INTRA-RETINAL CORRELATIONS INSTRUCT RETINOTOPY

In contrast to eye-specific segregation, local intra-retinal correlations induced by propagating activity are thought to be the primary feature instructing retinotopic refinement. During locally propagating activity, nearby RGCs are more correlated than distant RGCs and therefore information about retinotopy is contained within the retinal wave firing pattern (Eglen et al., 2003). The process of retinotopic refinement has been studied primarily in retinocollicular projections,

though recent progress in understanding the role of waves in retinotopic refinement of retinogeniculate projections has also been made.

Several of the mouse models described above have also identified key features of retinal waves that instruct retinotopic refinement. For example, restoring the overall level of retinal activity in β2KO mice using optogenetics or intraocular injections of CP-cAMP rescued impairments in eye-specific segregation, but not retinotopy (Zhang et al., 2011; Burbridge et al., 2014; **Figure 2D**), suggesting that local intra-retinal correlations rather than overall retinal activity instruct retinotopy. One of the most striking mouse models lacks β2-nAChR in the nasal and temporal retina but expression is maintained along the central dorso-ventral strip (*Retβ2-cKO*), which serves as an internal control (Burbridge et al., 2014). *In vivo* calcium imaging of retinocollicular axon terminals confirmed that these mice exhibit locally propagating waves in the central retina while waves are absent in the nasal and temporal portions of the retina. Retinocollicular projections emerging from temporal or nasal retina were diffuse, indicating a lack of retinotopic refinement, while projections emerging from central retina were normal. This finding indicates that local intra-retinal activity patterns play a central role in establishing retinotopy (**Figure 2C**).

The *Rx-β2cKO* retina discussed earlier exhibits lower overall activity, smaller waves with high local correlations, more variable inter-wave intervals, and lower wave amplitude as measured with calcium imaging. *Rx-β2cKO* mice have disrupted eye-specific segregation and normal retinotopy in the monocular zones, indicating that the restricted local propagation of waves is sufficient for retinotopic refinement. Interestingly, retinotopy of the binocular zones was largely impaired, indicating interdependence between eye-specific segregation and retinotopy in the binocular zone (Xu et al., 2015). A careful comparison of waves spatiotemporal patterns in various knockout models is discussed in this study.

The role of waves in the retinotopic refinement of retinogeniculate projections in ferrets has also seen recent advancements. Intraocular blockade of inhibition to enhance glutamatergic stage 3 retinal waves led to abnormally early retinotopic refinement of retinogeniculate projections, as measured anatomically and functionally (Davis et al., 2015). Based on *in vitro* experiments, blockade of inhibition increases the frequency of glutamatergic waves while maintaining local correlations (Kerschensteiner and Wong, 2008; Firl et al., 2013). These findings are rather surprising since it suggests that increased spontaneous activity prior to visual experience could potentially compensate for lack of visual experience.

It is important to note that retinal waves work in concert with molecular cues for establishing retinotopic maps (Pfeiffenberger et al., 2006; Cang et al., 2008; Ackman and Crair, 2014; Assali et al., 2014). Though recent progress in this field is too vast to review here, we want to make note of a recent study that implicates a role for retinal waves in establishing heterogeneity in visual map organization not only across animals but also across the two colliculi of a single animal. The study makes a strong case that spontaneous activity and molecular cues interact stochastically to form well-defined yet variable retinotopic maps (Owens et al., 2015).

In summary, the evidence suggests that in mice, overall activity levels and inter-eye competition are determinant for eye-specific segregation while local intra-retinal correlations determine retinotopy, with some interdependence of the two in the binocular zones of central retinal targets. In ferrets, there is additional evidence that local intra-retinal correlations also influence eye-specific segregation.

EMERGING ROLES OF RETINAL WAVES IN THE DEVELOPMENT OF THE VISUAL SYSTEM

Most of the research on how retinal waves influence visual system development has been focused on retinofugal projections. However, there is also an interest in understanding whether retinal waves drive other aspects of visual system development. Recent studies have elucidated emerging roles for retinal waves in the development of visual circuits. In one study it was found that disrupting retinal waves increased cortical neurogenesis in the primary visual cortex while enhancing waves decreased neurogenesis (Bonetti and Surace, 2010). Another study found that eliminating the input of retinal waves to the SC resulted in migration and connectivity deficits in inhibitory interneurons of the dLGN (Golding et al., 2014). It has also been shown that elimination of retinal inputs to central targets accelerates the cortical innervation of the dLGN, yet synapse maturation was unaffected (Seabrook et al., 2013). In addition, computational models have implicated slow features of retinal waves in the establishment of complex cells in primary visual cortex (Dähne et al., 2014), and in the establishment of initial biases in orientation maps in primary visual cortex (Hagihara et al., 2015). These discoveries suggest a broad involvement of developmental spontaneous activity in cell signaling processes that mediate development, differentiation, integration, and connectivity of neural circuits.

The refinement of strategies to precisely control neural activity *in vivo*, as well as a deeper understanding of the molecular mechanisms linking retinal waves to various developmental processes will continue to provide insights onto the various roles of spontaneous activity patterns in circuit development.

AUTHOR CONTRIBUTIONS

Both authors contributed to the writing of the manuscript. All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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frontiers in Neural Circuits

REVIEW
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Rules for Shaping Neural Connections in the Developing Brain

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It is well established that spontaneous activity in the developing mammalian brain plays a fundamental role in setting up the precise connectivity found in mature sensory circuits. Experiments that produce abnormal activity or that systematically alter neural firing patterns during periods of circuit development strongly suggest that the specific patterns and the degree of correlation in firing may contribute in an instructive manner to circuit refinement. In fish and amphibians, unlike amniotic vertebrates, sensory input directly drives patterned activity during the period of initial projection outgrowth and innervation. Experiments combining sensory stimulation with live imaging, which can be performed non-invasively in these simple vertebrate models, have provided important insights into the mechanisms by which neurons read out and respond to activity patterns. This article reviews the classic and recent literature on spontaneous and evoked activity-dependent circuit refinement in sensory systems and formalizes a set of mechanistic rules for the transformation of patterned activity into accurate neuronal connectivity in the developing brain.

Keywords: retinotectal, visual system, colliculus, optic tectum, activity-dependent, Hebbian plasticity, review, retinal ganglion cell

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INTRODUCTION

In this review article, we propose a detailed set of cellular rules that govern activity-dependent circuit refinement. This list of rules synthesizes what has been learned in the extensive experimental literature on the development of the visual system, with a strong emphasis on data obtained from live imaging of the retinotectal projection in fish and frogs. Because of their external development and largely translucent bodies, permitting high-resolution *in vivo* imaging of developing neurons and their associated glial cells, albino tadpoles of the African clawed frog (*Xenopus laevis*) and larval zebrafish (*Danio rerio*) in particular have been popular models for studying activity-dependent circuit development and remodelling. Moreover, unlike mammals, these animals rely extensively on vision for survival from very early developmental stages, and use this same visual information to direct circuit refinement.

While molecular guidance cues are critical for establishing the initial crude topographic projections from the eye to the brain, even greater precision of neuronal maps is achieved through the involvement of activity-dependent mechanisms. Because neighboring retinal ganglion cells (RGCs) in the eye are more likely to exhibit temporal correlation in their firing patterns than distantly separated RGCs, the pattern of firing of action potentials in the developing visual system contains important information about the relative position of the RGC somata in the retina. The system therefore is able to use patterned neural activity in RGCs to instruct the orderly mapping of their axons onto postsynaptic partners in the optic tectum. This results in a more

precise map of the retina onto the optic tectum. This review article will discuss classic studies and more recent experimental insights from *in vivo* imaging that reveal fundamental details about how activity-dependent structural and functional refinement takes place. The refinement of the retinotectal circuit achieves a remarkably accurate retinotopic representation that contributes to effective visual processing and ultimately to the generation of visually-guided behaviors. Here, we define refinement as the process of establishing precise anatomical wiring (i.e., the representation most closely reproducing the input space), which allows for the optimal function of neural circuits in the animal.

RETINOTOPIC MAPS AND EYE-SPECIFIC SEGREGATION IN THE DEVELOPING CENTRAL NERVOUS SYSTEM

In fish and frogs the RGCs project to at least 10 distinct tectal and pretectal arborization fields believed to mediate important behavioral responses such as eye movements and prey capture (Easter and Taylor, 1989; Burrill and Easter, 1994; Kubo et al., 2014; Semmelhack et al., 2014). By far, the most extensive projection terminates in the contralateral optic tectum, where the organization of axonal terminals reconstitutes a topographic map of the retina. The axons of RGCs whose somata are located in the temporal retina project to the rostral tectum, whereas RGCs residing in the nasal retina send their axons to the caudal tectum (Attardi and Sperry, 1963). Similarly, the dorsoventral axis of the retina is represented medio-laterally in the optic tectum. The retinocollicular projection in mammals forms a retinotopic map with a similar coordinate system in the analogous structure to the optic tectum, the superior colliculus (SC) (N.B., The term optic tectum is applied generically to represent the analogous retinorecipient structure in all vertebrates). The SC is involved in directing eye and head movements in mammals (Schiller, 1972).

Unlike amphibians, in which the retinofugal projection is almost exclusively contralateral, in mammals a fraction of the RGCs does not cross at the optic chiasm but projects to the ipsilateral hemisphere of the brain. This fraction ranges from 3% to 5% in mice, around 15% in ferrets to up to nearly 40% in humans (Petros et al., 2008). Interestingly, anterograde and retrograde tracing of RGCs also reveals a substantial transient ipsilateral projection during embryonic development in mammals and chicks, much of which is lost during subsequent maturation (Land and Lund, 1979; Dräger and Olsen, 1980; McLoon and Lund, 1982). In the mammalian brain, inputs from both eyes project to the deep part of the stratum griseum superficiale and to the stratum opticum of the SC. These binocular projections segregate into alternating eye-specific bands in the rostral colliculus (Godement et al., 1984). The more superficial part of the stratum griseum superficiale of the SC normally receives exclusively contralateral eye input. In addition to providing afferents to the SC, RGC axons also project to the visual thalamus in mammals (Sretavan and Shatz, 1984). A distinct visual processing pathway, the retinothalamic (retinogeniculate) projection terminates in both the ventral and dorsal lateral geniculate nuclei (LGN) in the thalamus. The dorsal LGN is thought to serve as the fundamental relay station through which visual information is passed to higher order cortical visual centers where increasingly complex features are extracted from visual scenes (Fellenman and Van Essen, 1991). The afferents in the LGN, much like in the SC, project retinotopically but segregate into eye-specific laminae (Linden et al., 1981).

This review article focuses primarily on the retinotectal projection and outlines the evidence supporting a specific set of rules for projection refinement based on activity-dependent cellular mechanisms involved in the establishment and refinement of the functional retinotopic map in the optic tectum. Experimental perturbations and live observations of labeled neurons in the developing brains of small transparent tadpoles and fish larvae constitute the main source of data that explain how map development proceeds at the cellular level. However, the same mechanisms responsible for retinotectal development in these simpler vertebrates are also likely to play essential roles in mammalian development. We have highlighted experiments from multiple species that have provided particularly important insights into projection refinement mechanisms throughout this review article. In addition, it is important to note that the most prominent activity-dependent stages of brain circuit refinement do not necessarily take place at the same time in development (e.g., the retinal projection to the SC achieves its mature organization earlier than that in the LGN) and therefore the rules that control retinotectal refinement may be fundamentally different, or manifest themselves differently, during later refinement events.

We begin by outlining a list of fundamental mechanistic events that the experimental evidence indicates are likely to occur during retinotectal map refinement. Each of these is elaborated in greater detail below.

RULES FOR RETINOTECTAL STRUCTURAL PLASTICITY

1. Molecular guidance cues provide information for coarse axonal targeting.
2. Inputs compete for available synaptic target space.
3. Axonal and dendritic arbors are highly dynamic, even after seemingly mature morphology is attained.
4. Patterned neuronal activity provides instructive cues that help refine inputs:
   (a) Synchronous firing stabilizes synapses and prolongs branch lifetimes while actively suppressing branch dynamics via N-methyl D-aspartate receptor (NMDAR)-dependent retrograde signaling (Hebbian mechanisms).
   (b) Asynchronous activity weakens synapses (LTD) and actively promotes axonal branch dynamics, including addition and elongation, as well as branch elimination (Stentian mechanisms).
5. In the absence of sensory input, correlated spontaneous firing provides surrogate patterned activity.
6. New axonal branch tips emerge near existing synapses.

7. Stronger synapses help stabilize the axons and dendrites on which they form (Synaptotropism).
8. Homeostatic mechanisms help maintain the overall level of functional synaptic input to the target.

1. MOLECULAR GUIDANCE CUES PROVIDE INFORMATION FOR COARSE AXONAL TARGETING

The classic experiments of Sperry (1963) studying the developing and regenerating retinotectal projection in fish and amphibians, led Sperry to propose the "Chemoaffinity Hypothesis" in which the molecular tagging of presynaptic and postsynaptic partners with a kind of address code guides the establishment of the retinotopic maps in the optic tectum. Sperry (1943) observed that following optic nerve section, regenerating RGC axons projected back to roughly correct, retinotopically appropriate sites in the optic tectum irrespective of whether the whole retina was still present, even if the eye had been rotated to provide erroneous information about the visual environment. Furthermore, even upon initial entry to the optic tectum, RGC inputs are already coarsely retinotopically distributed within the optic tract (Holt and Harris, 1983), suggesting the presence of molecular guidance cues throughout the developing visual system.

Concrete support for this model came initially with the identification of a candidate molecular activity expressed in membranes isolated from posterior optic tectal cells that could serve as one of Sperry's tags (Walter et al., 1987). Ultimately the molecule was identified to be a ligand of the EphA family of receptor tyrosine kinases, and together with related EphA binding molecules, this group of ligands was labeled as the ephrin-A family (Drescher et al., 1995; Feldheim and O'Leary, 2010). In mammals, there are nine members of the EphA subclass (EphA1-EphA8 and EphA10) and five members of the EphB subclass (EphB1-EphB4 and EphB6), the molecular properties and signaling of which have been exhaustively reviewed (see Egea and Klein, 2007). Studies in numerous species have demonstrated that EphA and ephrin-A members are expressed in complementary gradients across the retina and the tectum (Brennan et al., 1997; Monschau et al., 1997; McLaughlin et al., 2003a; Higenell et al., 2012). The expression levels of EphA are highest in temporal retina and decline in a graded manner toward the nasal retina; A decreasing gradient of ephrin-A from the caudal to the rostral tectum is also present. Ephrin-A and EphA family members are expressed at the surface of both RGC axons and tectal cells. Once bound, the receptor-ligand couple activates a signaling pathway that results in axon repulsion (Drescher et al., 1997). Therefore, temporal axons expressing high EphA levels avoid the caudal tectum where the ephrin-A levels peak.

Interestingly, the complementary expression gradients of ephrins and Ephs mean that they are co-expressed at differing ratios within cells across the retina and the tectum (Marcus et al., 1996; Rashid et al., 2005). Ephrin-A5 is expressed in a strong graded fashion (nasal > temporal) in the mouse retina (Suetterlin and Drescher, 2014). Thus, cis-signaling mediated by Eph-ephrin binding within the same RGC axon may serve to effectively sharpen the nasotemporal gradient of trans-neuronal ephrin signaling (Hornberger et al., 1999). Moreover, the high levels of ephrin-A5 expressed on nasal axons allows them to also participate in the repulsion of temporal axons that express high levels of EphA (Bonhoeffer and Huf, 1980; Raper and Grunewald, 1990; Suetterlin and Drescher, 2014).

Just as the interaction of EphA and ephrin-A mediates the mapping of the temporal-nasal axis of the retina along the rostral-caudal dimension of the tectum, the interaction of EphB with ephrin-B has been proposed to contribute to the dorsoventral mapping of RGC terminals along the mediolateral axis of the optic tectum. However, the underlying interaction appears to mediate axonal attraction rather than repulsion and may also involve reverse signaling from EphB to ephrin-B (Hindges et al., 2002; Mann et al., 2002). While the importance of EphA-ephrin-A signaling in establishing the anteroposterior axis of the retinotectal topographic map has been well validated with knock-out and misexpression studies (Feldheim and O'Leary, 2010), there remains some uncertainty about the specific roles played by EphB-ephrin-B signaling. In frogs, the actual gradient of expression during development is not consistent with that predicted by the cell biology. The proposed reverse signaling model, based on *in vitro* observations, suggested that dorsal RGCs expressing ephrin-B should be attracted to putative high levels of EphB in the ventral tectum (Mann et al., 2002). However, labeling the expression pattern of EphB in the *Xenopus* tadpole brain using ephrin-B-alkaline phosphatase fusion proteins revealed that, to the contrary, there is instead a dorsal > ventral expression gradient of EphB and no detectable ephrin-B gradient in the optic tectum (Higenell et al., 2012). Furthermore, the dorsoventral axis of the retinofugal projection appears to presort in the optic tract prior to encountering any gradient in the tectum, suggesting that EphB-ephrinB signaling may have a role to play in axon guidance in the optic tract (Plas et al., 2005).

2. INPUTS COMPETE FOR AVAILABLE SYNAPTIC TARGET SPACE

Aside from the chemoaffinity and chemorepulsive mechanisms that direct RGC axons to arborize roughly within topographically appropriate locations in the target, several lines of evidence indicate that the mapping of the projection is subject to an additional fundamental influence that is most likely independent of neural activity. There appears to be a competition for available target space that has the important consequence of rendering the retinotectal projection free of discontinuities and innervation gaps. One striking example of this phenomenon can be seen in the retinotectal projection of the ephrin-A2/A5 double knock-out mouse, which has severely disrupted mapping of RGC inputs along its rostrocaudal axis, such that injection of the anterograde tracer DiI into one site in the eye results in multiple discrete patches of axon terminal labeling along the entire axis instead of a focused single termination zone in the target as seen in wildtype animals (Feldheim et al., 2000). Despite the complete topographic disorganization of retinal inputs, bulk labeling the entire retinotectal projection by intraocular cholera

toxin B injection produces a uniform and uninterrupted pattern of afferent labeling across the entire area of the tectal neuropil which is indistinguishable from that in wildtype animals. Thus the ability of the inputs to occupy all available target space is unperturbed despite the absence of the ephrin-A signaling that is essential for ordering the map.

An extreme version of this phenomenon, which offers some insights into the importance of this competition over target space in map formation is the so-called "EphA ki/ki" transgenic mouse in which two overlapping gradients of EphA expression are induced in the retina, one normal and one elevated by uniform high expression of EphA3 in a subset of RGCs (Islet2+ cells) throughout the eye (Reber et al., 2004). In these mice, RGCs expressing the normal levels of graded EphA project to the tectum and form a complete, topographically ordered map that is restricted to the caudal half of the tectum. In the rostral tectum a second complete and well-ordered map is formed by the Islet2+ RGCs. This double map reveals that in distinction to the pure chemoaffinity model put forth by Sperry, it is relative, rather than absolute, levels of EphA receptor expression that organize the map. The fact that two full maps form in the space that normally accommodates one further supports the idea that maps can expand or contract as necessary to fill the available territory.

In animals capable of central nervous system regeneration like fish and frogs it has long been known that if half the retina is ablated and the remaining half allowed to regrow into the tectum, the resulting half-map expands to fill the territory formerly occupied by afferents from the intact retina (Schmidt and Easter, 1978). Similarly, ablation of part of the tectum results in a compressed retinotectal map that fits within the remaining area (Yoon, 1976; Schmidt and Coen, 1995). It appears that this process of map regulation, by which input and target size are matched, may occur without the benefit of patterned neural activity, as injection of the sodium channel blocker tetrodotoxin (TTX) failed to prevent the map rescaling (Meyer and Wolcott, 1987). These observations support the notion of activity-independent competitive interactions in the optic tectum, perhaps analogous to the competition of peripheral axons for nerve growth factor in the skin (Lewin and Barde, 1996). The reorganization of the map that occurs after ablations therefore appears to represent an independent influence that the system imposes on the afferents through competition for space.

Map plasticity has also been examined at the single axon level in a clever experiment in zebrafish in which a single arbor from a transplanted RGC is allowed to innervate the optic tectum of a *lakritz* mutant fish that is incapable of generating its own RGCs. This single axon is free to innervate its target in the complete absence of competition from other retinal afferents (Gosse et al., 2008). Interestingly, these axons managed to target their topographically appropriate termination zones in the tectum, but formed abnormally large terminal arbors. This result suggests that retinal axons do at least have a crudely defined inherent preferred termination zone within the target, presumably due to chemoaffinity cues, but that in the absence of competition for space, arbors can enlarge their coverage area, at least to a limited extent.

Is there a role for neural activity in the competition between afferents? This question was addressed in an experiment in which the Kir2.1 potassium channel which reduces neuronal excitability and firing was overexpressed in just a few RGCs together with GFP in zebrafish larvae (Hua et al., 2005). These silenced RGC axons failed to elaborate arbors in the optic tectum that were as large as those from control GFP-expressing neurons. But blocking all activity in the network by rearing the animals in TTX restored normal arbor size to the Kir2.1-expressing cells, indicating a competition based on overall activity levels can also regulate arbor elaboration. Electroporation to express Kir2.1 in RGCs in mice, produces a similar result, with axon arbor elaboration greatly reduced compared to control cells (Benjumeda et al., 2013).

Interestingly, partial ablation of the SC at birth in the Syrian hamster results in an enhancement in the steepness of the ephrin-A gradient in the remaining part of the colliculus that is consistent with the accompanying compression of the retinotectal map (Tadesse et al., 2013). Implantation of a slow-release Elvax polymer to deliver the NMDAR blocker amino phosphonovaleric acid (APV) to the SC during development fails to prevent map compression in response to partial SC ablation, as measured electrophysiologically (Huang and Pallas, 2001). Thus the compression of the map appears to depend more upon molecular than on activity-dependent cues. However, receptive field sizes are enlarged in these APV-treated animals, consistent with neural activity playing an important role in map refinement. Thus, the experimental evidence indicates that the rough retinotopic mapping of axon arbors, as well as map compression and expansion, is largely the result of activity-independent mechanisms, including guidance molecule expression and competition for space in the overall target structure. Axon arbor size, important for the precision of connectivity, is regulated by activity-dependent competitive interactions.

3. AXONAL AND DENDRITIC ARBORS ARE HIGHLY DYNAMIC, EVEN AFTER SEEMINGLY MATURE MORPHOLOGY IS ATTAINED

The remarkable potential for structural plasticity observed in the developing and regenerating retinotectal projections reflects the cellular mechanisms by which retinal axons ramify within the optic tectum. Static images of labeled cells, reconstructed from fixed histological specimens, reveal convoluted trajectory changes and the frequent presence of interstitial branches throughout the axonal arbor which hint at the fact that axon growth and arbor development result from a highly exploratory process involving extensive axon remodeling over time (Sakaguchi and Murphey, 1985; Nakamura and O'Leary, 1989; Cline and Constantine-Paton, 1990; Dhande et al., 2011). However, live imaging of axonal and dendritic remodeling in intact, transparent zebrafish and *Xenopus* embryos has revealed a far more dynamic reality in which axons are perpetually extending and retracting extensive interstitial branch tips to

probe the target area (O'Rourke and Fraser, 1990; Kaethner and Stuermer, 1992; Dingwell et al., 2000). In zebrafish, the process by which an axon arrives at and elaborates extensive branch tips within its final termination zone is not directed growth as one might find with chemoattraction, but rather what appears to be a process of random branch extension in which the overall progression of branch elongation and stabilization favors the future termination zone (Kita et al., 2015). The process is similar in *Xenopus* except that individual arbors occupy a relatively larger proportion of the total tectal neuropil from earlier stages, creating a situation in which the topographic map increases in precision with age, not only by restricting axonal branches to appropriate locations, but also by constant growth of the total retinorecipient field with age (Sakaguchi and Murphey, 1985). As the tectum expands by adding cells at its caudomedial pole, the RGC arbors adjust and improve their relative retinotopic order by gradually shifting their positions within the tectum. This creates a need for ongoing structural dynamism and plasticity at least until metamorphosis in order to optimize the map. The dendritic arbors of tectal neurons and even the filopodial processes extended by radial glial cells are similarly labile during this period, consistent with the notion that dynamic process remodeling can combinatorially increase the potential set of connections available for the network to sample and also reduce the steric interference that may result when multiple cells are actively rewiring within the same volume (Rajan et al., 1999; Chklovskii et al., 2004; Tremblay et al., 2009). Thus even in relatively mature tadpoles, in which most RGC axons have attained their mature size and complexity, time lapse imaging still reveals ongoing remodeling and exploratory probing at branch tips, albeit at considerably slower rates than are observed during the initial establishment of the retinotectal projection.

4. PATTERNED NEURONAL ACTIVITY PROVIDES INSTRUCTIVE CUES THAT HELP REFINE INPUTS

The relative contributions of molecular signaling vs. neuronal activity in topographic map establishment and refinement have long been a subject of debate. The apparent lack of a requirement for action potential firing in the initial establishment of retinotopy has renewed questions about the overall importance of activity in map formation (Harris, 1984; Stuermer et al., 1990; Benjumeda et al., 2013). In this section, we have made an effort to describe the various experimental approaches, covering nearly a half-century, that have contributed to the conclusion that patterned neuronal activity is indeed instructive for precise retinotopic map refinement. We further discuss recent efforts from *in vivo* imaging to dissect how specific properties of patterned neuronal activity instruct different aspects of retinotectal refinement.

Effects of Dark Rearing

Dark-rearing repeatedly has been shown to have no significant impact on topographic precision in the retinotectal projections in fish or amphibians, measured either electrophysiologically or morphologically. In the optic tectum of *Xenopus* frogs, dark-rearing produced no significant modifications in multiunit tectal receptive field sizes or in the laminar segregation of RGC inputs defined by specific stimulus selectivities (Keating et al., 1986). There are also no obvious alterations in the proper laminar targeting of RGC inputs within the superficial layers of the optic tectum in dark-reared compared to control zebrafish (Nevin et al., 2008). Furthermore, visual deprivation following optic nerve crush in adult goldfish does not impair the gradual sharpening of the initially diffuse termination field of regenerating retinal afferents into fine patches (Olson and Meyer, 1991). At first glance, these data appear consistent with the possibility that visual experience, and patterned neural activity, may not play a meaningful role in directing RGC axons to refine their projections to topographically appropriate tectal partners, and that the precise spatial organization of inputs in the tectum is exclusively determined by graded molecular guidance cues. However, it is critical to bear in mind that dark rearing does not necessarily deprive the visual system of all activity as spontaneous activity may be sufficient to provide the necessary activity-dependent cues needed for normal retinotopic map refinement. Work in the mammalian visual system certainly suggests that the maintenance of receptive field properties in the SC requires ongoing patterned visual input (Carrasco et al., 2005).

Blockade of Action Potential Firing

In contrast to dark-rearing, experiments using chronic pharmacological blockade of voltage-gated sodium channels with TTX can directly test the requirement for action potential firing in development. Schmidt and Edwards (1983) reported that, unlike with dark-rearing, intraocular injection of TTX during optic nerve regeneration in adult goldfish prevented the refinement of multiunit receptive field sizes in the TTX-treated animals. It also resulted in the degradation of precision in the anatomical projection (Meyer, 1983). At the single cell level, TTX treatment resulted in significantly enlarged regenerated axonal arbors, but failed to induce any detectable alterations in an intact projection (Schmidt and Buzzard, 1990).

In *Xenopus laevis* tadpoles, retinal action potential blockade with TTX leads to a rapid increase in axonal branch dynamics measured as number of branches added and lost per 2 h (Cohen-Cory, 1999). The TTX-treated arbors also undergo greater net growth and branch addition over 24 h. In zebrafish larvae, however, the size and topographic location of individual RGC terminal arbors is not altered when action potential firing is blocked and in macho mutant fish with reduced sodium channel activity during development, perhaps reflecting the relatively faster pace of development in this species (Stuermer et al., 1990; Gnuegge et al., 2001). Interestingly, however, both TTX treatment and the macho mutation result in greater divergence of the retinotectal axons as they project into the tectum from the same quadrant of the eye, suggesting that while individual axon arbors are morphologically normal, they fail to converge precisely within their correct termination zone.

In the mouse, silencing RGCs by *in utero* electroporation of Kir2.1 also does not prevent axonal pathfinding or targeting in the SC, but this manipulation does result in less elaborate, more diffusely arborizing axon terminals, indicative of a degraded retinocollicular map (Benjumeda et al., 2013).

Blockade of NMDA Receptors

Locally-correlated, patterned firing in the retina, whether mediated by visual stimuli or spontaneous retinal waves, carries information about the relative locations of RGCs with respect to one another that the system can use to instruct map refinement. The notion of correlated firing between pre- and postsynaptic cells leading to strengthening of connection efficacy was originally articulated by Canadian psychologist Hebb (1949) in the context of learning and memory. The idea of a "Hebb synapse" capable of modifying its synaptic strength in response to co-activation of pre- and postsynaptic partners is in fact born out by the basal occlusion by Mg^{2+} of the ion channel of NMDA receptors, the principal glutamate receptor type found at newly formed synapses. Only when the dual requirements of glutamate binding and simultaneous postsynaptic depolarization to relieve the Mg^{2+} block of the pore are satisfied can the NMDAR flux current (Nowak et al., 1984). This property of the NMDAR means that it can function as a molecular detector of correlated activity. The fact that NMDAR activity appears to be required for many forms of neural plasticity, including long-term potentiation of excitatory synaptic transmission is consistent with this role.

One of the first demonstrations that correlation detection by NMDARs likely contributes to retinotectal map refinement involved the implantation of a slow-release polymer to continuously deliver the NMDAR antagonist APV over the optic tectum in Rana pipiens frogs and tadpoles. After several weeks of tectal NMDAR blockade a focal injection of a retrograde tracer was made into the optic tectum in order to reveal the convergence of inputs from the eye, a measure of map refinement. Compared with sham treated control animals, the animals that had undergone tectal NMDAR blockade exhibited a pronounced degradation of input convergence resulting in less precise retinotectal maps (Cline and Constantine-Paton, 1989). A similar experiment performed on early postnatal rat SC gave consistent results, leading to many mistargeted retinocollicular axon terminals and disrupting the normal refinement of termination zones of RGC axons (Simon et al., 1992). Electrophysiological analysis of SC receptive fields in the Syrian hamster provided functional evidence that NMDAR blockade during development also prevents the normal refinement of receptive field size (Huang and Pallas, 2001). Thus, Hebb's postulate that "cells that fire together, wire together" indeed appears to be implemented by NMDARs, presumably acting as correlation detectors.

Dually Innervated Tectum

The normal retinotectal projection in fish and frogs is almost exclusively contralaterally projecting and monocular. Surgical ablation of one of the tectal lobes or surgical deflection of the optic fibers from one tectal lobe to the other forces both eyes to map onto a single lobe, resulting in a dually innervated tectum (Sharma, 1973; Levine and Jacobson, 1975; Springer and Cohen, 1981). Alternatively implanting a supernumerary third eye during embryogenesis results in a projection that must share the optic tectum with the animal's normal retinal inputs (Constantine-Paton and Law, 1978). The retinal afferents in dually innervated tectal lobes segregate into alternating ocular dominance bands, each dominated by inputs from one eye (Higenell et al., 2012). Because the tectum is normally monocular, it seems unlikely that any cryptic molecular patterning exists to program the segregation of retinal afferents into ocular dominance bands. Instead, it has been proposed that ocular dominance bands in the dually innervated tectum reflect a compromise between Sperry's chemoaffinity cues and a Hebbian convergence of co-active inputs. Presumably each eye expresses the full complement of graded molecular guidance cues and therefore their axons seek to target topographically appropriate sites in the optic tectum. When two such axons from the same eye terminate in the same part of the tectum, Hebbian mechanisms should facilitate their convergence, but in the case where two axons from different eyes attempt to terminate in the same location they may be forced apart by competitive mechanisms as a consequence of their poorly correlated firing patterns. Evidence that neuronal activity indeed mediates the segregation into ocular dominance bands comes from experiments in which the firing of action potentials in retinal axons in 3-eyed frogs was chronically blocked by TTX, which resulted in the desegregation of inputs into a uniform, overlapping field in the tectum (Reh and Constantine-Paton, 1985). Moreover, the activation of tectal NMDARs specifically is also required, as chronic delivery of APV from a slow-release polymer placed over the tectum also desegregated the afferents from both eyes (Cline et al., 1987). Though it remains formally possible that neural activity and tectal NMDAR activation are merely permissive for segregation into ocular dominance bands, at least no band-like pattern of ephrin-A expression in the tectum, which would foreshadow the formation of eye-specific bands, has been observed in the dually innervated tectum (Higenell et al., 2012).

Stroboscopic Rearing

Further evidence for activity patterns being instructive rather than merely permissive for activity-dependent retinotectal projection refinement comes from strobe-rearing experiments, in which an atypically high degree of correlation in the firing activity of RGCs is induced across the entire eye. The retinotectal projections in goldfish reared by stroboscopic illumination after hatching overlap substantially and fail to refine throughout development (Schmidt and Buzzard, 1993). Labeling of single RGCs in strobe-reared fish revealed axonal arbors that are long and diffusely branched without forming the characteristic dense clusters of branches at the termination zone multiunit receptive field maps in these animals also showed poor topographic refinement, with atypically large response fields. Regenerating projections exhibit a similar failure to refine under conditions of stroboscopic illumination (Schmidt and

Eisele, 1985). Interestingly, the effects of strobe rearing on ocular dominance bands in the dually innervated fish or frog tectum has not to our knowledge been reported, though differences in axonal path length from the two eyes could produce sufficiently asynchronous synaptic activation in the tectum to reinforce segregation. On the other hand, a study in mice, which normally do have binocular innervation of the SC, found that in animals that had experienced optogenetic simultaneous co-activation of the two eyes during the period of retinotectal axon ingrowth prior to eye-opening, ipsilateral eye afferents were no longer restricted to deeper tectal layers but instead appeared able to stabilize inputs within the more superficial layers where contralateral inputs normally terminate exclusively (Zhang et al., 2011).

HEBBIAN MECHANISMS: SYNCHRONOUS FIRING STABILIZES SYNAPSES AND PROLONGS BRANCH LIFETIMES WHILE ACTIVELY SUPPRESSING BRANCH DYNAMICS VIA NMDAR-DEPENDENT RETROGRADE SIGNALING

The amenability of the intact *Xenopus* tadpole retinotectal system to live imaging and whole-cell electrophysiology makes it an ideal system in which to ask questions about the fundamental cellular events that underlie Hebbian structural plasticity. The retinotectal synapse was one of the first synapses shown to exhibit spike-timing-dependent long-term potentiation (tLTP) and depression (tLTD) *in vivo* (Zhang et al., 1998; Tsui et al., 2010). During the period of retinotectal map refinement, repeated visual stimulation can be used to induce conditions favorable for tLTP and this results in a shift in receptive field structure toward the potentiated subfield (Vislay-Meltzer et al., 2006). As in many other brain areas, tLTP in the retinotectal system is pathway-specific and NMDAR-dependent. One advantage of tLTP as a model for experience-dependent plasticity is the fact that it is far more physiological than protocols like tetanic stimulation and therefore more likely to resemble the actual mechanisms by which sensory input and patterned activity alter synaptic strengths in the developing visual system. As in the hippocampal Schaffer collateral-CA1 synapse, induction and expression of retinotectal LTP are both mediated by signaling events in the postsynaptic cell, which involves activation of NMDARs, Ca^{2+} influx, activation of Ca-calmodulin kinase type II (CaMKII), and trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid type glutamate receptors (AMPARs) to the synapse (Wu et al., 1996; Mu and Poo, 2006). In order for these phenomena to be relevant to map refinement, however, such postsynaptic signaling must be able to drive changes in the presynaptic axons through the production of one or more retrograde signals that can act back on the presynaptic terminal.

Normal visual experience during the period of developmental refinement can activate postsynaptic NMDARs. Indeed, blockade of NMDARs by bath application of APV results in a rapid upregulation of presynaptic RGC axon branch dynamics visualized *in vivo* by confocal microscopy, with a greater number of new branch tips added and retracted at the axon terminal over minutes to hours (Rajan et al., 1999). Application of the non-competitive NMDAR blocker MK-801 in zebrafish at 3 days post-fertilization when the retinotectal projection is first established is reported to result in an overall expansion of RGC axon arbor size (Schmidt et al., 2000). However because NMDARs are present not only in postsynaptic tectal neurons, but also in the retina and potentially at presynaptic terminals of RGCs in the tectum (Corlew et al., 2008; Banerjee et al., 2016) pharmacological blockade of NMDARs is not conclusive evidence for the existence of retrograde signaling.

More conclusive evidence for a retrograde signal originating in the postsynaptic cell that can modify the growth of presynaptic axons comes from an impressive series of *in vivo* time lapse imaging studies in *Xenopus* tadpoles by Zou and Cline (1996). Viral overexpression in tectal neurons of a constitutively active truncated form of CaMKII (tCaMKII), which lacks the autoinhibitory regulatory domain, mimics the activation of CaMKII that takes place in LTP induction. Animals in which the postsynaptic tectal neurons, but not the presynaptic RGCs, were virally infected with tCaMKII showed the expected enhancement in synaptic AMPAR currents as NMDAR-only "silent synapses" matured *en masse* to become AMPAR-containing functional synapses (Wu et al., 1996). Interestingly, the RGC axon arbors were visualized in these animals and found to grow far less and exhibit much lower branch tip density than control cells, indicating the existence of a retrograde signal downstream of CaMKII activation that stabilizes existing branches and suppresses branch elaboration as it drives synaptic maturation.

The process of ocular dominance band formation in dually innervated fish and frog tectum (described above) almost certainly requires retrograde signaling, as the correlation detection is most likely performed by NMDAR activation in postsynaptic neurons. At the level of single axon branch dynamics, time-lapse imaging of *Xenopus* RGC axon arbors in dually innervated tectum reveals a preferential stabilization of branches that extend into same eye territory, compared to territory dominated by the other eye (Ruthazer et al., 2003). This preference is eliminated when NMDARs are pharmacologically blocked, a result that conforms with the idea that NMDARs mediate axon branch stabilization via retrograde signaling.

STENTIAN MECHANISMS: ASYNCHRONOUS ACTIVITY WEAKENS SYNAPSES (LTD) AND ACTIVELY PROMOTES AXONAL BRANCH DYNAMICS, INCLUDING BRANCH ADDITION, ELONGATION, AND ELIMINATION

To date, the most direct elucidation of how correlated firing among retinal afferents can instruct the refinement of the retinotectal map at the level of individual RGC axon branch dynamics has come from a study that took advantage of the fact that although the retinotectal projection in *Xenopus* tadpoles is almost purely contralateral, in the occasional animal one or two individual RGC axons can be found to project by

accident to the ipsilateral optic tectum (Munz et al., 2014). These misguided axons arborize and form synaptic contacts within the ipsilateral tectum, presumably responding to the same molecular cues that guide the contralateral RGC axons to form a crude map. This creates a unique experimental system in which, by visually stimulating the two eyes independently and systematically varying the degree to which stimulation is correlated between the two eyes, it becomes possible to directly test the Hebbian "fire together, wire together" hypothesis. Because the contralateral eye drives most of the inputs, a flash of light presented to that eye will cooperatively recruit activation of postsynaptic tectal neurons. In contrast, for the lone ipsilateral RGC to participate in firing the tectal neurons, it must fire at the same time as the contralateral inputs.

In his 1973 treatise on Hebbian plasticity, Gunter Stent argued that there must exist a complementary rule to Hebb's postulate to explain the case where a presynaptic axon repeatedly fails to excite a postsynaptic partner that is actively firing under the influence of another input (Stent, 1973). Stent proposed that this condition should be punitive, resulting in the weakening of that non-contributing input. This is sometimes referred to as the "Stentian extension" of Hebb's postulate. The lone ipsilaterally projecting RGC axon allows for both Hebbian and Stentian forms of correlation-based plasticity to be examined by applying synchronous or asynchronous stimulation to the two eyes.

Electrophysiological recordings from tectal neurons that receive synaptic input from both the ipsilateral and contralateral eyes revealed that the ipsilateral input maintains or even slightly increases its synaptic strength relative to the contralateral inputs when both eyes are stimulated together. However, when the two eyes were stimulated 1 s apart, the ipsilateral eye input, which by itself is usually not strong enough to drive the postsynaptic neurons to fire action potentials, very rapidly declines in synaptic strength and in many cases entirely loses its ability to evoke an AMPAR-mediated postsynaptic current, suggesting that a phenomenon like tLTD can be induced by asynchronous visual stimulation of the two eyes in this case.

In vivo multiphoton time-lapse imaging of the misdirected ipsilateral axon was also performed while concurrently presenting these same synchronous or asynchronous visual stimuli to the two eyes. Remarkably, asynchronous stimulation resulted in a rapid (within 30 min) and dramatic upregulation of new branch additions and a significant increase in branch tip elongation compared with axon dynamics during a preceding period of darkness. Elimination of branch tips was also enhanced, indicating that rather than producing a larger arbor, asynchronous stimulation makes the axon more dynamic and exploratory. Thus, asynchronous visual stimulation produced an enhancement in growth and dynamics akin to the effects of NMDAR blockade. This makes sense as it is unlikely that the lone ipsilateral axon would by itself be able to drive sufficient depolarization of the postsynaptic tectal cell to permit Ca^{2+} flux through NMDARs. Consistent with this notion, addition of MK801 to block NMDARs did not prevent the increased rate of branch additions in response to asynchronous stimulation. It is therefore possible that the source of the branch promoting signal may not be postsynaptic in origin, but could, for example be released by surrounding glial cells or come directly from nearby axon terminals.

In contrast, synchronous stimulation of the two eyes resulted in a rapid decrease in the rate of branch additions to levels seen in darkness. This decrease in branch dynamic behavior was completely prevented in the presence of MK801, or if tetanus toxin was expressed in the ipsilateral axon to render it incapable of releasing neurotransmitter, indicating that the activation of postsynaptic NMDARs likely leads to the release of a retrograde branch suppressing factor. In addition, branches that did form during synchronous stimulation had longer lifetimes on average that those that emerged during periods of asynchronous stimulation, indicating that they were more stable overall.

This experimental protocol tests the full range of growth responses that patterned activity might be able to induce, as it creates a set of extreme differences in firing correlation with synchronous stimulation resembling the conditions that might be produced by strobe rearing where all inputs are artificially correlated, and asynchronous stimulation creating a set of correlations that might only be found if the RGC were to ramify in an entirely inappropriate part of the tectum or what occurs in the dually innervated tectum. In the normal process of activity-dependent developmental refinement a typical axon might be expected to experience a more modest range of local correlation and asynchrony that would lead to a slight upregulation of exploratory branching and synapse disassembly on those branches that extend away from the proper termination zone (promoting them to keep growing until they land in more welcoming territory), and a stabilization and synaptic strengthening on those branches that extend into the appropriate part of the map where inputs with similar activity patterns converge (promoting consolidation and further synaptogenesis at this site). **Figure 1** portrays several plausible models for how these mechanisms could promote projection refinement.

5. IN THE ABSENCE OF SENSORY INPUT, CORRELATED SPONTANEOUS FIRING PROVIDES SURROGATE PATTERNED ACTIVITY

The pattern of action potential firing in the developing visual system contains information about the relative positions of the RGC somata in the retina and thus can instruct the precise mapping of the axons onto their postsynaptic partners in the optic tectum. Anamniotes, which include fish and amphibians, develop exclusively externally which allows for neuronal activity driven by the natural visual scenery (see review in this special topic issue by Pratt et al., 2016). Unlike fish and frogs, amniotes are hidden behind thick shells or develop *in utero*, which leads to a general deprivation of visual experience during the time when visual circuit refinement takes place. It is reasonable to

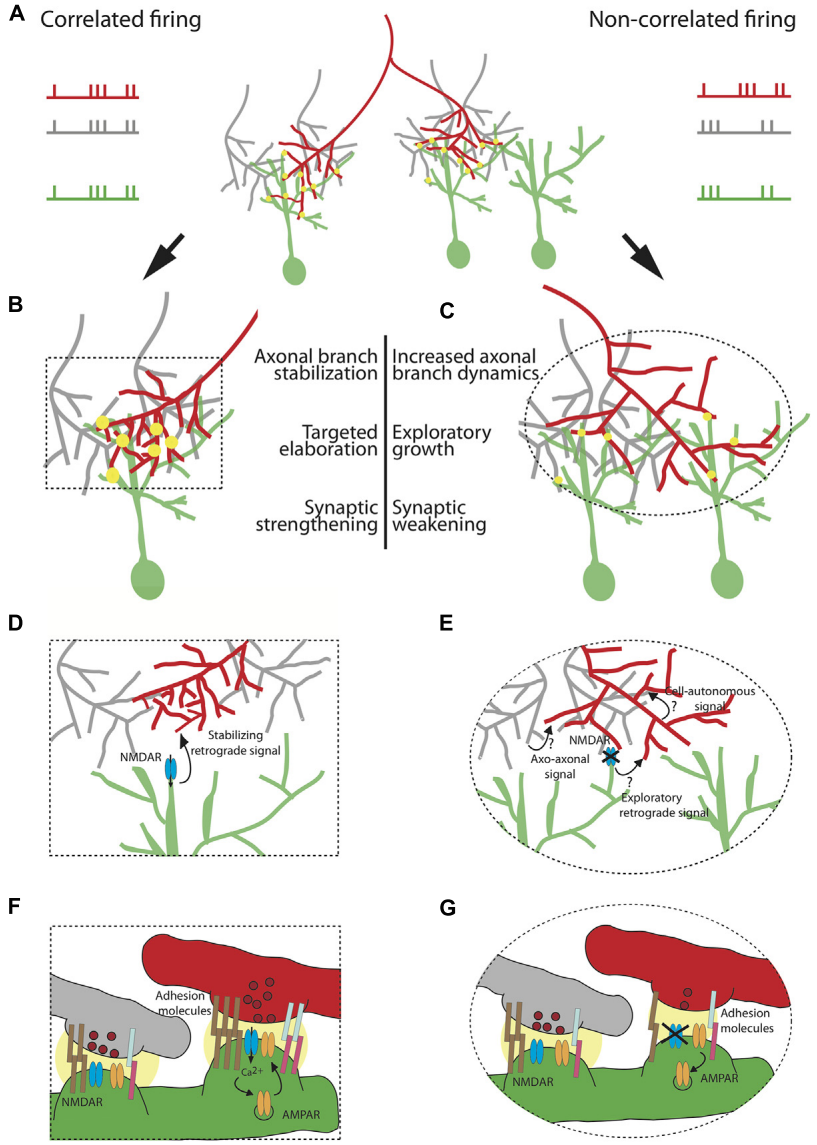

FIGURE 1 | Cellular and molecular mechanisms underlying the instructive role of patterned neuronal activity in retinotectal map refinement. A retinal ganglion cell (RGC) axon of interest (red) synapses onto tectal neuron dendrites (green) in **(A)** The red axon (left branch) is co-active with its neighboring RGCs (gray) and its firing pattern is therefore correlated with the firing pattern of the tectal neuron on which it synapses. The right branch of the red axon is not co-active with its neighbors and its firing pattern is not correlated with the firing pattern of its synaptic partner. Synapses are depicted as yellow circles. Synaptic strength is represented by the size of the yellow circle. **(B)** Correlated firing patterns of an RGC with its partnering tectal neuron instruct an increase in synaptic strength and stabilization of the axonal arbor allowing for local targeted arbor elaboration as new branches tend to form at existing synapses. **(C)** Non-correlated firing of an RGC with its neighboring axons and its postsynaptic partner instructs synaptic weakening and an increase in axonal branch dynamics, accompanied by exploratory growth in search of a better partner. The effects of patterned neuronal activity on structural remodeling and synaptic efficacy are schematized in **(D–G)**, respectively. **(D)** Shows a zoom-in of the area of the box in **(B)** depicting a "stabilizing retrograde signal" downstream of activation of N-methyl D-aspartate receptor (NMDAR) which encodes axonal branch stabilization and targeted elaboration. **(E)** Zoom-in of the ellipse in **(C)** Plausible mechanisms instructing axonal exploratory growth and branch destabilization due to the lack of correlated firing with the neighboring RGC inputs and the postsynaptic partner include: 1. Axo-axonal signal, released by the firing neighboring inputs (gray); 2."Exploratory retrograde signal", unmasked by the inactivation of NMDAR; 3. Cell-autonomous activity-dependent signal, released by the red neuron or acting intracellularly. **(F)** Zoom-in depicting molecular mechanisms underlying synaptic strengthening and stabilization of the red axon from **(B)**. Correlated firing of the RGC axon (red) with its neighboring axons (gray) and the tectal neuron (green) results in both release of glutamate containing vesicles (dark red) and postsynaptic depolarization. This satisfies the conditions required for release of the Mg^{2+} block from the channel pore of the NMDAR (blue), allowing for cation influx. Ca^{2+} triggers a molecular cascade resulting in insertion of more AMPAR (orange) in the membrane. A retrograde signal downstream of NMDAR encoding higher probability of vesicle fusion in the red axon (depicted as higher number of vesicles in dark red). Increase in synaptic efficacy is accompanied by strong homophilic (brown) and heterophilic interactions of adhesion molecules (light blue and pink). The strength of the interaction is represented by the number of pairs of adhesion molecules. **(G)** Zoom-in showing the change is synaptic efficacy in **(C)**. Non-correlated firing of the red axon with its neighbors and its partnering tectal neuron prevents opening of the channel pore of NMDAR, leading to AMPAR endocytosis, lower probability of glutamate release and weaker interaction of adhesive molecules.

speculate that spontaneous activity in the retina may have arisen as an evolutionary adaptation to provide replacement patterns in amniotes of neuronal activity that lower vertebrates are able to experience by visually interacting with their surrounding environment after hatching. The first evidence for locally correlated spontaneous activity in the fetal retinal came from extracellular retinal recordings made in rat pups while still attached to the uterus via the umbilical cord (Maffei and Galli-Resta, 1990). They have subsequently been confirmed and meticulously characterized using *in vitro* multielectrode array recordings and calcium imaging of retinal explants (Meister et al., 1991; Wong et al., 1993; Feller et al., 1996). These spontaneous activity patterns exhibits a high degree of local correlation in firing and consequently have been dubbed "retinal waves" (Meister et al., 1991). RGCs located in close proximity overlap their bursting activity in time, whereas RGCs that reside further away from each other are less likely to be co-active. This spatiotemporal pattern of RGC activity results from a local initiation of depolarization, which propagates to adjacent neurons spreading over long distances across the retina.

The definitive demonstration of this phenomenon was recently implemented in the intact mouse by loading RGCs out to their axon terminals with a calcium indicator to permit retinal waves in the eye to be detected by imaging their calcium transients in the SC (Ackman et al., 2012). This study revealed that the initiation point of the waves is biased to the ventrotemporal region of the retina, an observation that is particularly interesting in light of the fact that a locomoting fish or tadpole in the wild would typically experience natural visual stimuli as optic flow similarly sweeping from temporal to nasal retina, further arguing that retinal waves may have evolved as a replacement for natural vision prior to eye-opening. Retinal waves in zebrafish also stereotypically originate from the temporal retina (Zhang et al., 2016). In tadpoles, it has been shown directly that visual stimulation which includes optic flow in this more natural direction is far more effective at refining the retinotopic projection of RGCs than an identical amount of stimulation oriented in the opposite direction (Hiramoto and Cline, 2014). The mechanisms generating waves in the mammalian retina differ over development: embryonic type I waves depend on gap junctions; type II waves are initiated by starburst amacrine cells and spread through activation of nicotinic acetylcholine receptors; and type III waves utilize glutamate (Feller et al., 1996; Bansal et al., 2000; Torborg et al., 2005; see reviews in this special topic issue by Arroyo and Feller (2016) and Kerschensteiner (2016). In zebrafish, only one stage type of retinal waves has been described. They originate at the axonal terminals of bipolar cells and depend mostly on ionotropic glutamatergic receptors and gap junctions, with acetylcholine receptors likely having a modulatory role (Zhang et al., 2016).

While there is abundant evidence that patterned activity has an instructive role in topographic map refinement, an important remaining problem is to dissect the specific aspects of spontaneous activity that instruct retinocollicular refinement. Genetic deletion of the β2 nicotinic acetylcholine receptor subunit (β2-nAChR) in mice results in abnormal retinal waves with impaired spatiotemporal properties (Rossi et al., 2001; McLaughlin et al., 2003b; Chandrasekaran et al., 2005; Mrsic-Flogel et al., 2005). These perturbations result in severe defects in both the topographic refinement and eye-specific segregation in the SC and LGN. Unexpectedly, it was also reported that between-eye correlations in wave activity are enhanced in these β2-nAChR knock-out mice, which may partially account for the failure of eye-specific segregation (Burbridge et al., 2014).

Xu et al. (2011) examined a transgenic mouse line (β2(TG)nAChR) in which expression of the β2-nAChR was restored specifically in the RGCs in β2-nAChR knock-out animals. Although retinal waves in these mice occur with the same frequency and overall level of activity as in wild-type animals, their propagation is truncated and thus the correlation between the firing patterns of neighboring cells decreases steeply with distance. The very local correlations in RGC spiking are still intact, but the large-area within-eye correlations are lacking. These "smaller" retinal waves are sufficient to permit the refinement of retinotopy in the monocular zones of SC. Interestingly, however, RGC projections to the binocular zone fail to refine normally. This is apparently a result of an interaction between inputs from the two eyes, as monocular enucleation permits the full refinement of the remaining eye's afferents. Eye-specific segregation is also strongly disrupted in these mice.

They also tested animals (Rxβ2-cKO) in which β2-nAChR expression was conditionally deleted specifically in the retina (Xu et al., 2015). Much like the β2(TG)nAChR mice, Rxβ2-cKO mice exhibit only small residual retinal waves that have high local correlation but much lower long-distance correlations in firing. Retinotopy in Rxβ2-cKO mice was normal in the monocular SC, but eye-specific segregation was disrupted, phenocopying the β2(TG)nAChR animals. The rescued retinotopy and impaired eye-specific segregation in the SC compared to full β2-nAChR knock-outs suggest that topographic precision of the visual map depends primarily on local correlation in spiking patterns, whereas eye-specific segregation requires global within-eye correlations that differentiate the two eyes. It remains to be tested whether the abnormal between-eye correlations seen in full β2nAChR knock-out mice might also occur in β2(TG)nAChR or Rxβ2-cKO lines. This binocular correlation could perhaps help explain the failure of RGC inputs to refine topographically in the binocular zone of the SC, as inappropriately correlated inputs would be converging on postsynaptic cells in the SC.

Spontaneous retinal waves occurring before the onset of vision have been observed across numerous amniote species: turtle, chick, rat, mouse, ferret, cat, and monkey (Ackman and Crair, 2014). A form of retinal waves have also been described in zebrafish (Zhang et al., 2016). However they do not appear to be present in amphibians, which are able to rely on photoreceptor-driven vision from the onset of development of the retinotectal projection.

Zhang et al. (2016) have shown that retinal waves in zebrafish are restricted to a very short developmental window 2.5–3.5 days

post-fertilization (dpf). During this period RGCs begin to form synapses with postsynaptic tectal neurons (2 dpf) and visual behaviors such as prey capture and predator avoidance emerge shortly afterwards (4–6 dpf; Stuermer, 1988; Borla et al., 2002). Thus, these animals have a very short time to form a precise representation of the visual world necessary for survival. We can speculate that the available visually-driven and spontaneous activity might work in concert to provide adequate information for topographic map refinement (Zhang et al., 2010). Interestingly, Demas et al. (2012) used multielectrode arrays to record retinal activity across the eye, and discovered that rearing *Xenopus* tadpoles in complete darkness induces an increase in the amount of correlation in the spontaneous activity between neighboring RGCs. This observation supports the notion that the retinal circuitry may exhibit a tendency to favor retinal waves as a natural means of compensating for an absence of early visual stimulation. These critical discoveries finally helped make sense of several decades of earlier dark-rearing experiments in fish and frogs that had concluded that visual experience was dispensable for normal refinement of the developing retinotectal projection.

6. NEW AXONAL BRANCH TIPS EMERGE NEAR EXISTING SYNAPSES

At the same time as the retinotectal axons are dynamically remodeling by constant exploratory branch addition and withdrawal, fish larvae and tadpoles are already using their visual system to interact with the environment. Zebrafish are avid predators that can track and capture tasty paramecia and other organisms for food, a behavior that requires precise tectal function (Gahtan et al., 2005). This raises the paradox of how a functional circuit can at once be wired to reliably perform essential behavioral tasks while actively adding and eliminating synaptic contacts to refine connectivity. Insights into this process have come from time-lapse imaging of RGCs axons and tectal neurons expressing fluorescently labeled synaptic marker proteins to reveal synapse locations. This powerful approach was pioneered in the retinotectal system by Alsina et al. (2001) who expressed GFP-VAMP2 in *Xenopus* RGCs to reveal that many putative synaptic sites along the developing axonal arbor are added and eliminated rapidly over time, the rate of which can be regulated by Brain-Derived Neurotrophic Factor (BDNF) signaling. These authors made the important observation that new axonal branch tips almost always emerged from GFP-VAMP2 positive puncta, a finding that was later confirmed using a better targeted synaptic marker, synaptophysin-GFP (Meyer and Smith, 2006; Ruthazer et al., 2006). This result has profound implications as a mechanism for map refinement because it means that wherever a synapse strengthens (or weakens) through activity-dependent plasticity, it will be available (or not) to nucleate new branches from which new synapses can form. This constitutes a positive feedback loop that will lead to the targeted elaboration of axonal arbor at sites where that axon has formed effective, strong synaptic contacts and the scaling back of branch initiation at inappropriate sites where synapses may form transiently but are subsequently eliminated.

7. STRONGER SYNAPSES HELP STABILIZE THE AXONS AND DENDRITES ON WHICH THEY RESIDE (SYNAPTOTROPISM)

Postsynaptic dendritic growth and remodeling was studied in zebrafish tectal neurons co-expressing PSD-95-GFP to mark postsynaptic sites (Niell et al., 2004). These investigators made the critical observation that synaptic sites were fairly labile. They observed that the dendritic tree elaborated through a process of dynamic filopodial extensions followed rapidly by synapse formation. As synapses formed, those synapse-bearing branches became consolidated. Further branch extension then proceeded by building upon these more stable sites. Thus the presence of a synapse appears to confer stability onto the branch on which it forms, a phenomenon referred to as "synaptotropism" (Vaughn et al., 1988; Cline and Haas, 2008). Further support for the synaptotropic model of dendritic growth was solidified by experiments where synaptogenesis or synapse maturation respectively were prevented by blocking neurexin/neuroligin signaling or AMPAR trafficking in *Xenopus* tectal neurons, resulting in a failure to elaborate normal complex dendritic arbors (Haas et al., 2006; Chen et al., 2010).

Time lapse imaging of dsRed/synaptophysin-GFP-expressing RGC axons in zebrafish and *Xenopus* tadpoles showed that synaptotropism is equally applicable at the presynaptic side (Meyer and Smith, 2006; Ruthazer et al., 2006). Within minutes of axonal branch extension, synaptophysin-GFP puncta could be observed accumulating in the wake of the advancing growth cone. Some synaptic puncta were later lost while others became more mature over time, indicated by the bright accumulation of synaptophysin-GFP positive vesicles. When these branches later attempted to retract, the presence of a mature synaptic site conferred structural stability, preventing the branch from withdrawing beyond that site.

8. HOMEOSTATIC MECHANISMS HELP MAINTAIN THE OVERALL LEVEL OF FUNCTIONAL SYNAPTIC INPUT TO THE TARGET

Both the Hebbian and Stentian mechanisms in the context of changes in synaptic efficacy are inherently unstable. Correlated firing between a presynaptic neuron and its postsynaptic partner would induce synaptic strengthening as described above. An increase in synaptic efficacy would result in an even higher probability of correlation between the firing patterns of the pre- and postsynaptic neuron. Thus, applying only the Hebbian plasticity rules, the positive feedback loop would become unsustainable. Applying the same logic to Stent's extension to the Hebbian postulate, we will find ourselves

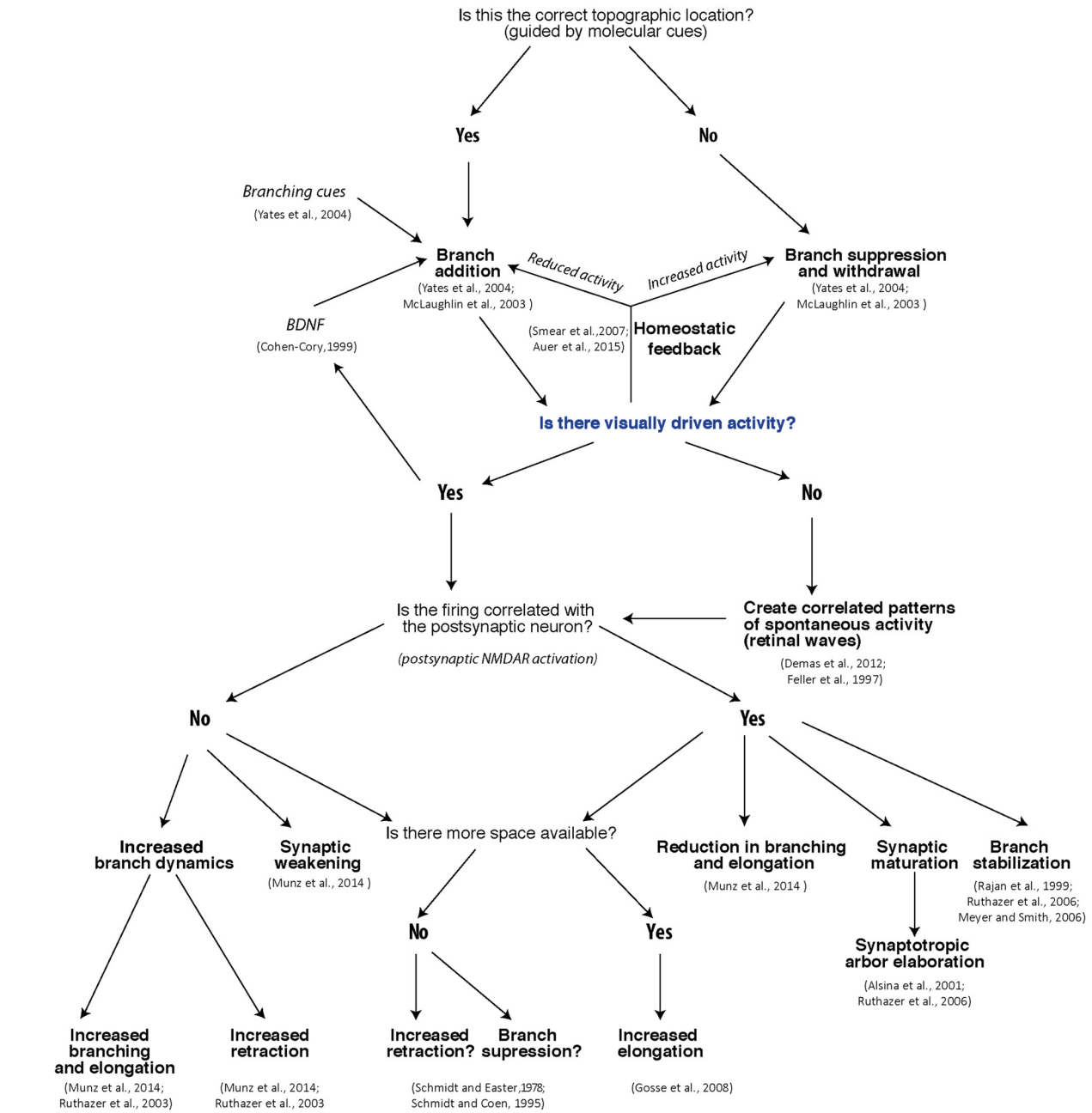

FIGURE 2 | Flow-chart of decisions RGCs face during retinotectal map formation and refinement.

in a similar situation where each time the synapse weakens, it will be less likely that the pre- and the postsynaptic firing patterns will be correlated. The brain overcomes this inherent instability by applying additional rules which ensure a healthy dynamic range of synaptic transmission within which bidirectional changes in synaptic efficacy can occur. These rules are referred to as homeostatic plasticity and have been extensively studied in the neocortex and the hippocampus (Bienenstock et al., 1982; Turrigiano and Nelson, 2004; Kaneko et al., 2008). An example of homeostatic regulation in the developing *Xenopus* retinotectal projection can be found in experiments where the intrinsic excitability of tectal neurons was manipulated either by overexpression of leak K^+ channel or by modifying synaptic efficacy by

application of a peptide that hinders AMPAR trafficking. Both manipulations lead to upregulation of voltage-gated Na^+ currents (Pratt and Aizenman, 2007), suggesting a homeostatic mechanism regulating intrinsic excitability that counteracts Hebbian/Stentian plasticity rules.

