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The various forms of neuroplasticity: Biological bases of learning and teaching

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Abstract Education is a socially structured form of learning. It involves the brains of different players – students, teachers, family members, and others – in permanent interaction. The biological set of mechanisms by which these brains receive, encode, store, and retrieve mutually exchanged information is called "neuroplasticity". This is the ability that enables developing and adult brains to react and adapt at different coexisting levels - from molecules to neurons, circuits, networks, persons, and societies. This article aims to discuss the major current concepts of neuroplasticity research to help policymakers, researchers, and educators bridge them to learning and teaching models and practices.

Keywords Synaptic plasticity · Developmental plasticity · Neuroeducation · Synaptogenesis · Long-distance plasticity · Circuit plasticity · Network plasticity

About learning, education, and the brain

Any time an animal interacts with the environment, some trace of this interaction remains stored in its brain for at least a brief period of time. The nature of the interaction and its impact on the animal's life will determine its significance. The significance for the animal, on the other hand, will regulate the duration of the trace maintenance within the brain (memory) and its eventual use for the benefit of the animal (retrieval to guide behavior). Memory, therefore, is the ability to encode, store, and retrieve information, while only the storage process is called "learning" (Klingberg 2013). Storage mechanisms and abilities

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are crucial for the survival of many species, and constantly undergo changes and updates in response to environmental demands (Bonin and De Koninck 2015).

The word “learning”, therefore, involves one subject with its brain capturing information from the environment, keeping it for some time, and eventually using it to orient subsequent behavior. The concept of learning largely overlaps that of memory, although we should distinguish them by considering memory as the whole process and learning as only the acquisition stage.

The environment most of the time includes other subjects with their own brains, so an interaction between brains takes place, and learning becomes a reciprocal exchange. In this context, the two brains learn at the same time. This is particularly important for humans, as we live in society, which means an active set of interactions between individuals. Reciprocal learning, therefore, is the most important process by which humankind builds mental improvement and progresses materially.

Learning can assume an infinity of forms in daily life (Meltzoff, Kuhl, Movellan, and Sejnowski 2009), some as simple as observing a still object; others very complex, as playing a musical instrument in synchrony with an orchestra. Newborns learn simple things, but they soon discover the best strategies to learn the most complex (and interesting!) things as well. They *learn to learn*.

In addition, given the complexity of the task of capturing the astounding amount of information available in the environment, human society has developed a structured, planned way to facilitate learning and created what has become known as “education” (Bruer 1997; Byrnes and Fox 1998).

Education, thus, is a socially structured way of learning and of learning to learn. It is also reciprocal because it involves at least two parts: learners and teachers. Learners are those who do not know something, while teachers possess the knowledge and are expected to transfer it to the learners. Thus, education is reciprocal, but not symmetrical. Of course, in modern civilization, teachers may design tools to substitute for them—books, kits, games, videos, and many other devices.

The asymmetrical, reciprocal interaction between learner and teacher is basically an interaction between two brains (Sigman, Peña, Goldin, and Ribeiro 2014). Both have to establish mental contact, using language (spoken or otherwise), sensory contact (visual, auditory, tactile), and synchronized motor behaviors to communicate efficiently (Friston and Frith 2015). Over time, the two interacting brains change each other, since they manage to convey and store information, one into the other. In order to achieve this gigantic task, brains make use of a very important property: neuroplasticity (Chang 2014).

Neuroplasticity may be defined as the ability of the brain to undergo temporary or permanent changes whenever it is influenced by other brains and by the environment. It exists in many different, simultaneous levels in all animals with neural systems (Rose 1976): molecular/cellular, inside neurons and glial cells; within the synapse between two neurons (microcircuit level); considering each neuron with its mesh of fiber branches terminating in different, other neurons (multicircuit level); and network level, regarding active, interactive brain regions of an individual. One may conceive an additional, even more complex, level—societal organization—encompassing human pairs and even groups (= interactive human brains).

In this article, we describe and briefly discuss these levels of neuroplasticity, aiming to uncover the mechanisms that interactive brains employ that underpin teaching, learning, education, and similar, related social processes. The greatest challenge is to translate the concepts and evidence reviewed here to educational practices. This is an endeavor just beginning in neuroscience. We give some examples to illustrate the potential of this translational approach.

Learning neurons

Neurons are able to learn—that is, to store information they receive from other neurons through synapses. This is perhaps the most reductionist level of analysis of educational phenomena.

It is well known that a nerve fiber transmits electrochemical information (nerve impulses) to a second neuron through the junction between them (the synapse). Besides the fast phenomena taking place by way of pre- and postsynaptic membrane potentials, a somewhat slower biochemical message may be conveyed within the communicating neurons towards their nuclei, to reach the genetic machinery therein (Kandel 2012; Salazar, Caldeira, Curcio, and Duarte 2015), and synthetize proteins which stabilize and strengthen the involved synapses.

The phenomena described above occur in two main physiological forms known as long-term potentiation (LTP) and long-term depression (LTD), which have been subjects of intensive research after the original description of the former by Bliss and Lomo (1973) in the hippocampus, and of the latter by Ito, Sakurai, and Tongroach (1982) in the cerebellum. Both LTP and LTD represent interactive, opposing mechanisms that modulate synaptic efficacy, and therefore contribute for a better tuning of information processing and storage. Together, they provide memory with flexibility, facilitating learning of novel data that may substitute obsolete, erroneous, or stressful information (Connor and Wang 2015).

Long-term potentiation and depression, as putative biological mechanisms of learning and memory, are electrophysiological phenomena that dissipate after a relatively short time. How, then, could long-term memory be explained as a result of learning? How can a highly entropic, electrobiological phenomenon be converted into a stable engram of memory, capable of retrieval anytime the person needs it?

The long-term mechanisms have been shown to involve the mobilization of the genetic machinery to provide new protein synthesis. From the nucleus, then, newly formed mRNAs and proteins are carried back to the originally activated synapses, which are thought to have been "tagged" by the previous synaptically-transmitted information (Frey and Morris 1997). In sequence, these proteins increase the adhesion between connected neurons, multiply the number of synapses between them, and therefore enroot the original information in the brain (Giese and Mizuno 2013).

