OPINION

Using genetic data in cognitive neuroscience: from growing pains to genuine insights

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Abstract | Research that combines genetic and cognitive neuroscience data aims to elucidate the mechanisms that underlie human behaviour and experience by way of 'intermediate phenotypes': variations in brain function. Using neuroimaging and other methods, this approach is poised to make the transition from health-focused investigations to inquiries into cognitive, affective and social functions, including ones that do not readily lend themselves to animal models. The growing pains of this emerging field are evident, yet there are also reasons for a measured optimism.

The field of cognitive neuroscience, which studies the neurobiological mechanisms that underlie mental function, increasingly incorporates genetic data. The aim of this hybrid approach is to relate variation in specific genes to variation in brain activity and psychological phenotypes, including cognitive, affective and social information processing. A tacit promise of 'cognitive-neurogenetic' investigations (FIG. 1) is that they will provide a newly mechanistic type of insight into the genomic and molecular-biological construction of psychological phenotypes[1,2], and this insight will be relevant not just to basic science but also to health and education[3]. In the causal chain from gene to protein to mental function, brain activity is likely to be considered a key intermediate that can help bridge the gap between genes and behaviour.

In this Perspective we critically assess the extent to which the intermediate-phenotype strategy has fulfilled this potential, on the basis of recent progress[2,4]. We detail how further advancement in the field will depend on further developing the theory and methods for defining cognitive phenotypes, analysing complex genetic and neural networks, and characterizing gene expression and function at the molecular level. The growing pains of an emerging field are evident in the literature — for example, there have been various failures to replicate findings. Yet some developments and specific examples indicate the potential of genetic data to inform key questions in cognitive neuroscience.

Focus on the phenotype

Research that aims to integrate genetic and cognitive-neuroscience data seeks to provide an increasingly detailed understanding, at the genetic and molecular levels, of psychological phenotypes. However, even the most precise molecular–genetic data cannot be useful if the phenotypes are not well defined. Thus, cognitive-neurogenetic studies are only as good as their ability to measure mental phenotypes validly and specifically; clear psychological theory and rigorous psychometrics are essential[5]. In particular, describing and parsing the components of psychological functions will require well-developed behavioural tasks to ensure that the components that are being investigated are the ones that are actually being measured.

A close characterization of a phenotype comes only with effort, and brain-based intermediate phenotypes (for example, phenotypes that are based on data from neuroimaging and electrical recordings) are no exception. If anything, the demands of brain imaging and electrical recording impose further constraints, making task development difficult: once an appropriate challenge paradigm has been identified or developed, subjects must be able to perform it in the constrained testing environment (for example, a functional MRI (fMRI) scanner limits options for overt responding, movement, task pacing, social interaction and task duration). At least one control condition is generally essential for interpretation of the results; often several are required in order to adequately control for nonspecific effects or alternative interpretations.

Because such requirements can change the psychological demands and psychometric properties of a given task, tasks that are used to assay intermediate phenotypes need to be carefully developed and evaluated. The attention-networks test (ANT)[1] provides a good example of a task that has been developed on the basis of cognitive theory, further validated using brain imaging, and then used to investigate gene–brain–behaviour relationships. The ANT is a good measure of the executive-control, orienting and alerting components of attention[6] (for example, the executive-control measure has a test–retest reliability of 77% and a heritability of 89%[7]) and is effective in parsing these components in an individual[1,6]. Imaging studies of brain activity during performance of the ANT[8] have revealed dissociable networks for the three components of attention (as delineated in Posner's "three-network model" of attentional systems)[1,9–11] (FIG. 2). A gene-association study showed modest associations between executive control of attention and single-nucleotide polymorphisms (SNPs), including in the monoamine oxidase-A (*MAOA*) and the dopamine D4 receptor (*DRD4*) genes[12]. Differences between individuals in the activation of the anterior cingulate cortex (ACC) during performance of the ANT were correlated with variation in the *MAOA* gene[13] (FIG. 2), and this finding has been independently replicated and extended[14]. Correlations with behaviour were weaker.

