Lineage divergence of activity-driven transcription and evolution of cognitive ability

Giles E. Hardingham, Priit Pruunsild, Michael E. Greenberg and Hilmar Bading

Abstract | Excitation–transcription coupling shapes network formation during brain development and controls neuronal survival, synaptic function and cognitive skills in the adult. New studies have uncovered differences in the transcriptional responses to synaptic activity between humans and mice. These differences are caused both by the emergence of lineage-specific activity-regulated genes and by the acquisition of signal-responsive DNA elements in gene regulatory regions that determine whether a gene can be transcriptionally induced by synaptic activity or alter the extent of its inducibility. Such evolutionary divergence may have contributed to lineage-related advancements in cognitive abilities.

The basic brain structures and anatomical connectivities that enable perception, motor function, social behaviour and cognitive skills are similar between mice and humans. However, in the 80–100 million years since the last common ancestor of these two species existed, human brain complexity and circuit organization have diverged substantially from those of mice, coinciding with the development of superior intellectual abilities in humans[1–3]. The prevailing view is that in mammals, such phenotypic divergence has been driven primarily by changes in the relative expression levels of genes[4], rather than by differences in their coding regions. This hypothesis, which is over 40 years old[5], is grounded in the observation that there are substantial biological differences between species, despite conservation of protein sequences[5], and is supported by recent genome-wide comparative transcriptome analyses that have demonstrated human-specific gene expression networks in the brain and elsewhere[6–8].

The evolutionary changes in gene expression that have supported progression towards an increased cognitive repertoire in humans may have been caused by alterations in regulatory DNA regions, such as promoters and enhancers. Indeed, one study demonstrated that forebrain-specific enhancers that are active in early development show high levels of evolutionary conservation, whereas enhancers that are active after mid-gestation do not[9]. The results of this genome-wide study of the preservation of enhancer activities suggest that conserved core gene expression patterns (such as those that control cortical neuron identity[10]) give way to later, species-specific specializations in gene expression.

An area that has received less attention in the context of evolutionary divergence and mammalian brain development is the impact of signal-regulated changes in the transcriptome on the construction of neuronal networks during development and on adaptive brain functions in the adult. Signal-regulated transcription is a basic feature of synaptically activated neurons that allows the conversion of short-lasting electrical events into long-lasting structural and functional alterations. For example, gene expression induced by synaptic activity supports the stabilization of synapses, allows for maintenance of changes in synaptic efficacies, leads to adjustments of metabolism and boosts neuronal survival. The principal mechanisms underlying signal transduction from the synapse to the nucleus in rodent neurons are well understood, and the most crucial target DNA regulatory elements and their cognate transcription factors, which are responsible for mounting the gene expression responses, have been identified (BOX 1).

Whole-transcriptome studies using rodent neurons have identified several hundred activity-responsive genes[11–14]. However, these results are difficult to extrapolate to humans because 80 million years is sufficient time to have allowed substantial divergence of transcription factor binding sites in gene promoters[15]. Given that experimental alterations in the regulatory region of a gene can dramatically change its responsiveness to neuronal activity[16–19], it is expected that these naturally occurring, lineage-related promoter variations may also translate into altered signal-evoked transcriptional responses and, thus, are likely to change the nature of the neurons' adaptive response to synaptic activity. This article provides an overview of recent evidence[20–22] that the responsiveness to neuronal activity of the genome has indeed changed during evolution. We discuss both the underlying mechanisms and potential functional implications for the evolution of cognitive abilities.

Activity-regulated transcription

Comparing mouse and human neurons. Three recent studies set out to probe the transcriptional response to neuronal activity of human neurons and to compare it to that of mouse neurons[20–22]. Albeit using different cell sources and activation paradigms, all three studies demonstrated the conserved activity responsiveness of human orthologues of many well-studied activity-regulated mouse genes, including classical immediate-early genes. This is in line with the concept of a largely generic and conserved neuronal activity-dependent transcriptional programme[23]. However, all three studies also uncovered evolutionary divergence of activity-regulated transcriptomes.

In their study, Qiu *et al.*[20] compared cortical neurons derived from human embryonic stem cells (ESCs) with mouse primary cortical neurons or cortical neurons derived from mouse ESCs and showed that the extent to which the mRNA levels of orthologous human and mouse genes changed in response to membrane

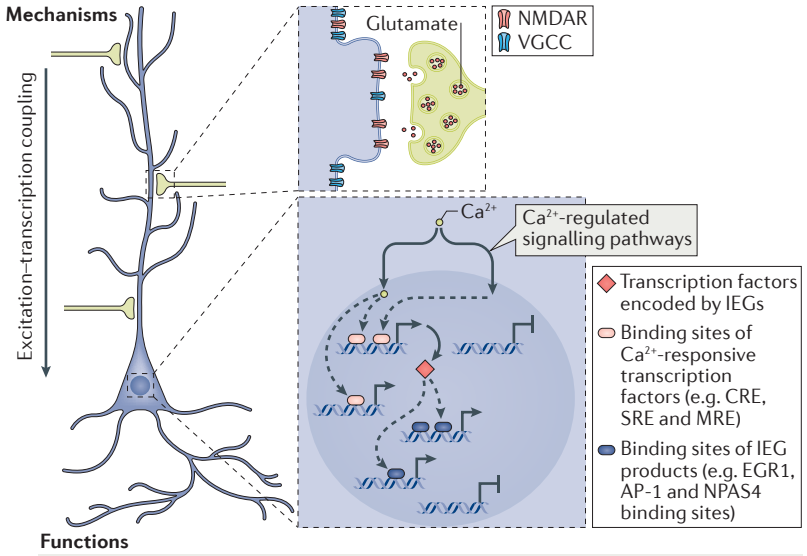
Box 1 | **Mechanisms and functions of neuronal activity-dependent transcription**

Transcriptional responses to electrical activity were first described in the rat pheochromocytoma cell line PC12, where membrane depolarization and Ca^{2+} influx evoked by elevated extracellular K^+ concentration trigger induction of *Fos*[66,67]. Experiments with networks of rat primary hippocampal neurons identified Ca^{2+} as the principal second messenger that operates via distinct synapse-to-nucleus signalling pathways and links neuronal excitation to transcriptional regulation[16]. The type of transcriptional response — that is, the nature of the target genes and the temporal profile of their induction — is influenced by the pattern of electrical activity, the spatial properties of the Ca^{2+} signal, the degree of Ca^{2+} release from internal stores, the route of Ca^{2+} entry and even the oscillatory frequency of the Ca^{2+} signals[23,68–70].