By constant use of these mechanisms, a neuron is able to learn. This means that it is able to capture information from other neurons and store it for some time. Eventually, the stored information becomes decoded into efferent signals derived from the neuron to reach other ones in different places of the brain.

Learning circuits

Neurons are able to learn, but they do not do it alone; a good number of synaptically connected neurons are involved in even the simplest form of learning. They establish circuits with many other neurons and with glial cells (Diniz, Matias, Garcia, and Gomes

2014; Fields et al. 2014), and it is these multicellular circuits that become the basic repository of the stored information characteristic of learning.

The arrangement in the typical cerebral cortex or hippocampal neuron enables one single neuron to receive information from thousands of other neuronal cells and to feedback itself and other surrounding neurons with copies of its output information. A single cortical neuron, therefore, is not alone in the place; rather, it establishes a complex circuitry throughout, by which information is actively transmitted and where neuroplasticity mechanisms occur in joint interaction. A small volume of one cubic millimeter of neocortex may host some hundred thousand neurons, and up to fifteen hundred million synapses (da Costa and Martin 2013). Furthermore, researchers have often identified neuronal modules (e.g., columns) structurally and functionally, either with a uniform (Rockel, Hiorns, and Powell 1980) or a nonuniform (Herculano-Houzel, Collins, Wong, Kaas, and Lent 2008) cellular composition. These modules are regarded as processing units of the brain, with somewhat canonical circuits therein, capable of performing similar computations (Douglas and Martin 2004). It is conceivable that they represent a microcircuit unit of memory processing in the brain.

In addition to modules with a cellular composition acquired during development, in some particular regions involved with learning, neuronal precursors in specific niches proliferate to generate novel cohorts of neurons, not only during development but also throughout adulthood. This phenomenon is called “neurogenesis”, meaning neuronal production by stem cells situated in the surroundings; studies have reported this occurrence for the hippocampus not only in experimental rodents (Kempermann, Gast, Kronenberg, Yamaguchi, and Gage 2003; Ninkovic, Mori, and Götz 2007) but also in humans (Spalding et al. 2013). Other regions, such as the neocortex, have been subjects of hot controversy concerning the existence (or not) of adult neurogenesis in animals (Bandeira, Lent, and Herculano-Houzel 2009; Rakic 1985), which has not been confirmed in humans (Bhardwaj et al. 2006). In any case, studies have shown that new neurons migrate to the right place in the hippocampal dentate gyrus, mature, and integrate into the circuits that underpin memory mechanisms (Aimone et al. 2014). So, neuronal proliferation maintained through adulthood is one of the mechanisms that may explain learning at the cellular level of its expression. In addition, learning itself boosts neurogenesis (Opendak and Gould 2015), what creates a virtuous cycle for optimization of this phenomenon.

Recent technology—such as optogenetics—for identifying active neurons in living rodent brains allows a positive, gain-of-function proof of the existence of learning neuronal ensembles, specifically related to fear-memory acquisition and retrieval (Liu et al. 2012). Optogenetics allows specific neurons to fluoresce when they are active, and therefore makes them visible at the microscope. Studies show that some fluorescent neuronal ensembles represent the circuits activated during learning, since they could be reactivated by light exposure after that, and be related to retrieval of the previous experience stored in them. They would represent the memory engram—that is, the particular memory trace stored in the brain after the animal experiences fear (Liu, Ramirez, Redondo, and Tonegawa 2014).

Glial cells, especially those called “astrocytes”, have an important role in expanding and delimiting the territory of these circuits. They are strategically positioned within neuronal circuits to couple synapses into a large spatial domain that becomes capable of storing much more information than the unidirectional pathways of synaptic communication would allow (Fields et al. 2014). It is estimated that a single astrocyte in the human cerebral cortex would be able to integrate input from about 2 million synapses (Oberheim, Wang, Goldman, and Nedergaard 2006). This spatial arrangement in the brain is thought to be important when perceptual learning depends on the context surrounding the perceived target. The established functions of astrocytes—of controlling extracellular K^+ concentrations, removing/delivering excitatory neurotransmitters from/to the synaptic cleft, and providing fast, synchronizing communication via gap junctions—are compatible with this local network control, necessary for time regulation of the flow of information (Froes and Menezes 2002). Bernardinelli et al. (2014) have shown that, similar to neurons, astrocytes undergo structural changes after LTP, increasing their volume coverage of synaptic circuits and long-term stability of synaptic contacts.

Learning networks

If conceived of as micro- and multicircuit functions, learning and memory would be limited to specific functional domains. However, each single episode involving learning in humans requires the interplay of an enormous diversity of functions. Take, for instance, a little girl trying to learn how to write. While sitting at a desk, she has to coordinate posture with the movement of her arm and fingers in order to hold the pencil and display the right symbols on the piece of paper. Furthermore, she must control her performance visually, listen and attend to the commands of the teacher, understand their meaning, think about them, and transform her thoughts into written words. Practically all functional domains of the brain are involved in the task.

Therefore, it is more realistic to conclude that learning involves not only a few neurons and synapses, and not only neuron-glial circuits within a brain module, but a set of different brain areas that constitute networks (Gilmore, Nelson, and McDermott 2015). Brain areas are connected by long-distance tracts of white matter, so if we wish to understand the plasticity mechanisms of complex behaviors, we should take into account the possibility that it occurs within these long pathways and not only within small modules—a concept we call “long-distance plasticity” (Tovar-Moll et al. 2014).

Functional networks are found in the human brain that are synchronously active even in the absence of any specific tasks, as is the case of the default mode system (DMN) (Greicius, Krasnow, Reiss, and Menon 2003), recorded when subjects lay in the MR machine with no specific task in mind. The DMN is very consistent among different subjects, and researchers hypothesize it to represent a network devoted to wandering thoughts (Fox, Spreng, Ellamil, Andrews-Hanna, and Christoff 2015).