Further evidence for homeostatic regulation of the retinotectal circuit during development was obtained using zebrafish *blumenkohl* mutants (*blu*). The *blu* mutation disrupts vglut2a, encoding a vesicular glutamate transporter homologous to the mammalian VGLUT2 (Smear et al., 2007). *Blu* mutants exhibit a decrease in TTX miniature EPSC (mEPSC) amplitudes in tectal neurons, suggesting a reduction in glutamate concentration per vesicle. Interestingly, mEPSC frequency in mutant animals is increased, consistent with a higher probability of glutamate vesicle release or with upregulation in the number of release sites. These observations allude to a compensatory mechanism that helps normalize glutamatergic transmission in these animals. In accordance with this homeostatic regulation, the RGC arbors in the *blu* mutant zebrafish are larger, spanning a greater area of the optic tectum and tectal neurons exhibit larger receptive fields. In tectal *blu* animals neurotrophin-3 (NT-3) protein levels are upregulated in the optic tectum, suggesting that it could act as a homeostatic retrograde signal through TrkC receptor promoting axonal branch elaboration (Auer et al., 2015).

SUMMARY

Thanks to many decades of experimentation on retinotectal development in models ranging from fish to mammals, in conjunction with modern technology permitting live imaging of developing axons in the intact animal, we now have a much clearer understanding of the mechanisms that regulate the developmental fine-tuning of the retinotectal map. The decisions faced by a growing retinotectal axon are summarized in the form of a flow chart in **Figure 2**. While it is likely that other brain regions will apply slightly different strategies for activity-dependent refinement, the rules we have outlined here should prove a useful template for further investigation.

AUTHOR CONTRIBUTIONS

This article was written jointly by EK and ESR in consultation with MM. Figures were made by EK and MM.

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frontiers in Neural Circuits

REVIEW
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An Evolutionarily Conserved Mechanism for Activity-Dependent Visual Circuit Development

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Neural circuit development is an activity-dependent process. This activity can be spontaneous, such as the retinal waves that course across the mammalian embryonic retina, or it can be sensory-driven, such as the activation of retinal ganglion cells (RGCs) by visual stimuli. Whichever the source, neural activity provides essential instruction to the developing circuit. Indeed, experimentally altering activity has been shown to impact circuit development and function in many different ways and in many different model systems. In this review, we contemplate the idea that retinal waves in amniotes, the animals that develop either in ovo or utero (namely reptiles, birds and mammals) could be an evolutionary adaptation to life on land, and that the anamniotes, animals whose development is entirely external (namely the aquatic amphibians and fish), do not display retinal waves, most likely because they simply don't need them. We then review what is known about the function of both retinal waves and visual stimuli on their respective downstream targets, and predict that the experience-dependent development of the tadpole visual system is a blueprint of what will be found in future studies of the effects of spontaneous retinal waves on instructing development of retinorecipient targets such as the superior colliculus (SC) and the lateral geniculate nucleus.

Keywords: visual system plasticity, retinal waves, Hebb

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INTRODUCTION

Spontaneous neural activity, defined here as self-generated electrical activity that is not driven by afferent input, exists in many amniote sensory systems during their development. This activity provides important instructions for circuit development and maturation. For example, spontaneous activity of the cochlear inner hair cells promotes the maturation of central auditory pathways before hearing onset in mammals (Wang and Bergles, 2015), spontaneous firing in olfactory sensory neurons is required for the formation of the olfactory sensory map (Yu et al., 2004; Lorenzon et al., 2015), and spontaneous retinal waves in the developing visual system, prior to visual experience, drive topographic map formation in downstream targets such as the superior colliculus (SC) and lateral geniculate nucleus (Torborg and Feller, 2005). In amphibian larvae, whose development is completely external, visual stimuli, instead of spontaneous retinal waves, drives retinal ganglion cells (RGCs) and this activity is known

to instruct many aspects of development of this circuit (Sin et al., 2002; Ruthazer et al., 2003; Dong et al., 2009; Xu et al., 2011; Udin, 2012; Hiramoto and Cline, 2014). In this review, we discuss the role of activity in the development of topographic maps, neuronal structure and function and the maturation of neuronal circuits in the developing visual system. We first focus on the role of spontaneous retinal waves in amniotes, how they could be an evolutionary adaptation to developing on dry land in eggs or *in utero*, and recent findings about the consequence of these waves on their downstream targets. Next, we discuss the development of the amphibian visual system, and how the instructional activity in RGCs is generated by visual stimuli from the environment rather than retinal waves. We provide a comprehensive summary of the consequences of visual experience on the development of this circuit, underscoring both the importance of neural activity in circuit development and the advantages of the tadpole model for the study of circuit development. Lastly, we mention striking similarities between activity-dependent processes in the amphibian retinotectal circuit and those in non-sensory regions of the developing mammalian brain, suggesting that the fundamental mechanisms by which visual activity drives circuit development in tadpoles are conserved throughout the CNS of many species.

Retinal Waves are Expressed in Amniotes But not in Non-Amniotes

While spontaneous retinal waves have been well described and studied in embryonic retinas of amniotes such as turtles (Sernagor and Grzywacz, 1996, 1999), chicks (Wong et al., 1998), ferrets (Meister et al., 1991; Wong et al., 1993), rodents (Torborg and Feller, 2005; Ackman et al., 2012) and primates (Warland et al., 2006), this spontaneous patterned activity is not present in the retina of amphibians (Demas et al., 2012) nor fish (Kolls and Meyer, 2002). This dichotomy suggests the intriguing possibility that retinal waves are an evolutionary adaptation in response to the transition from life in the water to life on the land, when the transparent jelly coat of the aquatic anamniote embryo was replaced by a hard opaque shell for birds and reptiles, or a uterus for mammals. Developing in ovo or *in utero* is well-suited for survival on dry land, but these protective environments keep the embryo literally in the dark, devoid of visual stimuli during periods of brain development when neurons are extending processes and establishing nascent connections, and when circuit connectivity is being refined. In contrast, the development of aquatic amphibians and fish, from fertilization onwards, takes place externally, with embryos surrounded by nothing more than a transparent coat of jelly and larvae being exposed to complex sensory environments. This means that these anamniote embryos and larvae are always exposed to the external visual scene. Natural visual stimulation of the photoreceptors and retinal interneurons activates RGCs and transmits activity to retinal axons in targets as soon as synapses are formed (Holt and Harris, 1983). Consequently, spontaneous retinal waves are not needed to activate RGCs and convey patterned activity to the central retinal targets in anamniotes. In fact, one might anticipate that retinal waves in the presence of natural visual stimuli would likely interfere with important instructional information provided by environmental visual cues.

We suggest that, for amniotes, retinal waves could be an evolutionary adaptation to developing in the absence of patterned visual stimulation and serve as a proxy for visual experience (natural vision) in anamniotes (**Figure 1**). If this were true, it would be expected that retinal waves and visual experience would share common functions in the context of visual system development. In fact, emerging evidence indicates parallels between the role of visual stimulation in anamniotes and retinal waves in amniotes.

The Function of Retinal Waves in Amniotes

In the absence of external visual stimuli, amniotes are born or hatch with an impressive amount of their visual system already wired and capable of detecting and processing visual information. Although earlier *in vitro* and *in vivo* electrophysiological studies revealed that RGCs are spontaneously active (Mastronarde, 1983; Galli and Maffei, 1988) it was not until rather recently that bona fide waves have been recorded *in vivo* using calcium imaging (Ackman et al., 2012). The ability to visualize retinal waves *in vivo* makes it possible to address, directly, fundamental questions about the function of retinal waves, in particular, how these waves may contribute to developmental events in the RGC targets. Ackman et al. (2012) imaged retinal waves in mice *in vivo*, from the RGC somata to their axon terminals in the SC, and found that the spontaneous retinal waves drive the same spatiotemporal pattern of wave activity in their postsynaptic SC targets. In other words, the waves in the postsynaptic SC neurons match the RGC waves in space and time. This suggests that patterned spontaneous activity generated in the retina provides a template of patterned activity that could instruct the development of higher-order circuits in the visual system (Ackman et al., 2012). This study also demonstrated that retinal waves have defined—not random—initiation sites: retinal waves are initiated in the ventro-temporal retina, and they tend to propagate toward dorso-nasal retina. Similarly, waves in the retinal axons within SC initiate in the rostral-medial region of the SC and propagate to the caudal-lateral region, indicative of the topographic organization of the retinocollicular projection that forms based on instructive signals from spontaneous retinal waves prior to vision in amniotes (Torborg and Feller, 2005). In addition, by imaging calcium transients in SC neurons, it is clear that waves of retinal activity drive postsynaptic collicular activity. Ackman et al. (2012) interpret these data as a way in which retinal waves contribute to the development of direction selectivity in collicular neurons as well as higher order neurons in cortex.

These studies demonstrate that retinal waves expressed in amniotes are essential for supplying the specific temporal

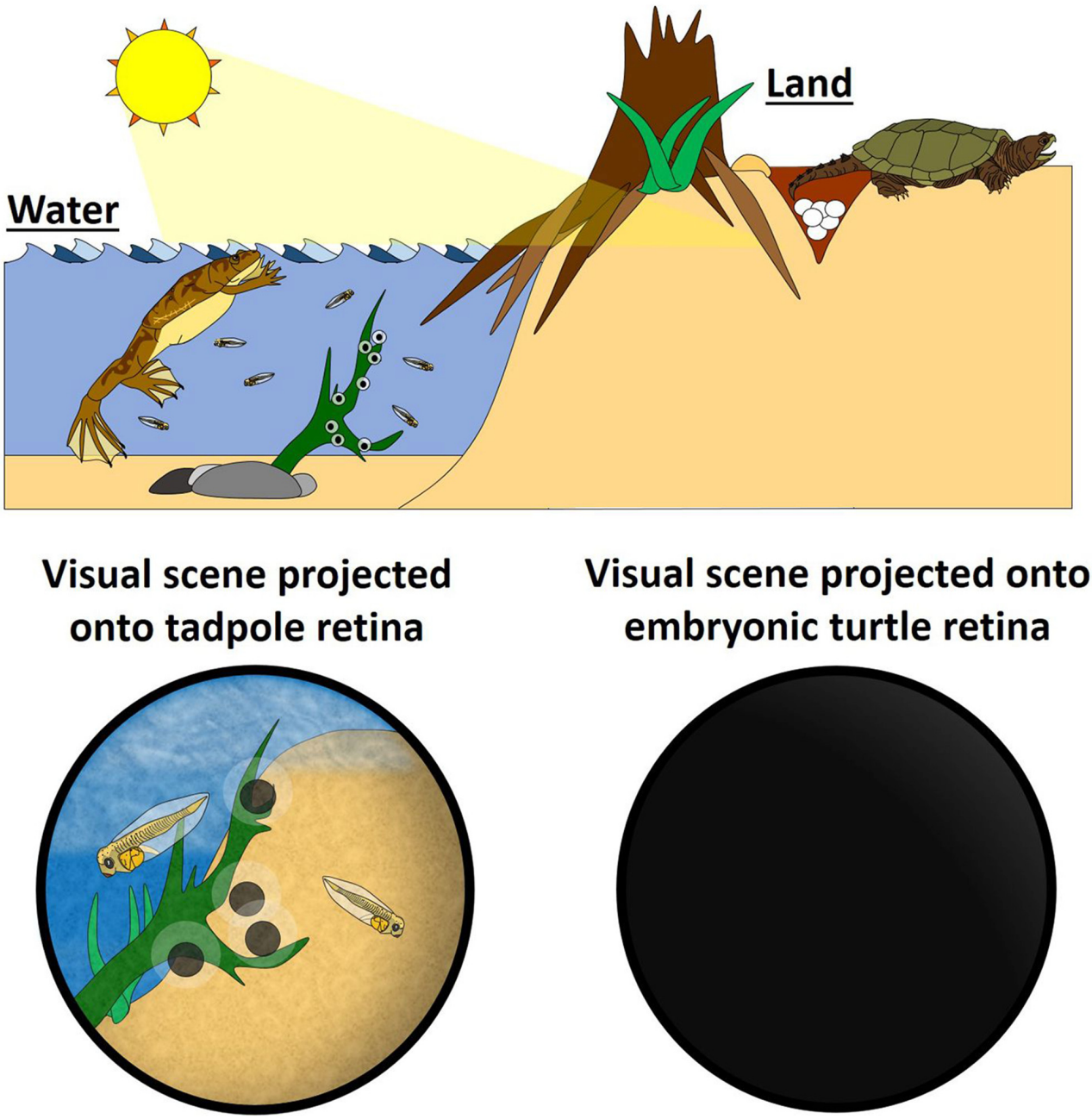

FIGURE 1 | Retinal waves could be an evolutionary adaptation for visual systems developing in the absence of visual stimuli. (Left) In the water, fertilization of fish and aquatic amphibians takes place externally, so the embryos develop with the benefit of direct sensory stimuli. This natural visual stimulation activates retinal ganglion cells (RGCs), providing the activity for activity-dependent mechanisms that instruct the formation and maturation of the visual system. Retinal waves are not needed to generate RGC activity in anamniotes, and, consistent with this, the anamniote retina does not express retinal waves. (Right) On land, development of amniotes—by definition—takes place *in utero* or in ovo, and so these embryos do not experience natural visual scenes. RGCs still get activated, however, by self-generating spontaneous waves of activity *(Schematic by Harley Yerdon).*

and spatial patterns of activity to the RGCs and thereby, via correlation-based mechanisms, guiding RGC inputs to precise postsynaptic targets. Likewise, in the anamniote, visual experience supplies a similar type of patterned activation of RGCs such that neighboring RGCs are most correlated and the further apart they are, the less they are correlated. The similar roles of retinal waves in amniotes and visual stimuli in anamniotes are highlighted in the next section as we describe the role of visual stimuli in the developing visual system of the *Xenopus* tadpole.

Function of Vision in the Amphibian Embryo

Meanwhile, back in the water, the visual systems of amphibians and fish are developing, forming topographic maps, refining receptive fields, and building circuits to detect and process visual information—all in the absence of spontaneous retinal waves (**Figure 2**). Visual responses can be observed in Xenopus tadpoles as soon as RGC axons reach the optic tectum and begin forming synapses onto dendrites of tectal neurons, which happens at developmental stage 39/40, only 4–5 days postfertilization (dpf; Holt and Harris, 1983). Below, we review several consequences of visually driven activity on the development and function of the immature retinotectal circuit.

Development and Refinement of The Topographic Retinotectal Map

One consequence of visually-driven activity is the development and refinement of a retinotopic projection in the optic tectal neuropil. Studies by Holt and Harris (1983) indicate that the first visual responses that can be recorded in the optic tectum already have a crude spatial organization, suggesting that retinal axons may form a rough topographic map as soon as retinal afferents innervate tectal neurons. Experiments, largely in chicks and amniotes, provided evidence that gradients of cell surface ligands and receptors located on RGCs and central retinal targets guide retinal axons to topographically matched target locations (reviewed in McLaughlin et al., 2003; Feldheim and O'Leary, 2010), and recent work showed that similar gradients are present in frog and tadpole optic tecta (Higenell et al., 2012). Reh and Constantine-Paton (1984) published two landmark articles: one demonstrated that individual RGC axon arbors shift their positions within the optic tectum as the retina and optic tectum enlarge during development to maintain a refined retinotopic projection. The second showed that blocking action potential activity traveling from the retina to the tectum disorganized the retinotectal projection (Reh and Constantine-Paton, 1985). Even before the discovery of spontaneous retinal waves (Meister et al., 1991), studies showing that activity in neighboring RGCs was highly correlated (Mastronarde, 1989) together with the studies from Reh and Constantine-Paton showing that blocking action potential activity from the retina to targets, provided critical support for the idea that patterned retinal input instructed the development of topographic visual projections by regulating the termination site of axons in the target. Subsequent work indicated that tectal N-Methyl-D-aspartate (NMDA) receptor activity is required for the development and maintenance of organized retinotectal projections (Cline et al., 1987; Cline and Constantine-Paton, 1989; Ruthazer et al., 2003). Synthesizing this body of work with work from other systems led to the idea that retinal input, be it from natural visual input in anamniotes or from spontaneous retinal waves in amniotes, instructs the development of organized visual projections (Udin and Fawcett, 1988; Constantine-Paton et al., 1990).

A core element of this conceptual framework is that correlated activity in neighboring afferents is detected by postsynaptic NMDA receptors based on principles of Hebbian plasticity models and spike timing dependent plasticity (STDP), suggesting that STDP-based mechanisms might refine the topographic map. Cellular mechanisms underlying topographic map refinement can be evaluated by examining dynamic rearrangements of retinotectal axon arbors *in vivo* (Ruthazer et al., 2003; Munz et al., 2014). These types of studies have been instrumental in identifying rules by which correlated activity governs axon remodeling underlying topographic map plasticity, as reviewed by Kutsarova et al. (2016). Topographic map refinement can also be read out as a refinement of the size of visual receptive fields in tectal neurons, and this refinement is thought to occur by engaging long-term potentiation and depression synaptic plasticity mechanisms (Ruthazer and Aizenman, 2010). Several experiments lay the groundwork for this important cross-cutting concept. In the first *in vivo* demonstration of STDP, Zhang et al. (1998) used a stimulation electrode to activate RGCs and postsynaptic recordings in tectal neurons, to show that activation of presynaptic RGC inputs could induce either LTP or LTD, depending on the timing of the incoming RGC action potential relative to the depolarization of the postsynaptic tectal neuron. Furthermore, a repetitive dimming light stimulus to the eye also induced LTP of retinotectal synapses in the contralateral tectum (Zhang et al., 2000).

Between stages 44 and 49, experience-dependent refinement of the retinotectal projection decreases receptive field size (Tao and Poo, 2005; Dong et al., 2009). This may occur by STDP-based mechanisms (Tao et al., 2001), although STDP of retinotectal synapses cannot be induced throughout this developmental period (Tsui et al., 2010), suggesting that other mechanisms contribute to receptive field and topographic map refinement (Ruthazer and Aizenman, 2010). Indeed, brief training with visual experience induces transcriptional and translational changes that affect visual responses and visually evoked behaviors (Dong et al., 2009; Schwartz et al., 2009, 2011; Shen et al., 2014), suggesting that further exploration will reveal additional cellular and molecular mechanisms regulating topographic map refinement and the development of neuronal response properties. As discussed in more detail below, refinement of visual receptive fields and the topographic map is necessary for tadpoles' visually guided avoidance responses.

The receptive fields of tadpole tectal neurons display robust forms of activity-dependent plasticity. At developmental stage 45, tectal neurons are not direction selective, meaning that they respond equally to all moving stimuli, regardless of the direction of movement of the visual stimulus. However, training tectal neurons by projecting unidirectional moving bars of light onto the retina induces direction selectivity in tectal neurons for the trained direction (Engert et al., 2002). In other words, after unidirectional training, neurons respond best to the trained direction. It is interesting to note that this training-induced directional selectivity involved an asymmetric shift in the neuron's receptive field, with

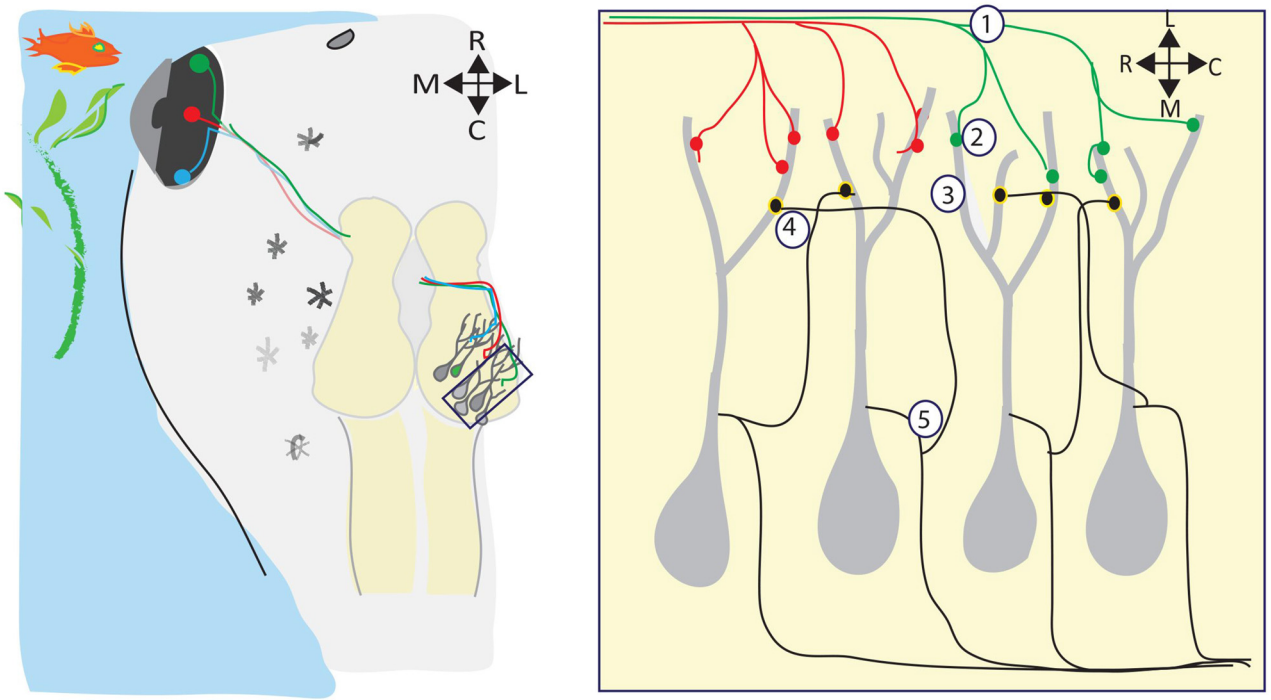

FIGURE 2 | Visual experience regulates many aspects of retinotectal circuit development. (Left) The retinotectal circuit is comprised of the RGCs in the eye which project their axons to the optic tectum, the primary visual processing area in tadpoles and frogs. Retinotectal axons extend in the optic tract across the ventral midline of the brain, and then extend dorsally and caudally to their final destination, the contralateral optic tectum. Here, RGC axons branch out and form synapses with postsynaptic tectal neurons, forming a topographic map of visual space within the tectal neuropil such that near-neighbor RGCs synapse with near-neighbor tectal neurons. Visual stimuli in the tadpole's environment activate the RGCs. During retinotectal circuit development, this visually-driven activity refines the retinotopic map (1), drives the development and maturation of the complex postsynaptic tectal neuron dendritic arbors (2), regulates the maturation and stabilization of retinotectal synapses (3), refines the microcircuitry within the tectum (4), and sets the level of intrinsic excitability expressed by tectal neurons (5).

new responsiveness to earlier-activated bar locations (Engert et al., 2002) suggestive of a STDP-type of plasticity in action.

Several studies indicate that STDP mechanisms distort visual receptive field properties and topographic projections of sensory input (Engert et al., 2002; Fu et al., 2002; Sundberg et al., 2006; Lim et al., 2010). The fact that receptive field properties and topographic maps are relatively stable suggests that mechanisms other than STDP might function to limit receptive field and map distortion. Indeed, a recent study demonstrates that the natural visual experience in response to optic flow from the constant forward swimming motion of tadpoles instructs the refinement of the retinotectal topographic map (Hiramoto and Cline, 2014). We noticed, as you could too, that tadpoles always swim forward, producing a constant source of anterior to posterior visual stimulation in the retina. This would produce a constant sequence of RGC activity from temporal to nasal retina. Rearing animals for 4 days under conditions in which the only visual experience they received was anterior to posterior moving bar stimulus resulted in the development of a refined retinotopic projection, whereas rearing animals with posterior to anterior moving bar stimulus prevented the refinement of the retinotectal projection. Temporal RGCs terminate in rostral tectum and RGCs in incrementally more nasal positions along the temporal-nasal axis terminate in correspondingly more caudal positions in the optic tectum (**Figure 3**). This suggested that the sequence of activity in temporal to nasal RGCs in response to the anterior to posterior moving stimuli might organize the RGC axons along the rostrocaudal axis of the tectum. Further analysis indicated that the axons of RGCs that were active earlier than converging RGC axons would shift their positions to more rostral tectal locations and that RGC axons that were active later than converging inputs would shift to more caudal tectal locations. The *in vivo* imaging protocols used in this study even provided the spatial and temporal resolution to show that the positions of individual axons could be arbitrarily shifted along the rostrocaudal tectal axis by changing the relative sequence of activity in the RGCs. Overall, this study supports a model in which the spatial location of objects in the visual field is encoded in the temporal sequence of RGC activity as the objects move in an anterior to posterior direction across the retina, and that this temporal sequence of RGC activity is then transformed into the spatial arrangement of RGC axon arbors within the target optic tectum. The spatial to temporal to spatial (STS) transformation of information operates throughout the temporal to nasal axis of the retina

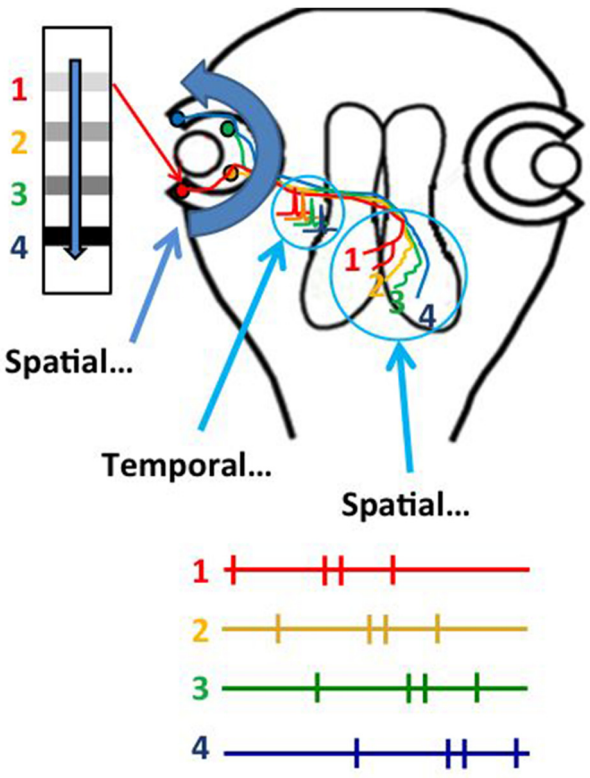

FIGURE 3 | Experience-dependent refinement of the retinotectal map occurs by a spatial to temporal to spatial (STS) transformation of visual information. Natural anterior to posterior motion of objects in the visual scene, or optic flow, activates RGCs from a temporal to nasal sequence, transforming spatial information in the visual scene into temporal information in the sequence of action potential activity in RGCs. The temporal information in firing sequence is transformed into an ordered spatial distribution of RGC axons in the tectal neuropil, based on a rule in which axons that fire first occupy more rostral positions within the tectal target. The STS mechanism ensures that retinal inputs map topographically across the entire target area.

and the rostral to caudal axis of the tectum, suggesting that one critical function of the STS mechanism may be to calibrate sensory information from the periphery to the target area devoted to that sensory projection. Importantly, this mechanism would this explain how sensory maps are customized to each individual, and accommodate individual differences in physical dimensions or positions of the sensory periphery. In addition, this mechanism may also underlie plasticity of topographic projections in response to changes in the sensory periphery or central targets (Garraghty and Kaas, 1992), as shown for instance with retinal scotomas (Gilbert, 1992; Gilbert and Wiesel, 1992), loss of digits or limbs, or stroke (Nudo and Friel, 1999), as well as classic studies on retinotectal map plasticity in which removal of half a retina or half the tectum results in expansion and compression, respectively, of the retinotopic projections (Udin and Fawcett, 1988). STDP and STS likely operate in concert, with STDP-based mechanisms allowing critical rapid modifications in neuronal response properties and STS maintaining a scaled topographic projection across the available target space.

The STS mechanism likely operates in amniotes as well as anamniotes, but in amniotes it is the temporal to nasal direction of spontaneous waves (Stafford et al., 2009; Ackman et al., 2012), rather than anterior to posterior motion of natural optic flow, that organizes the rostrocaudal mapping of retinal afferents in the SC, as well as the topographic projections in higher order visual centers that are likely organized by propagating spontaneous waves originating in the retina (Stafford et al., 2009; Ackman et al., 2012; Ackman and Crair, 2014; Burbridge et al., 2014).

Structural and Functional Development of Tectal Neurons

A second consequence of sensory experience is the effect on the structural and functional development of tectal neurons and their connectivity in nascent circuits. Although the majority of experimental work on this topic in *Xenopus* has been done by manipulating visual inputs to the tectum, mechanosensory experience, which enters the tectum from the hindbrain (Deeg et al., 2009; Hiramoto and Cline, 2009), is also likely to play a significant role in governing the development of tectal cell structure, function and connectivity. Because of their transparency at early developmental stages, their external development, and the ease with which *in vivo* time-lapse imaging, electrophysiology and gene manipulation can be accomplished, *Xenopus* tadpoles have been a particularly valuable experimental system in which to investigate neuronal development in intact developing animals.

Single cell labeling of optic tectal cells followed by *in vivo* time-lapse imaging showed that tectal neuron dendrites go through a rapid phase of growth, lasting several days, followed by a plateau in growth rate (Wu et al., 1999; Cline, 2001). Although one could imagine that dendritic arbor growth occurs by lengthening pre-existing branches and adding new branches, collecting *in vivo* time-lapse images at relatively short intervals, such as every 10–30 min over several hours, indicated that dendritic arbor growth occurs by dynamic addition and retraction of branches. Furthermore, net growth or net retraction of the entire arbor structure occurs as a result of relatively more branch additions and extensions than branch retractions, or conversely more retractions than additions, respectively (Dailey and Smith, 1996; Rajan and Cline, 1998; Haas et al., 2006; Cline and Haas, 2008; Ewald et al., 2008). It is interesting to note that the branch dynamics underlying arbor growth persist in mature neurons when the arbor structure is stable, albeit at a slower rate (Wong and Ghosh, 2002; Lee et al., 2006, 2008; He et al., 2016), suggesting that mechanisms that regulate developmental dendritic dynamics also regulate dendritic structural plasticity in mature neurons.

During the initial period of dendrite elaboration, analysis of individual neurons showed considerable spatial and temporal heterogeneity in dendritic arbor growth patterns. The developing dendritic arbors in some neurons would show a rapid spurt of growth and then remain stable for a period before they resumed

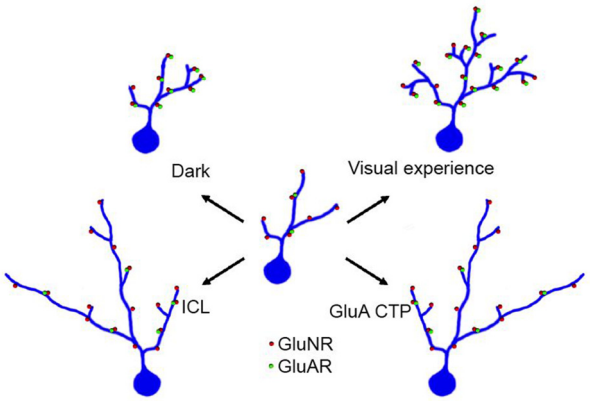

FIGURE 4 | Visual experience enhances dendritic arbor development through effects on excitatory and inhibitory synaptic transmission. An immature optic tectal neuron is schematized in the center of the figure and structural changes in dendritic arbor in response to different conditions are shown radiating from the center. Under conditions in which tadpoles receive visual stimulation, optic tectal neurons elaboration complex dendritic arbors and their excitatory synapses mature and increase in strength by increasing the ratio of AMPA/NMDA type glutamate receptors. Raising tadpoles in the dark decreases elaboration of the dendritic arbor. Expressing GluA C terminal peptides (GluA CTP) impairs AMPA receptor trafficking, decreases excitatory synapses and decreases complexity of dendritic arbors. Expressing intracellular loop (ICL), which impairs $GABA_AR$ residence at synapses and decreases inhibitory synaptic inputs onto tectal neurons, also decreases complexity of dendritic arbors.

growth. Others elaborated one region of their dendritic arbor at the same time that other regions remained stable or were retracted (Rajan and Cline, 1998; Wu and Cline, 1998, 2003; Rajan et al., 1999; Wu et al., 1999). Optic tectal neurons receive glutamatergic and GABAergic synaptic input even as they elaborate their dendritic arbors (Wu et al., 1996; Akerman and Cline, 2006). We suspected that the spatial and temporal growth heterogeneity might be readouts of activity-dependent signaling that affected branch dynamics. Indeed this idea was supported by experiments showing that blocking NMDA receptors decreased dendritic arbor growth by altering branch dynamics in newly differentiating tectal neurons (Rajan and Cline, 1998; Rajan et al., 1999). By contrast, blocking α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors or action potential activity in these relatively immature neurons had no significant effect (Rajan and Cline, 1998). These observations suggested that the glutamatergic retinal inputs might be regulating dendritic arbor growth of postsynaptic tectal neurons by regulating branch dynamics via signaling through synapses. Electrophysiological recordings from tectal neurons demonstrated that relatively immature tectal neurons respond to retinal axon stimulation and that transmission at their glutamatergic synapses was predominantly mediated by NMDA receptor conductances and that AMPA receptors were trafficked into synapses by a Calcium/calmodulin-dependent protein kinase II (CaMKII)-dependent mechanism (Wu et al., 1996; Wu and Cline, 1998). As neurons mature, their dendritic arbors become more complex and transmission at their glutamatergic synapses becomes stronger through the addition of AMPA receptors. Consistent with this synaptic maturation profile, pharmacologically blocking AMPA receptors selectively interferes with dendritic arbor elaboration in more mature neurons (Rajan and Cline, 1998). Furthermore, interfering with AMPA receptor trafficking, by manipulating CaMKII function (Wu et al., 1996; Wu and Cline, 1998), or by expressing a peptide corresponding to the C-terminal of GluA subunits, called CTP, drastically altered dendritic arbor growth (Haas et al., 2006).

Experiments in which single tectal neurons were imaged in intact animals before and after brief 4 h periods during which animals were either kept in the dark or exposed to a motion stimulus, provided direct demonstration of the role of visual experience on dendritic arbor growth (Sin et al., 2002). Dendritic arbor growth rates were relatively low over the 4 h period in the dark and increased significantly over the 4 h period with visual stimulation. This imaging protocol allows comparison of growth rates over time in individual neurons with and without visual stimulation and therefore provides greater power to detect experience-dependent changes in structural plasticity and to identify cellular and molecular mechanisms regulating experience-dependent dendritic arbor development.

A core element of mechanisms underlying experience-dependent dendritic arbor development is the regulation of glutamate receptor trafficking. Visual experience increased the strength of retinotectal glutamatergic synapses by increasing the contribution of AMPA receptors, or the AMPA/NMDA ratio, at synapses (Engert et al., 2002; Haas et al., 2006; Shen et al., 2011). Blocking AMPA receptor trafficking by expression of CTP blocks visual experience-dependent dendritic arbor growth (**Figure 4**; Haas et al., 2006). This in turn predicts that manipulating synaptic proteins that affect AMPA receptor trafficking will also affect dendritic arbor elaboration and specifically experience-dependent dendritic arbor elaboration. This prediction has been borne out in recent studies of the transmembrane AMPA receptor regulatory proteins (TARPS), a family of proteins, which regulate AMPA receptor trafficking and modulate their function (Chen et al., 2000). A subset of type I TARPs enhance AMPA receptor trafficking to postsynaptic sites and also regulate activity-dependent dendritic arbor development in cortical pyramidal neurons (Hamad et al., 2014). Conversely, CPG15, aka neuritin, an activity-induced growth factor (Nedivi et al., 1996; Fujino et al., 2003; Harwell et al., 2005; Javaherian and Cline, 2005) increases AMPA receptor trafficking into retinotectal synapses and dramatically increases dendritic arbor elaboration and retrogradely increases elaboration of presynaptic retinal axon arbors (Nedivi et al., 1998; Cantallops et al., 2000).

Other postsynaptic density proteins, including ion channels, cell adhesion molecules, cytosolic signaling proteins, cytoskeletal proteins and scaffolding proteins (Kim and Sheng, 2004; Sheng and Hoogenraad, 2007) may affect NMDA or AMPA receptor mediated synaptic transmission and thereby affect experience-dependent dendritic arbor growth. This generalization is important because it suggests that mechanistic understanding

of the assembly, function and stability of synapses will in turn identify mechanisms that affect dendritic arbor development and circuit connectivity in the developing brain. In particular, a significant number of postsynaptic density proteins are candidate disease genes for neurodevelopmental disorders such as autism and schizophrenia (Ebrahimi-Fakhari and Sahin, 2015), supporting the idea of neurodevelopment origins of complex neurological diseases that manifest at later life stages.

It is interesting that activity-dependent mechanisms can also restrict dendritic arbor growth. For instance, electrophysiological experiments of synaptic maturation in *Xenopus* tectal optic neurons indicate that increased α-CaMKII activity both increases glutamatergic synaptic strength (Wu et al., 1996) and stabilized dendritic arbor structure by reducing rates of branch additions and retractions (Wu and Cline, 1998). α-CaMKII, a multifunctional calcium and calmodulin-dependent kinase, acts downstream of synaptic activity-dependent increases in calcium to regulate synaptic strength (Lisman et al., 2002) and cytoskeletal dynamics (McVicker et al., 2015), for instance via GTPases (Sin et al., 2002; Ghiretti et al., 2014). Mechanisms limiting neuronal arbor size are less well studied than those that enhance arbor growth, but are under active investigation as reviewed in Koleske (2013).

Although studies of sensory experience-dependent development have focused research on excitatory synaptic input mediated effects on dendrite development, inhibitory synaptic activity driven by sensory input also regulates dendritic arbor development. The roles of inhibitory GABAergic or glycinergic synaptic transmission in regulating dendritic arbor development depends on the expression of chloride transporters and therefore whether the transmitter depolarizes or hyperpolarizes the postsynaptic neuron. Activation of ionotropic type A GABA receptors ($GABA_AR$) in young neurons increases process outgrowth and synaptogenesis, possibly mediated by GABA—induced excitation (Barbin et al., 1993; Ben-Ari, 2002; Cancedda et al., 2007). Blocking inhibitory GABAergic transmission in preparations containing mature neurons increases process outgrowth (Wayman et al., 2006) by increasing activity indirectly. Similarly, glycinergic transmission affects dendritic arbor development, both at early stages of development, when it is depolarizing (Maric et al., 2001; Tapia et al., 2001), and later, when glycinergic transmission is inhibitory (Sanes and Chokshi, 1992; Sanes et al., 1992; Sanes and Hafidi, 1996). Blocking glycinergic input with strychnine increased dendritic arbor size, suggesting that the normal function of inhibitory input is to restrain dendrite growth. Although these experiments indicate that inhibitory transmission affects dendritic arbor development, the experiments produce circuit-wide effects on activity levels that confound the interpretation of changes in neuronal structure (Ben-Ari et al., 1989; Chen et al., 1996; Tapia et al., 2001).

One useful strategy to study the effects of inhibitory synaptic input on neuronal development and function is to express a peptide corresponding to the intracellular loop of γ2 subunit of $GABA_AR$, called intracellular loop (ICL), which prevents γ2 subunit—containing $GABA_AR$ from anchoring at synapses (Alldred et al., 2005; Christie et al., 2006) and allows cell autonomous manipulations of inhibitory input. Electrophysiological experiments show that ICL decreased inhibitory synaptic inputs in neurons that expressed ICL but not in untransfected neurons or those expressing a mutant ICL, called mICl, and furthermore that ICL increased the ratio of excitatory to inhibitory synaptic activity in ICL-expressing neurons. Time-lapse 2 photon images of optic tectal neurons *in vivo* collected at daily intervals showed that ICL-expressing neurons have less elaborate dendritic arbors that span a larger area of the tectal neuropil compared to controls. Images collected at shorter intervals indicated that the decrease in arbor branches arose from a decrease in the numbers of new branch additions to the arbors (Shen et al., 2009), rather than in increase in branch retractions as seen when AMPA receptor trafficking into synapses was disrupted (Haas et al., 2006). Decreasing inhibitory input, which likely increased the balance of excitation to inhibition, blocked the visual-experience dependent increase in dendritic arbor complexity. These results suggest that a change in the balance of excitatory to inhibitory inputs disrupts dendritic arbor development. Given the current evidence that the balance of excitation to inhibition is critical for normal brain function, and that neurodevelopmental disorders, such as autism spectrum disorders disrupt the balance of excitation to inhibition (Gatto and Broadie, 2010; Paluszkiewicz et al., 2011; Calfa et al., 2015), it will be of great interest to determine how changes in the relative balance of excitatory to inhibitory synaptic inputs affect signaling pathways and cellular machinery that regulate dendritic arbor development.

Development and Maturation of Local Tectal Circuitry

A third effect of visual experience on the development of the visual system in tadpoles is the maturation of local tectal microcircuitry. In addition to direct activation of tectal neurons, visually driven RGC input also activates local recurrent microcircuitry within the tectum (Pratt et al., 2008; Xu et al., 2011). Relatively long lasting, and capable of eliciting the firing of multiple action potentials in a given tectal neuron, this recurrent activity adds a temporal dimension to the visual response. Although the exact function of the polysynaptic recurrent activity is not completely understood, it likely codes for different aspects of the visual stimuli and/or response, similar to the recurrent activity in the SC (Sparks, 1986; Moschovakis et al., 2001). Another possibility is that recurrent activity maintains neurons at relatively depolarized potentials and thereby boosts their ability to respond to incoming input (Haider et al., 2007). Like the monosynaptic response, the local polysynaptic activity undergoes activity-dependent refinement between stages 44 and 49. Refinement of the local microcircuitry is characterized by visually-evoked responses becoming more compressed and occurring closer in time to the preceding monosynaptic response (Pratt et al., 2008). Dampening RGC input by blocking both NMDA and calcium-permeable AMPA

receptors during this time resulted in responses that were similar to those seen in stage 44 circuits, suggesting that retinal input contributes to the maturation of microcircuitry (Pratt et al., 2008). It is interesting to note that visual experience-dependent maturation of temporal response properties in local tectal circuitry occurs by STDP rules. This was shown in an isolated brain preparation and in intact tadpoles. In the isolated brain preparation, pairs of stimuli were delivered to the retinal inputs, so that the second stimulus was timed to occur in the midst of the recurrent portion of the response activated by the first stimulus. This stimulus condition shifted the temporal properties of the recurrent activity in accordance with STDP rules. When tadpoles were exposed to pairs of visual stimuli with different interstimulus intervals for 4 h, the temporal properties of recurrent tectal activity were also shifted, as seen in the *ex vivo* brain preparation. This important observation indicated that the visual system connections and therefore visual system responses in intact animals are "trained" to respond optimally to the temporal properties of predominant stimuli.

The spatial pattern of connectivity of optic tectum microcircuits is also affected by visual experience. This was demonstrated by bulk-loading tectal neurons with calcium indicators so that calcium transients could be imaged simultaneously in a large population of neurons. Activating RGC inputs with a whole field light on stimulus to the retina demonstrated that the degree of correlated activity across the tectum significantly increased between developmental stages 44 and 49. Furthermore, this increased spatial correlation depends on visual experience, as it is almost completely eliminated by dark rearing (Xu et al., 2011). These experiments indicate that the development of both the spatial and temporal features of tectal circuit responses are experience-dependent.

Synaptic input from the retina can also regulate a tectal neuron's intrinsic excitability—the ease in which a neuron fires action potentials. Because recurrent activity is generated by local tectal-tectal connections, the intrinsic excitability of the individual tectal neurons greatly impacts the strength and pattern of this local activity (Dong and Aizenman, 2012). Furthermore, the long range projections of tectal neurons provides afferent input to the brainstem, which is then relayed ultimately to spinal cord circuits to elicit a swimming response (Khakhalin et al., 2014). Therefore, changes in intrinsic excitability would be expected to impact both the local tectal microcircuitry, as well as the downstream target circuits. Between developmental stages 45 and 49, the number of synapses, and so the overall amount of synaptic drive received by tectal neurons, increases dramatically (Pratt and Aizenman, 2007). The developmental increase in synaptic drive received by tectal neurons triggers a compensatory response in their intrinsic excitability. In other words, as synaptic drive increases, intrinsic excitability decreases. Importantly, dampening the increase in synaptic drive by expressing a truncated AMPA receptor subunit prevented the decrease in intrinsic excitability. In fact decreasing synaptic input causes a significant increase in intrinsic excitability, illustrating that intrinsic excitability adjusts bidirectionally in response to changes in synaptic drive, and not the other way around (Pratt and Aizenman, 2007). Similarly, 4 h of enhanced visual experience induces a decrease in synaptic drive by activating polyamine blockade of current through AMPA ion channels—a protective mechanism in times of synaptic over-activation (Bell et al., 2011). This downregulation of synaptic drive increases intrinsic excitability, which overall, is thought to increase the signal to noise ratio (Aizenman et al., 2003).

Perhaps the most enchanting demonstration of the effect of visual experience on tectal neuron action potential firing is a study by van Rheede et al. (2015). First, the authors establish that at early larval stages 42–44, the time in development when RGC input has just started to form nascent synapses onto tectal neurons, a large fraction of tectal neurons do not fire action potentials in response to a "light-off" stimulus projected onto the retina. Interestingly, these neurons can fire action potentials in response to current injection, but they don't fire in response to visually-driven input. The non-spiking neurons can be converted to spiking neurons with 15 min of visual conditioning, consisting of a drifting bar of light (van Rheede et al., 2015). Better yet, non-spiking neurons can be converted to spiking neurons by showing the tadpole underwater scenes from the documentary "*Planet Earth*" (BBC). When tadpoles are shown a black scene, non-spiking neurons are not converted to spiking ones. The mechanism underlying the conversion to spiking involves changes in synaptic strength, while no changes in intrinsic excitability were detected. Overall, during development of the retinotectal circuit, the input provided by RGC activation shapes the functional development of tectal microcircuitry, making it more consistent and faster.

CONCLUSION

Visual experience plays a critical function in the development and maturation of the visual circuitry in anamniotes, including the development of the topographic retinotectal projection, retinotectal synaptic properties, tectal neuronal morphological development, as well as broader properties of tectal circuitry including connectivity underlying recurrent activity. Together, these studies provide strong evidence that sensory input drives the development and maturation of diverse synaptic, neuronal and circuit properties. These events likely require changes in gene expression and translation. Although, we did not review studies on activity-induced gene transcription or translation, hundreds of transcripts are known to be regulated by activity (Nedivi et al., 1993; Loebrich and Nedivi, 2009), and analysis of many activity-regulated transcripts has shown they affect nervous system development (Loebrich and Nedivi, 2009). Similarly, protein translation can also be regulated by activity, with an effect on visual system plasticity (Shen et al., 2014). Given the functional parallel between spontaneous waves of activity in amniotes and the role of visual experience in visual system development in non-amniotes, it seems likely that spontaneous waves of activity propagated from the retina throughout the visual system (Ackman and Crair, 2014) will have widespread

repercussions for visual system development in these systems. Finally, recent studies have demonstrated striking parallels in activity-dependent cellular and molecular mechanisms governing synaptic and circuit maturation in non-sensory brain circuits (Kozorovitskiy et al., 2012) as we have reviewed above, suggesting that the fundamental mechanistic principles of brain circuit development identified in the developing *Xenopus* visual system are evolutionarily conserved and apply broadly to brain circuit development across phyla and brain regions.

AUTHOR CONTRIBUTIONS

KGP, MH and HTC discussed the concept. KGP and HTC co-wrote the article.

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Emergence of Selectivity to Looming Stimuli in a Spiking Network Model of the Optic Tectum

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The neural circuits in the optic tectum of Xenopus tadpoles are selectively responsive to looming visual stimuli that resemble objects approaching the animal at a collision trajectory. This selectivity is required for adaptive collision avoidance behavior in this species, but its underlying mechanisms are not known. In particular, it is still unclear how the balance between the recurrent spontaneous network activity and the newly arriving sensory flow is set in this structure, and to what degree this balance is important for collision detection. Also, despite the clear indication for the presence of strong recurrent excitation and spontaneous activity, the exact topology of recurrent feedback circuits in the tectum remains elusive. In this study we take advantage of recently published detailed cell-level data from tadpole tectum to build an informed computational model of it, and investigate whether dynamic activation in excitatory recurrent retinotopic networks may on its own underlie collision detection. We consider several possible recurrent connectivity configurations and compare their performance for collision detection under different levels of spontaneous neural activity. We show that even in the absence of inhibition, a retinotopic network of quickly inactivating spiking neurons is naturally selective for looming stimuli, but this selectivity is not robust to neuronal noise, and is sensitive to the balance between direct and recurrent inputs. We also describe how homeostatic modulation of intrinsic properties of individual tectal cells can change selectivity thresholds in this network, and qualitatively verify our predictions in a behavioral experiment in freely swimming tadpoles.

Keywords: looming detection, optic tectum, collision avoidance, recurrent networks, sensorimotor transformation, intrinsic excitability, homeostatic plasticity, visual development

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INTRODUCTION

Spontaneous neural activity plays a key role in the developing nervous system. In the visual system of vertebrates, spontaneous activity generated both in the retina and in retinorecipient structures is critical for organizing early experience and facilitating the developmental refinement of neural circuitry (Pratt et al., 2016). However, spontaneous activity also places serious constraints on the normal processing of sensory stimuli by adding varying amounts of neural noise, and can therefore affect how the organism interacts with its environment. How then does a developing nervous system balance the need to sustain spontaneous activity with the ability to effectively process and

react to external sensory stimuli? Here we examine the process of collision detection in Xenopus laevis tadpole tectum, in which activity generated through recurrent connectivity (Pratt et al., 2008; Liu et al., 2016) can interact with waves of evoked visual responses, potentially leading to differential reactions to different visual stimuli (Khakhalin et al., 2014).

Detection of visually expanding, or looming stimuli is ubiquitous across the animal world, and is critical for both navigation and predator avoidance (Sun and Frost, 1998; Preuss et al., 2006; Liu et al., 2011; Herberholz and Marquart, 2012; Vagnoni et al., 2012). Research suggests that different animals may rely on different types of computations to detect looming stimuli: it was proposed that in birds collision detection may be achieved through spatial integration of appropriately directed edge movements in different parts of the visual field (Frost and Sun, 2004). In other animals, such as adult Ranid frogs (Ishikane et al., 2005; Kuras et al., 2006; Kang and Li, 2010; Baranauskas et al., 2012), collision detection seems to rely on competitive temporal inactivation of inputs from OFF-detectors in a retinotopic system. Yet other species either combine spatial motion integration and competitive inactivation, as in the case of locusts (Gabbiani et al., 2002; Peron and Gabbiani, 2009; Fotowat et al., 2011), or have several distinct startle systems, some relying on motion processing, and some on visual OFF detectors, as it was described in fruit-flies (Card and Dickinson, 2008; Fotowat et al., 2009; de Vries and Clandinin, 2012; Schilling and Borst, 2015). For many popular experimental species however, including Zebrafish larvae and *Xenopus* tadpoles, the exact mechanisms of collision detection are not yet clear (Khakhalin et al., 2014; Temizer et al., 2015; Dunn et al., 2016). Similar to larval Zebrafish that perform three types of evasive maneuvers in response to different visual stimuli (Burgess and Granato, 2007; Bianco et al., 2011; Dunn et al., 2016), *Xenopus* tadpoles can execute either fast randomized escapes or slow course corrections (Khakhalin et al., 2014), suggesting that different competitive collision detection mechanisms may be at play.

As in Zebrafish (Dunn et al., 2016), detection of looming stimuli in tadpoles relies on the circuitry in the optic tectum (OT) (Dong et al., 2009; Khakhalin et al., 2014). It is known that principal neurons in the tectum receive strong recurrent excitation (Pratt et al., 2008; Liu et al., 2016) that supports spontaneous neuronal activity during development (Pratt and Aizenman, 2007; Imaizumi et al., 2013; James et al., 2015). Principal tectal neurons also demonstrate prominent and rapid inactivation of spiking (Aizenman et al., 2003; Ciarleglio et al., 2015), which allowed us to suggest that together these two phenomena may underlie, or at least contribute to collision detection (Khakhalin et al., 2014). We hypothesized that in the presence of strong recurrent connections, rapidly inactivating networks would naturally discriminate in favor of expanding stimuli, reminiscent of dendritic competition, and spike-frequency accommodation in looming-selective neurons in insects (Peron and Gabbiani, 2009). At the same time, strong recurrent connections may present a challenge for a behaving animal, as spontaneous recurrent activity can overpower both sensory inputs and computed pre-motor outputs. The developing brain is thus faced with the problem of finding a proper balance between recurrent and sensory inputs to each neuron, keeping spontaneous activity at the levels appropriate for circuitry development, and stimulus detection.

In this study we describe an informed spiking model of the *Xenopus* tectum based on the recent detailed cell-level description of this region (Ciarleglio et al., 2015), and test whether the activation of this network may support collision detection. As the exact topology of recurrent connections in the tectum is not known, we compare several hypothetical internal connectivity profiles, and make tentative predictions about which of these profiles are more likely to be utilized by real tadpoles. We also study how the relative strength of recurrent and sensory inputs affect generation of spontaneous activity and stimulus selectivity, and investigate the robustness of stimulus selectivity in recurrent networks to different levels of spontaneous neural noise.

RESULTS

The Computational Model

To keep the model computationally efficient, we represented each tectal neuron as a one-compartmental cell with spiking governed by a system of two ordinary differential equations: a quadratic differential equation with hard reset for voltage, and a linear differential equation for slow outward currents, similar to classic hybrid models with reset (Izhikevich, 2003, 2010). Compared to many other neural cells types however, principal neurons in the tadpole tectum typically produce very few spikes in response to both *in vitro* current injections (Ciarleglio et al., 2015) and *in vivo* visual stimulation (Khakhalin et al., 2014), yet show little frequency accommodation, presumably due to strong inactivation of Na^+ voltage-gated channels. To approximate this spiking behavior, we adjusted the model by introducing several tuning parameters and a non-linear dependency between the input current in the cell and the change in cell potential (see Methods). These adjustments ensured that model neurons ceased spiking even in response to strong current injections (**Figure 1A**), and showed little frequency adaptation (**Figure 1B**).

To populate the network with appropriate cell types, we reanalyzed the data from Ciarleglio et al. (2015), and classified 104 biological cells recorded in stage 48–49 naïve tadpoles according to the maximal number of spikes they produced in response to step current injections. To simplify model tuning, we did not attempt to replicate the full gradient of neuronal excitability profiles, but classified biological cells from Ciarleglio et al. (2015) into four representative spiking phenotypes (**Figure 1C**): low-spiking cells that produced at most one spike; 3-spike cells (that produced 2 or 3 spikes); 5-spike cells (from 4 to 7 spikes), and highly spiking cells (8–11 spikes; 10 on average). For each cell group, we then collected three types of statistics: the number of spikes generated in response to whole-cell step current injections of different amplitudes (20–120 pA); the first spike latency in response to a 100 pA step current injection, and the inter-spike interval for 100 pA current injection. We then manually tuned four model neurons (**Figure 1D**), ensuring that they reproduce spike counts (**Figure 1E**), latencies,

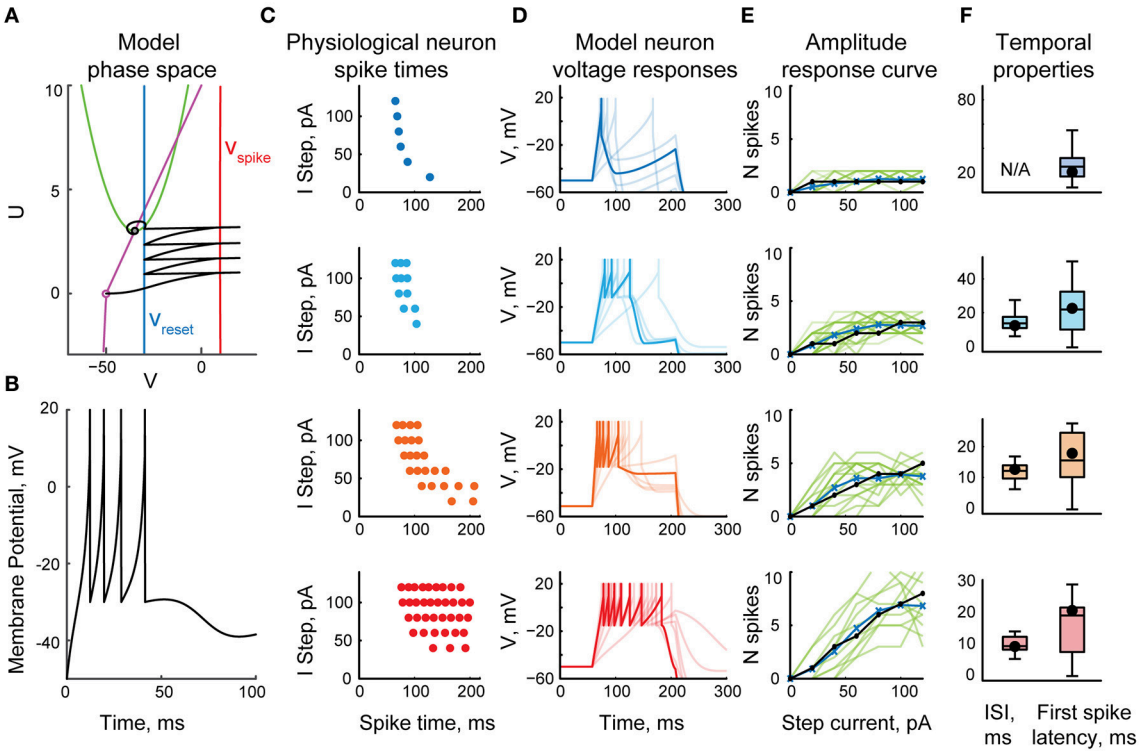

FIGURE 1 | The fast-inactivating spiking model of a tectal neuron. (A) The phase space of a system of two differential equations representing a spiking neuron, showing a sample trajectory in this space (black), two nullclines (purple and green), and the values involved in potential reset during spiking (V_{spike} in red, and V_{reset} in blue). **(B)** A typical response of a model neuron to a step current injection. **(C)** Spike-time rasters of four representative physiological neurons from Ciarleglio et al. (2015) as they spike in response to current clamp steps of amplitudes from 20 to 120 pA. **(D)** Voltage traces of four model neurons in response to current steps of amplitudes from 20 to 120 pA. Responses to 80 pA current step are highlighted. **(E)** Input-output curves, showing the number of spikes generated by neurons in response to current step injections of different amplitudes, for four representative spiking groups separately. Response curves of individual biological neurons from Ciarleglio et al. (2015) are shown in green, averages for biological neurons in blue, model neuron responses in black. **(F)** Distributions of first spike latencies (left) and first-to-second inter-spike intervals (right) during responses of biological neurons to step current injections of 100 pA, with similar values for model neurons superimposed on them (black dots).

and inter-spike intervals (**Figure 1F**) observed in physiological experiments.

The model tectal network consisted of 400 cells arranged in a 20 × 20 grid; the cells were randomly assigned one of four cell types (1-, 3-, 5-, or 10-spike-generating cells) in the proportions observed in stage 49 tadpoles (20, 25, 40, and 15% respectively; Ciarleglio et al., 2015). This network received excitatory inputs from 400 retinal ganglion cells (RGCs) arranged in a similar grid. All RGCs were assumed to be OFF cells, and once activated, each RGC produced a train of four spikes with random inter-spike intervals; the distribution of these inter-spike intervals was adjusted to approximate both experimentally observed spiking of individual RGCs (Demas et al., 2012; Miraucourt et al., 2016), and bulk summary responses in the optic nerve during *in vivo* visual stimulation (Khakhalin et al., 2014). Projections from the RGC layer formed a "blurred" retinotopic map in the OT layer (**Figure 2A**), with a stride of 5 grid cells, and connection strength decreasing with distance.

As the topology of recurrent connections in the tadpole tectum is not known (Pratt et al., 2008; Liu et al., 2016), we considered three possible configurations (**Figure 2B**): **uniform**, with random connections across the entire network and uniformly distributed connection weights; **local**, with connections spanning 5 nearby cells in each direction, and with average strength decreasing with distance; and **scale-free**: a small-world network with a few strongly connected hub cells linking the entire network in a set of connected clusters (Barabasi and Albert, 1999). All connectivity profiles were normalized by synaptic strength, so that the average sum of synaptic inputs received by tectal cells was same in each network type, regardless of its topology. Both feedforward RGC-OT and recurrent OT-OT connections were modeled as conductance-based excitatory synapses with exponential decay, and dynamics approximating physiological data from Xu et al. (2011), Khakhalin et al. (2014), Ciarleglio et al. (2015). See Methods for more details on the model construction and parameter validation.

