Perinatal exposure to a noncoplanar polychlorinated biphenyl alters tonotopy, receptive fields, and plasticity in rat primary auditory cortex

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Noncoplanar polychlorinated biphenyls (PCBs) are widely dispersed in human environment and tissues. Here, an exemplar noncoplanar PCB was fed to rat dams during gestation and throughout three subsequent nursing weeks. Although the hearing sensitivity and brainstem auditory responses of pups were normal, exposure resulted in the abnormal development of the primary auditory cortex (A1). A1 was irregularly shaped and marked by internal nonresponsive zones, its topographic organization was grossly abnormal or reversed in about half of the exposed pups, the balance of neuronal inhibition to excitation for A1 neurons was disturbed, and the critical period plasticity that underlies normal postnatal auditory system development was significantly altered. These findings demonstrate that developmental exposure to this class of environmental contaminant alters cortical development. It is proposed that exposure to noncoplanar PCBs may contribute to common developmental disorders, especially in populations with heritable imbalances in neurotransmitter systems that regulate the ratio of inhibition and excitation in the brain. We conclude that the health implications associated with exposure to noncoplanar PCBs in human populations merit a more careful examination.

autism | brain development | cortical maps | environment | sensory systems

Polychlorinated biphenyls (PCBs) are organic compounds that were manufactured and used mostly as coolants and lubricants from the 1930s until 1977, when they were banned because of a range of environmental concerns. Despite the ban, PCBs persist widely in environmental samples, and high levels of residues have been detected in many lakes and rivers, including, for instance, in fish in Michigan's Fox River (1) and San Francisco Bay sediments in California (2). PCBs accumulate in fat and affect the food chain primarily, but not solely, through consumption of game fish (3). Early toxicology studies of PCBs focused mostly on a subset of PCBs known as coplanar PCBs, which have ≤1 *ortho*-chlorine substitutions, are dioxin-like, and act on the aryl hydrocarbon receptor. This family of PCBs disrupts endocrine pathways, is carcinogenic, and is strongly toxic to the immune and reproductive systems. Although most studies focused primarily on coplanar PCBs, it recently has been shown that noncoplanar PCBs are particularly stable and predominate in environmental human tissue samples over their coplanar counterparts (4). This finding has contributed to a renewed scientific interest in the potential biological hazard of this structural class of PCBs. Noncoplanar PCBs have two or more *ortho*-chlorine substitutions and very weak or no aryl hydrocarbon receptor-binding activity. Recent studies find that noncoplanar PCBs are potent sensitizers of ryanodine receptor (RyR) calcium channels (5–9) and, as such, affect Ca^{2+} release from intracellular stores (7–10) and influence the cellular response to both chemical and electrical signals (11, 12). RyRs, previously thought to exist only in muscle tissue, now are documented in both pre- and postsynaptic terminals in neurons in many brain areas (13–15); RyRs were shown to affect neuronal excitability (13, 15–22) and synaptic plasticity, modulating both long-term potentiation and long-term depression (13, 16–19). RyR activation probably is involved in syntilla generation (20), regulation of inhibitory circuitry (21–25), and neuronal exocytosis (26, 27), as well as in modulation of levels of BDNF (17) and acetylcholinesterase (28), both of which strongly influence learning and plasticity.

Indeed, exposure to noncoplanar PCBs has been shown to reduce the brain's production of dopamine in tissue culture and nonhuman primate brains (29, 30), degrade hippocampal function in culture (31, 32), alter behavior in rats (33, 34), and negatively impact general indices of neuropsychological functioning in exposed children (35). Collectively, these demonstrations of the effects of exposure to noncoplanar PCBs on neurological mechanisms raise serious concerns about their more subtle consequences on proper brain development. Yet, to date, despite accumulating evidence of biotoxic effects and documented environmental prevalence and persistence, no *in vivo* cortical effects of exposure to noncoplanar PCBs have been documented. Of particular interest is the effect of PCB exposure on the development of sensory systems, which are the first to mature in the cortex. Evidence of abnormal sensory development would indicate a likely profound and pervasive effect of exposure.

This study focused on one specific noncoplanar PCB, 2,2′,3,5′,6-pentachlorobiphenyl (PCB95), which is representative of these environmentally ubiquitous toxicants. PCB95 was chosen because of its prominent prevalence in environmental samples (1, 2), its complete lack of aryl hydrocarbon receptor activity (I.N.P. and M. S. Denison, unpublished data), and because it appears to have the highest potency measured so far among PCB congeners in altering RyR function and cellular signaling events in neurons (9).

