Transformation of cortical and hippocampal neural circuit by environmental enrichment

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Published as:

Hirase H, Shinohara Y (2014) Transformation of cortical and hippocampal neural circuit by environmental enrichment. Neuroscience 280:282-298.

doi:10.1016/j.neuroscience.2014.09.031

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Key words: enriched environment, cerebral cortex, hippocampus, neuropil, spines, glia, gamma oscillations

Abbreviations: ACC, anterior cingulate cortex; AMPA, alpha-amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; cAMP, cyclic adenosine monophosphate; ChAT, choline acetyltransferase; ECM, extracellular matrix; ECS, extracellular space; EE, enriched environment / environmental enrichment; GABA, gamma-aminobutyric acid; GAD65, glutamate decarboxylase 65;GDNF, glial cell line-derived neurotrophic factor; GFAP, glial acidic fibrillary protein; GPCR, G-protein coupled receptor; IGF-1, insulin-like growth factor-1; KO, knock out; LFP, local field potential; LTP, long-term potentiation; NGF, nerve growth factor; NMDA, N-methyl-D-aspartate; NT-3, neurotrophin-3; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PV, parvalbumin; tPA, tissue-type plasminogen activator; SSRI, selective serotonin reuptake inhibitor; VGlut, vesicular glutamate transporter; vGAT, vesicular GABA transporter; VIP, vasoactive intestinal peptide;

Abstract

It has been half a century since brain volume enlargement was first reported in animals reared in an enriched environment (EE). As EE animals show improved memory task performance, exposure to EE has been a useful model system for studying the effects of experience on brain plasticity. We review EE-induced neural changes in the cerebral cortex and hippocampus focusing mainly on works published in the recent decade. The review is organized in three large domains of changes: anatomical, electrophysiological, and molecular changes. Finally, we discuss open issues and future outlook toward better understanding of EE-induced neural changes.

Introduction

The plastic nature of the brain allows animals to change their behavior to adapt to their environment. Remarkably, a given animal's capacity for both plasticity and successful behavioral adaptation is greatly influenced by its postnatal experience. For example, animals nurtured in a housing condition with environmental enrichment (EE, enriched environment) develop enhanced memory and learning abilities compared with those with standard caging conditions (van Praag et al., 2000; Nithianantharajah and Hannan, 2006; Simpson and Kelly, 2011, for reviews). EE is achieved across three axes. First, EE contains a larger habitable area in which physical objects like toys, tunnels, and running wheels are placed to promote animals' sensory and motor experience. Second, these objects are changed regularly, so as to keep animals' curiosity and voluntary exploration high. Third, animals are housed in groups to promote social interactions. Animals are typically reared in EE for a few to several weeks, during which these components are thought to synergistically influence brain plasticity. Additionally to cognitive enhancement effects, EE rearing has gained growing attention in the recent decade as it has been shown to have resilient, mitigating and sometimes recuperating effects in various neurological conditions including Alzheimer's disease, Huntington's disease, Rett Syndrome, and stroke (Nithianantharajah and Hannan, 2006; Pang and Hannan, 2013, for reviews).

Past studies have demonstrated that EE induces visible structural and functional changes in the brain. Description of enlarged brain volume and increased of dendritic morphological complexity in the cerebral cortex and hippocampus date back to 1960s (Bennett et al., 1964; Diamond et al., 1964) and detailed anatomical studies carried out in the following years confirmed these results. Another significant structural change is enhanced adult neurogenesis in the dentate gyrus of the hippocampus (Kempermann et al., 1997). While the contribution of neurogenesis to improved memory task performance remains controversial (Bruel-Jungerman et al., 2005; Meshi et al., 2006; Kerr et al., 2010; Bednarek and Caroni, 2011; Akers et al., 2014), these newly generated cells certainly innervate their target cells thus reorganizing the neural circuit.

Molecular genetics and physiological recording techniques have progressed tremendously since the days EE-induced structural changes were first noticed. Almost every cell type can now be molecularly labeled and manipulated by clever combinations of transgenic mice and recombinant viruses. Moreover, the activity of these cells can be manipulated by optogenetics or pharmacogenetics. *In vivo* two-photon microscopy and high-density extracellular electrophysiology provide means to observe dynamic changes of neural structure and activity, respectively. With these methodological advancements, the next likely step to advance our knowledge on experience-dependent modification of brain capacity is to understand the neuropil dynamism caused by EE exposure. This review aims to discuss recent progress on the neuropil reorganization and associated molecular changes triggered by EE exposure during juvenility to adulthood.

Recent views of neuropil

The term "neuropil" traditionally refers to the commingled substrate consisting of axons, dendrites, and glial cells typically observed in the gray matter of the central nervous system **(Figure 1)**. Serial electron microscopic reconstruction of neuropil, particularly by use of block face scanning electron microscopy, has made it possible to efficiently quantify the composition of neuropil. For instance, Mishchenko et al. (2010) reported that in rat hippocampal CA1 stratum radiatum neuropil, axons and dendrites occupy nearly 50% and 40% of the volume, leaving 8% for glial processes and the rest for the extracellular space (ECS). It has been suggested that ECS fraction assessed by electron microscopy could be underestimated as cells swell during the fixation process (Van Harreveld et al., 1965). In fact, other works by electron microscopy using rapid-freezing samples, *in vivo* iontophoretic or optical measurements estimated the ECS fraction to be 15-20% (reviewed in Sykova and Nicholson, 2008).

While the volume of axons and dendrites are comparable, individual axons are smaller in volume than dendritic processes. Intricate axonal innervation in the neuropil often results in several axons

being within reach of a single dendritic spine (Stepanyants et al., 2002; Mishchenko et al., 2010). Of note, the average number of reachable axons from a spine is a few times higher in primates than in rodents, suggesting a higher degree of freedom for connectivity modification of the neural circuit (Escobar et al., 2008).

The majority of cortical and hippocampal synapses are excitatory asymmetrical synapses characterized by the presence of electron-dense postsynaptic density (PSD) in the electronmicrograph. The PSD is packed with proteins for synaptic signal transduction, such as glutamate receptors, calcium/calmodulin-dependent kinase 2, actin, PSD95 and shank family proteins. The ratio of symmetrical and asymmetrical (i.e. inhibitory *vs.* excitatory) synapses is roughly 1/9 and is similar among mammalian species. The density of synapses are estimated to be $1.1/\mu m^3$ in human temporal cortex, $1.4/\mu m^3$ in rat hindlimb somatosensory cortex, $2.9/\mu m^3$ in mouse barrel cortex, and $2.5/\mu m^3$ in mouse visual cortex (DeFelipe et al., 2002). Mouse synapses are generally more compact than those of humans or rats, with the mean cross section length being about 75% of rat or human synapse (DeFelipe and Fariñas, 1992; DeFelipe et al., 2002). Remarkably, the numerical density of neurons decreases by several fold in humans compared with that of mice, implying that human neurons receive more synapses on average and that neuropil represents a larger proportion in humans.