Activation in Response to Visual Stimuli

In computational experiments, the RGC layer simulated responses to virtual "visual stimuli" that were modeled after behaviorally relevant stimuli from Khakhalin et al. (2014). These included a full-field dark "**flash**"; a linearly expanding

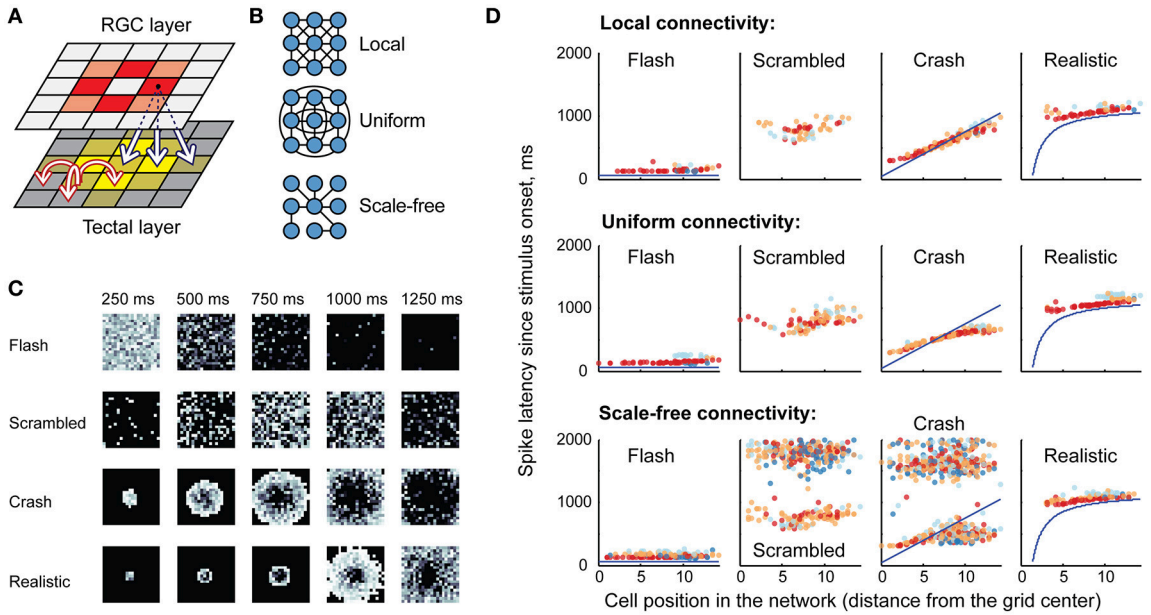

FIGURE 2 | Network model, retinal inputs, and network responses. (A) The basic topology of the model network: a grid of retinal ganglion cells (RGCs) made a "blurred" retinotopic projection to the tectal layer (black arrows), with recurrent connections within the tectal layer (red arrows). Different colors schematically show relative activation of RGCs and tectal cells during visual stimulus processing. **(B)** Three possible options for the recurrent intra-tectal connectivity: local (neurons are connected only to their neighbors); uniform (connections between any two neurons are equally probable); and scale-free (small-world network with highly connected hub cells). **(C)** Snapshots of RGC layer spiking, representing four visual stimuli: instantaneous full-field **flash**; randomly rearranged (**scrambled**) looming stimulus; linearly expanding looming stimulus (**crash**); and **realistic** non-linearly expanding looming stimulus. Each square shows a "still frame" from a dynamic response, taken in 250 ms increments after the stimulus onset (0 ms); with more recent spikes within each 250 ms window shown in lighter shades of gray. **(D)** Sample rasters of spiking responses in the tectum, generated for different recurrent connectivity profiles (rows), and different visual stimuli (columns). The horizontal axis presents model cell positions as distance from the 20 × 20 grid center; vertical axis shows spike latency; blue lines for crash and realistic stimuli show the theoretical time at which each tectal cell is directly engaged by the visual stimulus through the corresponding retinal cell. Cells of different spiking phenotypes are shown in different colors, from most spiky (red) to least spiky (dark blue).

looming "**crash**"; a looming stimulus with its pixels randomly spatially rearranged on the 20 × 20 grid ("**scrambled**"), and a geometrically realistic looming stimulus with faster, non-linear hyperbolic dynamics ("**realistic**"). The spiking of RGC layer neurons over time is shown in **Figure 2C**.

In the tectal layer (**Figure 2D**) "flashes" evoked rapid, short responses, mostly mediated by high-spiking cells (red). "Scrambled" stimuli produced delayed and more prolonged responses with higher involvement of medium-spiky cells (orange and blue), but also lacking spatial organization, while "crashes" and "realistic" collisions created spatially organized waves of excitation that propagated through the tectum, from its center to the periphery. We observed that scale-free connectivity (bottom row of **Figure 2D**) easily gave rise to spontaneous epileptiform activity (clouds of points on the top of each raster plot, corresponding to ongoing spontaneous spiking), while activity in local and uniformly-connected networks tended to "die out" even in the absence of inhibition.

We found that the relative number of spikes generated in response to each type of stimulus (**Figures 3A–C**), as well as median latencies of neuronal responses (**Figures 3A–C**), matched the results of biological experiments (**Figures 3A,C,D**; data from Khakhalin et al. (2014). Linear looming "crashes" produced the highest total network activation, followed by spatially disorganized stimuli ("scrambled" in this study; "ramp" and "grid" in Khakhalin et al., 2014), and finally followed by synchronous "flashes." The average number of spikes generated by neurons in the model was lower than that observed in physiological experiments (1.4 for "crash" in the model; 6.2 for "crash" in Khakhalin et al. (2014), for which we can offer several potential explanations (see Discussion).

The higher total spike-output in response to looming stimuli, compared to full field "flashes," was primarily due to the stronger contribution of medium-high spiking neurons (orange in **Figure 3B**) that were mostly silent after "flashes," but spiked in response to slower stimuli. In line with this observation, the relative preference for "crash" over "flash" in model data correlated with neuronal spikiness (**Figure 3D**). We verified this prediction by re-analyzing experimental data from Khakhalin et al. (2014), and found that in our *in vivo* experiments selectivity of individual neurons for "crash" over "flash" also correlated with their spikiness (**Figure 3E**, $r = 0.4$, $p = 1e{-}3$, $N = 55$; data from Khakhalin et al. (2014).

Parametric Analysis

We then investigated how the relative strength of recurrent connections and retinal inputs in the tectum affected network activation and selectivity for different visual stimuli. For each

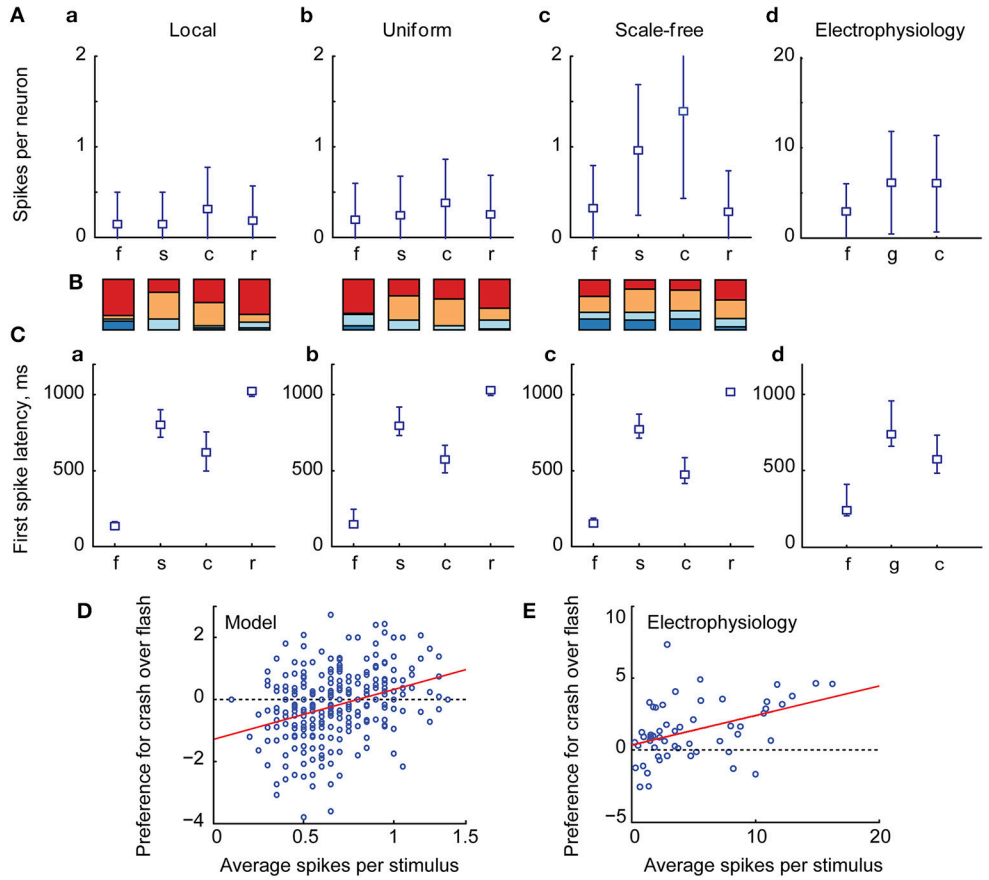

FIGURE 3 | The summary statistics of spiking output in the tectum. (A) Averages (markers) and standard deviations (error bars) of the number of spikes per neuron generated by the tectum during responses to different visual stimuli, in models with different recurrent connectivity profiles **(A–C)**, and in physiological data [**D**, data from Khakhalin et al. (2014) **Figure 4**]. Here "f" stands for "flash," "s" for "scrambled," "c" for "crash," "r" for realistic, and for physiological data "g" stands for "grid" (a stimulus that can be considered analogous to the "scrambled" stimulus from the model); responses were respectively modeled or recorded for 2 s after stimulus onset. Both in computational and biological experiments looming stimuli evoked stronger responses than an instantaneous flash (paired *t*-test $p = 5e{-}66$, $n = 400$ for the model, $p = 1e{-}5$, $n = 56$ in physiological experiments; significant after Bonferroni correction), while spatially disarranged stimuli evoked intermediate responses **(B)**. The relative contribution of different neuronal spiking phenotypes to model responses, measured as the total number of spikes generated by all 10-spike (red), 5-spike (orange), 3-spike (light blue), and 1-spike (dark blue) neurons. Medium-spiking neurons were more involved in responses to slow than to fast stimuli **(C)**. The median and inter-quartile ranges of first spike latencies during tectal responses to different stimuli, in model networks with different connectivity profiles **(A–C)**, and in biological experiments **(D)**, data from Khakhalin et al. (2014), not previously presented). The model successfully predicted typical latencies observed in physiological experiments (all pairwise comparisons between responses to different stimuli $p < 1e{-}6$, paired *t*-test, significant after Bonferroni correction). **(D)** The average number of spikes generated by model neurons across all four visual responses correlated ($r = 0.31$) with their preference (Cohen d effect size) for looming stimuli (crashes) over flashes; regression line shown in red **(E)**. A similar analysis for the physiological data from Khakhalin et al. (2014) verified this prediction, as spikier neurons preferred looming stimuli to flashes ($r = 0.42$).

connectivity configuration we considered a family of models, with differently scaled weights of direct and recurrent inputs. Each network model was defined by two scaling coefficients: SR for RGC inputs, and ST for recurrent intratectal connections. With SR = 1 and ST = 0 there were no functional recurrent currents, and all drive to tectal cells came from RGCs, reaching on average 180 pA at peak (a rather high number, compared to *in vivo* average peak value of 70 pA in Xu et al. (2011), and 30 pA in Khakhalin et al. (2014). Conversely, with SR = 0 there were no inputs from the retina, while with SR = ST the average strengths of direct and recurrent connections were equal.

We ran the model 25 times for each combination of SR and ST, and calculated total amount of spikes generated in response to each stimulus in each experiment. The average values of total spike output are shown at (**Figure 4A**), encoded by color, with lighter colors representing stronger spike outputs. We then used signed *F*-values to quantify statistical reliability of stimulus preference across multiple experimental runs, and compared responses to different visual stimuli for each point in the (SR, ST) parametric space (**Figure 4B**). This calculation divided the (SR, ST) space into regions statistically selective for "flashes" (shown in blue in **Figure 4B**); selective for looming stimuli (red), and non-selective regions (white). We found that

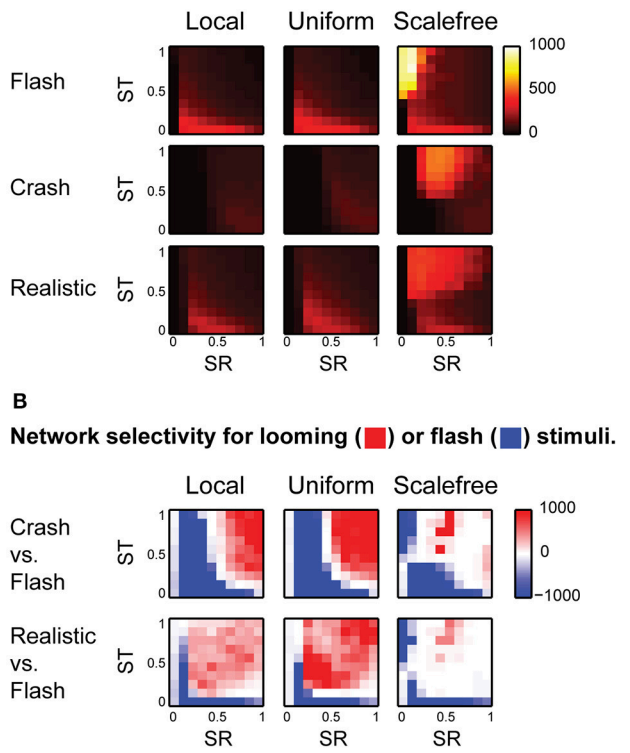

FIGURE 4 | The effect of balance between recurrent and direct inputs to the tectum on stimulus selectivity (A). The total number of spikes (shown as pixels of different color, from black to white) generated in model networks with different strength of direct (horizontal axes) and recurrent (vertical axes) synaptic inputs, for different stimulus types (rows), and recurrent network topologies (columns) **(B)**. The comparison of looming stimuli responses to full-field flash responses. Here color encodes the reliability (signed *F*-value) of getting a stronger total network response to either looming (red), or flash (blue) stimulus, for different strengths of direct (SR) and recurrent (ST) synaptic inputs. Networks with strong direct and recurrent synaptic inputs are selective for looming stimuli (red in the top right corner), while weakly connected networks are selective for full-field flashes (blue crescents in the left lower corner). This pattern is present, but less pronounced for fast looming stimuli, and in scale-free networks.

for both "local" and "uniform" recurrent connectivity profiles most of the parametric space was selective to looming stimuli (red in **Figure 4B**), while models with underpowered RGC-OT or OT-OT synaptic connections were selective for full field flashes (blue crescents in bottom left corners in **Figure 4B**). The parametric space for "scale-free" connectivity looked somewhat similar, but less selective, as networks were easily overpowered by epileptiform activity (of a kind shown earlier in **Figure 2D**, bottom row). The effects of spontaneous epileptiform activity can also be seen in respective spike-output heat maps (**Figure 4A**, third column), as yellow and orange areas of high total spiking at the top of each heat map (corresponding to regions of strong recurrent connectivity, ST $>$ 0.5). Overall, our results suggest that in the absence of neuronal noise, the balance between recurrent and direct inputs in tectal networks does not have to be tight, as the networks are naturally selective to looming stimuli, provided that both direct and recurrent inputs are strong enough.

Effects of Spontaneous Neuronal Noise

We then investigated the sensitivity of looming stimuli detection in our model to the level of background neural noise. We made every tectal neuron in the model spontaneously fire action potentials at frequencies from 0 to 0.3 Hz, and analyzed these "noisy" networks in the same way as we analyzed the original network. The results of these experiments are presented in (**Figure 5A**): as the level of neuronal noise increased (from top rows down in **Figure 5A**), red areas, representing tuning parameter combinations that kept the network selective for looming stimuli, shrunk, and then disappeared altogether. From the shape of red looming-selective regions in the second and third rows of (**Figure 5A**) we can see that for moderate levels of neuronal noise (0.05–0.10 Hz) collision detection happened only when the strength of recurrent connections offered a trade-off between the absence of temporal integration for low values of ST, and susceptibility to epileptiform spontaneous activity for large values of ST. Moreover, as the levels of noise increased, the areas of selectivity for slow "crashes" and fast "realistic" collisions became increasingly non-overlapping, suggesting that for high levels of neuronal noise the balance of recurrent and direct connections may be important not just for enabling collision detection, but also for tuning it to specific temporal dynamics of the stimulus.

To quantify the range of "valid" (SR, ST) combinations that kept the network tuned for looming detection under conditions of high neuronal noise, for each heat map from (**Figure 5A**) we measured the relative size of the looming-selective region in the (SR, ST) parametric space, using an arbitrary threshold of 10 for the *F*-value. We found (**Figure 5B**) that selectivity areas reduced in size with increasing levels of neuronal noise, and that the uniformly connected network (red) was most robust, followed by locally-connected network (blue), while scale-free network was very sensitive to noise due to its propensity to spontaneous activity (green). These results suggest that in realistic conditions of non-zero neuronal noise, the balance of recurrent and direct inputs in the tectum may require a tight homeostatic control, and that the level of inflexibility in tuning increases with the amount of spontaneous activity in the system.

Effects of Sensory Experience

Finally, we looked into the effects of sensory experience on spontaneous activity and collision detection in the tectum. We updated the model to mimic the changes in the tectum of *Xenopus* tadpoles in response to strong, prolonged visual stimulation (Aizenman et al., 2003; Ciarleglio et al., 2015), and analyzed this "overstimulated" tectal network in the same way as we analyzed the "naïve" network. We changed the distribution of spike phenotypes to 5, 30, 20, and 45% for 1, 3, 5, and 10-spike-generating cells respectively (Ciarleglio et al., 2015); decreased all synaptic currents by 25% (Aizenman et al., 2002); and introduced 30% inward rectification for synaptic currents (Aizenman et al., 2002). The parametric space analysis showed that in overstimulated networks the selectivity for slow

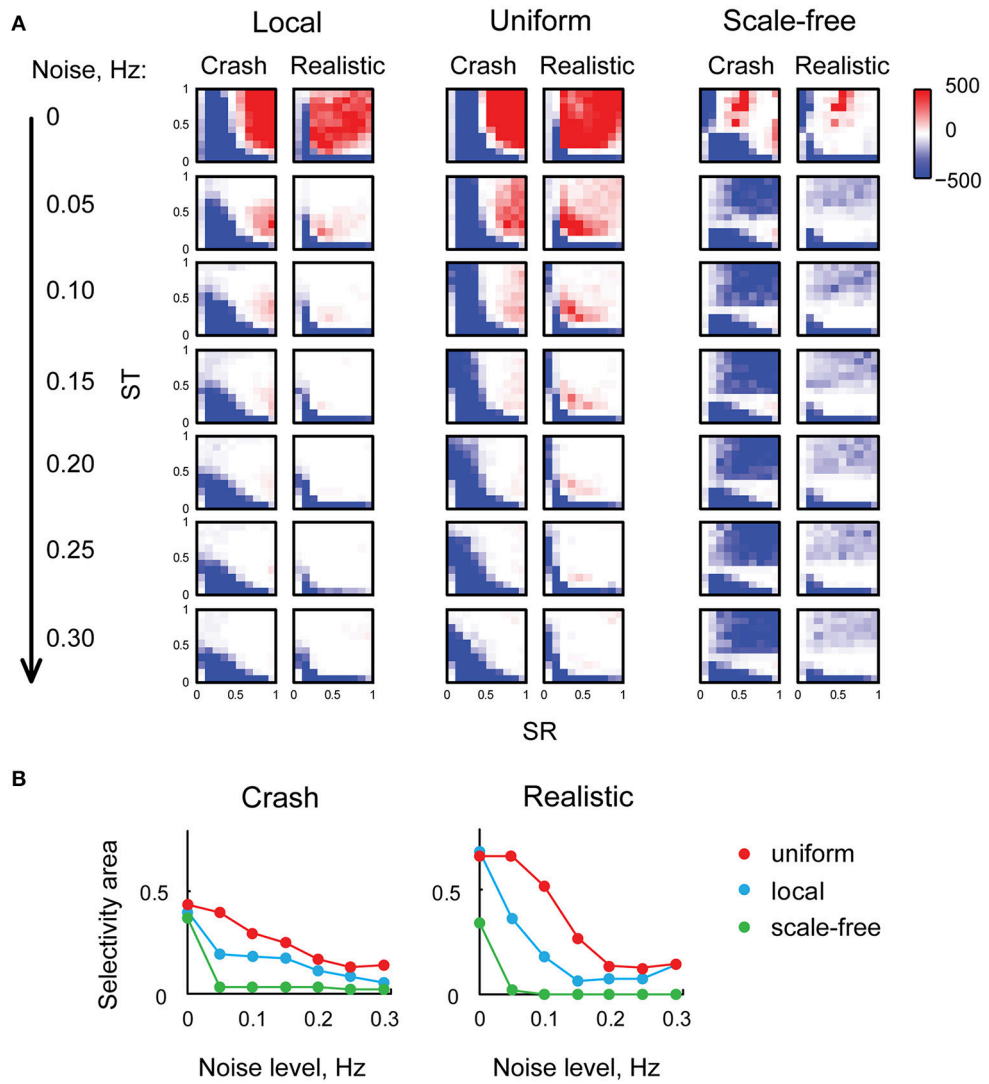

FIGURE 5 | Neuronal noise reduces selectivity to looming stimuli, and makes the balance of direct and recurrent inputs more important. (A) Selectivity charts in the (SR, ST) space for different connectivity profiles and two types of looming stimuli (columns), shown for different levels of spontaneous neural noise (rows). As the levels of noise increase (top to down across the panel), the areas of selectivity for looming stimuli become smaller. **(B)** The share of the parametric space selective to looming stimuli (with arbitrary threshold of $F = 10$), as a function of neural noise level, for two types of looming stimuli. The share of (SR, ST) combinations allowing looming stimuli detection goes down as noise levels increase, yet uniformly connected network (red) is less sensitive to background noise than either local (blue) or scale-free (green) networks.

"crashes" was lost, except for areas of extreme synaptic strength (**Figure 6A**), while selectivity for fast collisions remained largely unaffected (**Figure 6B**).

From these results, we predicted that after prolonged stimulation with light flashes tadpoles would retain avoidance of fast collisions, but would no longer respond to slow moving objects. We performed a series of behavioral experiments in freely swimming stage 49 Xenopus tadpoles, and found that after prolonged visual stimulation the avoidance of large (11 mm in diameter) slow (1.4 cm/s) black circles was indeed significantly impaired (from 0.73 ± 0.22 to 0.51 ± 0.23, Mann-Whitney $P = 0.003$; **Figure 6C**), while it was previously shown, using a slightly different, but conceptually similar experimental protocol, that the avoidance of smaller (8 mm) black circles moving at faster speeds (3 cm/s) is not affected by prolonged sensory stimulation (Dong et al., 2009).

DISCUSSION

In this study we show that a simple experimentally inspired retinotopic network of inactivating spiking neurons can naturally function as a detector of looming stimuli, provided that recurrent excitation in the network is strong enough, and the network is not overpowered by spontaneous neural noise. Notably, this selectivity for looming stimuli occurs even in the absence of inhibition, and without contribution from specialized motion

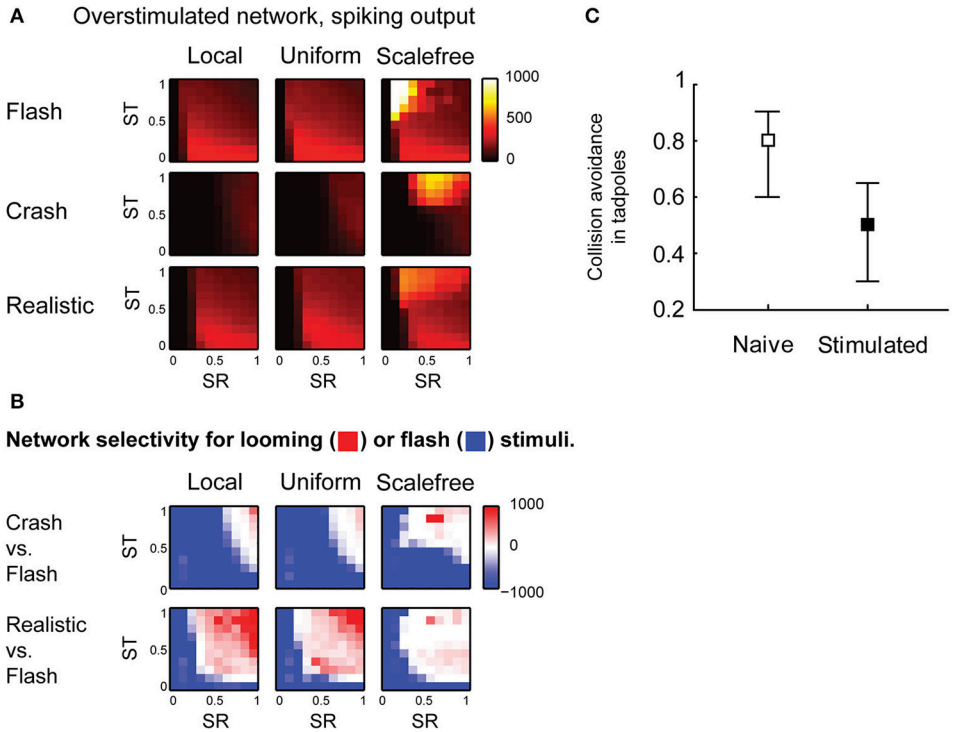

FIGURE 6 | Effects of sensory experience on looming stimulus detection. (A) Compared to naïve networks shown in **Figure 4**, overstimulated networks spike more in response to fast stimuli ("flash" and "realistic" looming), but not in response to slow "looming" stimuli. Color shows the total number of spikes generated by the network in response to a stimulus **(B)**. Unlike naïve networks, overstimulated networks are not selective for slow looming stimuli (large blue areas in the first row), but retain selectivity for fast "realistic" looming stimuli (red areas in the second row) **(C)**. In behavioral experiments, after prolonged strong visual stimulation tadpoles don't perform avoidance maneuvers in response to slow-moving objects (Mann-Whitney $P = 0.003$).

or expansion detectors. Importantly, our model replicates some of the key physiological and behavioral features of looming stimulus detection in the tectum of *Xenopus* tadpoles, potentially including effects of strong visual stimulation on collision avoidance.

Our results indicate a possibility of functional convergent evolution between looming detection in vertebrates and insects, as in our model a retinotopic layer of rapidly inactivating neurons seemed to discriminate looming stimuli in a way that is fundamentally similar to how spike frequency adaptation and competitive inhibition make it happen in the dendritic tree of looming-selective DCMD neurons in locusts (Gabbiani et al., 2002; Peron and Gabbiani, 2009). In both cases, inactivation-like processes (recurrent excitation and sodium channel inactivation in *Xenopus*; spike frequency adaptation and spatially localized shunting inhibition in insects) introduce a competition mechanism that discriminates against stimuli that are either too synchronous and localized or develop too slowly. To evoke the highest total spike-output, in both insects and tadpoles, the sensory input should develop as a wave of increasing strength, running slow enough to allow temporal summation (Gabbiani et al., 2004; Khakhalin et al., 2014), yet fast enough to get ahead of competitive inactivation in the retinotopic system (Peron and Gabbiani, 2009; Jones and Gabbiani, 2010). These local inactivation mechanisms therefore may link temporal and spatial properties of the sensory stimulus, providing a simple and efficient mechanism for stimulus recognition.

The discrepancy between the average number of spikes observed in this model study (about one or two) and during physiological *in vivo* loose cell-attached recordings (5–12 spikes; Khakhalin et al., 2014), which happened despite a careful and meticulous calibration of cell intrinsic properties we undertook in this study, can be explained by several possible effects. One potential difference between the studies of neuronal spiking in whole cell (Ciarleglio et al., 2015) and loose cell-attached modes (Khakhalin et al., 2014) is that spike-triggering currents arrive at different compartments within the neuron: in the soma in case of whole-cell studies, but at the dendritic tree during synaptic stimulation. Because of that, our model, which was based on the data from Ciarleglio et al. (2015), might have underestimated the effects of active dendritic integration that may in fact take place in the optic tectum (Bollmann and Engert, 2009; Felch et al., 2016). Another complication of whole-cell studies is that during the recording, messenger molecules may be washed out from the cytoplasm of the neuron (Zhang et al., 1998; Khakhalin and Aizenman, 2012), potentially triggering changes in intrinsic properties and loss of spikiness (Staley et al., 1992). Although in Ciarleglio et al. (2015) intrinsic properties were typically recorded first, immediately following the entry to the cell, this effect might have contributed to lower estimations of cell spikiness in this

study. Further, during data acquisition for (Khakhalin et al., 2014), cells that were silent to both "crash" and "flash" after one or two presentations were not included in the analysis, introducing a selection bias, which was not present in this study.

Our study suggests that looming stimulus detection can be supported by very different recurrent connectivity profiles, including spatially disorganized random uniform networks. It may mean that at least for the purposes of collision detection, the refinement of recurrent connections in the tectum may be not as critical as the refinement of input retinotectal projections (Dong et al., 2009), although it may still be critical for computing appropriate directional motor responses during avoidance of slow-moving objects, as described in Khakhalin et al. (2014). The best robustness to spontaneously generated neural noise was observed in a spatially unorganized uniformly connected network, which intuitively lies "in between" a local recurrent network and a scalefree network in terms of how strongly on average it connects neural cells from different parts of the model tectum. (A formal justification for this claim can be achieved through a calculation of the average distance between two randomly selected nodes in the network of N nodes, which is typically the largest for a locally connected network (it grows with N as a power of N), intermediate in a random network (grows slower, as ln N), and is the smallest (grows as ln ln N) in the scale-free network we used (Barabási, 2014, ch. 4, p. 21). In effect, for connectivity profiles, as well as for synaptic scaling parameter ST that defined the strength of recurrent connections, it was the "middle solution" that offered most robust collision detection under conditions of moderate neural noise, while both weakly connected and hyperconnected networks failed. This finding suggests that developing tectal networks in *Xenopus* tadpoles may employ a yet undescribed homeostatic mechanism to balance relative abundance and strength of direct and recurrent synaptic connections in principal tectal cells, keeping it in the range that supports collision avoidance computations. Our modeling data also suggests that collision detection may be further improved by recurrent feedback inhibition (Khakhalin et al., 2014; Liu et al., 2016), especially for networks that are tightly connected and susceptible to epileptiform activity, or when the level of spontaneous neuronal noise in the system is high. We hypothesize that delayed feedback inhibition may act as a safeguard, temporally limiting recurrent activity in the network, and thus allowing for strong integration between direct and recurrent inputs within the window before the inhibition onset, without the risk of succumbing to epileptiform activity.

Overall, we demonstrated that recurrent networks with inactivation can indeed underlie collision detection, and that appropriate calibration and tuning of recurrent and direct inputs to the tectum becomes more important as the levels of noise generated by spontaneous activity in the network increase.

METHODS

Spiking Cell Model

Our spiking cell model is governed by two ordinary differential equations: a quadratic differential equation with hard reset for voltage (V), and a linear differential equation for slow outwards currents (U):

$$\begin{cases} \frac{dV}{dt} = \frac{1}{C} \cdot \left(k_1 (V - V_r)(V - V_{th}) - U + IM\right) \\ \frac{dU}{dt} = a(k_2 (V - V_r) - U) \end{cases} \quad (1)$$

Here V represents cell membrane potential (the value of V is dimensionless, but can be interpreted as membrane potential in mV); U (also dimensionless) approximates both activation of slow K+ channels and inactivation of Na+ channels; V_r stands for resting membrane potential (typically −50); V_{th} represents voltage-gated Na+ channels threshold potential (a-value in the −5 to −20 range, depending on the cell type); C is a tuning parameter similar to cell membrane capacitance (large values of C make cells spike slower); I is the external current injected in the cell (in pA), and M represents the current adjustment for leak and space clamp effects.

The parameter a controls the speed of inactivation (U), and flips between two values, depending on whether the voltage is increasing or decreasing, to better represent the dynamics of recovery of physiological neurons from Na channels inactivation, as observed in Ciarleglio et al. (2015) during responses to cosine current injections:

$$a = \begin{cases} a_1, \text{ if } \frac{dV}{dt} > 0 \\ a_2, \text{ otherwise} \end{cases} \quad (2)$$

Of parameters $k1$ and $k2$, the latter was made dynamically dependent on the value of external current injected in the cell (I):

$$k_1 = \frac{-4L}{(V_{th} - V_r)^2}$$
$$k_2 = \begin{cases} b & \text{if } 2 \cdot \frac{L+I}{V_{th} - V_r} > b \\ 0.2 & \text{if } 2 \cdot \frac{L+I}{V_{th} - V_r} < 0.2 \\ 2 \cdot \frac{L+I}{V_{th} - V_r} & \text{otherwise} \end{cases} \quad (3)$$

Here L and b are tuning parameters. With these adjustments, compared to the original Izhikevich model (Izhikevich, 2003), the parabolic and linear nullclines of the phase portrait (**Figure 1A**) always intersect: as the parabolic nullcline moves up during positive current injections, the linear nullcline changes its slope, always passing through the lower point of the parabola. As a result, the phase space retains a stable attractor for the phase trajectory, ensuring that even for high currents the neuron never switches to regular spiking, but generates a limited near-constant number of spikes (**Figure 1B**). Moreover, unlike in bursting and thalamo-cortical varieties of the Izhikevich model, where fast inactivation is achieved by an increase in the value of U, our neurons showed very little spike-frequency adaptation, with almost constant inter-spike intervals within a burst, as it is the case in real physiological neurons (Ciarleglio et al., 2015).

The equations were solved using an Euler method with time step dt of 0.1 ms, except for the hard spiking reset that was implemented algorithmically:

$$\text{if } V > V_{spike}, \text{ then : } \begin{cases} V \leftarrow V_{reset} \\ U \leftarrow U + d \end{cases} \quad (4)$$

where tuning parameter d contributes to inactivation speed. Here and below, all modeling and analysis of results were performed in Matlab (MathWorks Ltd., Natick, MA).

Cell Model Tuning and Calibration

We manually tuned four model spiking cells to represent electrophysiological subtypes of tectal cells observed in Ciarleglio et al. (2015). We also selected a representative physiological cell for each group to serve as a general visual guide (cell ids: 28,003, 9004, 1104, and 9003 from Ciarleglio et al. (2015) respectively). For each of four cell types, our goal was to make the model match as well as possible the mean number of spikes for different injected currents, and the median latency and inter-spike intervals, or at least be within one standard deviation from it. The results of this manual tuning process are shown in **Figures 1E,F**.

To make sure that model cells respond adequately to dynamic inputs, we compared spiking of model and biological cells in response to cosine current injections of different frequencies (data not shown, but see (Ciarleglio et al., 2015) for details of the protocol). As each model cell spiked in a deterministic fashion, we had to introduce noise in each of the tuning parameters before visually comparing average behavior of model cells to that of biological cells. This rough comparison prompted us to introduce different speeds for spiking inactivation and recovery (parameters a_1 and a_2 in the model above), as depolarization-associated inactivation of spiking in biological cells was typically much slower than recovery during in-between hyperpolarization periods.

The final set of model parameters for the four representative cell types were chosen as follows:

Parameter	1-spike cell	3-spike cell	5-spike cell	10-spike cell
1/C	0.1036	0.0451	0.0444	0.0513
V_r	−50	−50	−51.1765	−50
V_{th}	−20	−14.1176	−6.353	−14.8235
V_{spike}	9.5294	10	10	10
V_{reset}	−12	−12	−18.0588	−15.0588
M	0.34	0.5918	1.3406	0.5682
a_1	0.022	0.02	0.0068	0.0106
a_2	0.33	1.2	0.374	0.848
L	−1.4118	−2.3824	−8.0294	−3.2647
d	50	20.6	31.4	6.1579

Network Population

The model tectal network consisted of 400 cells arranged in a 20 × 20 grid. A fixed share of these cells was assigned each of the four subtypes, according to the actual distribution of spikiness in biological stage 49 tadpoles (Ciarleglio et al., 2015): 20%, 25%, 40% and 15% respectively for naïve animals (default state of the network, **Figures 2–5**), and 5%, 30%, 20% and 45% for the "overstimulated" network (**Figure 6**). The cell types were randomly permuted for each model run.

Synaptic Transmission

Synaptic connections were modeled as an excitatory conductance-based transmission with exponential decay of conductance over time after each pre-synaptic spike:

$$\frac{dG_i}{dt} = -\frac{G_i}{\tau} + q \cdot \sum_j w_{ij} S_j \tag{5}$$

$$I_i = G_i \cdot (E - V) \tag{6}$$

where G_i represents synaptic conductance of cell i at this moment of time; S—the vector of pre-synaptic cell spike-trains, with each spike treated as a delta-function, w—the matrix of synaptic input weights (synaptic strengths); q—scaling sensitivity of this neuron type to synaptic inputs (set to 2.5, 2, 1.5, and 1.5 for neurons of 4 spiking types respectively); τ—synaptic conductance decay (25 ms); I—synaptic current; E—excitatory reversal potential (0 mV), and V—cell membrane potential at this moment of time. We found that with these values of synaptic parameters, the model produced subthreshold postsynaptic potentials that were very similar in shape to average postsynaptic potentials observed in biological experiments in response to synchronous activation of visual inputs (Ciarleglio et al., 2015). We also found that in response to suprathreshold synchronous synaptic stimulation of biologically reasonable strength, our model cells on average produced the same number of spikes (from 1 to 10) that they produced in response to current injections. As dynamic properties of recurrent intra-tectal synapses are not yet described in the literature, we modeled them in the same way as synapses from the retina. Our model did not include effects of short-term pre-synaptic plasticity, such as paired-pulse facilitation or paired-pulse depression.

For the model of overstimulated tectal network (**Figure 6**) synaptic transmission was further adjusted: all synaptic conductances (q) were reduced by 25%, and inward rectification was introduced (Aizenman et al., 2002), reducing synaptic currents by further 30% if the postsynaptic cell has a positive membrane potential:

$$\begin{cases} I_i = 0.7 \cdot G_i\,(E - V)\,, & \text{if } V > 0 \\ I_i = G_i\,(E - V)\,, & \text{otherwise} \end{cases} \tag{7}$$

Projections from RGCs to OT

The model retina consisted of 400 "retinal ganglion cells" (RGCs), arranged in a 20 × 20 square matrix, and producing trains of spikes in response to "visual stimulation" (see below). Each RGC cell was connected to a square spanning 5 cells in each direction from its "precise projection" in the tectal retinotopic network. It led to a total projection size of about one half tectal network width, roughly matching projection size in real *Xenopus* tectum (Shen et al., 2011). The strength of synaptic inputs within this projection square was inversely proportional to the Euclidian distance between each tectal cell location (i, j) and the "precise projection" point (ip, jp):

$$w_{RT} = \frac{w_{max}}{1 + \sqrt{(i - ip)^2 + (j - jp)^2}} \tag{8}$$

OT Recurrent Connectivity

In the model tectum, we tested three different connectivity profiles: uniform connectivity, in which each tectal cell was equally probable to get connected to every other tectal cell; local, in which only neighboring tectal cells were connected, and scalefree, which gave the model properties of a small world network. All three connection profiles were calibrated to deliver similar total drive to the tectal cells (see below).

For the **uniformly connected** OT network, we first created a random adjacency (connectivity) matrix, with every tectal neuron connected to every other tectal neuron, and synaptic weights w_{ij} uniformly distributed between 0 and 1. We then removed self-connections, calculated the sum of all inputs received by each tectal cell (total synaptic drive), and divided all input weights for this cell to its total synaptic drive, thus scaling the total sum of inputs received by each cell to 1:

$$\text{for each i}: \quad \sum_j w_{ij} = 1 \tag{9}$$

For the **locally connected** OT network, we first connected all neurons randomly and uniformly, as described above, and removed all self-connections. We then scaled all weights between tectal neurons based on the Euclidian distance between them along the rectangular grid, making the average weight linearly increase from zero for cells separated by 5 or more grid steps, to strong connections between immediately neighboring cells:

$$w = \xi \cdot \begin{cases} 1 - \frac{D}{5}, & \text{if } D \le 5 \\ 0, & \text{otherwise} \end{cases} \tag{10}$$

were D is the distance between cells, $D = \sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2}$, and ξ is a random variable uniformly distributed on [0, 1]. The width of 5 cells in each direction was chosen to match the width of retinotectal connections seen in Shen et al. (2011), and seems to roughly replicate the direct measurements of tectal activation observed after local release of glutamate in the tectum (Carlos Aizenman, unpublished data), which is the best guess we can make in the absence of published observations. In the same way as it was done for the uniform network, we then calculated the total sum of inputs received by each cell, and scaled inputs by this number, ensuring that each cell in the network received the same total synaptic drive, regardless of the size and position of its recurrent connectivity "watershed."

For the **scale-free** tectal network, we followed the version of Barabasi algorithm (Barabasi and Albert, 1999) as implemented in the SFNG Matlab script (George, 2006). After the scale-free connection graph was constructed, the weights of all established connections were randomized with a uniform distribution between 0 and 1, and scaled (normalized) for each cell in the same way as for uniform and local connectivity profiles.

Balancing Retinal and Recurrent Inputs

As relative strength of direct and recurrent inputs to tectal cells in real *Xenopus* tecta are not known, we created a family of model networks with different average strengths of retinotectal and recurrent inputs, and performed a hyperparameter analysis for this family of models. We multiplied all normalized synaptic weights by two scaling factors: SR for retinal inputs, and ST for tecto-tectal recurrent inputs. For SR = 1 the total synaptic current in each model cell during full-field synchronous visual stimulation reached 180 pA, which was 2–3 times higher than highest amplitudes of total synaptic currents recorded *in vivo* in *Xenopus* tadpole tectum (Xu et al., 2011; Khakhalin et al., 2014), and was similar to strongest synaptic currents ever recorded in tectal cells in response to optic chiasm stimulation (160–250 pA in selected cells in Ciarleglio et al. (2015). As in our model, for SR = 1 this extreme current was experienced by all cells in the tectum, we could be sure that for SR = 1 the retinal inputs were too strong (stronger than in a biological tectum), and so the "realistic" SR value would lie somewhere between 0 and 1.

As isolated recurrent tectal currents are not well studied in the biological tectum, we linked recurrent scaling coefficient ST to the value of SR, in such a way that for ST = SR the total recurrent drive experienced by tectal cells during massive spiking in the tectum would be on average the same as for the retinotectal sensory drive. A practical interpretation of ST values is therefore similar to that for SR: for ST = 0 all recurrent connections were eliminated, while for ST = 1 recurrent connections were obviously exaggerated, suggesting that the unknown "realistic" value of ST would lie somewhere in the [0, 1] region. For **Figures 2**, **3** we used values of SR = ST = 0.5, which produced visual responses similar to that in Khakhalin et al. (2014), and spontaneous recurrent events similar in strength to spontaneous barrages in James et al. (2015). For hyperparameter search in **Figures 4**–**6**, the values of SR and ST were sampled between 0 and 1 in steps of 0.1.

Visual Stimuli

The model retina was presented with four different black-and white (binary) virtual visual stimuli. In case of instantaneous full-field **flash**, the entire visual field went black at moment $t = 0$. For **crash**, or linear looming stimulus, a black circle grew from the center of the visual field and onto the edge, with the radius of the circle linearly increasing with time over a course of 1 s, as in Khakhalin et al. (2014). For **scrambled**, first a crash stimulus was calculated, and then 400 pixels of which it consisted were randomly rearranged (we used a different random permutation in each computational experiment, but the permutation was fixed during the experiment itself). Finally, a **realistic** stimulus was also looming, but with the relative radius of the visual stimulus increasing hyperbolically, to reproduce a perspective projection during a frontal collision with a flat object:

$$R = \sqrt{2n} \cdot \frac{1 - v}{1 - vt} \tag{11}$$

where n is the number of RGC neurons (400); v is a dimensionless approach speed (0.1), and time t changed from 0 to 1 s.

RGC Spiking

All retinal cells (RGCs) were modeled as "off" cells, with simple one-pixel receptive fields, together representing the 20 × 20 virtual black-and-white visual stimuli. The change of

a virtual pixel from white to black triggered a "response" in the corresponding RGC. Each response consisted of 4 spikes, reflecting the average number of spikes recorded in loose cell-attached recordings from individual RGCs in Xenopus tadpoles (Demas et al., 2012; Miraucourt et al., 2016). The latency of the first spike was distributed normally with the mean of 50 ms and standard deviation of 17 ms, while the inter-spike intervals followed a gamma-distribution with mean of 50 ms and standard deviation of 20 ms. With these parameter values, the superposition of spike-trains generated by the model retina in response to full-field flashes well reproduced the average response in the optic nerve in response to full field stimulation (Khakhalin et al., 2014).

Spontaneous Activity

When studying the effects of noise on stimulus selectivity (**Figure 5**) we also introduced background spontaneous spiking in the tectal network. In these computational experiments each cell was equally likely to generate a "spontaneous spike" at each time tick, with frequencies ranging from 0 to 0.3 Hz. These "spontaneous spikes" were not modeled fully, and did not affect the instantaneous membrane potential V of the cell itself, but triggered postsynaptic potentials in all cells that received innervation from the spiking cell, according to the weight of this connections, in the same way as it would have happened for a "normal," evoked spike. Note that this approach to modeling of spontaneous neuronal noise may somewhat exaggerate the network effects of it, as it does not take into account the inactivation of sodium channels after each spontaneous spike. We also made all neuronal types generate the same amount of spontaneous spikes, even though in a biological network high-spiking neurons may generate more spontaneous activity than low-spiking neurons.

Analysis

For the analysis of looming stimulus selectivity regions in the (SR, ST) parametric space (**Figures 4–6**), we ran the model 25 times for each recurrent connectivity profile, stimulus type, and the combination of SR and ST parameters. In each run all other parameters of the network were randomized (cell types assignments, synaptic connectivity weights, RGC spiking patterns, and background spiking, where applicable). For each model run we calculated the total number of spikes generated by the network during 2 s of visual stimulus processing (**Figures 4A**, **6A**), and used signed *F*-values (the share of variance in total spiking responses *S*, explained by the stimulus type as a factor, taken with the sign of average response difference between two stimuli) as a measure of statistical reliability of looming stimulus selectivity:

$$F = \text{sign}\left(\overline{S_1} - \overline{S_2}\right) \cdot \frac{explained\ variance}{unexplained\ variance} = \quad (12)$$

$$= \text{sign}\left(\overline{S_1} - \overline{S_2}\right) \cdot \frac{var\left(\left[\,\overline{S_1}\ \ \overline{S_2}\,\right]\right)}{var\left(\left[\,S_1 - \overline{S_1}\ ,\ S_2 - \overline{S_2}\,\right]\right)} \cdot \frac{N-1}{N-2}$$

where S_1 and S_2 are random variables representing total network responses to two different stimulus types, each value obtained in a different model run; square brackets stand for vector concatenation (as in Matlab notation), and N is the total sample size for values compared:

$$N = length\,(S_1) + length\,(S_2) = 25 + 25 = 50 \quad (13)$$

For the analysis of noise influence (**Figure 5**) we used an arbitrary threshold of $F = 10$ to classify noisy networks with different scaling parameters combinations as either selective for looming stimuli or not, similar to how it is shown as differently colored regions in **Figure 4B**.

When Cohen d effect size is reported, it was calculated as $d = \Delta m/s$, where Δm is the difference of means, and s is a pooled standard deviation across both groups.

Behavior

All animal experiments were performed in accordance with Brown University Institutional Animal Care and Use Committee standards, and were approved by the committee. For behavioral experiments, *Xenopus* tadpoles were raised as described previously (Ciarleglio et al., 2015) until they reached developmental stage 48–49 (Nieuwkoop and Faber, 1994). At this point they were either put to experiment directly from the incubator ("naïve" group), or were first stimulated by lines of green LEDs flashing in sequence at 1 Hz for 4 h (Aizenman et al., 2003; Dong et al., 2009; Ciarleglio et al., 2015). One by one, tadpoles were then placed in a Petri dish, and each of them was presented with a black circle 11 mm in diameter projected on the floor of the chamber. Every 30 s the circle was sent toward the tadpole at a speed of 1.4 cm/s, to elicit a collision avoidance response, as described in Khakhalin et al. (2014). Tadpole behavior was recorded with a video camera, tracked in Noldus EthoVision XT (Noldus Information Technology, Leesburg, VA, USA), and analyzed offline. Each tadpole was presented with 8–10 stimuli (average of 9.8), and avoidance responses were counted. Trajectory analysis showed that neither average distance (1.5 ± 0.7 cm), nor average speed of successful avoidance responses (4.0 ± 3.8 cm/s) were different between naïve and overstimulated tadpoles (Mann-Whitney $p = 0.8$ and 0.2 respectively), suggesting that we observed a true change in collision detection and avoidance responsiveness in the sensory and sensorimotor regions of the brain, and not a difference in avoidance maneuver implementation.

AUTHOR CONTRIBUTIONS

EJ and AK designed the model and ran the computational experiments. CR designed, ran and analyzed the behavioral experiments. CA and AK oversaw the project, contributed to the overall experimental design and interpretation. All authors contributed to the manuscript.

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REVIEW
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The Wiring of Developing Sensory Circuits—From Patterned Spontaneous Activity to Synaptic Plasticity Mechanisms

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In order to accurately process incoming sensory stimuli, neurons must be organized into functional networks, with both genetic and environmental factors influencing the precise arrangement of connections between cells. Teasing apart the relative contributions of molecular guidance cues, spontaneous activity and visual experience during this maturation is on-going. During development of the sensory system, the first, rough organization of connections is created by molecular factors. These connections are then modulated by the intrinsically generated activity of neurons, even before the senses have become operational. Spontaneous waves of depolarizations sweep across the nervous system, placing them in a prime position to strengthen correct connections and weaken others, shaping synapses into a useful network. A large body of work now support the idea that, rather than being a mere side-effect of the system, spontaneous activity actually contains information which readies the nervous system so that, as soon as the senses become active, sensory information can be utilized by the animal. An example is the neonatal mouse. As soon as the eyelids first open, neurons in the cortex respond to visual information without the animal having previously encountered structured sensory input (Cang et al., 2005b; Rochefort et al., 2011; Zhang et al., 2012; Ko et al., 2013). *In vivo* imaging techniques have advanced considerably, allowing observation of the natural activity in the brain of living animals down to the level of the individual synapse. New (opto)genetic methods make it possible to subtly modulate the spatio-temporal properties of activity, aiding our understanding of how these characteristics relate to the function of spontaneous activity. Such experiments have had a huge impact on our knowledge by permitting direct testing of ideas about the plasticity mechanisms at play in the intact system, opening up a provocative range of fresh questions. Here, we intend to outline the most recent descriptions of spontaneous activity patterns in rodent developing sensory areas, as well as the inferences we can make about the information content of those activity patterns and ideas about the plasticity rules that allow this activity to shape the young brain.

Keywords: spontaneous activity, developmental biology, visual system development, auditory system development, synaptic plasticity, plasticity mechanisms

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PATTERNS OF SPONTANEOUS ACTIVITY IN THE DEVELOPING SENSORY SYSTEM

Non-evoked activity has been described throughout developing sensory systems, in visual (Torborg and Feller, 2005), auditory (Clause et al., 2014), somatosensory (Allène et al., 2008) and olfactory (Yu et al., 2004) circuits in rats and mice. It also appears across species, having been characterized in ferrets (Chapman, 2000) and cats (Godecke et al., 1997) as well as humans (Colonnese et al., 2010). The exact properties of the activity patterns can vary widely in terms of duration, spread or cell participation, and the various guises of spontaneous activity have been the topic of several excellent reviews (Blankenship and Feller, 2009; Allene and Cossart, 2010; Kerschensteiner, 2014; Kirkby et al., 2013; Ackman and Crair, 2014). Technological advances during the last decade have allowed imaging of spontaneous activity in the live animal, confirming that several types of spontaneous activity patterns exist during development in sensory regions *in vivo*.

It is possible that the different spatiotemporal properties of spontaneous activity lend themselves to performing distinct functions during development. For instance, a different type of activity may be required to produce sensory maps whilst another causes synaptic homeostasis. Though we have not yet reached a full understanding of the connection between the various types of spontaneous activity and their functions, some general patterns in the characteristics of spontaneous activity have already emerged and we can speculate on the potential consequences of their properties.

First the mechanism with which activity travels across the network can change with age. The earliest forms of spontaneous activity are often dependent on the direct exchange of electrical or chemical signals through gap-junction connections. The expression of gap-junctions between excitatory cortical cells reduces with age, until absent at P17 in the rat (Peinado et al., 1993). During this reduction, chemical synapses take over neuronal signaling. Both gap-junction mediated and chemical synaptic activity can activate specific subsets of cells, potentially creating ensembles (Yuste et al., 1992; Allène et al., 2008; Siegel et al., 2012).

Second, besides the mechanism of transfer, the size of the area activated by a spontaneous event can vary, between small local events capable of producing cortical columns (Yuste et al., 1992) or large-scale events, recruiting many cells over greater areas of the cortex (Adelsberger et al., 2005; Kirmse et al., 2015). Intuitively, the number of co-active cells have consequences for the potential function of activity, as correlated activity amongst neighboring cells in the retina or cochlea could pass on the spatial information necessary to precisely pattern sensory maps.

Around the onset of sensation, neural activity gradually changes from infrequent, high amplitude bursts, with a high number of participating cells, to an almost continuous active system with low-participation rates and reduced calcium amplitude, indicating a reduced firing rate (Rochefort et al., 2009; Siegel et al., 2012). This reduction of participation rates (Golshani et al., 2009), or "sparsification" occurs as the system shifts from a preparatory role of wiring the brain, to functional processing when the animal needs to make sense of its environment. As sparseness of encoding is associated with extracting statistics from data rather than a piece by piece replication of the input (Olshausen and Field, 2004), this could reflect the onset of visual processing and the cessation of developmental patterning (Rochefort et al., 2009). Additionally, sparse encoding is an energy efficient representation, given the smaller number of activated neurons at any one time (Olshausen and Field, 2004). Early development seems to disregard this efficiency by using high activation rates, perhaps suggesting a developmental advantage of inclusive activations despite the required energy. As the animal matures, the activity in the sensory areas gradually contains more information as activity begins to become modulated by external stimuli and vigilance state (Colonnese et al., 2010).

Here, we outline current knowledge on spontaneous activity generation and propagation during the first two postnatal weeks in the visual, auditory and somatosensory systems in mice and rats (**Figure 1**).

The Visual System

In rodents, the eyelids do not open until P14. Throughout these first two postnatal weeks, pacemaker cells in the retina fire spontaneously, depolarizing sequentially in waves of correlated activity that travel across the retina (as reviewed in Torborg and Feller, 2005). Activity from the retina can propagate to the superior colliculus (SC; Ackman et al., 2012) and through the lateral geniculate nucleus (LGN) of the thalamus to the primary visual cortex (V1), as the frequency of spontaneous activity in V1 drops when the eye is removed (Siegel et al., 2012). *In vivo* calcium imaging of both SC and V1 simultaneously demonstrated that the location of origin and direction of travel of the waves is matched between these areas, confirming that waves of spontaneous activity could indeed convey information about the spatial properties of the retina to the visual cortex (Ackman et al., 2012). These retinally-driven events can be identified as those in which only a subset of cells (20–80%) are active. Events in which more than 80% of cells are active are unaffected by retinal enucleation, indicating a distinct, perhaps cortical, source (Siegel et al., 2012).

During the end of the second postnatal week, the activity becomes more frequent, less correlated between cells and the amplitudes of intracellular calcium events decrease (Rochefort et al., 2009). In accordance with the imminent onset of visual input, the visual system becomes responsive to light flashes from P8. Colonnese et al. (2010) defined an early "bursting phase" in rats before eye-opening, between P8 and P11, where a bright light flash evokes a bursting pattern through the closed eyelid. During this phase, the cortical burst responses evoked by an identical stimulus vary greatly in response amplitude, the time of onset after the stimulus and the number of spikes fired. This variation in the response pattern does not depend on whether the animal is awake or sleeping. From P12, 2 days before eye

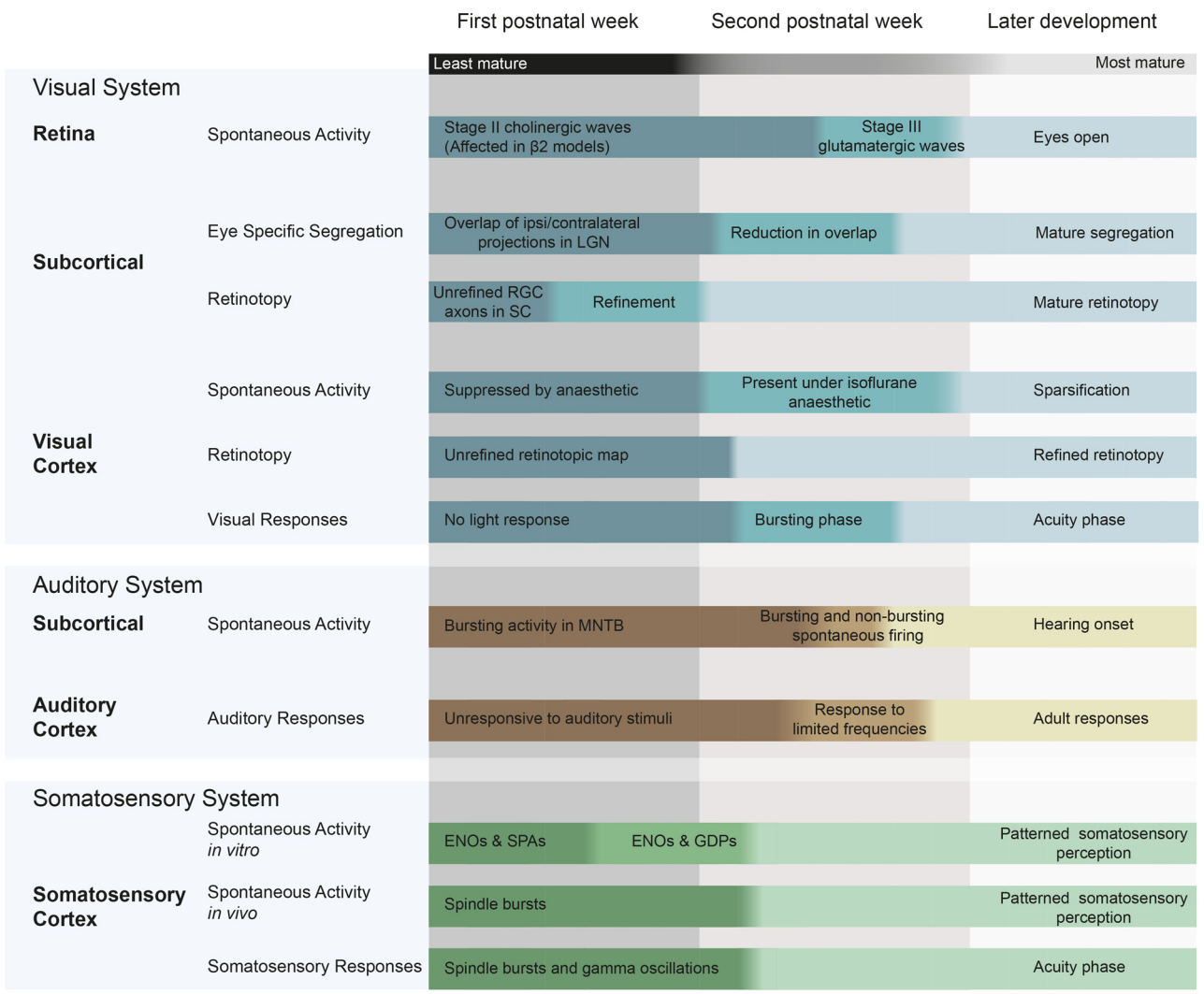

FIGURE 1 | Spontaneous and evoked activity during early postnatal development in mice and rats, and the changes in patterning that occur during this time. Visual system: (McLaughlin et al., 2003; Cang et al., 2005a; Firth et al., 2005; Torborg and Feller, 2005; Demas et al., 2006; Rochefort et al., 2009; Colonnese et al., 2010; Siegel et al., 2012). Auditory system (Geal-Dor et al., 1993; Kandler and Gillespie, 2005; Sonntag et al., 2009; Tritsch et al., 2010; Froemke and Jones, 2011) Somatosensory system (Minlebaev et al., 2007; Allène et al., 2008; Allene and Cossart, 2010; Colonnese et al., 2010). ENO, early network oscillations; SPA, synchronized plateau assemblies; GDPs, giant depolarizing potentials; RGC, retinal ganglion cell; LGN, lateral geniculate nucleus; MNTB, medial nucleus of the trapezoid body; SC, superior colliculus.

opening, the next phase begins where cortical responses to a stimulus become consistent and these responses are modulated by the vigilance state of the animal.

The Auditory System

In the cochlea, inner hair cells show spontaneously generated calcium action potentials before hearing onset. The initiation of these action potentials is triggered by glia-like inner supporting cells through a remarkable mechanism. These cells release ATP, activating a flow of chloride out of supporting cells through TMEM16A channels. Water and potassium follow the chloride efflux, leaving the supporting cell and causing transient osmotic shrinkage. The high extracellular potassium depolarizes inner hair cells (Wang et al., 2015), triggering glutamate release and producing bursts of action potentials in spiral ganglion cells. At this synapse, NMDA receptors act as an amplification mechanism, prolonging post-synaptic currents and enhancing depolarizations in spiral ganglion cells, enabling their fast spontaneous firing rate. This NMDA-dependent increased excitability also controls how many cells are activated by each inner hair cell depolarization, as pharmacologically blocking NMDA receptors reduces the number of spiral ganglion cells that participate in each event (Zhang-Hooks et al., 2016). Such peripherally generated activity can propagate via the auditory nerve to the rest of the auditory system, as action potentials in the medial nucleus of the trapezoid body (MNTB) and the inferior

colliculus were abolished by removal of the cochlea (Tritsch et al., 2010). For an extensive overview of spontaneous activity in the developing auditory system see Wang and Bergles (2015).

Before P11, a combination of various mechanical factors prevent hearing, though bone conducted stimuli cause an auditory brainstem response from P7 (Geal-Dor et al., 1993). The brainstem tonotopic map can respond to the mature range of frequencies by P14 (Friauf, 1992). The auditory cortex also develops quickly at P10, the cortex does not respond to tone stimuli. At P11, A1 responds only to high-intensity stimuli between 6 and 10 KHz (de Villers-Sidani et al., 2007), and it reaches the adult level of responsiveness at P14. During this time, A1 greatly increases in size and represents an increasing range of frequencies and intensities. For a review of this development, see Froemke and Jones (2011).

The Somatosensory System

The somatosensory system matures earlier than the visual and auditory systems in rodents. The onset of both hearing and sight occurs after birth, allowing postnatal *in vivo* experiments during which measured activity cannot be directly evoked though external stimuli and therefore can be classified as spontaneous. In contrast, the somatosensory cortex responds to sensory stimulations from P2, creating some difficulty when measuring its spontaneous activity *in vivo*—particularly in awake animals where somatosensory inputs cannot be fully prevented. After P8, the somatosensory cortex enters the "acuity phase" where stimuli cause reliable responses (Colonnese et al., 2010).

In cultured slices of the somatosensory cortex, both gap junction mediated and synaptic forms of spontaneous activity have been described, including gap-junction mediated synchronized calcium plateau assemblies, NMDA dependent early network oscillations (ENO) and GABAergic giant depolarizing potentials (Allene and Cossart, 2010). Similar activity patterns occur in the somatosensory cortex *in vivo*. There, early gamma oscillations (EGOs) can be measured during the first postnatal week. These short oscillations typically remain within one cortical barrel and rely on rhythmic input from the thalamus (for a review on EGOs see Khazipov et al., 2013). Together with spindle bursts of around 10 Hz, measured between P0 and P8 *in vivo* (Khazipov et al., 2004; Yang et al., 2009), EGOs form a young sensory response during the first postnatal week. This underlines the precocious maturation of the somatosensory cortex in comparison to the visual and auditory cortices, though spindle bursts can occur in the absence of sensory inputs (Khazipov et al., 2004).

SPONTANEOUS ACTIVITY HELPS WIRE THE DEVELOPING SENSORY SYSTEM

The above descriptions paint a picture of spontaneous activity as a pervasive phenomenon during development, but do not directly demonstrate its function. Blocking spontaneous activity often causes severe disruptions of the organization of sensory areas, indicating its importance for precise wiring of the developing brain (Cang et al., 2005b; Chandrasekaran et al., 2005). This importance is underlined by the observation that spontaneous activity patterns are different in neurodevelopmental disorders such as Fragile X syndrome (Gonçalves et al., 2013).

Since early experiments in which spontaneous activity was eliminated, our grasp on the rules which underlie the patterning of the neonatal brain has greatly increased. This is mainly due to technical improvements which have allowed subtle and specific manipulations of spontaneous activity rather than overall elimination. These exciting experiments aimed to clarify the information content of these waves and which characteristics (for instance, their timing, spatial properties, frequencies or wave amplitudes) are important for their function. Thanks to this recent work, we can begin to sketch out the role of spontaneous activity, more clearly delineating which processes do and which do not rely on intrinsically generated activity.