We show here that exposure of rats to PCB95 *in utero* and throughout the postnatal nursing epoch resulted in a grossly distorted development of the primary auditory cortex (A1).

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Abbreviations: PCB, polychlorinated biphenyls; A1, primary auditory cortex; RyR, ryanodine receptor; PCB95, 2,2′,3,5′,6-pentachlorobiphenyl; RF, receptive field; ABR, auditory brainstem responses; CF, characteristic frequency; EPSC, excitatory postsynaptic current; IPSC, inhibitory postsynaptic current; P*n*, postnatal day *n*.

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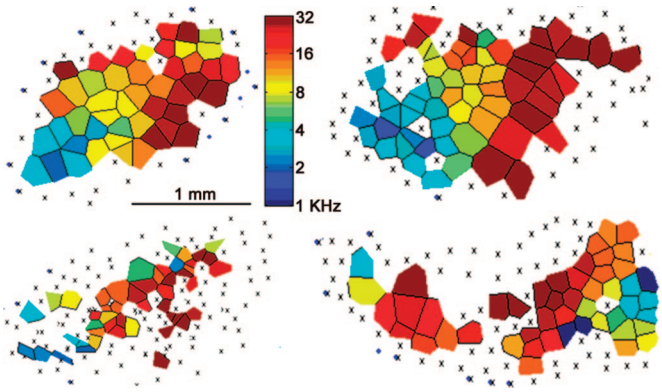

Fig. 1. Exposure to PCB95 alters A1 maps. (*Upper Left*) Tonotopic map from a typical control rat pup. (*Upper Right*, *Lower Left*, and *Lower Right*) Examples of maps from PCB95-exposed rat pups. × indicates an unresponsive site. Color bar, CF (kilohertz).

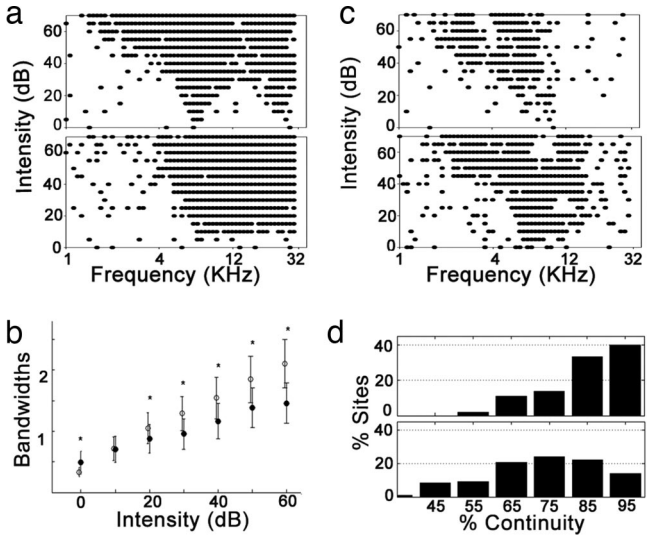

Fig. 2. Exposure to PCB95 reduces RF selectivity. (*a*) Examples of a double-peaked (*Upper*) and a rectangular (*Lower*) RF. (*b*) Mean bandwidths (octaves) as a function of attenuation (decibels). Open circles, control rats; filled circles, PCB95-exposed rats. *, $P < 0.01$. (*c*) Examples of RFs with unresponsive sites. (*d*) For control rats, >75% of the recording sites had continuous RFs (at least 85% uninterrupted by holes) (*Upper*), versus only 35% of sites in PCB95-exposed rats (*Lower*) ($P < 0.001$).

Distortions occurred in A1 response characteristics, topography, tonotopic gradients, and receptive fields (RF). There were altered correspondences of the relative strengths of excitation and inhibition within A1 circuitry, and critical period plasticity in A1 was sharply disrupted. These findings occurred in the setting of normal thresholds, magnitudes, and latencies of auditory brainstem responses (ABRs) to tones and clicks. Similarly, evoked cortical neuronal response had consistently normal thresholds. Such findings suggest that developmental exposure to certain noncoplanar PCBs interferes with normal cortical development in the absence of significant peripheral deficits, possibly through the disruption of RyR activity in the brain.