The evolutionary conservation of the mechanisms underlying activity-regulated transcription (see the figure) was already apparent in early experiments[16] in which human *FOS* and mutated versions of its promoter were transfected into rat primary hippocampal neurons in order to map the activity-controlled DNA motifs that are targeted by Ca^{2+}-regulated signalling pathways. This experiment showed that the initiators of activity-dependent transcription are synaptic NMDA receptors (NMDARs) and voltage-gated Ca^{2+} channels (VGCCs)[22,70]. Synapse-to-nucleus communication is mediated by Ca^{2+} itself, which can invade the cell nucleus and activate nuclear Ca^{2+}/calmodulin-dependent protein kinases and phosphatases. Ca^{2+} also activates protein-based signalling pathways, including those mediated by the extracellular-signal-regulated kinase–mitogen-activated protein kinase (ERK–MAPK) cascade[71], Jacob (also known as NMDAR synaptonuclear signalling and neuronal migration factor)[72] and CREB-regulated transcription co-activator 1 (CRTC1)[73].

Whether or not transcription-regulating Ca^{2+} signalling pathways induce expression of a gene depends on the presence of binding sites for Ca^{2+}-responsive transcription factors in its promoter or enhancers. Examples of regulatory DNA motifs that are responsive to synaptic activity are indicated in the figure by coloured boxes; they include the cAMP response element (CRE), the serum response element (SRE) and the myocyte-specific enhancer factor 2 (MEF2) response element (MRE). Such DNA regulatory elements are often found in the promoters of immediate-early genes (IEGs), which explains the robust induction of these genes by synaptic activity. Many IEGs encode transcription factors, such as FOS (a component of the activator protein 1 (AP-1) complex), early growth-response protein 1 (EGR1) and neuronal PAS domain-containing protein 4 (NPAS4), which in turn bind their cognate target DNA elements and take part in producing the late phase of the activity-induced gene expression programme[23,62,70].

Excitation–transcription coupling is conserved from invertebrates to vertebrates and plays a key role in translating synaptic activity into physiological responses in the developing and the adult brain[74–77]. Many cell biological processes, including dendritic outgrowth and arborization, synapse formation and maturation, network excitatory–inhibitory balance, synaptic plasticity, neuronal survival and metabolic homeostasis are controlled by activity-dependent gene expression[23,62,78–80]. Moreover, a number of neurodevelopmental disorders, particularly autism spectrum disorders, have been associated with mutations in genes that are known to affect synaptic-activity-dependent transcription in mouse models[81,82].



depolarization was similar. However, the correlations were far from perfect, suggesting that there are quantitative differences in activity-dependent transcriptional responses between the two species, with some genes being more (and others less) responsive to activity in human neurons than in mouse neurons.

As a means of evoking neuronal activity, Ataman *et al*[21]. also induced membrane depolarization, in this case of primary cells derived from human fetal brain and of mouse or rat primary neurons. Pruunsild *et al.*[22], on the other hand, triggered excitatory synaptic activity within networks of human neurons derived from induced pluripotent stem cells (iPSCs) or within networks of co-cultured human iPSC-derived neurons and mouse primary neurons by the administration of a GABA type A receptor antagonist together with a weak K^+ channel blocker. This stimulation protocol is often used for gene expression analyses in rodent neuronal networks[24]. In both studies, the comparison of transcriptional responses in human and mouse neurons revealed remarkable differences in the kinetics of transcriptional induction for a number of human and mouse gene orthologues, even though the mechanisms that mediate synapse-to-nucleus communication and regulate the genomic response in human neurons were shown to involve the evolutionarily highly conserved nuclear Ca^{2+}/calmodulin kinase pathway[22,23].

In addition to these quantitative findings, all three studies revealed qualitative differences between the human and mouse activity-regulated transcriptomes[20–22]. For example, some human activity-regulated genes, such as the protein-coding gene *ZNF331* (REFS 21,22) and the genes producing the non-coding RNAs LINC00473 (REFS 21,22) and BRE-AS1 (REF. 22), lack a mouse orthologue, either because it has been lost in the mouse (in the case of *ZNF331*) or because it has been acquired in humans (in the cases of *LINC00473* and *BRE-AS1*). Other genes, such as *OSTN* (which encodes osteocrin; also known as musclin)[21], *CAMTA1* (which encodes calmodulin-binding transcription activator 1)[20,22] and *TUNAR*[20,22], are present both in the human genome and in the mouse genome but are activity-regulated in human neurons only[20–22] (TABLE 1).

Divergent promoter architectures. The lineage-specific differences in responses to neuronal activity of gene orthologues described above could arise either owing to divergence in the genes' regulatory

Table 1 | **Genes with divergent activity responsiveness in human and mouse neurons**