Astrocytes are the most numerous and represent the largest volume among glial cells in mammalian neuropil. Gray matter astrocytes have a highly ramified bushy structure. The astrocytic microprocesses ensheath synapses to maintain a healthy extracellular environment, via mechanisms such as glutamate uptake and potassium buffering. They are also considered to function as a physical barrier to prevent crosstalk (e.g., transmitter spillover) between synapses (Nedergaard and Verkhratsky, 2012). Unlike in the cerebellum where the astrocytic (Bergmann glia) coverage of Purkinje cell synapses is virtually 100%, the coverage of spines in the cortex is less complete with ~30% of the synapses contacted by astrocytes (Spacek, 1985). In rat hippocampus CA1 stratum radiatum, one study calculated that 57% of the synapses had astrocytic contact (Ventura and Harris, 1999). Furthermore, the astrocytic contact depended on the morphology of the synapse: 88% of perforated synapses had astrocytic contact while the contact likelihood was substantially less (52%) for non-perforated synapses. As perforated synapses are generally larger, the fraction of astrocytic contact is lower in perforated synapses (34%) than non-perforated synapses (43%). Perisynaptic astrocytic coverage is biased to the postsynaptic side (Lehre and Rusakov, 2002) and spines with astrocytic contact tend to be larger in size and have more surface areas exposed to the extracellular environment (Witcher et al., 2007), possibly allowing neurotransmitter spillover or gliotransmission reception. Astrocytes are distributed in a tile-like pattern with minimal territorial overlaps amongst themselves (Bushong et al., 2002; Ogata and Kosaka, 2002). It has been estimated that a single rodent astrocyte can cover in the range of 100,000 synapses whereas a human astrocyte can cover an order of magnitude higher number of synapses (Oberheim et al., 2009). Another important glia cell type is the microglia which is the immune cell of the brain. Although their numerical and volumetric representation is much less than astrocytes, *in vivo* imaging of cortical microglia revealed that these cells are highly motile (Davalos et al., 2005; Nimmerjahn et al., 2005). Importantly, microglia have been reported to engulf synapses during postnatal development to refine neural circuitry (Tremblay et al., 2010) and shown to be important in neural circuit development and plasticity (Wake et al., 2013, for review). Intriguingly, one recent study proposed that astrocytes are also capable of engulfing synapses (Chung et al., 2013).

In addition to the cellular substrates, modern views incorporate the ECS and extracellular matrix (ECM) as part of neuropil. The ECS is modulated by neural activity and has a large impact on extracellular molecular diffusion including volume transmission of subcortical neuromodulators (Sykova and Nicholson, 2008; Vizi et al., 2010). For example, in conditions with increased neural activity, astrocytes can swell due to potassium uptake leading to narrowing of the ECS (Andrew and Macvicar, 1994; Holthoff and Witte, 1996, 2000; MacVicar et al., 2002).

The ECM is composed of collagens, proteoglycans and glycoproteins (reviewed in Dityatev and Schachner, 2003; Dityatev et al., 2010) and interacts with cell surface proteins. The ECM has been

recognized to be important for neural circuit development, for instance, acting as axon guidance cues (Dodd and Jessell, 1988) and known to mature with neural circuit development. Previously, maturation of the ECM was found to stabilize synaptic AMPA receptors by inhibiting lateral diffusion (Frischknecht et al., 2009). A net-like ECM structure, so called perineuronal net, is prominent around GABAergic interneurons (Celio et al., 1998). The maturation of the perineuronal net has been demonstrated to be one of the key determinants for ceasing the critical period of the visual cortex (Pizzorusso et al., 2002).

Anatomical Changes

As manifested in cortical volume changes, the neuropil of EE cortex and hippocampus undergo a major structural reorganization. Here, we review EE-induced microscopic changes in the neuropil by featuring works published mainly after 2000. We classify the anatomical changes into four not-mutually-exclusive categories: postsynaptic, presynaptic, glial, and extracellular changes. Enhancement of neurogenesis in the hippocampal dentate gyrus by EE (Kempermann et al., 1997) is also a significant modification of the neuronal circuit. Interested readers are referred to excellent review articles published elsewhere (for instance, van Praag et al., 2000; Nithianantharajah et al., 2004; Olson et al., 2006; Zhao et al., 2008).

Postsynaptic changes

A number of studies have been published on postsynaptic changes after EE owing to the conspicuous structure of dendritic spines, the main excitatory synapse type in the cerebral cortex and hippocampus. EE-induced spine density increases have been noted for a few decades in the cortex and hippocampus (e.g., Turner and Greenough, 1985; Moser et al., 1994). Although spine increase is found across species, including primates (Kozorovitskiy et al., 2005), there is considerable variability in the degree of increase across cortical studies, with some reporting no significant increases (e.g., Diamond et al., 1975). It is possible that the variability may come from subtle differences in housing conditions. Additionally, Kolb and colleagues found that dendritic proliferation and spine number depend on the branch type (i.e. apical *vs*. basal dendrites), age and gender (Kolb et al., 2003).

In a recent study by Jung and Herms (2014), chronic imaging of pyramidal cell morphology in mouse somatosensory cortex was performed to investigate if EE indeed increases spine density within a single animal. According to this work, chronic exposure to EE results in a 30% (basal dendrites of layer 2/3) to 40% (apical dendrites of layer 5) spine density increase on dendrites at three to four months of age, confirming previous results obtained by histological methods (Johansson and Belichenko, 2002; Leggio et al., 2005; Gelfo et al., 2009). An increase in spine density can also be induced by exposing mice to EE at adult ages but to a lesser degree (20 to 30%). In another chronic imaging study, Yang and colleagues (2009) have demonstrated that in addition to spine formation elevation, spine elimination is enhanced in the somatosensory cortex in a sensory-enriched standard-sized housing condition. The spine elimination enhancement occurs several days after the spine formation enhancement, hinting that enriched experience promotes a general increase in spine turnover. EE-induced spine increases also occurs in the mortor cortex. This is not surprising since exposure to EE leads unavoidably to increased motor exercise. Intriguingly, mice which experienced an EE with an emphasis on motor activity promotion exhibit a different topological pattern of spinogenesis from those which experienced repetitive motor skill training. While both have increased spinogenesis, newly generated spines in the latter tend be spatially clustered (Fu et al., 2012). Such clustered formation of spines could reflect multiple postsynaptic contacts to a common axon or postsynaptic contacts of functionally similar and spatially close axons, although these are not mutually exclusive. On the other hand, EEs feature multiplexed modalities, possibly making the axonal input to postsynaptic neurons randomized on the dendritic geometry. As the neurotrophic factors that lead to spino-/synaptogenesis (e.g., insuline-like growth factor (IGF-1) or brain-derived neurotrophic factor (BDNF), discussed later) are common to both motor exercise and EE, it is conceivable that axonal configuration and activity pattern are key factors for the

topology of spinogenesis.

In rodent hippocampus, some studies report dendritic spine density and/or tissue spine density increases after EE (Moser et al., 1994; Rampon et al., 2000b; Kondo et al., 2012; Malik and Chattarji, 2012) while another study reports only volumetric increases (Li et al., 2013). Indeed, similar to the cortex (Leggio et al., 2005; Gelfo et al., 2009), EE hippocampal principal cells are reported to have more intricate dendritic arborizations (Faherty et al., 2003). Remarkably, at least in CA1 stratum radiatum, the spine density change has been noted to depend on the hemispheric laterality (Shinohara et al., 2013), supposing that mixture of the left and right hippocampi would obscure EE induced effects. Moreover, spines of EE CA1 pyramidal cells have higher density of non-perforated synapses (Rampon et al., 2000b), which suggests that the new spines form smaller and possibly weaker synapses. These synapses could be overlooked when quantification is assessed with light microscopy of Golgi-stained samples, especially in mice which have smaller synapses to begin with. Interestingly, while NMDA receptors (NMDA-Rs) play a pivotal role in long-term potentiation (LTP) and associated spine changes in CA3-CA1 synapses, NMDA-R knockout (KO) mice exhibit similar spine increases after EE (Rampon et al., 2000b). This result raises the possibility that EE-induced spine increase is mediated by globally secreted factors which will be discussed later.