Results

Significant abnormalities were documented in A1 of pups of PCB95-exposed dams (see *Methods*) at the intracellular, RF, and topographical levels.

Disrupted A1 Organization and Altered RF Properties in PCB-Exposed Animals. In a series of earlier reports from this laboratory, we have documented the response characteristics and boundaries of the normal A1 obtained by densely sampling neuronal responses with microelectrode recording in the middle cortical layers hundreds of times (36–40). To map A1, we plotted for each neuron its characteristic frequency (CF), the frequency to which neurons respond at lowest sound intensity. Neurons at sampled locations within the functionally defined A1 were only rarely ineffectively excited by brief tones. In striking contradistinction, in PCB95-exposed rats, A1 maps had irregular shapes, could have abnormal topographic organization, and invariably were marked by sites at which neurons could not be excited by simple tonal stimuli (Fig. 1). Additionally, neurons in the PCB95 group, on average, were less selective than neurons in the control group at low sound intensities. In 13% of the A1 RFs measured in PCB-exposed animals, there was a clear lack of frequency-selectivity, i.e., they had multiple peaks (Fig. 2*a Upper*) or were flat-peaked (Fig. 2*a Lower*), versus 3% in control animals exposed to vehicle alone ($P < 0.001$). This situation was reversed at higher intensities, where bandwidths at high sound intensities were consistently narrower in PCB-exposed versus control animals (Fig. 2*b*).

Furthermore, RFs usually are continuous, i.e., domains of frequency intensity within the RF boundaries over which neurons were excitable uninterrupted. When we define a continuous RF as one in which at least 85% of the area enclosed by the edges of the RF elicits a response, we find that 55% of RFs recorded in PCB-exposed rats, compared with 25% in control rats ($P <$ 0.001), were marked by discontinuities, i.e., domains of frequency intensity within the RF boundaries over which neurons were excitable either not at all or only very weakly. These nonresponsive "holes" in RFs were highly replicable, persisting through repeated sampling. Typical examples are shown in Fig. 2*c* and data-summarized in Fig. 2*d*. Although fewer cortical sites were excited and activity was restricted to smaller cortical areas in PCB95-exposed rats, recording locations that were responsive showed no group difference in response parameters such as onset latencies and spike discharge rates.

Excitatory and Inhibitory Currents Are Only Weakly Correlated in PCB-Exposed Animals. To investigate whether the abnormal RFs in PCB-exposed rats could be a consequence of alterations of inhibitory versus excitatory synaptic inputs, whole-cell voltage-clamp recordings were obtained from A1 neurons *in vivo* in anesthetized PCB-exposed rats to directly measure the balances of excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) that contributed to defining the RF. The best frequencies for the tone-evoked EPSCs and IPSCs were well matched in control animals, as previously reported (41, 42) (Fig. 3*a*). This balance was disrupted in PCB95-exposed animals (Fig. 3*b*). The mean linear correlation coefficient r between the frequency-matched excitation and inhibition was 0.76 ± 0.04 ($n = 16$) in controls and 0.35 ± 0.10 ($n = 10$, difference $P < 0.01$) in exposed rats (Fig. 3*c*).

In PCB-exposed animals, IPSCs tended to be maximal near the excitatory peak overall, albeit less reliably. More significantly, there was great heterogeneity in the magnitudes of currents away from the best frequency relative to normal responses. Off-center inhibitory currents variably were markedly deregulated, often being unusually large or small and well outside of the ranges recorded for control neuron samples (Fig. 3*d*). However, there was no systematic increase or decrease in either excitation or inhibition for particular frequencies; i.e., we observed no selective alteration to responses evoked by either high- or low-frequency tones in PCB-exposed animals ($P >$ 0.85). Finally, we examined the monotonicity of frequency tuning in control and PCB-exposed neurons. In control cells