Further validation of the ANT task has come from comparison with other, related

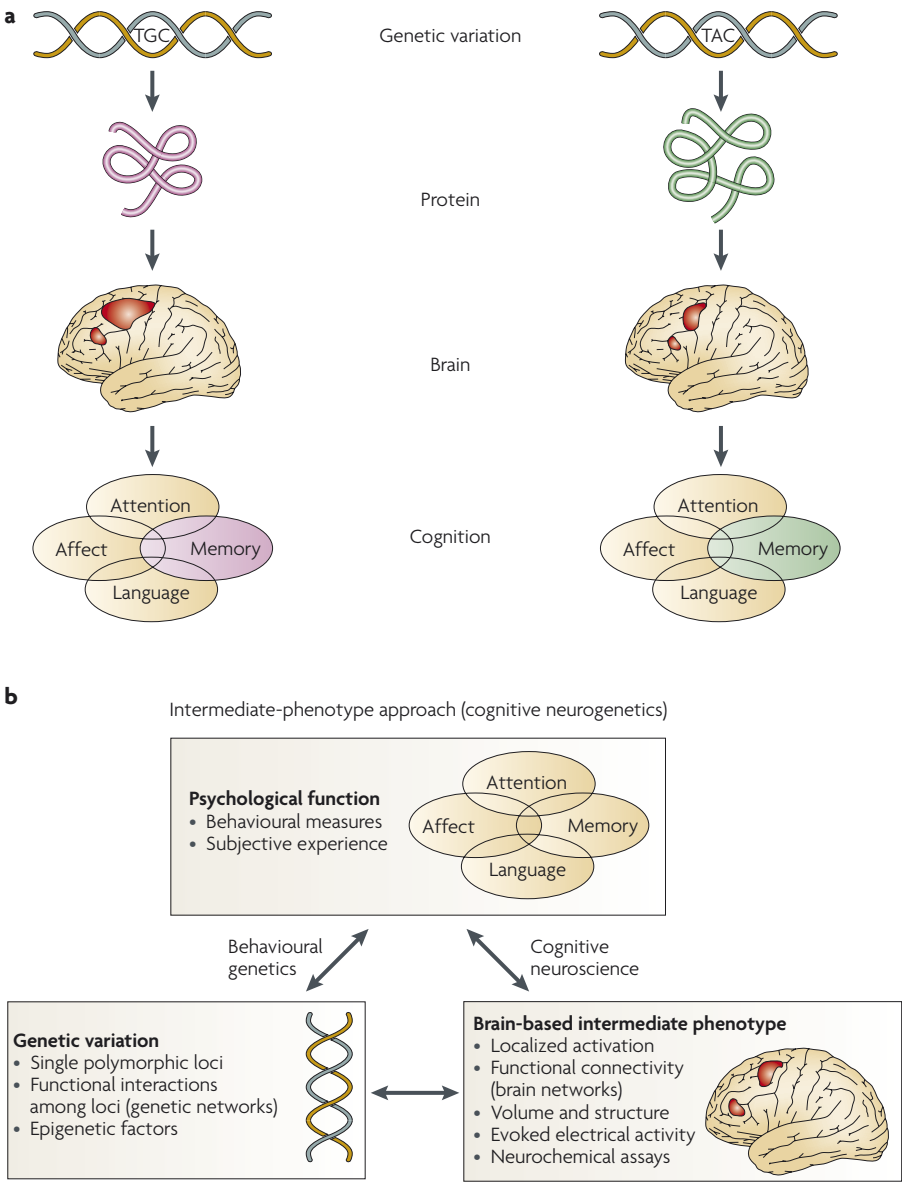

Figure 1 | **Integrating cognitive neuroscience and molecular genetics through the intermediate-phenotype approach. a** | Variations in genes can lead to variations in cognitive function, but there are many steps in between. First, a polymorphic gene can encode different gene products (proteins). These proteins might function differently. This difference in molecular function could be reflected in different levels or localizations of neural activity during a particular cognitive task; these can be measured using neuroimaging or other techniques. Finally, differences in neural activity might be reflected in differences in cognition. **b** | A number of subdisciplines study the different links in the causal chain from gene to cognition. Cognitive neuroscience aims to associate brain activity with mental functions. Cognitive neurogenetics, in which genetic analysis is applied to cognitive neuroscience, seeks to identify points of variation in the genome that can be linked to cognitive functions through intermediate neural characteristics. Thus, this approach would identify patterns of brain activity or connectivity that are associated with the cognitive function of interest and that vary as a function of the specified genetic variation. This also typically involves using behavioural-genetic analysis to test whether behavioural measures of the psychological function of interest are influenced by the specified genetic variable. A relationship between genetic variation and a brain-based phenotype in the absence of a brain–behaviour association can also be informative. In particular, this sort of finding can identify effects of genetic differences on neural processes (for example, neural processing efficiency) that could not otherwise be directly observed; these differences are often not reflected in behavioural data owing to compensatory factors (such as motivation and cognitive strategy). A gene–behaviour association in the absence of a detected mediatory neural mechanism can also be informative; for example, it can indicate that genetic differences that affect the cognitive function of interest might act through neural processes that are not typically studied in relation to that function.

tasks. The executive-control component of attention is an important aspect of working memory[15,16]. Genes that are associated with executive attention are also associated with working memory, and this provides a degree of convergent validity for the ANT. For example, during tasks that demand executive control of attention, carriers of the Met allele at the Val158Met polymorphism in the catechol-*O*-methyltransferase (*COMT*) gene show lower activity in the ACC than carriers of the Val allele and perform better[17–19], suggesting that the Met allele is associated with more efficient neural processing during such tasks. However, the data that relate genetic variation to behaviour have been less consistent than the data that link genetic variation to individual differences in neural