Studies of brain networks involved in learning tasks have multiplied in recent years, after the improvement of morphological and functional neuroimaging methods (Chang 2014). Most works compare subjects who learned a certain skill—such as playing a musical instrument, performing a sport, and other tasks—with control subjects having no similar training. Studies have described a number of morphological and functional differences in specific brain areas and attributed these to training (see Jancke 2009 for a review). Most of these studies are cross-sectional: they examine subjects at a particular age, after a certain amount of training, and compare them with paired controls. However, how can one be sure that “trained” subjects were not actually born with differentiated brain areas that allowed them to excel in their tasks? What is cause, what is consequence? The gold standard for more robust conclusions are the longitudinal studies, which examine subjects at sequential time points during training, or at least before and after training. Fortunately (although fewer such studies are currently available), they ascertain that training induces plastic changes in the brain. This is true for music (Hyde et al. 2009), hearing (Herdener et al. 2010), juggling (Draganski et al. 2004), rugby (Sekiguchi et al. 2011), dance (Cross, Kraemer, Hamilton, Kelley, and Grafton 2009), and many other tasks and skills.

These studies contribute to identifying brain areas that are changeable by learning of different tasks. They represent, therefore, a kind of "cartographic" effort that singles out specific active brain regions for each kind of training.

A more complete approach shows brain regions (networks) in cooperative, or at least synchronized, activity during particular tasks related to learning. One interesting example is the "parietal memory network" proposed by Gilmore and his colleagues (2015). A number of studies (e.g., Shirer, Ryali, Rykhlevskaia, Menon, and Greicius 2012; Yeo et al. 2011), found that the regions that make part of this network display a synchronized activity as detected by fMRI. In addition, meta-analyses have revealed that these same regions, jointly, are activity-related to memory-retrieval success (Kim 2013)—that is, to the ability to recognize items learned in the past, as compared to new items. One might conclude that this set of regions represents a network for memory retrieval in the human brain. A recent work has added to our knowledge of memory circuits, revealing an unexpected role for the DMN in emotional autobiographic memory recall (Bado et al. 2014), thus broadening its function as previously described.

Investigators report different networks for different tasks, including such high-complexity functions of cultural determination as reading, writing, and arithmetic (Dehaene and Cohen 2007). Networks for these complex functions are particularly interesting because they cannot be explained by evolutionary selection: humans developed written language very recently (about 5,500 years ago) and arithmetic even more recently (about 2,500 years)—time periods too short for biological selection to exert its pressure. For this reason, they are considered cultural products and, therefore, learned during postnatal development (Dehaene and Cohen 2007). In adults, a set of cortical regions in the left hemisphere is consistently activated when researchers ask subjects to read while having their brain activity recorded by MRI. In kindergartners (nonreaders), electrophysiological data (Maurer et al. 2006) have shown that reading function is bilateral at first but becomes unilateral (usually left-sided) after schooling provides reading competence. Interestingly, other functions (e.g., face recognition) activate some components of this network in illiterate subjects (Dehaene et al. 2010)—which means that plasticity is determinant in this case since the same cortical regions can either be "occupied" by face recognition or by grapheme recognition when one is learning to read. Furthermore, the reading network is consistent cross-culturally, with similar topography in the brains of Japanese-, Chinese-, and Roman-alphabet readers (Bolger, Perfetti, and Schneider 2005). We note, in this context, that reading is a complex function involving not only a perceptual component specialized in identifying graphemes (Jobard, Crivello, and Tzourio-Mazoyer 2003) but also others in charge of coordinated eye movements and attentional focus (Vogel et al. 2013). This is why many cortical regions are activated simultaneously when investigators demand a reading task of subjects under fMRI acquisition.

The most accepted interpretation for functional networks is that synchronized regions are connected anatomically, either directly or indirectly. Therefore, it is conceivable to think of them as long-distance circuits formed during development and subjected to environment-driven plasticity. In consonance with this view, most investigators believe that complex mammalian brains might have evolved a highly efficient structural connectivity (connectome) that enables vast functional network frames (Sporns and Kotter 2004).

Long-distance circuits compose an important sector of all brain areas: the white matter. Large amounts of nerve fibers form this sector, all of them connecting neurons at different distances. White matter differs from gray matter, where neuronal bodies are clustered within the modules referred to above. White matter includes fibers directed to many brain areas and that compose the main channels whereby neurons conduct information to other neurons at a distance.

Take the example of the corpus callosum. This large tract makes part of the white matter that specifically connects one hemisphere with the other (Shen et al. 2015). Evidence indicates that it includes about 200 million fibers (Aboitiz, Scheibel, Fisher, and Zaidel 1992), interconnecting cortical regions of many functional domains: visual, auditory, emotional, cognitive, mnemonic, and many others. The corpus callosum starts to form in humans at about 12 weeks of gestation, simultaneously with other fiber tracts within the white matter (Rakic and Yakovlev 1968). We are starting to know in detail the mechanisms that govern the complex ability of individual fibers—callosal and many others—to elongate in the right direction, making turns and giving off branches, in order to find their exact targets throughout the brain (Chédotal and Richards 2010; Fenlon and Richards 2015). Axonal growth and guidance involve a great number of molecular and cellular signals expressed in the right places at the right moments. This is true of callosal fibers, which emerge from some pyramidal cells in the cerebral cortex of each hemisphere, grow toward the white matter where they bifurcate or directly deflect toward the midline (Garcez et al. 2007), and, upon crossing there, restart the journey to end in the homotopic mirror regions in the opposite hemisphere (Fenlon and Richards 2015).

Callosal fibers are instrumental in enabling humans to have complex interactions between both sides of our bodies and in providing communication between regions of the brain in each hemisphere, which look similar but function differently (Gazzaniga 2005). It is by way of the corpus callosum that we are able to associate speech with prosody (Sammler, Kotz, Eckstein, Ott, and Friederici 2010), for example. By "speech", we mean rational ideas conveyed through sounds that anatomical structures in our throat and face produce. Speech always conveys a rational meaning that derives from our thoughts. However, emotions modulate speech in a way that sometimes entirely changes the meaning of a sentence's cold, rational content. Just think about "yes" and "no". We have all experienced situations in life when "yes" meant "no", and vice versa. This emotional modulation of the meaning of speech is effected by intonation, facial mimics, and gestures that accompany speech sounds. Both speech and prosody are lateralized in the brain, usually the former in the left hemisphere, and the latter in the right. A typical function of the corpus callosum is to integrate prosody with speech—that is, left hemisphere with right hemisphere networks (Sammler et al. 2010).