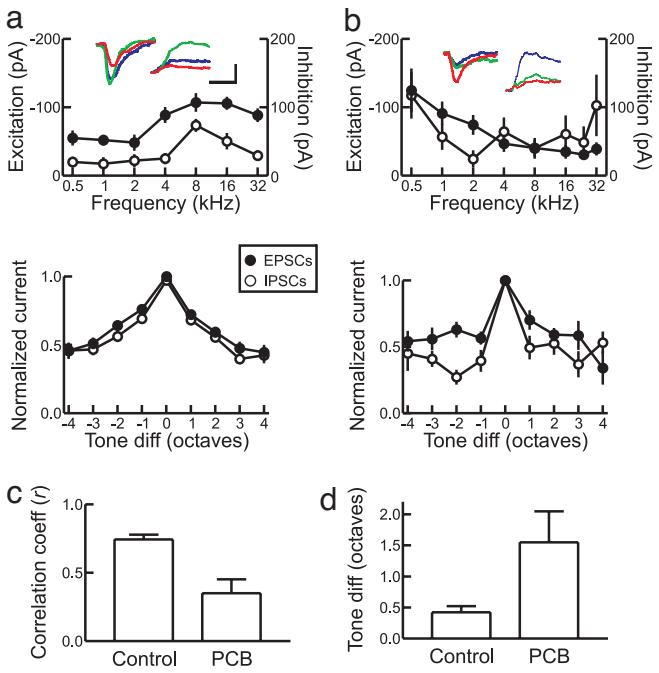

Fig. 3. Excitatory–inhibitory balance is disrupted in PCB95-exposed animals. (*a*) Frequency tuning of excitatory (filled circles) and inhibitory (open circles) currents in control animals. Symbols indicate peak tone-evoked synaptic currents. (*Upper*) Currents from one A1 neuron. (*Insets*) Representative EPSCs and IPSCs (red, 2 kHz; green, 8 kHz; blue, 32 kHz). (Scale bar: 50 pA, 50 ms.) (*Lower*) Cotuning and balance of mean (±SEM) currents, normalized to peak excitatory and inhibitory responses and centered on the best excitatory frequency of each cell ($n = 16$). (*b*) As in *a* but for PCB-exposed animals ($n = 10$). (*c*) Linear correlation coefficient *r* of peak excitation and inhibition. In control animals, tuning of excitation and inhibition is highly correlated, but that correlation is reduced in PCB-exposed animals. (*d*) Absolute difference in octaves between best frequency of excitatory and inhibitory currents. In control animals, the best frequency for inhibition tends to occur at or near the best frequency for excitation. In PCB-exposed animals, the difference between excitatory and inhibitory best frequencies is larger.

[supporting information (SI) Fig. 6*a*], there was a general monotonic decrease, i.e., negative linear correlation in tone-evoked response amplitude from the best frequency for both excitation (r: -0.62 ± 0.08) and inhibition (r: -0.49 ± 0.11). PCB-exposed neurons (SI Fig. 6*b*), however, were less monotonic; excitation was weakly anticorrelated with relative tone frequency (r: -0.31 ± 0.18), and inhibition was essentially uncorrelated with frequency (r: -0.03 ± 0.12).

Disrupted Exposure-Based Plasticity. In normal rat pups, passive exposure to daily epochs of a pulsed tonal stimulus results in the enlargement of the A1 cortical zone representing that frequency (37). By contrast, exposure to noise frustrates developmental progression and degrades cortical RFs (39). The abnormal features of A1 development that result from PCB95 exposure suggested that the mechanisms of developmental plasticity might be abnormal in these animals. To evaluate that possibility, rats in two additional series of PCB95-exposed pups also were exposed during the auditory critical period (37, 39) to (*i*) tonal stimuli or (*ii*) modulated noise stimuli. Both cases then were contrasted with similar sound-exposure-driven plasticity in control pups that were reared in the same distorted sound environments.

PCB exposure had a strong impact on this crucial early-stage plasticity. Results differed between tone-exposed and noise-exposed rats. Of five PCB95 tone-exposed rats, one showed clear evidence of positive plasticity expressed as a modest tone-

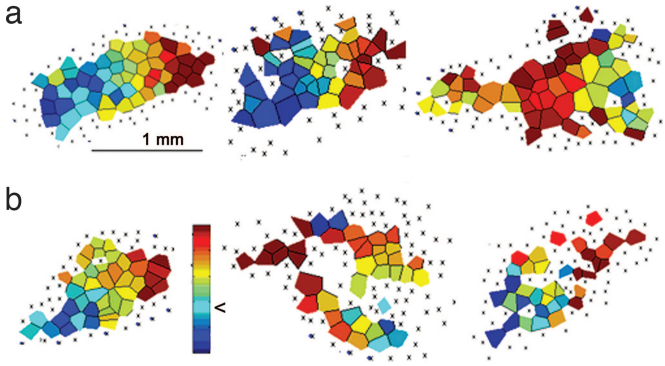

Fig. 4. Disrupted plasticity in PCB95-exposed animals. (*a*) (*Left*) Control rat exposed to pulsed tone (4 KHz, marked on color bar in *b* by arrowhead). (*Center* and *Right*) Examples of PCB95 rats exposed to the same pulsed tone. Some plasticity was evident only in one of the five rats in the group (*Center*). (*b*) (*Left*) Control rat exposed to pulsed noise. (*Center* and *Right*) Examples of PCB95 rats exposed to same pulsed noise, resulting in extensive discontinuities.

