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Review

Cite this article: Edelstein L, Smythies J.

2014 The role of epigenetic-related codes in neurocomputation: dynamic hardware in the brain. *Phil. Trans. R. Soc. B* **369**: 20130519. <http://dx.doi.org/10.1098/rstb.2013.0519>

One contribution of 19 to a Theme Issue 'Epigenetic information-processing mechanisms in the brain'.

Subject Areas:

neuroscience, cellular biology, molecular biology, physiology

Keywords:

neurocomputation, exosomes, microRNAs, epigenetics, neural modality, synaptic plasticity

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The role of epigenetic-related codes in neurocomputation: dynamic hardware in the brain

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This paper presents a review of recent work on the role that two epigenetic-related systems may play in information processing mechanisms in the brain. The first consists of exosomes that transport epigenetic-related molecules between neurons. The second consists of homeoproteins like Otx2 that carry information from sense organs to primary sensory cortex. There is developing evidence that presynaptic neurons may be able to modulate the fine microanatomical structure in the postsynaptic neuron. This may be conducted by three mechanisms, of which the first is well established and the latter two are novel. (i) By the well-established activation of receptors that trigger a chain of signalling molecules (second messengers) that result in the upregulation and/or activation of a transcription factor. The two novel systems are the exosome system and homeoproteins. (ii) Exosomes are small vesicles that are released upon activation of the axon terminal, traverse the synaptic cleft, probably via astrocytes and are taken up by the postsynaptic neuron. They carry a load of signalling proteins and a variety of forms of RNA. These loads may then be transported widely throughout the postsynaptic neuron and engineer modulations in the fine structure of computational machinery by epigenetic-related processes. (iii) Otx2 is a transcription factor that, *inter alia*, controls the development and survival of PV+ GABAergic interneurons (PV cells) in the primary visual cortex. It is synthesized in the retina and is transported to the cortex by a presently unknown mechanism that probably includes direct cell-to-cell transfer, and may, or may not, include transfer by the dynein and exosome systems in addition. These three mechanisms explain a quantity of data from the field of de- and reafferentation plasticity. These data show that the modality of the presynaptic neuron controls to a large extent the modality of the postsynaptic neuron. However, the mechanism that effects this is currently unknown. The exosome and the homeoprotein hypotheses provide novel explanations to add to the well-established earlier mechanism described above.

1. Introduction

Until recently, the computational role of the brain was seen mainly in terms of rapid electrical and electrochemical events such as spike coding, local field potentials and electroencephalography rhythms [1,2]. Pioneer work on the idea that neurons themselves might change while carrying out their computational functions, in more extensive ways than simply by changing their interconnections and circuitry, was carried out by Eric Kandel [3]. He showed that environmental stimuli activate receptors linked to complex signalling chains, such as those employing cyclic adenosine monophosphate and mitogen-activated protein kinase that terminate by producing transcription factors such as CREB-1, CREB-2 and CPEB, that modulate the synthesis of proteins by the genome in functions such as learning and memory. Since then many other such systems have been discovered.

Over the past decade, this focus has greatly expanded with the discovery of the role that exosomes and their cargoes play in neuronal function [4–6], and

the role that homeoproteins play in the adult brain [7–10]. We are now beginning to appreciate the possibility that the pre-synaptic neuron, via homeoproteins and exosomes, may change the internal computational machinery of the postsynaptic neuron by epigenetic-related mechanisms in remarkable ways. This development took place in two distinct yet interrelated fields—clinical neurology and cell biology.