Spine size is considered to be a predictor of synaptic strength. Landers and colleagues (2011) performed comprehensive analysis of spine size in mouse barrel cortex by serial electron microscopy following ‘naturalistic’ EE. This study showed that excitatory synapses on spines and dendritic shafts were both increased after EE. Notably, the distribution of synapse size, as assessed by the PSD area, was similar to what was observed in control mice. We also find that the nature of distribution of PSD area does not substantially change for CA1 stratum radiatum spines (Shinohara et al., 2013). It is conceivable that there is a mechanism to renormalize the synaptic strength to keep the excitability of neurons to a certain level, as seen in CA1 neurons’ firing rates after exposure to novel environments (Hirase et al., 2001).

EE also increases inhibitory synapses. According to a recent study by Donato and colleagues (2013), GABAergic terminals on CA3 parvalbumin (PV) cell dendrites increased by more than 50% after three weeks of EE while the number of excitatory terminals remaind similar. The increase of GABAergic terminals on CA3 PV cells includes those from vasoactive intestinal peptide (VIP) positive interneurons (Donato et al., 2013). In the cortex, an increase of inhibitory synapses occurs preferrentially on spines, while inhibitory synapses on the dendritic shaft showed only marginal increases (Landers et al., 2011). Inhibitory axons that innervate spines are known to arise from various subclasses of interneurons (Kubota et al., 2007). Whether specific subclasses of interneurons (eg. PV *vs.* VIP) exhibit distinct innervation patterns by EE exposure remains to be addressed. It is noted that in cats, one study reported that EE results in cortical volume expansion without changing the density of the excitatory synapse, resulting in an increase of the total number of excitatory synapses. As for inhibitory synapses, the number of synapses per neuron remains similar while the synapse size is enlarged, resulting in possible increase of inhibition to counterbalance the enhanced excitatory input (Beaulieu and Colonnier, 1987, 1988).

Presynaptic changes

Presynaptic structural changes also occur after EE, however they are more sparsely characterized compared to the postsynaptic alternations. In a classical study by Sirevaag and Greenough (1987), they reported that EE rat cerebral cortex has 20% more synaptic boutons than rats reared in isolation. Using an enzyme-linked immunosorbent assay (ELISA), Nithianantharajah and colleagues (2004) found that EE for 30 days in young rats increased synaptophysin, a major synaptic vesicle glycoprotein, as well as the PSD protein PSD95 in multiple brain areas including the cortex, hippocampus, thalamus and hypothalamus, but not in the cerebellum. These results suggest that more functional synapses are formed after EE in these areas. Furthermore, it has been reported that the cortex of mature rats kept in EE contains a higher amount of synaptophysin than the age-matched control (Saito et al., 1994). This increase of synaptophysin was later shown to be accompanied by a concomitant increase of the packing density of synaptic vesicles, but not by an increase of

presynaptic boutons (Nakamura et al., 1999). An increase of synaptophysin in the frontoprietal cortex and hippocampus has been shown to occur in aged EE mice as well (Frick and Fernandez, 2003).

Recently, more detailed morphological analyses of presynaptic terminals were conducted by Caroni's group. The study investigated giant boutons formed by the mossy fibers of dentate granule cells onto CA3 pyramidal cells at different stages of EE exposure. They found that the mossy terminals become larger in volume and more intricate in morphology with following a month of EE. This morphological transformation was distinct from age-dependent volume increase of mossy terminals (Galimberti et al., 2006; Bednarek and Caroni, 2011). The morphological change of mossy terminals includes active zone rearrangement and postsynaptic structural changes that are dependent on the F-actin binding protein beta adducin (Bednarek and Caroni, 2011). Additionally, Wnt7 signaling from post-synaptic CA3 neurons has been shown to be necessary and sufficient for the increase of mossy fiber-CA3 synapse number (Gogolla et al., 2009). The beta adducin-dependent mechanism of synapse rearrangement has been also shown for CA1 pyramidal cell spines (Bednarek and Caroni, 2011). Possible distinct mechanisms of structural rearrangements after learning and exposure to EE are discussed in a recent Caroni's review (Caroni et al., 2012). According to their hypothesis, increased baseline levels of synapse turnover as a result of EE may augment the magnitude of learning-induced spine gains and losses, as the formation of stable synapses requires both pre- and postsynaptic mechanisms.

EE also enhances the expression of motor proteins necessary for axonal transport. Hirokawa's group reported that EE upregulates KIF1A, a kinesin superfamily motor protein expressed in axons (Kondo et al., 2012). Moreover, the brain-derived neurotrophic factor (BDNF) was shown to increase the KIF1A expression and KIF1A-mediated axonal transport. In the same study, KIF1A-mediated transport was shown to be critical for EE-induced learning enhancement and spine density increase. Although a stringent assessment of causality of BDNF and KIF1A remains to be determined, the study showed that BDNF increase starts in the first week of EE exposure, while KIF1A increases occur from the second week, supporting the idea that the BDNF increase is upstream of KIF1A-mediated changes (Kondo et al., 2012). Of note, KIF1A has been demonstrated to be the primary motor protein that transports BDNF-containing dense-core vesicles to presynaptic terminals (Lo et al., 2011). Therefore, it is conceivable that BDNF secretion is increased via the BDNF-KIF1 positive feedback loop (Kondo et al., 2012).

Glial changes

Early reports already pointed to increases in glia numbers (Diamond et al., 1966; but see Jones and Greenough, 1996; or Ehninger and Kempermann, 2003 for more recent assessments) and glial nucleus volume (Sirevaag and Greenough, 1987) in the cortex after EE. Glia proliferation has also been reported in the hippocampus (Steiner et al., 2004; Ziv et al., 2006). In both cortex and hippocampus, physical exercise (wheel running) seemed to have a higher influence on gliogenesis (Ehninger and Kempermann, 2003; Steiner et al., 2004). Electronmicroscopic observation showed that astrocytic processes have more contacts with synaptic structures in the visual cortex of EE rats (Jones and Greenough, 1996). This change may be related to LTP-like synaptic plasticity, in which similar morphological changes were described several hours after the induction (Wenzel et al., 1991). Remarkably, EE exposure for 7 days after ischemia enhances the proliferation of astrocytes and NG2-positive glial cells (Komitova et al., 2006). The NG2-positive cells were found to be immunoreactive for BDNF and hence possibly exert beneficial effects for recuperation and plasticity of poststroke brain damage.

Aside from the functions as support cells, astrocytes have been demonstrated to play an important role in inducing LTP-like synaptic plasticity via elevation of the intracellular calcium level *in vitro* (Henneberger et al., 2010) and *in vivo* (Takata et al., 2011). As calcium-dependent gliotransmission has been supposed to enhance the plasticity and astrocytic contact to neurons is recognized to promote synaptogenesis (Hama et al., 2004) and maturation of dendritic spines (Haber et al., 2006; Nishida and Okabe, 2007), astrocytic microprocess positioning relative to a synapse is likely to be an

important factor to determine the susceptibility of synaptic plasticity (i.e., metaplasticity). Remarkably, a line of evidence suggests that astrocyte calcium signaling is also involved in microprocess morphology in the hippocampus (Tanaka et al., 2013) and cerebellum (Iino et al., 2001; Saab et al., 2012), namely in both brain areas compromised calcium signaling results in retraction of perisynaptic astrocytic microprocesses. Interestingly, the astrocytic gap junction proteins connexin 30 has been shown recently to modulate perisynaptic astrocytic microprocess morphology (Pannasch et al., 2014). Connexin 30 was shown to be elevated after 2 weeks of EE (Rampon et al., 2000a) and positively regulated by neural activity (Roux et al., 2011). Identification of the molecular cascades that bridges astrocytic calcium signaling, connexin 30, and microprocess dynamics could provide a major mechanism for astrocytic involvement of experience-dependent plasticity.