specific A1 expansion (Fig. 4*a Center*), as was recorded in tone-exposed controls and as previously established (37). The other four animals showed no map enlargement at the exposure frequency. Overall, tone-evoked reorganization of A1 was greatly attenuated in the pups of PCB95-treated dams. By contrast, critical period exposure to modulated noise stimuli, which by itself is known to degrade normal response selectivity and sound frequency representation, intensified the plasticity-driven deficits in response selectivity and cortical representation. Gross discontinuities and extensive topographic disruptions within A1 maps were more evident in pulsed-noise-exposed PCB95 rats than in any other group (Fig. 4*b*). Discontinuities were a prominent feature of normal-noise-exposed rats, as has been described (39). This effect was exacerbated in the PCB95 group (SI Fig. 7*a*). In control animals reared in pulsed noise, 45% of sampled neurons had discontinuous RFs, whereas in PCB95 rat pups, that percentage increased to >70% ($P < 0.002$). In other respects, neuronal responses in the two noise-exposed groups (controls and PCB95) were similar. Bandwidths were wider than in normal rats (SI Fig. 7*b*). Abnormally unselective and double-peaked RFs were recorded from >20% of sampled sites in both groups.

Tonotopic Organization. In A1 of normal animals, CF increases monotonically in the posterior-to-anterior direction. We quantified frequency organization (tonotopy) by using a two-dimensional tonotopy index (see Fig. 5*a* and legend). All of the rats in control groups had normal tonotopic progressions. However, eight rats (half the rats in the PCB95 group) had abnormal tonotopic progressions (Fig. 5*b*) that, to various degrees, were disorganized or reversed. Abnormal tonotopic progression was documented in approximately half of the pups from all three PCB-exposed groups, regardless of auditory experience.

Hearing Sensitivity Is Normal in PCB95 Rats. To assess the hearing of PCB95-exposed rats relative to normal controls, we measured ABR latencies and thresholds for responses evoked by clicks (see *Methods*). Thresholds and waveforms of the PCB95-exposed rats to clicks and to tone pips of all frequencies showed no statistical difference from controls (SI Fig. 8). Results were independent of the auditory experience of the rats. Those results were confirmed further by threshold measurements derived from cortically recorded RFs. CF thresholds of PCB95-exposed rats did not differ from controls across the entire frequency spectrum, regardless of auditory experience. Although behavioral

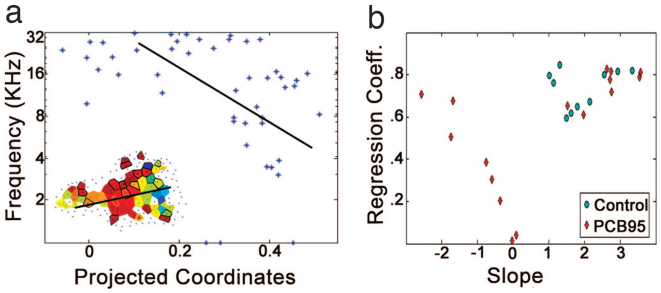

Fig. 5. Abnormal tonotopy was observed in half of the PCB95-exposed animals. (*a*) To compute tonotopy, we defined the map's major axis by using linear regression, then projected all points onto this axis as a dimensionality reduction illustrated in *Inset*. We then plotted frequency as a function of distance along the tonotopic axis and fitted a regression line to this plot. (*b*) Examples from all three control groups (blue circles) have positive slopes, indicating normal tonotopic progressions, and high regression coefficients, indicating a good fit of the data. However, in the PCB95-exposed groups (red diamonds), only 8 of the 16 PCB95 rats were clustered with the control group. The other eight had near zero or negative slopes. A low regression coefficient indicates that tonotopic progression generally was disorganized. A negative slope combined with a high regression coefficient (three rats) indicates a reversed tonotopic progression, as in *a Inset*.

outcomes of the abnormal auditory maps we observed were not explored, it is likely that, despite the apparently normal hearing, the affected rats would exhibit, among other PCB-related deficits, abnormal learning of more complex auditory sounds (such as sweeps or complex tone sequences) or gating abnormalities.