First, we should clarify what we mean by ‘epigenetics-related’. Traditionally, ‘epigenetics’ has been used to indicate the study of heritable changes in gene activity that are not caused by changes in the DNA sequence, as well as the study of stable, long-term alterations in the transcriptional potential of a cell that are not necessarily heritable. This may involve modulations of DNA and histones by reactions such as methylation and acetylation. Many workers today use a more extended version of the term ‘epigenetics’ in which the activity of many types of non-coding RNAs (that act at the post-transcriptional level) is included together with accessory transporting agents such as exosomes. This seems justifiable as the whole system works in a coordinated fashion. The latter reactions are dynamic rather than permanent or semi-permanent. However, for clarity, our referees suggested that we should give the action of non-coding RNAs and ancillary agents, such as exosomes, a different label. Therefore, we have used the term ‘epigenetic-related’ to cover these latter usages. This paper will discuss epigenetic-related topics.

We present here an extension of a previous hypothesis formulated by other workers that relates to the functional significance of the mRNAs, microRNAs, trophic factors and morphogens carried by exosomes across neuronal synapses. This previous hypothesis had suggested that these agents, forming the cargo of exosomes, modulated activities at the particular synapse they traversed [4,5]. Our present hypothesis suggests that these agents may traffick throughout the post-synaptic neuron, where they may modulate the functional microstructure of interior mechanisms involved in information processing. The paper further discusses the hypothesis that homeoproteins like Otx2 may play a part in this epigenetic system relating to parvalbumin (PV) neurons in the cortex [11].

2. The developments in clinical and experimental neurology

We now know that the modality of a sensory neuron is determined by its source of afferents and that the processing mechanisms in neurons belonging to different modalities are different. The degree to which this is maintained during later stages of growth by any signalling molecules and epigenetic-related factors transmitted from the presynaptic to the postsynaptic neuron is currently unknown. However, under extreme conditions of massive deafferentation and reafferentation, this system can undergo more extensive changes [12–14]. For example, in experiments on blind subjects skilled in Braille, transcranial magnetic stimulation of neurons located in the visual cortex results in a somatosensory, and not a visual, experience [15]. The number of cortical sites inducing tactile sensations appears to be related to the number of hours of Braille reading per day, Braille reading speed and dexterity. In these cases, a number of differentiated ‘visual’ cells are ‘taken over’ by the somatosensory system, and instead start to process somatic information. This activity generates somatosensory sensations in consciousness (i.e.

sensations that the fingers are being touched) in place of the normal visual sensations. The authors concluded [15, p. 198]: ‘Our data show that the qualitative character of the subject’s experience is not determined by the area of cortex that is active (cortical dominance), but by the source of input to it (cortical deference)’.

Another source of information about sensory modality determination stems from sensory input rerouting experiments—diverting the sensory inflow of one system to the cortex of another system by neonatal diversion of, for example, retinal axons to the auditory thalamus (i.e. cross-modal rewiring). This operation leads to profound changes in diverse components of cortical circuitry, at both the anatomical and functional level [16,17]. At a functional level, a deafferented cortex (e.g. the visual cortex in the blind) can take over functions of another sensory modality (e.g. hearing), but only if the functions of the two are homologous. For example, spatial hearing functions are improved but not tone discrimination [18]. If the auditory cortex is deafferented as in the deaf, then visual spatial discrimination is improved, but not colour functions. Heron *et al.* [19] suggest that dedicated asynchrony mechanisms interact with spatially selective mechanisms early in both visual and auditory sensory pathways.

These three sources of evidence indicate that neurons themselves, not just their interconnections, are plastic. These changes would appear to be the result of some signalling system carried by afferent axons. The implication is that these axons are carrying a code which is most probably contained either in the spatio-temporal patterns of its spike trains [6,20], by some form of molecular signal, or both. The identity of this molecular code is currently limited to the first mechanism described earlier. This paper will be concerned with presenting two novel possible further candidates for this role.

3. The developments in cell biology

The classical view in neuroscience has been that neurons communicate only via synapses, which may be chemical or electrical gap junctions, and via paracrine mechanisms involving signalling cascades and protein messengers. Then almost contemporaneously two additional mechanisms were discovered—exosomes and Otx2-like homeoproteins.