Human gene	Function and/or relevance	Fold change in expression in response to activity		Refs
		Human neurons	Mouse neurons	
ADRA1B	Associated with attention-deficit–hyperactivity disorder	4.3*	0.9*	20,22,84
ATP1B3	Associated with absence epilepsy	3.5*	1.2*	20,21,85
BRE-AS1[‡,§]	Not known	26.6[‖]	NA	22
CAMTA1	Associated with episodic memory performance and intellectual disability	1.5[¶]	0.7[¶]	20,22,32,86
CCNH	Associated with brain tumour risk	3.7*	0.9*	20,21,87
CENPN	Involved in regulation of chromosome segregation	2.5[#]	0.8[#]	20,21,88
CTNNAL1	Involved in modulation of Rho GTPase signalling	4.6*	1.3*	20,21,89
DNMBP	Associated with Alzheimer disease and memory performance in aged rats	2.3*	0.9*	20,22,90,91
DUSP3	Involved in modulation of ERK–MAPK signalling	2.2*	1.1*	20,21,92
ETS2[§]	Implicated in apoptosis of neurons in Down syndrome	3.8*	1.1*	20,93
GNB4	Mutations cause Charcot–Marie–Tooth disease	2.2[#]	0.9[#]	20,21,94
GREM2	Associated with Warburg Micro syndrome	8.1[#]	1.4[#]	20,21,95
HIC1[§]	Involved in regulation of reelin receptor genes	6.5[¶]	1.3[¶]	22, 29
LINC00473[‡,§]	Involved in regulation of IEGs	48.7[¶]	NA	21,22,96
MAFG	Loss of function causes neuronal degeneration in mice	2.4*	1.1*	20,21,97
NKD2	Role in negative regulation of Wnt signalling	5.4*	1.1*	20,22,98
OSTN[§]	Contributes to negative regulation of activity-dependent dendritic growth	102.9[#]	ND	21
RTL1	Potential proneural functions in the developing telencephalon	4.4[¶]	1.3[¶]	20,22,99
TUNAR	Involved in regulation of pluripotency and neural lineage commitment	3.8*	1.0*	20,22,100
ZNF331[‡,§]	Tumour suppressor function	4.9**	NA	21,22,101

The table shows examples of genes that were transcriptionally induced by activity in human neurons but have mouse orthologues that displayed no induction, significantly weaker induction or different induction kinetics in mouse neurons. Because different activity-inducing stimulation paradigms and durations were used in different studies, only the highest fold change detected in human neurons (together with the corresponding change in mouse neurons) is shown. ERK–MAPK, extracellular-signal-regulated kinase–mitogen-activated protein kinase; IEGs, immediate-early genes; NA, not applicable; ND, not detected. *Experiment involved 3 h membrane depolarization. [‡]No orthologue in rodents. [§]Experimentally validated at the level of promoter activity. [‖]Experiment involved 1 h evoked excitatory synaptic activity. [¶]Experiment involved 4 h evoked excitatory synaptic activity. [#]Experiment involved 6 h membrane depolarization. **Experiment involved 1 h membrane depolarization.

regions or to differences in the cellular environment and signal-processing machinery of the neuron. A compelling experiment by Qiu *et al.* strongly supports the idea that the divergence in genes' regulatory regions is the predominant factor[20]. By using neurons derived from the Tc1 transchromosomic mouse strain, which carries a copy of human chromosome 21 (HSA21), they studied HSA21 genes and their mouse orthologues side by side in the same cellular environment. The results of this experiment broadly recapitulated the differences that were observed in separate human and mouse neuron preparations[20], providing strong evidence that changes in DNA sequence must, at least partly, underlie the differential activity responsiveness of the human and mouse orthologues. However, the existence of as yet undiscovered activity-responsive factors that are unique to the order Primates or to the human lineage cannot be ruled out as an additional contributor to this evolutionary divergence.

The three studies[20–22] suggested that acquisition of signal-regulated DNA elements in gene regulatory regions underlies the evolutionary divergence of activity responsiveness. Among the regulatory elements that were shown to have been acquired by promoters during evolution are binding sites for activator protein 1 (AP-1), myocyte-specific enhancer factor 2 (MEF2) and early growth-response protein 1 (EGR1)[20–22]. MEF2 is regulated directly by Ca^{2+} signalling pathways, whereas EGR1 and AP-1 (heterodimers of the FOS and/or JUN families) are themselves transcriptionally induced by electrical activity. Thus, these DNA elements can confer direct or indirect activity responsiveness onto a gene.

The promoter of human *ETS2*, which was found to be more strongly induced by activity than its mouse counterpart, was revealed to have gained three activity-responsive AP-1 sites in evolution, two being primate-specific and one being hominid-specific[20]. The promoter of *OSTN*, a gene robustly induced in human neurons but not in mouse or rat neurons, was shown to have become activity-responsive in the primate lineage by gaining binding sites for MEF2 (REF. 21). As a result of the presence of an EGR1 binding site in the human promoter that is absent in the mouse promoter, *HIC1* displayed sustained induction by activity in human neurons but only transient induction in mouse[22]. Thus, transcriptional inducibility by synaptic activity has changed during evolution through the emergence of signal-regulated DNA elements in the genes' regulatory regions.

It is unknown whether these divergent promoter architectures evolved through natural selection or result from genetic drift — a process characterized by random fluctuations in the number of gene variants in a population. Nevertheless, there is good evidence that remodelling of gene regulatory regions is a general feature of molecular evolution[25]. Gains, and also losses, of activity-responsive regulatory elements probably occurred in other lineages and contributed to the divergence of activity-dependent transcriptomes between species. The extent to which there are human lineage-specific differences remains a matter of further investigation. As noted above, the critical activity-responsive promoter element in *OSTN* is present in other primates, as are two of the sites in the *ETS2* promoter. In addition to targeting specific examples, however, it would be instructive to perform side-by-side analyses of neurons derived from human, monkey (such as rhesus macaque) and ape (such as chimpanzee) iPSCs. Assessment of the activity-dependent transcriptomes across these closely related primate species, as well as in rodents, would clarify whether any leap in divergence occurred after the human lineage separated from that of other primates or happened earlier, after primates and rodents diverged.

Functional impact of divergent gene inducibility. Studies of *OSTN* have provided insight into the potential functional consequences of a lineage-specific gain of neuronal activity-responsive DNA motifs in gene regulatory regions. *OSTN* was shown to be induced by activity *in vivo* in the primate lineage in experiments that manipulated the sensory input to the macaque visual cortex by monocular deprivation[21]. Activity-dependent expression of *OSTN* in layer IV of the primary visual cortex as well as across the multimodal parietal cortex pointed towards a potentially widespread role for this activity responsiveness in development. Indeed, its cortical expression in human peaks in mid-late gestation and remains high through childhood[26]. Overexpression and knockdown studies *in vitro* identified *OSTN,* previously known to have functions in muscle and bone in mice[27,28], as a negative regulator of dendritic growth and arborization in primate neurons[21]. This suggests that *OSTN* has been repurposed, through a primate-specific remodelling of its regulatory region, to serve a role in the experience-dependent organization of cortical networks in primates.