Callosal fibers usually employ the excitatory neurotransmitter glutamate to communicate with postsynaptic neurons at the opposite hemisphere. However, most often the postsynaptic neurons are GABAergic, inhibitory neurons, and the net effect of callosal activation is the inhibition of the opposite, homotopic cortical area (Rock and Apicella 2015). Research has strongly associated the amount of interhemispheric inhibition with learning and neuroplasticity. An example is the corpus callosum of musicians, reportedly shown to display stronger inhibitory effects over the opposite hemisphere than does that of nonmusicians. Among musicians, the effect is greater for string players than for piano players (Vollmann et al. 2014). A more drastic example is that of single-limb amputees, who often present with a phantom-limb syndrome (somatic sensations, including pain, experienced as coming from the absent limb). Chen, Cohen, and Hallett (2002) have shown that, for lack of contralateral suppression, the sensorimotor body map at the hemisphere contralateral to the amputation is altered, with a larger representation of the remaining parts adjacent to the missing limb. Together with this phenomenon, the corpus callosum shows signs of microstructural disorganization, which could be related to lower inhibition, causing plastic changes in the functional topographic map contralateral to amputation, or even in both hemispheres (Simões et al. 2012).

Another example of interhemispheric communication potentially subject to long-distance plastic changes is the ability we all have to identify, by hand palpation with closed eyes, an object placed in one of the hands. We can correctly name the object and point to it (with the opposite hand) from a set of pictures of similar objects (Reed, Klatzky, and Halgren 2005).

These are all functions of the corpus callosum that gradually mature during brain development. However, what happens when developmental disorders block callosal fibers from crossing the midline? One would think that all interhemispheric communication should be interrupted, since the corpus callosum is absent. However, Saul and Sperry (1968) revealed a paradoxical situation in a human subject born without the corpus callosum (callosal dysgenesis): besides being cognitively preserved, she did not display the classical syndrome of hemisphere disconnection typical of adult patients who underwent surgical transection of the corpus callosum for therapeutic reasons (Sperry 1968). One hypothesis to explain the phenomenon became more relevant recently: the possibility that anomalous tracts form in these brains, some of them crossing through other commissures in the ventral forebrain or the midbrain. Callosal dysgenesis, then, represents an interesting model to evaluate the ability of white-matter tracts to undergo radical changes in their trajectory, thereby generating entirely different networks by long-distance plasticity. We have studied some of these cases, and could unravel a vast anomalous circuitry established by misdirected callosal fibers (Tovar-Moll et al. 2007, 2014). Some of the anomalous tracts identified in these subjects connected homotopic cortical regions involved in object recognition, albeit crossing the midline in lower levels of the nervous system. We showed that, despite the anomalous trajectories, these tracts could be attributed a compensatory role in integrating the cortical areas of both hemispheres devoted to object recognition, and thus allow the subjects to perform normally in that function. We reasoned that the environment, as much as genetic determinants, may largely change the human brain connectome during development—a phenomenon we called "long-distance plasticity" (Tovar-Moll et al. 2014).

Learning brains

In social settings, learning takes place between interacting individuals. This means that reciprocal interchanges between brains during communication or educational activities essentially operate through neuroplasticity mechanisms, which allow storing, decoding, and modulating the content of the exchanged information.

For this reason, experiments exploring interactions between subjects require simultaneous recordings of brain activity in two (or more) interacting individuals and are of great value in understanding the underlying mechanisms of learning and education. Anders and collaborators (2011), for instance, have analyzed fMRI brain activity of male subjects asked to interpret the emotional content of the videotaped face of their female partners. Since there was high consistency of brain areas activated by the same emotions between the female senders and the male perceivers ("shared emotion network"), it was possible to predict the corresponding brain regions related to each emotional face display. Face displays are commonly associated to speech, and make part of prosody, together with hand

and body gestures, as mentioned previously. Thus, it would be relevant to study how the linguistic interactions between teachers and students (speech + prosody) take place in an educational setting. Stephens, Silbert, and Hasson (2010) achieved a close approximation when they tackled verbal communication directly—by analyzing the brain activity of a person telling a story to an audio recorder, and comparing it to that of another who listened to the recorded story. They also demonstrated that the storyteller and the listener had coordinated activation of homologous brain areas, sharing the same brain space for production and comprehension of linguistic messages. This approach left unanswered, however, the important question of what are the differences in brain processing between the emitter (e.g., the teacher) and the receiver (e.g., the student), since communication activates the same brain areas in both of them.

More recently, Kuhlen, Allefeld, and Haynes (2012) advanced this topic further; they discerned coordination between activities of nonhomologous cortical regions, as well. In addition, they could find similarities in the EEG patterns of listeners who attended the same stories, suggesting a correlation between the activity and the content processing of the stories by the listeners' brains. The researchers used high-density EEG recording to improve the technique's spatial resolution. The authors interpreted the differential topographic pattern of coordinated activation in the brains of listeners to be related to the involvement of areas associated with retrieving information from memory, as other studies suggested (Amodio and Frith 2006). Based on these pioneering analyses, Frith (2007) identified an assemblage of regions that would constitute our "social brain".

Most of these works, however, are limited because they explore the social dimension of human nature by recording only one person at a time. In interactive situations, on the other hand, each person modifies the other's perceptions and reactions on a real-time basis (Konvalinka and Roepstorff 2012). Therefore, social interaction between persons and their brains are neither univocal nor static. Quite the opposite, they are reciprocal and highly dynamic: information flows bidirectionally between the two interacting brains, and continuously change their functional and morphological underpinnings. Therefore, to understand processes that require mutual or multi-individual interactions such as social perception, social emotions, learning, and cooperation, we need novel systems or methodologies allowing investigation of the dynamic nature of these interactions (McCabe, Houser, Ryan, Smith, and Trouard 2001).