activation[20]; this illustrates the value of the brain-based intermediate-phenotype approach. A relatively strong gene–brain finding has demonstrated, for example, that the effect of *COMT* genotype on ACC activation is strongest at the highest levels of attentional-control demand[17]. The effects of *COMT* genotype on executive attention have been framed under the dopamine signal-to-noise ratio hypothesis[18,21], and carriers of the Val allele might have increased 'noise' levels surrounding peaks of activation in the ACC and elsewhere in the prefrontal cortex (PFC)[18].

Genes other than *COMT* also affect attentional brain networks. Indeed, several other dopamine-system-related polymorphisms have been implicated in the executive-control component of attention — through performance measures, evoked potentials and functional imaging of ACC activation[13,22–26]. A number of genes that are not involved in the dopamine system have been associated with the orienting and alerting components of attention and their associated brain networks, including the glutamatergic[27], GABA (γ-aminobutyric acid)-ergic[28], serotonergic[29] and cholinergic[30–33] systems (FIG. 2). Cholinergic-system-related genes are neurophysiologically plausible mediators of attention, as acetylcholine has a role in activating parietal attention networks in response to salient stimuli[34,35]. Indeed, two cholinergic receptor genes have been associated with attentional orienting, as indexed by response time and by brain-based intermediate-phenotypic measures obtained by electrical recording and brain imaging[30–33]. Together these findings reinforce the separable-networks model of attention[10,11,36], and they enrich it by identifying separable genetic influences that bear on distinct brain networks.

The ANT is an example of careful task design and validation, and its use has demonstrated how clear demarcations between cognitive phenotypes and brain-based intermediate phenotypes can be successfully mapped onto different clusters of genetic variation. Other cognitive functions have been genetically parsed with similar success. For example, testing people with variations in *COMT* (at the Val158Met polymorphism) revealed that dopamine is implicated in the cognitive components (such as temporal updating) and in the network components (such as activity in the dorsolateral PFC) of working memory[37]. Furthermore, genetic information has revealed differences between aspects of reward-based learning that are striatum-dependent (probabilistic and negative-reinforcement learning, which are influenced by variations in *DARPP32* and *DRD2*, respectively) and aspects that are PFC-dependent (behavioural flexibility, which is influenced by variations in *COMT*)[38]. Note that a role for dopamine in negative-reinforcement-based learning had previously been controversial. In the context of complementary imaging work[39], Frank and colleagues used genetic information to make a compelling case for a role for dopamine, thus showing that genetic data can be used to inform cognitive neuroscience and not just to recapitulate its conclusions.