The functions of several other genes regulated by neuronal activity in humans but not in mice (TABLE 1) suggest further mechanisms by which synaptically activated human neurons may mount a physiological response that is distinct from that of mouse neurons. One example is *HIC1*, which has been shown to repress the transcription of the reelin receptor genes *LRP8* (also known as *APOER2*) and *VLDLR*[29]. The sustained induction of *HIC1* expression by synaptic activity in human neurons[22] could provide control of reelin signalling[30] that is different from that in mouse neurons, where *Hic1* is induced transiently[22]. Another example is CAMTA1, a Ca^{2+}-sensitive transcription factor that is involved in cognitive functions. *CAMTA1* polymorphisms in humans correlate with performance levels in psychological tests[31], and haploinsufficiency of *CAMTA1* results in non-progressive cerebellar ataxia with mental retardation[32]. In mice, *Camta1* knockdown leads to impairments in the formation of long-term memory[33], and loss of *Camta1* causes ataxia and cerebellar atrophy[34]. The function of CAMTA1 in both human and mouse neurons is regulated through its interaction with Ca^{2+}/calmodulin[35]. However, in human neurons, activity can also alter the rate of *CAMTA1* transcription[22], adding another level of *CAMTA1* regulation.

Implications for brain function

The discovery of operational changes in activity-regulated transcription in evolution caused by lineage-related divergence in promoter architectures provides the mechanistic basis for a new conceptual framework that may help us to understand the evolution of cognitive abilities. According to this hypothesis, species-specific activity-regulated transcriptomes may both specify the construction of neuronal networks during development and determine their capacity for structural and functional plasticity in the adult. This could contribute to the advancement of cognitive abilities during evolution and the development of the intellectual power of humans.

Many neuronal activity-regulated genes are expressed with a human-specific temporal profile in the brain[36]. Moreover, disturbance of this human-specific gene expression programme is associated with autism spectrum disorder (ASD)[37], consistent with experience-dependent gene expression playing a role in both circuit development and cognition. These observations support the view that activity-regulated transcription may be a source of the dissimilarities in brain development and function of humans compared with other species. It is known that activity-induced control over brain development begins prenatally. For example, cortical neuron migration, and probably gyrogenesis as well, is regulated by synaptic inputs and NMDA receptor-induced Ca^{2+} signalling[38,39], which also strongly activates gene expression[16]. Evolutionary divergence of the activity-dependent transcriptome could affect sensory-input-mediated maturation of brain networks. This may represent a major functional consequence of promoter divergence in addition to its impact on the responses of mature circuits to neuronal activity. The neuronal structure-related function of particular genes, such as *OSTN*[21], provides an example of how species-dependent activity-driven gene regulation could affect dendritic arborization and network architecture, which have been linked to cognitive abilities[40]. However, the evolutionary adjustments that bring about the progression of the intellectual capacities of the brain are probably the result of the combined effects of alterations in the activity responsiveness of many genes.

Until now, the genetic bases of vocal learning, neuronal metabolism[41–43] and, especially, expansion and convolution of the neocortex[44–47] have been the centre of attention in searches for the evolutionary changes that underlie the cognitive capacity of primates and the pre-eminence of the human brain. There is plenty of evidence that alterations in developmental gene regulation programmes and gene expression patterns in the brain are major sources of this phenotypic divergence[1,8,48]. Evolutionary advances, such as the emergence of lineage-specific genes or gene copies[49–51], species-dependent gene regulatory changes[52,53] and cortical region-specific patterns of gene expression[54], are likely to have provided a basis for the increase in cortex size and the concurrent elaboration of its structure that, in the primate lineage, are driven in part by the evolvement of neuronal progenitor proliferative capacity[55,56]. The new findings described above[20–22] provide an additional mechanism for human brain evolution in which the evolution of a particular regulatory property of neuronal genes (namely, the responsiveness of the genome to synaptic activity) may have expanded the capacity of the brain from being able to execute core cognitive tasks, such as learning and memory, to permit highly advanced functions, such as language and theory of mind (FIG. 1).