At the cellular scale, EE hippocampal astrocytes have higher levels of glial acidic fibrillary protein (GFAP), the main intermediate filament protein of astrocytes (Viola et al., 2009; Sampedro-Piquero et al., 2014). Moreover, EE hippocampal astrocytes show more ramified and well-defined GFAP patterns. While GFAP patterns do not strictly define the morphology of astrocytes (e.g., Mishima and Hirase, 2010), such enhancement of GFAP patterns could be indicative of elevated trafficking of molecules for neuron-glia interactions. Accordingly, it is not clear if the tiling layout or the domain of astrocytes overlaps changes after EE. A recent report shows that diffusion tensor magnetic resonance imaging (MRI) can detect structural changes in the dorsal dentate gyrus after two hours of a spatial memory task in rats. Intriguingly, GFAP pattern changes were observed in the same area along with enhanced immunolabeling of synapsin and BDNF (Sagi et al., 2012). As dentate granule cell axons form a well-defined projection pathway to CA3, morphological changes, especially the volume change, of astrocytes may have a large impact in the ECS diffusion anisotropy. Molecular genetic studies that assess the causal relationship between EE-induced GFAP and astrocytic morphology changes are expected.

Promotion of myelination by EE has also been recognized for several decades (Szeligo and Leblond, 1977; Sirevaag and Greenough, 1987). Recently, two studies showed that rearing environment during early post-weaning period has influence on myelination in the prefrontal cortex and the change persists in the adulthood (Liu et al., 2012; Makinodan et al., 2012). In the hippocampus of middle aged EE rats, increases of length and volume of myelinated fibers were reported by Qiu and colleagues (2012). These experiments suggest that rearing environment influences neural circuitry by modulating axon conductance fidelity and velocity.

Extracellular space and extracellular matrix changes

Literature on the impact of EE on brain ECS and ECM is rather limited at this time point. A very recent report shows that the activity of matrix metalloproteinase-9, a key enzyme that converts proBDNF to BDNF in the ECS, is elevated by ~50% after seven weeks of EE after weaning (Cao et al., 2014) in rat hippocampus. As far as experience-dependent physical ECS change is concerned, diffusion measurements by real-time iontophoresis in aged rats revealed that superior learners of the Morris water maze have larger ECS volume fractions than inferior learners in the hippocampus (Syková et al., 2002). Similar measurements on EE animals would be interesting.

In monocularly deprived rats, the recovery of vision in EE leads to reorganization of the visual cortical circuit by concomitant reduction of GABAergic inhibition and perineuronal nets (Sale et al., 2007). Although the causal relationship of these changes has not been fully resolved, one study shows that mice with reduced GABA (i.e. GAD65-KO) have compromised ocular dominance plasticity and diminished activity of tissue-type plasminogen activator (tPA), a serine protease that controls a cascade of extracellular proteolytic activities after monocular deprivation (MD) (Mataga et al., 2002). Indeed, it has been shown that removal of ECM by the bacterial enzyme chondroitinase ABC reactivates cortical plasticity (Pizzorusso et al., 2002) and chondroitinase ABC treatment promotes hippocampal and cortical spine motility in organotypic slices (Orlando et al., 2012) or *in vivo* (de Vivo et al., 2013), supporting the idea that maturation of ECM makes the neural circuit less

plastic. Importantly, hippocampal tPA activity was shown to be elevated after EE in mice (Horii-Hayashi et al., 2011; Obiang et al., 2011) and a subsequence increase of BDNF was observed (Obiang et al., 2011; but also see Cao et al., 2014). While these results suggests a permissive role of extracellular proteases for synaptic remodeling, ECM molecules such as reelin, tenascin C, and hyaluronic acid promote LTP (Kochlamazashvili et al., 2010). The dual role of the ECM (Dityatev et al., 2010) will need to be further examined in the context of EE.

Electrophysiological changes

Quite obviously, EE-induced morphological changes of neuropil imply concomitant physiological changes. As EE-reared animals show enhanced learning and cognition, synaptic plasticity and cellular response to stimuli have been investigated by a number of research groups. Synaptic transmission and plasticity after EE have been studied with acute cortical or hippocampal slices. Functional changes of neural circuitry are commonly addressed with LTP type synaptic plasticity in acutely prepared slices, a synaptic model implied in memory formation and learning (Rioult-Pedotti, 2000; Pastalkova et al., 2006; Whitlock et al., 2006).

Neocortex

EE-induced physiological changes have been reported in multiple regions of the sensory cortex. EE during young adult period enhances the sound-evoked field potential response in the primary auditory cortex and the neurons become more responsive to the volume and more selective for the tone (Engineer et al., 2004). Recordings from EE auditory cortex slices show an elevation of glutamatergic transmission in layer 2/3 without significant changes in GABAergic transmission (Nichols et al., 2007). Notably, the field response change is reversible by later exposure to standard environment. Likewise, standard environment rats that are exposed to EE in later adulthood had comparable enhancements of the auditory response (Engineer et al., 2004). These changes occur in the time course of less than two weeks. EE enhances paired pulse depression in the primary auditory cortex, hinting to an increase in presynaptic release probability (Percaccio et al., 2005). Interestingly, the response enhancement is greater in the posterior auditory field, an auditory association area contingent to the primary auditory cortex (Jakkamsetti et al., 2012). Enrichment of the auditory experience, but not of social experience or increased exercise, is demonstrated to be the primary factor of the neural response enhancement in the primary auditory cortex (Percaccio et al., 2007). In the same study, it was also suggested that cholinergic input may not play a crucial role as a similar degree of enhancement was seen in animals with partial immunotoxic ablation of cholinergic neurons. However, it remains possible that residual cholinergic projection was sufficient to coordinate the EE-induced plasticity. Another possibility is that the effect of other subcortical neuromodulators may have elevated to compensate the reduction of cholinergic modulation.

The somatosensory cortex undergoes analogous EE-induced changes to the auditory cortex. Coq and Xerri (1998) reported that receptive fields become more sharply tuned and the cortical area representing somatosensation expands in young adult EE rats. In rat barrel area, the action potential discharge response of layer 2/3 neurons, but not of layer 4, become more selective to the principal whisker (Polley et al., 2004). In a similar recent study, the authors report that the response become enhanced in layer 2, 3, 4, and 5 neurons (Alwis and Rajan, 2013), although the enhanced response is most notable in layer 2 and 3. According to this work, the latency to the peak firing rate remains unaltered, suggesting that the locus of change is within the cortex (Alwis and Rajan, 2013). In mice, a brief rhythmic (10 minute at 8Hz) stimulation of whiskers elevates the whisker-evoked response in the barrel cortex in layers 2/3 and 4. Mégevand and colleagues (2009) showed that the whisker-evoked response is similarly increased after three weeks of EE. Moreover, they found that the brief rhythmic stimulation does not enhance the whisker-evoked response in EE mice, suggesting that neuropil modifications occurred in the granule and supragranular layers to occlude the whisker-evoked plasticity.