The Visual System

As described above, one major source of spontaneous activity in the visual system is the retina. The wave characteristic of spontaneous activity in the developing retina provides spatiotemporal information—as the wave travels, neighboring retinal ganglion cells (RGCs) will fire in turn, passing on information about their spatial relationship in the temporal properties of the wave. When a mouse opens its eyes at around P14, neurons in the V1 have already been thoroughly organized according to the spatial structure of the retina; V1 shows retinotopic maps, eye-specific segregation and orientation tuning of individual neurons (Smith and Trachtenberg, 2007; Rochefort et al., 2011; Ko et al., 2013).

In the visual system, RGCs representing adjacent parts of the visual field project to neighboring cells in the visual cortex, resulting in a retinotopic map. Initially, the retinotopic maps set out in the visual system are instructed by molecular guidance cues, such as ephrins- the ligands of the Eph receptor tyrosine kinases, which guide projections from the LGN to V1 (Cang et al., 2005a). These cues appear in a gradient across the target area, giving some directional information that results in a coarse retinotopic organization. This initial targeting is activity independent (Benjumeda et al., 2013). Futher fine-tuning of these connections, then occurs through both pruning of excessive connections and increasing arborization within the correct termination zones (Simon and O'Leary, 1992). Anatomically, refinement is measured through labeling of projections and determining the size of the area in which they terminate. This refinement can also be measured functionally, by determining the size of the regions within the higher visual areas that are activated by a given stimuli. It is important to study refinement at both the single cell an population level, as a population that represents a large part of the visual field can be made up of many neurons that are each broadly-tuned, or a group of individually finely-tuned neurons with large variation in receptive fields (Mrsic-Flogel et al., 2005).

Retinotopy and Eye Specific Segregation

One of the most common models of disrupted retinal activity is the β2 global knock-out mouse. These mice lack the β2 subunit of the nicotinic acetylcholine receptor. They have altered retinal activity during the first postnatal week and in turn, disrupted patterning of higher visual areas. In contrast to wild-type, in which a clear wave-front travels over the retina in a successive activation of ganglionic cells, the β2 global knock-out shows almost simultaneous activation of much larger groups of neurons. These waves are gap-junction dependent and of lower frequency and amplitude when compared to controls. When dye injections are used to visualize geniculocortical projections, the termination areas are larger in β2 knock-out mice than in wild-type (Cang et al., 2005b), and this corresponds to less fine-tuned functional retinotopy in the SC (Mrsic-Flogel et al., 2005), LGN and V1 (Grubb et al., 2003; McLaughlin et al., 2003; Cang et al., 2005b). Additionally, the segregation of terminals from the ipsilateral and contralateral eyes (eye-specific segregation) is disrupted. The propagation of activity through the visual system also seems to be changed, as the SC shows "extra", short waves that do not correspond to retinal activity (Burbridge et al., 2014).

Retinotopic Patterning Requires Locally Correlated Activity

One major limitation of the β2 knock-out mouse is that both the frequency and the spatial properties of retinal activity are different to wild-type mice (**Figure 2**), making it difficult to tease apart the relative influence of each factor on the phenotype. Several variations of the β2 mouse have been used to more specifically manipulate activity patterns by varying either the frequency or the spatial spread of waves (for an excellent review of the consequences of many of these disruptions see Kirkby et al., 2013). These experiments have allowed us to link specific properties of spontaneous activity to higher area patterning. The first conclusion we can begin to draw is that retinotopy seems to depend on local correlations within retinal waves and not on firing rate. Retinotopy is disrupted in mouse models with a large retinal wave, where large areas of the retina become correlated. In contrast, genetic models in which local correlations between neighboring cells are maintained consistently have accurate retinotopy (Xu et al., 2011, 2015). The mutant mouse Rx-β2cKO has a lower than wild-type firing frequency, but shows normal retinotopy and restoring the firing rate of retinal waves in the β2 knock-out to wild-type levels does not save the retinotopic map (Burbridge et al., 2014; **Figure 2**).

Recently, Burbridge et al. (2014) used a Ret β2-cKO mouse (**Figure 2**) as an elegant demonstration of the link between retinal waves and higher area retinotopy. In this mouse, the β2 subunit is knocked out selectively in temporal and nasal areas of the retina, locally canceling wave activity during stage II cholinergic waves. The rest of the retina, along the dorso-ventral axis, showed clearly propagating waves. This is reflected in the SC at P8, where terminations from the altered, naso-temporal retina spread over a larger area than those from the non-expressing areas. Overall, it seems that retinal waves contain local spatial information about the retina essential for normal retinotopy, indicating an instructive role for spontaneous activity. However, Zhang et al. (2012) debate the role of spatial properties in retinotopic mapping. They used channelrhodopsin in RGCs to create artificial retinal waves in which all simulated cells fired simultaneously, removing the local spatial information. They report very little effect of this stimulation on contralateral axon retinotopy in the SC. However, as the stimulation occurred at P9, it is likely that some retinotopy had been set up before the time of stimulation. A learning rule put forward by Butts et al. (2007) postulates that the initial strength of connections will bias subsequent activity competition in favor of the more strongly connected wiring, perhaps explaining why retinotopy was not reorganized by synchronous stimulation occurring after the circuit was established. Indeed, stronger stimulation may have strengthened these connections, reinforcing the existing map (Kirkby et al., 2013).

Eye-Specific Segregation Depends on Firing Frequency and Inter-Eye Synchronicity

In both the LGN and SC, projections from both eyes initially terminate in partially overlapping areas (Demas et al., 2006). During the first two postnatal weeks, these terminations are refined, clearly dividing where inputs from each eye are segregated and where they are combined to produce binocular vision. In contrast to retinotopy, this eye-specific segregation does depend on the firing rate of spontaneous activity, as restoring the firing rate of the retina in whole body β2 knock-out mice using CPT-cAMP improves segregation (Burbridge et al., 2014). Results from the Rx-β2cKO mouse confirm this, as their lower frequency of relatively normal activity results in selective disruption of eye-specific segregation (Xu et al., 2015), **Figure 2**. Overall firing rate cannot be the only important factor, as mice with wild-type frequency, but spatially smaller waves (β2 (TG)) have disrupted eye-specific segregation (Xu et al., 2011). It therefore seems that spatial information is important, but at a different scale than for retinotopy; rather than local correlations amongst neighboring cells, the overall area activated by each wave may be essential in eye-specific segregation. There may be an activity threshold for segregation, which can either be reached by frequent or by large-scale activity.

Zhang et al. (2012) also used their protocol for optogenetic activation of RGCs to test the role of spontaneous activity in eye-specific segregation of the SC and LGN. The more the stimuli overlapped between the eyes, the more the disruption in eye-specific segregation worsened. Synchronous stimulation could also disrupt segregation even after eye-specific segregation, indicating an important role for retinal waves not only in creating but also in maintaining segregation, as has been previously reported (Demas et al., 2006). Asynchronous stimulation of the eyes (with more than 100 ms difference) did not disrupt segregation, suggesting a sub-second resolution of this competition. It is usually assumed that, as retinal waves arise spontaneously, retinal or SC waves from both eyes are not synchronized. However, Ackman et al. (2012) found that 15%

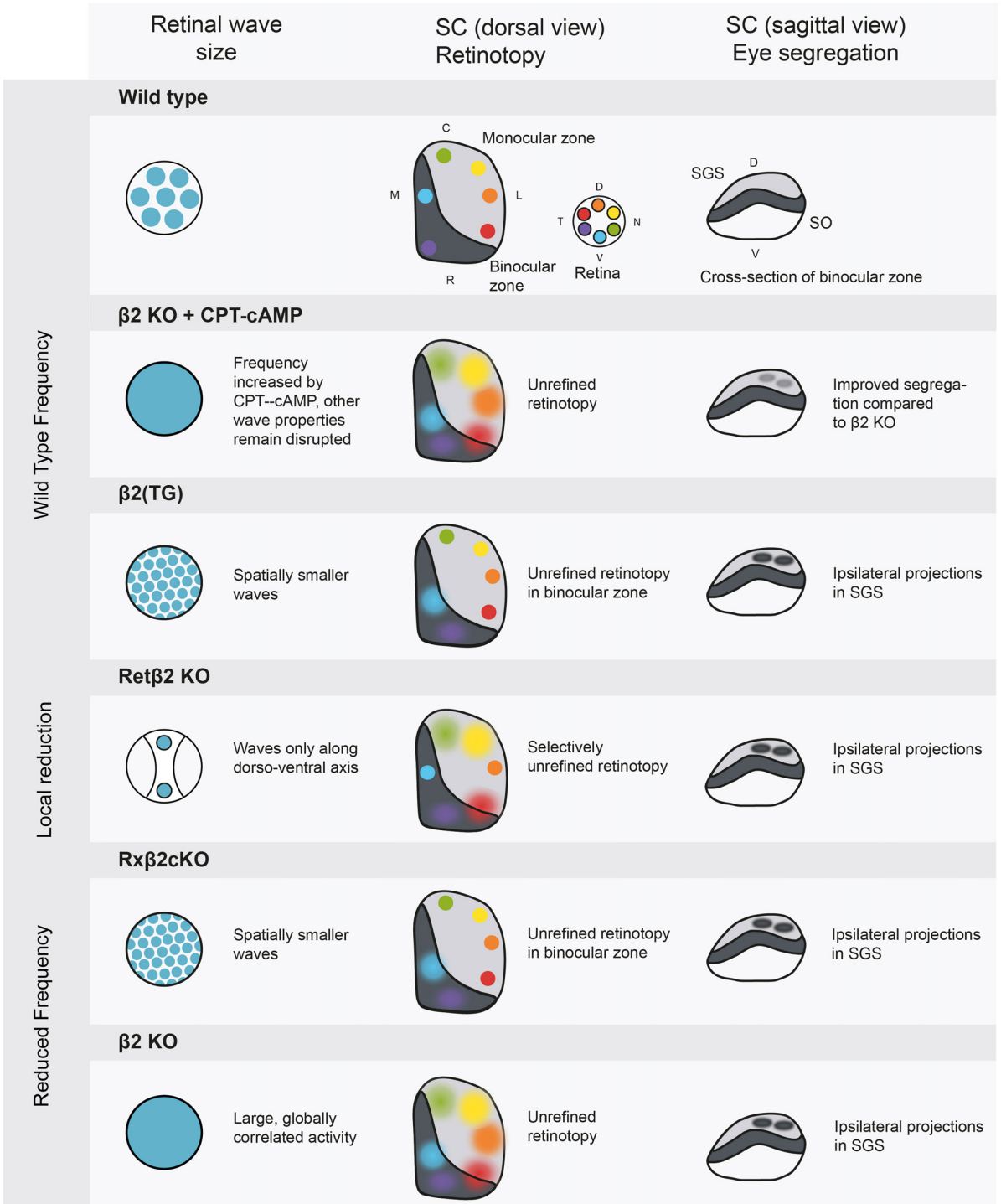

FIGURE 2 | Manipulations of spontaneous activity frequency and wave size and the consequences for retinotopy and eye-specific segregation in the SC. The SC has a binocular and a monocular region, and contains a retinotopic map in a mirror image of the retina. The most superficial layer of the SC is the stratum griseum superficial (SGS), which is targeted only by axons from the contralateral eye. The stratum opticum (SO) contains ipsilateral projections in wild type animals. *Wild type*: retinal activity in the wild type mouse. β2 knockout+ cAMP-CPT: this manipulation increases frequency to wild type levels (Burbridge et al., 2014), rescuing eye specific segregation but not retinotopy. β2 (TG): truncated waves as in the β2 (TG) mouse disturb eye-specific segregation (Xu et al., 2011). Retβ2-KO: partially disrupting wave activity also has spatially selective consequences for retinotopy (Burbridge et al., 2014). Rxβ2-KO: reducing wave frequency and size disturbs segregation (Xu et al., 2015) and retinotopy in the binocular zone. β2 KO: the whole body β2 knockout has low frequency activity over large areas of the retina, leading to unrefined retinotopy and disrupted eye specific segregation. SGS, stratum griseum superficiale; SO, stratum opticum; D, dorsal; V, ventral; C, caudal; R, rostral; T, temporal; N, nasal.

of retinal waves are temporally matched between the eyes and proposed that descending synchronized inputs or synaptic interaction between the eyes mediated this synchronicity. As mentioned above, Burbridge et al. (2014) report that the β2 knock-out mouse has a much higher correlation of wave activity between the two retinas, which could contribute to the reduced segregation seen in this model.

The retina has different stages of wave activity, starting with gap-junction coupled stage I waves and maturing to cholinergic stage II waves from P0. From P11, glutamatergic stage III waves take over (Firth et al., 2005). The retinal wave stages have different activity properties, the importance of which is not fully understood. Xu et al. (2016) report that when stage II waves are disrupted, eye-specific segregation is affected. This disruption can be rescued by a period of stage III waves. However, when stage II waves persist throughout the second postnatal week (i.e., the system does not transition into stage III glutamatergic waves) it does not affect segregation in the LGN or SC (Xu et al., 2016). It seems that stage II waves are important for segregation, and that activity during the second postnatal week (but not specifically the properties of stage III waves) can still influence this segregation.

The difference between retinotopy and eye-specific segregation is not a complete opposition; the sensory system must be able to form and maintain both patterns simultaneously and problems with one can interrupt with the other. Excellent examples of this occur in the Rx-β2cKO and β2(TG) mice models, which both have normal retinotopy in monocular regions, but disrupted retinotopic mapping in binocular regions of the SC. It seems that the localized problems with retinotopy are a consequence of problematic eye-specific segregation. The strongest evidence for this, is the observation that when one eye is removed at birth, therefore removing inter-eye competition, all retinotopy from the remaining eye stayed intact in both the SC and LGN (Xu et al., 2011, 2015). When both eyes are present, but eye-specific segregation does not occur, the unpruned ipsilateral projections convey out-of-sync spontaneous activity, that may disrupt the retinotopic map.

Direction and Orientation Selectivity

Some RGCs respond specifically to one of four cardinal directions. These RGCs are contacted by starburst amacrine cells whose synapses are, at first, uniformly present over the dendrites. During the second postnatal week, direction selectivity emerges as GABAergic inhibitory current increases on the side of the cell opposite to the preferred direction, likely due to a selective increase of synaptic strength (Wei et al., 2010). The development of direction selectivity of the retinal cells occurs independently of spontaneous activity, as intraocular injections of muscimol or gabazine (selective GABA-A receptor agonist and antagonist, respectively), administered between P6 and P12, did not alter this developmental trajectory (Wei et al., 2010).

Despite not being required for setting up the direction selectivity in the retina, spontaneous activity traversing the system could contain the information needed to pattern the cortex according to RGC direction selectivity. Additionally, in mouse V1, neurons are tuned to direction from eye-opening. This early selectivity is independent of any visual experience as it is not prevented by dark-rearing (Rochefort et al., 2011), suggesting that spontaneous activity may mediate orientation and direction selectivity. Surprisingly, a recent study found that blocking spontaneous activity during development in mice did not reduce orientation selectivity (Hagihara et al., 2015). Spontaneous activity was blocked in L2/3 of the visual cortex, by expressing the inward rectifying potassium channel Kir 2.1 through *in utero* electroporation. Expression of Kir 2.1 in only a small subset of L2/3 neurons (4.6% in central V1, through to 30% in anterior V1) was enough to significantly reduce synchronized activity in both L2/3 and L4. Surprisingly, when the visual responses of these neurons were measured in adult mice, they were equally responsive and selective to visual stimuli with different orientations as neurons in the control animals. This also occurred when animals were reared in darkness. The role of spontaneous activity may vary per brain region, as SC cells in the β2 knock-out mouse have reduced orientation and direction selectivity (Wang et al., 2009).

The Role of Spontaneous Activity in Visual System Patterning Varies Between Species

Much of the work done in spontaneous activity has focused on rats and mice, but it is vital to note the important experiments carried out *in vivo* in the ferret. Many of the same patterning processes as in the mouse also take place in neonatal ferrets, which open their eyes after the fourth postnatal week. However, the significant spatiotemporal properties and the permissive or instructive nature of spontaneous activity may vary between species. Spontaneous activity is clearly important in the ferret visual system, as it is able to drive refinement. When Davis et al. (2015) pharmacologically increased the frequency of spontaneous activity between P15 and P25, at the stage of glutamatergic retinal waves, they were able to actually accelerate the normal refinement of LGN receptive fields. After eye opening, the animals with more retinal waves had smaller receptive fields than saline controls.

One clear difference between the ferret and mouse is in eye-specific segregation. Ablating starburst amacrine cells in the ferret retina reduced the size of retinal waves and inter-cell correlations, but did not prevent normal eye-specific segregation (Speer et al., 2014). This is in contrast to the mouse, where smaller retinal waves did disrupt the accurate segregation of ipsi- and contralateral projections, although it is not straightforward to directly compare wave size between species.

In the ferret, neurons responding to orientation have been found from P23, before eye opening. During the following 3 weeks, more neurons become responsive to the orientation and the average selectivity of the responsive population increases (Chapman and Stryker, 1993). This maturation depends on neuronal activity; orientation tuning at 6 weeks old was somewhat reduced after artificially correlated activity was produced through stimulation of the optic nerve (Weliky and Katz, 1997). If activity is silenced through TTX application between postnatal weeks 4 and 7, orientation tuning fails to mature beyond the level found at 4 weeks of age. Preventing visual experience through binocular eyelid suturing also greatly

impairs the development of orientation selectivity, but not to the same extent as when all activity is blocked (Chapman and Stryker, 1993), suggesting a role for spontaneous activity. Cells in the ferret V1 are not direction selective at eye opening, but become selectively responsive to direction over the next few days. Importantly, visual experience is required for this maturation (Li et al., 2006). Seemingly in contrast, in the mouse, Hagihara et al. (2015) found that the proportion of responsive and orientation-selective cells is mature at eye opening. This is in line with findings from Ko et al. (2013) but unlike Rochefort et al. (2011) where a gradual increase in responsiveness and selectivity was reported. At eye opening, there is a bias for cells to respond to lines with a 90° angle (Rochefort et al., 2011; Hagihara et al., 2015). This bias is equalized after eye opening, which depends on activity but, critically, does not require visual experience (Hagihara et al., 2015). It seems that the development of direction and orientation tuning is challenging to directly compare between the ferret and mouse. One possible cause for these discrepancies is a different developmental time course; the effect of manipulations such as TTX administration could depend greatly on how much patterning has already occurred. The underlying question is whether the same developmental processes occur in these animals. Though it seems likely that the computation of orientation selectivity is comparable across species (Kondo and Ohki, 2016), the differences in the organization (mice, for instance, lack orientation columns) may lead to different requirements when wiring up the brain.

Auditory System

There are very clear parallels between the development of the visual and auditory system, such as the need for maps (tonotopic or retinotopic) and the combination and segregation of inputs from the left and right sensory organs. Throughout the auditory system, a tonotopic map is maintained, organizing projections depending on the sound frequency they represent.

In terms of spatiotemporal characteristics of spontaneous activity, auditory activity may contain equivalent information to visual retinal waves; not only are they grouped into bursts that synchronize tonotopically similar cells (Kandler et al., 2009), in chick embryos these bursts also contain information about the frequency sensitivity of the hair cells (Lippe, 1995).

Information from the cochlea enters the ventral cochlear nucleus via the auditory nerve. Subcortically, the lateral superior olive (LSO) encodes the inter-aural sound amplitude differences, required for auditory localization, by receiving excitatory inputs from the ipsilateral ear and inhibitory inputs from the contralateral ear via the MNTB (Kandler and Gillespie, 2005). To identify the important properties of spontaneous activity in the subcortical auditory system, Clause et al. (2014) worked with a mouse model in which the α9 subunit of the Nicotinic acetylcholine (nAch) receptor was knocked out. The inner hair cells are transiently innervated by cholinergic fibers from the medial olivocochlear bundle (for a discussion of transient synaptic connections in spontaneous activity during development, see Blankenship and Feller, 2009). When the α9 subunit is knocked out, this results in bursts of activity at the same overall firing frequency, but organized into shorter bursts with more action potentials per individual burst. Deleting the α9 subunit led to less refined tonotopy—the LSO was targeted by a larger region of the MNTB, and received many more connections. The overall amount of inhibition received was the same, as each connection had weaker synapses than in wild type. It is striking that a subtle change in the temporal properties of cochlear spontaneous activity, whilst preserving the overall firing rate, led to such disruption of developmental organization. This result suggests that the auditory system, similar to the visual system, is sensitive to the information content of spontaneous activity.

In contrast to subcortical areas, the auditory cortex is very immature at the time of hearing onset (P11). At this time, only a small area of the auditory cortex shows tuned responses, selectively to frequencies of around 7 Hz. Besides tuning, the latency between stimulus onset and cortical response is longer than in the adult. The tonotopic map matures quickly, reaching its adult size and mapping at P13-P14, whereas response latencies take longer to mature (Froemke and Jones, 2011). As A1 shows little patterning before hearing onset, this may indicate that spontaneous activity has a relatively small role in auditory cortical development when compared to the auditory brainstem or visual cortex.

PLASTICITY MECHANISMS IN SPONTANEOUS ACTIVITY

The above studies describe changes in development caused by spontaneous activity. We do not yet fully understand the mechanisms that guide these changes—which electrical signals, chemical factors and plasticity rules determine how altered temporal or spatial patterns can change the organization of the network. For instance, we do not have a clear idea of the signals received by a cell that cause the synaptic elimination during retinotopic refinement. Only by directly observing the plasticity mechanisms at work at these synapses can we really link the information content of the activity to the structural and functional changes it causes.

Different plasticity mechanisms exist and may function side-by-side. Both refinement and homeostasis occur in many manipulations of activity which result in unrefined axonal projections—these neurons have larger termination areas, made up of more individual fibers than in wild type. When these projections are functionally tested, the overall innervation strength is similar, as each individual axon has a weaker effect on the postsynaptic cells (Clause et al., 2014; Lee et al., 2014). This suggests an interesting homeostatic plasticity mechanism—a system is in place to ensure that the overall projection strength is maintained. A similar pattern of many, weaker synapses is found in the auditory brainstem of the Cav1.3 knock-out mouse (Hirtz et al., 2012).

Long-Term Potentiation (LTP) and Long Term Depression (LTD)

Classically, the Hebbian postulate that neurons that fire together and wire together has been thought to underlie the developmental shaping of the higher areas by peripherally

generated spontaneous activity. The repeated firing of presynaptic neurons together with the cells to which they project, strengthen those feedforward connections. In addition, lateral connections between postsynaptic cells that are simultaneously depolarize become potentiated. Conversely, when a postsynaptic cell fires an action potential without presynaptic glutamate release the strength of the connection decreases—therefore an existing strong connection can indirectly decrease the strength of other inputs by causing asynchronous action potentials.

There is empirical evidence for Hebbian learning during sensory development. Žiburkus et al. (2009) used 50 Hz spike trains in bursts of 1 s to mimic retinal activity. This stimulus could induce LTD at the rat retinogeniculate synapse *in vitro*, but only up until P14, after which the same protocol induced synaptic potentiation. Lee et al. (2014) examined the same synapse in mice, using a model with altered AMPA receptors. This mouse lacks the major histocompatibility complex (MHC) class 1 immune proteins H2-K^b and H2-D^b ($K^bD^{b-/-}$), which does not affect their retinal waves but does impair eye-specific segregation in the thalamus (**Figure 3**). In healthy animals, the convergence from the retina to the LGN is developmentally reduced until only 1–3 RGCs project to each postsynaptic cell, but the $K^bD^{b-/-}$mouse does not show this reduction in projection number. In these animals, LTP could be induced normally, through pairing LGN cell depolarization with a presynaptic 10 Hz activity train. However, when pre- and postsynaptic stimulations were offset in time in an attempt to cause LTD, the synapses did not weaken. This imbalance towards LTP was due to the high calcium permeability of the AMPA receptors in the $K^bD^{b-/-}$ mouse—when their permeability was reduced, LTD could be induced. Restoring H2-D^b only to neurons rescued the phenotype, indicating that the protein specifically plays a role in neurons rather than in a systemic immune response. Interestingly, the overall amount of excitation received by each postsynaptic cell was the same—the higher number of terminating fibers was compensated for by each fiber having a weaker synaptic strength, implying that there is a homeostatic mechanism at work. These results suggest a strong link between the ability for synapses to weaken through LTD and the removal of excessive axons.

Similar refinement is necessary in the auditory system. As the LSO grows in size between P4 and P12, the projecting axons from the MNTB expand to compensate for this, maintaining a stable projection size. This growth also occurred in α9 knockout animals, where the temporal properties of cochlear spontaneous activity were altered (Clause et al., 2014). At P12, measurements of bouton spread over the tonotopic axis were made, an anatomical measurement that reflects how much of the tonotopic map the observed neuron can innervate. These measurements were indistinguishable from wild-type (**Figure 3**). Functional recordings, however, showed some differences. The total strength of the whole projection was the same, but was made up of more, individually weaker connections in the α9 knockout, reminiscent of the homeostasis reported in the $K^bD^{b-/-}$mouse (Lee et al., 2014). The apparent contradiction of unrefined functional, but refined anatomical measurements, might be explained through the existence of silent synapses in the wild type animal. These synapses would show up in anatomical measurements of boutons, but not in functional analyses. In both WT and α9 knockout animals, new boutons were selectively added to the center of tonotopic receptive fields even before hearing onset. After hearing begins, spatially uniform synaptic pruning takes place, expressively described by the authors as a "sinking iceberg" model. This form of refinement also did not occur in the α9 knockout, resulting in less specific anatomical and functional innervation at P21. This suggests that spontaneous activity plays a role in both (un)silencing synapses and anatomical pruning, though not necessarily at the same time. These extensive changes in functional and anatomical refinement are particularly fascinating given that the change in spontaneous activity was relatively minor: the overall activity level was the same, but each burst (which occurred more frequently) was shorter. Typically, functional differences in synaptic strength are quickly converted into structural changes such as bouton elimination. However, the time offset between functional and structural refinement suggests that this could be an excellent model for separating synaptic plasticity mechanisms. It seems that functional synaptic strengthening and anatomical elimination are not always a package deal, and that different rules and mechanisms underlie each phenomenon. The use of the α9 knockout has opened up a new way of investigating auditory development, raising many new questions. For instance, the MNTB-LSO projection is inhibitory, which may affect the plasticity mechanisms at work. To strengthen the link between this model and the extensive literature in the visual system, it will be interesting to see how much of the tonotopic map is activated by waves in the α9, perhaps allowing the comparison with retinal wave size in the β2 variants.

LTD and LTP learning could build up some aspects of the maps necessary for sensory processing. However, there are some results that cannot be explained by classic Hebbian learning, clearly set out in Kirkby et al. (2013). Additionally, synaptic organization is observed at a subcellular level (see below), which cannot directly be explained through this mechanism. In order to really understand the rules that work together to build young brains, we need a more thorough understanding of the various types of plasticity mechanisms.

Gap Junctions and Connection Specificity

Early in development, network activity relies heavily on coupling through gap junctions, which permits the creation of assemblies (Yuste et al., 1992) that can become active simultaneously (Kandler and Katz, 1998), for review see Niculescu and Lohmann (2013). Because their cytoplasm is directly linked, neurons can share electrical signals and exchange small molecules (Shimizu and Stopfer, 2013). As the animal develops, gap junctions disappear and signaling is fully taken over by mature chemical synapses, through which cells signal to each other using neurotransmitters. It is not yet known how the network shifts between these two types of connections. Important studies concerning this change have been focused on clonally related cells, which are neurons that originate from divisions of the same precursor cell. Not only are these clones more likely to

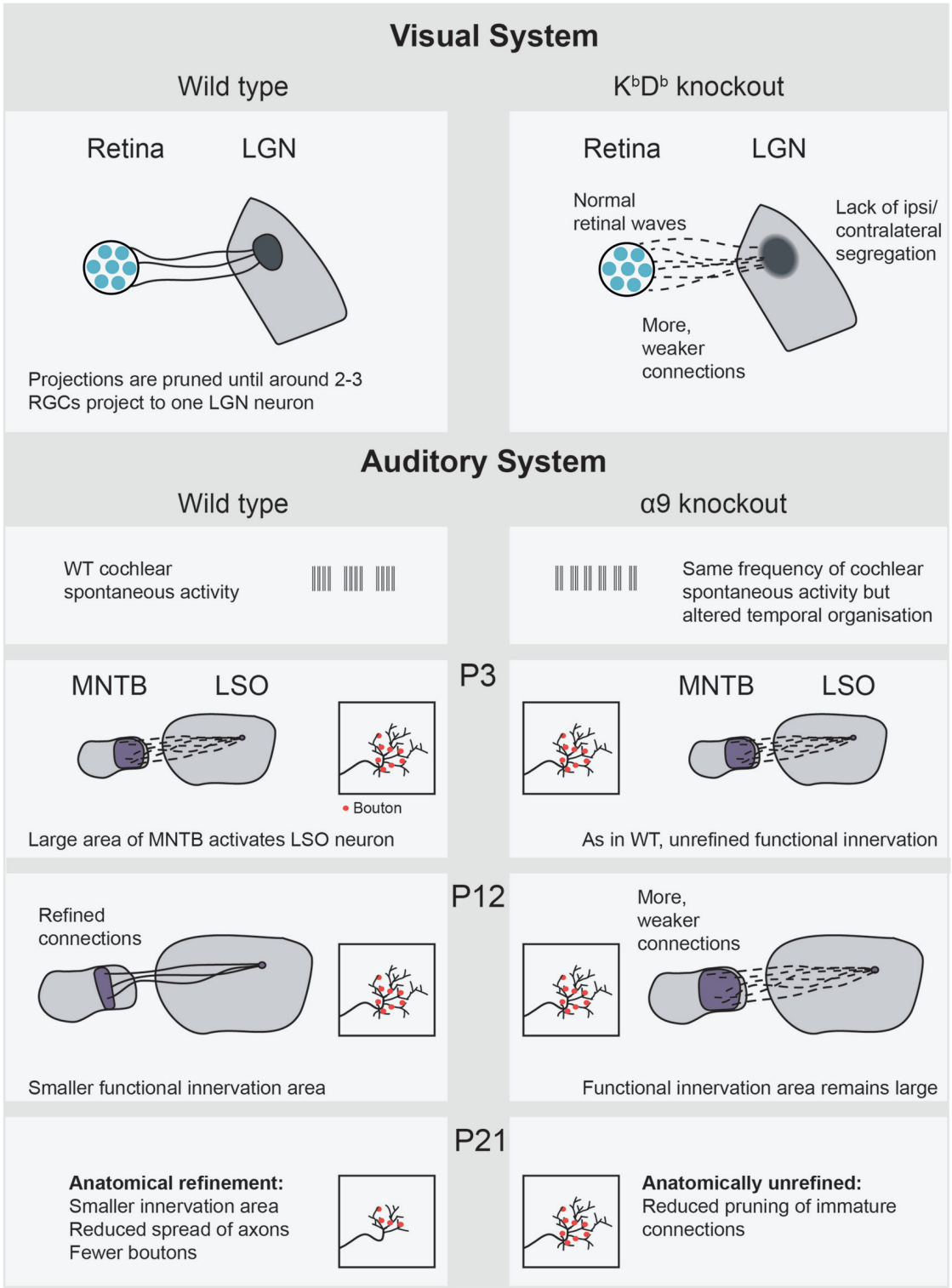

FIGURE 3 | Recent empirical evidence for Hebbian and homeostatic plasticity mechanisms mediated by spontaneous activity. The $K^bD^{b-/-}$ knockout (Lee et al., 2014) shows lack of LTD and altered retinogeniculate projections, in which many weak projections connect the retina to the LGN. In the auditory system, the MNTB-LSO projection in the α9 subunit knockout (Clause et al., 2014) shows both functional and anatomical consequences of altered cochlear spontaneous activity, occurring at different postnatal ages. LGN, Lateral geniculate nucleus; RGC, retinal ganglion cell; WT, wild-type; MNTB, medial nucleus of the trapezoid body; LSO, lateral superior olive; P3, postnatal day 3.

be connected by gap junctions than non-clonally related cells during postnatal days 1–6 (P1–6), they are also more likely to form chemical synapses in animals from P9 (Yu et al., 2009, 2012), and this preference relies on gap junctions (Yu et al., 2012). It is possible that the repeated correlated firing of cells during spontaneous activity maintains the pattern of cell connections set up by gap junction coupling whilst the network changes to rely on chemical synapses. A recent article modeled a potential link between early gap junction connections and later chemical synapses (Ko et al., 2013). In this model, the electrical coupling provided by gap junctions increased the likelihood that linked cells would fire action potentials simultaneously. Because of this co-activity, clonally related cells were more likely to fire in response to the same set of feedforward inputs, stabilizing the same presynaptic connections according to the Hebbian postulate. Given that clonally related cells show similar orientation preferences (Li et al., 2012; Ohtsuki et al., 2012), these findings may have significant consequences for our understanding of visual development. It is important to consider that blocking spontaneous activity during the age at which the system transitions from gap-junction to chemical synapse signaling did not prevent normal orientation tuning (Hagihara et al., 2015). Together, these studies emphasize the importance of future studies to outline where spontaneous activity is, and is not, necessary for sensory development.

Dendritic Organization

In recent years, there has been a surge in our understanding of the computational power of a neuron. The classic description of a neuron is as a linear integrator, summing inputs evenly regardless of their position along the dendritic tree. Recently, experimental evidence has come to support the idea that dendritic compartments can act as computational units, integrating inputs in a non-linear fashion (Poirazi and Mel, 2001), for review see Govindarajan et al. (2006); Larkum and Nevian (2008); Branco and Häusser (2010) and Winnubst and Lohmann (2012). Spatially clustered synapses can exert increased influence on cell output when they are simultaneously active by generating NMDA dependent "dendritic spikes"—large events whose charge exceeds the linear summation of the synapses involved. For this to have functional advantages, strategic organization of synapses along the dendrite is required. Such dendritic specificity and the implications for the output of the cell has been demonstrated in adults (Lavzin et al., 2012; Sheffield and Dombeck, 2015).

During spontaneous activity in development, synapses along the dendrite that are closer together (<12 μm) are more likely to be active simultaneously. This organization disappears quickly when spontaneous activity is blocked (Kleindienst et al., 2011). An "out of sync, lose your link" plasticity rule underlies this organization, as synapses that show low synchronicity to their neighbors become depressed through a significantly decreased transmission efficiency (Winnubst et al., 2015). It is essential to understand how these changes, induced by activity patterns, are signaled to individual synapses. It seems likely that proBDNF, acting on the $p75^{NTR}$ receptor, acts as a local "punishment factor" for synapses with low co-activity levels (Winnubst et al., 2015).

Spatial clustering was found in both the visual cortex *in vivo* and the hippocampus *in vitro*. Though there are many similarities between the mechanisms of clustering these two areas, they have different temporal characteristics—a burst in the hippocampus lasts only around 400 ms, whereas bursts in the visual cortex have a longer duration of around 2 s. Interestingly, this was reflected in the plasticity rules guiding synaptic depression. When probing the time window during which two synapses were considered coactive, the hippocampus showed a much shorter integration window, of 400 ms, whereas in V1, depression of synapses was prevented if they were coactive within 2 s. Burst duration could be an important property of spontaneous activity, linking together only cells that are active within a certain time window.

It is possible that clustering of synchronized inputs and similar new plasticity rules could work together with Hebbian mechanisms to provide a range of options for patterning the developing brain. For instance, the depression of out-of-sync synapses is a tempting rule to apply to eye-specific segregation, where competition-based elimination takes place. However, we have little empirical evidence of how this might occur. As current techniques now allow us to directly measure changes at the level of the "nuts and bolts" of the developing brain, we are set to begin to really understand the rules according to which the nervous system is built.

AUTHOR CONTRIBUTIONS

AHL and CL wrote the manuscript.

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frontiers in Neural Circuits

REVIEW
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Spontaneous Neuronal Activity in Developing Neocortical Networks: From Single Cells to Large-Scale Interactions

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Neuronal activity has been shown to be essential for the proper formation of neuronal circuits, affecting developmental processes like neurogenesis, migration, programmed cell death, cellular differentiation, formation of local and long-range axonal connections, synaptic plasticity or myelination. Accordingly, neocortical areas reveal distinct spontaneous and sensory-driven neuronal activity patterns already at early phases of development. At embryonic stages, when immature neurons start to develop voltage-dependent channels, spontaneous activity is highly synchronized within small neuronal networks and governed by electrical synaptic transmission. Subsequently, spontaneous activity patterns become more complex, involve larger networks and propagate over several neocortical areas. The developmental shift from local to large-scale network activity is accompanied by a gradual shift from electrical to chemical synaptic transmission with an initial excitatory action of chloride-gated channels activated by GABA, glycine and taurine. Transient neuronal populations in the subplate (SP) support temporary circuits that play an important role in tuning early neocortical activity and the formation of mature neuronal networks. Thus, early spontaneous activity patterns control the formation of developing networks in sensory cortices, and disturbances of these activity patterns may lead to long-lasting neuronal deficits.

Keywords: development, cerebral cortex, subplate, spontaneous activity, somatosensory cortex, columnar organization, rodent, human

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INTRODUCTION

Neuronal populations have the ability to self-organize into networks that promote the generation of spontaneous, correlated neuronal activity already at earliest developmental stages. Isolated cortical neurons in dissociated cultures generate after a few days *in vitro* spontaneous action potentials and intracellular calcium transients at irregular intervals (Ramakers et al., 1990; Opitz et al., 2002; Sun et al., 2010). With the developmental shift from electrical to chemical synaptic transmission and increasing axonal connectivity, developing neuronal cultures generate repetitive burst discharges, which are then synchronized over large fractions of the culture. At this developmental stage, spontaneous activity is organized in repetitive spike patterns, with a subgroup of neocortical neurons exhibiting a high degree of synaptic

inputs and outputs ("hub" neurons; Sun et al., 2010). A similar maturation of spontaneous burst activity can be also observed in organotypic neocortical slice cultures (Gorba et al., 1999; Baker et al., 2006). Intriguingly, these activity patterns generated autonomously by the self-organization of networks from isolated cortical neurons resemble many basic properties of spontaneous network activity observed in the immature neocortex *in vivo*.

In vivo, the early postnatal development of spontaneous activity in sensory neocortical areas has been studied in various mammalian species, including mice, rats, ferrets, monkeys and humans. The patterns of spontaneous synchronized network activity in these different species show surprising similarities when the developmental status of the neocortical network is taken into account (for review see Khazipov and Luhmann, 2006). Since rodents offer the advantage of a rich repertoire of experimental manipulations and read-outs, experimental studies in these species provided a better understanding of the early development of physiological and pathophysiological properties of the cerebral cortex in humans. At the same time, these studies offer the opportunity to obtain evidence for the causal relationship between network activity and the structural and functional development of the cerebral cortex.

Both *in vitro* and *in vivo* results strongly suggest that spontaneous synchronized burst activity represents a functional hallmark of developing neocortical networks. In addition, theoretical considerations, experimental evidence and clinical findings suggest that such spontaneous, correlated activity is fundamental for the functional maturation of sensory cortices (Thivierge, 2009; Ben-Ari and Spitzer, 2010; Kirkby et al., 2013; Rahkonen et al., 2013; Levin, 2014). This review aims to provide an update on the properties of spontaneous activity patterns in sensory neocortical areas during early stages of development and how these patterns are generated. Subsequently, we will discuss the physiological relevance of these early activities and how pathophysiological disturbances in spontaneous activity may alter the maturation of cortical networks. Since the developing cerebral cortex shows prominent anatomical and physiological changes during late prenatal and early postnatal stages, we will first briefly summarize the structure of the developing cerebral cortex.

THE STRUCTURE OF THE DEVELOPING CEREBRAL CORTEX

Although the overall structural and functional development of the cerebral cortex during early stages is similar in different mammalian species ranging from mouse to human, the time points and periods of distinct developmental processes (e.g., neurogenesis, migration, differentiation, synaptogenesis, apoptosis) differ due to large differences in gestation periods (for review see Molnár et al., 2006; Molnár and Clowry, 2012). In most species the six-layered cerebral cortex is generated during prenatal and early postnatal stages following an inside first—outside last pattern. Thus, neurons in layer (L) 6 are born first in the ventricular zone (VZ) and migrate to the pial surface to split the primordial plexiform layer (PPL) into the superficial marginal zone (MZ) and the profound subplate (SP; **Figure 1**). Neurons in L3 and L2 are generated later and migrate through the lower layers, which are populated with postmigratory neurons that display more mature properties. Two populations of very early generated and transient neurons fulfill important roles in corticogenesis (for review see Luhmann, 2013): (1) Cajal-Retzius neurons (CRNs) are located in the MZ (which later becomes L1), and control radial neuronal migration (for review see Kirischuk et al., 2014); and (2) subplate neurons (SPNs) are located between the White matter (WM) and L6, playing important roles in early thalamocortical circuits and the maturation of the neocortical architecture (for review see Kanold and Luhmann, 2010). CRNs as well as SPNs are among the earliest generated forebrain neurons and show relatively mature functional properties in the newborn rodent cortex, such as repetitive action potential discharges and prominent synaptic inputs. It has been suggested that highly connected SPNs may act as amplifiers and hub neurons in early neocortical networks (Luhmann et al., 2009; Kanold and Luhmann, 2010). When all neocortical layers have been generated (in rodents at postnatal day [P] 4–5, in full-term human infants shortly before birth), most CRNs and a substantial fraction of SPNs disappear and the developing neocortical networks undergo extensive experience-dependent reorganization during the subsequent critical period for the different sensory systems. While virtually all CRN have been shown to perish by apoptosis (Chowdhury et al., 2010), the fate of SPN is a matter of discussion (Marx et al., 2015).

SPONTANEOUS NEOCORTICAL ACTIVITY AT PRENATAL AND EARLY POSTNATAL STAGES

Prominent spontaneous activity can be observed in the neocortex at surprisingly early developmental stages, both at the single cell level as well as at the network level (**Figure 2**). Spontaneous calcium transients have been reported in mouse neocortical slices already at embryonic day (E) 16 (Corlew et al., 2004), i.e., 4–5 days before natural birth of the mouse and at a time point when the six neocortical layers have not even been formed. This low frequency (<1 min^{-1}) activity is correlated between a large set of neurons, is sensitive to tetrodotoxin (TTX, a blocker of voltage-gated sodium channels), and relies on voltage-gated calcium channels, indicating that electrical activity with subsequent calcium influx is essential for its occurrence (Corlew et al., 2004). At this developmental stage spontaneous calcium transients can also be observed in the proliferative epithelium of the VZ (**Figure 2A**). These calcium transients are independent of electrical activity, glutamate or GABA receptors and require calcium release from intracellular stores (Owens et al., 2000). They are most probably representing spatially restricted, slowly propagating calcium waves, which are mediated by connexin hemichannels and purinoceptors (Weissman et al., 2004).

Already at the first postnatal day (P0), a repertoire of large scale network events appear in the immature neocortex. The so-called cortical early network oscillations (cENOs)

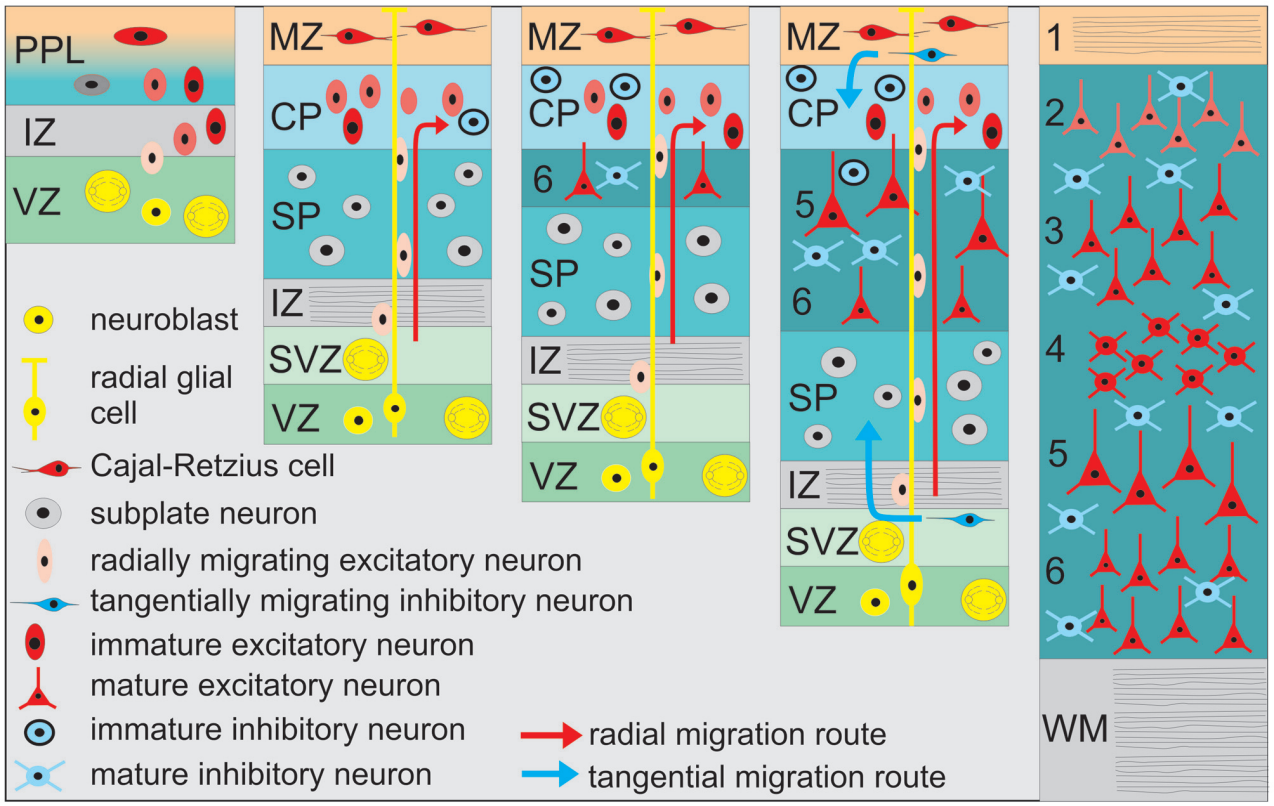

FIGURE 1 | Schematic diagram illustrating the basic principles of neocortical development. The earliest cohort of generated neurons forms the primordial plexiform layer (PPL), which includes Cajal-Retzius (CR) and subplate neurons (SPNs). Later generated neurons migrate along the processes of radial glial cells and split the PPL into the superficial marginal zone (MZ) and the SP. Later born neurons migrate toward the pial surface and detach from radial glial processes at the border of the MZ into the cortical plate (CP), thus establishing the inside first—outside last orientation of the neocortex. IZ, Intermediate zone; VZ, Ventricular zone; SVZ, Subventricular zone; WM, White matter. Neocortical layers are numbered by 1–6.

have been found in neocortical slice preparations of newborn rats (**Figure 2C**). These spontaneous and TTX-sensitive cENOs usually start in the posterior cerebral cortex, occur approximately every 2 min and propagate with ~2 mm/s over the whole cortex to the anterior pole (Garaschuk et al., 2000). At all ages cENOs are completely and reversibly blocked by AMPA and NMDA receptor antagonists. Using large acute brain slice preparations Namiki et al. (2013) demonstrated that a comparable activity pattern in P1–P6 rats is triggered by a population of L3 neurons, that are autonomously active. Spontaneous calcium waves (reflecting the correlated activity of a few thousand cells, and resembling the *in vitro* cENOs) have been also observed *in vivo* under non-anesthetized conditions (Adelsberger et al., 2005).

Using calcium imaging in combination with single-cell and field potential recordings, Allène et al. (2008) demonstrated by using somatosensory neocortical slices from E20 to P9 rats that cENOs are developmentally followed by another distinct spontaneous activity pattern, the so-called cortical giant depolarizing potentials (cGDPs, **Figure 2E**). They appear around P4–P5 and differ from cENOs by: (1) their higher occurrence (~8 min^{-1}); (2) their substantially faster kinetics; and (3) they are only partially affected by AMPA/NMDA receptor antagonists, but depend mainly on depolarizing $GABA_A$ receptor-mediated transmission (Allène et al., 2008). GDPs have been initially observed and extensively studied in the hippocampus of newborn rodents (Ben-Ari et al., 1989), and later also in hippocampal slices from fetal monkeys (Khazipov et al., 2001; for review see Ben-Ari, 2014). As a conclusion it has been postulated that, in the neocortex, spontaneous cGDPs synchronize localized neuronal assemblies.

At a later developmental stage, at around P10–P11, so-called slow activity transients (SATs) have been recorded in the visual cortex of non-anesthetized rats before eye opening (Colonnese and Khazipov, 2010). SATs are long (~10 s) and large [>1 mV in local field potential (LFP) recording] spontaneous events produced by the summation of rapid oscillatory bursts (15–30 Hz). In the cerebral cortex SATs spread horizontally and locally synchronize network activity via the rapid oscillations. SATs have been also recorded with direct current (DC) coupled EEG recordings from sleeping preterm human babies (Vanhatalo et al., 2005). These SATs, which in humans are mostly confined to the prenatal stage, are slow and large (up to 0.8 mV) voltage deflections which nest oscillatory activity up to 30 Hz (Tolonen et al., 2007).

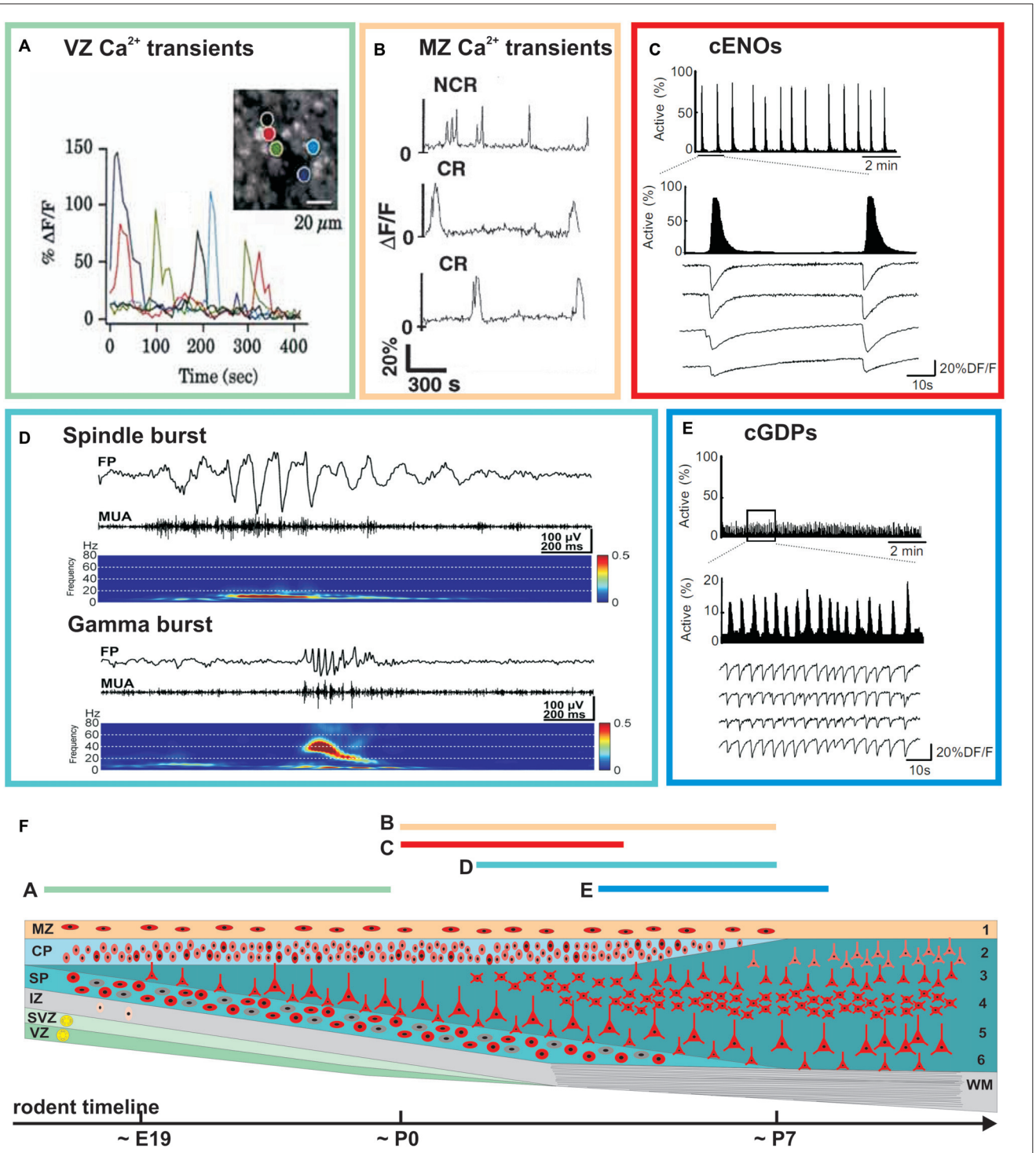

FIGURE 2 | Examples of spontaneous activity patterns at specific early ontogenetic stages (A–E) and schematic illustration of the developmental trajectory (F). Rodent timescale included below based on Ignacio et al. (1995). The approximate occurrence of these events is indicated by the color-coded bars and corresponding letters. **(A)** Correlated and uncorrelated slow calcium transients occurring in the VZ of a E15 rat cortex (modified from Owens et al., 2000). **(B)** Spontaneous calcium transients of CR and non-Cajal-Retzius (NCR) neurons in the MZ of a postnatal rat neocortex (modified from Schwartz et al., 1998). **(C)** Calcium imaging reveals spontaneous cortical early network oscillations (cENOs) in P3 rat neocortical neurons. **(D)** Both spindle and gamma bursts occurring spontaneously in the somatosensory cortex (S1) of a P3 rat (modified from Yang et al., 2009). **(E)** In P6 rat neocortical slices, cENOs are replaced by cortical giant depolarizing potentials (cGDPs; **C** and **E** modified from Allène et al., 2008). See main text for details.

Whereas the events described so far mainly cover activity patterns which propagate over large-scales, *in vivo* electrophysiological recordings from rodent cortex revealed local and distinct spontaneous activity patterns synchronizing only confined neuronal networks (**Figure 2D**). A typical example of such local spontaneous activity in the newborn (∼P3) rodent cerebral cortex are gamma oscillations, which appear spontaneously every 10–30 s, have a duration of 100–300 ms and a frequency of 30–40 Hz (Yang et al., 2009; Minlebaev et al., 2011; for review see Khazipov et al., 2013). Gamma oscillations are restricted to local functional columns. Inhibitory synaptic transmission plays only a minor role in their generation, suggesting that they are functionally distinct from the typical gamma oscillation observed in mature neocortex (Khazipov et al., 2013). A second typical pattern is the spindle bursts, i.e., local and short network oscillations in a frequency range of 10–20 Hz (**Figure 2D**), which have been recorded in visual and somatosensory cortical areas of newborn rats (Khazipov et al., 2004; Hanganu et al., 2006; Minlebaev et al., 2007; Yang et al., 2009, 2013; for review see Yang et al., 2016). They occur spontaneously every ∼10 s and have a duration of 0.5–3 s.

In addition to these activity patterns described mainly in the cortical plate (CP) and/or cortical layers 2–6, correlated network activity has been also found in the MZ of rodent perinatal neocortex (**Figure 2B**). In this layer spontaneous calcium transients occur at a low frequency (<0.5 min^{-1}) and are uncorrelated, but yet reveal an underlying network of connected neurons (Schwartz et al., 1998). The correlation between individual neurons is abolished in the presence of TTX and by inhibition of AMPA, NMDA or GABA-A receptors (Aguiló et al., 1999).

In summary, electrophysiological and imaging studies in different neocortical areas of various mammalian species have revealed a rich repertoire of spontaneous activity patterns, which are present during distinct phases of late prenatal and early postnatal development (**Figure 2F**). Notably, these patterns mostly develop from repetitive activity (cENOs and cGDPs) to more complex activity motives. In the next paragraphs, we will review our current understanding on the cellular elements and the mechanisms underlying these neocortical activity patterns.

CELLULAR ELEMENTS UNDERLYING SPONTANEOUS NEOCORTICAL ACTIVITY AT PRENATAL AND EARLY POSTNATAL STAGES

A number of reports identified specific neuronal populations that are suited to generate these spatially and temporally distinct activity patterns in the developing neocortex. Spontaneous calcium transients in the proliferative epithelium of the VZ shown in acute cortical preparations from embryonic rats and mice are probably generated in proliferating radial glial cells (Owens et al., 2000; Weissman et al., 2004). Already at E16 some neurons in the mouse neocortex show high-frequency repetitive action potential discharges and spontaneous glutamatergic and GABAergic synaptic inputs (see Figure 4 in Kilb et al., 2011). Such early pioneer populations may underlie the occurrence of TTX-sensitive activity transients in the embryonic neocortex (Corlew et al., 2004).

The cellular mechanisms underlying the activation of the MZ has been studied in tangential slice and whole-hemisphere preparations of newborn rat cerebral cortex using optical imaging and patch-clamp recordings. These experiments showed that the major neuronal class involved in these activity transients are CRNs (Schwartz et al., 1998; Aguiló et al., 1999). Pharmacological experiments revealed that the correlation between individual CRNs observed during spontaneous calcium transients is abolished in the presence of TTX and by inhibition of AMPA, NMDA or GABA-A receptors, indicating their dependence on synaptic transmission (Aguiló et al., 1999). On the other hand, the frequency of these spontaneous calcium transients is unaffected by TTX or inhibition of GABA and glutamate receptors (Schwartz et al., 1998; Aguiló et al., 1999), suggesting that additional mechanisms drive these events. One possible candidate is the nonsynaptically released neurotransmitter taurine, which has been shown to mediate the propagation of excitation in the MZ via glycine receptors (Qian et al., 2014) and which excites CRNs (Kilb et al., 2002). A similar role of taurine has been described in the CP, where taurine selectively excites GABAergic interneurons in the CP, which enhance network excitability by excitatory GABAergic postsynaptic potentials (Sava et al., 2014). However, as the synaptic targets of neocortical CRNs have not been functionally identified yet (Kirischuk et al., 2014), the role of CRNs in the generation or transmission of spontaneous activity remains unclear.

Another transient cell population which drives the activity in the developing neocortical network are SPNs. It is well documented in different species and in various sensory neocortical areas that during early development SPNs receive a strong thalamocortical synaptic input mediated by AMPA and NMDA receptors (Friauf et al., 1990; Friauf and Shatz, 1991; Hanganu et al., 2001, 2002; Hirsch and Luhmann, 2008; Zhao et al., 2009). Furthermore, SPNs receive different neuromodulatory (e.g., cholinergic) inputs and are well integrated in a dense network of intracortical connections (for review see Kanold and Luhmann, 2010). Activation of nicotinic receptors (Hanganu and Luhmann, 2004), muscarinic receptors of predominantly m1/m5-subtype (Hanganu et al., 2009), glycine receptors (Kilb et al., 2008) and GABA-A receptors (Hanganu et al., 2001, 2002) causes an excitation of SPNs, which may elicit a local and transient network oscillation in a frequency range of 10–20 Hz (Dupont et al., 2006; Hanganu et al., 2009). This activity pattern, related to spindle bursts at the network level, depends on an intact functional SP (Dupont et al., 2006; Yang et al., 2009; Tolner et al., 2012). Since SPNs neurons are capable to intrinsically discharge at 10–20 Hz when activated by cholinergic mechanisms (Hanganu et al., 2009) and are electrically coupled in a columnar manner to neighboring and developing CP neurons via neuronal, connexin-36 containing gap junctions (Dupont et al., 2006),

it has been suggested that SPNs act as local amplifiers of this early cortical activity (for review see Luhmann et al., 2009). The interplay between phasic and tonic synaptic activation elicits a 10–20 Hz oscillatory response in SPNs, which synchronizes the activity of a local, columnar network, thereby producing spindle bursts.

GENERATION OF SPONTANEOUS NEOCORTICAL ACTIVITY—MORE THAN ONE CIRCUIT AND ONE BRAIN REGION!

The question how and where early neocortical activity patterns are generated is still debated. Clearly, the mechanisms underlying network activity critically depend on the developmental stage, and in rodents, which show a fast development during the perinatal phase, the mechanisms of activity generation change within 2–3 days (for review see Allene and Cossart, 2010; Kilb et al., 2011). Synchronized network activity may either be triggered by a specific brain region, circuit or a discrete subset of pacemaker neurons, or may emerge during early development as an intrinsic property of the network.

Pacemaker properties within cortical networks have been identified in slice cultures of the mouse neocortex, which reveal synchronized spontaneous activity propagating as a wave from a ventrolateral pacemaker region (Lischalk et al., 2009). However, activity originating from this ventrolateral region is itself often triggered by preceding activity in the septal nuclei (Conhaim et al., 2010). These spontaneous waves occur in organotypic slice cultures between E18 and P12 and show prominent developmental changes in their transmitter dependence and propagation patterns (Conhaim et al., 2011). Both *in vitro* and *in vivo* studies have shown that synchronized spontaneous activity in the neocortex during the late embryonic and early postnatal phase often depends on gap junctional coupling (Yuste et al., 1995; Kandler and Katz, 1998; Owens and Kriegstein, 1998; Sun and Luhmann, 2007; Yang et al., 2009) with a contribution of neuronal, connexin-36 containing electrical synapses (Dupont et al., 2006; Wagner and Luhmann, 2006; Hanganu et al., 2009; for review see Uhlén et al., 2015). A role of gap junctions, including connexin-36, has also been demonstrated recently in the generation of spontaneous depolarizations in human fetal cortex during the second trimester of gestation (Moore et al., 2014). Computational studies have shown that gap junction-coupled networks can produce a wide range of spontaneous activity patterns (Kepler et al., 1990; Sherman and Rinzel, 1992; Bennett and Zukin, 2004; Tseng et al., 2008; Uhlén et al., 2015). However, for the interpretation of experimental studies using gap junction blockers it should be noted that some compounds are not very specific and have a number of side effects, such as inhibiting NMDA receptors (Chepkova et al., 2008) or voltage-gated calcium channels (Vessey et al., 2004).

It remains to be studied in more detail whether developing neocortical neurons possess intrinsic pacemaker properties to generate and drive distinct synchronized activity patterns (Luhmann et al., 2003; Sun et al., 2012). Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels constituting the molecular substrate of hyperpolarization-activated current (I_h) could potentially fulfil an important functional role (for review see Bender and Baram, 2008), as e.g., clearly demonstrated for thalamic relay neurons expressing HCN channels (for review see Pape, 1996).

Some early generated neocortical neurons may have the intrinsic capability to function as pacemakers. CRNs in newborn rodent cerebral cortex are functionally characterized by a prominent I_h (Kilb and Luhmann, 2000) and a low voltage-activated calcium channel (Kirmse et al., 2005). However, CRNs are insufficiently connected with other neocortical neurons to drive and synchronize early activity patterns (for review see Kirischuk et al., 2014; Luhmann et al., 2014). Further, in CRNs I_h does not contribute to spontaneous membrane potential shifts (Kilb and Luhmann, 2000). More likely, highly connected neurons with widespread axonal connectivity play a key role in synchronizing the activity of developing neocortical neurons. Such hub neurons, consisting of a subpopulation of GABAergic interneurons, have been found in developing hippocampal networks (Bonifazi et al., 2009; for review see Cossart, 2014). In developing cultured neocortical networks, synchronous oscillatory activity is driven by highly connected, large GABAergic preplate neurons, resembling SPNs (Voigt et al., 2001). Experimental data obtained from *in vitro* slice and *in vivo* preparations indicate that SPNs are capable to drive early synchronized network in newborn rodent cortex (Dupont et al., 2006; Hanganu et al., 2009; Tolner et al., 2012; Moore et al., 2014). SPNs may "amplify" their synaptic inputs and transmit the resulting oscillatory burst activity in a local columnar manner to the developing CP above (for review see Luhmann et al., 2009; **Figure 3**). Experiments in which the ablation of the SP by p75-immunotoxin abolished spontaneous spindle bursts and the columnar organization of the barrel cortex provided additional evidence for a causal role of SPNs in the generation of spontaneous oscillations and the neocortical architecture (Tolner et al., 2012).