Notably, gene–behaviour associations can be task-specific, even if the neural substrate is similar between tasks — not all tasks function as equivalent 'reflex hammers' to activate the same neural circuitry in the same way. For example, a study[40] investigated the association of the *COMT* Val158Met genotype with performance in two tasks that both depend on the dorsolateral PFC (as shown by lesion studies[41,42]) but that differ in their sensitivity to dopamine levels in the PFC (as shown by pharmacological manipulations[43,44]). *COMT* genotype was differentially associated with task performance, underscoring the degree to which subtle distinctions in behavioural tasks can be crucial for detecting genetic contributions to a given phenotype. Once such subtleties are appreciated, a major benefit is that they allow well-matched control conditions to be developed.

For various psychological constructs it has been informative to assess the extent to which human 'mental architecture', as revealed through behavioural analyses, is reflected at a neural level. Testing for a convergence of evidence from psychological and neural approaches is worthwhile because agreement across levels is not assured: divergence (when convergence fails and a similar understanding of the nature or the structure of the system is not reflected at different levels of description) is possible and typically is even more informative than convergence. In addition to psychological and neural data, genetic data are yet a third source of variation that can provide converging or diverging evidence for a given parsing of mental processes. The fact that multiple genes often contribute to a psychological function does not imply that genetic data cannot meaningfully dissociate or parse different psychological functions — far from it, as the above examples make clear. Rather, dissociations are informative, but they must be understood in the context of multiply-determined effects[4]. A full understanding of such complexity necessitates a systems approach.

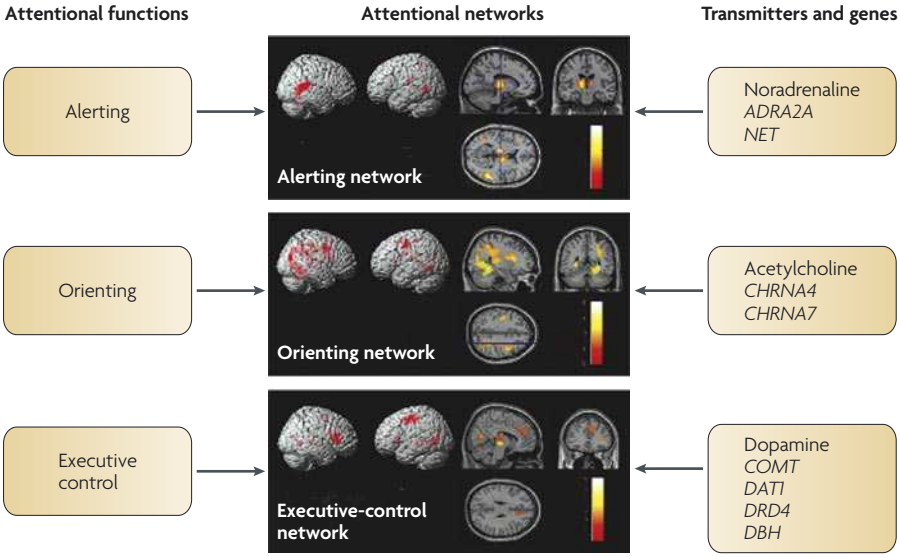

Figure 2 | **Attention-related candidate genes validated using the Attention Network Test (ANT).** In order to generate genetic markers for cognitive studies — in this case for studies on different aspects of attention — one relies on several sources of evidence. First, neuroimaging and lesion data point to separable neural networks that carry out different aspects of attention. Second, pharmacological manipulations demonstrate that noradrenergic modulation can influence the efficiency of the alerting network, whereas cholinergic modulation and dopaminergic modulation can influence orienting and executive control of attention, respectively. Finally, one can consider areas where neural-network activation overlaps with patterns of gene expression. It is possible that when all three sources of evidence converge, variations in gene sequences should correlate with individual differences in patterns of brain activity when subjects use a specific neural network. Brain-imaging data have shown that executive control of attention is mediated by brain regions that are targets of dopamine innervation, such as the frontal midline areas, the lateral prefrontal areas and the basal ganglia. Other neuromodulatory systems have also been explored using cognitive or behavioural genetics. ADRA2A, adrenergic receptor, α2a; CHRNA4, cholinergic receptor, nicotinic, α4; CHRNA7, cholinergic receptor, nicotinic, α7; COMT, catechol-*O*-methyltransferase; DAT1, dopamine transporter; DBH, dopamine β-hydroxylase; DRD4, dopamine receptor D4; NET, norepinephrine transporter. Figure reproduced, with permission, from REF. 132 © (2005) Academic Press.