(a) Exosomes

About a decade ago, the discovery was made that most cells, including both presynaptic and postsynaptic neurons, bud off exosomes. These exosomes transport protein transcription factors, various types of RNA including mRNA and non-coding RNAs, in particular miRNAs, between cells that may induce phenotypic changes in recipient neurons [21–24].

Three groups of workers have suggested that exosomes and their cargoes play a role in neuronal communication and function:

- (i) The INSERM research group at the University of Grenoble [4,25,26] suggests that exosomes may have a regulatory function at synapses and may present an ideal mechanism for the interneuronal transfer of information. They report that exosomal release is regulated by K^+ -induced depolarization and that mature cortical neurons in culture release exosomes from the somatodendritic compartment of

neurons. This process is modulated by glutamatergic synaptic activity, suggesting a physiological role.

- (ii) Smalheiser [5] has suggested that exosomes are involved in much transsynaptic activity, and that exosomal secretion of proteins and RNAs may be a fundamental mode of communication within the nervous system. He based his hypothesis on the observation that exosomes contain a mixture of proteins and RNAs, including mRNAs and microRNAs [27,28].
- (iii) At the neuromuscular junction of *Drosophila*, the signalling morphogenetic protein Wg is transferred to the interior of exosomes (having budded off from multivesicular bodies) through binding to the exosomal protein Evi ([29]: see their fig. 1).

Microvesicles are a similar species that are formed by membrane blebbing. Furthermore, vesicle-free extracellular RNAs bound to lipoproteins (such as Argonaute 2 and others [30–33]) may have an important signalling function. However, this review will focus on exosomes where the bulk of research has been done. Alvarez *et al.* [34] have published a good paper presenting data that support the importance of exosomes in information transfer in the brain. MiRNAs primarily regulate gene expression at the post-transcriptional level. However, miRNAs can also regulate gene expression at the epigenetic level [35–39]. Furthermore, miRNAs and RNAi processing machinery are present in the nucleus [40–42]. For an excellent review of the involvement of miRNAs in the brain, see Fineberg [43]. A paper by Varela *et al.* [44] explains the logic of miRNA involvement in epigenetic gene regulatory mechanisms (namely targeted post-transcriptional regulation of an epigenetic modifier gene).

4. Our hypothesis relating to particular epigenetic-related factors

Our major hypothesis suggests that epigenetic-related factors play a major role in the processes by which the presynaptic neuron controls the development and function of numerous facets of the functional neuroanatomy of the postsynaptic neuron. The principle agents in this are exosomes that carry loads of protein transcription factors and a wide variety of RNAs between cells. Minor players are homeoproteins, especially *Otx2*, that have a more specialized function.

(a) Exosomes

The researchers quoted in the previous section limited their hypotheses of the function of the exosome system to local retrograde activity at the synapses on which the exosomes attach (save a brief mention by von Bartheld *et al.* [45]). We suggest that this function may extend beyond the synapse to include anterograde activity in the entire postsynaptic neuron [6,46]. In a recent review, Vlassov *et al.* [47, p. 945] state,

Given that exosomes are able to be endocytosed into the endosomal system of recipient cells, it seems likely that, following uptake, exosomes could fuse with the limiting membrane of endosomes to deliver their content into the host cell cytoplasm in a reversal of their formation process.

From the host cytoplasm, a system of carrier molecules could handily and widely deliver exosomes and their cargoes to the rest of the postsynaptic neuron. There is some evidence to

suggest that they do. Tian *et al.* [48] isolated exosomes from PC12 cells, and labelled them with a lipophilic dye and an amino-reactive fluorophore. The exosomes were then incubated with PC12 cells. Live-cell microscopy revealed that the exosomes were taken up into the cells via the endocytosis pathway, enclosed in vesicles and transported to the perinuclear region. This transport may have been mediated by the cytoskeleton. If this process occurs in neurons, then that would support our hypothesis.