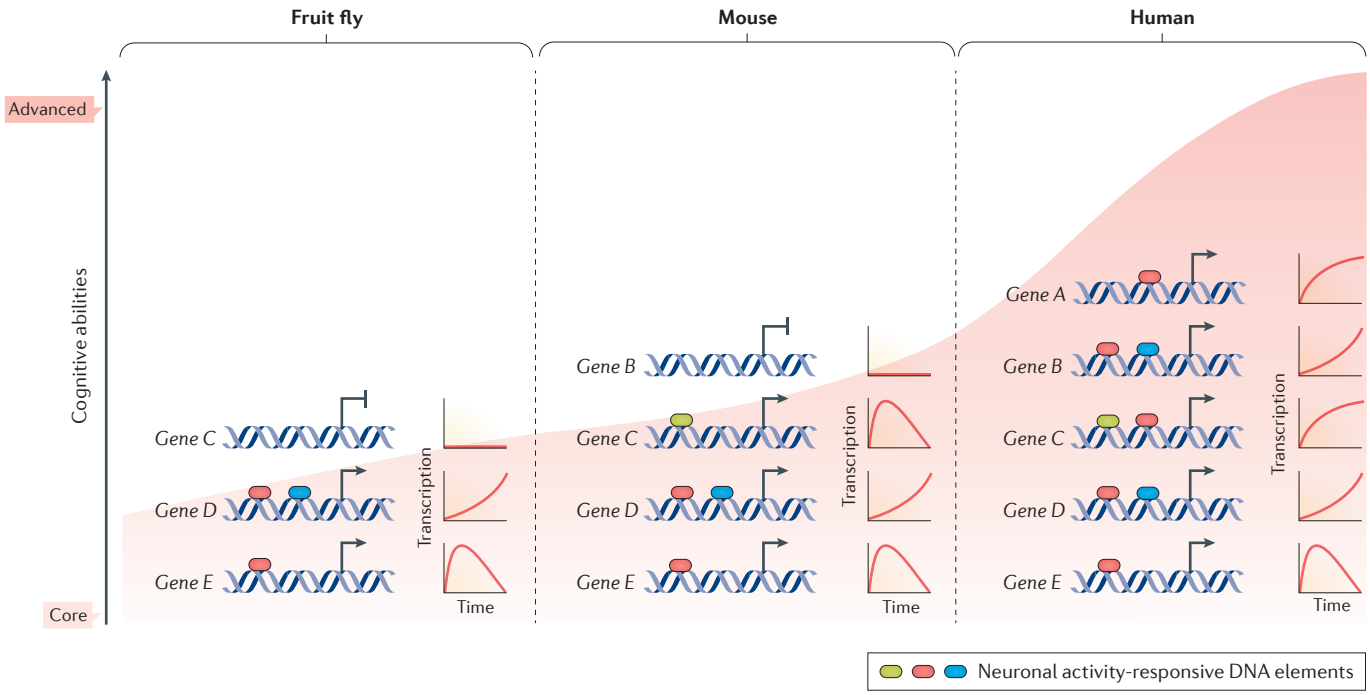


Figure 1 | **Linking evolutionary remodelling of promoter architectures to altered activity-dependent gene expression and cognitive abilities.** The figure shows a schematic representation of hypothetical gene orthologues present in one, two or all depicted species — fruit fly, mouse and human — together with graphs illustrating their transcriptional response to synaptic activity. Orthologous genes are presented in parallel and are named *gene A*, *gene B*, *gene C* and so on. According to our hypothesis, gaining activity-responsive DNA elements (depicted as coloured boxes) in regulatory regions of orthologous genes in one lineage either confers inducibility (defined as a synaptic-activity-regulated increase in the rate of transcription, which is indicated by arrows; blunt arrows denote that the gene is not inducible) upon that gene, as shown for *gene B* (which is present in mouse and human but gains inducibility in the human lineage) or alters the kinetics and/or magnitude of its responsiveness to synaptic signals, as shown for *gene C* (which is inducible in both the mouse and human lineages but demonstrates different response kinetics). The emergence of activity-regulated lineage-specific genes (such as *gene A*, present only in the human) increases the evolutionary divergence of activity-regulated transcriptomes between species. Combined, this may contribute to lineage-specific advancements in cognitive abilities. For simplicity, gains of activity-responsive genes are shown only for the mouse and human. The fruit fly is included as an example of an invertebrate that uses excitation–transcription coupling via evolutionarily conserved nuclear Ca^{2+}-regulated mechanisms for core cognitive functions[83]. This schematic illustration is not intended to be comprehensive and categorical, and it does not include all possible scenarios by which activity responsiveness may be conferred upon a gene (for example, through the loss of a repressive DNA element) and does not imply that advancement of cognitive abilities is necessarily linear.

Progression towards a superior performance level may rest on altered (perhaps increased) network complexity that is empowered by the expansion of brain size[57] as well as by the implementation of particular structural measures and plasticity capacities. Such features of neuronal circuits have been implicated in the evolution of the human brain[58–61] and, moreover, are the prototypical targets for regulation by neuronal activity-induced genes[23,62].

Finally, it should also be noted that changes in the architecture of gene regulatory regions may not only affect intellectual capacities but also increase the vulnerability of neurons to conditions that initiate degenerative processes. A common pathology in a broad spectrum of excitotoxicity-associated neurodegenerative diseases, including stroke, Alzheimer disease, Huntington disease and amyotrophic lateral sclerosis, is a transcriptional shut-off mechanism that is triggered by increased extrasynaptic NMDA receptor signalling[63,64]. The cAMP response element (CRE) binding protein CREB, the prototypical activity-regulated transcription factor[23,62], is a prime target of this shut-off mechanism[65]. Thus, acquisition by the human genome of new CREs that bind CREB and may confer new responses to synaptic activity could also render the human transcriptome more susceptible to deregulation in disease.

Concluding remarks

The coupling of neuronal excitation to gene transcription is mechanistically highly conserved from invertebrates to mammals and triggers largely generic gene expression responses. However, during evolution, the responsiveness of certain promoters has evolved through the acquisition of DNA motifs that serve as genomic targets of transcription-regulating synapse-to-nucleus signalling pathways. The specificity of transcriptional profiles evoked by synaptic inputs in different species, genera or other taxonomic ranks may contribute to differences in the construction and functioning of neuronal networks, providing a mechanism that fuels the advancement of cognitive abilities in evolution. This hypothesis could be tested; for example, mutations could be experimentally introduced into putative activity-responsive promoter elements that are present in the human genome but not the mouse, and the effect of these mutations on functional responses to electrical activity in human neurons could be probed. Alternatively, mouse promoters could be altered to resemble the architecture of their human orthologues. Such promoter-editing studies may shed light on facets of brain development, cognitive function and disease that are unique to humans.

Giles E. Hardingham is at the UK Dementia Research Institute at The University of Edinburgh, Edinburgh Medical School, 47 Little France Crescent, Edinburgh EH16 4TJ, UK.

Priit Pruunsild and Hilmar Bading are at the Department of Neurobiology, Interdisciplinary Center for Neurosciences (IZN), Heidelberg University, 69120 Heidelberg, Germany.

Michael E. Greenberg is at the Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, USA.