Discussion

Previous studies of PCB toxicity have identified that developmental exposure to a complex mixture of coplanar and noncoplanar PCBs known as Aroclor 1254 produces cochlear hair loss that caused peripheral hearing impairment (43, 44), which has been attributed in part (45) to reductions in thyroid hormone (hypothyroidism). The present study showed that developmental exposure to a noncoplanar PCB can disrupt the topographic organization and the critical period plasticity that underlies normal postnatal auditory system development in the absence of detectable peripheral hearing impairment. The organization of A1, and presumably of other cortical areas, was significantly altered in rat pups after *in utero* and postnatal PCB95 exposure through nursing. Changes were evident (*i*) in overall A1 organization, including tonotopy and A1 topography; (*ii*) in RF alterations, such as patchy RFs as assessed with extracellular recording; (*iii*) in changes in the balance of excitatory and inhibitory inputs as assessed with *in vivo* whole-cell recording; and (*iv*) in the ability of auditory cortex to plastically reorganize after changes in the acoustic environment, thus significantly impacting critical period plasticity. Persistent alterations in RyR densities and/or activity in cortical preparations have been documented as a result of perinatal exposure to PCB95 (34). These changes, although extensive, may well lie within the scope of interference with RyR activity, given the multitude of roles of RyRs described in the literature. The irregularities observed in topographical organization indicate that chemical and electrical signaling processes thought to regulate tonotopic order and cortical field relationships were disrupted by developmental exposure to PCB95. PCB95 affects intracellular calcium channels (7, 9, 10), which play an important role in tonotopic map formation (46), and the developmental organization and segregation of the barrel field in the somatosensory cortex of mice in which LMO4 was deleted in the cortex was severely disrupted (47). The LMO4 transcription complex is a mediator of calcium-dependent transcription in cortical neurons; calcium influx via voltage-sensitive calcium channels and NMDA receptors contributes to synaptically induced LMO4-mediated transactivation. Thus, the LMO4 transcription complex is an example of how abnormal calcium activity could affect thalamocortical patterning of cortical sensory maps. It also has been shown that the calcium-induced dendritic growth is regulated by activation of a transcriptional program that involves calmodulin kinase IV and cAMP response element-binding protein (CREB)-mediated signaling to the nucleus (48), further underscoring the role of calcium in map formation. It is hypothesized that this impact we observed on the ordered organization of effects in A1 may occur through an activity-dependent bias that, along with other mechanisms such as chemical guidance molecules (49, 50), contributes to map formation and maintenance. Further studies are needed to more fully elucidate the relationship by which RyR dysfunction may alter aspects of neuronal growth and synapse formation. The effects on cortical plasticity are most likely the outcome of abnormal long-term potentiation and long-term depression, for which RyRs are important (13, 17–19). The abnormal inhibition to excitation ratios that we observed are probable outcomes of disrupted RyR signaling on inhibitory circuitry. Indeed, PCB95 has been shown to significantly enhance Ca^{2+} signals elicited by NMDA and AMPA glutamate receptor activation, without altering basal Ca^{2+} levels (10, 11). Finally, unresponsive holes in RFs might result from a number of potential mechanisms, including a shift in the relative timing of excitation and inhibition or in imbalance in the relative degree of excitation and inhibition (nonmonotonic E–I relationship). A selective decrease in excitation, or an increase in inhibition, or both, could lead to a hole by reducing the spiking output of a neuron at a spectrally restricted region of the tonal RF. For instance, holes could be the direct result of overactive local inhibitory circuits, whereas poorer than normal response selectivity is consistent with a lack of focused inhibition.