Effects of EE on precocious visual development and recovery from amblyopia have been

investigated by the group of Maffei and comprehensive reviews have been published (for instance, Sale et al., 2009, 2014). The followings focus on EE-induced electrophysiological changes in adult visual cortex. MD in adulthood (i.e. after the critical period) does not make a shift in ocular dominance in the binocular zone of the visual cortex. Sale and colleagues reported that MD during EE exposure in adulthood can induce ocular dominance plasticity (Sale et al., 2007). Baroncelli and colleagues (2010) reported that cortical increase of serotonin is a key factor for this EE-induced plasticity, which is accompanied by a reduction of GABAergic inhibition, an increase of BDNF, and enhanced white-matter induced LTP and LTD (Baroncelli et al., 2010). These results are in good agreement with an earlier report from the same group that an selective serotonin reuptake inhibitor (SSRI) fluoxetine reinstates cortical plasticity in adults (Maya Vetencourt et al., 2008). EE exposure in adults (postnatal 90 days) for fifteen days results in enhanced thalamocortical transmission and LTP (Mainardi et al., 2010).

Hippocampus

The basal transmission of CA3-CA1 synapses (Schaffer collaterals) have been reported to increase in one study (Foster and Dumas, 2001), while other studies reported little change (Duffy et al., 2001; Artola et al., 2006; Li et al., 2006) or mixed results (Irvine and Abraham, 2005). This variability could be due to age, gender, and strain/species differences. There is, however, a general agreement that EE enhances LTP at the Schaffer collaterals (Duffy et al., 2001; Artola et al., 2006; Huang et al., 2006; Li et al., 2006, 2013; Buschler and Manahan-Vaughan, 2012; Malik and Chattarji, 2012). Recent whole cell patch clamp studies demonstrated that the frequency of miniature EPSCs is elevated in EE CA1 pyramidal cells while the amplitude is unchanged (Malik and Chattarji, 2012; Li et al., 2013). Together with the previous sharp electrode experiment which showed that unitary EPSPs do not change significantly in EE rats (Foster and Dumas, 2001), these results are in line with the anatomical finding that the number of spines increases whereas the spine size distribution change is modest, if any. Moreover, there was little change in the miniature EPSC amplitude or presynaptic release probability in EE hippocampal slices, suggesting that excitatory synapses increased in number rather than strengthening of individual synapses (Malik and Chattarji, 2012, but see Artola et al., 2006). One study reported that EE rat CA1 additionally has enhanced long-term depression, proposing that the range of population level synaptic efficacy becomes larger (Artola et al., 2006). On the other hand, the basal synaptic transmission of the perforant path (entorhinal cortex - dentate gyrus synapse) is increased in EE animals (Green and Greenough, 1986; Foster et al., 1996), although LTP induction is unaltered (Feng et al., 2001; Irvine et al., 2006), if not occluded (Foster et al., 1996). Of note, one study reported that prolonged long-term (more than three months) exposure to either EE or impoverished condition has little influence on basal synaptic transmission or LTP (Eckert et al., 2010). This result supports the notion that EE has a precocious effect of brain maturation (Cancedda et al., 2004) and by the end of the prolonged environmental exposure, non-EE animals catch up in terms of brain maturity.

In addition to synaptic changes, the excitability of neurons changes by EE. A short exposure (2-7 days) to EE of young adult rats leads to increased propagation of dendritic sodium spikes in a subset of CA1 pyramidal cell dendrites (Makara et al., 2009). Barium sensitive potassium current is shown to be involved in this plasticity. Moreover, CA1 pyramidal cells of EE rats become more excitable in response to current injection due to a lower spike threshold (Malik and Chattarji, 2012). EE CA1 pyramidal cells exhibit a reduced after-hyperpolarization potential AHP (Kumar and Foster, 2007; Malik and Chattarji, 2012). Incidentally, a similar reduced AHP is observed in CA1 pyramidal cells in animals after recent acquisition of learning (Coulter et al., 1989; Moyer Jr. et al., 1996). As AHP is mediated by calcium-activated potassium conductance (e.g., SK channels), a potassium channel reorganization conceivably underlie the altered excitability of EE neurons.

Network dynamics

How are neuropil changes reflected in population dynamics of neural activity? Compared with the rich amount of the research on LTP, this question has just begun to be addressed. One study has

reported that the local field potential (LFP) coupling of the primary visual cortex and secondary motor cortex, which are synaptically connected, are reduced after EE. (Di Garbo et al., 2011). On the other hand, EE strengthens the LFP cross-correlation coupling between the visual cortex and auditory cortex in a frequency and age dependent manner (Mainardi et al., 2014). The reason for different outcomes of the two studies is yet to be elucidated. One possible interpretation is that the connection between the motor and sensory areas could be sparser than that between the two sensory areas, hence the enhancement of functional connectivity was more difficult to be detected in the former. Moreover, as cortical and hippocampal neuronal discharge activities are modulated with gamma oscillations during cognitive processing (Engel et al., 2001; Buzsáki et al., 2012), investigations of LFP at this frequency band might have unveiled more information. For instance, the gamma oscillation power increases during theta states in EE rats (Shinohara et al., 2013). In the same study, we found that the interhemispheric coherence of gamma oscillations increases in EE rats. Activation of NMDAR is thought to be involved in this enhancement of gamma oscillations as chronic administration of ketamine blocks the phenomenon.

Molecular/epi-genetic changes

Neuromodulators

Subcortical neuromodulators exert significant influences on animals' mood and behavior. The receptors for subcortical neuromodulators are predominantly G-protein coupled receptors (GPCRs) and have complex intracellular signal transduction in neurons and glia. Many GPCRs have been demonstrated to be involved in synaptic plasticity and learning. As EE animals become more tolerant to stress and demonstrate enhanced learning abilities, subcortical neuromodulator systems are likely to be a subject of modification. Despite such expectations, published results on various neuromodulator systems are mixed, perhaps due to technical difficulties to accurately determine the *in situ* concentration. We summarize EE-induced neuromodulator changes below.

The activity of choline acetyltransferase (ChAT), an essential enzyme to synthesize acetylcholine, is found to be elevated by maze training in EE rat hippocampus, anterior cortex and the caudate (Park et al., 1992). On the other hand, a study by Del Arco and colleagues does not detect a significant difference in acetylcholine level in the prefrontal cortex (Del Arco et al., 2007b). A recent rat study reported that prolonged exposure to EE (twenty months) results in more numerous cholinergic cells in the nucleus basalis of Meynert and medial septum which sends cholinergic projections to the cortex and hippocampus, respectively (Harati et al., 2013). However, an earlier study by an independent group reports an apparent decrease of the basal acetylcholine level in the prefrontal cortex after twenty four months of EE in rats (Segovia et al., 2008b).

Naka and colleagues reported that the basal noradrenaline (norepinephrine) level increases by ~30% in the Parieto-temporo-occipital cortex by EE, while a smaller increases in the frontal cortex and hippocampus did not reach statistical significance (Naka et al., 2002). Indeed, a recent study shows that there is a marked increase of the beta-2 adrenergic receptor in the hippocampus of EE mice (Li et al., 2013). In rat prefrontal cortex, no significant changes of noradrenaline were reported among EE, standard, and isolated rearing conditions, while there is a marked decrease in the ventral striatum in the isolated rearing group (Brenes et al., 2008).