However, SPNs are probably not the generators of the spontaneous neocortical activity, as demonstrated in diverse species and different cortical areas. For example, *in vivo* recordings in newborn rats have shown that spindle bursts in visual cortex are driven by spontaneous activity in the retina, the so-called retinal waves (Hanganu et al., 2006). Similar results have been obtained in the barrel cortex of newborn rats *in vivo*, where spontaneous spindle bursts and gamma oscillations are both reduced in their occurrence by about 50% when the electrical activity in the sensory periphery is silenced by injection of lidocaine into the contralateral whisker pad (Yang et al., 2009). Not only in newborn rats, but also in premature human neonates the spontaneous activity in sensory cortical areas is driven by activity of the sensory periphery (for review see Colonnese and Khazipov, 2012). SATs synchronize 87% of the spontaneous activity in the visual cortex and are eliminated following enucleation (Colonnese and Khazipov, 2010), providing further support that retinal waves drive the spontaneous activity in the very immature visual cortex. In mice, Zhang et al. (2011) used optogenetic techniques to directly

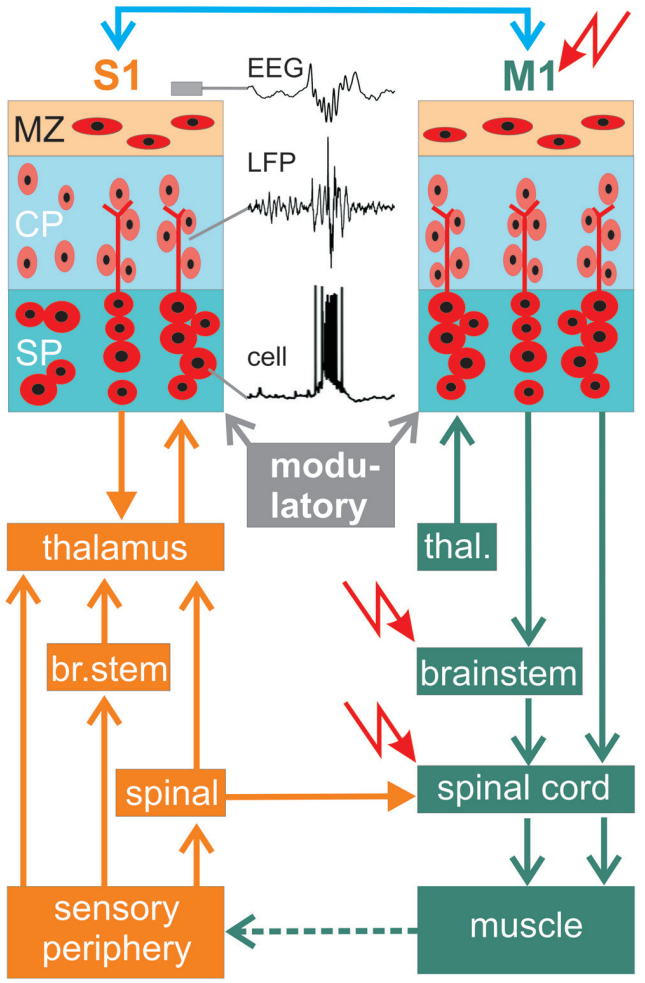

FIGURE 3 | Summary diagram showing connectivity between primary motor cortex (M1) and a primary sensory cortex area (here primary somatosensory cortex, S1) during early development. Location of potential central pattern generators (CPGs) are indicated by ⚡. Traces in S1 illustrate spontaneous activity of a SP neuron, local field potential (LFP) activity in the CP and EEG recording on the cortical surface. Blue line at top indicates reciprocal corticocortical connections between S1 and M1.

manipulate retinal activity *in vivo* and demonstrated that the synchrony and precise temporal pattern of retinal activity is required for the development of eye-specific segregation and retinotopy. It is less clear whether activity in the sensory periphery also triggers early network activity in the auditory cortex. Inner hair cells in the cochlea of mice can generate spontaneous calcium action potentials as early as E17 (Marcotti et al., 2003). This early spontaneous activity of cochlear hair cells is triggered by a fluid secretion mechanism in adjacent glia-like support cells (Wang et al., 2015). The output of neighboring inner hair cells is synchronized via ATP-dependent signaling and triggers a theta-like burst of action potentials in auditory nerve fibers (Tritsch et al., 2007). *In vivo* recordings in the auditory brainstem suggest that inner hair cells act as pacemakers for spontaneous burst activity in more central auditory neurons before hearing onset (Tritsch et al., 2010). Taken together, these observations indicate that a substantial proportion of spontaneous activity observed in the developing sensory neocortex is caused by events in the sensory periphery or intermediary relay stations.

The activity from the sensory periphery (retina, cochlea) is transmitted to the cortex via direct connections to the thalamus (e.g., retino-geniculate in the visual system) or indirectly via the midbrain or brainstem (as in the auditory and somatosensory system, respectively; **Figure 3**). However, spontaneous activity arising from the sensory periphery lacks rapid oscillations, suggesting that the typical frequency of spindle bursts and gamma oscillations arise centrally. In the somatosensory system of newborn rodents, both activity patterns with their typical frequencies can be recorded already in the thalamus and synchronize a single thalamic barreloid with the corresponding neocortical barrel (Minlebaev et al., 2011; Yang et al., 2013). A corticothalamic feedback loop then modulates the thalamic network activity as demonstrated in newborn rats (Yang et al., 2013) and developing ferrets (Weliky, 1999).

Whereas the origin of spontaneous activity in the visual and auditory cortices may arise in the retina and sound-insensitive cochlea, respectively, the situation in the somatosensory system is more complex, because somatosensation (e.g., pain perception) is already present at birth (Mazzuca et al., 2011) and somatosensory activity is closely associated with motor activity. Newborn rats show during active (or REM) sleep, a behavioral state that predominates in early human infancy, hundreds of thousands of skeletal muscle twitches each day, which may be triggered by spontaneous activity in the spinal cord, brainstem or motor cortex (M1). The somatosensory feedback resulting from these spontaneous movements subsequently activates the somatosensory cortex (S1; **Figure 3**). In the somatosensory system of the neonatal rat *in vivo*, spatially confined spindle bursts in primary S1 are selectively triggered in a somatotopic manner by spontaneous muscle twitches (Khazipov et al., 2004). In human fetal development, spontaneous movements can be observed by ultrasound *in utero* already at the beginning of the second trimester, at a time point when the neocortical network has not been formed and is governed by a prominent SP (for review see Kostovic and Judas, 2010; Judaš et al., 2013). As an experimental model to study the development and the relationship between the sensory periphery and the cerebral cortex the whisker-to-barrel-cortex pathway in rodents proved to be most valuable, since here each whisker is represented in a somatotopic manner in the contralateral barrel cortex (for review see Feldmeyer et al., 2013). Rapid and asynchronous whisker movements in neonatal rats appear during active sleep and are related to cortical barrel-specific activity (Tiriac et al., 2012, 2014). These spontaneous whisker twitches appear before they are needed for behavior (active whisking during exploration) and are generated by central pattern generators (CPGs) located in the spinal cord, brainstem or primary M1 (red arrows in **Figure 3**).

Spontaneous activity is already present in the embryonic spinal cord and characterized by highly rhythmic episodes

of motor neuron bursting activity that may drive muscle contractions. In the spinal cord of the mouse, spontaneous activity appears at E12.5, in the rat at E13 (for review see Moody and Bosma, 2005). During early stages, spontaneous activity depends on the excitatory action of GABA and glycine in embryonic spinal cord networks (for review see Sibilla and Ballerini, 2009). At this stage, developing spinal motor circuits are highly sensitive to the frequency and pattern of spontaneous activity, and drugs that alter this activity may cause developmental defects (Kastanenka and Landmesser, 2010). The excitatory action of both neurotransmitters is developmentally downregulated by a decrease in the expression levels of the neuron-specific potassium-chloride co-transporter type 2 (KCC2), reducing the intracellular chloride concentration and leading to a shift of equilibrium potential for chloride ions towards more negative values (Stil et al., 2011). Beside the spinal cord, the brainstem is another candidate to function as a potential CPG for spontaneous motor activity and movements during embryonic development (for review see Nakamura and Katakura, 1995; **Figure 3**). Facial motor neurons evoking rhythmic whisker movements have been detected in the brainstem and are part of a whisking CPG (Hattox et al., 2003). These whisking motor neurons receive synaptic inputs from the whisker representation in neocortical M1 (Hattox et al., 2002; Haiss and Schwarz, 2005; Cramer and Keller, 2006; Friedman et al., 2006), suggesting that more than one circuit and more than one brain region control the motor activity and the generation of movements. Interestingly, in this network electrical stimulation of even a single pyramidal cell in L5 of M1, or a single neuron in the facial nucleus, can elicit whisker movements (Brecht et al., 2004). Whereas activity in M1 activates brainstem reticular nuclei containing whisker premotor neurons mediating whisker protraction, activity in S1 excites premotor neurons in brainstem spinal nucleus trigeminalis interpolaris inducing a whisker retraction (Matyas et al., 2010; for review see Petersen, 2014).

However, these observations on the motor control of whisker movements have been obtained in adult rodents and it is less clear whether the M1 can elicit movements and subsequently an activation of the somatosensory system also in neonatal cortex. Anatomical studies in rodents have demonstrated the presence of corticomotor neuronal projections to the spinal cord before birth, but whether M1 drives muscle activity (e.g., in the form of twitches) in newborns is less clear. Direct electrophysiological proof for the functional role of this pathway comes from *in vivo* studies on newborn rats. Focal electrical stimulation of L5 in M1 at frequencies resembling the gamma oscillations (40 Hz) and spindle bursts (10 Hz) reliably elicited movements (An et al., 2014). About one quarter of the spontaneous gamma oscillations and spindle bursts in M1 triggered movements, which subsequently elicited gamma and spindle activity in S1 (An et al., 2014). This activity is most likely generated by the M1—brainstem/spinal cord—peripheral sensor—S1 pathway (**Figure 3**). A substantial proportion (∼40%) of the spontaneous movements preceded the activity in M1 and blockade of the periphery by local lidocaine injection reduced the occurrence of gamma and spindle bursts by ∼40% (An et al., 2014), indicating that motor-sensory interactions contribute to gamma and spindle burst activity in M1 of newborn rodents.

It has been shown in the mouse whisker system that the functional interaction between M1 and S1 does not depend on thalamic connections (Zagha et al., 2013), suggesting direct corticocortical connections between M1 and S1 (blue in **Figure 3**). Neuroanatomical tracing studies have demonstrated reciprocal connections between S1 and M1 (Aronoff et al., 2010; Mao et al., 2011). In preterm neonates simultaneous EEG and EMG measurements followed by Granger causality analysis have shown that M1 drives muscle activity, suggesting that corticomuscular communication in humans begins to develop during the late prenatal and neonatal stage (Kanazawa et al., 2014). As in developing neuronal networks of rodents, it is suggested that spontaneous activity in immature human cerebral cortex at some time point also depends on a high intracellular chloride concentration and an excitatory GABA action. SATs in human cerebral cortex show a decline by the time of normal birth. In age-matched fetal brain tissue, this decrease in SATs is correlated with a developmental up-regulation of KCC2 (Vanhatalo et al., 2005). Whether GABA may also have an inhibitory action at this early developmental stage, as recently suggested by Kirmse et al. (2015), remains to be studied in more detail in newborn awake animals by the use of non-invasive techniques addressing the influence of GABA on single cell firing and on network activity.

PHYSIOLOGICAL ROLE OF SPONTANEOUS ACTIVITY AND PATHOLOGICAL CONSEQUENCES OF DISTURBANCES IN EARLY NETWORK ACTIVITY

An increasing amount of experimental data strongly indicates that spontaneous activity during very early ontogenetic stages is not simply an epiphenomenon of developing neuronal networks when they become electrically active, but rather plays important roles in various physiological processes in embryonic and early postnatal neocortical networks. One of the first developmental processes influenced by spontaneous neuronal activity is neurogenesis. Spontaneous retinal waves drive synchronized activity in the embryonic visual cortex of mice and modulate corticogenesis. Pharmacological inhibition of retinal waves increases neurogenesis and causes alterations in neocortical layering (Bonetti and Surace, 2010). Calcium waves in neuroproliferative radial glial cells in the VZ may be also directly involved in the regulation of neurogenesis, as: (i) the number of cells involved in them as well as the frequency and amplitude of calcium waves directly correlate to the proliferation in the VZ; and (ii) suppression of calcium waves drastically reduces proliferation in the VZ (Weissman et al., 2004). Further, even very basic morphogenic factors like sonic hedgehog are directly regulated by electrical activity (Belgacem and Borodinsky, 2015). Finally, the neurotransmitter identity of neurons can also be altered by electrical activity (Borodinsky

et al., 2004). These examples indicate, that electrical activity strongly influences the generation and identity of neurons (for review see Kilb et al., 2011; Yamamoto and López-Bendito, 2012).

The overall number of neurons in the brain is determined by the number of generated neurons in relation to the number of dying neurons. Cell death is a fundamental physiological process in the developing brain (for review see Kuan et al., 2000) and is also modified by electrical activity. Already 6 h of spontaneous activity blockade induces a 2.5-fold increase in the number of neurons undergoing programmed cell death in neocortical cultures and organotypic slice cultures (Heck et al., 2008). Pharmacological interference with glutamatergic receptors has a similar impact on apoptosis, and blockade of GABA-A receptors causes a ∼50% increase in cell death (Ikonomidou et al., 1999; Heck et al., 2008). Drugs that increase GABA-A receptor function (ethanol, antiepileptic drugs) also cause a prominent rise in apoptosis during early development (Ikonomidou et al., 2000; Ikonomidou and Turski, 2010). Lebedeva et al. (2015) recently demonstrated in rats that between P4 and P7, at the peak of ethanol-induced apoptosis, ethanol not only strongly suppressed spontaneous gamma and spindle bursts, but also sensory-evoked bursts and motor activity. These data indicate that any drug that modifies physiological activity patterns during early development may have an immediate impact on apoptosis.

The effect of GABA on apoptosis is cell type specific, as recent experiments revealed that inhibition of depolarizing GABAergic responses prevented apoptosis of CR neurons, while death rates of CP neurons were unaltered under this condition (Blanquie et al., 2016). Direct proof that the pattern of spontaneous activity controls the extent of programmed cell death comes from *in vitro* studies using multi-electrode arrays (MEAs) to record the activity in developing neocortical cultures. A reduction or delay in caspase-3 dependent apoptosis and an overall increase in neuronal survival could be observed in cultures showing high-frequency burst activity (Golbs et al., 2011), indicating that the physiological activity patterns observed in perinatal cerebral cortex *in vivo* have an impact on the control of cell survival vs. cell death. Brain-derived neurotrophic factor (BDNF) and activation of phosphatidylinositol 3-kinase (PI3K) and its downstream effector Akt are key downstream elements in the activity-dependent control of apoptosis (Wagner-Golbs and Luhmann, 2012). But also other survival-promoting pathways can be activated depending on the pattern of electrical activity or the site of calcium entry (for review see Hardingham and Bading, 2010; Bell and Hardingham, 2011). The ratio between cell death and cell survival determines brain volume. Studies in the developing human brain using EEG and quantitative magnetic resonance imaging have documented that an increased brain activity in the first postnatal days correlates with a faster growth of brain structures during subsequent months until term age. Particularly subcortical structures grew faster in babies with more SAT events (Benders et al., 2015).

Neuronal migration is another important process occurring in the cerebral cortex mostly during embryonic and early postnatal development (dependent on the species). Neuronal migration in the developing neocortex depends critically on the appropriate level of spontaneous activity (Bando et al., 2016). Spontaneous rhythmic intracellular calcium transients control neuronal migration (for review see Komuro and Kumada, 2005) and the two main cortical neurotransmitters, GABA and glutamate, have both a strong influence on migration (for review see Luhmann et al., 2015). The growth and differentiation of neuronal dendrites and axonal projections is also influenced by spontaneous activity (for review see Chen and Ghosh, 2005; Zheng and Poo, 2007; Yamamoto and López-Bendito, 2012). In neocortical cultures spontaneous synchronous network activity converts within a few minutes silent synapses to active synapses by incorporating AMPA receptors into the postsynaptic membrane (Voigt et al., 2005). Although a complex spatio-temporal pattern of transcription factors and intercellular communication mediated by constitutive secretion of transmitters or growth factors play an important role in the early development of thalamocortical axonal connections (for review see Molnár et al., 2003), spontaneous activity clearly contributes to the formation and refinement of topographic maps (for review see Hanganu-Opatz, 2010). A close interaction between spontaneous electrical activity and the expression of transcription factors has been demonstrated in the embryonic spinal cord, where blocking or slowing of bursting activity induces a downregulation of LIM homeodomain transcription factors (Hanson and Landmesser, 2004). In the mouse retinotectal system spontaneous retinal activity controls ephrinA-mediated responses and is required for the development of the retinotopic map and the elimination of exuberant retinal axons (Nicol et al., 2007).

Blocking spontaneous retinal activity in ferrets, which are born in a very immature state, during very early stages of development (P1–P10) caused a persistent disorganization of ocular dominance columns and a pronounced increase in receptive field size of neurons in primary visual cortex (Huberman et al., 2006). These data suggest that spontaneous retinal activity present before the onset of vision is required for the normal development of the columnar architecture (for review see Ackman and Crair, 2014). In newborn rat S1, spindle bursts and gamma oscillations originating from thalamic relay neurons synchronize local neocortical network in functional pre-columns (Minlebaev et al., 2011; Yang et al., 2013), indicating that these patterns of early activity might play an instructive role for the generation of the neocortical columnar architecture. This suggestion was corroborated by the observation that the attenuation of oscillatory activity after an ablation of SPNs affect the formation of barrels in the S1 (Tolner et al., 2012).

Neuronal activity, including spontaneous and sensory evoked activity, directly regulates axon myelination (Demerens et al., 1996; Barrera et al., 2013). This effect is partly mediated by an activity dependent differentiation of oligodendrocyte precursor cells via activation of adenosine receptors (Stevens et al., 2002). In addition, a vesicular, extrasynaptic release of the neurotransmitter glutamate from active axons can directly

induce myelin formation in differentiated oligodendrocytes (Wake et al., 2015), via an NMDA receptor mediated and Fyn-kinase dependent release of translational repression (White and Krämer-Albers, 2014). Finally, in the early postnatal period it was recently shown that peripheral-driven neuronal activity also regulates vessel development and patterning in the developing rodent brain (Lacoste et al., 2014; Whiteus et al., 2014).

Further evidence for an important function of spontaneous activity in developing neocortical networks also comes from clinical studies. In humans, abnormal neocortical activity patterns recorded during perinatal stages predict the further development and the outcome in the following years. In extremely low gestational age infants, abnormalities in magneto-encephalography recorded somatosensory evoked magnetic fields at term age are associated with adverse neurodevelopment at 2 years of age (Rahkonen et al., 2013). Recently, Vanhatalo et al. (2005) have demonstrated that spontaneous bursts recorded with EEG in preterm infants exhibit scale-free properties and provide prognostic value of brain activity in the subsequent days (Iyer et al., 2015). A pilot study from the same group addressed the important question whether SATs in preterm babies are affected by drugs (phenobarbital, fentanyl, theophylline) that are routinely used in neonatal intensive care units. Although the visual EEG interpretation did not reveal any drug effects, advanced time-series analyses demonstrated that all drugs examined had an effect on spontaneous brain activity and may interfere with further development (Malk et al., 2014). In this context it is intriguing to note that both *in vitro* and *in vivo* experiments in rodents demonstrated that experimentally induced inflammation induces a rapid modification in the properties of spontaneous (spindle and gamma burst) activity, which subsequently causes an increase in programmed cell death (Nimmervoll et al., 2013). This study provides first evidence that altered neuronal activity may also contribute to the deleterious effects of inflammatory events in the immature human brain (Hagberg and Mallard, 2005).

CURRENT CHALLENGES AND FUTURE DIRECTIONS

Spontaneous activity in immature neocortical networks plays important roles during early and subsequent development. Although experimental animal studies and clinical data from preterm infants provided over the last decade a large amount of interesting and important results, a number of key questions remain to be addressed in the near future:

1. Does spontaneous neocortical activity fulfill a more general role in controlling activity-dependent processes or does a specific activity pattern have a distinct role during a certain stage of development in controlling a specific process?
2. What is the impact of disturbances in spontaneous activity caused by genetic, intrinsic (e.g., intrauterine infection, inflammation, hypoxia) or extrinsic factors (e.g., maternal medication or drug abuse) on different activity-dependent processes during corticogenesis? What are the long-term effects of these disturbances?
3. What are the characteristics of the normal spontaneous neocortical activity in the EEG recorded from full-term neonates and preterm infants? Which parameters can be extracted from the EEG to evaluate and quantify the spontaneous activity patterns? We need this information to evaluate the (patho-)physiological neuronal state of newborn and particular preterm babies during critical stages of their development.

AUTHOR CONTRIBUTIONS

HJL, AS, J-WY, VR-P, MCS, SK and WK wrote the article and generated the figures.

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Cardiac Arrest-Induced Global Brain Hypoxia-Ischemia during Development Affects Spontaneous Activity Organization in Rat Sensory and Motor Thalamocortical Circuits during Adulthood

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Normal maturation of sensory information processing in the cortex requires patterned synaptic activity during developmentally regulated critical periods. During early development, spontaneous synaptic activity establishes required patterns of synaptic input, and during later development it influences patterns of sensory experience-dependent neuronal firing. Thalamocortical neurons occupy a critical position in regulating the flow of patterned sensory information from the periphery to the cortex. Abnormal thalamocortical inputs may permanently affect the organization and function of cortical neuronal circuits, especially if they occur during a critical developmental window. We examined the effect of cardiac arrest (CA)-associated global brain hypoxia-ischemia in developing rats on spontaneous and evoked firing of somatosensory thalamocortical neurons and on large-scale correlations in the motor thalamocortical circuit. The mean spontaneous and sensory-evoked firing rate activity and variability were higher in CA injured rats. Furthermore, spontaneous and sensory-evoked activity and variability were correlated in uninjured rats, but not correlated in neurons from CA rats. Abnormal activity patterns of ventroposterior medial nucleus (VPm) neurons persisted into adulthood. Additionally, we found that neurons in the entopeduncular nucleus (EPN) in the basal ganglia had lower firing rates yet had higher variability and higher levels of burst firing after injury. Correlated levels of power in local field potentials (LFPs) between the EPN and the motor cortex (MCx) were also disrupted by injury. Our findings indicate that hypoxic-ischemic injury during development leads to abnormal spontaneous and sensory stimulus-evoked input patterns from thalamus to cortex. Abnormal thalamic inputs likely permanently and detrimentally affect the organization of cortical circuitry and processing of sensory information. Hypoxic-ischemic injury also leads to abnormal single neuron and population level activity in the basal ganglia that may contribute to motor dysfunction after injury. Combination of deficits in sensory and motor thalamocortical circuit function may negatively impact

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sensorimotor integration in CA survivors. Modulation of abnormal activity patterns post-injury may represent a novel therapeutic target to improve neurologic function in survivors.

Keywords: spontaneous activity, correlation, ischemic injury, sensory thresholds, developmental period

INTRODUCTION

Acute brain injury (e.g., hypoxia-ischemia due to stroke or cardiac arrest [CA]) disrupts sensory processing, motor function, cognition and behavior in survivors (Volpe et al., 1984; Graves et al., 1997; Schreckinger et al., 2007). Injury-induced alterations include inflammation, astrocyte and microglial activation, excitotoxicity, axonal and dendritic remodeling and changes in intrinsic and synaptic neural function (Wong et al., 1993; Barone and Feuerstein, 1999; Werner and Engelhard, 2007; Winship and Murphy, 2008, 2009). While injury-induced structural and functional changes will impact sensory and motor function and sensorimotor integration, the developmental time point at which injury occurs may independently affect functional maturation of neural circuitry. Spontaneous and evoked activities are both critical for normal development; therefore, injury affecting both will disrupt normal development. In order to develop better therapeutic and rehabilitative interventions, we must understand how neural injury during development affects maturation and functional organization of neuronal circuits in adulthood.

Evidence that ongoing spontaneous activity during development exerts a lasting effect on the organization and function of neuronal circuits in adulthood has emerged from both sensory and motor systems. Before the onset of visual experience, retinal ganglion cells fire spontaneous bursts of action potentials that contribute to coherent propagating waves of activity across the retina (Wong et al., 1993). These waves can engage burst-dependent Hebbian synaptic plasticity rules to refine retinogeniculate synapses (Butts et al., 2007). Ongoing spontaneous activity in the brain can predict a large degree of stimulus response variability (Arieli et al., 1996; Petersen et al., 2003) and can act to gate cortical responses to sensory stimuli (Luczak et al., 2013). Spontaneous correlations in inhibitory and excitatory neural activity in the cortex mediate stimulus-response decorrelation via a disynaptic feedforward inhibitory circuit (Middleton et al., 2012). Spontaneous sequences of activity contain signatures of sensory evoked responses (Han et al., 2008; Luczak et al., 2009; Berkes et al., 2011; Eagleman and Dragoi, 2012; Luczak and MacLean, 2012) and sensory activity can stabilize neural assemblies through reinforcement of patterned synaptic connectivity (Litwin-Kumar and Doiron, 2014). In the motor system, corticospinal axons in the spinal cord remodel their arbors in response to changes in activity of primary sensory afferents (Jiang et al., 2016). In the developing motor cortex (MCx), spontaneous gamma and spindle bursts correlate with motor and sensory inputs associated with movement (An et al., 2014).

In this study, we used two previously collected data sets from rats that underwent asphyxial CA during development to explore further the impact of injury on the relationship between spontaneous and evoked activity in the somatosensory system (Shoykhet et al., 2012) and on the variability and firing modes in the thalamic entopeduncular nucleus (EPN) in the motor system (Aravamuthan and Shoykhet, 2015). The EPN is located slightly ventral to the ventrobasal complex containing ventroposterior medial nucleus (VPm) and represents a rodent homolog of human and monkey Globus Pallidus pars interna (GPi). EPN, like GPi, sends output from the basal ganglia to the motor thalamus and the MCx. Abnormal activity patterns in GPi and other basal ganglia nuclei are associated with movement abnormalities in Parkinson's disease and in dystonia. In the somatosensory thalamus, spontaneous activity of thalamocortical neurons correlated with specific aspects of their evoked responses in sham but not in injured rats. In the EPN, CA-induced hypoxia-ischemia followed by months of recovery was still associated with higher variability of spontaneous firing and increased frequency of bursting. Altogether, the findings from our analysis highlight that key features of spontaneous activity organization in sensory and motor brain areas remain persistently altered months after global brain hypoxic-ischemic injury. These alterations may significantly impact development, organization and function of thalamocortical circuit that underlie sensory processing, sensorimotor integration, cognition and behavior.

MATERIALS AND METHODS

The analyses in this manuscript utilize data acquired during two previously published studies (Shoykhet et al., 2012; Aravamuthan and Shoykhet, 2015). The experimental procedures are briefly described below.

Animals

All animal procedures were approved by the Institutional Animal Care and Use Committees at the University of Pittsburgh and at Washington University in St. Louis. For recordings in the VPm, male Sprague-Dawley rats were housed with their mother until postnatal day 30 (P30). Food and water were provided *ad libitum*. A total of 32 rats were used in this study. For neurophysiologic experiments, the rats underwent either CA ($n = 7$) or sham ($n = 9$) intervention at P17–19 (see below, CA and resuscitation in "Materials and Methods" Section). After CA, the rats were further divided into early and late groups. Electrophysiological experiments were performed on these groups 2–3 days and 6–8 weeks, respectively, after injury or sham treatment. The early group consisted of six sham and three injured rats, the late group of three sham and four injured rats. The experimenter was blind to the injury status of the rats

at the time of the recordings and data analyses. There were no grossly observable differences in appearance or behavior between injured and sham rats in either the early or the late groups. Specifically, all rats in the study groomed, whisked, ate and drank. There were no differences in posture or gross locomotion in the cage, and no obvious differences in anesthetic requirement or physiologic parameters during the recordings. For recordings in the EPN and the MCx, adult Long-Evans rats of both sexes were used. There were eight rats each in the CA and sham groups in this experiment. CA and sham procedures at P17–19 were identical to those used in VPm recordings. Rats were allowed to mature until 6–8 months of age prior to EPN/MCx recordings.

Cardiac Arrest and Resuscitation

The model of developmental asphyxial CA has been described in detail previously (Fink et al., 2004). Briefly, P17–19 rats underwent tracheal intubation and placement of femoral artery and venous lines under isoflurane/nitrous oxide anesthesia. The rats were mechanically ventilated under neuromuscular blockade. Arterial blood pressure, electroencephalogram and electrocardiogram were continuously monitored and recorded. Core body temperature was maintained constant at 37°C with a servo-controlled heating blanket. The anesthetic was then briefly washed out with room air, and rats were disconnected from the ventilator. Asystole ensued within 60–90 s of apnea and was allowed to continue for 9 min in rats used for VPm recordings and 9.5 min for rats used in EPN/MCx recordings. The rats were then resuscitated with mechanical ventilation using 100% oxygen, intravenous epinephrine, sodium bicarbonate and manual chest compressions. Upon return of spontaneous circulation, the rats additionally received a 20 ml/kg bolus of 5% dextrose in normal saline intravenously to prevent dehydration. After ∼2–3 h, mechanical ventilation was discontinued, the rats were extubated, arterial and venous lines were removed and wound margins were sutured. All wound margins were infiltrated with lidocaine. The rats were observed for an additional 1 h in a chamber with 100% oxygen to mimic a clinical scenario and then returned to the mother.

Surgical Preparation for Electrophysiological Recordings

Surgical procedures in developing animals have been described previously (Shetty et al., 2003; Shoykhet et al., 2003; Shoykhet and Simons, 2008). Either 2–3 days (early) or at least 6 weeks (late) after asphyxial CA, rats were anesthetized with isoflurane, and underwent: (1) tracheotomy; (2) placement of an external jugular venous and femoral arterial catheters; and (3) craniotomy. A steel post was affixed to the skull with dental acrylic to allow holding the rat's head without pressure points for the remainder of the experiment. The craniotomy (∼2 mm^2) was performed through the skull overlying the appropriate region of interest. Dura mater was left intact. A ground screw was placed through the skull and fixed with dental acrylic. After completions of all surgical procedures, the rat was transferred to the vibration isolation table and placed in a custom-made head holder. Mechanical ventilation using a volume-controlled Inspira ventilator (Harvard Apparatus) was initiated using tidal volumes of ∼8 ml/kg and rates of 100 and 70 breaths/min in younger and older rats, respectively. Neuromuscular blockade was initiated with a bolus dose of pancuronium bromide (∼1 mg/kg) and maintained with a continuous infusion of pancuronium (0.8 $mg \cdot kg^{-1} \cdot h^{-1}$) in 5% dextrose/0.9% sodium chloride for the remainder of the experiment. Isoflurane anesthesia was then discontinued, and the rat was transitioned to fentanyl analgesia using continuous fentanyl infusion at ∼8–10 $\mu g \cdot kg^{-1} \cdot h^{-1}$. At these doses, the rats enter a state of dissociative analgesia without compromise of thalamocortical network dynamics observed under anesthesia (Simons et al., 1992).

The rat's physiologic state during the recording session was continuously monitored as described previously (Shoykhet and Simons, 2008). Briefly, temperature was maintained at 37°C using a servo-controlled heating blanket (Harvard Apparatus) and, in young rats, a 20W DC lamp. Intra-arterial pressure and heart rate (HR) were monitored using a blood pressure monitor (WPI Inc.) connected to the arterial line via a pressure transducer. If mean arterial pressure (MAP) did not remain in the developmentally appropriate range, experiments were discontinued. Adequate analgesia was assured throughout the recording session by monitoring pupillary constriction and by maintaining a lack of MAP and HR elevation in response to gentle touch. At the end of the recording session, the rats were deeply anesthetized with 5% isoflurane in room air and perfused transcardially for cytochrome oxidase (CO) histochemistry. Recording electrode tracks were visible in thionin or Nissl stained brain slices (Aravamuthan and Shoykhet, 2015).

Electrophysiological Recordings

Extracellular recordings were obtained using stainless steel microelectrodes (6–8 ΩM impedance at 1 kHz; FHC, Bowdoinham, ME, USA). The electrode was advanced perpendicularly to the pia using a hydraulic micropositioner (David Kopf Instruments, Tujunga, CA, USA). Entry into VPm was signaled by an abrupt increase in spontaneous and evoked neural activity both in sham and in injured animals. In both groups, VPm was somatotopically organized, individual units were easily isolated and there were no silent zones. Signals were initially bandpass filtered between 300 Hz and 10 KHz and amplified 10- to 100-fold using a DC-powered preamplifier (Grass Instruments). The resulting signal was further filtered using a 60 Hz notch and a bandpass filter (BAK Electronics) and digitized using a PCI-MIO-16E4 data acquisition board (National Instruments) connected to a personal computer.

For EPN and MCx recordings, the rats were transferred from the surgical table to a stereotaxic frame (David Kopf Instruments). Electrodes in MCx were positioned at a 20° angle to pial surface through a craniotomy (1.5 mm rostral to bregma and 3.0 mm lateral to midline) and manually advanced to a depth of 1 mm with a fine 3-axis micropositioner (David Kopf Instruments) to target cortical Layer V. Electrodes for

EPN recordings were advanced perpendicularly to pial surface through a craniotomy (2.3 mm caudal to bregma and 3.0 mm lateral to midline) with a hydraulic microdrive (David Kopf Instruments). EPN location was determined via its position directly ventral to VPm and ventral posterolateral nucleus (VPl). After traversing VPm and VPl, relative quiescence indicated passage through the internal capsule, followed by an abrupt increase in activity indicating entry into EPN. For both MCx and EPN recordings, the raw wide-band signal was passed through a 1× headstage, digitized at 40 kHz/channel, and digitally filtered between 1 and 300 Hz for local field potential (LFP) recordings and between 300 Hz and 10 kHz for spike trains (Plexon Inc., Dallas, TX, USA). Continuous wide band, LFP and spike train signals were stored in .plx format for off-line analyses.

Whisker Stimulation and Data Acquisition

Facial vibrissae were deflected using a multi-angle piezoelectric stimulator (Simons, 1983) controlled by custom-written LabView software. The stimulator was attached to the whisker 5 mm from the face in young rats and ∼10 mm from the face in adults. The stimuli consisted of a hold-ramp-hold pat- tern, delivered over a total of 500 ms. The stimulator was calibrated to deflect the whisker at ∼125 mm/s; the deflection amplitude was 0.5 mm in young rats and 1 mm in adults, resulting in equivalent angular deflection (∼5.7°) in all age groups. The whisker was deflected in eight standard directions, with deflection in each direction repeated 10 times for a total of 80 stimuli per whisker. Deflections were interleaved in a random manner. All sensory-evoked responses in this study were calculated by averaging individual trial responses to all eight whisker deflection angles, while the study that previously presented the thalamocortical unit data analyzed only responses to the best angle of deflection (Shoykhet et al., 2012). The 25 ms value of the counting window during the deflection onset (and offset) was chosen to cover the transient increase and return to near baseline levels after onset, while excluding an excessive number of plateau (or spontaneous) spikes that would dilute the true response values. The plateau counting window of 125 ms was chosen because it covers a period of the sustained plateau response in relative equilibrium after recovery from the onset increase in activity.

Data were collected for 500 ms; action potential time stamps were collected with 100 μs resolutions. Action potential waveforms were digitized at 32 kHz and stored for off-line analyses. Off-line, the action potential waveforms were sorted using custom written software that allowed for isolation of single-unit recordings based on a combination of waveform parameters, including principal component analysis, deflection slopes and amplitude of peaks and valleys. To confirm further the single-unit nature of the recordings, sorted spikes were examined using an interspike interval (ISI) histogram; only recordings with <1% of the sorted spikes within the absolute refractory period (1 ms) were used in the analyses.

For simultaneous EPN and MCx recordings, 5–10 min of spontaneous activity for each EPN unit were recorded, and 300 s of data free from artifacts was used for analyses. Single units were sorted using Plexon Offline Sorter (Plexon Inc.) based on visualization of 3D clusters in principal component space. All isolated units had an absolute refractory period >1 ms. Since all neurons in EPN are GABA-ergic (Kha et al., 2000), we did not analyze individual waveforms to differentiate between excitatory and inhibitory cells (Simons, 1978; Bruno and Simons, 2002).

Statistical Treatment of the Data

Alpha-trimmed means were used to compare average values among populations. An α-trimmed mean is defined as the sample mean derived from the set of observations n from which κ largest and smallest values have been removed, and where κ is the next largest integer of $\alpha \times n$ (Fisher and van Belle, 1993). α-trimmed means are more robust with respect to the effect of outliers, and statistical analyses using α-trimmed means become more conservative due to a reduction in the degrees of freedom and inclusion of only the most frequently observed values in the calculations. For statistical comparison of mean values between groups that are defined by injury (sham or CA) and time point (early or late) we used a two-way ANOVA followed by multiple pairwise comparisons using Tukey's honest significant difference (HSD) test ("anova2" and "multcompare" in Matlab). Unless otherwise stated, *indicates $p < 0.05$, **indicates $p < 0.01$ and ***indicates $p < 0.001$. The correlation was calculated using Spearman's correlation coefficient. Spearman's correlation is the Pearson's correlation coefficient of the indexed ranks of two data sets. This measure is robust to the influence of statistical outliers. Power spectra were calculated using Welch's method of power spectral estimation and the mean-square coherence ("pwelch" in Matlab). The pairwise comparison of Sham vs. CA data in the EPN single unit recordings was performed using either a student t-test or Wilcoxon Rank Sum test after normality of the data was determined using the Liliefors test for normality.

RESULTS

Magnitude and Variability of Whisker Somatosensory Thalamic Neuron Spontaneous and Sensory-Evoked Activity

We used extracellular recordings of action potentials of thalamic barreloid (VPm) neurons and quantified spontaneous activity and responses to whisker deflection sensory stimuli. Poststimulus time histograms show rapid increases in neural firing in response to whisker deflection onsets and offsets in recordings from four groups of rats: sham-early, CA-early, sham-late and CA late (**Figure 1A**). We quantified whisker deflection-evoked responses by calculating the evoked spike rates averaged in the 25 ms period after whisker deflection. A two-way ANOVA was conducted to understand the effects of ischemic injury and age on response and variance. We found that the effect of both injury ($F_{(1,109)} = 5.13$, $p = 0.024$) and age ($F_{(1,109)} = 4.0$, $p = 0.046$) were significant (**Figure 1B**). *Post hoc* pairwise comparisons using Tukey's HSD test revealed significant differences between evoked firing rates of sham-early neurons (38.9 ± 3.2 Hz) and CA-early neurons (57.2 ± 6.1 Hz, $p = 0.031$), as well

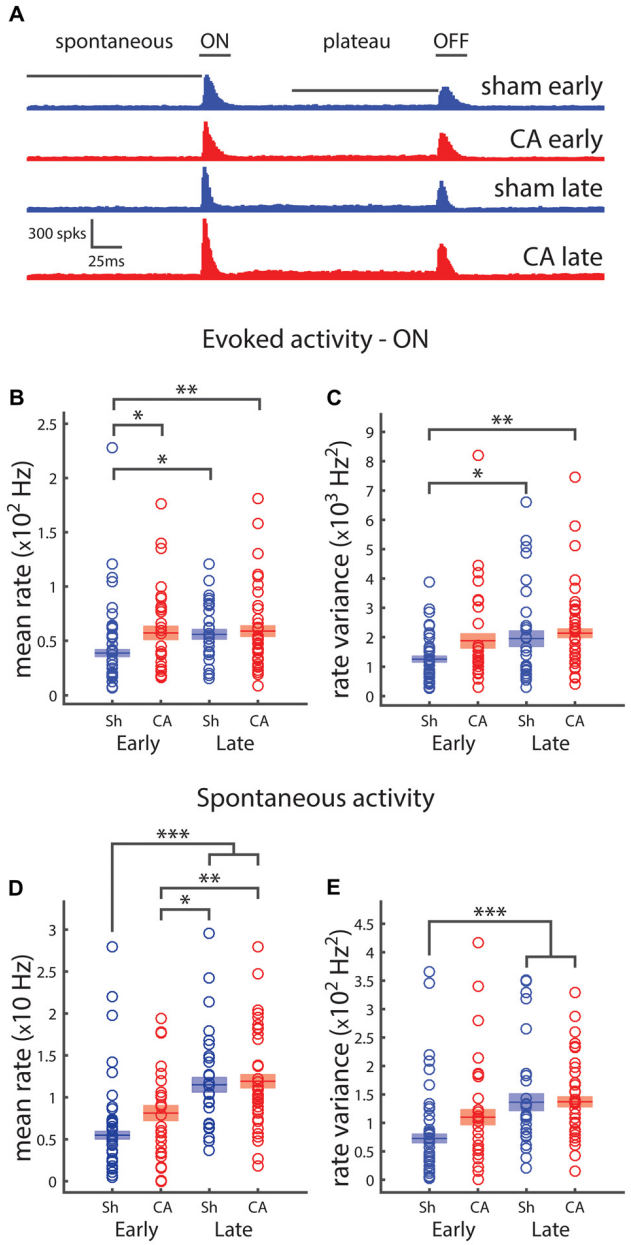

FIGURE 1 | Spiking activity of thalamocortical neurons depends on development and is affected by ischemic injury. (A) The poststimulus time histogram (PSTH) for whisker ramp and hold deflection stimuli responses of sham (blue) and cardiac arrest (CA; red) neurons during early and late periods. The horizontal bars indicate the time periods over which spikes are counted for response counts in spontaneous, ON, plateau and OFF periods. **(B)** Average firing rates for evoked whisker deflection (onset: ON) response. Early sham responses are significantly smaller than all other group averages. **(C)** Average firing rate variance of whisker responses reveal that early sham responses are less variable than both late responses. **(D)** Firing rates during the 150 ms spontaneous period preceding deflection. Early sham and early CA firing rates are smaller than both late groups. **(E)** Firing rate variance of whisker responses reveals that early sham spontaneous activity is less variable that both late groups. Horizontal bars and rectangular shaded regions in **(B–E)** indicate mean ± S.E.M. of the α-trimmed data sets. *Indicates $p < 0.05$, **indicates $p < 0.01$ and ***indicates $p < 0.001$.

as between sham-late neurons (55.9 ± 4.5 Hz, $p = 0.048$) and CA-late neurons (58.9 ± 4.8 Hz, $p = 0.008$; **Figure 1B**). The firing rate variability of whisker-evoked responses was quantified by calculating the trial-to-trial rate variance in the 25 ms post-stimulus period. There were significant effects of both injury ($F_{(1,109)} = 4.71$, $p = 0.032$) and age ($F_{(1,109)} = 6.65$, $p = 0.011$) on variance (**Figure 1C**). *Post hoc* comparisons revealed significant pairwise differences between sham-early neuronal firing rate variance (13.2 ± 1.2 × 10^2 Hz^2) and sham-late (21.9 ± 3.5 × 10^2 Hz^2) and CA-late (24.1 ± 2.7 × 10^2 Hz^2) firing rate variances (**Figure 1C**). The neural activity of VPm neurons during spontaneous conditions were quantified by calculating the average spike firing rate in the 150 ms period before whisker deflection. The effects of both injury and age were significant: $F_{(1,109)} = 4.22$, $p = 0.042$ and $F_{(1,109)} = 43.4$, $p = 1.6e^{-9}$, respectively (**Figure 1D**). There were significant pairwise differences between sham-early spontaneous rates (5.49 ± 0.45 Hz) and sham-late (11.5 ± 0.9 Hz, $p = 4e^{-7}$) and CA-late (11.9 ± 0.8 Hz, $p = 1e^{-8}$) firing rate variances. Additionally, there were significant pairwise differences between CA-early (8.13 ± 0.89 Hz) and sham-late ($p = 0.017$) and CA-late ($p = 0.003$) spontaneous firing rates (**Figure 1D**). We only observed a main effect of age on spontaneous firing rate variances: $F_{(1,109)} = 17.4$, $p = 6e^{-5}$ (**Figure 1E**). Pairwise significance differences were observed between sham-early (7.26 ± 0.76 × 10^1 Hz^2) and sham-late (14.5 ± 1.7 × 10^1 Hz^2, $p = 3e^{-4}$) and CA-late (15.2 ± 1.1 × 10^1 Hz^2, $p = 1e^{-4}$) firing rate variances (**Figure 1E**).

The response of VPm neurons to ramp and hold stimuli exhibits an elevated plateau of activity during the sustained whisker deflection and a transient response to the deflection offset, when the stimulator returns the whisker to its resting position (OFF responses, Simons and Carvell, 1989). We examined how OFF and plateau responses depend on exposure to brain hypoxia-ischemia and on the developmental period. Two-way ANOVA revealed a significant effect of injury on OFF whisker-deflection response firing rates: $F_{(1,109)} = 9.5$, $p = 0.003$ (**Figure 2A**). Pairwise *post hoc* comparison revealed significant differences between sham-early OFF firing rates (30.9 ± 2.8 Hz) and CA-early (48.7 ± 5.1 Hz, $p = 0.006$) and CA-late (46.3 ± 3.7 Hz, $p = 0.012$) offset response firing rates (**Figure 2A**). Similarly, there was a significant effect of injury on OFF firing response variance: $F_{(1,109)} = 10.6$, $p = 0.002$ (**Figure 2B**). We observed pairwise significant differences between sham-early (13.9 ± 1.5 × 10^2 Hz^2) and CA-late (23.6 ± 3.0 × 102 Hz^2, $p = 0.006$) OFF response rate variance (**Figure 2B**). When we examined whisker plateau activity (in between whisker deflection onset and offset, **Figure 1A**), we found that both injury and age had an effect on plateau firing rates (**Figure 2C**). *Post hoc* pairwise comparisons revealed significant differences between sham-early (6.8 ± 0.8 Hz) and sham-late (18.1 ± 2.0 Hz, $p = 3e^{-6}$) and CA-late (20.9 ± 1.9 Hz, $p = 5e^{-9}$) firing rate responses. There were also significant difference responses between CA-early responses (11.6 ± 1.1 Hz) and sham-late ($p = 0.029$) and CA-late ($p = 3e^{-4}$) firing rates.

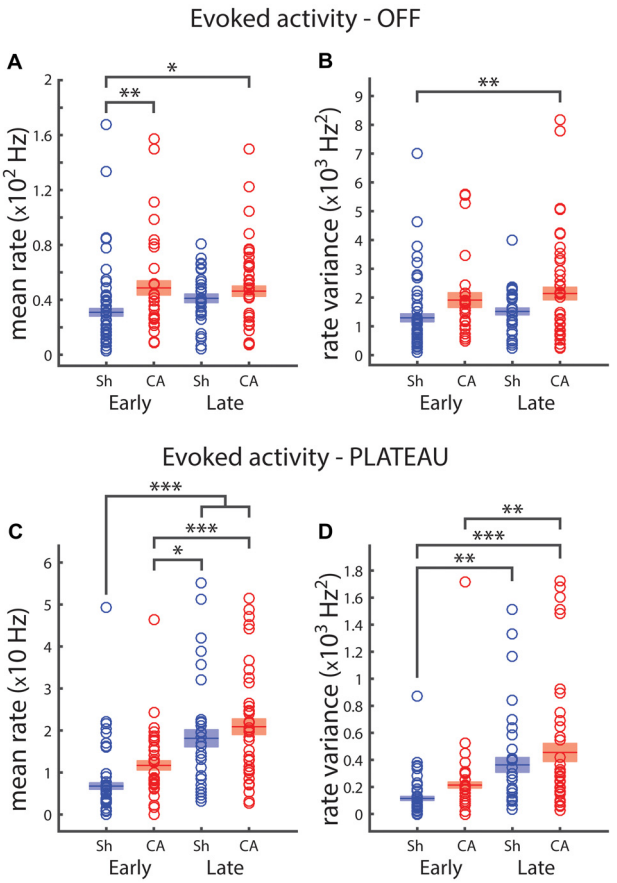

FIGURE 2 | Plateau and OFF responses depend on development and are affected by ischemic injury. (A) Early sham average OFF response rate is significantly lower that both CA groups. **(B)** Firing rate variance of OFF whisker responses reveals that early sham response is less variable that late CA response. **(C)** Firing rates for the 125 ms plateau period preceding whisker offset. Early sham rate is smaller than both late group rates; early CA rate is smaller than both late group rates. **(D)** Early sham plateau responses are less variable than both late group responses; early CA firing rate variance is smaller than late CA firing rate variance. Horizontal bars and rectangular shaded regions indicate mean ± S.E.M. of the α-trimmed data sets. *Indicates $p < 0.05$, **indicates $p < 0.01$ and ***indicates $p < 0.001$.

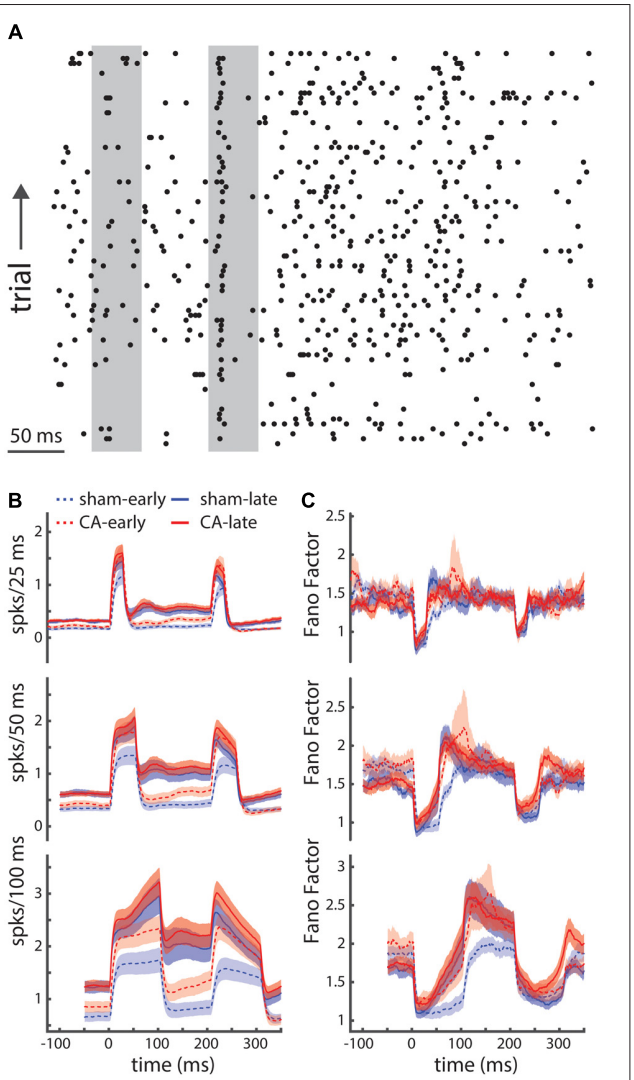

FIGURE 3 | Relative spike-count variability is unique for early sham rats. (A) A spike raster plot displaying the relatively high trial-to-trial variability of thalamocortical neurons in the ventral posterior medial (VPm) nucleus. **(B)** Time dependent firing rates are calculated by convolving spike counts with sliding square-wave windows of different widths (25 ms, 50 ms and 100 ms; top-bottom). **(C)** The time-dependent Fano factor (spike count variance divided by mean count), using different counting windows (top-bottom: 25 ms, 50 ms, 100 ms), reveals stimulus dependent reduction in spike count variability. Using a large counting window (100 ms) we are better able to estimate the Fano factor and start to see systematic differences in the pattern of relative variability of sham-early spiking activity compared to other groups: both CA-early and late groups. Shaded regions indicate s.e.m.

Both injury and age had an effect on plateau firing rate variance: $F_{(1,109)} = 4.31$, $p = 0.04$ and $F_{(1,109)} = 28.2$, $p = 6e^{-7}$, respectively. There were significant pairwise differences between sham-early ($12.3 \pm 1.8 \times 10^1$ Hz^2) and sham-late ($41.1 \pm 7.1 \times 10^1$ Hz^2, $p = 0.001$) and CA-late ($48.6 \pm 8.0 \times 10^1$ Hz^2, $p = 1e^{-6}$) plateau firing rate variances. There was also a significant difference between CA-early ($21.7 \pm 2.4 \times 10^1$ Hz^2) and CA-late ($p = 0.002$) plateau rate variance (**Figure 2D**).

We can see that both the amplitude and relative variability of spike counts are different between spontaneous periods and stimulus-evoked periods (**Figure 3A**: left and right gray bars, respectively). Our approach to examining the state-dependent nature of the spike count variability involved using a sliding counting window. We examined spike counts using sliding counting windows of different widths (**Figure 3B**). The sliding nature of the window allows us to examine variations in mean spike counts that may change on timescales smaller than the window itself. However, counts in time points that are closer than the counting window width will be correlated. We used these time-dependent spike count profiles and their corresponding spike count variances to estimate the Fano-factor. The Fano factor of a point process is the ratio of the count variance to mean count in a given window. We show Fano factor as a function of time for different

count windows (**Figure 3C**). As observed in other sensory neurons, we see that the Fano factor decreases with whisker deflection onset. Fano factors increase with the counting window width (top-bottom) and the sensory dependent reduction becomes more apparent with large counting windows (e.g., 100 ms shown in the bottom panel). What also becomes apparent is the unique time course of the stimulus-evoked reduction in the Fano-factor from sham-early neurons. While the Fano factor for CA-early, sham-late and CA-late neurons initiates a return to baseline shortly after the stimulus-evoked minimum, the Fano-factor for sham early neurons remains low for a time period on the order of ∼50 ms. As the counting windows used to reveal this different behavior is large (100 ms) this span of persistently low Fano factor includes both whisker deflection evoked spikes and early plateau spikes. This result implies that the neural activity of sham-early neurons may exhibit a unique form of stimulus-dependent reduction of neural variability that is absent in CA-early neurons. This contrast may have differential consequences on how thalamocortical activity engages cortical circuits during important developmental periods.

Correlation of Average Spontaneous and Sensory-Evoked Activity

To understand the functional connection between spontaneous activity and sensory activity in the whisker somatosensory thalamus, we calculated the correlation coefficient between observed spontaneous spike counts and whisker-evoked spike counts. The mean spontaneous rate and evoked rate across a population of sham-early VPm neurons is positively correlated ($\rho = 0.342$, $p = 0.028$; **Figure 4A**), while spontaneous and evoked rates are uncorrelated for CA-early neurons ($\rho = 0.167$, $p = 0.394$; **Figure 4B**). A similar spontaneous-evoked correlation is observed for late animals: spontaneous and evoked spike rates are correlated for sham-late VPm neurons ($\rho = 0.452$, $p = 0.013$; **Figure 4C**) and uncorrelated for CA-late neurons ($\rho = 0.115$, $p = 0.494$, **Figure 4D**). Firing rate variance and mean firing rates observed from neural recordings in the brain generally exhibit a positive increasing relationship (Goris et al., 2014). To confirm that the correlation of spontaneous and whisker-evoked firing rate variance exhibits the same dependence as on mean firing, we additionally calculated the correlation coefficient between these measures from our data sets. The spontaneous and evoked firing rate variances of sham-early VPm neurons are positively correlated ($\rho = 0.553$, $p = 2e^{-4}$; **Figure 4E**) and uncorrelated for CA-early neurons ($\rho = 0.092$, $p = 0.638$; **Figure 4F**). A similar spontaneous-evoked correlation structure is observed for late animals: spontaneous and evoked firing rates are correlated for sham-late VPm neurons ($\rho = 0.556$, $p = 0.002$; **Figure 4G**) and uncorrelated for CA-late neurons ($\rho = -0.033$, $p = 0.845$, **Figure 4H**).

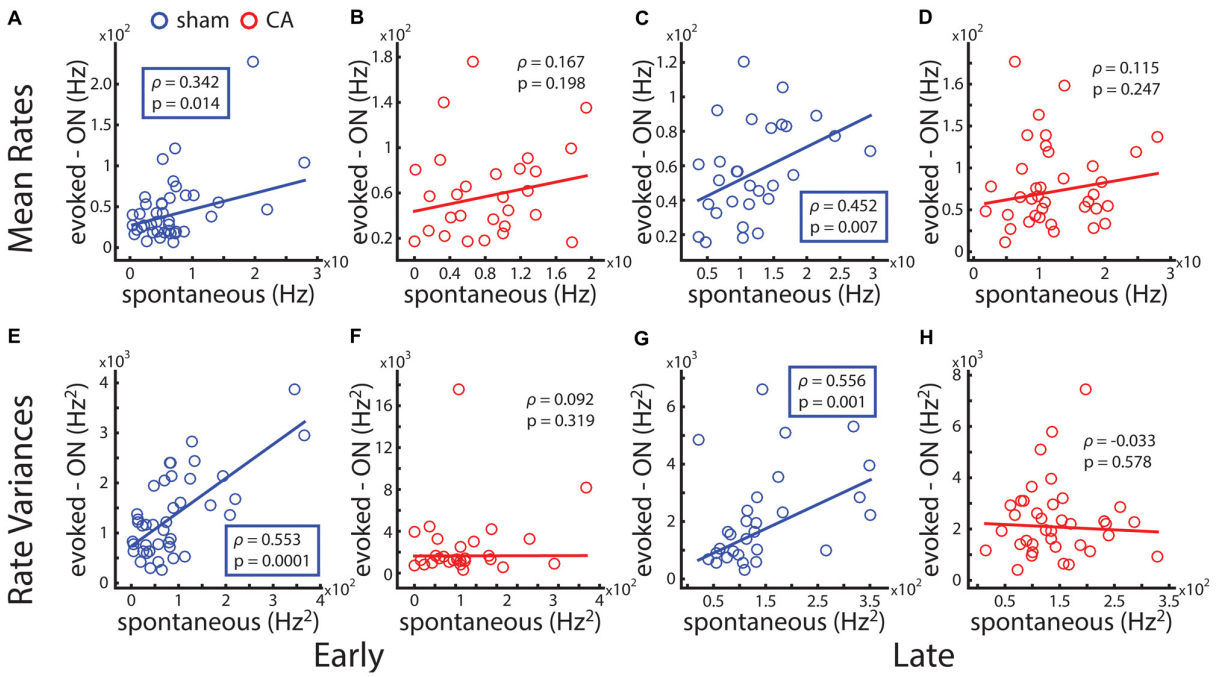

FIGURE 4 | Correlation of spontaneous and deflection-evoked activity is affected by ischemic injury. (A) The average level of spontaneous activity a thalamocortical neuron exhibits and its average whisker evoked stimulus response count is positively correlated across the population of neural recordings in early sham rats. **(B)** Spontaneous-evoked rate correlation is not significant for early CA rats. **(C)** Spontaneous-evoked rate correlation is positive and significant for late sham rats. **(D)** Spontaneous-evoked rate correlation is not significant for late CA rats. **(E)** Spontaneous-evoked variance correlation is positive and significant for early sham rats. **(F)** Spontaneous-evoked variance correlation is not significant for early CA rats. **(G)** Spontaneous-evoked variance correlation is positive and significant for late sham rats. **(H)** Spontaneous-evoked variance correlation is not significant for late CA rats.

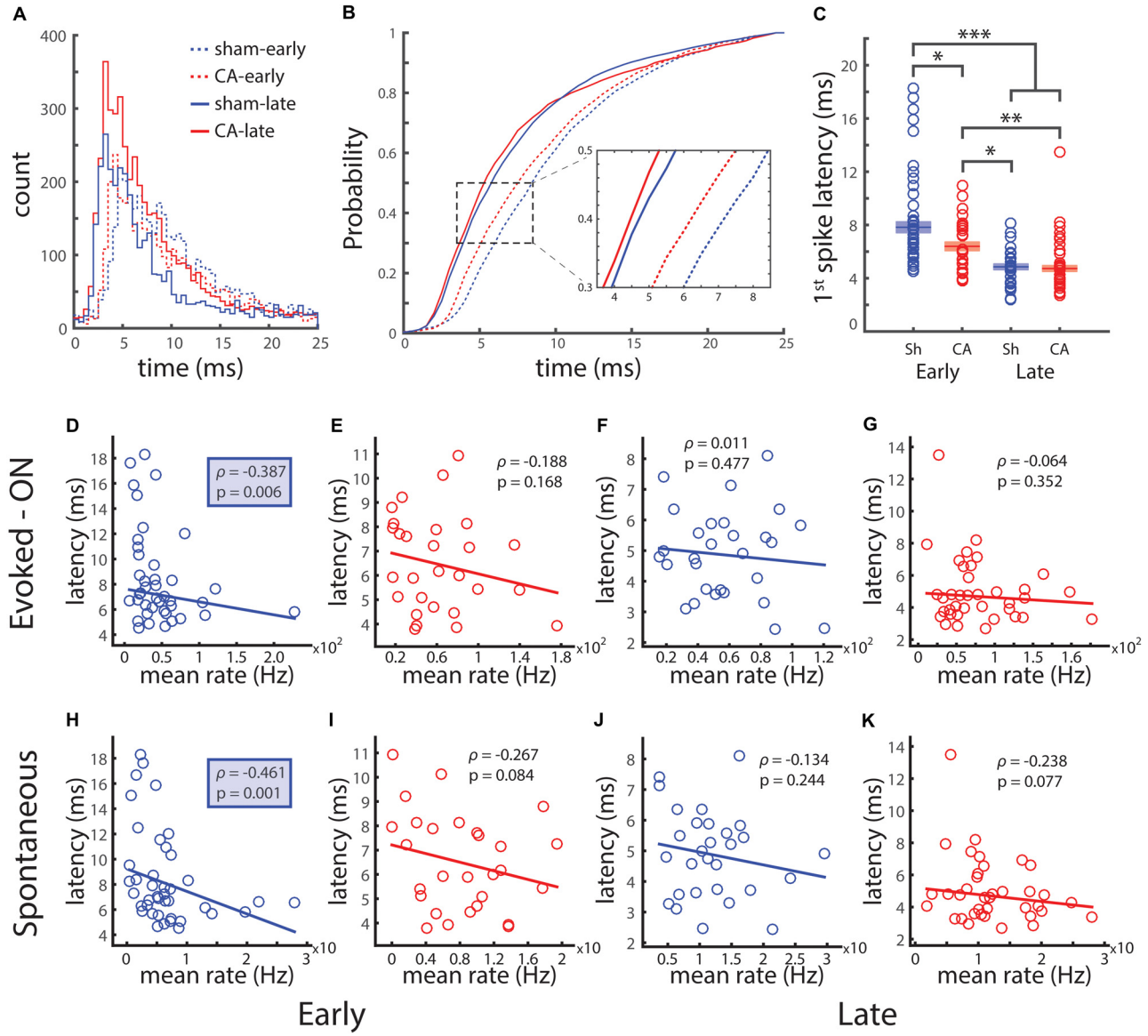

FIGURE 5 | Spike timing of whisker-evoked responses depends on development and is affected by ischemic injury. (A) PSTHs for the 25 ms whisker deflection ON period. **(B)** The cumulative distribution of spike times during the 25 ms ON period. Zoom: the population average time to 40% of spikes shows that the same proportion of spikes occurs earlier for late groups compared to early groups and for CA-early compared to sham-early. **(C)** The latency between whisker deflection onset and the first spike in trials with non-zero response is significantly longer for sham-early vs. all other groups, and CA-early vs. late groups. **(D)** The first spike latency and evoked spike count is negatively correlated for the early sham group. **(E–G)** First spike latency and evoked count are uncorrelated for CA-early and both late groups. **(H)** First spike latency and spontaneous spike count are negatively correlated for sham-early rats. **(I–K)** First spike latency and spontaneous spike count are not significantly correlated for early CA and both late groups. *Indicates $p < 0.05$, **indicates $p < 0.01$ and ***indicates $p < 0.001$.