There is now considerable evidence to indicate that microRNAs modulate a number of neuronal functions. For example:

- spine numbers and synapse formation are controlled in an activity-regulated manner by miR-485 [49];
- transcription of the microRNA miR-335 is promoted by naturally evoked synaptic activity at the climbing fibre-Purkinje cell synapse in the mouse cerebellar flocculus [50];
- neuronal activity regulates spine formation, in part, by increasing miR-132 transcription, which in turn activates a Rac1-Pak actin remodelling pathway [51];
- microR-181a activity in primary neurons, induced by dopamine signalling, is a negative post-transcriptional regulator of GluA2 expression [52];
- using a mouse line with a conditional neuronal deletion of *Dgcr8* (a microRNA biogenesis protein predicted to process microRNAs exclusively) Hsu *et al.* [53] produced evidence that some microRNAs govern essential aspects of inhibitory transmission and interneuron development in the mammalian nervous system;
- Genneböck *et al.* [54] and de Jong *et al.* [55] show that particular modes of stimulation of parental cells (by platelet-derived growth factor and hypoxia, respectively) induce changes in the transcriptional contents of secreted exosomes. This offers new mechanisms of modulation of computational activities in neurons by exosome loads; and
- microRNA function is required for neurite outgrowth of mature neurons in the mouse postnatal cerebral cortex [56].

The designs of most studies of microRNAs in neurons have concentrated on the effects of these agents on the processes within the same neuron that produced them. However, the evidence now suggests that it is possible that microRNAs, as well as other signalling agents, may be exported from a presynaptic neuron by synaptic, or perisynaptic, transfer via the exosome system, to induce extensive structural and functional changes in the entire postsynaptic neuron.

(i) Additional data relating to exosomes

In a recently published study of thalamus-specific double knockout (*ThVGdK*) mice by Li *et al.* [57], thalamocortical glutamate neurotransmission in somatosensory cortex was silenced or knocked-out, thereby disabling the release of glutamate from synaptic vesicles. This resulted in a failure to construct barrel cortex columns and induced severe defects in both cortical-layer-four lamination and in the morphology of spiny stellate cells. One mechanism responsible for this finding may be that exosome secretion is powerfully activated by glutamate activity at both the NMDA and AMPA receptors [58]. Thus, it would appear likely that, in the genetically engineered glutamate-silenced mice of Li *et al.* [57], the release of exosomes is severely reduced. This would suspend a major source of epigenetic-related factors controlling the structure and function of the postsynaptic neuron. Another

link between glutamate activity and exosomes is provided by Frühbeis *et al.* [58]. They showed that activity-dependent release of the neurotransmitter glutamate triggers oligodendroglial exosome secretion mediated by Ca^{2+} entry through oligodendroglial NMDA and AMPA receptors. In turn, neurons internalize the released exosomes by endocytosis [59].

(b) Homeoproteins

Basic work on this system started about a decade ago mainly by a group at the Collège de France [7–10]. The latest information on this system is contained in Prochiantz *et al.* [60] in this volume. The homeoprotein transcription factor Otx2 is synthesized in the retina and trafficks to the visual cortex where it controls the development and function of PV cells, in particular engineering their critical periods for plasticity. The molecule has the remarkable property of being able to cross a cell membrane without using any energy-consuming mechanism like endo- or exocytosis.

Desgent & Ptito [11] note that auditory thalamic afferents, redirected into the visual system of enucleated hamsters, engineer auditory cortex-like distribution patterns of PV + GABAergic interneurons (INs) and their projections in the primary visual cortex. They suggest that this might be due to the action of a not yet identified homologue of the homeoprotein transcription factor Otx2 carried by these afferents. Presumably, a similar change would also apply if the visually active homeoprotein Otx2 itself were to be redirected into auditory cortex, which should then develop visual cortex-like patterns of INs. This might offer a complementary way of transporting particular transcription factors to engineer plasticity, in addition to the epigenetic factors carried by exosomes that we have discussed earlier in this paper.