In rat visual cortex, EE in adulthood increases the visual cortical serotonin level, which is upstream of BDNF increase and GABA reduction (Baroncelli et al., 2010). Similarly, a significant increase in the serotonin is observed in EE rat prefrontal cortex (Brenes et al., 2008). In mice, however, no changes were observed in the serotonin level after EE in the cerebral cortex or hippocampus (Naka et al., 2002). Serotonin receptor type 1A mRNA increases in the EE rat hippocampus whereas serotonin receptor type 2A or 2C does not show significant changes (Rasmuson et al., 1998).

While many subcortical neuromodulators are strengthened, published studies suggest that the basal dopamine level is relatively inert to EE in the prefrontal cortex, striatum, nucleus accumbens

(Naka et al., 2002; Zhu et al., 2004; Segovia et al., 2008a) or even decreases in these areas (Bowling et al., 1993; but also see Segovia et al., 2010). Working memory tasks are not affected by EE (Segovia et al., 2008a). However, under an acute mild stress condition, EE rats have lower increase of the dopamine level in the prefrontal cortex (Segovia et al., 2008a). D1 expression is attenuated in EE prefrontal cortex (Del Arco et al., 2007a). Another study shows that D1 or D2 expression levels remains similar in standard housing and EE conditions (Li et al., 2013). Mesolimbic dopamine transporter system is reported to be attenuated by EE (Bezard et al., 2003) in mice, however, a rat study suggests that the surface expression of dopamine transporter decreases in the prefrontal cortex, but not in the striatum or nucleus accumbens (Zhu et al., 2005).

Growth factors

Growth factors are indispensable for neurogenesis, neural circuit development, and synaptic plasticity (Segal, 2003; Zhao et al., 2008). As EE promote neurogenesis and learning, its influence on growth factors, including nerve growth factor (NGF), BDNF, and insulin-like growth factor (IGF), has been a subject of high interest. Accordingly, NGF, BDNF, and neurotrophin-3 (NT-3) transcripts are reported to increase in rat cortex and hippocampus after EE (Falkenberg et al., 1992; Torasdotter et al., 1996, 1998). At the protein level, NGF and its receptors were first identified to increase by EE in the rat cortex and hippocampus (Mohammed et al., 1993; Pham et al., 1999a, 1999b). A later study by Ickes and colleagues (2000) reported that one year of EE resulted in increasing NGF and BDNF levels in the cerebral cortex, hippocampus, basal forebrain and hindbrain of the rat. The same study also reported that NT-3 level increases in the cerebral cortex and basal forebrain. In each case, the neurotrophin level, which was detected by ELISA on respective tissue, showed several fold increase (Ickes et al., 2000). EE for four weeks in young adulthood restores the number of BDNF expressing cells in the auditory cortex of rats which were exposed to noise during juvenility (Zhu et al., 2014). Additionally, EE also enhances the transcript and protein of glial cell-derived neurotrophic factor (GDNF), which promotes the survival of neurons (Young et al., 1999).

IGF-1 is another growth factor that influences neurogenesis and cell proliferation. EE has been demonstrated to transiently increase the number of IGF-1 positive neurons and promotes maturation of perineuronal nets during the critical period in the visual cortex (Ciucci et al., 2007). Moreover, chronic infusion of IGF-1 to the visual cortex mimics the precocious development (Ciucci et al., 2007). EE exposure up to postnatal day 12 (i.e., before eye-opening) upregulates the IGF-1 receptor, but not glucocorticoid receptors or BDNF at the adult stage (postnatal day 60), whereas EE exposure up to postnatal day 60 increases IGF-1R, glucocorticoid receptors, and BDNF (Baldini et al., 2013). This result suggests that BDNF increase may be related to visual and other sensory modalities that integrate to cognition of external environments. The increase of IGF-1 by early exposure to EE has been shown to be the key step to modulate the anxiety level (Baldini et al., 2013). The source of increased IGF-1 could be extracerebral. Serum IGF-1 has been demonstrated to be taken up by neurons across wide spread brain areas after one hour of treadmill running (Carro et al., 2000). Moreover, the same study demonstrated that intracarotid injection of IGF-1 results in increases in BDNF transcripts, similar to the increase observed by exercise. IGF-1 entry to the brain is the key trigger for BDNF transcription, as systemic blockade of IGF-1 by antibody abolishes BDNF increase by exercise (Ding et al., 2006). A later study shows that the blood-brain permeability for IGF-1 is increased in areas with elevated neural activity (Nishijima et al., 2010). These lines of experiments elucidate the finding that EE or tactile stimulation by massaging during infantile leads to precocious effects in cortical development (Cancedda et al., 2004; Guzzetta et al., 2009), as motor activity in infants triggers synchronized neural activities in the brain (Khazipov et al., 2004).

As described above and elsewhere, voluntary exercise also enhances animal's learning ability and neurogenesis (van Praag et al., 1999a, 1999b). As enriched rearing environments typically contain running wheels, voluntary exercise is a significant component of EE. Indeed several studies have reported that both BDNF (Berchtold et al., 2001) and IGF-1 (Carro et al., 2000; Trejo et al., 2001) protein levels are increased in the brain after voluntary exercise. Although EE and voluntary exercise are considered to be similar in this regard, NT-3 levels are differentially affected in that exercise decreases hippocampal NT level (Johnson et al., 2003). Glia proliferation has been markedly

elevated by voluntary exercise (Ehninger and Kempermann, 2003; Steiner et al., 2004). Such difference may contribute differential patterns of neuronal proliferation seen in pure voluntary exercise and EE (Olson et al., 2006).

Synaptic molecules

As EE increases spines, synaptic molecules also undergo changes in expression. For instance, Tsien's group has shown that two weeks of EE increases the expression of GluR1 AMPA receptor subunit and NR2B NMDA-R subunit, while the NR1 NMDA-R subunit expression remains unchanged using mouse forebrain tissue homogenate samples that included the cortex, hippocampus and amygdala. A recent paper demonstrated that EE increases the protein expression of NR2A and phosphorylated GluR1 in hippocampal synaptosomes, while NR2B and GluR1 remain unchanged (Li et al., 2013). This discrepancy may come from tissue differences in that various cortical and limbic areas cannot be generalized, or there is a differential modulation of synaptic and extrasynaptic receptors and synaptosome preparation more precisely addresses the synaptic receptor changes.

In mouse visual cortex, EE tended to increase the expression of GluR1 in tissue homogenate (Restivo et al., 2005). Rats which were exposed to noise environment during juvenility and later exposed to EE for four weeks (noise-EE) express more NR2A and NR2B than noise-standard or naive rats in auditory cortical tissue homogenate (Zhu et al., 2014). As for presynaptic molecules, two weeks of EE upregulates intracortical and thalamocortical vesicular glutamate transporters (VGlut1 and VGlut2, respectively) and downregulates vesicular GABA transporters (vGAT) in adult rats (Mainardi et al., 2010). Synaptophysin is increased in EE mouse cortex and hippocampus (Nithianantharajah et al., 2004; Lambert et al., 2005; Kondo et al., 2012). While these changes are likely to be associated with the increased number of synapses by EE, biochemical assays cannot determine if such changes also take place within a single synapse. An electron or super-resolution microscopic observations (for example, electronmicroscopy on freeze-fracture replica labeled synapses (Masugi-Tokita et al., 2007)) is necessary for the evaluation of synapse-level molecular changes.