Correspondence to H.B.
bading@nbio.uni-heidelberg.de

doi:10.1038/nrn.2017.138
Published online 23 Nov 2017

1. Geschwind, D. H. & Rakic, P. Cortical evolution: judge the brain by its cover. *Neuron* **80**, 633–647 (2013).
2. Izpisua Belmonte, J. C. *et al.* Brains, genes, and primates. *Neuron* **86**, 617–631 (2015).
3. Kaas, J. H. The evolution of brains from early mammals to humans. *Wiley Interdiscip. Rev. Cogn. Sci.* **4**, 33–45 (2013).
4. Necsulea, A. & Kaessmann, H. Evolutionary dynamics of coding and non-coding transcriptomes. *Nat. Rev. Genet.* **15**, 734–748 (2014).
5. King, M. C. & Wilson, A. C. Evolution at two levels in humans and chimpanzees. *Science* **188**, 107–116 (1975).
6. Konopka, G. *et al.* Human-specific transcriptional networks in the brain. *Neuron* **75**, 601–617 (2012).
7. Nord, A. S., Pattabiraman, K., Visel, A. & Rubenstein, J. L. Genomic perspectives of transcriptional regulation in forebrain development. *Neuron* **85**, 27–47 (2015).
8. Silbereis, J. C., Pochareddy, S., Zhu, Y., Li, M. & Sestan, N. The cellular and molecular landscapes of the developing human central nervous system. *Neuron* **89**, 248–268 (2016).
9. Nord, A. S. *et al.* Rapid and pervasive changes in genome-wide enhancer usage during mammalian development. *Cell* **155**, 1521–1531 (2013).
10. Shim, S., Kwan, K. Y., Li, M., Lefebvre, V. & Sestan, N. Cis-regulatory control of corticospinal system development and evolution. *Nature* **486**, 74–79 (2012).
11. Kim, T. K. *et al.* Widespread transcription at neuronal activity-regulated enhancers. *Nature* **465**, 182–187 (2010).
12. Spiegel, I. *et al.* Npas4 regulates excitatory-inhibitory balance within neural circuits through cell-type-specific gene programs. *Cell* **157**, 1216–1229 (2014).
13. Zhang, S. J. *et al.* Decoding NMDA receptor signaling: identification of genomic programs specifying neuronal survival and death. *Neuron* **53**, 549–562 (2007).
14. Zhang, S. J. *et al.* Nuclear calcium signaling controls expression of a large gene pool: identification of a gene program for acquired neuroprotection induced by synaptic activity. *PLoS Genet.* **5**, e1000604 (2009).
15. Villar, D., Flicek, P. & Odom, D. T. Evolution of transcription factor binding in metazoans - mechanisms and functional implications. *Nat. Rev. Genet.* **15**, 221–233 (2014).
16. Bading, H., Ginty, D. D. & Greenberg, M. E. Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. *Science* **260**, 181–186 (1993).
17. Hong, E. J., McCord, A. E. & Greenberg, M. E. A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. *Neuron* **60**, 610–624 (2008).
18. Robertson, L. M. *et al.* Regulation of *c-fos* expression in transgenic mice requires multiple interdependent transcription control elements. *Neuron* **14**, 241–252 (1995).
19. Hardingham, G. E., Chawla, S., Johnson, C. M. & Bading, H. Distinct functions of nuclear and cytoplasmic calcium in the control of gene expression. *Nature* **385**, 260–265 (1997).
20. Qiu, J. *et al.* Evidence for evolutionary divergence of activity-dependent gene expression in developing neurons. *eLife* **5**, e20337 (2016).
21. Ataman, B. *et al.* Evolution of osteocrin as an activity-regulated factor in the primate brain. *Nature* **539**, 242–247 (2016).
22. Pruunsild, P., Bengtson, C. P. & Bading, H. Networks of cultured iPSC-derived neurons reveal the human synaptic activity-regulated adaptive gene program. *Cell Rep.* **18**, 122–135 (2017).
23. Bading, H. Nuclear calcium signalling in the regulation of brain function. *Nat. Rev. Neurosci.* **14**, 593–608 (2013).
24. Hardingham, G. E., Arnold, F. J. & Bading, H. Nuclear calcium signaling controls CREB-mediated gene expression triggered by synaptic activity. *Nat. Neurosci.* **4**, 261–267 (2001).
25. Taylor, M. S. *et al.* Heterotachy in mammalian promoter evolution. *PLoS Genet.* **2**, e30 (2006).
26. Kang, H. J. *et al.* Spatio-temporal transcriptome of the human brain. *Nature* **478**, 483–489 (2011).
27. Moffatt, P. & Thomas, G. P. Osteocrin — beyond just another bone protein? *Cell. Mol. Life Sci.* **66**, 1135–1139 (2009).
28. Subbotina, E. *et al.* Musclin is an activity-stimulated myokine that enhances physical endurance. *Proc. Natl Acad. Sci. USA* **112**, 16042–16047 (2015).
29. Dubuissez, M. *et al.* The Reelin receptors ApoER2 and VLDLR are direct target genes of HIC1 (Hypermethylated In Cancer 1). *Biochem. Biophys. Res. Commun.* **440**, 424–430 (2013).
30. Forster, E. *et al.* Emerging topics in Reelin function. *Eur. J. Neurosci.* **31**, 1511–1518 (2010).
31. Miller, L. A. *et al.* CAMTA1 T polymorphism is associated with neuropsychological test performance in older adults with cardiovascular disease. *Psychogeriatrics* **11**, 135–140 (2011).
32. Thevenon, J. *et al.* Intragenic CAMTA1 rearrangements cause non-progressive congenital ataxia with or without intellectual disability. *J. Med. Genet.* **49**, 400–408 (2012).
33. Bas-Orth, C., Tan, Y. W., Oliveira, A. M., Bengtson, C. P. & Bading, H. The calmodulin-binding transcription activator CAMTA1 is required for long-term memory formation in mice. *Learn. Mem.* **23**, 313–321 (2016).
34. Long, C. *et al.* Ataxia and Purkinje cell degeneration in mice lacking the CAMTA1 transcription factor. *Proc. Natl Acad. Sci. USA* **111**, 11521–11526 (2014).
35. Finkler, A., Ashery-Padan, R. & Fromm, H. CAMTAs: calmodulin-binding transcription activators from plants to human. *FEBS Lett.* **581**, 3893–3898 (2007).
36. Liu, X. *et al.* Extension of cortical synaptic development distinguishes humans from chimpanzees and macaques. *Genome Res.* **22**, 611–622 (2012).
37. Liu, X. *et al.* Disruption of an evolutionarily novel synaptic expression pattern in autism. *PLoS Biol.* **14**, e1002558 (2016).