What are some possible implications of these results? First, a question arises as to whether or not the exposure levels we used were realistic. Based on existing studies by others (51), it is safe to assume that our pups had plasma PCB levels of 50–1,000 ppb, which, although very high, is of the same order of magnitude of total PCB levels for populations near severely contaminated sites (52). Our rat pups attained such levels of contamination solely via exposure *in utero* and nursing. Indeed, the primary source of exposure for infants is through breast milk. Although some studies have shown a decrease in breast milk concentrations of PCBs since the 1970s (53, 54), they are limited by differences in analytical techniques as well as the number and especially types of congeners measured, with the focus usually being on coplanar PCBs rather than on their more stable and prevalent counterparts noncoplanar PCBs, which predominate in breast milk samples (55). Although concentrations of noncoplanar PCBs in breast milk may or may not be decreasing, the proportion of infants being breastfed is on the rise, according to the Centers for Disease Control and Prevention (www.cdc.gov/pednss/how_to/interpret_data/case_studies/breastfeeding/when.htm); generally, both the number of breastfed infants and the duration of the breastfeeding periods are significantly higher today relative to 30 years ago (56). In parallel, studies show that although infants in exposed populations have plasma PCB concentrations that are correlated with those of their mothers, there is as much as a 6.6-fold increase of plasma PCB concentrations in infants who were breastfed for more than 3 months relative to infants who were not breastfed (52). This finding, along with the sharp increase in breastfeeding numbers and duration, makes it particularly pertinent to study the breast milk concentration of potentially harmful substances such as noncoplanar PCBs. Although numerous studies have shown that breastfeeding of infants is superior to existing alternatives, these data suggest the possibility that, in extreme cases, for mothers with high levels of

exposure to PCBs and/or other closely related toxicants that bioaccumulate in breast milk, lactational exposure of genetically at-risk infants may in fact not be in the infant's best interest. Although the PCB95 exposure in our rats was on the high end of the cumulative PCB exposure spectrum in humans, it is important to keep in mind that in the genetically susceptible child (for example, see ref. 57), it is possible that significantly lower levels of exposure to an environmental trigger could magnify important deviations from normal development. How to identify infants who may be genetically at risk from such an exposure remains an open question, and scenarios such as genes that confer a higher risk of autism (57) merit further investigation. Presently, it is unknown whether the increase in breast-feeding rates and duration in as-yet-undefined but genetically at-risk populations could be correlated with incidence of some developmental disorders such as autism spectrum disorders (ASD), language impairments, or attention disorders (58, 59). Although to date no evidence exists to support such a scenario, our finding of an imbalance between excitation and inhibition, which is consistent with genetic (60–63), anatomical (64, 65), and electrophysiological (66–69) evidence of imbalance between excitation and inhibition as a core abnormality of autism, supports further investigations in this direction (70). Furthermore, it has been suggested that abnormal Ca^{2+} signaling, resulting from genetics or environmental factors, may be linked to ASD (71) through disruptions of critical developmental signaling pathways important in regulating neuronal migration, differentiation, and synaptogenesis. It also is interesting to note that children with Timothy Syndrome, a rare genetic disease in which Ca(v)1.2 calcium channels are operationally defective, frequently fall within the autism spectrum (72).

On the basis of these findings, we believe that noncoplanar PCBs and chemically related structures should hold a more prominent position on the candidate list for environmental factors that may contribute to gene–environment interactions with potentially negative consequences. Exploration of the possible biotoxic impacts of noncoplanar PCBs and their related chemical family on fetuses and infants still are incomplete and, given their prevalence in the environment, should be a high national and world-health priority.

Methods

All experimental procedures used in this study were approved by the University of California, San Francisco, Animal Care and Use Committees.

PCB95 Administration. Female rats (Sprague–Dawley) were administered 6 mg/kg per day PCB95 dissolved in corn oil (Wesson) and applied onto a corn flake (Nature's Path), from gestation day 5 to weaning at postnatal day 21 (P21). Dams always consumed the corn flake within 10 min of administration. Control groups were fed corn flakes with pure corn oil. In total, we mapped six rats in each of the normal auditory exposure groups (controls, PCB-exposed), five from each of the tone-reared groups (controls, PCB-exposed), and five from each of the noise-reared groups (controls, PCB-exposed). Controls pups were from a minimum of two separate litters, and PCB-exposed pups were sampled from three litters. Developmental exposure to PCB95 did not produce differences in litter size, sex ratio, or weight gain compared with the control group.

Auditory Exposure. All litters were reared in a sound-shielded (Acoustic Systems, Austin, TX), calibrated test chamber, with a 12-hour light/dark cycle controlled by a timer. At P9, before cochlea opening, an auditory stimulus (noise or tones) was turned on for noise/tone-exposed litter groups and remained on continuously up to P35–P40, past closure of auditory critical period. The setup is described in detail in refs. 38 and 39. Tone stimuli consisted of a 25-ms tone (5-ms ramps), and noise stimuli consisted of 50-ms noise pulses (5-ms ramps) with a 65–70 dB sound pressure level.