Multiple biochemical pathways are suggested for EE-induced synaptic changes. While they are not mutually exclusive, the relative prevalence remains to be clarified. For instance, EE upregulates cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB) and protein kinase C (PKC) (Paylor et al., 1992; Ickes et al., 2000; Williams et al., 2001; Mohammed et al., 2002). Additionally, cAMP-dependent protein kinase (PKA) has been shown to be critical for EE-induced LTP enhancement (Duffy et al., 2001). Neurogranin, a substrate of PKC that triggers alpha-Ca^{2+}/calmodulin-dependent protein kinase II (αCaMKII) and CREB pathways, has been shown to be upreglated by EE and deficiency of neurogranin abolishes LTP and spatial learning (Huang et al., 2006). As EE elevates αCaMKII and CREB protein amounts similarly in both WT and NG-KO mice, the permissive role of neurogranin in EE-enhanced plasticity and learning is demonstrated (Huang et al., 2006). Li and colleagues (2006) have shown that EE exposure during adolescence enhances LTP in CA1 by promoting a pathway involving cAMP and the mitogen-activated protein (MAP) kinase p38, however, the same paper shows that this pathway is not critical in LTP enhancement of adult EE.

Epigenetic modifications

As EE transforms structural and molecular organization of neuropil, epigenetic changes in neurons and glia are inevitably supposed to be occurring. Transcriptome analysis of mouse cerebral cortex shows that along with transcripts related to synaptic functions, several hours of EE enhances the transcription of DNA methyl-transferase by ten fold (Rampon et al., 2000a), which may reflect epigenetic modification. In fact, EE induces hippocampal and cortical acetylation and methylation of histones 3 and 4, indicating reorganization of the chromatin structure (Fischer et al., 2007). Moreover, administration of histone deacetylase inhibitors mimicked EE-induced enhanced learning (Fischer et al., 2007). Importantly, epigenetic modification is manifested in BDNF promoter regions after EE (Kuzumaki et al., 2011). Another work shows that IGF-1, a growth factor known to be elevated by EE, induces histone 3 and 4 acetylation in the mouse visual cortex, mimicking a similar

histone acetylation by EE (Wang et al., 2013). The molecular mechanism that bridges IGF-1 reception to the epigenetic changes of chromatin structure has not been identified to date.

Recently, the rearing environment has been demonstrated to enhance prefrontal cortical myelination by the interaction of epidermal growth factor receptor 3 and its ligand neuroregulin-1 (Makinodan et al., 2012). Although the downstream targets of this signaling cascade are yet to be identified, another group has reported immature chromatin structure in oligodendrocytes of animals raised in an isolated condition (Liu et al., 2012). Enlarged neuronal and glial nuclei reported in EE animals (Sirevaag and Greenough, 1987) possibly reflect epigenetic changes that takes place in the chromatin structure.

Post-transcription interference by microRNAs (miRNAs) is another epigenetic mechanism and has been implied in neurogenesis and synaptic plasticity (Fiore et al., 2008). However, several representative miRNAs influential for neurogenesis and synaptic plasticity including miR9, miR124a, and miR132 did not significantly change their expression levels following EE (Kuzumaki et al., 2011). Profiling studies using next-generation sequencers to identify EE-modulated miRNAs and their targets will be a powerful approach to examine epigenetics of experience-dependent neural circuit transformation.

Future perspectives

Here we reviewed anatomical and molecular changes of cortical and hippocampal neuropil induced by enriched rearing environment. As a substantial amount of research has been carried out by different groups, there is variation in both the EE protocol use and the species of animals examined. Despite the variability, there is a general consensus that EE animals have improved learning and memory ability with concomitant increase in neuropil complexity. Moreover, EE-induced maturation of neuropil provides a biochemical condition that favors LTP-type plasticity. Considering that the integrity of the neuropil is thought to be critical in maintaining healthy mental capacity (Selemon and Goldman-Rakic, 1999), and supposing that neuropil complexity of a brain region represents the computational capability of the neural circuit, it is conceivable that overall neuropil complexity predicts the intelligence of a being. As EE is a way to improve animals' learning ability that can be translated to humans, the field deserves further investigations. There are at least several open questions.

First, although many experiments demonstrate that cognitive enhancement occurs after EE, hardly anything is known about how neural code changes after EE **(Figure 2)**. Cortical and hippocampal neurons are supposed to fire synchronously in groups that code perceptually related entity. Such temporal binding of different sensory modalities and higher-order representations has been hypothesized to realize perception. Ample evidence from rodent and primate experiments suggests that neural activity is coordinated with gamma oscillations for perception, cognition, and conscious action. In the hippocampus of anesthetized EE rats, we find that gamma oscillations enhance during theta states which are thought to be analogous to awake brain states (Shinohara et al., 2013). While this is an interesting finding, experimental results from unanesthetized animals await for further confirmation and significance. Other studies covered in the section of network dynamics also addressed functional coupling of neural activity in different cortical areas. Despite these recent studies, enhancement in functional coupling of neural activity should ultimately be addressed by single-unit activity. For example, while PV-positive GABAergic interneurons plays a pivotal role in gamma oscillations (Cardin et al., 2009; Sohal et al., 2009) and EE induces gamma oscillation enhancement, EE attenuates PV-positive neuron activity (Donato et al., 2013). Chronic recording of individual neuron with subtype identification may shed light on how enhanced gamma synchrony is achieved by EE.

Second, the physiological and biochemical involvement of glial cells remains to be addressed. While existent studies have documented structural reorganization of astrocytes and oligodendrocytes, the degree to which these changes affect neuronal network operation remains to be elucidated. As

glial cells are in general electrophysiologically passive, the neuron-glia signaling could take form of GPCR signaling. Recent studies have shown that calcium signaling in astrocytes promote synaptic plasticity of glutamatergic signals (Rusakov et al., 2014, for a recent review). As astrocytes express GPCRs for subcortical neurotransmitter and subcortical neuromodulation is strengthened during EE, enhanced plasticity is conceivably in part aided by astrocytes (Hirase et al., 2014). Moreover, if astrocytic microprocesses actively modulate synaptic plasticity, cell surface molecules interacting between microprocesses and synapses may play an important role for structural rearrangement. Supportive experimental results for synaptic-activity-driven astrocytic microprocess motility and the role of astrocytic GPCR activation in stabilization of spines has been reported recently (Bernardinelli et al., 2014). Other possible glial mechanisms include microglial involvement of synapse turnover and improved action potential conduction fidelity by oligodendrocytes. Furthermore, T-lymphocytes have been shown to interact with microglia and boosts BDNF in EE dentate gyrus (Ziv et al., 2006). Future studies should address the time course and causality of neuron-glia interactions induced by EE. Molecular manipulation and chronic *in vivo* imaging of non-neural cells are likely to be pivotal to uncover the role of these quiescent cells in neuropil.

Next, we have very limited knowledge about the generality of neuropil changes in different cortical regions or layers, not to mention other parts of the brain. We reviewed that the dentate gyrus and CA1 have different metaplasticity for LTP after EE. Similarly, literature suggests that sensory cortical layer 2/3 becomes more plastic than layer 4 or 5 and that association areas are more plastic than primary sensory cortex. Despite this inhomogeneous expression of metaplasticity, there does not seem to be an attempt to relate the metaplasticity to neuropil changes. One intriguing possibility is that stress-induced steroid hormones, concordantly with subcortical neurotransmitters, play important roles in remodeling neuropil. For instance, chronic stress results in reduction of dendritic complexity in the hippocampus with a concomitant enhancement of dendritic complexity in the amygdala (Vyas et al., 2002). Indeed, an SSRI anti-depressant fluoxetine has been demonstrated to express similar metaplasticity as EE in the visual cortex (Maya Vetencourt et al., 2008). Considering the well-known negative correlation between corticosteroid level and serotonin receptor gene expression in the hippocampus (López et al., 1998), stress-induced hormones conceivably modulate neuromodulator receptors to affect metaplasticity. Regional differences of metaplasticity and neuropil changes could be a reflection of variability in neuromodulator innervation and corticosterone receptor expression. For instance, stress induces dendritic remodeling and spine increases in the basolateral amygdala (Vyas et al., 2002; Mitra et al., 2005), where the hippocampus would show atrophic effects. As we are at an entrance to the era of “big data”, neuroinformatic approaches to identify best-correlated set of gene expression (or molecular) changes and neuropil transformation may suggest a next step towards a unified mechanism of environmentally induced neuropil remodeling.