38. Luhmann, H. J., Fukuda, A. & Kilb, W. Control of cortical neuronal migration by glutamate and GABA. *Front. Cell Neurosci.* **9**, 4 (2015).
39. Sun, T. & Hevner, R. F. Growth and folding of the mammalian cerebral cortex: from molecules to malformations. *Nat. Rev. Neurosci.* **15**, 217–232 (2014).
40. Hemstedt, T. J., Bengtson, C. P., Ramirez, O., Oliveira, A. M. M. & Bading, H. Reciprocal interaction of dendrite geometry and nuclear calcium-VEGFD signaling gates memory consolidation and extinction. *J. Neurosci.* **37**, 6946–6955 (2017).
41. Enard, W. The molecular basis of human brain evolution. *Curr. Biol.* **26**, R1109–R1117 (2016).
42. Li, Q. *et al.* Mice carrying a human GLUD2 gene recapitulate aspects of human transcriptome and metabolome development. *Proc. Natl Acad. Sci. USA* **113**, 5358–5363 (2016).
43. Li, Q. *et al.* Changes in lipidome composition during brain development in humans, chimpanzees, and macaque monkeys. *Mol. Biol. Evol.* **34**, 1155–1166 (2017).
44. Florio, M., Borrell, V. & Huttner, W. B. Human-specific genomic signatures of neocortical expansion. *Curr. Opin. Neurobiol.* **42**, 33–44 (2017).
45. Dehay, C., Kennedy, H. & Kosik, K. S. The outer subventricular zone and primate-specific cortical complexification. *Neuron* **85**, 683–694 (2015).
46. Rakic, P. Evolution of the neocortex: a perspective from developmental biology. *Nat. Rev. Neurosci.* **10**, 724–735 (2009).
47. Fernandez, V., Llinares-Benadero, C. & Borrell, V. Cerebral cortex expansion and folding: what have we learned? *EMBO J.* **35**, 1021–1044 (2016).
48. Bae, B. I., Jayaraman, D. & Walsh, C. A. Genetic changes shaping the human brain. *Dev. Cell* **32**, 423–434 (2015).
49. Keeney, J. G. *et al.* DUF1220 protein domains drive proliferation in human neural stem cells and are associated with increased cortical volume in anthropoid primates. *Brain Struct. Funct.* **220**, 3053–3060 (2015).
50. Florio, M. *et al.* Human-specific gene *ARHGAP11B* promotes basal progenitor amplification and neocortex expansion. *Science* **347**, 1465–1470 (2015).
51. Ju, X. C. *et al.* The hominoid-specific gene *TBC1D3* promotes generation of basal neural progenitors and induces cortical folding in mice. *eLife* **5**, e18197 (2016).
52. Boyd, J. L. *et al.* Human-chimpanzee differences in a *FZD8* enhancer alter cell-cycle dynamics in the developing neocortex. *Curr. Biol.* **25**, 772–779 (2015).
53. Reilly, S. K. *et al.* Evolutionary genomics. Evolutionary changes in promoter and enhancer activity during human corticogenesis. *Science* **347**, 1155–1159 (2015).
54. de Juan Romero, C., Bruder, C., Tomasello, U., Sanz-Anquela, J. M. & Borrell, V. Discrete domains of gene expression in germinal layers distinguish the development of gyrencephaly. *EMBO J.* **34**, 1859–1874 (2015).
55. Otani, T., Marchetto, M. C., Gage, F. H., Simons, B. D. & Livesey, F. J. 2D and 3D stem cell models of primate cortical development identify species-specific differences in progenitor behavior contributing to brain size. *Cell Stem Cell* **18**, 467–480 (2016).
56. Mora-Bermúdez, F. *et al.* Differences and similarities between human and chimpanzee neural progenitors during cerebral cortex development. *eLife* **5**, e18683 (2016).
57. Herculano-Houzel, S. Neuronal scaling rules for primate brains: the primate advantage. *Prog. Brain Res.* **195**, 325–340 (2012).
58. Hill, J. *et al.* Similar patterns of cortical expansion during human development and evolution. *Proc. Natl Acad. Sci. USA* **107**, 13135–13140 (2010).
59. Gomez-Robles, A., Hopkins, W. D., Schapiro, S. J. & Sherwood, C. C. Relaxed genetic control of cortical organization in human brains compared with chimpanzees. *Proc. Natl Acad. Sci. USA* **112**, 14799–14804 (2015).
60. Kwan, K. Y. *et al.* Species-dependent posttranscriptional regulation of NOS1 by FMRP in the developing cerebral cortex. *Cell* **149**, 899–911 (2012).
61. Hage, S. R. & Nieder, A. Dual neural network model for the evolution of speech and language. *Trends Neurosci.* **39**, 813–829 (2016).
62. Greer, P. L. & Greenberg, M. E. From synapse to nucleus: calcium-dependent gene transcription in the control of synapse development and function. *Neuron* **59**, 846–860 (2008).
63. Hardingham, G. E. & Bading, H. Synaptic versus extrasynaptic NMDA receptor signalling: implications for neurodegenerative disorders. *Nat. Rev. Neurosci.* **11**, 682–696 (2010).
64. Bading, H. Therapeutic targeting of the pathological triad of extrasynaptic NMDA receptor signaling in neurodegenerations. *J. Exp. Med.* **214**, 569–578 (2017).
65. Hardingham, G. E., Fukunaga, Y. & Bading, H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat. Neurosci.* **5**, 405–414 (2002).
66. Greenberg, M. E., Ziff, E. B. & Greene, L. A. Stimulation of neuronal acetylcholine receptors induces rapid gene transcription. *Science* **234**, 80–83 (1986).
67. Morgan, J. I. & Curran, T. Role of ion flux in the control of *c-fos* expression. *Nature* **322**, 552–555 (1986).
68. Worley, P. F. *et al.* Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement. *J. Neurosci.* **13**, 4776–4786 (1993).
69. Dolmetsch, R. E., Xu, K. & Lewis, R. S. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* **392**, 933–936 (1998).
70. Hagenston, A. M. & Bading, H. Calcium signaling in synapse-to-nucleus communication. *Cold Spring Harb. Perspect. Biol.* **3**, a004564 (2011).
71. Bading, H. & Greenberg, M. E. Stimulation of protein tyrosine phosphorylation by NMDA receptor activation. *Science* **253**, 912–914 (1991).
72. Dieterich, D. C. *et al.* Caldendrin-Jacob: a protein liaison that couples NMDA receptor signalling to the nucleus. *PLoS Biol.* **6**, e34 (2008).