Continuous accumulation of Otx2 in PV cells may be necessary to sustain a state that limits further plasticity in the adult [8]. When this supply is cut off by deafferentation, and replaced by an input belonging to another sensory modality, this may allow the cell to undergo plastic changes organized perhaps by a new modality specific homeoprotein carried by the new input. Sugiyama *et al.* [7] have shown that Otx2, suffused into an immature visual cortex, is sufficient to promote the full growth and functionality of PV neurons. Therefore, the contribution of exosome loads must be something other than acting on PV cells in this way. However, cortical restructuring involves neuronal species other than PV cells, as in the case of microRNAs acting upon dendrites and spines listed above. Perhaps the microRNAs, etc., transferred by exosomes play a role in those reactions.

We suggest that it is possible that, early on, a modality specific homeoprotein, like Otx2 for vision, enters the sensory cortex and, by epigenetic and epigenetic-related mechanisms, specifies that piece of the cortex, in the case of Otx2, as generally visual. Later on the influx of exosomes may introduce further subspecification of more detailed visual mechanisms. An analogy in music might be a basic theme later developed by variations.

5. MicroRNAs and homeoproteins as carriers of an epigenetics-related neuroconstructional code (blueprint)

As traditionally described and noted above, the brain's computational code is composed of electrochemical events ('software').

However, we suggest that the brain may also possess an epigenetic-related constructional code that brings about changes in the neuroanatomical structures ('hardware') that conduct these computations. The code consists simply of the nature and quantity of the epigenetic-related material delivered by the afferent neuron, in particular microRNAs, carried by exosomes, and homeoproteins, whose mode of transport is currently not completely understood. In the case of a unisensory neuron (e.g. visual) A, the incoming mix of epigenetic-related factors travelling down the afferent axons will determine the modality specific details fA of its computational machinery, as evidenced by the data from deafferentation and reafferentation experiments. In the case of a unisensory neuron of another modality (e.g. audition) B, these details (fB) will differ, whereupon the machinery will be different. For multisensory neurons (e.g. bimodal), afferent axons belonging to each different modality will carry different combinations of these epigenetic-related agents. Therefore, within the multisensory neuron there will be a mix of A and B that might engineer the construction of computational machinery of a 'bound' format fC (fA + fB = fC). This may serve to determine the nature of the epigenetic-related factors that this bimodal neuron exports to various other neurons at higher levels. In this way, the precise nature of the transmitted epigenetic-related packets carries the information that A and B are bound together. This information is not used for computation *per se*, but to construct the appropriate mechanism to carry out computations in that particular modality or mixture of modalities involved.

In support of this hypothesis there have been several studies of regional patterns of microRNA distribution in the brain.

- Several miRNA families and clusters are differentially expressed between frontal cortex and the hippocampus [61].
- Bak *et al.* [62] report extensive regional differences in mouse brain between the cerebellum, medulla oblongata, hypothalamus, hippocampus and spinal cord.
- Ziats and Rennert [63] found differences in the expression of various microRNAs in the human prefrontal cortex (four areas), hippocampus and cerebellum during development and also between the sexes. The authors state 'However, unlike global gene expression patterns, miRNAs become more differentially expressed between brain regions over time, potentially driving regional specialization as the brain matures' [63, p. 850].
- A differential study by Pichardo-Casas *et al.* [64] found that miR-183, miR-200, miR-200a, miR-200b, miR-211 and miR-429 were exclusively and strongly expressed in the olfactory bulb. Others like miR-206 and miR-378 were selectively and strongly expressed in the cerebellum.
- Other regional differences are recorded by McNeill & Van Vactor [65], Pichardo-Casas *et al.* [64] and Kye *et al.* [66].