Real "two-brain" analysis (known as "hyperscanning") is only just beginning, due to the complexities both for designing a well-controlled experimental protocol of social interaction and for performing the real-time comparative signal processing of the interacting brains under different recording setups operated at the same time. This new approach has proven possible, either using EEG (Astolfi et al. 2011), magnetoencephalography (Hirata et al. 2014), fMRI (Montague et al. 2002), or functional near-infrared spectroscopy (fNIRS, Osaka et al. 2015). Using fMRI, for example, Krueger and collaborators (2007) explored the brain network dynamics of two strangers interacting online with one another in a sequential reciprocal trust game while their brains were scanned simultaneously. In addition, researchers used EEG/fNIRS to map the brain circuitry of two individuals interacting in a face-to-face dialogue (Jiang et al. 2012), as well as singing (Osaka et al. 2015) or playing musical instruments (Lindenberger, Li, Gruber, and Muller 2009).

Taken together, these studies show an interesting pattern of interbrain synchronization (especially related to engagement of frontal lobe networks), indicating a promise of hyperscanning techniques to understand the neuroplasticity mechanisms of sharing and exchanging information between brains/persons (Liu and Pelowski 2014). So far, although explored in social, neuroeconomic, and emotional context, this type of experimental design has been less employed in educational interactions. It is possible, in the near future, that technological improvements will make it possible to record from multiple brains under realistic settings.

Final remarks

We cannot, in this short review, cover the gigantic number of contributions on the various levels of neuroplasticity that may have potential impact on educational practices. Our aim, however, is to call attention to the importance of exploring this kind of translational research. Stokes (1997) proposed a new approach to health-directed translational research, by which policy-makers and institutions would give emphasis to research induced by its social use, in the belief that it could better connect the basic sciences with practical applications to health and disease. We propose that researchers target a similar approach to education; in such case, neuroscience—neuroplasticity, in particular—would play a crucial role.

The practical benefits of neuroscience research for education is a hot topic of discussion between neuroscientists and psychologists (see Bowers 2016, as a recent example). On one side, neuroscientists claim that knowledge about the brain during learning may inspire suggestions to accelerate literacy, reading speed, cognitive abilities such as attention, problem solving, and other skills (Sigman et al. 2014). At the other pole, psychologists argue that "the bridge is too far" (Bruer 1997), and that cognitive psychology should mediate relations between neuroscientific evidence and practice in schools. In our view, such territorial approach may sound like a fruitful academic discussion, but it does not help to build a bridge between the two sides. In fact, an effort toward exchange of expertise, common discussions, and some experimental applications could help speed up the development of new methodologies and improvement of educational policies in developed and developing countries. Moreover, it might have a greater impact in the latter, which need to move with maximal speed to reach acceptable levels of education among their populations. The same rationale is valid for children with learning disabilities: they need solutions as quickly as possible.

We give examples, among many, of evidence-based suggestions starting to ripen. Physical exercise is one such example. Studies have shown that exercise increases neurogenesis in the hippocampus of mice and men, benefitting cognition (Erickson et al. 2011; Pereira et al. 2007; van Praag, Kempermann, and Gage 1999), and even that this effect transmits to the adult offspring when their mothers exercise during pregnancy (Gomes da Silva et al. 2016). Implementation of daily aerobic exercise in schools, therefore, is an intuitive suggestion to educators that now has acquired a solid scientific basis. Another example is the effect of sleep on hippocampal plasticity and the consolidation of memory. Many experiments with animals and humans have documented plasticity upregulation and better memory consolidation (reviewed by Diekelmann and Born 2010; and Ribeiro 2012). In addition, human performance in many different cognitive tasks is improved after sleep (Beijamini, Pereira, Cini, and Louzada 2014; Wamsley, Tucker, Payne, and Stickgold 2010), as well as such socioemotional competences as insight (Wagner, Gais, Haider, Verleger, and Born 2004) and creativity (Ritter, Strick, Bos, van Baaren, and Dijksterhuis 2012). Therefore, it seems well grounded to suggest that educators implement a nap during the school day as an attempt to boost learning. In both of these examples, psychologists and teachers must themselves evaluate the efficacy of exercise and sleep at school, and scale their efforts to whole school networks only after these assessments.

In sum, we have very briefly explored various levels—from molecules to social settings—to illustrate how mutable the learning brain is in contact with the environment, which is also very broad and diverse, from the natural context to socially structured education in schools. These different levels coexist and, as suggested by Rose (1976), we must approach them with different degrees of reductionism, but connect them whenever possible to achieve a holistic perception of all the phenomena involved. We intended, therefore, to emphasize that not only are neurons and circuits mutable but also brain areas and interactive, whole brains. Understanding all these specific but connected levels of representation should better prepare educators to propose, implement, test, and map changes to educational processes and methodologies.

References

Aboitiz, F., Scheibel, A. B., Fisher, R. S., & Zaidel, E. (1992). Fiber composition of the human corpus callosum. *Brain Research, 598,* 143–153.

Aimone, J. B., Li, Y., Lee, S. W., Clemenson, G. D., Deng, W., & Gage, F. H. (2014). Regulation and function of adult neurogenesis: From genes to cognition. *Physiological Reviews, 94,* 991–1026.

Amodio, D. M., & Frith, C. D. (2006). Meetings of minds: The medial frontal cortex and social cognition. *Nature Reviews. Neuroscience, 7,* 268–277.

Anders, S., Heinzle, J., Weiskopf, N., Ethover, T., & Haynes, J. D. (2011). Flow of affective information between communicating brains. *Neuroimage, 54,* 439–446.

Astolfi, L., Toppi, J., Fallani, F. V., Vecchiato, G., Cincotti, F., Wilke, C. T., et al. (2011). Imaging the social brain by simultaneous hyperscanning during subject interaction. *IEEE Intelligence Systems, 26,* 38–45.

Bado, P., Engel, A., de Oliveira Souza, R., Bramati, I. E., Paiva, F. F., Basilio, R., et al. (2014). Functional dissociation of ventral frontal and dorsomedial default mode network components during resting state and emotional autobiographical recall. *Human Brain Mapping, 35,* 3302–3313.