Timing of Whisker Somatosensory Thalamic Neuron Sensory-Evoked Activity

The peri-stimulus time histograms (PSTHs) of whisker evoked responses (**Figure 5A**) and their corresponding cumulative probability distributions (**Figure 5B**) reveal an ordered relationship of response timing after whisker deflection. The inset shows that the average time it takes to observe 40% of the spikes in the counting window is smaller in both late populations, followed by CA-early neurons and then sham-early neurons. To quantify the relative distributions of early stimulus responses, we calculated the average first spike latencies in the 25-ms counting windows. Note that this measure differs from the response latency as calculated in a prior study (Shoykhet et al., 2012). The response onset latency in that study detects the first stimulus-dependent response that is statistically distinguishable from spontaneous activity, while the first spike latency we

calculate is more dependent on the temporal location of the highest slope of the PSTH. We performed two-way ANOVA to understand the influence of injury and age on first spike latency. There were significant effect of both injury and age on latency: $F_{(1,109)} = 5.94$, $p = 0.016$ and $F_{(1,109)} = 52.5$, $p = 7e^{-10}$, respectively. The interaction effect of injury and age on first spike latency was significant: $F_{(1,109)} = 4.18$, $p = 0.043$. *Post hoc* pairwise comparison of latencies revealed significant differences between sham-early (7.8 ± 0.4 ms) and CA-early (6.4 ± 0.3 ms, $p = 0.010$), sham-late (4.9 ± 0.2 ms, $p = 1e^{-8}$) and CA-late (4.7 ± 0.2 ms, $p = 4e^{-9}$) first spike latencies (**Figure 5C**). There were also significant differences between CA-early and sham-late ($p = 0.010$) and CA-late ($p = 0.002$) latencies.

We calculated the correlation of first spike latency and stimulus evoked spike count to see if there was a relationship between early stimulus timing and stimulus magnitude. The first-spike time and spike count for sham-early neurons were negatively correlated ($\rho = -0.387$, $p = 0.012$), while they were uncorrelated for CA-early ($\rho = -0.188$, $p = 0.335$), sham-late ($\rho = 0.011$, $p = 0.953$) and CA-late ($\rho = -0.064$, $p = 0.703$) neurons (**Figures 5D–G**). We saw that the magnitude of the sensory-evoked responses was correlated with the average spontaneous activity of that neuron (**Figure 4**). To see if the average first-spike latency of a neuron was also related to its level of spontaneous activity, we calculated the correlation coefficient of these two quantities. The first spike-latency and average spontaneous spike count for sham-early neurons were negatively correlated ($\rho = -0.461$, $p = 0.002$) while they were uncorrelated for CA-early ($\rho = -0.267$, $p = 0.168$), sham-late ($\rho = -0.314$, $p = 0.488$) and CA-late ($\rho = -0.238$, $p = 0.154$) neurons (**Figures 5H–K**). This unique relationship between initial response timing and both spontaneous and sensory evoked activity in early healthy thalamocortical neurons may play an important role in the transmission of thalamocortical activity during critical periods.

Activity in the Motor Thalamus and Motor Cortex

We also examined whether increased spontaneous firing rates of thalamic neurons represent a general feature of circuit reorganization following hypoxia-ischemia. As in the original study (Aravamuthan and Shoykhet, 2015), we observed decreased firing rates of EPN neurons in CA rats compared to sham rats (**Figure 6A**). However, variability in ISIs, measured by the coefficient of variation (CV = SD/mean), and skewness of the ISI histogram (ISIH; 3rd moment/SD^3) both increased in CA rats compared to sham rats (**Figures 6B,C**). The CV is a measure of spike train variability—low CVs (>1) reflect temporally regular spike train firing patterns and a Poisson spike train has a CV of 1 (Cox, 1962; Nawrot et al., 2008). CVs >1 can arise from larger membrane fluctuations (Middleton et al., 2003; Ackman et al., 2012; Siegel et al., 2012), fluctuating inputs and variable spike generating mechanisms (Churchland et al., 2006; Failor et al., 2010) or bursting spike activity (Tripp and Eliasmith, 2007; Failor

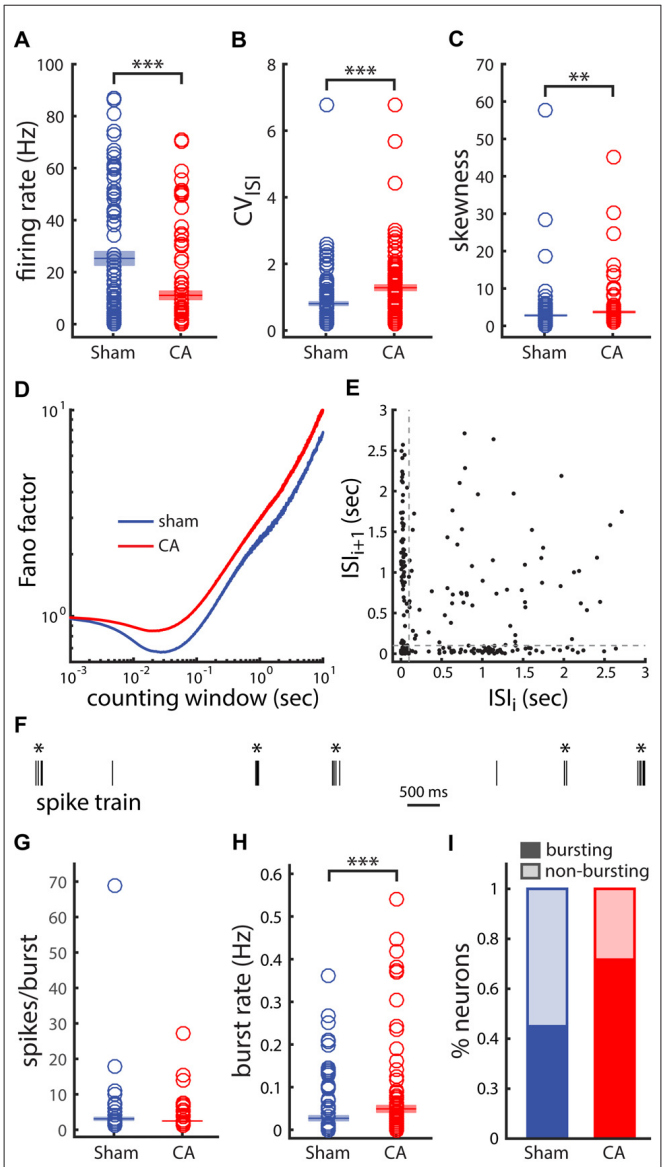

FIGURE 6 | Variability and burst firing of activity from neurons in the entopeduncular nucleus (EPN) of the basal ganglia is affected by ischemic injury. (A) Firing rate is lower in CA neurons than in Sham neurons as previously observed. **(B)** The coefficient of variation (CV) of the interspike intervals (ISIs) is larger in CA neurons than in Sham neurons. **(C)** The skewness (a measure of asymmetry) of the ISI histogram (ISIH) of CA neurons is larger than in Sham neurons. This asymmetry arises from a relatively larger, more distributed tail of ISI values in the CA neuron ISIHs. **(D)** The Fano-factor of spike counts as a function of counting window is larger for Sham neurons than CA neurons for the range of counting windows examined. **(E)** The ISI return map for an example CA neuron reveals burst-like firing in this case. The spread of points along the axes arise from a long ISI followed by a short ISI and vice versa. These ISI pair sequence arise at the initiating of a burst and at the end of a burst, respectively. **(F)** The spike train of the same example neuron shows clearly distinguishable burst and isolated spike events. **(G)** The number of spikes per burst in Sham and CA neurons is similar. **(H)** The burst rate averaged across all neurons in the groups is larger for CA neurons than for Sham neurons. **(I)** 45% of Sham neurons exhibit burst activity compared to 72% of CA neurons. *Indicates $p < 0.05$, **indicates $p < 0.01$ and ***indicates $p < 0.001$.

et al., 2010). The Fano factor of spike counts as a function of the counting window length is also higher at all time scales in CA compared to sham neurons (**Figure 6D**), further indicating increased relative spike count variability with injury. As the counting window approaches ∼0 both Fano factors converge to 1, the Fano factor of a Poisson process (Cox, 1962; Simons and Land, 1987; Shoykhet et al., 2005) and both curves have relative minima near the inverse of the mean firing rate (Carvell and Simons, 1996; Middleton et al., 2003). To further examine the ISI variability and confirm that the increase observed in CA neurons is related to burst firing, we examined more closely the ISI sequences from spike trains in each group. ISI return maps (scatter plot of ISIs vs. the ISIs immediately preceding them in sequence) can reveal temporal correlations and non-randomness in ISI sequences (Avila-Akerberg and Chacron, 2011). An example ISI return map from a CA spike train in our data set illustrates non-random correlation features consistent with burst firing. The elongated clusters near the axes correspond to sequential ISI pairs transitioning from long to short intervals during the commencement of a burst (or vice versa for the end of a burst), and the cluster near the origin illustrates sequential short intervals that occur within bursts (**Figure 6E**). For our dataset we used a lower bound threshold of 100 ms to determine inclusion of shorter intervals in bursts. Additionally, we set a higher threshold of 250 ms that set the requirement that intervals preceding and following a sequence of short intervals needed to be longer than this threshold in order for the sequence to be defined as a burst. A sample segment of the spike train from the same neuron shows the efficacy of these classification criteria to separate visibly distinguishable burst events (asterisks) from single isolated spike events (**Figure 6F**). We found that on average the number of spikes counted in each observed burst was not distinguishable between sham and CA neurons (sham: 4.10 ± 0.37, CA: 3.49 ± 0.23, $p = 0.106$; **Figure 6G**). However, when averaged across all neurons the rate of burst events firing (bursts/second) in sham neurons (0.027 ± 0.005 Hz) was lower than observed for CA neurons (0.049 ± 0.007 Hz, $p = 6.8e^{-4}$; **Figure 6H**). The proportion of Sham neurons that displayed burst firing was 0.45 (36/80) while the proportion of CA neurons that displayed burst firing was 0.722 (52/72; **Figure 6I**). A Chi-Squared test for equality of binomial proportions confirmed that the prevalence of burst firing differed significantly between sham and CA neurons in the EPN ($\chi^2 = 11.5$, $p = 0.0007$). These data indicate that the firing rates of EPN neurons, unlike those of VPm neurons, decrease in CA survivors, and suggest that post-injury functional abnormalities in the thalamus may be circuit-specific. Decreased firing rates in EPN after CA are associated with an increased spiking variability that is related to a change in burst firing prevalence.

Abnormalities of long-range circuit functional connectivity after CA are also evident in simultaneous LFP recordings in EPN and MCx (**Figures 7A–C**). We previously reported that coherence between EPN and MCx LFP is decreased in CA survivors. Here, we extend this finding further by demonstrating that the normalized total power of EPN LFP covaries with that of MCx LFP across multiple frequency bands in sham rats (**Figures 7D–G**). We did not observe such covariation in CA rats, however, except in the low frequency Θ range. Each data point represents a single average value from LFPs in multiple recording electrode placements in the same animal. Breakdown of covariance between EPN and MCx LFP's in CA rats occurs despite the fact that the overall LFP power remains similar between CA and sham rats in both brain locations. Abnormal brain rhythms and coherence of rhythms between different areas have been implicated in a number of neurological diseases (Babiloni et al., 2004; Sarnthein and Jeanmonod, 2007; Schulman et al., 2011; Ping et al., 2013). The relative independence of power levels in different bandwidths of LFP fluctuations between EPN and MCx may underlie some form of functional decoupling between these areas during development, which is supported by the previous finding that the temporal coherence between these areas is also reduced with ischemic-hypoxia induced injury (Aravamuthan and Shoykhet, 2015). This differential long-range organization may arise from the way the different levels of burst activity in EPN that we observed engages postsynaptic plasticity mechanisms in MCx.

DISCUSSION

Normal organization and function of information processing in the cortex relies on sensory-dependent experience, but equally importantly relies on network patterns of spontaneous activity during pre- and peri-critical developmental periods (Katz, 1993; Wong, 1993; Wong et al., 1995). Spontaneous thalamocortical activity is essential for engaging synaptic mechanisms that established the refined topical organization in cortical circuits (Crair and Malenka, 1995; Ackman et al., 2012; Siegel et al., 2012). Disruption of these mechanisms during different developmental periods by neuronal injury can have long lasting functional consequences as we have observed in a model of pediatric hypoxia-induced ischemic injury. We showed that the early neural injury resulted in changes of several aspects of stimulus response properties. Response properties of VPm neurons from older rats were different than younger rats and some of the differences between sham and CA neurons persisted into these late periods. In particular, we found that average sensory evoked responses in CA-early rat neurons were elevated to levels similar to late sham and CA rats, while spontaneous activity level of CA-early neurons were intermediate to those of sham-early and both late groups. We found that the timing of initial whisker deflection responses in CA-early neurons were accelerated relative to sham-early neurons but still slower than either late group. Notably, we observed correlations between spontaneous activity and sensory evoked activity (timing and amplitude) in sham groups, but not in early or late CA groups. We also studied neural activity in the basal ganglia and MCx of sham and CA rats. We found that neurons in the EPN in the basal ganglia had lower firing rates yet had higher variability and higher levels of burst firing after injury. Correlated levels of power in LFPs between the EPN and the MCx were also disrupted with injury.

The present study extends the findings of previous related studies from our group examining neural dysfunction after

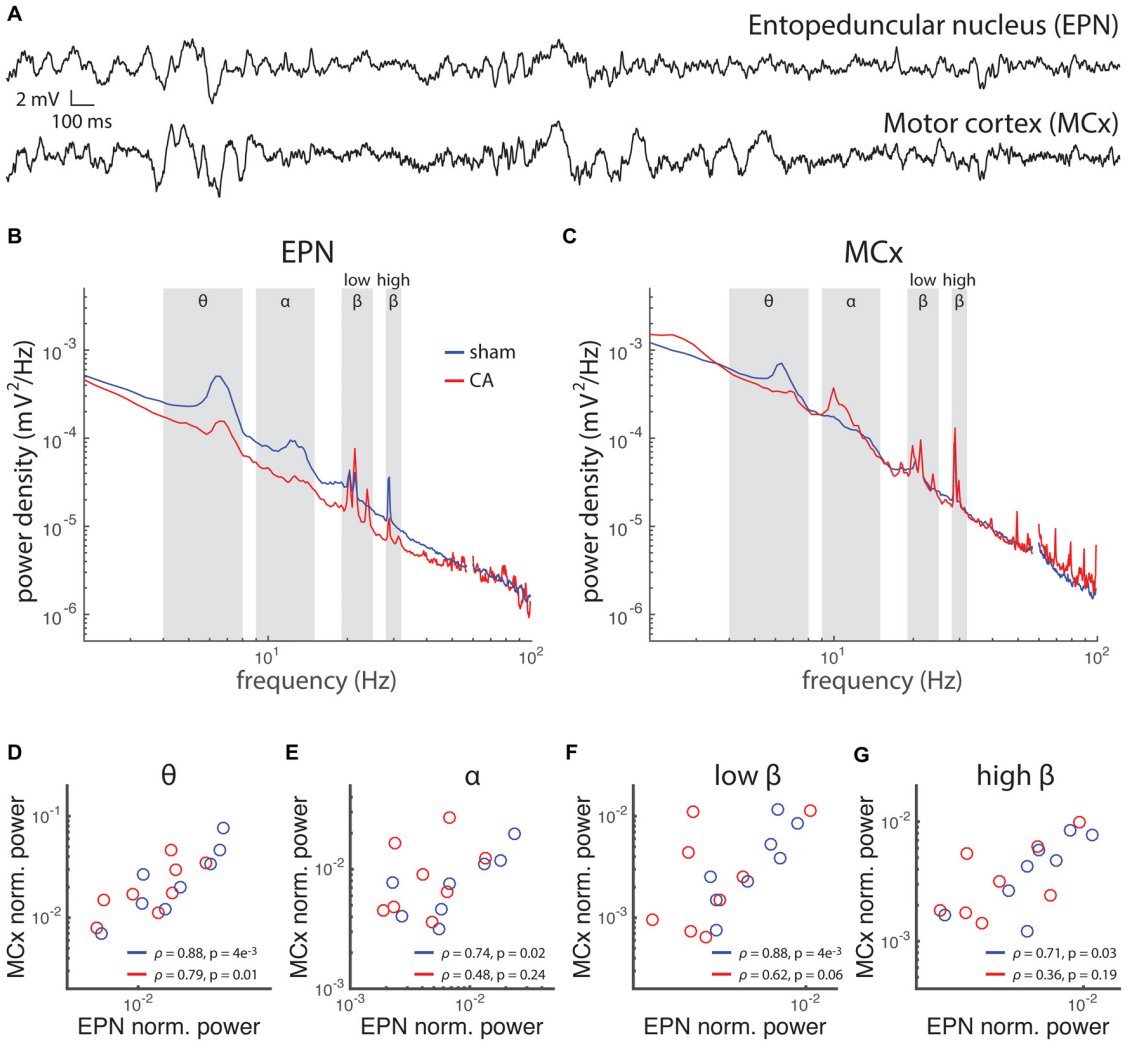

FIGURE 7 | Rhythmic activity and correlation between motor cortex (MCx) and basal ganglia is affected by hypoxic ischemic injury. (A) Local field potentials (LFPs) were simultaneously recorded in the EPN of the basal ganglia and the MCx in rats 6 weeks after injury or sham treatment. **(B)** The power spectral density of EPN LFPs in sham rats and CA rats exhibit spectral peaks at several frequencies, implying relatively more pronounced rhythms contributing to the LFP fluctuations at those frequencies. The frequency bandwidths are labeled: θ (4–8 Hz), α (9–15 Hz), low-β (19–25 Hz) and high-β (28–32 Hz). **(C)** The power spectral density of MCx LFPs exhibits relative peaks in the same frequency bandwidths of EPN LFPs. **(D–G)** Power in each bandwidth was averaged and normalized to the mean power across all frequencies. Average power in MCx was plotted against average power in EPN for each rat. The correlation between MCx and EPN mean power levels in different bandwidth was examined across the rats in our study by calculating the Spearman correlation coefficient of these two area-specific powers. We found that Sham rats had correlated MCx-EPN LFP power levels in the θ, α and low-β ranges, while CA rats had correlated MCx-EPN LFP power levels in the θ band only.

pediatric CA-induced global brain hypoxia-ischemia (Shoykhet et al., 2012; Aravamuthan and Shoykhet, 2015). The prior studies revealed deficits in the average firing rates under spontaneous and stimulus-evoked conditions of thalamocortical neurons. Our study additionally characterizes the trial-to-trial variability of these neurons. Variability of neural activity can have significant impact on its ability to drive postsynaptic targets (Salinas and Sejnowksi, 2000). Slow-temporal correlations in neuronal spike trains result in Fano factors larger than 1 that increase with the counting window width (Middleton et al., 2003; Goris et al., 2014). In multiple sensory and motor areas of the brain, Fano factors universally decrease with the onset of sensory stimulation or the onset of motor planning and behavior (Churchland et al., 2006, 2010). Any differences in spontaneous or sensory evoked activity with neural injury may reflect changes in variability that compromise normal circuit function. We have also shown how spontaneous and sensory evoked activity, as well as stimulus response timing, is correlated with one another in normal and injured brain states. The present study also characterizes burst activity in the EPN in disease and normal states. What still remains unclear is how the parallel forms of dysfunction that we observed in sensory and motor systems may be interacting

through development. Active whisking (motor) behavior can modulate how sensory information is processed (Fanselow and Nicholelis, 1999; Poulet and Petersen, 2008). Sensorimotor integration can occur at the level of the cortex (Ferezou et al., 2007), and behavior based on sensorimotor integration can exhibit deficits after ischemic injury (Hurwitz et al., 1991). Future studies are required to further understand that these injuries and age-related dysfunctions depend on one another.

Even though the functional differences we observe between neural activity in injured *vs*. sham rats in adulthood are fewer and smaller in magnitude than during early post-injury periods, the differences in activity we observe post-injury could have significant impact on sensory processing in the cortex. Altered thalamic input after CA likely has significant implications for experience-dependent plasticity in the developing cortex. In the rodent visual cortex, neonatal hypoxia-ischemia impairs ocular dominance plasticity following monocular deprivation (Failor et al., 2010). Concurrently, it alters the phenotype of inhibitory parvalbumin-expressing neurons and reduces overall activity levels. Thus, it has been suggested that neonatal hypoxia-ischemia impairs cortical plasticity by altering the development of inhibition in the visual cortex (Failor et al., 2010). Abnormal thalamic input, in turn, may contribute to dysfunctional cortical plasticity. In the rodent somatosensory system, altered sensory input during development disrupts maturation of inhibitory and excitatory cortical circuitry (Simons and Land, 1987; Shoykhet et al., 2005) and results in permanent behavioral deficits (Carvell and Simons, 1996). By analogy, abnormal thalamic input after CA may impact the functional maturation of cortical circuitry and result in long-lasting deficits. The central organization that we observed, reflected by the correlation of mean spontaneous activity and sensory-evoked activity magnitude and timing, likely has a significant impact on how spontaneous and sensory evoked activity of cortical neurons are correlated. This global change in population activity may affect the refinement of cortical circuits through the plasticity of recurrent synaptic activity during development. Further studies are required to assess functional changes in the somatosensory cortex in this ischemic injury model.

Ongoing spontaneous activity can engage short term plasticity of synaptic connections between neurons (Graham, 1977; Fujioka et al., 1994; Arbelaez et al., 1999; Reig et al., 2006; Choi et al., 2010) and may also be important for reinforcing stimulus-driven neural assemblies by engaging long term plasticity and homeostatic mechanisms (Myers and Yamaguchi, 1977; Pulsinelli and Brierley, 1979; Radovsky et al., 1997; Litwin-Kumar and Doiron, 2014). Subsets of neurons in the cortex that have higher spontaneous firing rates have specialized synaptic input composition that may increase their ability to exert strong excitatory influence on cortical activity (Myers and Yamaguchi, 1977; Pulsinelli and Brierley, 1979; Radovsky et al., 1997; Böttiger et al., 1998; Fink et al., 2004; Yassin et al., 2010). Spontaneously co-active sub networks of cortical neurons overlap with sensory evoked neuronal ensembles (Graham, 1977; Myers and Yamaguchi, 1977; MacLean et al., 2005). Spontaneous activity plays an important role in establishing and refining synaptic circuits in the brain during developmental periods. Based on our results we hypothesize that the covariation of spontaneous activity and stimulus-response amplitude and timing across a population of thalamic neurons may play an important role in the normal development of thalamocortical and intracortical synaptic circuits in sensory systems. The relationship between spontaneous and sensory-evoked activity, that CA neural activity lacks, may be important for reinforcing strong and organized thalamocortical sensory pathways during critical developmental periods. Additionally, burst firing may engage postsynaptic short-term plasticity mechanisms differently than the tonic or random Poisson firing at similar rates (Fortune and Rose, 2001; Middleton et al., 2011). All of these changes together may have important consequences on sensory processing and function of cortical neural networks in that it persist for much longer periods after early acute neural injury.

AUTHOR CONTRIBUTIONS

MS performed the experiments in this study. JWM performed the data analysis in this study. MS and JWM wrote the manuscript.

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frontiers in Neural Circuits

REVIEW
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Large Scale Cortical Functional Networks Associated with Slow-Wave and Spindle-Burst-Related Spontaneous Activity

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Cortical sensory systems are active with rich patterns of activity during sleep and under light anesthesia. Remarkably, this activity shares many characteristics with those present when the awake brain responds to sensory stimuli. We review two specific forms of such activity: slow-wave activity (SWA) in the adult brain and spindle bursts in developing brain. SWA is composed of 0.5–4 Hz resting potential fluctuations. Although these fluctuations synchronize wide regions of cortex, recent large-scale imaging has shown spatial details of their distribution that reflect underlying cortical structural projections and networks. These networks are regulated, as prior awake experiences alter both the spatial and temporal features of SWA in subsequent sleep. Activity patterns of the immature brain, however, are very different from those of the adult. SWA is absent, and the dominant pattern is spindle bursts, intermittent high frequency oscillations superimposed on slower depolarizations within sensory cortices. These bursts are driven by intrinsic brain activity, which act to generate peripheral inputs, for example via limb twitches. They are present within developing sensory cortex before they are mature enough to exhibit directed movements and respond to external stimuli. Like in the adult, these patterns resemble those evoked by sensory stimulation when awake. It is suggested that spindle-burst activity is generated purposefully by the developing nervous system as a proxy for true external stimuli. While the sleep-related functions of both slow-wave and spindle-burst activity may not be entirely clear, they reflect robust regulated phenomena which can engage select wide-spread cortical circuits. These circuits are similar to those activated during sensory processing and volitional events. We highlight these two patterns of brain activity because both are prominent and well-studied forms of spontaneous activity that will yield valuable insights into brain function in the coming years.

Keywords: cortex, development, barrel cortex, plasticity, voltage sensitive dye imaging

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INTRODUCTION

A key feature noted by early investigators of brain activity was that its pattern depends on behavioral state. The most dramatic shift in behavior occurs when falling asleep, when the brain becomes much less responsive to the external world and focuses inwards on an internal one. Sleep would suggest a brain at rest, although it remains active with a structured set of ongoing spontaneous activity patterns that do not rely on external sensory inputs. Less is known about this spontaneous activity compared to activity evoked by external stimulation given the challenges of recording and interpreting it. In contrast to events related to sensory inputs or motor outputs, ongoing spontaneous activity has no fixed point to which it can be referenced. Factors that influence it are less clear and thus harder to experimentally control. Most challenging, though, has been the lack of a clear and consistent framework through which to examine it (Raichle, 2011; Hutchison et al., 2013).

Converging lines of evidence show spontaneous brain activity during sleep reflects the communication of brain regions to process past experiences and mediate learning and memory in the adult brain (Wilson and McNaughton, 1994; Huber et al., 2006; Euston et al., 2007; Ji and Wilson, 2007; Rasch and Born, 2007; Han et al., 2008; Schwindel and McNaughton, 2011; Bermudez Contreras et al., 2013; Barnes and Wilson, 2014). In addition, unique forms of spontaneous activity are present during sleep in the immature brain. These activity patterns, like the adult brain activity discussed above, are not reflective of current sensory or motor processes involving the external world. Studying this activity has significant benefits in that its function can be more easily tested, by disrupting it and examining the subsequent deficits in the resulting adult brain. Doing so has revealed crucial roles for this early network activity in measurable processes such as the formation of ocular dominance columns in the visual thalamus (Torborg et al., 2005), refinement of corticospinal projections (Martin et al., 2007), maturation of spinal reflex circuits (Grillner, 2004), and modulating the machinery of synaptic release in the hippocampus circuit (Mohajerani et al., 2007). Furthermore, there is substantial overlap between the processes that underlie developmental plasticity and that plasticity which continues through life in response to learning or injury (Singer, 1995; Caleo and Maffei, 2002; Wolpaw, 2002; Carmichael, 2003; Uhlhaas et al., 2010), suggesting that lessons learned via the study of spontaneous brain activity during development are likely to prove useful in understanding the adult brain as well (Constantine-Paton et al., 1990; Murphy and Corbett, 2009; Ben-Ari et al., 2012).

In this review article, we discuss two prominent forms of spontaneous activity in sensory cortices during sleep or under light anesthesia, one found in adult brains and one in developing brains. First, we discuss recent findings related to the slow-wave activity (SWA), a 0.5–4 Hz oscillation in the sleeping adult brain which synchronizes sensory, motor and association cortices in non-REM (NREM) sleep (Contreras and Steriade, 1997). Second, we discuss spindle bursts, a pattern of intermittent fast oscillations at 5–25 Hz in the developing brain which play an important role in maturation of sensory systems (Khazipov and Luhmann, 2006; Blankenship and Feller, 2009; Blumberg et al., 2013; Cirelli and Tononi, 2015). In discussing how both patterns are generated and shaped by neural circuits, we hope to reinforce the concept that sleep-related activity within sensory systems and other regions of the brain are neither noise nor idle "holding-patterns", but rather are actively formed and deployed for specific purposes.

SLOW WAVE ACTIVITY

Since the earliest days of EEG recording, sleep was a major target of research (Adrian and Matthews, 1934; Berger, 1969). Researchers made rapid progress, identifying and categorizing major stages of sleep based on distinct brain activity as well as behavior (Hirshkowitz, 2000; Rasch and Born, 2013). This promoted a view of sleep related activity as a set of distinct and isolated rhythms. More recently, a unified view of brain oscillations has become prominent. In this view, different brain rhythms are seen as elements of a more global complex oscillation grouping slow and fast frequency events (Steriade, 2006).

In a series of three articles, Steriade et al. (1993a,b,c) described a slow ∼1 Hz oscillation that would support this view by providing a link between rhythms generated in the thalamus and their synchronous projection to the cortex during NREM sleep. Intracellularly, this oscillation was seen as a relatively rapid switch in the values of the membrane potential between silent (hyperpolarizing) and active (depoarizing by 7–10 mV) state. The two states are referred to as UP and DOWN states and the >1 Hz alternation between them is generally referred to as the *slow oscillation* (Van Someren et al., 2011). Usually, membrane potential fluctuations around the Up state are of higher amplitude, whereas the Down state is relatively free of membrane fluctuations (Steriade et al., 1993b). Its role in grouping faster rhythms within the thalamus and cortex, including delta- and spindle activity, has given rise to some confusion as to its naming, as it cannot fully be separated from the faster rhythms. Generally, the *term slow oscillation* refers to the <1 Hz component, while the term *SWA* to represent the broader collection of these rhythms and their power within the 0.5–4 Hz range (Mascetti et al., 2011).

The finding that cells from multiple regions of the cortex, along with subcortical structures, were synchronized by a single rhythm brought the newly described SWA a good deal of attention. Soon after its description in anesthetized cats, it was shown during natural sleep in cats (Steriade et al., 1996; Amzica and Steriade, 1998) and in humans (Achermann and Borbély, 1997; Simon et al., 2000) and it has since been demonstrated in rodents (Cowan and Wilson, 1994; Petersen et al., 2003; Doi et al., 2007; Ruiz-Mejias et al., 2011). Subsequent investigations have shown it exists in nearly all sensory, motor and association areas of the cortex (Steriade et al., 1993c; Ferezou et al., 2006; Mohajerani et al., 2013) and synchronizes the membrane potential of cells in different functional regions far removed from each other (Destexhe et al., 1999; Volgushev et al., 2006; Dickson, 2010).

PRECISE DISTRIBUTION OF SLOW-WAVE ACTIVITY WITHIN CORTICAL SENSORY SYSTEMS

More recently, a more precise distribution of SWA within specific cortical regions has been demonstrated (Genzel et al., 2014). In fact, intracranial depth EEG from regions across human brain showed many slow-waves are restricted to small regions of cortex, or begin regionally before traveling to broader areas (Nir et al., 2011; Botella-Soler et al., 2012). Furthermore, SWA can reflect the functional architecture of the brain; following learning tasks, for example, SWA can increase or decrease within localized functional systems (Huber et al., 2004, 2006; Iwasaki et al., 2004; Han et al., 2008; Hanlon et al., 2009; Bermudez Contreras et al., 2013).

We and others, over the past years, have sought to expand these findings to determine precisely how SWA relates to the functional architecture of the brain, in turn giving further insights to its function (Kenet et al., 2003; Ferezou et al., 2006; Han et al., 2008; Mohajerani et al., 2010, 2013; Antic et al., 2016). Our strategy has been to use Voltage-sensitive dye (VSD) imaging of the rodent cortex, which has allowed collection of cortical activity at the mesoscale (tens of millimeters) spatial resolution and high temporal sampling, from very large regions of the cortex (Grinvald and Hildesheim, 2004). Examples of this activity, in a lightly anesthetized mouse, are shown in **Figure 1** (adapted from Mohajerani et al., 2010). A crucial dependence on brain circuitry to shaping these patterns was demonstrated by the role of the corpus callosum in coordinating synchronous patterns of activity between hemispheres (Mohajerani et al., 2010).

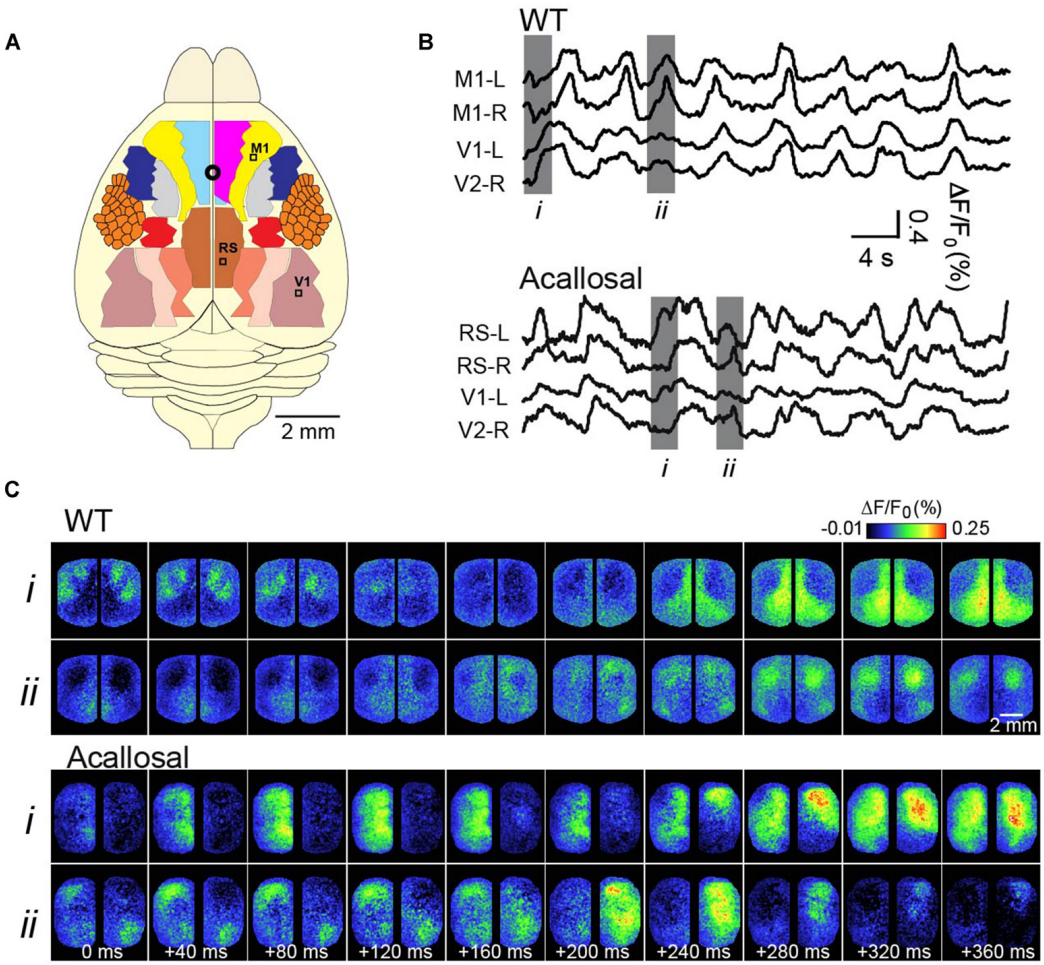

FIGURE 1 | Voltage-sensitive dye (VSD) imaging of slow-wave activity (SWA) in the adult anesthetized mouse. (A) Schematic shows different cortical regions of the bilateral craniotomy preparation. Location of bregma is indicated by a white circle. **(B)** Example traces showing VSD signal from selected cortical regions in wild-type (WT) and acallosal I/LnJ mice that lack corpus callosum. M1, primary motor cortex; V1, primary visual cortex; RS, retrosplenial cortex **(C)**. Montages showing VSD images at time indicated in gray (*i, ii*) in **(B)**. Without the interhemispheric connections carried in the corpus callosum in acallosal mouse, spontaneous slow wave activity was less synchronized between hemispheres compared to the wild type animal. A, anterior; R, right; L, left; P, posterior. Republished with permission of Society of Neuroscience, (Mohajerani et al., 2010); permission conveyed through Copyright Clearance Center, Inc.

We also studied the role of brain circuitry more precisely by examining how SWA is represented within functional cortical systems. Our strategy has been to collect long continuous stretches of cortical activity, and then to examine correlations of activity patterns between a functional region of interest and the remainder of the cortex (as shown in **Figures 2A,B**). In this way, subtle local patterns of activity emerge that are not necessarily distinguishable based on examination of raw signals. We have shown that, contrary to its early description as a global phenomenon that synchronizes broad regions of cortex, SWA activity has regional distribution which reflects the underlying axonal projections of cortex (Mohajerani et al., 2013; Vanni and Murphy, 2014). **Figure 2A** demonstrates this by showing a strong correlation of SWA within individual primary sensory regions or association regions. Indeed, we have shown that patterns of SWA can be temporally divided into combinations of activity within specific functional brain circuits (**Figures 2C,D**; Mohajerani et al., 2013).

We believe these results fit within a growing recognition that oscillations of SWA tap into functional networks of cortical cells (Castro-Alamancos, 2009; Harris and Thiele, 2011; Luczak and Maclean, 2012). This has been particularly well studied within sensory systems of the cortex. During the transition to UP states, the spatio-temporal pattern of spikes within a local network of cells is highly stereotyped. For example, the pattern of cell activation during UP states generated via thalamic simulation in slices (MacLean et al., 2005) or sensory stimulation *in vivo* (Luczak et al., 2009) is very similar to that of spontaneous UP states. On a larger scale of network also, slow-wave oscillations reflect functional connections with the visual (Kenet et al., 2003; Han et al., 2008; Mohajerani et al., 2013), somatosensory (Ferezou et al., 2006; Mohajerani et al., 2013) and

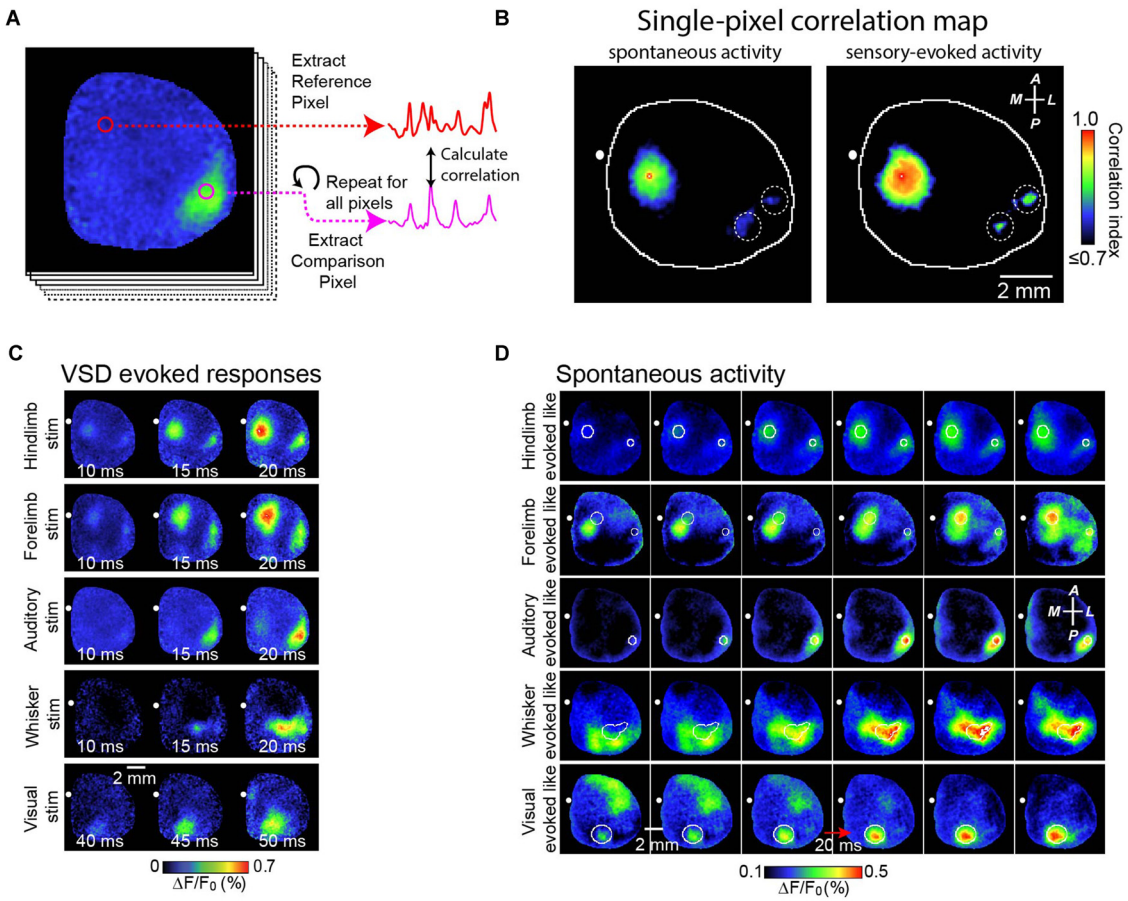

FIGURE 2 | Correlation maps show precise spatial layout of correlation of VSD signals from all pixels with VSD signal from each brain region. To analyze patterns of spontaneous slow wave activity quantitatively, correlation maps based on a seed pixel have been shown to be an effective measurement. **(A)** Shows an example of this calculation. In this example, a sequence of voltage sensitive dye images is shown at the left. Using a seed pixel within an area of interest (reference pixel; red circle), one could obtain the correlation map by calculating its correlation to all other pixels within the imaged cortex. **(B)** Representative correlation maps from a seed pixel located within the primary representation of Hindlimb somatosensory area generated from VSD signals during 300 s of cortical spontaneous activity (left) or after sensory stimulation of the contralateral forelimb (right). There was a distant correlation at HLS2, as indicated by white dashed circles. CC, correlation coefficients (color coded). **(C)** Representative montages show examples of the VSD response to five different forms of sensory stimulation. **(D)** Representative montages showing cortical slow wave activity that resembles sensory-evoked cortical activity shown in **(C)**. A, anterior; R, right; L, left; P, posterior. Figure is adopted and modified with permission from Mohajerani et al. (2013).

auditory (Farley and Noreña, 2013; Mohajerani et al., 2013) cortices.

FUNCTION OF SLOW-WAVE ACTIVITY

These specific spatial patterns of SWA suggest it may be related to known sleep roles in learning and memory formation and consolidation. These roles come from studies which show sleep results in enhanced memory retention (Barrett and Ekstrand, 1972; Fowler et al., 1973; Plihal and Born, 1997; Walker et al., 2002; Tucker et al., 2006; Gais et al., 2008), by showing that performance on memory tasks after sleep is proportional to the amount of NREM sleep (Stickgold et al., 2000a,b), and that enhancing SWA via external electrical stimulation improves subsequent performance on memory tasks (Marshall et al., 2006; Binder et al., 2014). In addition, SWA is increased during sleep following social challenges (Meerlo et al., 2001), verbal learning tasks (Mölle et al., 2004), procedural motor learning tasks (Huber et al., 2004; Hanlon et al., 2009), or exploration (Vyazovskiy et al., 2005), but decreased following sensory deprivation (Iwasaki et al., 2004; Huber et al., 2006).

We believe the effects of sleep and SWA on memory formation are consistent with an emerging consensus of SWA having an important role in synaptic remodeling, a molecular correlate of learning. There are two main models of what sleep-related synaptic remodeling represents: replay of neural patterns associated with learning to promote synaptic plasticity (Sutherland and McNaughton, 2000; Schwindel and McNaughton, 2011); and down-scaling of new synapses created during normal waking or learning, to maintain a consistent overall excitatory balance in the brain (Tononi and Cirelli, 2006; Diekelmann and Born, 2010). The first model, known as active system reconsolidation, rests on an idea advanced by Marr (1970), namely that memories are encoded in short-term representations during waking, before being permanently encoded during sleep, when the brain is free of ongoing processing needs. In particular, it posits that memories are encoded temporarily in the hippocampus during exploratory activity, and then being "replayed" and transmitted to the cortex during NREM sleep via fast hippocampal sharp wave ripples (SPW-R), all under the synchronizing action of the slow oscillation (Buzsáki, 1996, 2015; Mölle and Born, 2011). Evidence for this theory includes: (a) NREM sleep primarily (but not exclusively, for example see Huber et al., 2004) benefits hippocampal dependent declarative memory consolidation (Stickgold and Walker, 2007); (b) recordings showing that cortical and hippocampal sequences of neuronal firing during waking are replayed together during NREM sleep (Wilson and McNaughton, 1994; Qin et al., 1997; Ji and Wilson, 2007) but not during REM sleep (Siapas and Wilson, 1998; Kudrimoti et al., 1999; Wierzynski et al., 2009); (c) hippocampal SPW-R are present at the transition between cortical UP and DOWN states of the slow oscillation (Battaglia et al., 2004; Mölle et al., 2006) and are temporally linked with both spindles and delta waves (Sirota et al., 2003; Johnson et al., 2010); and (d) selectively suppressing hippocampal SPW-R reduces sleep-related improvements in spatial memory (Girardeau et al., 2009).

The second model, that of synaptic homeostasis, comes from the observation that while synaptic potentiation and formation may be an effective mechanism for learning-related plasticity in the brain, it must be balanced by equivalent reductions in synaptic strength to avoid excess excitation (Miller and Mackay, 1994; Turrigiano, 2008; Tononi and Cirelli, 2014). Unfettered increases in synaptic strength and number also place energy and size burdens on the brain (Howarth et al., 2012). Rather than balancing increasing and decreasing of synaptic strengths in parallel, the synaptic downscaling theory of sleep suggest that synaptic potential proceeds mostly unhindered during waking before being selectively downscaled during sleep (Tononi and Cirelli, 2001, 2014).

Evidence is accumulating rapidly in support of this theory, and includes the following: (a) genes associated with synaptic potentiation (e.g., Arc and brain-derived neurotrophic factor (BDNF) are decreased during sleep (Cirelli and Tononi, 2000), while those associated with synaptic depression (e.g., Calcium/Calmodulin Dependent Protein Kinase IV (CAMK4), calcineurin) are increased (Cirelli et al., 2004); (b) cortical excitability as measured via frequency of spontaneous mini-excitatory post-synaptic potentials (Liu et al., 2010), spiking rates (Vyazovskiy et al., 2009), size of cortical evoked potentials (Hulse et al., 2011), or responsiveness to transcranial magnetic stimulation (Huber et al., 2013), increases with time awake and decreases following sleep; (c) direct visualization of synapses in both flies (Bushey et al., 2011) and mice (Maret et al., 2011) show that waking is associated with increasing numbers of synaptic spines, while sleeping is associated with a decrease; and (d) artificially enhancing synaptic potentiation by infusion of BDNF enhances subsequent SWA, but not REM, sleep activity in a region restricted to the infusion (Faraguna et al., 2008).

As mentioned earlier in this review article, activity-dependent synaptic potentiation is a major factor in our understanding of learning and memory. Active-system consolidation, with its focus on coordinated replay of salient sequences of neural activity, might be expected to promote synaptic potentiation via Hebbian plasticity by repeatedly activation of pairs of neurons. This would be in keeping with the observed benefits of sleep on memory, discussed earlier in this section. On the other hand, synaptic homeostasis emphasizes synaptic weakening and an elimination of non-relevant potential accrued during waking. Can these opposite effects be reconciled?

In fact, neither model of the role of SWA suggests that it contributes directly to synaptic potentiation. It has been known for some time that LTP is poorly induced during SWA (Leonard et al., 1987; Bramham and Srebro, 1989) and genes associated with LTP are down-regulated during SWA (Thiel et al., 1994; Jones et al., 2001). Thus, instead of triggering LTP directly, replayed activity during SWA may "tag" particularly salient synaptic changes, preserving them on a background of overall synaptic down-regulation (Ribeiro et al., 2004). Although the molecular underpinnings of this "tagging" are not clear, they may include persistently elevated calcium levels in selected cells

(Rosanova and Ulrich, 2005; Diekelmann and Born, 2010). Alternatively, induction of some plasticity related genes, which respond strongly to pulsatile intracellular oscillation of calcium levels at SWS frequencies (Frey et al., 1993; Abel et al., 1997) could contribute (Sejnowski and Destexhe, 2000).

We believe recent results showing SWA reflects precise sensory circuits are consistent with both models. Important learning related changes can occur within very local cortical circuits (Komiyama et al., 2010; Fu et al., 2012) and local SWA within these circuits could facilitate this process, perhaps by permitting synchronized faster oscillation within components of the circuit (Yang et al., 2014). It bears noting that spontaneous activity within functional circuits may not have any particularly important function, but rather may simply reflect synaptic connectivity (Luczak and Maclean, 2012). However, localized changes in SWA that follow a specific learning task or sensory change suggest otherwise (Bermudez Contreras et al., 2013).

SPINDLE BURSTS—A KEY PATTERN OF SPONTANEOUS ACTIVITY IN THE DEVELOPING BRAIN

In the preceding section, we discussed patterns of activity in sensory systems of the brain when it is not required to be purposefully interacting with the environment because of sleep. During early life, the brain is also *less-able* to purposefully interact with the environment, as most sensory systems are not matured (Himwich, 1970; Jewett and Romano, 1972; Krug et al., 2001; Petersson et al., 2003; Colonnese et al., 2010), yet these systems display a variety of patterns of neural activity (Feller, 1999; Blankenship and Feller, 2009). There is a great deal of interest in this activity because it has been recognized to play essential roles in the normal development of the nervous system, acting in concert with genetically driven, molecular determinants of development (Goodman and Shatz, 1993; Tessier-Lavigne and Goodman, 1996; Chilton, 2006) to ensure formation of functional neural circuits (Pallas, 2001; Hanganu-Opatz, 2010; Blumberg, 2015).

A striking feature of the earliest recordable brain activity at approximately 24 weeks post-conception in humans, equivalent to approximately the time of birth in rats, is the lack of state-dependent differentiation. It does not display the defined patterns of continuous cortical activity that represent a particular behavioral state such as sleep or waking in the adult. Instead, the EEG consists mainly of bursts of high-amplitude undifferentiated waves, separated by periods of silence (Deza and Eidelberg, 1967; Anderson et al., 1985; Selton et al., 2000; Engel, 2008). Much of this early work on this pattern was spearheaded by French neonatologists and pediatric neurologists, led by Collette Dreyfus-Brisac, and the patterns are occasionally still referred to by their original French name, trace discontinue (discontinuous trace; Dreyfus-Brisac, 1978).

The absence of robust state-dependent patterns of EEG activity in early life does not mean that such states do not exist (Seelke and Blumberg, 2010). Some of the clearest transitions between states occur with regards to movements. As early as 10 weeks gestation in the human (Van Dongen and Goudie, 1980), and P2 (postnatal day 2) in the rat (Gramsbergen et al., 1970), alternating periods of quiescence and atonia, quiescence with general atonia but small multi-limb twitches, and large movements involving the whole body are seen (Corner, 1977; Robinson et al., 2000; Blumberg et al., 2005). These states are termed quiet sleep, active sleep and waking, respectively, and a consistent cycle of wake, quiet sleep, then active sleep can be seen in rats as young as P2 (Karlsson et al., 2004).

An important developmental milestone occurs at 28 weeks post-conception, when the human EEG remains discontinuous, but a prominent pattern of short 5–25 Hz bursts superimposed on a slower 0.5–2 Hz background emerges during active sleep. The dominance of this pattern is illustrated by its many descriptions in the early literature (Khazipov and Luhmann, 2006), including "spindle-shaped bursts of fast waves" (Ellingson, 1958), "spindle-like fast rhythms" (Watanabe and Iwase, 2008), and "fast activity" (Goldie et al., 1971). These are now referred to *delta brushes* (8–28 Hz) in the clinical setting (André et al., 2010) and usually *spindle bursts* in the research setting. Khazipov et al. (2004) were the first to demonstrate that spindles bursts are linked to peripheral inputs. Recording from the hind- and forelimb somatosensory cortex of rats during the first week of life, they found that spontaneous limb twitches, as well as limb stimulation, were followed 100–200 ms later by spindle bursts.

Analogous results were also shown in preterm human infants; the clinically well-known delta brush pattern in the somatosensory cortex follows spontaneous hand and foot movements (Milh et al., 2006). The delta (0.5–2 Hz) and spindle burst (5–25 Hz) differ in their source, the former being mostly representative of cutaneous inputs and the latter, representative of proprioceptive inputs (Marcano-Reik and Blumberg, 2008). Spindle bursts remain generally localized to the corresponding sensory system. Twitches in the fore- and hindlimb give rise to spindle bursts in the fore- or hindlimb sensory cortex, respectively (Khazipov et al., 2004; **Figure 3A**). Using voltage sensitive dye (VSD) imaging in cortex of rat pups, we have shown that highly specific patterns of activation across the cortex follow hindlimb or tail twitches (McVea et al., 2012; **Figures 3B,C**). Importantly, this characteristic generalizes across the cortex; spontaneous retinal activity (a prominent feature of the visual system prior to eye opening (Feller, 1999; Feller and Scanziani, 2005; Firth et al., 2005; Torborg et al., 2005), or optic nerve stimulation gives rise to spindle bursts in the visual cortex (Hanganu et al., 2006), whisker twitches or whisker stimulation give rise to spindle bursts in the whisker somatosensory cortex (Minlebaev et al., 2007; Yang et al., 2009; An et al., 2014). In all cases, disrupting ascending sensory inputs (via spinal transection, whisker pad anesthesia, or retinal inactivation) decreases but not completely stopped the incidence of spindle bursts significantly in the corresponding cortex.

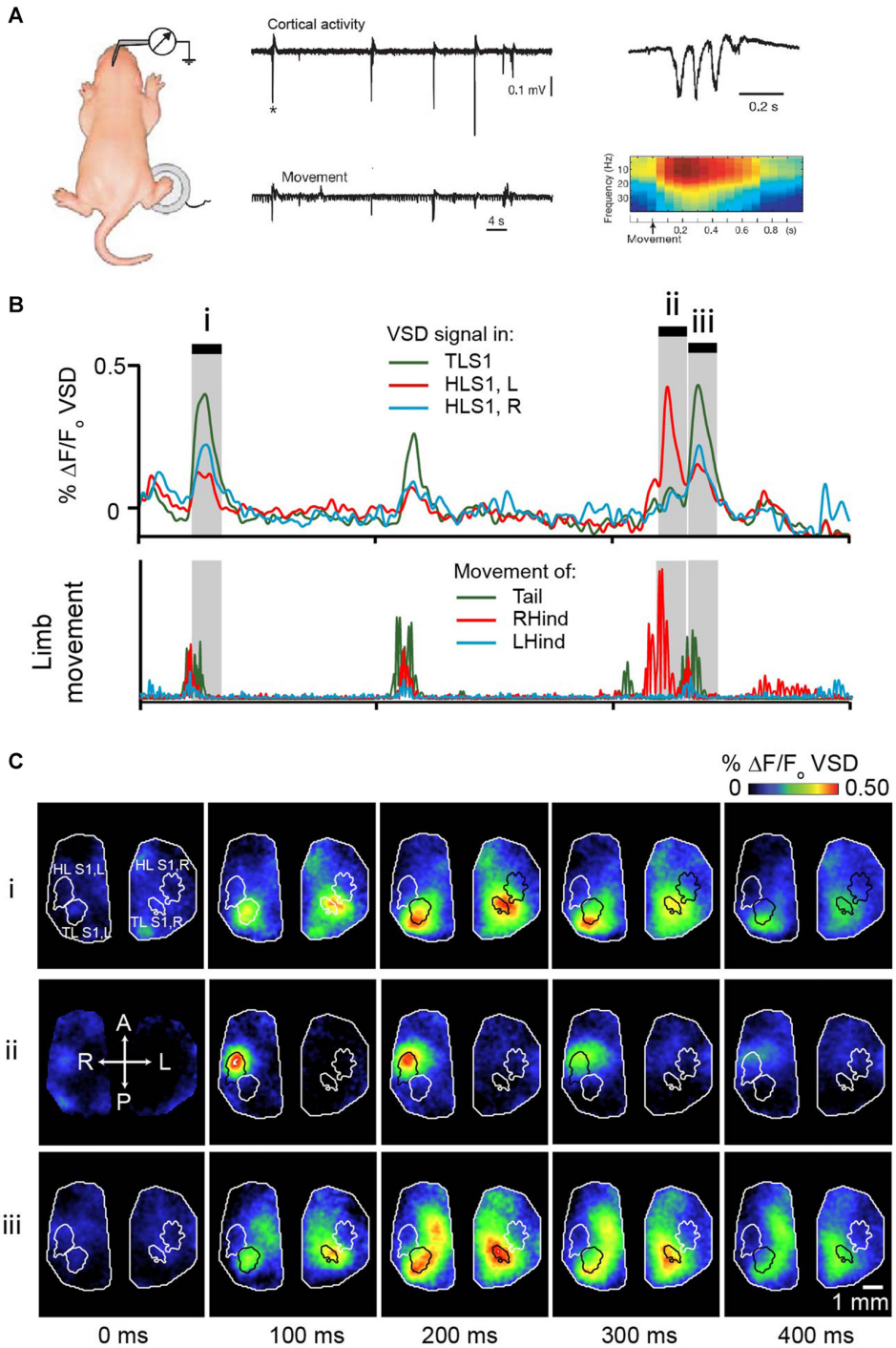

FIGURE 3 | Movement-triggered spindle bursts in primary somatosensory cortex of the newborn rat. (A) Simultaneous recordings of the intracortical field potential in left hindlimb somatosensory cortex (top trace) of a 2 days old (P2) behaving rat. The bottom trace is the actual right-hindlimb movements. Note that the cortical spindle bursts are correlated with the movements. Right, spindle-burst marked by asterisk is shown on an expanded timescale (top) and below is corresponding time-frequency analysis. Figure is adapted and modified from Khazipov et al. (2004), used with permission from Nature through RightsLink. **(B)** Bursts of activity in sensory cortices of a 5 days old rat pup following movements of the limbs or tail. Pup is lightly anesthetized with isoflurane to approximate active sleep. Top panel shows VSD signal from tail sensory cortex, and left and right hindlimb cortex. These brain regions are outlined in **(C)**. Bottom panel concurrent signal of limb and tail movement collected with high speed camera. **(C)** Montages showing cortical activity every 100 ms in shaded gray regions highlighted in **(B)**. Republished with permission of Society of Neuroscience, (McVea et al., 2012); permission conveyed through Copyright Clearance Center, Inc.

PURPOSE OF SPINDLE BURSTS

Although our understanding of the function of cortical activity in sleep during infancy remains limited, there are clues that spindle bursts play important developmental roles. Limb twitches during development have a clear instructive role to the nervous system. This was shown directly by manipulating the sensory feedback that arrives following a spontaneous twitch in 2-week old rat pups (Pearson et al., 1999). When an air puff was used to artificially stimulate the side of the tail opposite to the movement direction (a reversal of what would occur normally), the rat later made more errors in withdrawing from stimuli applied to the tail. The authors concluded that the sensory feedback provided by the twitch is used to aid in the maturation of pain-withdrawal reflexes. Nearly five decades ago, Roffwarg et al. (1966), suggested that high levels of active or REM sleep in early life could serve to provide the developing cortex with activity when little is available from the outside world. Could the spindle bursts that accompany the twitches of active sleep be a cortical manifestation of this hypothesis, and have an instructive role similar to that seen in the pain-withdrawal reflex?

It is well established that, although the gross patterning of the somatosensory cortex may occur independently of activity, refinement relies on activity in the sensorimotor system (Pallas, 2001; Price et al., 2006; Inan and Crair, 2007; Hanganu-Opatz, 2010; Kolb et al., 2012; Blumberg, 2015; Cirelli and Tononi, 2015). In particular, injuring or deafferenting a limb during the first few days of life in the rat results in a failure of the development of the corresponding sensory cortex, as well as changes in sensory regions of the remaining limbs (Wall and Cusick, 1986; Dawson and Killackey, 1987; Waters et al., 1990; Pearson et al., 1999). Transecting the sensory regions of the spinal cord confirms that this effect reflects loss of ascending sensory, particularly proprioceptive, inputs (Jain et al., 2003). While it is hard to link this process to a particular pattern of brain activity, the altered development of the sensory cortex is only seen if the sensory feedback is disrupted during the first week of life, which overlaps with the peak period of expression of muscle twitches and spindle bursts (Blumberg et al., 2015).

The role of ascending sensory information depends on functional NMDA receptors in the cortex (Iwasato et al., 2000), suggesting it acts via Hebbian learning mechanisms as in the visual system. There is a critical period during the first week of life in which alterations in sensory feedback become reflected in the sensory representation at the cortex (Jeanmonod et al., 1981; Simons et al., 1984; McCasland et al., 1992). Do these adaptive changes reflect a role of spindle bursts in the sensory cortex? There is indirect evidence that they do. The normal development (Fox et al., 1996; Iwasato et al., 2000; Dagnew et al., 2003; Lee et al., 2005) of the barrel cortex, as well as the plastic responses following whisker removal (Schlaggar et al., 1993), depends on glutamatergic transmission via NMDA receptors. A second requirement for the normal barrel pattern is the intact subplate (Tolner et al., 2012). These same factors are necessary for the expression of spindle bursts (Minlebaev et al., 2008; Tolner et al., 2012). There is also indirect evidence from the visual system that spindle bursts may guide development. Spindle bursts in the visual cortex result from ascending sensory activity from spontaneous waves of retinal activity (Hanganu et al., 2006). As previously mentioned, it is well recognized that retinal waves are essential to the normal development of the visual system (Wong, 1999; Firth et al., 2005; Torborg et al., 2005). Much of this research has focused on the impact of retinal activity on retino-thalamic maturation, but it is clear that it impacts primary and even secondary cortical regions as well (Ackman et al., 2012). In particular, blocking retinal waves and presumably the subsequent cortical spindle bursts during the first week of life prevents normal ocular dominance column and receptive field formation in ferrets (Huberman et al., 2006; Alme et al., 2010) as well as normal precise connections from thalamus to the visual cortex in mice (Cang et al., 2005). Ablation of the subplate, also necessary for spindle bursts, also prevents the normal development of the visual cortex (Ghosh and Shatz, 1994; Kanold et al., 2003). These changes are not reversed by subsequent normal visual inputs (Hooks and Chen, 2007), again implying a critical function for spindle-burst associated patterns of activity in early life.