Another unresolved question is how EE-induced structural changes are related to functional alternation of the neural circuit. Studies on cortical spine turnover by EE report that the morphological changes are discernible within a few days (Yang et al., 2009; Fu et al., 2012; Jung and Herms, 2014). On the other hand, hippocampal gamma oscillations are enhanced at least a few weeks after EE exposure (Shinohara et al., 2013), which incidentally is similar to the time course of hippocampal neurogenesis (Kee et al., 2007; Tashiro et al., 2007). These scattered pieces of results could favorably be interpreted as structural plasticity precedes circuit function maturation; however, more rigorous assessments are necessary.

Finally, functional lateralization of the cerebral cortex is distinct for higher order functions particularly in humans (Toga and Thompson, 2003) and may be correlated to general intelligence. Human MRI studies show that experience induces structural asymmetry in the hippocampus (Maguire et al., 2000; Boyke et al., 2008). EE or infantile tactile stimulation influence on brain asymmetry has been noticed half a decade ago in experimental animals (Diamond et al., 1964; Denenberg et al., 1978). In mice, hippocampal CA3-CA1 ipsi- and contralateral connections are asymmetrically arranged in terms of postsynaptic glutamate receptor subtype distribution (Shinohara and Hirase, 2009, for a review). While a genetic component is partially responsible for the

hippocampal asymmetry (Kawakami et al., 2008), our recent study demonstrate that EE during juvenility and young adulthood significantly affects functional and anatomical interhemispheric asymmetry in rats. The cellular and molecular mechanism that leads to the experience-induced hippocampal asymmetry is largely unknown, except that NMDARs are likely to be involved (Shinohara et al., 2013). While very little is known about experience-dependent development of functional asymmetry in rodent cerebral cortex, experience-dependent functional lateralization was reported in the anterior cingulate cortex (ACC) for an observational fear learning task (Kim et al., 2012). As the ACC neurons have been reported to induce lateralized morphological changes by environmental stimuli (Perez-Cruz et al., 2009), it may display comparable hemisphere-biased changes by EE. Future investigation is expected to identify how EE and neuropil transformation contribute to functional specialization and asymmetry of the brain.

Acknowledgements

The authors wish to thank Drs. Youichi Iwai, Thomas McHugh, Kazuhito Nakao, Susumu Takahashi, and Mika Tanaka for comments and suggestions on earlier versions of this manuscript. This work was supported by the RIKEN Brain Science Institute and JSPS KAKENHI grant numbers 26117520, 26282222, 23115522, and 23590273.

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Figure 1

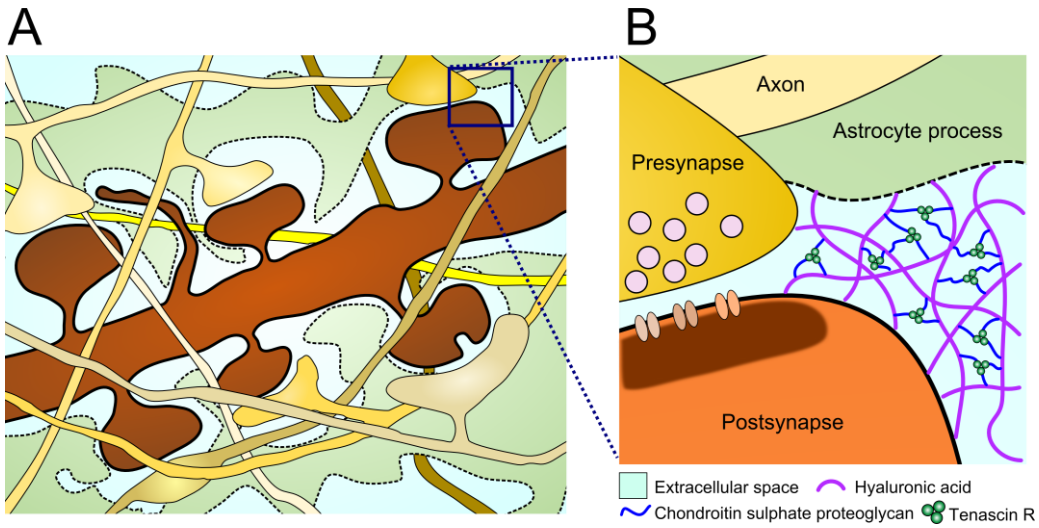

Figure 1: A simplified representation of neuropil. (A) The main cellular constituents of neuropil are dendritic segments (brown object with thick outline - a dendritic branch with spines and a filopodia), axons (yellowish structures with thin outline), and surrounding astrocytic microprocesses (green structures with dashed outline). (B) In addition, the extracellular space (ECM, cyan background) and extracellular matrix, which consists of a meshed network organizations consisting of hyaluronic acid, chondroitin sulfate proteoglycan, tenascin R, along with collagen and other glycoproteins such as laminin and fibronectin (not shown in this diagram).

Figure 2

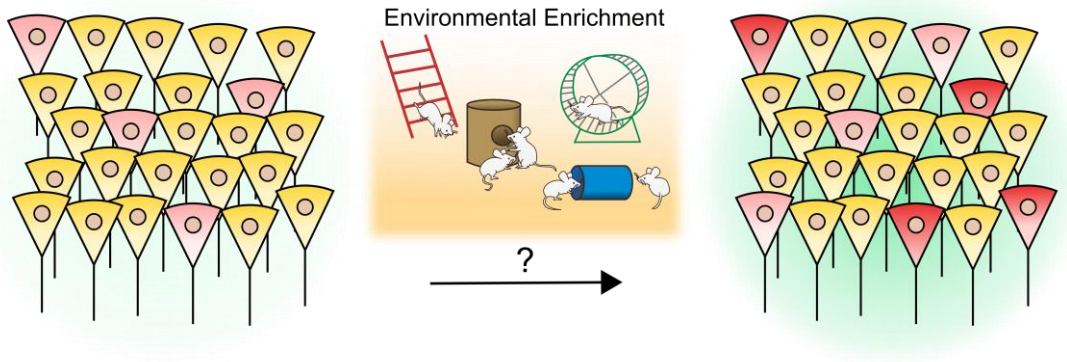

Figure 2: Reorganization of neural code after EE is virtually unknown. LFP alternations (e.g., gamma oscillation enhancement) predict that EE reorganizes neural activity. Such a change could be realized by (1) an increase in the number of active (pink and red cells) cells, (2) elevated discharge activity of neurons (red cells); (3) alternations in diffusible molecules including volume-transmitted neuromodulators, neurotrophic factors and steroids (represented as ambient green background); (4) temporal synchrony enhancement of the active cells by strengthened excitatory connections or rhythmic inhibitory interneuronal activity (not illustrated); (4) glial changes (not illustrated); and more.