ABRs. ABR testing was conducted in a sound-attenuated chamber (Acoustic Systems). ABRs were recorded with silver-wire electrodes (0.13-mm diameter; A-M Systems, Sequim, WA) threaded through the skin at three locations: the positive electrode was placed directly posterior to the left (stimulated) ear over the bulla, the negative electrode was placed over the vertex, and the ground electrode was placed 1–2 cm posterior to the right (unstimulated) ear. ABR signals were acquired, filtered, amplified, and analyzed with equipment and software manufactured by Tucker-Davis Technologies (Alachua, FL). Click and tone pip stimuli were presented with a custom-made tube speaker inserted into the ear. Tone pips (1.2-ms duration, 0.2-ms raised cosine ramps) were presented at 2, 4, 7, 14, and 28 kHz. Acoustic calibration was performed with a microphone (Brüel and Kjær, Nærum, Denmark) before each recording session to ensure that tones and clicks were presented at equivalent loudness with minimal (<1%) total harmonic distortion. Click thresholds were determined by presenting 500 click stimuli (10 per s) at 50 dB and reducing the sound level in 5-dB steps until the response pattern was no longer visible. Tone thresholds were determined by using 10-dB steps and averaging over 1,000 stimulus presentations. Auditory thresholds were defined as the lowest sound intensity capable of eliciting a response pattern characteristic of that seen at higher intensities. Thresholds were compared for each stimulus type with ANOVA statistics. All recording and analysis was performed blind with respect to exposure. Rats were lightly anesthetized with 60 mg/kg pentobarbital i.p. during the procedure and recovered upon completion.

Mapping. Mapping methods are described in detail in refs. 37 and 39. Frequency–intensity response areas were reconstructed in detail by presenting 60 pure-tone frequencies (1–30 kHz, 25-ms duration, 5-ms ramps) at 8 to 15 sound intensities to the contralateral ear at a rate of 2 stimuli per s by using a calibrated sound delivering system with a custom-made tube speaker inserted into the ear canal. Sound intensities ranged from 0 to 70 dB sound pressure level to objectively define a frequency–intensity response area and thus the RF. In later experiments (70% of experiments), the stimulus set was presented twice to ascertain the stability of abnormalities such as holes and nonselective RFs. Broad RFs that showed no distinct threshold minimum were classified as nonselective. In those cases, upper and lower RF edges were selected, and the geometric mean was used as CF estimate. In the case of double-peaked RFs, the peak occurring at the lower intensity of the two was chosen as the CF. The average sampling grid unit was 175–225 μm. A full A1 map usually consisted of 60–100 penetrations. A1 was defined in this study by short-latency (7- to 30-ms) responses and areas of good responses to low as well as high frequencies. Mapping was done within 1–14 days of ABR testing

Whole-Cell Recordings. Whole-cell recordings were performed on female littermates of the mapped rats at adulthood. A1 first was located by coarse mapping with a tungsten electrode. Whole-cell voltage-clamp recordings were obtained from neurons located 400–800 μm beneath the cortical surface. We reduced cortical pulsation with 4% agarose. The recording pipette (5–9 MΩ) contained 125 mM Cs-gluconate, 5 mM TeaCl, 4 mM MgATP, 0.3 mM GTP, 10 mM phosphocreatine, 10 mM Hepes, 0.5 mM EGTA, 3.5 mM QX-314, and 2 mM CsCl (pH 7.2). In some experiments, K-gluconate-based internal solution was used. Recordings were made with an Axopatch 200B (Molecular Devices, Sunnyvale, CA). Signals were sampled at 10 kHz and filtered at

5 kHz. Resting membrane potentials (-65.5 ± 11.7 mV SD) were measured in current-clamp after break-in. Auditory stimuli were delivered into the left ear canal by a tube sealed to a calibrated speaker. Stimuli consisted of a pseudorandom sequence of pure tone pips from 0.5 to 32 kHz on a logarithmic frequency scale at 50–75 dB sound pressure level, each with a 50-ms duration and 3-ms cosine rising and falling phases, at an interstimulus interval of 1 Hz. To determine the peak EPSC amplitude, we took the mean of a 1- to 2-ms window centered on the absolute peak. For IPSC peak amplitude, we used a 10-ms window.

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