In summary, cortical development depends on activity, which refines neural connections via NMDA-receptor mediated by Hebbian mechanisms. One source of this activity is via ascending sensory inputs, and the spindle bursts generated by these inputs also depend on NMDA receptors. An intriguing possibility that follows is that spindle bursts are an essential pattern of activity that underlies the prolific activity dependent plasticity of early life, perhaps by synchronizing related groups of pre- and post-synaptic neurons (Mohajerani and Cherubini, 2006; Mohajerani et al., 2007). Falling spindle burst activity with increasing age could contribute to the closure of pre-critical periods for cortical plasticity (Feldman, 2001; Hensch, 2004; Erzurumlu and Gaspar, 2012; Kolb et al., 2012) and the beginning of the critical period in which the sensory inputs tune up the existing circuits. Processes other than the decrease in spindle bursts are also likely to influence the decreasing plasticity of the system in question. For example, NMDA receptor subunit expression undergoes substantial changes during the first week of life (Monyer et al., 1994), to configurations which are less favorable to synaptic potentiation (Erisir and Harris, 2003). Nevertheless, it is reasonable to suggest that spindle bursts represent the network correlate of NMDA receptor mediated activity-dependent plasticity in the developing cortex.

CONCLUSIONS

In this review article, we have discussed two forms of spontaneous cortical activity present during sleep within sensory systems. In the adult, SWA is a prominent pattern activity seen during sleep or light sedation. Previously, it was thought to be a global phenomenon that synchronizes large swathes or cortex and sub-cortical regions. However, as new ideas about its role in scaling synapses within local systems have emerged, evidence has concurrently been gathered to show SWA has

localized and regional features that reflect underlying functional cortical circuits. In the developing brain, spindle bursts are the dominant feature of sleep activity. In contrast to the adult brain, they rely only minimally on intrinsic brain circuitry and depend on ascending sensory inputs, which themselves are spontaneously generated by the developing nervous system. The precise roles for spindle bursts remain unclear, but their contribution in synaptogenesis and circuit connectivity is unquestionable.

We do not, however, claim any particular functional links between these two forms of activity. In fact, the independence of these patterns is a key message regarding the spontaneous activity of the brain. We believe it is neither noise nor a wasteful consequence of brain structure; it is an useful activity, purposefully generated, and used for specific and definable purposes.

AUTHOR CONTRIBUTIONS

DAM and MHM wrote the manuscript, material in this manuscript was used in DAM's PhD thesis who was supervised by THM. THM edited the manuscript.

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MINI REVIEW
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Spatial diversity of spontaneous activity in the cortex

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The neocortex is a layered sheet across which a basic organization is thought to widely apply. The variety of spontaneous activity patterns is similar throughout the cortex, consistent with the notion of a basic cortical organization. However, the basic organization is only an outline which needs adjustments and additions to account for the structural and functional diversity across cortical layers and areas. Such diversity suggests that spontaneous activity is spatially diverse in any particular behavioral state. Accordingly, this review summarizes the laminar and areal diversity in cortical activity during fixation and slow oscillations, and the effects of attention, anesthesia and plasticity on the cortical distribution of spontaneous activity. Among questions that remain open, characterizing the spatial diversity in spontaneous membrane potential may help elucidate how differences in circuitry among cortical regions supports their varied functions. More work is also needed to understand whether cortical spontaneous activity not only reflects cortical circuitry, but also contributes to determining the outcome of plasticity, so that it is itself a factor shaping the functional diversity of the cortex.

Keywords: spontaneous activity, cortex, fixation, slow oscillations, attention, plasticity, reinforcement learning

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Introduction

The neocortex is a layered sheet across which a basic organization is thought to widely apply (Douglas et al., 2003). Excitatory and inhibitory connectivity within each layer is local (Perin et al., 2011; Levy and Reyes, 2012), and excitatory information flows into layer 4 of the cortex, to the superficial layers, then the deep layers (Thomson and Lamy, 2007). The local connectivity within a layer and vertical information flow across layers enables the heuristic of a columnar unit of computation repeated across the cortical sheet, historically termed a "minicolumn".

However, the basic organization is only an outline which needs adjustments and additions. For example, layer 6 also receives direct thalamic input, such that its latencies in the cat primary auditory cortex can be shorter than those of the superficial layers (Atencio et al., 2009), and even comparable in rodent primary auditory and somatosensory cortices to those of layer 4 (Sugimoto et al., 1997; Constantinople and Bruno, 2013). The modifications to the basic organization must vary spatially, because cortical areas differ in cytoarchitecture, and receive different inputs (Markov et al., 2014; Oh et al., 2014). Some areas may deviate substantially from the basic organization. For example, the presence of layer 4 in motor cortex is debated (Kaneko, 2013). The idea of a basic organization is thus widely acknowledged, but its most fruitful definition and range of applicability remain open (Harris and Shepherd, 2015). Computational models support the notion that varied repetition of a basic organization can explain a wide range of cortical functions (Buonomano and Merzenich, 1995; Ardid et al., 2007; Serre et al., 2007; Bengio et al., 2015).

Spontaneous activity is neural activity that is present even when all of a set of conventional variables are held constant, as is typically done in a reference or baseline state. It indicates initial variability, which together with the dynamics determines response variability (Kisley and Gerstein, 1999; Curto et al., 2009), and may influence plasticity (Legenstein et al., 2008; Toyoizumi et al., 2013; Chaisanguanthum et al., 2014). Spontaneous activity depends on behavioral state, and is present except in the most pathological conditions (Buzsáki, 2006; Wang, 2010; Ganzetti and Mantini, 2013). Across wide swaths of cortex, activity observed electroencephalographically (EEG) or via local field potential (LFP) in a quieter behavioral state (which may serve as a baseline state) often exhibits lower frequency power, which diminishes in a more active behavioral state; there may also be increased higher frequency power in the more active state (Buzsáki, 2006; Harris and Thiele, 2011). For example, the preponderance of slow oscillations (0.5–4 Hz) in deep non-rapid-eye-movement sleep decreases and is accompanied by increased alpha power (8–12 Hz) when a person awakens (Brown et al., 2010). Analogously, alpha power often decreases upon sensory stimulation or movement initiation (Buzsáki, 2006), while gamma power (30–80 Hz) often increases with alertness, visual stimulation or attention (Gray et al., 1989; Fries et al., 2001; Buzsáki, 2006; Harris and Thiele, 2011). Because the EEG and LFP represent many neurons, the decrease in lower frequency power suggests that sensory stimulation or attention desynchronizes the lower frequency "noise" correlations of nearby neurons, as has been widely observed (de Oliveira et al., 1997; Fries et al., 2001; Kohn and Smith, 2005; Cohen and Maunsell, 2009; Mitchell et al., 2009; Oram, 2011; Smith and Sommer, 2013; Tan et al., 2014). Crucially, models of a small patch of a cortical layer, based on data from cortical slices (**Figure 1A**), have a robust regime in which external excitation shifts the network from synchrony to asynchrony (**Figure 1B**) and increases the frequency at which synchrony peaks (van Vreeswijk and Sompolinsky, 1998; Brunel, 2000; Mehring et al., 2003; Renart et al., 2010; Tan et al., 2014). Thus, key aspects of a common pattern in the variety of spontaneous activity occurring with shifts in behavioral state are captured by a basic organization.

However, because the basic organization needs modification according to cortical area, spontaneous activity is presumably spatially diverse, even within a behavioral state. For example, primate prefrontal cortex (PFC) neurons have more spines than primary visual cortex (V1) neurons (Elston, 2000). By assuming that spine number indicates recurrent connectivity strength, Chaudhuri and colleagues argue that PFC and V1 spontaneous activity differ (Chaudhuri et al., 2015). This possibility can be understood by noting that a variant of the basic organization with stronger recurrent connections and more slowly decaying synapses suggested by prefrontal N-methyl D-aspartate receptor 2B subunits (Wang et al., 2013) exhibits the shift from synchrony to asynchrony, but with autocorrelations in the asynchronous state that decay more slowly (**Figure 1C**; Chaudhuri et al., 2015; Harish and Hansel, 2015).

Accordingly, this review surveys laminar and areal diversity in cortical spontaneous activity that might be expected because there is structural and functional diversity across the cortex. Behavioral state affects spontaneous activity, so we use it to structure our comparisons. We shall be interested in the cortical distribution of spontaneous activity within particular behavioral states, as well as how the distribution is affected by shifts in behavioral state. We begin with broadly-defined behavioral states that are nonetheless sufficiently specified for some spatial comparisons: fixation, an alert state in which gaze is held steady; as well as quieter states of sleep and anesthesia in which slow oscillations are present. Fixation defines a behavioral state broadly, because it can be performed in various attentional contexts. Hence, we will go on to consider more refined behavioral state specifications by examining the effects of attention, anesthesia and plasticity on the cortical distribution of spontaneous activity.

Laminar and Areal Diversity of Spontaneous Activity During Fixation

Spontaneous activity in macaque V1 varies by layer during fixation. It alternates between low and high, being low in layers 2/3, 4B and 5, and high in layers 4A, 4C and 6 (Poggio et al., 1977; Snodderly and Gur, 1995; Gur et al., 2005). Additionally, layer 2 has more spontaneous activity than layer 3 (Gur and Snodderly, 2008). Spontaneous activity is affected by ambient light level (Kayama et al., 1979), but its laminar pattern is similar in dark and light (Snodderly and Gur, 1995). Stimulus-evoked cross-correlations vary with V1 layer, but spontaneous cross-correlations do not (Hansen et al., 2012).

The expectation that spontaneous activity is spatially diverse is based in part on functional differences among cortical areas, which may therefore indicate features useful for characterizing the spatial diversity of spontaneous activity. A feature that functionally distinguishes cortical areas is the time scale over which a stimulus affects subsequent activity. Sensory cortical responses prominently decay nearly to baseline within several hundred milliseconds after the end of a stimulus, although appropriate tests show that longer time scales are also present (Fishman et al., 2001; Super et al., 2001; Micheyl et al., 2005). In contrast, the firing rates of prefrontal neurons can remain elevated for several seconds after the end of a stimulus, during the delay period of a working memory task (D'Esposito and Postle, 2015), with many features of the delay period activity compactly captured by attractor networks (Wimmer et al., 2014).

Are the different time scales that functionally distinguish cortical areas reflected in spontaneous activity? The autocorrelation of spontaneous activity decays more slowly in the frontal eye field (FEF) than in visual area V4 (Ogawa and Komatsu, 2010). Across neurons in the lateral parietal area (LIP), the time scale of autocorrelation decay correlates positively with the selectivity of delay period activity for target location (Nishida et al., 2014). Furthermore, the autocorrelation of fluctuations of spontaneous activity from the trial average decays more slowly from the medial temporal area (MT) to LIP to the lateral PFC

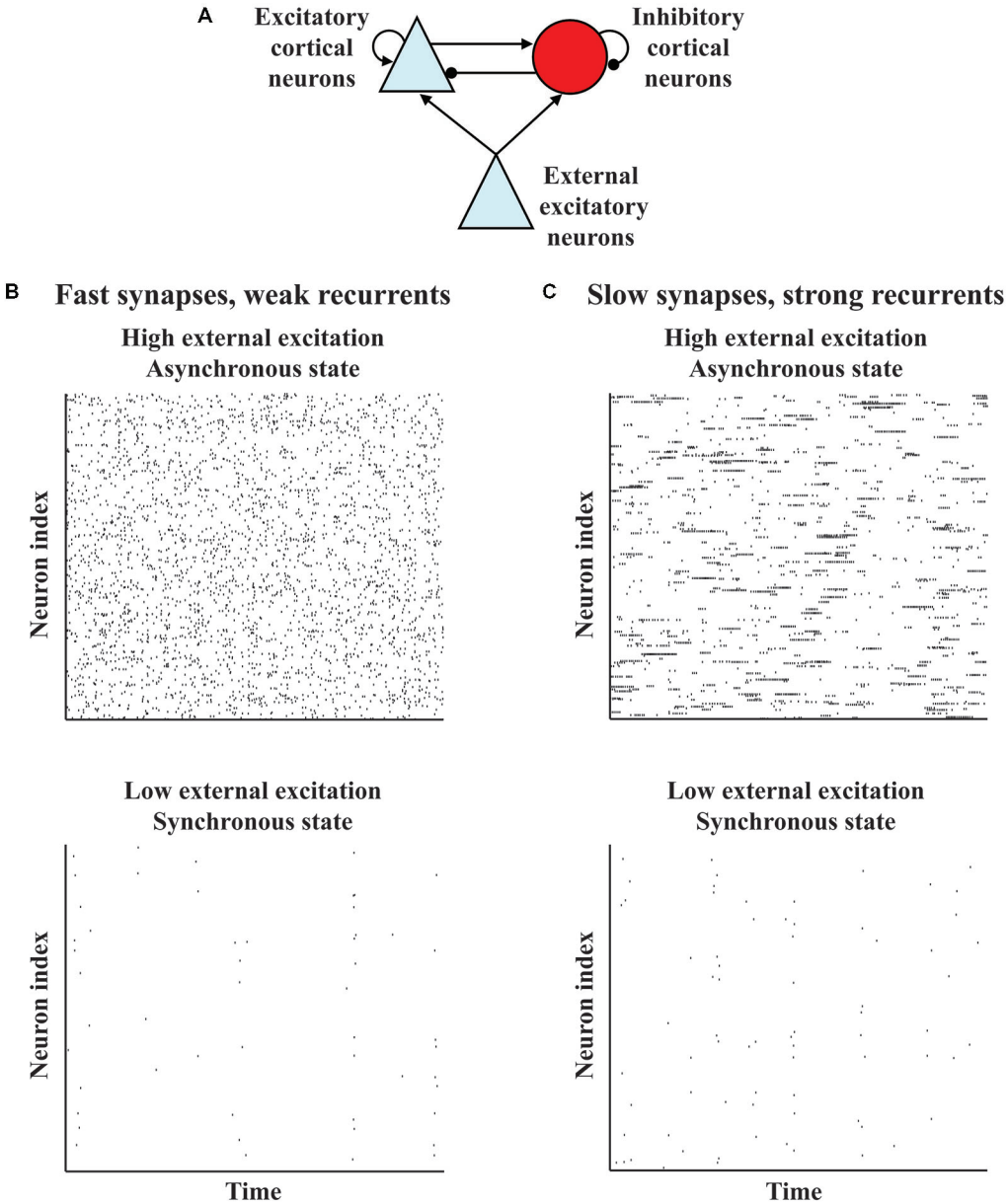

FIGURE 1 | Variant models of a basic organization show variants of a common behavior in which external excitation shifts the network from a synchronous to an asynchronous state. (A) Basic organization of models of a small patch of a cortical layer. The model networks contain recurrently connected excitatory and inhibitory neurons which receive external excitation. **(B)** Rasters indicating spike times of neurons from a model network (van Vreeswijk and Sompolinsky, 1998; Brunel, 2000; Mehring et al., 2003; Renart et al., 2010). The neurons show some sychronization at low external excitation, but become asynchronous at high external excitation. **(C)** Rasters indicating spike times of neurons from a variant model network with stronger recurrent connections and more slowly decaying synapses (Harish and Hansel, 2015). Like the model network in **(B)**, the neurons of the variant show some synchronization at low external excitation, but become asynchronous at high excitation. However, this variant model network differs from the model network in **(B)**, because each neuron of this variant in the asynchronous state has periods of sustained firing such that the autocorrelation of the neuron decays more slowly.

(LPFC) and orbitofrontal cortex (OFC) to the anterior cingulate cortex (ACC; Murray et al., 2014). Across LIP, LPFC and ACC, the time scale over which the autocorrelation of fluctuations decays correlates positively with the time scale over which a reward in one trial influences neural activity in subsequent trials (Murray et al., 2014). The variety of time scales may be due to each area's position in the cortical hierarchy and factors intrinsic to each area (Murray et al., 2014; Chaudhuri et al., 2015).

It is important to keep in mind that the context in which fixation is performed affects spontaneous activity (Andersen et al., 1990; Colby et al., 1996). However, the details of the above-mentioned studies indicate that the laminar differences in V1 and

the slower decay of autocorrelations at higher cortical levels are robust across fixation in several contexts.

Laminar and Areal Diversity of Slow Oscillations

Cortical slow oscillations are present during deep non-rapid-eye-movement sleep (Steriade et al., 2001; Buzsáki, 2006), ketamine and urethane anesthesia (Fuster et al., 1965; Fox and Armstrong-James, 1986; Metherate and Ashe, 1993; Steriade et al., 1993), and in cortical slices (Sanchez-Vives and McCormick, 2000). During slow oscillations, the EEG exhibits large amplitude fluctuations and the membrane potentials of cortical neurons alternate between depolarized "up" states and hyperpolarized "down" states (Fuster et al., 1965; Metherate and Ashe, 1993; Steriade et al., 1993). Increased activity during a down-to-up transition first occurs in layer 5 in cortical slices (Sanchez-Vives and McCormick, 2000), in the deep layers of the auditory cortex of urethane-anesthetized rats (Sakata and Harris, 2009) and the suprasylvian areas of ketamine-anesthetized and sleeping cats (Chauvette et al., 2010). In the sensory-motor areas of urethane-anesthetized mice slow oscillations are more greatly attenuated by suppressing the deep than the superficial layers (Beltramo et al., 2013). The prominence of the deep layers in slow oscillations in slices and *in vivo* suggests they are generated by common mechanisms, but the extent to which this is the case remains unclear (Crunelli et al., 2014). In contrast to the aforementioned studies, current source density analyses of recordings during sleep from frontal and parietal cortical areas of patients with drug-resistant focal epilepsy suggest that the superficial cortical layers are important for generating slow oscillations (Csercsa et al., 2010).

There is considerable diversity in the synchrony between slow oscillations in different cortical areas within the same hemisphere of urethane-anesthetized mice, although bilaterally corresponding regions are more synchronous (Mohajerani et al., 2010). Such diversity is also present during and varies over the course of human sleep, with fewer cortical regions exhibiting slow waves later into sleep (Nir et al., 2011). Asynchrony may be due to a consistent non-zero phase difference, or a lack of any consistent phase relationship. Both possibilities occur. In ketamine-anesthetized guinea pigs, slow oscillations in corresponding frequency regions of different tonotopic areas are coherent, having consistent phase differences that parallel the sound-evoked latencies of the various areas, whereas slow oscillations of different frequency regions are incoherent (Farley and Noreña, 2013). Slow oscillations thus resemble spontaneous gamma activity in reflecting auditory tonotopic organization (Fukushima et al., 2012).

Spontaneous activity can also distinguish between tonotopic and non-tonotopic areas, as the tonotopic primary auditory and non-tonotopic dorsoposterior (DP) fields of ketamine-anesthetized mice are distinguished by prominent spontaneous pulses in DP that can be entrained by sound (Stiebler et al., 1997; Joachimsthaler et al., 2014). Slow oscillations in ketamine-anesthetized mice further distinguish the PFC from the primary visual, somatosensory, and motor cortices, as PFC slow oscillations have faster down-to-up state transitions, higher firing rates during up states, and more regular cycles (Ruiz-Mejias et al., 2011).

Modulation of Spontaneous Activity by Attention and Anesthesia

Each previous section focused on spontaneous activity within a particular broadly-defined behavioral state. Since spontaneous activity is modulated by behavioral state, it is also interesting to compare its distribution across behavioral states. Accordingly we turn now to the effects of varying attentional context and anesthetic depth on the distribution of spontaneous activity in the cortex.

An animal may perform a reference behavior such as fixation following various cues, each of which sets an attentional context by signaling information about the task to be performed after the reference behavior. The neural activity during the reference behavior is conventionally called "spontaneous" or "baseline" activity (Luck et al., 1997; Chawla et al., 1999; Recanzone and Wurtz, 2000). Its distribution can be cue-dependent. For example, after monkeys viewed a cue indicating a particular visual location, baseline activity differentially increased in extrastriate visual neurons selective for the cued location (Luck et al., 1997; Recanzone and Wurtz, 2000). Similarly, after human subjects received a cue indicating either motion or color (Chawla et al., 1999), target location or color (Giesbrecht et al., 2006), a particular stimulus modality (Saupe et al., 2009; Langner et al., 2011) or object category (Puri et al., 2009), baseline activity differentially increased in cortical areas selective for the cued feature. The tasks in these studies involved attention as well as working memory, and the cue-dependence of baseline activity is thought to involve top-down signals from frontal and parietal cortical areas (Beck and Kastner, 2009).

Anesthetic depth also modulates the distribution of spontaneous activity. Several anesthetics cause EEG anteriorization in humans, in which EEG power shifts from posterior to anterior electrodes (Brown et al., 2010). Propofol anteriorization occurs mainly in the alpha range (Feshchenko et al., 2004; Purdon et al., 2013). Propofol has been proposed to increase anterior alpha power by potentiating GABAergic inhibition in the frontal thalamocortical network, and to decrease posterior alpha power by inhibiting the hyperpolarization-activated current I_h in the posterior thalamic network (Vijayan et al., 2013). Isoflurane, sevoflurane and halothane act like propofol on the model and were accordingly predicted to cause alpha anteriorization (Vijayan et al., 2013). Sevoflurane does cause the predicted alpha anteriorization, but with a theta coherence not observed with propofol (Akeju et al., 2014). The spatial modulation of spontaneous activity by isoflurane anesthesia has been studied in ferrets (Sellers et al., 2013). Spontaneous LFP in V1 of awake ferrets in a dark room exhibited a spectral peak near 18 Hz in the deep layers, which shifted towards 10 Hz with increasing isoflurane concentration. In comparison, spontaneous LFP in the PFC exhibited increased

power at all frequencies in all layers, and developed a spectral peak near 10 Hz in layer 4 and the deep layers with increasing isoflurane concentration. At the highest concentration the 10 Hz peak was abolished in V1 but sustained in the PFC, a pattern reminiscent of alpha anteriorization in humans. Anteriorization and the diversity of autocorrelation time scales (discussed in the section on fixation) both indicate differences between occipital and frontal areas, but it remains to be understood whether they are due to the same differences in circuitry.

Modulation of Spontaneous Activity by Plasticity

Longer lasting circuit modifications are likewise reflected in spontaneous activity. For example, coincident tone presentation and nucleus basalis stimulation, which alters auditory cortical frequency selectivity and enhances perceptual learning (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998; Reed et al., 2011), increases the spontaneous firing rates of neurons in the primary auditory cortex and posterior auditory field of pentobarbital-anesthetized rats (Puckett et al., 2007). Increased spontaneous firing rates have also been observed in PFC neurons of macaques that had learned a working memory task (Qi et al., 2011). The increased rates were accompanied by decreased baseline variability (Qi and Constantinidis, 2012b), and decreased correlations between fluctuations from the trial-averaged baseline firing of neurons separated by 0.5–1 mm (Qi and Constantinidis, 2012a). The changes occurred mainly in neurons that responded during stimulus-presentation or delay periods of the learned task. On a much larger spatial scale, changes in spontaneous correlations between visual and fronto-parietal areas following visual perceptual learning were revealed by functional magnetic resonance imaging of the blood-oxygen level-dependent (BOLD) signal in humans, and demonstrated to correlate with learning (Lewis et al., 2009). Alterations in the spatial distribution of spontaneous activity in pathologies such as spatial neglect following stroke (He et al., 2007b) and tinnitus following hearing loss (Weisz et al., 2005; Vanneste et al., 2011) have also been identified, and used to help develop candidate treatments (He et al., 2007a; Langguth et al., 2013).

Does the spatial diversity of spontaneous activity merely reflect structural and functional diversity across the cortex, or might it also have functional significance, perhaps contributing to the functional diversity? The latter possibility is hinted at by evidence that spontaneous activity contributes to determining the outcome of plasticity. For example, stimulation delivered in urethane-anesthetized rats to a cortical column increased its spontaneous correlation with a reference column only if the stimulation was in sync with spontaneous activity in the reference column (Erchova and Diamond, 2004), a result consistent with Hebbian plasticity (Fregnac et al., 1988; Jackson et al., 2006). Correlations between spontaneous activity characteristics and the rate or outcome of learning have been demonstrated in mice (Lin et al., 2013), rats (Arduin et al., 2013), monkeys (Sadtler et al., 2014), and humans (Freyer et al., 2013), suggesting some role for spontaneous activity in plasticity. Spontaneous activity can be an obstacle to long-lasting memories (Fusi et al., 2005). Reinforcement learning, on the other hand, is trial-and-error learning requiring variability, and may benefit from spontaneous activity (Mazzoni et al., 1991). Importantly, reinforcement learning can be implemented with biologically plausible models of synaptic plasticity in cortical networks (Bakin and Weinberger, 1996; Kilgard and Merzenich, 1998; Reynolds et al., 2001; Legenstein et al., 2008; Gavornik et al., 2009; Bourjaily and Miller, 2011; Rombouts et al., 2015). It is worth noting that learning may depend nonmonotonically on neural variability, and that neural covariability can be key (Legenstein et al., 2008, 2010). Intriguingly, a model incorporating a learning rule proposed to explain shifts of excitation and inhibition reported in a reinforcement learning paradigm (Froemke et al., 2007; Vogels et al., 2011) suggests that patterned spontaneous activity can stabilize memories (Litwin-Kumar and Doiron, 2014).

Summary and Future Directions

Let us summarize with an eye towards avenues for exploration. We have seen that cortical spontaneous activity is spatially diverse even within a behavioral state, a reflection of structural and functional diversity across the cortex. We noted theoretical proposals for the synaptic and intrinsic mechanisms underlying the diversity in function and spontaneous activity, which has been surveyed to date largely by observations of extracellular electromagnetic fields or blood-oxygenation. Further tests of the models are needed, and may be provided, for example, by intracellular recordings of membrane potential. During fixation, membrane potential in macaque V1 is far from spike threshold with non-Gaussian fluctuations (Tan et al., 2014). In contrast, some models of frontal areas predict that membrane potential hovers near spike threshold during fixation (Wang, 1999; Lundqvist et al., 2010; Vijayan et al., 2013; Chaudhuri et al., 2015; Harish and Hansel, 2015), resembling that observed in awake, but non-behaving cats (Steriade et al., 2001). Tests of such possible differences in membrane potential between V1 and frontal areas during fixation would help us understand how differences in circuitry among cortical regions supports their varied functions.

We also saw that behavioral state shifts and plasticity cause momentary or longer-lasting circuit changes that are reflected by the spatial distribution of spontaneous activity. Indeed, it is because spontaneous activity is affected by behavioral state and cortical location that this review has been structured according to states, such as fixation by behaving macaques, that are probably sufficiently specified for the diversity due to cortical location to be reproducibly observed. With respect to further characterization of the spatial diversity by intracellular recording of membrane potential, McGinley and colleagues have recently performed such recordings in mice trained to perform tone detection (McGinley et al., 2015). They show that measurements of pupil diameter characterize behavioral state sufficiently to predict when task performance is best, so trained mice may provide another valuable experimental paradigm in which behavioral state can be sufficiently specified for investigating

spatial diversity in spontaneous activity. Interestingly, the mice performed best when spontaneous membrane potential in the auditory cortex was relatively hyperpolarized (McGinley et al., 2015), resembling that in V1 during successful fixation by macaques (Tan et al., 2014).

Finally, we noted that spontaneous activity and the associated response variability could contribute to determining the outcome of plasticity. Neuromodulators like acetylcholine therefore perhaps affect plasticity not only by gating it (Gu, 2002; Chubykin et al., 2013; Chun et al., 2013), but also by modulating spontaneous activity and response variability (Zinke et al., 2006; Goard and Dan, 2009; Zhou et al., 2011). There is evidence that variability is needed for or enhances some forms of motor learning in people (Sans-Muntadas et al., 2014; Taylor and Ivry, 2014) and song birds (Woolley and Kao, 2015). Consequently, more work seems warranted to understand whether the variability associated with cortical spontaneous activity has a role in cortical plasticity, so that it is itself a factor shaping the functional diversity of the cortex.

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Frequency-specific alternations in the amplitude of low-frequency fluctuations in chronic tinnitus

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Tinnitus, a phantom ringing, buzzing, or hissing sensation with potentially debilitating consequences, is thought to arise from aberrant spontaneous neural activity at one or more sites within the central nervous system; however, the location and specific features of these oscillations are poorly understood with respect to specific tinnitus features. Recent resting-state functional magnetic resonance imaging (fMRI) studies suggest that aberrant fluctuations in spontaneous low-frequency oscillations (LFO) of the blood oxygen level-dependent (BOLD) signal may be an important factor in chronic tinnitus; however, the role that frequency-specific components of LFO play in subjective tinnitus remains unclear. A total of 39 chronic tinnitus patients and 41 well-matched healthy controls participated in the resting-state fMRI scans. The LFO amplitudes were investigated using the amplitude of low-frequency fluctuation (ALFF) and fractional ALFF (fALFF) in two different frequency bands (slow-4: 0.027–0.073 Hz and slow-5: 0.01–0.027 Hz). We observed significant differences between tinnitus patients and normal controls in ALFF/fALFF in the two bands (slow-4 and slow-5) in several brain regions including the superior frontal gyrus (SFG), inferior frontal gyrus, middle temporal gyrus, angular gyrus, supramarginal gyrus, and middle occipital gyrus. Across the entire subject pool, significant differences in ALFF/fALFF between the two bands were found in the midbrain, basal ganglia, hippocampus and cerebellum (Slow 4 > Slow 5), and in the middle frontal gyrus, supramarginal gyrus, posterior cingulate cortex, and precuneus (Slow 5 > Slow 4). We also observed significant interaction between frequency bands and patient groups in the orbitofrontal gyrus. Furthermore, tinnitus distress was positively correlated with the magnitude of ALFF in right SFG and the magnitude of fALFF slow-4 band in left SFG, whereas tinnitus duration was positively correlated with the magnitude of ALFF in right SFG and the magnitude of fALFF slow-5 band in left SFG. Resting-state fMRI provides an unbiased method for identifying aberrant spontaneous LFO occurring throughout the central nervous system. Chronic tinnitus patients have widespread abnormalities in ALFF and fALFF slow-4 and slow-5 band which are correlated with tinnitus distress and duration. These results provide new insights on the neuropathophysiology of chronic tinnitus; therapies capable of reversing these aberrant patterns may reduce tinnitus distress.

Keywords: chronic tinnitus, Frequency, ALFF, fALFF, fMRI

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INTRODUCTION

Chronic subjective tinnitus is a common hearing disorder often described as a buzzing, sizzling or ringing sensation that occurs in the absence of an objective sound. Among adults roughly 12% experience tinnitus, but the prevalence rises to 40–50% among military combat personnel exposed to intense noise (McFadden, 1982; Meikle, 1997; Cave et al., 2007; Hébert et al., 2013). In severe cases, chronic tinnitus can disrupt sleep and cause emotional distress and concentration difficulties (Leske, 1981; Lockwood et al., 2002). Since the phantom sound is often perceived in the impaired ear, tinnitus was originally believed to originate in the cochlea from aberrant hyperactivity in the auditory nerve. However, since surgical section of the auditory nerve fails to eliminate or reduce tinnitus (Dandy, 1941; Baguley et al., 1992; Jackler and Whinney, 2001), the hunt for the neural generators of tinnitus has shifted to the central nervous system. On the basis of previous electrophysiological and neuroimaging studies (Lockwood et al., 1998; Kaltenbach et al., 2005), tinnitus is now believed to be generated by aberrant neural activity in the central nervous system through such mechanisms as spontaneous hyperactivity, increased burst firing, heightened neural synchrony, aberrant tonotopic maps, abnormal coupling in distributed neural networks involving both auditory and non-auditory structures and aberrant gating of conscious perception (Henry et al., 2014). While there is support for each of these, the exact mechanisms responsible for the multifaceted dimensions of tinnitus (e.g., loudness, spectral profile, tinnitus severity, and distress) remain unclear.

Since the first human electroencephalographic (EEG) recordings in the 1920s, there has been a growing interest in neural oscillatory activity which occurs over a broad frequency range (0.05–500 Hz; Gomez et al., 2004; Kuo and Yang, 2009). The oscillations occurring in neural networks extending over large area of the brain are thought to play critical roles in perception, attention, memory and cognition and disruptions in these oscillatory patterns have been linked to neurological disorders such as epilepsy, autism, and tinnitus (Buzsáki and Draguhn, 2004). More recently, spontaneous low frequency oscillations (LFO) in the 0.01–0.1 Hz range were discovered in blood oxygen level-dependent (BOLD) signals measured during resting-state functional magnetic resonance imaging (fMRI; Biswal et al., 1995). These LFO are considered physiologically meaningful, are correlated with certain components of low frequency EEG (Penttonen and Buzsáki, 2003; Mantini et al., 2007) and are thought to contribute to long-range functional connectivity (Penttonen and Buzsáki, 2003). Resting-state fMRI has proved to be a useful noninvasive technique to assess normal and pathological brain function (Fox and Raichle, 2007; Zhang and Raichle, 2010; Schmidt et al., 2013).

Most studies to date have demonstrated disrupted functional connectivity in tinnitus using resting-state fMRI (Husain and Schmidt, 2014). Functional connectivity describes the temporal synchrony or interregional cooperation between two or more spatially separate regions. Abnormal resting-state functional networks have been found in tinnitus, such as the auditory network (Burton et al., 2012; Kim et al., 2012; Maudoux et al., 2012a; Schmidt et al., 2013), default mode network (DMN; Schmidt et al., 2013; Chen et al., 2015a,b), dorsal attention network (DAN; Burton et al., 2012; Schmidt et al., 2013), ventral attention network (VAN; Burton et al., 2012). Nonetheless, these findings are based on investigations of LFO from the perspective of temporal synchrony or interregional cooperation, but fail to consider the amplitude of alternations in local or regional neuronal activity. To identify local brain areas with abnormal activity, our group used the amplitude of low-frequency fluctuations (ALFF; Biswal et al., 1995; Zang et al., 2007) to identify specific brain regions with hyper- and hypoactive BOLD signal in patients with chronic tinnitus. Importantly, our patients had normal hearing thresholds up to the extended high frequencies and no evidence of hyperacusis (Chen et al., 2014). The ALFF values (0.01–0.08 Hz) in tinnitus patients were significantly increased in several brain regions including the middle temporal gyrus (MTG), superior frontal gyrus (SFG), and angular gyrus (AG), but were decreased in the visual cortex and thalamus. Further correlation of ALFF with tinnitus features indicated that abnormal ALFF patterns in tinnitus were linked to the duration and severity of tinnitus.

Most resting-state fMRI studies have examined tinnitus-related neural activity in a broad, low frequency band between 0.01 and 0.1 Hz (Biswal et al., 1995). Some studies, however, have found that aberrant patterns of intrinsic brain activity are restricted to specific frequency bands; these sub-bands may be generated by distinct oscillators with unique properties and physiological functions some of which are related to the alpha band in EEG (Penttonen and Buzsáki, 2003; Buzsáki and Draguhn, 2004). By decomposing resting-state fMRI LFO into four distinct frequency bands [Slow-5 (0.01–0.027 Hz), Slow-4 (0.027–0.073 Hz), Slow-3 (0.073–0.198 Hz), and Slow-2 (0.198–0.25 Hz)], Zuo et al. (2010) showed that gray matter (GM)-associated LFO amplitudes primarily occurred in the slow-4 and slow-5 frequency bands. Frequency-dependent changes in the ALFF/fALFF have been found in various neuropsychiatric diseases such as amnestic mild cognitive impairment (Han et al., 2011), schizophrenia (Yu et al., 2014), Parkinson's disease (Hou et al., 2014), and epilepsy (Wang et al., 2014). It has been confirmed that the slow-4 band has greater test–retest reliability for LFO amplitude measures and more reliable BOLD fluctuation amplitude voxels than the slow-5 band (Zuo et al., 2010), and the functional brain networks derived in the slow-4 are more reliable than those in slow-5 (Liang et al., 2012). Moreover, the slow-5 showing higher power localizes more within DMN regions such as the middle frontal gyrus (MFG) and precuneus, while the slow-4 exhibiting less power is more robust in midbrain and basal ganglia (Zuo et al., 2010; Baria et al., 2011; Han et al., 2011), indicating that individual frequency bands might be linked to specific properties (Buzsáki and Draguhn, 2004). Therefore, these researches suggest that the inherent brain functional activity is sensitive to specific frequency bands. Although abnormal LFO measures were observed in chronic tinnitus patients, an important and potentially interesting question is whether the abnormalities in the LFO signal occur within all frequency bands or only certain bands, i.e., frequency specific alternations.

To address this issue, we compared the changes of LFO amplitudes in chronic tinnitus patients using two resting-state fMRI metrics: ALFF and fractional ALFF (fALFF). ALFF measures the total power within a broad frequency range (typically 0.01–0.10 Hz; Zang et al., 2007) while fALFF measures the power within specific frequency bands divided by the total power in the entire frequency range, i.e., frequency specific, normalized amplitudes that reflect the relative contribution of each band to the total power (Zou et al., 2008). We hypothesized that (1) aberrant LFO amplitudes in tinnitus may depend on the specific frequency bands, especially the slow-4 and slow-5 bands that were mainly associated with neuronal oscillations, and that (2) these frequency specific abnormalities would be correlated with unique tinnitus characteristics such as tinnitus duration or distress.

MATERIALS AND METHODS

Subjects

All subjects provided written informed consent before their participation in the study protocol, which was approved by the Research Ethics Committee of the Affiliated Zhongda Hospital of Southeast University.

This study was conducted from September 2011 to September 2013. A total of 82 subjects including 41 chronic tinnitus patients and 41 healthy controls were recruited through community health screenings and newspaper advertisements. The tinnitus patients and healthy subjects were group-matched in terms of age, sex, and education. Two tinnitus patients were subsequently excluded because of they exceeded the limits of head motion during MR scanning. Sixteen patients reported a predominantly left-sided tinnitus, 13 a predominantly right-sided tinnitus, and 10 patients described their tinnitus as bilateral or originating within the head. All subjects were right handed and completed at least 8 years of education. The patients ranged from 20 and 70 years of age (41.5 ± 14.6 years), with tinnitus duration of 6–120 months (36.9 ± 36.4 months). The severity of tinnitus and related distress were assessed by the Iowa version of the Tinnitus Handicap Questionnaires (THQ; Kuk et al., 1990). Hearing thresholds were determined by puretone audiometry (PTA) examination. All participants had normal hearing (thresholds <25 dB HL) in the 10 frequencies from 250 Hz to 16 kHz. There were no significant differences in auditory thresholds between the tinnitus and control groups. None of the participants had accompanied symptoms such as depression and anxiety according to the Self-Rating Depression Scale (SDS) and Self-Rating Anxiety Scale (SAS; overall scores <50, respectively; Zung, 1971, 1986). Participants were excluded from the present study if they suffered from pulsatile tinnitus, hyperacusis or Meniere's diseases, or if they had a past history of severe smoking, alcoholism, brain injury, stroke, Alzheimer's disease, Parkinson's disease, epilepsy, major depression, or other neurological or psychiatric disorders that could affect cognitive function, major medical illness (e.g., anemia, thyroid dysfunction and cancer), MRI contraindications, or severe visual loss. The characteristics of the chronic tinnitus patients and healthy subjects are summarized in **Table 1**.

MRI Acquisition

Magnetic resonance imaging data were acquired at the Radiology Department of Zhongda Hospital using a 3.0 T MRI scanner (Siemens MAGENETOM Trio, Erlangen, Germany). Head motion and scanner noise were reduced using foam padding and earplugs. The earplugs (Hearos Ultimate Softness Series, USA) were used to attenuate scanner noise by approximately 32 dB. Subjects were instructed to lie quietly with their eyes closed without falling asleep, not think of anything in particular, and avoid any head motion during the scan. Functional images were obtained axially using a gradient echo-planar imaging sequence as follows: repetition time (TR) = 2000 ms; echo time (TE) = 25 ms; slices = 36; thickness = 4 mm; gap = 0 mm; field of view (FOV) = 240 mm × 240 mm; acquisition matrix = 64 × 64; and flip angle (FA) = 90°. The resting-state fMRI scan took 8 min and 6 sec. Structural images were acquired with a T1-weighted 3D spoiled gradient-echo sequence as follows: TR = 1900 ms; TE = 2.48 ms; slices = 176; thickness = 1 mm; gap = 0 mm; FA = 90°; acquisition matrix = 256 × 256; FOV = 250 mm × 250 mm. The structural sequence took 4 min and 18 sec.

Functional Data Preprocessing

Functional data analyses were conducted using Data Processing Assistant for Resting-State fMRI (DPARSF) programs (Chao-Gan and Yu-Feng, 2010), based on statistical parametric mapping (SPM8[1]) and resting-state fMRI data analysis toolkits (REST[2]). A total of 240 volumes were scanned, and the first 10 volumes were discarded to allow for signal equilibrium of the initial magnetic resonance signals and adaptation of the subjects to the circumstances. The remaining 230 consecutive volumes were used for data analysis. Subsequently, the following procedures were conducted in order: slice-timing adjustment, realignment for head-motion correction, spatial normalization to the Montreal Neurological Institute (MNI) template (resampling

[1]http://www.fil.ion.ucl.ac.uk/spm

[2]http://www.restfmri.net

TABLE 1 | Characteristics of tinnitus patients and healthy controls.

	Tinnitus patients (n = 39)	Healthy controls (n = 41)	p-value
Age (year)	41.5 ± 14.6	46.0 ± 12.2	0.137
Gender (male: female)	24:15	21: 20	0.352
Education levels (years)	10.7 ± 1.9	11.1 ± 1.5	0.545
Tinnitus duration (months)	36.9 ± 36.4	–	–
THQ score	43.5 ± 21.3	–	–

Data are represented as mean ± SD. THQ, tinnitus handicap questionnaire.

voxel size = 3 mm × 3 mm × 3 mm) and smoothing with an isotropic Gaussian kernel (FWHM = 6 mm), detrending and filtering (0.01–0.08 Hz). Any subjects with a head motion >2.0 mm translation or a 2.0° rotation in any direction were excluded.

ALFF and fALFF Calculation

Amplitude of low-frequency fluctuation/fALFF was calculated using REST software. ALFF values were calculated through procedures described previously (Zang et al., 2007). Briefly, time courses were first converted to the frequency domain using a Fast Fourier Transform. The square root of the power spectrum was computed and then averaged across a predefined frequency interval. This averaged square root was termed ALFF at a given voxel, and was further divided by the individual global mean ALFF value to reduce the global effects of variability across participants. fALFF is the fraction of ALFF in a given frequency band divided by the ALFF over the entire frequency range (0–0.25Hz) detectable in the signal (Zou et al., 2008). The full frequency range (0–0.25 Hz) of ALFF was divided into five different bands: slow-6 (0–0.01 Hz), slow-5 (0.01–0.027Hz), slow-4 (0.027–0.073 Hz), slow-3 (0.073–0.198 Hz), and slow-2 (0.198–0.25 Hz), (Buzsáki and Draguhn, 2004; Zuo et al., 2010). The signals of slow-6, slow-3, and slow-2 were discarded because they mainly reflect very low frequency drift, white matter (WM) signals, and high-frequency physiological noises, respectively, (Biswal et al., 1995; Zuo et al., 2010). Slow 4 and slow 5 were used for further analyses.

Structural Data Analyses

To exclude the effect of structural damage on ALFF measurements, we performed a voxel-based morphometry (VBM) approach to compute the GM volume of each subject using DPARSF software. Briefly, cerebral tissues were segmented into GM, WM, and cerebrospinal fluid and were then normalized to the MNI space using a unified segmentation algorithm (Ashburner and Friston, 2005). T1 images were normalized to the MNI template using affine linear registration followed by Gaussian smoothing (FWHM = 6 mm). GM and WM volumes were calculated by estimating these segments. Brain parenchyma volume was calculated as the sum of GM and WM volumes.

Statistical Analysis

Differences in demographic data between tinnitus patients and healthy controls were analyzed using between-group *t*-test for means and χ^2-test for proportions (statistical significance was set at $p < 0.05$) using SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). To determine the effects of group, frequency band and interaction between frequency band and group on ALFF/fALFF, we performed a two-way repeated-measures analysis of variance (ANOVA) on a voxel-by-voxel basis with group (tinnitus patients and healthy controls) as a between-subject factor and frequency band (slow-4 and slow-5) as a repeated-measures variable using SPM8 software. All the statistical maps were corrected for multiple comparisons to a significance level of $p < 0.05$ by combining the individual voxel $p < 0.05$ with cluster size >3591 mm^3 based on Monte Carlo simulations (Ledberg et al., 1998; Han et al., 2011).

Further correlative analyses between ALFF/fALFF values of significantly different brain regions and tinnitus characteristics were performed on the tinnitus groups by extracting the most significantly different frequency between groups using REST software. Then, Pearson's correlation coefficients between abnormal ALFF/fALFF values and each clinical characteristic were analyzed using SPSS software. Partial correlations were calculated after correction for age, sex, and education. Given the large age range of the subjects in the current study, we performed a multiple regression in SPM8 software to investigate the potentially altered brain regions accompanied with increased age in each group.

RESULTS

Structural Data

Table 2 presents the comparisons of the whole-brain volumes (GM volume, WM volume, and brain parenchyma volume) between the tinnitus patients and the healthy controls. The results showed that there were no significant changes in GM and WM volumes between the two groups ($p > 0.05$).

ALFF Analyses

First, we examined the effect of tinnitus on ALFF values. Main effects from the two-way repeated-measure ANOVA are shown in **Figure 1**. Brain regions showing a significantly larger ALFF in tinnitus patients compared to healthy controls (TIN > HC, red colors) were seen in right SFG, right MTG, right AG, and left inferior frontal gyrus (IFG). Brain regions in which ALFF values were larger in healthy controls than in tinnitus patients (TIN < HC, blue colors) were found in bilateral middle occipital gyrus (MOG), (**Figure 1A** and **Table 3**).

Next, we examined the data to determine the effect of slow-4 and slow-5 bands of ALFF (**Figure 2**). Brain regions showing a significantly larger slow-4 ALFF compared to slow-5 ALFF were found in the pons, midbrain, striatum, thalamus, hippocampus, and cerebellum (Slow 4 > Slow 5, red colors). In contrast, the values of slow-5 ALFF that were significantly larger than slow-4 ALFF occurred in the MFG, supramarginal gyrus (SMG), posterior cingulate cortex (PCC), and precuneus (Slow 5 > Slow 4, blue colors). In addition, we observed a significant interaction between frequency band and groups in the right orbitofrontal

TABLE 2 | Comparisons of brain volumes in tinnitus patients and healthy controls.

	Tinnitus patients ($n = 39$)	Healthy controls ($n = 41$)	*p*-value
GM	585.82 ± 24.86	576.93 ± 23.90	0.107
WM	539.31 ± 27.39	535.83 ± 30.50	0.594
Brain parenchyma	1118.33 ± 32.26	1112.27 ± 40.36	0.461

Data are presented as mean ± SD. GM, gray matter; WM, white matter.

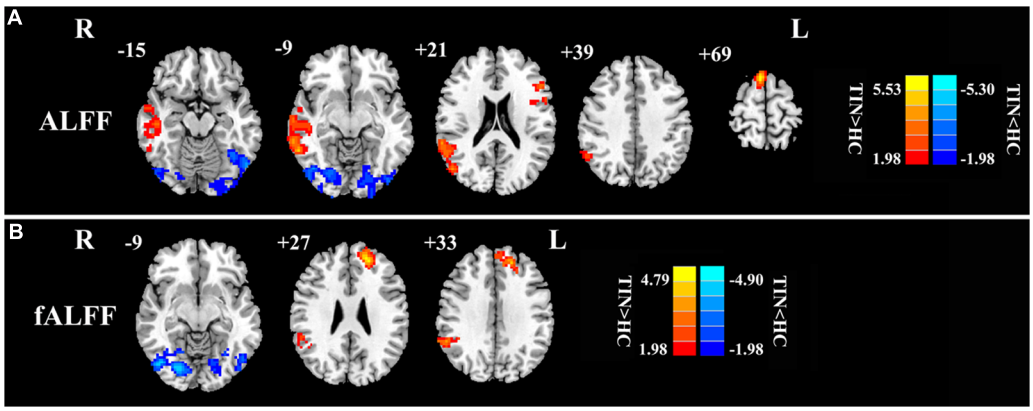

FIGURE 1 | The main effect of group on: (A) ALFF and (B) fALFF. Red/hot colors represent higher ALFF (*t*-values 1.98 to 5.53; red to yellow, respectively) or fALFF (*t*-values 1.98 to 4.79; red to yellow, respectively) in the tinnitus group than in the control group while blue/cold colors represent lower ALFF (*t*-values –1.98 to –5.30; dark blue to light blue, respectively) or fALFF (*t*-values –1.98 to –4.90; dark blue to light blue, respectively) in the tinnitus group than controls. Results obtained by a two-way repeated-measure ANOVA. AlphaSim corrected $p < 0.05$ (individual voxel threshold $p < 0.05$ with a minimum cluster size of 3591 mm^3). **Tables 3** and **4** show regions where significant increases and decreases occurred.

cortex (OFC; $p < 0.05$, AlphaSim corrected, **Figure 3A** and **Table 3**). **Figure 4A** showed the ALFF values of each significant brain regions between tinnitus patients and healthy controls in slow-4 and slow-5 frequency bands.

fALFF Analyses

For the fALFF analyses that only included the slow-4 and slow-5 band, the brain regions showing a significant main effect/difference between tinnitus patients and controls (**Figure 1B** and **Table 4**) including the left SFG, right SMG (TIN > HC, red colors), and the bilateral MOG (TIN < HC, blue colors). The general locations of the main effects for slow-4 and slow-5 fALFF (**Figure 5**) were very similar to that in ALFF (**Figure 2**). In addition, we also observed significant interaction between frequency bands and groups in the right OFC ($p < 0.05$, AlphaSim corrected, **Figure 3B** and **Table 4**). **Figure 4B** showed the fALFF values of each significant brain regions between tinnitus patients and healthy controls in slow-4 and slow-5 frequency bands.

TABLE 3 | Result of group × frequency ANOVA of ALFF.

Brain region	BA	Peak MNI coordinates *x, y, z* (mm)	Peak *t*-value	Voxels
Main effect of group				
R middle temporal gyrus	21	60, –51, –9	4.9609	907
L inferior frontal gyrus	45	–51, 24, 21	4.0804	282
R angular gyrus	39	45, –75, 39	4.6951	143
R superior frontal gyrus	8	6, 15, 69	6.2468	783
B middle occipital gyrus	19	–54, –66, –15	–4.1240	472
Group × frequency interaction				
R orbitofrontal cortex	25	12, 18, –18	3.1698	375

Corrected threshold of $p < 0.05$ determined by Monte Carlo simulation indicating a significant difference. BA, Brodmann's area; MNI, Montreal Neurological Institute; L, left; R, right; B, bilateral.

Correlation Analysis Results

We calculated the correlations between aberrant slow-4 and slow-5 bands in ALFF/fALFF and tinnitus characteristics to determine if there were any significant relationships. In tinnitus patients, slow-4 ALFF in right SFG (**Figure 6A**, left panel, $r = 0.446$, $p = 0.007$) and slow-4 fALFF in left SFG (**Figure 6A**, right panel, $r = 0.466$, $p < 0.005$) were positively correlated with THQ scores. Moreover, slow-5 ALFF in right SFG (**Figure 6B**, left panel, $r = 0.544$, $p = 0.001$) and slow-5 fALFF in left SFG (**Figure 6B**, right panel, $r = 0.526$, $p = 0.001$) were positively correlated with tinnitus duration. The ALFF activity in right SFG was not correlated with the fALFF in left SFG (Supplementary Figure S1). The other significant increases or decreases in ALFF or fALFF values were not significantly related to THQ scores or tinnitus duration (**Table 5**). We also did not observe any significant brain regions affected by increase of age in each group. In addition, the tinnitus severity was not significantly correlated with tinnitus duration through a multiple regression analysis (Supplementary Figure S2).

DISCUSSIONS

To our knowledge, this is the first study to examine changes of LFO amplitudes (ALFF and fALFF) in chronic tinnitus at two different frequency bands (slow-4 and slow-5 bands). We found significant differences between tinnitus patients and matched controls in ALFF/fALFF slow-4 and slow-5 bands in several brain regions. The ALFF and fALFF in the slow-4 band were higher in the midbrain, basal ganglia, hippocampus and cerebellum, and lower in the MFG, SMG, PCC, and precuneus, in comparison to that in slow-5. In addition, the right OFC exhibited significant interaction in ALFF/fALFF between frequency bands and groups.

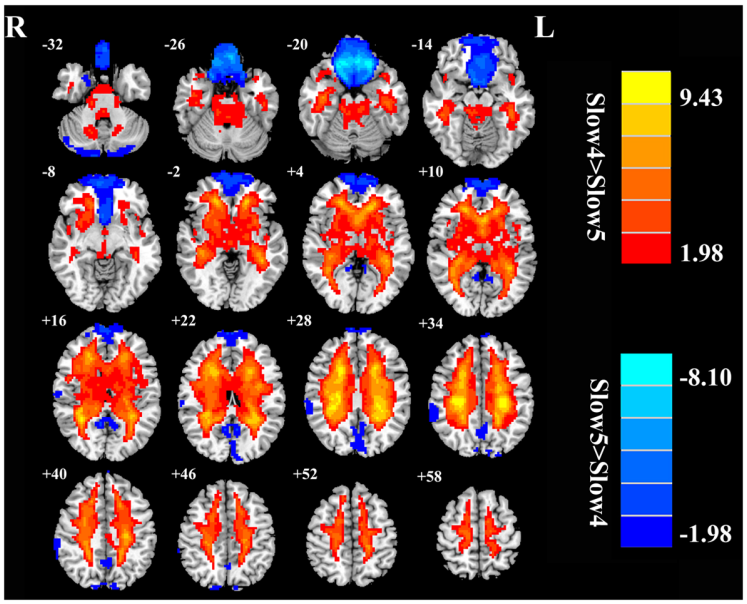

FIGURE 2 | The main effects of frequency band on ALFF. Red/hot colors identify brain regions where slow-4 ALFF values were significantly greater than slow-5 ALFF values (*t*-values 1.98 to 9.43; red to yellow, respectively). Blue/cold colors identify brain regions where the slow-5 ALFF values were significantly greater than the slow-4 ALFF band values (*t*-values –1.98 to –8.10; dark blue to light blue, respectively). Results were obtained by a two-way repeated-measure ANOVA. AlphaSim corrected $p < 0.05$ (individual voxel threshold $p < 0.05$ with a minimum cluster size of 3591 mm^3).

Importantly, aberrant ALFF/fALFF activity in the SFG at slow-4 and slow-5 bands was correlated with tinnitus duration and tinnitus distress. Our results suggest that the abnormal spontaneous LFO amplitudes in chronic tinnitus patients are frequency dependent.

In the current study, two different kinds of metrics (ALFF and fALFF) were used to investigate the changes of LFO amplitudes in tinnitus patients in slow-4 and slow-5 bands. Surprisingly, the abnormalities of both ALFF and fALFF activity were similar in many brain regions, especially in the SFG, which was significantly correlated with the tinnitus characteristics. ALFF is calculated as the sum of amplitudes within a specific low frequency range (0.01–0.1 Hz) while fALFF is calculated as a fraction of the sum of amplitudes across the entire frequency range (0–0.25 Hz) detectable in a given signal (Zang et al., 2007; Zou et al., 2008). These two are promising quantitative methods for measuring the LFO amplitudes and detecting spontaneous brain activity. Although both ALFF and fALFF are sensitive mostly to signal from GM region, ALFF is more prone to noise from physiological sources, particularly near the ventricles and large blood vessels, and these are attenuated in fALFF (Zou et al., 2008; Zuo et al., 2010). Anyway, both algorithms have been applied to evaluate the LFO amplitudes of normal and pathological brains (Yang et al., 2007; Zang et al., 2007; Han et al., 2011; Yu et al., 2014). However, the increased specificity to the GM signal for fALFF compared to ALFF may suggest favoring the former, but will reduce test–retest reliability, making fALFF intrinsically less reliable (Arndt et al., 1991; Zuo et al., 2010). Thus, in order to maximize the reliability across subjects while providing sufficient specificity to examine individual differences, it is probably most advisable to report findings with both measures (Zuo et al., 2010). Further studies will be required to determine which method is more effective and better for identifying aberrant spontaneous LFO amplitudes in tinnitus remains an important question for future work.

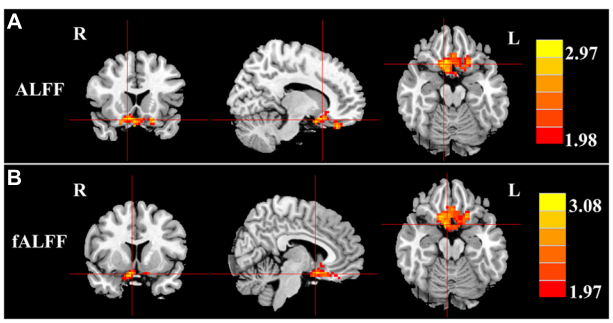

FIGURE 3 | Interaction between frequency bands and tinnitus vs. control groups on (A) ALFF values and (B) fALFF values in the orbitofrontal gyrus. Red/hot colors identify brain regions where there was a significant interaction effect on ALFF (*t*-values 1.98 to 2.97; red to yellow, respectively) or fALFF (*t*-values 1.97 to 3.08; red to yellow, respectively). Results were obtained by a two-way repeated-measure ANOVA and *post hoc* test. AlphaSim corrected $p < 0.05$ (individual voxel thresholds $p < 0.05$ with a minimum cluster size of 3591 mm^3).

Our group effect analyses showed that the ALFF was higher in right SFG, right MTG, left IFG, and right AG while the fALFF was higher in left SFG and right SMG, but both were

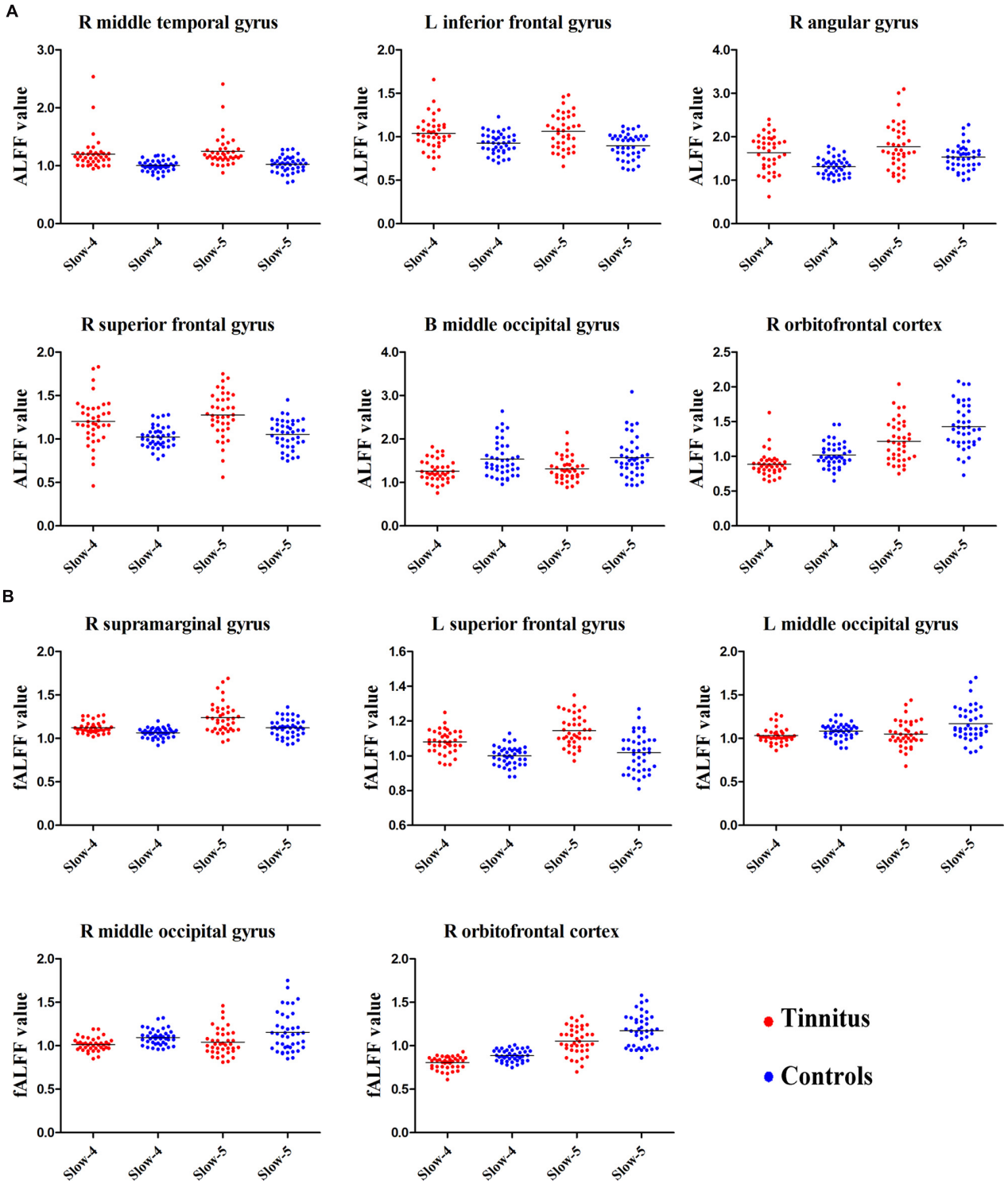

FIGURE 4 | The scatter plot showed the significant brain regions of abnormal (A) ALFF or (B) fALFF between tinnitus patients and healthy controls in slow-4 and slow-5 frequency bands. The red dots represented tinnitus patients; the blue dots represented healthy controls. L, left; R, right; B, bilateral.

lower in bilateral MOG in tinnitus patients than in healthy controls (**Figure 1**; **Tables 3** and **4**). These results are consistent with our earlier report using ALFF in the typical frequency band (0.01–0.08 Hz; Chen et al., 2014). Using single photon emission computed tomography (SPECT) and positron emission tomography (PET), previous studies have demonstrated that

TABLE 4 | Result of group × frequency ANOVA of fALFF.

Brain region	BA	Peak MNI coordinates *x, y, z* (mm)	Peak *t*-value	Voxels
Main effect of group				
R supramarginal gyrus	40	57, –48, 33	3.6976	260
L superior frontal gyrus	10	–21, 45, 27	5.3486	781
L middle occipital gyrus	19	–51, –69, –9	–3.3196	269
R middle occipital gyrus	19	42, –72, –9	–4.8310	437
Group × frequency interaction				
R orbitofrontal cortex	25	6, 9, –18	3.3001	314

Corrected threshold of $p < 0.05$ determined by Monte Carlo simulation indicating a significant difference. BA, Brodmann's area; MNI, Montreal Neurological Institute; L, left; R, right.

tinnitus patients exhibited hypermetabolism and hyperperfusion in the MTG (Mirz et al., 1999; Plewnia et al., 2007b; Farhadi et al., 2010), AG (Mirz et al., 1999; Plewnia et al., 2007a; Song et al., 2012), and SMG (Mirz et al., 1999; Plewnia et al., 2007a) confirming the involvement of these structures for tinnitus. More importantly and in a broader context, these brain regions are components of the DMN. The DMN, which consists of nodes in the MTG, MFG, PCC, precuneus, and inferior parietal lobe, is most active at rest and shows reduced activity when a subject enters a task-based state involving attention or goal-directed behavior (Raichle et al., 2001; Mantini et al., 2007). Using resting-state fMRI, several studies have found disrupted spontaneous neuronal activity and functional connectivity in the DMN regions in tinnitus patients (Burton et al., 2012; Maudoux et al., 2012b; Schmidt et al., 2013; Chen et al., 2014, 2015a,b; Zhang et al., 2015). Thus, we speculate that the phantom sound of tinnitus, particular severe cases that cause anxiety or distress, may disrupt the resting-state DMN. However, the source or type of aberrant spontaneous neuronal activity within DMN regions due to tinnitus remains unknown.