Bandeira, F., Lent, R., & Herculano-Houzel, S. (2009). Changing numbers of neuronal and non-neuronal cells underlie postnatal brain growth in the rat. *Proceedings of the National Academy of Sciences of the United States of America, 106,* 14110–14113.

Beijamini, F., Pereira, S. I. R., Cini, F. A., & Louzada, F. M. (2014). After being challenged by a video game problem, sleep increases the chance to solve it. *PLoS ONE, 9*(1), e84342.

Bernardinelli, Y., Randall, J., Janett, E., Nikonenko, I., Konig, S., Jones, E. V., et al. (2014). Activity-dependent structural plasticity of perisynaptic astrocytic domains promotes excitatory synapse stability. *Current Biology, 24,* 1679–1688.

Bhardwaj, R. D., Curtis, M. A., Spalding, K. L., Buchholz, B. A., Fink, D., Björk-Eriksson, T., et al. (2006). Neocortical neurogenesis in humans is restricted to development. *Proceedings of the National Academy of Sciences of the United States of America, 103,* 12564–12568.

Bliss, T. V., & Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate gyrus of the anesthetized rabbit following stimulation of the performant path. *Journal of Physiology, 232,* 331–356.

Bolger, D. J., Perfetti, C. A., & Schneider, W. (2005). Cross-cultural effect on the brain revisited: Universal structures plus writing system variation. *Human Brain Mapping, 25,* 92–104.

Bonin, R. P., & De Koninck, Y. (2015). Reconsolidation and the regulation of plasticity: Moving beyond memory. *Trends in Neurosciences, 38,* 336–344.

Bowers, J. S. (2016). The practical and principled problems with educational neuroscience. *Psychological Review., 123,* 600–612.

Bruer, J. T. (1997). Education and the brain: A bridge too far. *Education Research, 26,* 4–16.

Byrnes, J. P., & Fox, N. A. (1998). The educational relevance of research in cognitive neuroscience. *Educational Psychology Review, 10,* 297–342.

Chang, Y. (2014). Reorganization and plastic changes of the human brain associated with skill learning and expertise. *Frontiers in Human Neuroscience, 8,* 35.

Chédotal, A., & Richards, L. J. (2010). Wiring the brain: The biology of neuronal guidance. *Cold Spring Harbor Perspectives in Biology, 2,* a001917.

Chen, R., Cohen, L. G., & Hallett, M. (2002). Nervous system reorganization following injury. *Neuroscience, 111,* 761–773.

Connor, S. A., & Wang, Y. T. (2015). A place at the table: LTD as a mediator of memory genesis. *The Neuroscientist, 22,* 359–371.

Cross, E. S., Kraemer, D. J., Hamilton, A. F., Kelley, W. M., & Grafton, S. T. (2009). Sensitivity of the action observation network to physical and observational learning. *Cerebral Cortex, 19,* 315–326.

Da Costa, N. M., & Martin, K. A. (2013). Sparse recognition of brain circuits: Or, how to survive without a microscopic connectome. *Neuroimage, 80,* 27–36.

Dehaene, S., & Cohen, L. (2007). Cultural recycling of cortical maps. *Neuron, 56,* 384–398.

Dehaene, S., Pegado, F., Braga, L. W., Ventura, P., Nunes Filho, G., Jobert, A., et al. (2010). How learning to read changes the cortical networks for vision and language. *Science, 330,* 1359–1364.

Diekelmann, S., & Born, J. (2010). The memory function of sleep. *Nature Reviews. Neuroscience, 11,* 114–126.

Diniz, L. P., Matias, I. C. P., Garcia, M. N., & Gomes, F. C. A. (2014). Astrocytic control of neural circuit formation: Highlights on TGF-beta signaling. *Neurochemistry International, 78,* 18–27.

Douglas, R. J., & Martin, K. A. C. (2004). Neuronal circuits of the neocortex. *Annual Reviews of Neuroscience, 27,* 419–451.

Draganski, B., Gaser, C., Busch, V., Schuierer, G., Bogdahn, U., & May, A. (2004). Neuroplasticity: Changes in grey matter induced by training. *Nature, 427,* 311–312.

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., et al. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences of the United States of America, 108,* 3017–3022.

Fenlon, L. R., & Richards, L. J. (2015). Contralateral targeting of the corpus callosum in normal and pathological brain function. *Trends in Neurosciences, 38,* 264–272.

Fields, R. D., Araque, A., Johansen-Berg, H., Lim, S.-S., Lynch, G., Nave, K.-A., et al. (2014). Glial biology in learning and cognition. *Neuroscientist, 20,* 426–431.

Fox, K. C., Spreng, R. N., Ellamil, M., Andrews-Hanna, J. R., & Christoff, K. (2015). The wandering brain: Meta-analysis of functional neuroimaging studies of mind-wandering and related spontaneous thought processes. *Neuroimage, 111,* 611–621.

Frey, U., & Morris, R. G. (1997). Synaptic tagging and long-term potentiation. *Nature, 385,* 533–536.

Friston, K. J., & Frith, C. D. (2015). Active inference, communication and hermeneutics. *Cortex, 68,* 129–143.

Frith, C. D. (2007). The social brain? *Philosophical Transactions of the Royal Society of London Biological Sciences, 362,* 671–678.

Froes, M. M., & Menezes, J. R. L. (2002). Coupled heterocellular arrays in the brain. *Neurochemistry International, 41,* 367–375.

Garcez, P. P., Henrique, N. P., Furtado, D. A., Bolz, J., Lent, R., & Uziel, D. (2007). Axons of callosal neurons bifurcate transiently at the white matter before consolidating an interhemispheric projection. *European Journal of Neuroscience, 25,* 1384–1394.

Gazzaniga, M. S. (2005). Forty-five years of split-brain research and still going strong. *Nature Reviews. Neuroscience, 6,* 653–659.

Giese, K. P., & Mizuno, K. (2013). The roles of protein kinases in learning and memory. *Learning and Memory, 20,* 540–552.

Gilmore, A. W., Nelson, S. M., & McDermott, K. B. (2015). A parietal memory network revealed by multiple MRI methods. *Trends in Cognitive Sciences, 19,* 534–543.