73. Ch'ng, T. H. *et al.* Activity-dependent transport of the transcriptional coactivator CRTC1 from synapse to nucleus. *Cell* **150**, 207–221 (2012).
74. Montarolo, P. G. *et al.* A critical period for macromolecular synthesis in long-term heterosynaptic facilitation in Aplysia. *Science* **234**, 1249–1254 (1986).
75. Nguyen, P. V., Abel, T. & Kandel, E. R. Requirement of a critical period of transcription for induction of a late phase of LTP. *Science* **265**, 1104–1107 (1994).
76. Tully, T., Preat, T., Boynton, S. C. & Del Vecchio, M. Genetic dissection of consolidated memory in *Drosophila*. *Cell* **79**, 35–47 (1994).
77. Chew, S. J., Mello, C., Nottebohm, F., Jarvis, E. & Vicario, D. S. Decrements in auditory responses to a repeated conspecific song are long-lasting and require two periods of protein synthesis in the songbird forebrain. *Proc. Natl Acad. Sci. USA* **92**, 3406–3410 (1995).
78. Bell, K. F. & Hardingham, G. E. The influence of synaptic activity on neuronal health. *Curr. Opin. Neurobiol.* **21**, 299–305 (2011).
79. Leslie, J. H. & Nedivi, E. Activity-regulated genes as mediators of neural circuit plasticity. *Prog. Neurobiol.* **94**, 223–237 (2011).
80. Alberini, C. M. & Kandel, E. R. The regulation of transcription in memory consolidation. *Cold Spring Harb. Perspect. Biol.* **7**, a021741 (2014).
81. West, A. E. & Greenberg, M. E. Neuronal activity-regulated gene transcription in synapse development and cognitive function. *Cold Spring Harb. Perspect. Biol.* **3**, a005744 (2011).
82. Ebert, D. H. & Greenberg, M. E. Activity-dependent neuronal signalling and autism spectrum disorder. *Nature* **493**, 327–337 (2013).
83. Weislogel, J. M. *et al.* Requirement for nuclear calcium signaling in *Drosophila* long-term memory. *Sci. Signal.* **6**, ra33 (2013).
84. Mick, E. *et al.* Family-based genome-wide association scan of attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* **49**, 898–905.e3 (2010).
85. von Deimling, M. *et al.* Gene expression analysis in untreated absence epilepsy demonstrates an inconsistent pattern. *Epilepsy Res.* **132**, 84–90 (2017).
86. Huentelman, M. J. *et al.* Calmodulin-binding transcription activator 1 (*CAMTA1*) alleles predispose human episodic memory performance. *Hum. Mol. Genet.* **16**, 1469–1477 (2007).
87. Rajaraman, P. *et al.* Polymorphisms in apoptosis and cell cycle control genes and risk of brain tumors in adults. *Cancer Epidemiol. Biomarkers Prev.* **16**, 1655–1661 (2007).
88. Hellwig, D. *et al.* Dynamics of CENP-N kinetochore binding during the cell cycle. *J. Cell Sci.* **124**, 3871–3883 (2011).
89. Park, B. *et al.* Association of Lbc Rho guanine nucleotide exchange factor with α-catenin-related protein, α-catulin/CTNNAL1, supports serum response factor activation. *J. Biol. Chem.* **277**, 45361–45370 (2002).
90. Kuwano, R. *et al.* Dynamin-binding protein gene on chromosome 10q is associated with late-onset Alzheimer's disease. *Hum. Mol. Genet.* **15**, 2170–2182 (2006).
91. Casoli, T. *et al.* Dynamin binding protein gene expression and memory performance in aged rats. *Neurobiol. Aging* **33**, 618.e15–618.e19 (2012).
92. Patterson, K. I., Brummer, T., O'Brien, P. M. & Daly, R. J. Dual-specificity phosphatases: critical regulators with diverse cellular targets. *Biochem. J.* **418**, 475–489 (2009).
93. Wolvetang, E. J. *et al.* Overexpression of the chromosome 21 transcription factor Ets2 induces neuronal apoptosis. *Neurobiol. Dis.* **14**, 349–356 (2003).
94. Soong, B. W. *et al.* Exome sequencing identifies *GNB4* mutations as a cause of dominant intermediate Charcot-Marie-Tooth disease. *Am. J. Hum. Genet.* **92**, 422–430 (2013).
95. Arroyo-Carrera, I. *et al.* Deletion 1q43-44 in a patient with clinical diagnosis of Warburg-Micro syndrome. *Am. J. Med. Genet. A* **167A**, 1243–1251 (2015).
96. Reitmair, A., Sachs, G., Im, W. B. & Wheeler, L. C6orf176: a novel possible regulator of cAMP-mediated gene expression. *Physiol. Genom.* **44**, 152–161 (2012).
97. Katsuoka, F. *et al.* Small Maf compound mutants display central nervous system neuronal degeneration, aberrant transcription, and Bach protein mislocalization coincident with myoclonus and abnormal startle response. *Mol. Cell. Biol.* **23**, 1163–1174 (2003).
98. Wharton, K. A. Jr., Zimmermann, G., Rousset, R. & Scott, M. P. Vertebrate proteins related to *Drosophila* Naked Cuticle bind Dishevelled and antagonize Wnt signaling. *Dev. Biol.* **234**, 93–106 (2001).
99. Seibt, J. *et al.* Expression at the imprinted Dlk1-Gtl2 locus is regulated by proneural genes in the developing telencephalon. *PLoS ONE* **7**, e48675 (2012).
100. Lin, N. *et al.* An evolutionarily conserved long noncoding RNA TUNA controls pluripotency and neural lineage commitment. *Mol. Cell* **53**, 1005–1019 (2014).
101. Yu, J. *et al.* Zinc-finger protein 331, a novel putative tumor suppressor, suppresses growth and invasiveness of gastric cancer. *Oncogene* **32**, 307–317 (2013).

Author contributions
H.B., G.E.H. and P.P. contributed equally to researching the data for the article and making substantial contributions to discussion of content. H.B., P.P. and G.E.H. wrote the article. H.B, G.E.H., P.P. and M.E.G. contributed to reviewing and/or editing of the manuscript before submission.

Competing interests statement
The authors declare that there are no competing interests.