In the case of homeoproteins, each sensory modality may be organized by its own specific homeoprotein [11]. There is some evidence to suggest that the unknown auditory homologue of the visual Otx2 may be *Dix1*. This homeoprotein is a transcription factor that regulates the development, migration and survival of cortical interneurons. Conditional ablation of *Dix1* resulted in disturbances in processing of auditory stimuli [65]. Other homeoproteins that may cross membranes in the manner employed by Otx2 may be Nkx3 [67] and Hoxc8 [68]. However, since Otx2 is found in other cortical areas besides visual (A Prochiantz 2013, personal

communication), it is also possible that Otx2 has this function in these other cortical areas as well.

The manner of travel of Otx2 between the retina and the visual cortex has not as of yet been fully explained. One hypothesis is that it uses the cell-to-cell method described above throughout—or in part. It is difficult, however, to think how this cell-to-cell method could operate in axons. But the molecules might be transferred by the slow route of axonal transport in which numbers of individual protein molecules are packaged into bodies called multiprotein complexes and transported at a rate of around 8 mm d^{-1} [69]. Alternatively, they could be loaded into vesicles and transported by the fast route using dynein motors and a microtubule track at a speed of up to 200 mm d^{-1} . The Otx2 molecule could cross a synapse by the cell-to-cell mechanism via the astrocytes that embed the synapse. Or it could be taken up by the exosome system and transported by that. A third possible route might be to use the mechanism employed by botulinum toxin by binding to recycling synaptic vesicles [70]. Only further experiments could settle this issue.

6. Experiments to test and use our hypothesis

The epigenetic-related codes within different parts of the brain could be read by determining the identity and quantity of its individual signalling proteins, including homeoproteins and nucleic acids as listed in this paper. It is possible that each modality area will have its own unique pattern. Modern techniques are capable of identifying the individual constituents within exosomes. For example, Hooper *et al.* [71] report that they detected, within exosomes released from microglia by Wnt3a, 45 different proteins associated with cellular architecture, metabolism, protein synthesis and protein degradation, including beta-actin, glyceraldehyde-3-phosphate dehydrogenase, ribosomal subunits and ubiquitin. In our case, a detailed study of the identity and quantities of the payload of neurons and exosomes from different brain regions might give information as to the functional connectivity of those areas. The technology to accomplish this task has already been described by He *et al.* [72]. They reported finding the expression of a large fraction of known miRNAs

with distinct profiles in glutamatergic and GABAergic neurons, as well as subtypes of GABAergic neurons, in mouse cerebellum and cortex. Another useful experiment would be to determine whether exosomes carried by afferent cortical axons carry any homeoproteins in their cargoes. Further experiments on the role of exosomes in glutamate knock-out phenomena are indicated.

7. Conclusion

Our present hypothesis relating to exosomes involves a simple logical step from a previous hypothesis and offers an explanation for a large and growing body of heretofore unassimilated data. The deafferentation and reafferentation observations demonstrate that afferent nerves carry codes that produce extensive structural and functional changes in the postsynaptic neuron. Some of these codes may be electrical, but others may be molecular. Results from cell biology studies reveal that exosomes do in fact transport and deliver across synapses precisely the type of molecule necessary to carry such a molecular code. This would seem to be an unlikely coincidence. Furthermore, homeoproteins carried by the afferent axons to the sensory cortex provide another mechanism to modulate modality specificity.

One referee invited us to develop our theme that the brain modifies its hardware as a means of modifying its software for the benefit of neuroscientists and computer scientists. We do not see the process as modifying the software exactly: rather that the brain appears to be able, to some extent, to change its structural hardware, so as to process variations of software the better. This hardware particularly involves the modulation of the fine detail of the microanatomy of dendritic spines by epigenetic-related miRNAs, as presented elsewhere in this volume by Smalheiser [73]. We hope that computer scientists will develop more details of how this modulation could take place.

The brain may be a computer, but, if it is, then it seems to be one with a most unusual property—the innate ability to restructure its own hardware in order to optimally process the dynamic and ever-changing inflow of software.

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