Gomes da Silva, S., Almeida, A. A., Fernandes, J., Lopim, G. M., Cabral, F. R., Scerni, D. A., et al. (2016). Maternal exercise during pregnancy increases BDNF levels and cell numbers in the hippocampal formation but not in the cerebral cortex of adult rat offspring. *PLoS ONE, 11*(1), 0147200.

Greicius, M. D., Krasnow, B., Reiss, A. L., & Menon, V. (2003). Functional connectivity in the resting brain: A network analysis of the default mode hypothesis. *Proceedings of the National Academy of Sciences of the United States of America, 100,* 253–258.

Herculano-Houzel, S., Collins, C. E., Wong, P., Kaas, J. H., & Lent, R. (2008). The basic nonuniformity of the cerebral cortex. *Proceedings of the National Academy of Sciences of the United States of America, 105,* 12593–12598.

Herdener, M., Esposito, F., di Salle, F., Boller, C., Hilti, C. C., Habermeyer, B., et al. (2010). Musical training induces functional plasticity in human hippocampus. *Journal of Neuroscience, 30,* 1377–1384.

Hirata, M., Ikeda, T., Kikuchi, M., Kimura, T., Hiraishi, H., Yoshimura, Y., et al. (2014). Hyperscanning MEG for understanding mother-child cerebral interactions. *Frontiers in Human Neuroscience, 8,* 118.

Hyde, K. L., Lerch, J., Norton, A., Forgeard, M., Winner, E., Evans, A. C., et al. (2009). Musical training shapes structural brain development. *Journal of Neuroscience, 29,* 3019–3025.

Ito, M., Sakurai, M., & Tongroach, P. (1982). Climbing fibre induced depression of both mossy fibre responsiveness and glutamate sensitivity of cerebellar Purkinje cells. *Journal of Physiology, 324,* 113–134.

Jancke, L. (2009). The plastic human brain. *Restorative Neurology and Neuroscience, 27,* 521–538.

Jiang, J., Dai, B., Peng, D.-L., Zhu, C.-Z., Liu, L., & Lu, C.-M. (2012). Neural synchronization during face-to-face communication. *Journal of Neuroscience, 32,* 16064–16069.

Jobard, G., Crivello, F., & Tzourio-Mazoyer, M. (2003). Evaluation of the dual route theory of reading: A metanalysis of 35 neuroimaging studies. *Neuroimage, 20,* 693–712.

Kandel, E. R. (2012). The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. *Molecular Brain, 5*, 14.

Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M., & Gage, F. H. (2003). Early determination and long-term persistence of adult-generated new neurons in the hippocampus of adult mice. *Development, 130,* 391–399.

Kim, H. (2013). Differential neural activity in the recognition of old versus new events: An activation likelihood estimation meta-analysis. *Human Brain Mapping, 34,* 814–836.

Klingberg, T. (2013). *The learning brain*. New York: Oxford University Press.

Konvalinka, I., & Roepstorff, A. (2012). The two-brain approach: How can mutually interacting brains teach us something about social interaction? *Frontiers in Human Neuroscience, 6,* 215.

Krueger, F., McCabe, K., Moll, J., Kriegeskorte, N., Zahn, R., Strenziok, M., et al. (2007). Neural correlates of trust. *Proceedings of the National Academy of Sciences of the United States of America, 104,* 20084–20089.

Kuhlen, A. K., Allefeld, C., & Haynes, J.-D. (2012). Content-specific coordination of listeners' to speakers' EEG during communication. *Frontiers in Human Neuroscience, 6,* 266.

Lindenberger, U., Li, S., Gruber, W., & Muller, V. (2009). Brains swinging in concert: Cortical phase synchronization while playing guitar. *BMC Neuroscience, 10,* 22.

Liu, T., & Pelowski, M. (2014). Clarifying the interaction types in two-person neuroscience research. *Frontiers in Human Neuroscience, 8,* 276.

Liu, X., Ramirez, S., Pang, P. T., Puryear, C. B., Govindarajan, A., Deisseroth, K., et al. (2012). Optogenetic stimulation of a hippocampal engram activates fear memory recall. *Nature, 484,* 381–385.

Liu, X., Ramirez, S., Redondo, R. L., & Tonegawa, S. (2014). Identification and manipulation of memory engram cells. *Cold Spring Harbor Symposia on Quantitative Biology, 79,* 59–65.

Maurer, U., Brem, S., Kranz, F., Bucher, K., Benz, R., Halder, P., et al. (2006). Coarse neural tuning for print peaks when children learn to read. *Neuroimage, 33,* 749–758.

McCabe, K., Houser, D., Ryan, L., Smith, V., & Trouard, T. (2001). A functional imaging study of cooperation in two-person reciprocal exchange. *Proceedings of the National Academy of Sciences of the United States of America, 98,* 11832–11835.

Meltzoff, A. N., Kuhl, P. K., Movellan, J., & Sejnowski, T. J. (2009). Foundations for a new science of learning. *Science, 325,* 284–288.

Montague, P. R., Berns, G. S., Cohen, J. D., McClure, S. M., Pagnoni, G., Dhamala, M., et al. (2002). Hyperscanning: simultaneous fMRI during linked social interactions. *Neuroimage, 16,* 1159–1164.

Ninkovic, J., Mori, T., & Götz, M. (2007). Distinct modes of neuron addition in adult mouse neurogenesis. *Journal of Neuroscience, 27,* 10906–10911.

Oberheim, N. A., Wang, X., Goldman, S., & Nedergaard, M. (2006). Astrocytic complexity distinguishes the human brain. *Trends in Neuroscience, 29,* 547–553.

Opendak, M., & Gould, E. (2015). Adult neurogenesis: A substrate for experience-dependent change. *Trends in Cognitive Sciences, 19,* 151–161.

Osaka, N., Minamoto, T., Yaoi, K., Azuma, M., Shimada, Y. M., & Osaka, M. (2015). How two brains make one synchronized mind in the inferior frontal cortex: fNIRS-based hyperscanning during cooperative singing. *Frontiers in Psychology, 6,* 1811.