Several studies have reported that the frontal cortex plays a pivotal role in tinnitus (Jastreboff, 1990; Lanting et al., 2009; Vanneste et al., 2012). Consistent with this view we found increased ALFF/fALFF in the SFG. Besides the significant group difference, significant positive correlations were observed between increased ALFF/fALFF in bilateral SFG and tinnitus duration or tinnitus distress in both slow-4 and slow-5 bands (**Figure 6**). Other neuroimaging studies have identified abnormalities in the frontal cortex associated with tinnitus (Lanting et al., 2009; Schecklmann et al., 2013; Chen et al., 2014). Rauschecker et al. (2010) found structural and functional differences between tinnitus patients and controls in ventromedial prefrontal cortex; these differences were related to the subjective loudness of tinnitus (Leaver et al., 2011). Using PET, a significant increase in metabolism was seen in the SFG of tinnitus (Mirz et al., 2000a), some of whom could modulate the loudness of their tinnitus (Lockwood et al., 2001). In several task-based fMRI studies, the SFG was found to be activated in healthy subjects by aversive acoustic stimuli, suggesting that the negative affect associated with tinnitus may involve this area of the cortex (Mirz et al., 2000b). These results are consistent with greater activation in the STG of tinnitus patients during a pitch-discrimination task in an emotional context (Wunderlich et al., 2009). While it is difficult to establish conclusive interpretations from the correlation results, we speculate that the

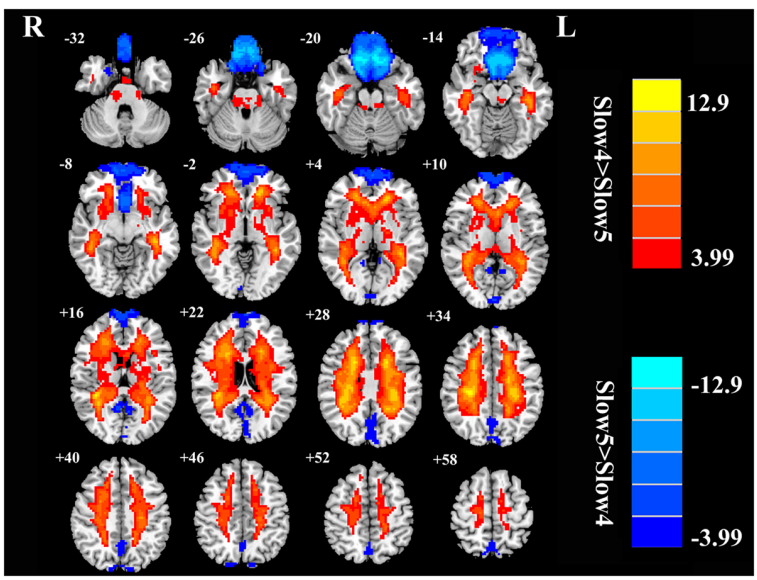

FIGURE 5 | The main effects for frequency band on fALFF. Red/hot colors identify brain regions where slow-4 fALFF values were significantly greater than slow-5 fALFF values (*t*-values 3.99 to 12.9; red to yellow, respectively). Blue/cold colors identify brain regions where slow-5 fALFF values were significantly greater than slow-4 fALFF values (*t*-values –3.99 to –12.9; dark blue to light blue, respectively). Results were obtained by a two-way repeated-measure ANOVA. AlphaSim corrected $p < 0.05$ (individual voxel thresholds $p < 0.05$ with a minimum cluster size of 3591 mm^3).

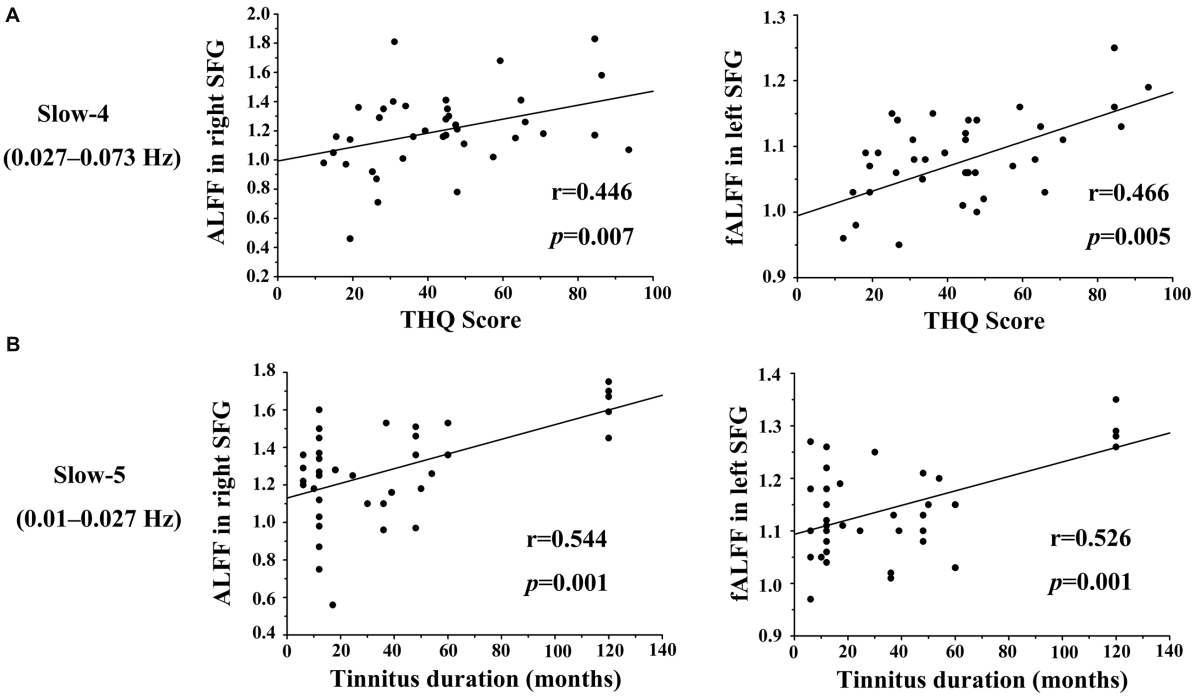

FIGURE 6 | Significant correlations observed between the ALFF/fALFF in two frequency bands vs. THQ score and tinnitus duration. (A) Slow-4 ALFF values in right SFG (left) and fALFF values in left SFG (right) were positively correlated with THQ scores ($r = 0.446$, $p = 0.007$; $r = 0.466$, $p = 0.005$). **(B)** Slow-5 ALFF values in right SFG (left) and fALFF values in left SFG (right) were positively correlated with tinnitus duration ($r = 0.544$, $p = 0.001$; $r = 0.526$, $p = 0.001$).

integration of multi-sensory information in tinnitus perception may be influenced by enhanced LFO amplitudes in the SFG.

We also observed reduced ALFF/fALFF activity in bilateral MOG in both frequency bands, which is compatible with previous fMRI studies showing aberrant function in visual network in tinnitus patients (Burton et al., 2012; Maudoux et al., 2012b; Chen et al., 2014, 2015a,b). The multisensory connections between auditory and visual regions make it possible for external acoustic stimuli or the internal phantom sound of tinnitus to alter the brain activity in the visual areas (Wang et al., 2008; Cate et al., 2009; Kayser et al., 2009). Thus, decreased LFO amplitudes may be due to increased attention devoted to processing a phantom sound which decreases neural synchrony or interaction with the visual system (Meredith et al., 2012; Araneda et al., 2015). These results suggest that further work is needed to determine how the phantom sound of tinnitus disrupts the normal audio–visual interactions.

Our frequency selective analyses showed that both ALFF and fALFF were higher in the slow-4 band than in the slow-5 band mainly in subcortical regions that included the pons, midbrain, striatum, thalamus, and hippocampus, but were lower in several DMN regions including the MFG, SMG, PCC, and precuneus (**Figures 2** and **5**). Although the origins, relation, and specific physiological functions of slow-4 and slow-5 frequency bands have not been fully clarified, neighboring frequency bands within the same neuronal network may compete or interact with each other (Engel et al., 2001). Previous studies suggest that LFO amplitudes arise from spontaneous neural activity which generated their rhythmic patterns through information exchange with neighboring brain regions to facilitate specific response characteristics (Biswal et al., 1995). The period of an oscillation is constrained by the size of neuronal pool engaged in a given cycle since most neuronal connections are local (Csicsvari et al., 2003; Buzsáki and Draguhn, 2004). Increasing evidences indicate that slow-4 band ALFF is greater than the slow-5 band in subcortical regions (Zuo et al., 2010; Han et al., 2011; Hou et al., 2014; Yu et al., 2014). However, slow-5 band ALFF has higher amplitude than slow-4 band mainly in the DMN (Zuo et al., 2010; Han et al., 2011; Wei et al., 2014). The lower frequency band has the highest power, and localizes mainly to prefrontal, parietal, and occipital cortex; the higher frequency band has less power and localizes mainly in subcortical structures (Baria et al., 2011). Buzsáki and Draguhn (2004) noted that the cortical regions have relatively large-amplitude low-frequency neural oscillations and that contribute to long distance connections in large networks such as the DMN. Subcortical regions have higher frequency, fast local events that are modulated by widespread slow oscillations. Csicsvari et al. (2003) suggested that cortical regions exert strong descending influences over subcortical regions in keeping with the large number of descending projections from auditory cortical regions to brainstem (Mellott et al., 2014). Taken together, these results suggest that it may be informative

TABLE 5 | Correlation coefficients between aberrant ALFF/fALFF in slow-4/slow-5 bands and tinnitus characteristics.

Brain region	Tinnitus duration	THQ score
(I) ALFF	**Slow-4/Slow-5**	**Slow-4/Slow-5**
R superior frontal gyrus	0.178/**0.544***	**0.446***/0.145
R middle temporal gyrus	–0.090/–0.061	0.030/0.126
L inferior frontal gyrus	–0.080/–0.175	0.018/–0.017
R angular gyrus	–0.039/–0.014	–0.019/0.043
B middle occipital gyrus	–0.009/0.117	–0.070/–0.094
(II) fALFF		
L superior frontal gyrus	0.102/**0.526***	**0.466***/0.193
R supramarginal gyrus	–0.186/–0.129	–0.149/0.190
L middle occipital gyrus	0.065/0.118	0.049/–0.124
R middle occipital gyrus	0.147/0.214	–0.026/0.020

*Correlations were controlled for age, sex, and education. *P < 0.05. L, left; R, right; B, bilateral. Bolded values represent the significant correlation coefficients.*

to investigate tinnitus-related neural alterations in specific frequency bands in cortical areas to determine if they influence or are correlated with frequency specific changes in the brainstem, i.e., cortical modulation of brainstem activity. The slow-4 band is more sensitive to detect abnormalities of intrinsic brain activity in the subcortical regions while the slow-5 band is more sensitive for the DMN regions in tinnitus patients compared to the other bands. Such frequency specific fluctuations will help understand the neuropathological basis underlying tinnitus and monitor disease progression in future studies.

The role of the OFC in tinnitus remains poorly understood. However, previous neuroimaging studies have identified alterations in the OFC of tinnitus patients in terms of the functional coupling of long-range cortical networks (Schlee et al., 2009; Vanneste et al., 2012; Song et al., 2014). The OFC is considered part of the reward system (Tremblay and Schultz, 1999; Rolls, 2004; Kringelbach, 2005) and in this capacity it may contribute to the aversive aspects of tinnitus. Interestingly, according to our interaction results (**Figure 3**; **Tables 3** and **4**), LFO amplitudes in right OFC were modulated not only by disease but also by frequency band. Future study with combination of fMRI and electrophysiological methods would be helpful for understanding the underlying neural mechanisms of the interaction in temporal and frequency domains.

Tinnitus is often perceived as tonal or narrow band noise suggesting that the neural generator for tinnitus resides within a discrete tonotopic region in the auditory pathway. However, we observed functional changes in non-tonotopic beyond the auditory pathway such as SFG, which is involved in self-awareness (Goldberg et al., 2006), and OFC. How could these non-auditory areas contribute to tonal tinnitus? The prefrontal cortex and the OFC are associated with auditory attention (Blood et al., 1999; Voisin et al., 2006) and appear to exert broad inhibitory control over primary auditory cortex and other central auditory structures (Knight et al., 1989; Angrilli et al., 2008); this allows for a broad, generalized top-down modulation of auditory processing allowing the system to focus on different features of the acoustic scene or internal states (Norena et al., 1999; Goldberg et al., 2006). Dysregulation of an expansive, top-down inhibitory mechanisms could "open the gate" (Rauschecker et al., 2010), allowing narrowband or wideband aberrant tinnitus signals within the auditory pathway to become more salient and enter consciousness (Knight et al., 1989; Goldberg et al., 2006).

We failed to observe significant differences in whole brain GM volume as well as regional GM volume in any of the regions showing altered ALFF/fALFF in tinnitus patients. In contrast, previous studies have reported increases and decreases in GM volume in auditory and non-auditory regions in tinnitus patients (Adjamian et al., 2014). However, the changes in GM volume seen in these tinnitus patients were typically correlated with hearing loss particularly when testing was extended beyond 8 kHz. Since our subjects had normal hearing out to 16 kHz and no evidence of hyperacusis; thus likely accounts for the lack of change in GM volume in our tinnitus subjects. An alternative possibility is that our analytical methods are not sensitive enough to detect the structural differences between neuroanatomical structures in our tinnitus patients versus controls. In any case, our results suggest that frequency-specific alterations in ALFF/fALFF can occur prior to any obvious structural changes in tinnitus patients with normal hearing and absence of hyperacusis.

Close inspection of **Figure 6B** reveals five subjects in which tinnitus duration exceeded 100 months and these five had ALFF and fALFF values that were equal to or slightly larger than the range of values of subjects that shorter tinnitus durations. This raises the possibility that these five subjects were so-called outliers and largely responsible for the significant correlation in **Figure 6B**. While we have not *a priori* reason for excluding the data from these five subjects, or any other subjects at the other extreme end of the distribution (e.g., small ALFF or fALFF values and short tinnitus duration), we nevertheless recomputed the correlations between tinnitus duration and ALFF or fALFF in the SFG, and found positive but not significant correlations. Since the correlations were no longer significant, one interpretation of these results is that tinnitus duration must exceed some critical duration (>60 months) in order for ALFF or fALFF to increase significantly in the SFG, i.e., there may be a critical duration for such effects to occur.

Several factors limit the generalizability and interpretation of our results. First, due to the relatively small sample size, we did not sort the fMRI data into left, right or bilateral-sided tinnitus to see whether and how the laterality of tinnitus contributed to the lateralization of neural effects. Therefore, additional studies are required with a larger sample size of different lateralization of the tinnitus percept. Second, although

we have attempted to minimize the amount of scanner noise with earplugs, the subjects cannot be completely prevented from hearing sounds generated by the scanner would activate the auditory pathway thereby disrupting the pattern of neural activity that would occur in a quiet environment (Logothetis et al., 2009). The acoustic characteristics of the noise generated by the MRI scanner may influence the magnitude and location of slow-4 and slow-5 ALFF/fALFF activity. Moreover, tinnitus patients could have been experiencing their tinnitus perception during the scan, which could affect the fMRI results. We did not ask the subjects if they experienced tinnitus during the scan because instructing tinnitus patients to listen to their tinnitus would have activated the attentional networks. A post-scan questionnaire will be included to determine whether the tinnitus perception is experienced in the scanner in order to identify its potential effect on brain activity in our future experiments. Finally, our study only investigated between-group ALFF/fALFF changes in the slow-4 and slow-5 frequency bands. Further investigations exploring the ALFF/fALFF changes in all frequency sub-bands and their relationships with clinical tinnitus characteristics could reveal additional insights on the neural basis of tinnitus (Baliki et al., 2011; Baria et al., 2011).

CONCLUSION

In this study, chronic tinnitus patients with normal hearing exhibited abnormal LFO amplitudes in several brain regions including the DMN regions, prefrontal cortex and visual cortex. Enhanced ALFF/fALFF values in bilateral SFG were positively correlated with tinnitus duration and tinnitus distress at both slow-4 and slow-5 bands. Furthermore, the significant interaction in the OFC indicated that LFO amplitudes abnormalities in chronic tinnitus were frequency-dependent. Our results suggest that frequency-specific analysis of ALFF/fALFF could provide new and novel insights related to various auditory and non-auditory dimensions of tinnitus.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fncir.2015.00067

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frontiers in Neural Circuits

REVIEW
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Animal models of spontaneous activity in the healthy and impaired auditory system

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Spontaneous neural activity in the auditory nerve fibers and in auditory cortex in healthy animals is discussed with respect to the question: Is spontaneous activity noise or information carrier? The studies reviewed suggest strongly that spontaneous activity is a carrier of information. Subsequently, I review the numerous findings in the impaired auditory system, particularly with reference to noise trauma and tinnitus. Here the common assumption is that tinnitus reflects increased noise in the auditory system that among others affects temporal processing and interferes with the gap-startle reflex, which is frequently used as a behavioral assay for tinnitus. It is, however, more likely that the increased spontaneous activity in tinnitus, firing rate as well as neural synchrony, carries information that shapes the activity of downstream structures, including non-auditory ones, and leading to the tinnitus percept. The main drivers of that process are bursting and synchronous firing, which facilitates transfer of activity across synapses, and allows formation of auditory objects, such as tinnitus.

Keywords: spontaneous firing rate, neural synchrony, burst firing, auditory nerve, inferior colliculus, auditory cortex, noise trauma, tinnitus

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Spontaneous Neural Activity; Noise or Information Carrier?

Spontaneous neural activity is very often considered as neural noise that sets limits on sensory performance. This neural noise idea has been the basis for the optimal processor model in psychoacoustics that typically worked on activity in auditory nerve fibers (Green, 1964; Siebert, 1965) and tried to extract the stimulus-induced activity from the spontaneous noise in these fibers. The concept of internal noise—albeit not limited to spontaneous firing—is fundamental in signal detection theory (Green and Swets, 1966). The alternative, already mentioned in Rodieck et al. (1962), investigating spontaneous firings in the cochlear nucleus, is that spontaneous firing is a carrier of information. The difference in these two hypotheses becomes visible when the effects of stimuli are taken into account. If spontaneous activity acts as noise one expects any stimulus induced activity to be additive to the spontaneous one. In contrast, when the spontaneous activity acts as carrier of information, one expects stimuli to modulate the spontaneous firings, i.e., a multiplicative action. I will compare the normal spontaneous activity in auditory nerve fibers (ANFs) and in primary auditory cortex (AI) with respect to these two hypotheses.

Auditory Nerve Firings

The neural noise hypothesis for spontaneous activity in auditory nerve fibers was apparently boosted by Kiang et al.'s (1965) extensive studies on ANF activity in the cat, including spontaneous firing properties. The inter-spike interval distribution of spontaneous firings in ANFs strongly

suggested an underlying Poisson process with dead time (the refractory period). Geisler et al. (1985) also found that for high spontaneous firing rate (SFR) ANFs in cat the mean of the inter-spike intervals was nearly equal to their standard deviation (SD). Siebert (1965), referring to Kiang et al. (1965), wrote: "Recent electrophysiological studies of the activity in response to acoustic stimuli of single primary afferent neurons in the VIIIth nerve of mammals strongly suggest that the spike activity is inherently stochastic. . . . Since the only way in which auditory information can reach the more central parts of the nervous system is via the VIIIth nerve, the effective "neural noise" implied by such stochastic "coding" of auditory information must set some sort of limits on auditory discriminations."

A decade later, Liberman (1978) raised cats in a soundproof room to exclude all potential causes from noise exposure on ANF firing properties. He demonstrated different synaptic noise sources reflected in "units with spontaneous rates greater than 18 spikes/s (sp/s) comprise a distinct and homogeneous group with respect to threshold. The suggestion that the units with rates below 18 sp/s fall into two threshold classes rather than one is most convincing when the overall (hearing) sensitivity of the experimental animals is exceptionally good." Later on, using intracellular labeling, a relation was found between SFR and ANF diameter in the cat (Liberman, 1982; Liberman and Oliver, 1984). Systematic differences were also found (Merchan-Perez and Liberman, 1996) "in synaptic ultrastructure among fibers of the three SFR groups: with decreasing SFR, the size and complexity of the synaptic body (a presynaptic specialization characteristic of the peripheral afferent synapses in all hair cell systems and some other peripheral receptors) tend to increase, as does the associated number of synaptic vesicles." These correspondences (see also Jackson and Carney, 2005) suggested that the difference in SFR is functional, making it unlikely that it is just neural noise. Augmenting this was the finding that ANFs with different SFR tended to project to different cell groups in the anteroventral cochlear nucleus (AVCN). Liberman (1991) described it as: "the small cell cap was almost exclusively innervated by low- and medium-SFR fibers, i.e., those with the highest acoustic thresholds. Within anterior AVCN, spherical-cell innervation was seen from all SFR groups, whereas almost all multipolar cell innervation was from low- and medium-SFR fibers. In the posterior AVCN, multipolar-cell innervation was equally likely from all SFR groups, whereas globular cells were preferentially contacted by high-SFR fibers."

Javel et al. (1988) showed for high SFR ANFs of the cat that the phase-locking (vector strength) of firings to the frequency of a tone showed a nearly 20 dB lower detection threshold than found on the basis of increases in driven firing rate. The latter, however, correlated better with behavioral thresholds. This suggests that the SFR can be modulated even by a sub-threshold stimulus, clearly in contradiction to the notion that SFR in auditory nerve fibers is noise, and supporting an information carrier function.

Auditory Cortex

Spontaneous Firing Rates

Spontaneous activity in cat auditory cortex was initially examined in paralyzed cats (Goldstein et al., 1968). Spontaneous activity was varied with some cells (n = 41) having SFR <1 sp/s but in a few others (n = 2) >35 sp/s, the remaining 60 cells had SFRs between 1–35 sp/s. Eggermont and colleagues performed studies on spontaneous activity in AI of ketamine-anesthetized cats. In a series of studies (Eggermont, 1992, 1994; Ochi and Eggermont, 1996; Eggermont and Kenmochi, 1998; Kimura and Eggermont, 1999; Valentine and Eggermont, 2001; Noreña and Eggermont, 2003), comprising a total of 2028 units, we found in each study units with SFRs between 0.02 and 30 sp/s, and with a mean between 1.9–3.5 sp/s. I will present these findings in more detail.

Eggermont (1992) recorded at least 15 min of spontaneous activity from each of 312 neurons in 9 adult ketamine-anesthetized cats from all layers of primary auditory cortex, and studied their pair-wise cross-correlation. The findings were described as: "for the 181 single-electrode pairs the percentage of unilateral excitation pairs (42%) was about the same as the percentage of common input pairs (38%), For the 77 unilateral excitation pairs a presynaptic spike produced on average 0.4 postsynaptic spikes, with 61 values <0.5 and only 16 above that value. The values >0.5 were consistently found in cases where the postsynaptic neuron was bursting. For the 297 dual-electrode pairs all but one of the 184 significant correlations were indicative of common input." In a subsequent study in ketamine-anesthetized cats, Eggermont (1994) investigated the effect of stimulation on the correlation strength. This study clearly indicated that the differences in cross-correlation strength between spontaneous and stimulus-driven activity could not be explained by an additive effect of the stimulus-induced correlations onto those of the spontaneous correlations, as is generally assumed by the "shift-predictor" correction procedure (Perkel et al., 1967). In this procedure, one spike train is shifted by one or more stimulus periods, and the resulting cross-correlogram is then interpreted as stimulus correlation and subtracted from the non-shifted correlogram. In our study, the remaining correlation after correction was typically smaller than the spontaneous cross-correlation. The reason may be that stimulation suppresses the SFR, and this in turn may lead to lower cross-correlation strength (see below; Britvina and Eggermont, 2008). This suggests that in auditory cortex the correlated spontaneous activity cannot be characterized as noise.

Spontaneous Burst Firing

Eggermont et al. (1993) then described the occurrence of spontaneous burst firing in keteamine-anesthetized cat AI. Bursts with durations less than 50 ms were "characterized by relatively well-defined intervals between the first two spikes (3–15 ms) in the burst followed by intervals with large spread (range 4–50 ms) and increasing modal interval value. The typical five-spike template that described a spontaneous burst in adult cat AI featured spikes at 0, 3.3, 14.6, 27.2, and 34.8 ms, (0 indicating the start of the burst). Bursts with fewer spikes showed larger intervals between the first three spikes." The probability of occurrence of isolated spikes, pairs, triplets, etc. showed a power-law dependence on SFR with a coefficient that was significantly lower than expected under Poisson firing conditions. A subgroup or neurons with the highest SFRs showed firing behavior close to Poisson and they showed less bursting. Also in the cat,

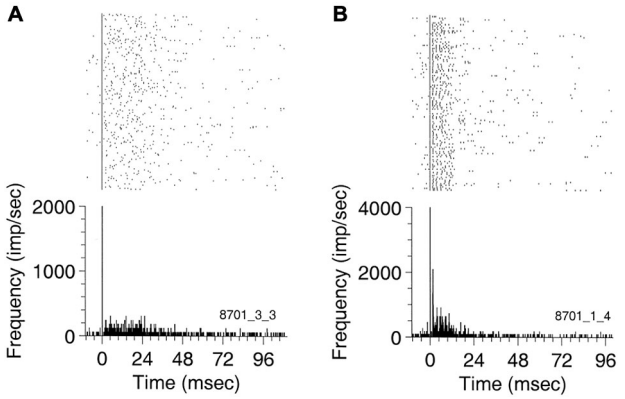

FIGURE 1 | Peri-event rasters for (A) a non-stereotyped bursting unit recorded in AI, and (B) a stereotyped burster recorded in ANTERIOR AUDITORY FIELD (AAF) simultaneously with the previous unit. The rasters and the PSTH were triggered on the first spike of the bursts. Ten ms of spontaneous activity are shown before the first burst spike. The burst in **(A)** has a duration of about 50 ms. The AAF unit in **(B)** exhibits a well-defined ISI of 2 ms (bin width 0.5 ms) and has an average duration of about 25 ms. Reprinted from Hearing Research, Vol 154, Pamela A Valentine, Jos J Eggermont, Spontaneous burst-firing in three auditory cortical fields: its relation to local field potentials (LFP) and its effect on inter-area cross-correlations. Pages 146–157, Copyright 2001, with permission from Elsevier.

Valentine and Eggermont (2001) found "burst-firing occurred in 85% of 371 units studied, and in 48 (15%) thereof there were at least 100 bursts per 15 min. Neurons in AI were bursting at a significantly higher rate, but with fewer spikes per burst, than units in (secondary auditory cortex) AII". In addition, we found that burst firing was not synchronized across cortical areas, so that it cannot attributed to a general cortical state characterized by spindling induced by the ketamine anesthesia. Only a few stereotyped bursting neurons were found, notably in anterior auditory field (AAF; **Figure 1**).

Effects of Stimulation on Spontaneous Cortical Activity

Eggermont (2006) recorded spontaneous and stimulus-driven spiking activity from auditory cortex in ketamine-anesthetized cats using multi-electrode arrays. Cross-correlograms were calculated for spikes recorded on separate microelectrodes, and corrected for mean SFR and effects of local field potential (LFP)-spindling activity. A pair-wise cross-correlation matrix was constructed for the peak values of the correlograms. Hierarchical clustering was then performed on the cross-correlation matrix for silence and five stimulus conditions. These neuron clusters reflected the firing synchrony across cortical patches of several mm^2 in area. The most striking result was that the cluster locations and size were very similar for spontaneous activity and multi-tone-stimulus evoked activity.

Britvina and Eggermont (2008) found that in ketamine-anesthetized cat AI, multi-frequency tonal stimulation suppressed spontaneous spindle waves, as shown by the decrease of spectral power within the spindle frequency range during stimulation as compared with the previous silent period (**Figure 2**). They showed that the percentage suppression was independent of the power of the spontaneous spindle waves, and that the suppression of spindle power occurred within one spindle period ($\leq$150 ms) after stimulus onset. The finding that spontaneous spindle oscillations can be modulated by stimuli suggests that this spontaneous LFP activity cannot be considered as "noise".

In neocortex alternating "DOWN" states with near absence of spontaneous activity and "UP" states of persistent spontaneous firing occur. Luczak et al. (2007) simultaneously recorded populations of 50–200 cortical neurons in layer V of anesthetized (urethane or ketamine) and awake rats. Each neuron displayed a bursting spike pattern during these spontaneous UP states. Spike timing in these bursts was most precise during the first $\sim$100 ms after UP state onset, and became more variable as the UP state continued. In auditory cortex, these bursts had a stereotyped make up, that was similar between UP state onset periods and stimulus onset periods, but the rate and also precise relative timing of spikes varied between stimuli (Luczak et al., 2013), suggesting a modulatory function of stimulation on the bursting pattern. The finding by Chen et al. (2013) that "spontaneously-recurring UP states evoked in these (dendritic) spines "patterned" calcium activity that may control consolidation of synaptic strength following epochs of sensory stimulation" also indicates that spontaneous activity in cortex is not noise.

All in all, these studies suggest that in auditory cortex, just as in auditory nerve fibers, the spontaneous firing activity is not just neural noise but plays a dominant part in information processing.

Anesthesia Effects on Spontaneous Firing

Anesthesia will affect SFRs in a way that depends on depth and type of anesthesia. Barbiturates and urethane have a strong effect, ketamine very little. In contrast to studies that measure anesthesia effects on stimulus-evoked activity, there are only a few studies that compared SFRs under anesthesia and awake. Torterolo et al. (2002) reported that pentobarbital anesthesia significantly reduced the SFR in the IC of guinea pigs from (mean $\pm$ SEM) 17.8 $\pm$ 1.7 sp/s in the awake state to 6.6 $\pm$ 0.6 sp/s. These SFRs in the awake state are similar to those measured under ketamine-xylazine anesthesia in the ICC of guinea pigs (mean $\pm$ SD): 19.4 $\pm$ 19.9 sp/s (Syka et al. (2000). Ter-Mikaelian et al. (2007) recorded from inferior colliculus and auditory cortex (AI) in ketamine anesthetized, and awake gerbils. They found no significant difference in SFR in the awake or anesthetic state in either IC (Mann–Whitney U test, one-tailed, $p = 0.2161$; $n = 94$; median, 3.8 sp/s anesthetized and 2.4 awake) or AI (Mann–Whitney U test, one-tailed, $p = 0.1343$; $n = 74$; median, 1.6 sp/s anesthetized and 1.0 awake). Huang et al. (2013) compared response properties in rat AI in urethane and ketamine anesthesia. They found that the mean SFR was 2.5 $\pm$ 0.6 sp/s under urethane, and 5.6 $\pm$ 1.2 sp/s under ketamine/xylazine ($p = 0.028$, Kruskal-Wallis test).

Anesthesia also changes the prevalence and properties of burst firing in neocortex. Ketamine anesthesia results in spindle-like LFP activity in the 5–15 Hz range in cat auditory

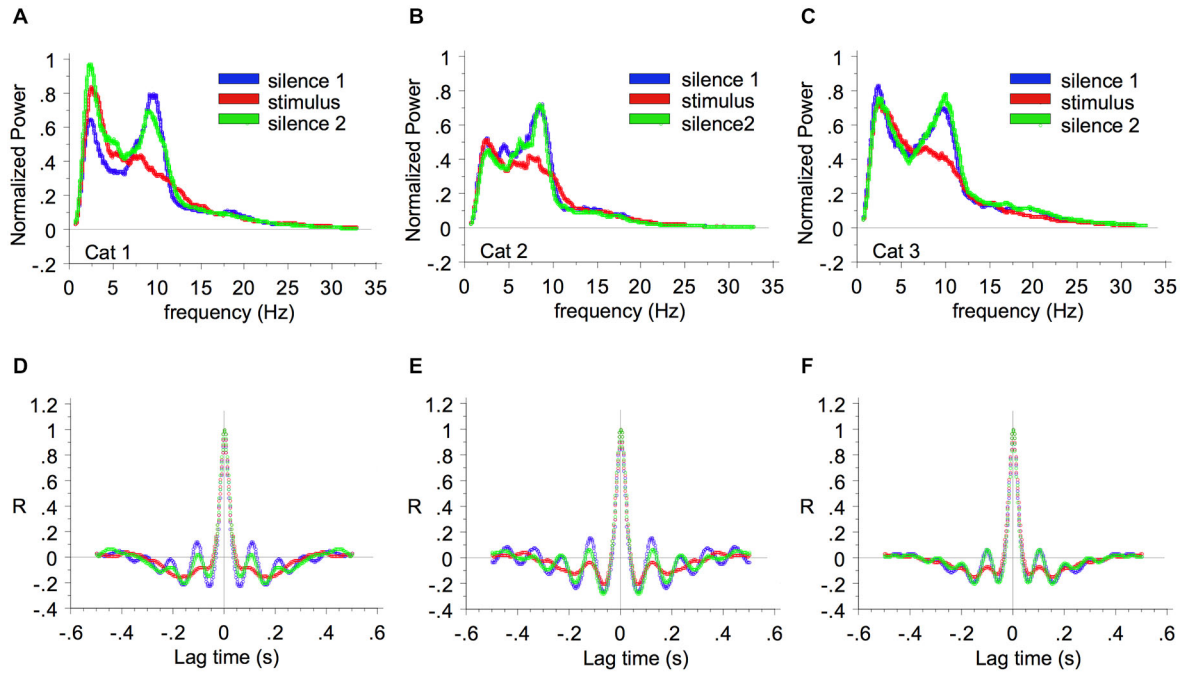

FIGURE 2 | Suppression of spindle wave power by multi-frequency sound. The average LFP power spectra are shown for cat 1 **(A)**, cat 2 **(B)**, and cat 3 **(C)**. The power during the first 15-min period of silence, the subsequent 15-min period of multi-frequency stimulation, and the second 15-min period of silence are represented by blue, red, and green lines respectively. Parts **(D)**, **(E)**, and **(F)** show the corresponding auto-correlation functions. Reprinted from Neuroscience, Vol 151, Tatiana Britvina, Jos J Eggermont, Multi-frequency auditory stimulation disrupts spindling activity in anesthetized animals. Pages 888–900, Copyright 2008, with permission from IBRO (2008).

cortex, depending on the depth of the anesthesia (Britvina and Eggermont, 2008), and is synchronized with bursts of action potentials. Erchova et al. (2002) recording from rat somatosensory cortex during light, intermediate and deep levels of urethane anesthesia. At all levels, spontaneously action potential firing at a single electrode tended to be clustered into "bursts". With increasing level of anesthesia, burst firing became more prominent (duration increased from 81 ms to 160 ms) and rhythmic. Burst frequency decreased from 1.6 to 0.4 burst/s and fewer spikes occurred outside bursts, leading to a decrease in the overall SFR from 5.9 to 2.8 sp/s.

Effects of Noise-Induced Hearing Loss

Hearing loss is frequently accompanied by tinnitus. As tinnitus may be a consequence of increased SFR (Eggermont and Roberts, 2004; Roberts et al., 2010) the study of SFR at various locations in the auditory system has met with increasing interest in the last decade. Here I follow part of the narrative from Chapter 7 in "The Neuroscience of Tinnitus" (Eggermont, 2012) while adding more recent findings. The issue I will address: Is increased SFR in tinnitus "noise"?

Auditory Nerve Fibers

Let us first look at the effects of noise-induced hearing loss (NIHL) on SFRs in auditory nerve fibers. Liberman and Kiang (1978) "exposed cats for 1–4 h to narrow band or broadband noise with levels between 100–117 dB SPL, and recorded ANF activity at 15–305 days after the trauma. Frequency regions with unaffected thresholds typically showed the normal bimodal distribution of SFRs. For units in the hearing loss region, the SFR distribution had lost its normal bimodal appearance. There was a low-SFR region <20 sp/s and a high-SFR region from 20–100 sp/s mostly with SFRs between 10–40 sp/s. Units that had become unresponsive to sound generally showed spontaneous bursting or no spontaneous activity at all. An important finding is that SFRs were hardly ever increased after noise trauma (Liberman and Kiang, 1978).

The Auditory Brainstem

Superficial multi-unit recordings, likely from fusiform cells, in the dorsal cochlear nucleus (DCN) of hamsters with 10 kHz, 125 dB SPL, 4 h induced noise trauma (Kaltenbach et al., 1998, 2000) showed strongly increased SFR. No correlation between SFR increase and hearing loss was found. In these hamsters, mean SFRs increased from below normal levels at day 2 post-exposure to higher than normal levels at day 5. The mean SFR continued to increase gradually over the next 6 months. Hamsters exposed to a lower level sound (10 kHz, 80 dB SPL, 4 h), showed multi-unit SFRs in the DCN that were already increased above control levels at 1 h post-exposure and significantly increased at 2 days after exposure (Kaltenbach et al., 2005). Recording from single units of the DCN in hamsters,

Finlayson and Kaltenbach (2009) showed average SFRs of 8.7 sp/s in controls and 15.9 sp/s after exposure to a 10 kHz tone at a level of 115 dB SPL for 4 h. The highest increases in SFR were found in the fusiform cell layer. Approximately half of the increase in SFR in exposed animals was accounted for by an increase in bursting activity. This effect may only be transient; SFRs were significantly higher than normal at 1 week following noise damage, whereas at 2 weeks post-noise damage SFRs were no longer significantly different from control (Shore and Zhou, 2006). This may not be in agreement with the long lasting effects in superficial recordings shown by Kaltenbach et al. (2000), which were also attributed to fusiform cells. Removing spontaneous input to the DCN in hamsters by cochlear ablation 30 days after the exposure had no significant effect on SFRs, suggesting that the increased SFRs at that time were intrinsically generated (Zacharek et al., 2002). This is consistent with findings by Koerber et al. (1966) showing that SFR in DCN of normal hearing cats did not change after cochlear ablation.

Ma and Young (2006) exposed cats to a 250 Hz band of noise centered at 10 kHz that was presented at 105–120 dB SPL for 4 h. After a one-month recovery period, neural activity was recorded in the DCN of a decerebrated preparation, which eliminates corticofugal activity towards the DCN among other effects. The threshold shift, determined from CAP audiograms, showed a sharp threshold elevation of about 60 dB for neurons with CFs above the 5–10 kHz lower-edge frequency of the hearing loss. In contrast to the above-described results in hamsters that were subjected to a similar exposure level and duration, SFRs in fusiform cells with elevated thresholds were not increased over those in unexposed animals. This could suggest a species difference as the recovery period is in the range where increased SFRs were seen in hamsters. As I remarked earlier (Eggermont, 2012): "The different delays between the exposure and the recording may have had an effect as well; Shore and Zhou (2006) showed that there was only a transient elevation for 1–2 weeks after the trauma. Finally, the recovery of the cats could have been in a noisy acoustic environment, which may have prevented the increase in SFR (see Noreña and Eggermont, 2005, 2006)."

Vogler et al. (2011) exposed guinea pigs for 2 h to a 10 kHz tone presented at 124 dB SPL. After a 2-week recovery period, the mean SFR in the VCN (VCN) of noise-exposed ears (N = 189) was significantly elevated (about a factor two) compared to sham controls (N = 143). This was more evident in primary-like and onset types of neurons. In addition, mechanical damage to the high frequency region of the cochlea (N = 258) showed similar results as noise exposure, suggesting that it is the hearing loss and not the induction method that causes the SFR changes.

The Inferior Colliculus

Ma et al. (2006) exposed CBA/J mice for 1 h to a 0.5 oct. band of noise centered around 16 kHz, 103 dB SPL. Bilaterally exposed mice fairly shortly after the exposure showed increases in the SFR of neurons in the central nucleus of the inferior colliculus (ICC) with tuning near the exposure frequency. However, the median SFR (6.0 sp/s) was not significantly different from controls, who had SFRs between 0 and 30 sp/s, with a median SFR = 4.1. No changes in burst-firing activity in the ICC were found for bilateral exposed mice. However, they reported changes in temporal aspects of firing in the protected ear after unilateral exposure. The contralateral ICC showed increased median ISIs, reduced SFR, and a significant increase in burst firing (Ma et al., 2006).

Vogler et al. (2014), using the same exposure as in the VCN (Vogler et al., 2011), also showed increased SFR in the ICC in regions corresponding to the frequencies at which there was peripheral hearing loss (12–20 kHz). Most unit types, with the exception of onset cells, showed a significantly increased mean SFR. Thus, in contrast to findings in the VCN, hyperactivity in the ICC was not confined to a particular cell type. This was confirmed by Ropp et al. (2014) in Sprague-Dawley rats who showed in ICC a median pre-trauma SFR = 10.4 sp/s and a post-trauma one of 14.1 sp/s. They found that abnormal SFRs were restricted to target neurons of the VCN. So one wonders what increased SFR in the DCN has to do with tinnitus. Nearly identical patterns of hyperactivity were observed in the contralateral and ipsilateral ICC. The elevation in SFR was found for frequencies well below and above the region of maximum hearing loss.

As in the DCN, acoustic trauma (10 kHz tone at 124 dB SPL for 1 h) in guinea pigs did not immediately resut in SFR changes in the ICC. Two weeks recovery after acoustic trauma resulted in more neurons with high SFR compared to control animals, and a significant increase in the average SFR from control (mean = 1.2 sp/s) values (Mulders and Robertson, 2009). Surprisingly, subsequent cochlear ablation, cochlear cooling or kainic acid infusion in the cochlea, resulted in statistically significant decreases in the average SFR in ICC, from 4.5 to 1.4 sp/s in the animals recorded 1 week post exposure and from 7.5 sp/s in the animals that were recorded from more than 4 weeks after the exposure. Thus, at all recovery times (up to 4 weeks) after the exposure, the increased SFR disappeared when cochlear input to the ICC was destroyed. These data suggested that the hyperactivity in the ICC after acoustic trauma was dependent on activity in the contralateral cochlea. The findings also suggest that the ICC SFR is not dependent on the activity in the DCN, which is not affected by ablation 30 days after induction of the noise trauma (Zacharek et al., 2002).

Corroborating evidence (Mulders et al., 2010) came from electrically stimulating the olivocochlear bundle in noise-exposed animals, which is know to decrease ANF activity, and this also resulted in a decrease of the exposure-enhanced SFR in the ICC. There is a transition period between an amplification of peripheral input to intrinsic generation of SFRs in the ICC. Robertson et al. (2013) observed that a "spontaneous afferent drive from the cochlea itself is necessary for the maintenance of hyperactivity up to about 8 weeks post cochlear trauma. After 8 weeks however, ICC hyperactivity becomes less dependent on cochlear input, suggesting that central neurons transition from a state of hyperexcitability to a state in which they generate their own endogenous firing".

Except one study (Ma et al., 2006), none of the midbrain studies reported on temporal aspects of spontaneous firing.

Thalamus and Cortex

Immediately Post-Trauma

Kimura and Eggermont (1999) recorded simultaneously from units in primary auditory cortex, AAF and secondary auditory area of ketamine-anesthetized cats before and immediately after a 30 min exposure to a 93–123 dB SPL pure tone. The frequency of the trauma tone was set 0.5 octave above the highest CF found for the three simultaneous recordings, to investigate effects of diminished lateral inhibition from neurons tuned to frequencies >0.5 oct. above the CFs of the recorded neurons (**Figure 3**). SFRs increased significantly in AI (from 0.54 to 1.08 sp/s), did not change in AAF (from 0.98 to 0.84 sp/s), and decreased significantly in AII (from 1.22 to 0.76 sp/s). The changes in spontaneous activity as a result of the pure-tone trauma stabilized within a few min after the trauma and for at least up to 30 min.

Changes in the neural activity in cat AI occurring within a few hours after a 1-h exposure to a 120-dB SPL pure tone (5 or 6 kHz) were further assessed by recording, with two 8-microelectrode arrays, from the same sorted-unit clusters before and after the trauma (Noreña and Eggermont, 2003; Noreña et al., 2003). Immediately after the exposure, the SFR was not significantly changed (**Figure 4**). Significant increases in SFR did occur after at least 2 h post trauma.

Spontaneous burst firing in AI was affected by this noise exposure (Noreña and Eggermont, 2003). In total, 497 SU spike trains were analyzed; the Poisson-surprise method (Legéndy and Salcman, 1985), at a surprise value >10 detected bursting activity in 468 of them (94%). **Figure 5** illustrates the averaged data for the percentage of time of burst firing (A), the number of spikes per burst (B), the mean burst duration (C) and the mean ISI within a burst (D), at pre- and post-trauma conditions as a function of the frequency band. One observes that the trauma induced an immediate and transitory change in burst-firing properties in the three frequency bands (Be, below the trauma tone frequency (TTF); Ab1, within one oct. above the TTF; Ab2, >1 oct. above the TTF). There was only a significant change in the number of spikes per burst, which was increased immediately after the trauma in Be ($P < 0.01$) and Ab1 ($P < 0.001$) groups. However, when the three frequency bands were combined, unpaired t-tests revealed a significant increase in the percentage of time of burst-firing ($P < 0.0001$), number of spikes per burst ($P < 0.0001$), mean burst duration ($P < 0.0001$) and mean ISI within a burst ($P \leq 0.001$), immediately after the trauma ("After1" condition). The burst properties returned to normal a few hours after the trauma ("After2" condition). However, as we have seen SFRs increased significantly (**Figure 4**) in the After2 condition. Thus, burst firing and SFRs are not correlated, in fact are negatively correlated.

Spontaneous neural synchrony between spike firing of two neurons is also affected by noise exposure

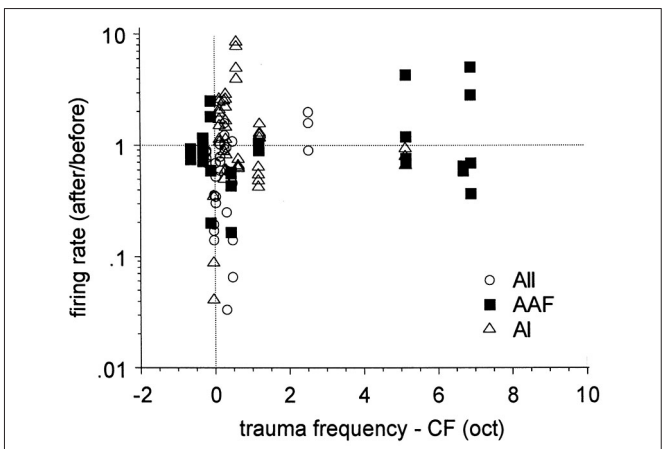

FIGURE 3 | Effects of pure-tone trauma on single-unit spontaneous firing rates (SFR). Shown is the ratio of the firing rates after and before the trauma as a function of the separation (in octaves) of CF and trauma tone frequency (TTF). Reprinted from Hearing Research, Vol 154, Makiko Kimura, Jos J Eggermont, Effects of acute pure tone induced hearing loss on response properties in three auditory cortical fields in cat. Pages 146–162, Copyright 1999, with permission from Elsevier.

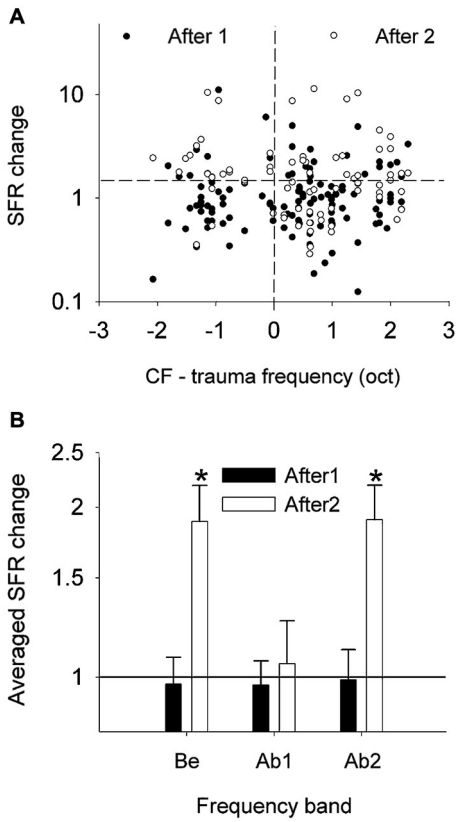

FIGURE 4 | Effects of the acoustic trauma on the SFR. (A) Changes in SFR (as a ratio of post/pre) immediately (filled circles) and a few hours (open circles) after the acoustic trauma as a function of the difference in pre-trauma CF and the TF. **(B)** SFR changes averaged (geometric mean) into three frequency bands (±S.E.M., *$P < 0.05$). Be, CF below the TTF. Ab1, CF-TTF ≤1 octave. Ab2, CF-TTF >1 octave. Reprinted from Hearing Research, Vol 183, A.J. Noreña, J.J. Eggermont, Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. Pages 137–153, Copyright 2003, with permission from Elsevier.

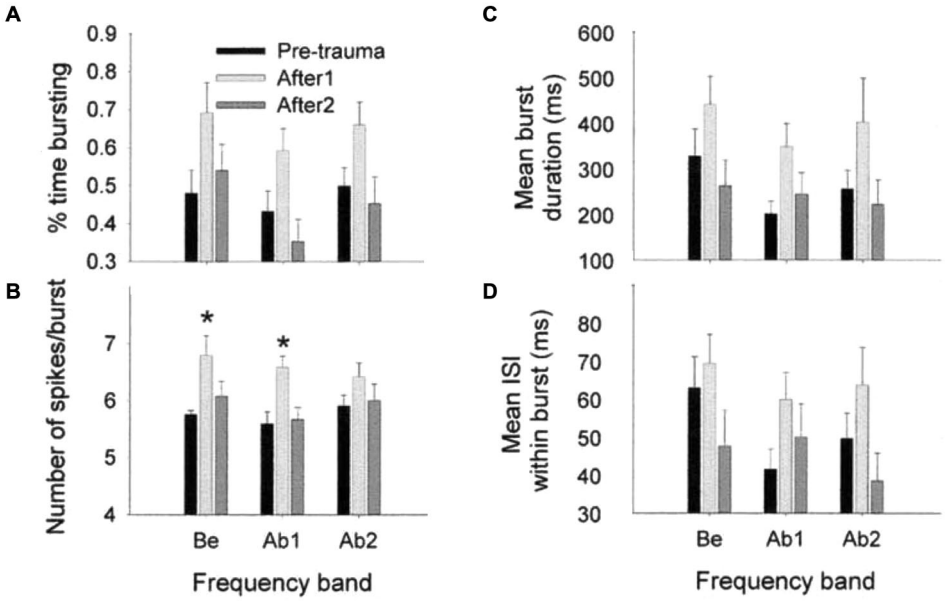

FIGURE 5 | Effects of an acoustic trauma on burst-firing properties. The proportion of time of burst-firing **(A)**, the number of spikes by burst **(B)**, the mean burst duration **(C)**, and the mean ISI within a burst **(D)** are shown as a function of the frequency band, in the pre-trauma, After1 and After2 conditions (±S.E.M., $^*P < 0.01$). Be, CF below the TTF. Ab1, CF-TTF ≤1 octave. Ab2, CF-TTF >1 octave. Reprinted from Hearing Research, Vol 183, A.J. Noreña, J.J. Eggermont, Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. Pages 137–153, Copyright 2003, with permission from Elsevier.

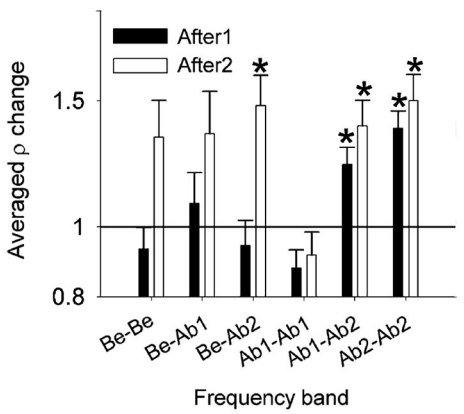

FIGURE 6 | Effect of the acoustic trauma on the cross-correlation coefficient ρ. Changes in ρ averaged (geometric mean) into six frequency bands, immediately (After1) and a few hours (After2) after the acoustic trauma (±S.E.M., $^*P < 0.0083$). Immediately after the acoustic trauma (black bars), one notes that ρ is significantly increased in the Ab2- Ab2 group. Be, CF below the TTF. Ab1, CF-TTF ≤1 octave. Ab2, CF-TTF >1 octave. Reprinted from Hearing Research, Vol 183, A.J. Noreña, J.J. Eggermont, Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. Pages 137–153, Copyright 2003, with permission from Elsevier.

(Noreña and Eggermont, 2003). **Figure 6** shows the ratio between post- and pre-trauma cross-correlation coefficient (ρ) averaged (geometric mean) into six groups. One observes that ρ is increased *immediately* after the tone exposure by a factor of about 1.25 for Ab1-Ab2 ($P = 0.0008$) and 1.4 for Ab2-Ab2 groups ($P < 0.0001$). For the After2 condition (well after the trauma), one observes that ρ is significantly increased for the Be-Ab2 ($P = 0.001$), Ab1-Ab2 ($P = 0.0004$) and Ab2-Ab2 groups ($P < 0.0001$). Again, SFRs increased significantly in the After2 condition over a wider frequency range compared to the changes in ρ.

Chronic Changes Post-Trauma

Kalappa et al. (2014) exposed rats unilaterally for 1 h to a 1 oct. band of noise centered on 16 kHz, and with a peak level of 116 dB SPL. Behavior and electrophysiology testing was done 2 months following exposure. At that time there was no remaining hearing loss. Single units in the medial geniculate body (MGB) in awake animals with behavioral (gap-startle test) signs of tinnitus showed significantly enhanced SFR from 4.7 sp/s in controls to 9.1 sp/s after noise exposure. In the gap-startle test, where a gap in a broad-band or narrow-band noise functions as a pre-pulse inhibitor for a startle inducing sound, tinnitus is assumed to fill the gap and (partly) abolishes the inhibitory function of the gap on the startle response (Turner et al., 2006). Burst firing showed a significant increase after noise exposure in: (1) the mean number of bursts per minute (9.2 in control vs. 37.1 in exposed); (2) mean number of spikes in a burst (5.1 in control vs. 6.1 exposed); and (3) mean burst duration (60 ms in control vs. 90 ms after noise exposure). These elevated patterns of neuronal activity and altered bursting showed a significant positive correlation with animals' scores on the gap-startle test.

Komiya and Eggermont (2000) exposed kittens to a 126 dB SPL tone of 6 kHz for 1 h at both 5 and 6 weeks of age, and recorded from primary auditory cortex 7–16 weeks after the exposure. Single-unit SFRs were significantly higher in reorganized tonotopic map regions (mean 2.3 sp/s) than in unaffected regions (mean 1.4 sp/s). For the littermate controls the mean SFR was 1.3 sp/s and was not CF dependent. Burst firing was not significantly affected by the exposure. Seki and Eggermont (2003) again found increased single-unit SFRs in reorganized tonotopic map regions (mean 3.2 sp/s) compared to the neurons in the non-reorganized map regions (mean 1.8 sp/s) in the same animals and in controls (mean 1.9 sp/s). In these reorganized map regions the peak cross-correlation coefficients were also increased relative to those for unit pairs in the non-reorganized parts. Using the same exposure paradigm, Noreña and Eggermont (2005, 2006) showed again that NIHL and recovery in quiet induces reorganization of the tonotopic map in cat AI. Here the frequencies above 10–15 kHz were no longer represented. In addition the exposure increased the multi-unit SFR (∼7 sp/s and a factor 2 larger than in control cats) and neural synchrony (by a factor 1.2) in the reorganized part of AI.

Engineer et al. (2011) induced noise trauma by exposing rats to 1 h of 115-dB SPL, octave-band noise centered at 16 kHz. This resulted in about 15–20 dB permanent hearing loss at 11 weeks post trauma in the frequency region between 4 and 32 kHz. At that time, the tonotopic map was reorganized, and the average multi-unit SFR was significantly increased from 14.3 sp/s to 17.7 sp/s. The degree of synchronization between spontaneous multiunit firings recorded at nearby sites was significantly increased as well.

Role and Function of Spontaneous Activity

Changes in the Role of SFR from Auditory Nerve to Cortex

Does the fact that spontaneous inter-spike-intervals in ANFs have a Poisson-like (mean interval equals its variance) structure, whereas in auditory cortex there is a hyperexponential distribution (Eggermont, 1990; Eggermont et al., 1993) with a variance about twice the mean (at least in primary visual cortex; Vogels et al., 1989), indicate a different role of spontaneous activity along the auditory pathway? To evaluate this we will also address whether increased SFR in tinnitus functions as "noise" as implied by the rationale for the gap-startle test (Turner et al., 2006).

First of all, we have to consider if is the designation of spontaneous activity as information carrier or as neural noise is dependent on the level of SFR. In auditory nerve fibers, high SFRs are strongly correlated with low thresholds, i.e., with increased sensitivity. For these units, a low-frequency pure tone can modulate the SFR especially at low sound levels as reflected in the phase-locking of the firings to the tone-period (Javel et al., 1988). This indicates a multiplicative action of the stimulus, suggesting that the spontaneous activity in an information carrier. However, at higher stimulus levels spikes are added to the spontaneous activity. A multiplicative action is hardly possible for the high-threshold, low-SFR ANFs and here stimulation dominantly adds spikes. Considering low SFR as noise, however, is stretching the definition of noise. It is also known that these low-SFR ANFs do not contribute to the CAP even at high stimulus levels (Bourien et al., 2014) and do not affect CAP threshold. These low-SFR units have also been described as vulnerable to noise exposures producing temporary threshold shifts (TTS), which after some delay result in high-threshold ANF loss (Kujawa and Liberman, 2009). This has been suggested (Hickox and Liberman, 2014) to result in increased central gain and through this modulation results in steeper rate-intensity functions and increased SFR. However, they also stated that: "Gap PPI tests" often used to assess tinnitus, revealed limited gap detection deficits in mice with cochlear neuropathy only for certain gap-startle latencies, inconsistent with the presence of tinnitus "filling in the gap."

Litvak et al. (2003) suggested: "Spontaneous activity helps to faithfully encode stimulus waveforms in the temporal discharge patterns of sensory neurons by allowing these waveforms to be represented by small modulations of ongoing activity. Such modulation coding lowers threshold and mitigates the distortions caused by refractoriness in single neurons. Spontaneous activity may also desynchronize stimulus-driven activity across neurons in a population, thereby allowing a volley principle to operate when the stimulus period is shorter than the neural refractory period. In this view, noise resulting from random spontaneous activity is the price paid for the lower thresholds neural population."

Is the SFR dependent on the recording site along the auditory pathway? In controls the SFR decreases from ANF (only the

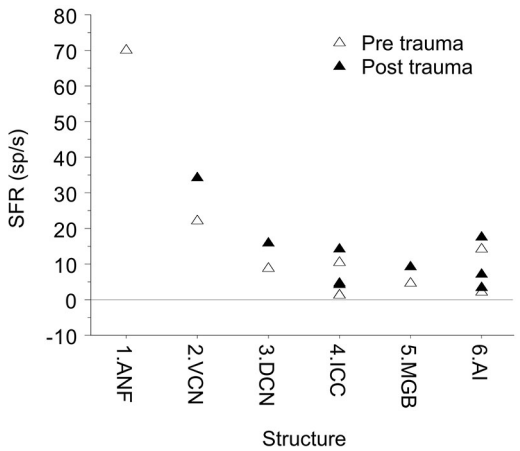

FIGURE 7 | Mean SFR vs. location in the auditory system. The data shown are from hamster DCN 8.7 sp/s (Finlayson and Kaltenbach, 2009). From barbiturate guinea pig VCN, 22 sp/s (Vogler et al., 2011). From barbiturate guinea pig ICC, 1.2 sp/s (Mulders and Robertson, 2009) and 1.4 sp/s (Vogler et al., 2014), and from ketamine rat ICC, 10.4 sp/s (Ropp et al., 2014). In medial geniculate body (MGB) of awake Sprague-Dawley rats the SFR was 4.7 sp/s (Kalappa et al., 2014). In auditory cortex, SFRs ranged from 1.9 sp/s (Seki and Eggermont, 2003), to 3.5 sp/s (Noreña and Eggermont, 2003) in ketamine anesthetized cat AI. In pentobarbital anesthetized Sprague-Dawley rats they were surprisingly high at 14.3 sp/s, potentially resulting from multi-unit activity (Engineer et al., 2011).

mean of high SFRs shown) to in particular the ICC and MGB and then increases somewhat in some auditory cortex recordings, likely because they reflect multi-unit activity in contrast to single-unit activity at more peripheral levels (**Figure 7**). Could one thus hypothesize that the information carrier function of spontaneous activity be limited to the in general high-SFR in auditory nerve and brainstem and functions only as noise in ICC, MGB and auditory cortex? That also affects how one interprets the recent finding of Buran et al. (2014) that "auditory cortex spontaneous discharge rate can be modulated transiently during task performance, thereby increasing the signal-to-noise ratio and enhancing signal detection." Clearly, stimuli or tasks modulate the spontaneous activity suggesting the information carrier model, but it also increases the signal-to-noise ratio implying that the spontaneous activity is basically noise.

The Role of Bursting and Neural Synchrony

After noise trauma, it is often observed that the increase in SFR (**Figure 7**, full symbols) is accompanied by increased burst firing and increased neural synchrony. Burst firing is uncommon in normal ANFs but is found after noise trauma (Liberman and Kiang, 1978), and also in the DCN (Finlayson and Kaltenbach, 2009) and in the MGB (Kalappa et al., 2014). Ma et al. (2006) did not find any changes in burst-firing activity in the ICC. In AI, changes in burst firing were only transient (and only reflected in the number of spikes in a burst; Noreña and Eggermont, 2003). Bursting was positively correlated with SFR in DCN (after PTS) and MGB (after TTS) and negatively in ANF as it occurred only when the thresholds were very elevated. Noise induced hearing loss very often results in tinnitus as well. This raises the question if increased SFR and burst firing have a causal role in tinnitus generation, which requires at least that increased burst firing is correlated with tinnitus. Only in the DCN and MGB was burst firing correlated with behavioral signs of tinnitus.

These two distinct views of spontaneous activity, either as unwanted noise or as information carrier, may thus determine how one views the neural mechanisms of tinnitus. If one considers spontaneous activity in the auditory system as unwanted noise, the favored concept about tinnitus is likely that it results from too much noise. The suggested neural substrate will then be increased SFR in the auditory system. On the other hand, if one considers spontaneous activity as the information carrier of the brain, sound modulates this firing rate and reorganizes it. In this model external sound can suppress tinnitus. Tinnitus in this model results from increased neural synchrony, i.e., the pathology also reorganizes the spontaneous firing times either in the form of serial correlations, i.e., burst firing, or as parallel correlations, i.e., as synchronous firing among neurons (Eggermont and Tass, 2015).

Both serial and parallel synchrony will enable efficient synaptic transmission and may amplify each other. Eggermont et al. (1993) phrased it as: "Burst firing in neocortex tends to be a communal event; when a neuron is firing in bursts there is a large probability that another adjacent neuron is also firing in bursts (Noda and Adey, 1970). Bursts occurrences of two or more simultaneously recorded neurons often appeared to be temporally close, especially between pairs of neurons recorded by the same electrode (Legéndy and Salcman, 1985). . . . In a structure such as the neocortex where the connection strengths between pyramidal cells is on average only 0.05 (Abeles, 1982; Eggermont, 1992) burst firing could act as an amplification mechanism of neural activity that could ensure faithful transmission across a synapse. . . . The timing intervals of increased firing rate for neighboring neurons will overlap to an extent determined by burst duration, and during this interval there will be a tendency to synchronize the events. Co-occurrences of bursts in neurons could therefore recruit those neurons into functional assemblies in a way analogous to the modification of excitatory transmission postulated by Hebb (Neven and Aertsen, 1992)." Both types of enhanced synchrony then may form "objects" of the increased SFRs, which depending on top-down modulating factors, may be experienced as "tinnitus". This again implies that spontaneous activity is an information carrier.

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