Pereira, A. C., Huddleston, D. E., Brickmann, A. M., Sosunov, A. A., Hen, R., McKhann, G. M., et al. (2007). An *in vivo* correlate of exercise-induced neurogenesis in the adult dentate gyrus. *Proceedings of the National Academy of Sciences of the United States of America, 104,* 5638–5643.

Rakic, P. (1985). Limits of neurogenesis in primates. *Science, 227,* 1054–1056.

Rakic, P., & Yakovlev, P. I. (1968). Development of the corpus callosum and cavum septi in man. *Journal of Comparative Neurology, 132,* 45–72.

Reed, C. L., Klatzky, R. L., & Halgren, E. (2005). What vs. where in touch: An fMRI study. *Neuroimage, 25,* 718–726.

Ribeiro, S. (2012). Sleep and plasticity. *Pflugers Archiv–European Journal of Physiology, 463,* 111–120.

Ritter, S. M., Strick, M., Bos, M. W., van Baaren, R. B., & Dijksterhuis, A. (2012). Good morning creativity: Task reactivation during sleep enhances beneficial effect of sleep on creative performance. *Journal of Sleep Research, 21,* 643–647.

Rock, C., & Apicella, A. J. (2015). Callosal projections drive neuronal-specific responses in the mouse auditory cortex. *Journal of Neuroscience, 35,* 6703–6713.

Rockel, A. J., Hiorns, R. W., & Powell, T. P. (1980). The basic uniformity in structure of the neocortex. *Brain, 103,* 221–244.

Rose, S. (1976). *The conscious brain*. New York: Vintage Books.

Salazar, I. L., Caldeira, M. V., Curcio, M., & Duarte, C. B. (2015). The role of proteases in hippocampal synaptic plasticity: Putting together small pieces of a complex puzzle. *Neurochemical Research, 41,* 156–182.

Sammler, D., Kotz, S. A., Eckstein, K., Ott, D. V., & Friederici, A. D. (2010). Prosody meets syntax: The role of corpus callosum. *Brain, 133,* 2643–2655.

Saul, R. E., & Sperry, R. W. (1968). Absence of commissurotomy symptoms with agenesis of the corpus callosum. *Neurology, 18,* 307.

Sekiguchi, A., Yokoyama, S., Kasahara, S., Yomogida, Y., Takeuchi, H., Ogawa, T., et al. (2011). Neural bases of a specific strategy for visuospatial processing in rugby players. *Medicine and Science in Sports and Exercise, 43,* 1857–1862.

Shen, K., Misic, B., Cipollini, B. N., Bezgin, G., Buschkuehl, M., Hutchinson, R. M., et al. (2015). Stable long-range interhemispheric coordination is supported by direct anatomical projections. *Proceedings of the National Academy of Sciences of the United States of America, 112,* 6473–6478.

Shirer, W. R., Ryali, S., Rykhlevskaia, E., Menon, V., & Greicius, M. D. (2012). Decoding subject-driven cognitive states with whole-brain connectivity patterns. *Cerebral Cortex, 22,* 158–165.

Sigman, M., Peña, M., Goldin, A. P., & Ribeiro, S. (2014). Neuroscience and education: Prime time to build the bridge. *Nature Neuroscience, 17,* 497–502.

Simões, E. L., Bramati, I., Rodrigues, E., Franzoi, A., Moll, J., Lent, R., et al. (2012). Functional expansion of sensorimotor representation and structural reorganization of callosal connections in lower limb amputees. *Journal of Neuroscience, 32,* 3211–3220.

Spalding, K. L., Bergmann, O., Alkass, K., Bernard, S., Salehpour, M., Huttner, H. B., et al. (2013). Dynamics of hippocampal neurogenesis in adult humans. *Cell, 153,* 1219–1227.

Sperry, R. W. (1968). Hemisphere deconnection and unity in conscious awareness. *American Psychologist, 23,* 723–733.

Sporns, O., & Kotter, R. (2004). Motifs in brain networks. *PLoS Biology, 2,* e369.

Stephens, G. J., Silbert, L. J., & Hasson, U. (2010). Speaker-listener neural coupling underlies successful communication. *Proceedings of the National Academy of Sciences of the United States of America, 107,* 14425–14430.

Stokes, D. E. (1997). *Pasteur's quadrant: Basic science and technological innovation*. Washington, DC: Brookings Institution Press.

Tovar-Moll, F., Moll, J., de Oliveira-Souza, R., Bramati, I., Andreiuolo, P. A., & Lent, R. (2007). Neuroplasticity in human callosal dysgenesis: A diffusion tensor imaging study. *Cerebral Cortex, 17,* 531–541.

Tovar-Moll, F., Monteiro, M., Andrade, J., Bramati, I. E., Vianna-Barbosa, R., Marins, T., et al. (2014). Structural and functional brain rewiring clarifies preserved interhemispheric transfer in humans born without the corpus callosum. *Proceedings of the National Academy of Sciences of the United States of America, 111,* 7843–7848.

Van Praag, H., Kempremann, G., & Gage, F. H. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience, 2,* 266–270.

Vogel, A. C., Church, J. A., Power, J. D., Miezin, F. M., Petersen, S. E., & Schlaggar, B. L. (2013). Functional network architecture of reading-related regions across development. *Brain and Language, 125,* 231–243.

Vollmann, H., Ragert, P., Conde, V., Villringer, A., Classen, J., Witte, O. W., et al. (2014). Instrument specific use-dependent plasticity shapes the anatomical properties of the corpus callosum: A comparison between musicians and non-musicians. *Frontiers in Behavioral Neuroscience, 8,* 245.

Wagner, U., Gais, S., Haider, H., Verleger, R., & Born, J. (2004). Sleep inspires insight. *Nature, 427,* 352–355.

Wamsley, E. J., Tucker, M. A., Payne, J. D., & Stickgold, R. (2010). A brief nap is beneficial for human route-learning: The role of navigation experience and EEG spectral power. *Learning and Memory, 17,* 332–336.

Yeo, B. T., Krienen, F. M., Sepulcre, J., Sabuncu, M. R., Lashkari, D., Hollinshead, M., et al. (2011). The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *Journal of Neurophysiology, 106,* 1125–1165.

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