Taking a systems approach

Like brain regions, genes do not operate in isolation. As well as being subject to complex environmental influences[45,46], multiple genes affect the function of a given neural network. For this reason, a systems approach to the investigation of genetic variations and neural substrates will be necessary to understand the neurobiological processes that underlie cognition. It will not be sufficient merely to identify specific brain regions with functions that are associated with specific genes (although this might often be a crucial early step). In this section, we discuss four issues that need to be taken into account in a systems approach to integrating genetic and cognitive-neuroscience data: first, genes typically have multiple functional polymorphisms; second, genes affect neural networks, not just isolated brain regions; third, a single gene can be involved in multiple cognitive, affective, sensory and motor processes (pleiotropy); fourth, mental functions involve the products of many different genes (polygenicity).

Multiple functional polymorphisms in a single gene. The first issue is that a single gene is itself a complex system. Multiple sources of functional variation can exist in a single gene. Often researchers focus on a single polymorphism, because its functional consequences have already been demonstrated and because the existing literature has focused on it. However, multiple polymorphisms can exist in non-coding and coding regions of a single gene, and all can have functional consequences. (This highlights the potential importance of haplotypes, which are patterns of alleles at multiple sites in a single gene or chromosome.) One should therefore analyse multiple polymorphisms in a single gene if possible. For example, Meyer-Lindenberg *et al.*[47] examined the combined effect of SNPs at three different loci in *COMT* on neural activity during an N-back working-memory task. Consistent with previous findings[48], Val-allele load (0, 1 or 2 Val alleles) at the Val158Met locus was predictive of prefrontal activation, as measured by fMRI. However, exploratory multi-polymorphic analysis indicated that the strongest predictor of prefrontal activation was actually a combination of variants that included all three polymorphic loci in the *COMT* gene. This analysis also revealed a complex, non-additive interaction between the Val158Met SNP and one of the other *COMT* SNPs (in the P2 promoter region).

Genes affect networks rather than isolated brain regions. Another important issue is that genes affect neural networks, not just single brain regions. This highlights the importance of sophisticated neuroimaging analysis. One technique for examining neural networks is connectivity analysis (FIG. 3). An excellent example of the use of connectivity analysis comes from research that explores the link between the serotonin (also known as 5-hydroxytryptamine (5-HT)) transporter (5-HTT) gene (*SLC6A4*) and negative affect: the short allele of the 5-HTT-linked polymorphic region (5-HTTLPR) of *SLC6A4* is poorly transcribed, resulting in reduced transporter density and altered serotonergic transmission[49,50]. Consistent with a role for serotonin in the emotional and behavioural regulation and control of negative affect[51], the short allele has been associated with high levels of negative affect and anxiety-related traits[52–54]. Additionally, carriers of the short allele show increased amygdala activity in response to facial expressions of negative affect and other negatively valenced images or words[55–57]. This stable, trait-like reactivity of the amygdala to affective stimuli[58,59] might suggest that amygdala reactivity is a mediator for the association between the 5-HTTLPR short allele and trait negative affect, but actually a more complex picture has emerged from connectivity analyses. The 5-HTTLPR short allele is associated with altered functional connectivity between the amygdala, the perigenual anterior cingulate cortex (pACC)[60] and the medial PFC[61], all of which are involved in processing and regulating negative affect. Crucially, it is the functional connectivity between the amygdala and the pACC, rather than amygdala reactivity in isolation[55,56], that mediates the relation between 5-HTTLPR and trait negative affect[60]. This highlights the importance both of connectivity-analysis strategies that focus on neural networks and of considering regulatory processes as cognitive mechanisms.

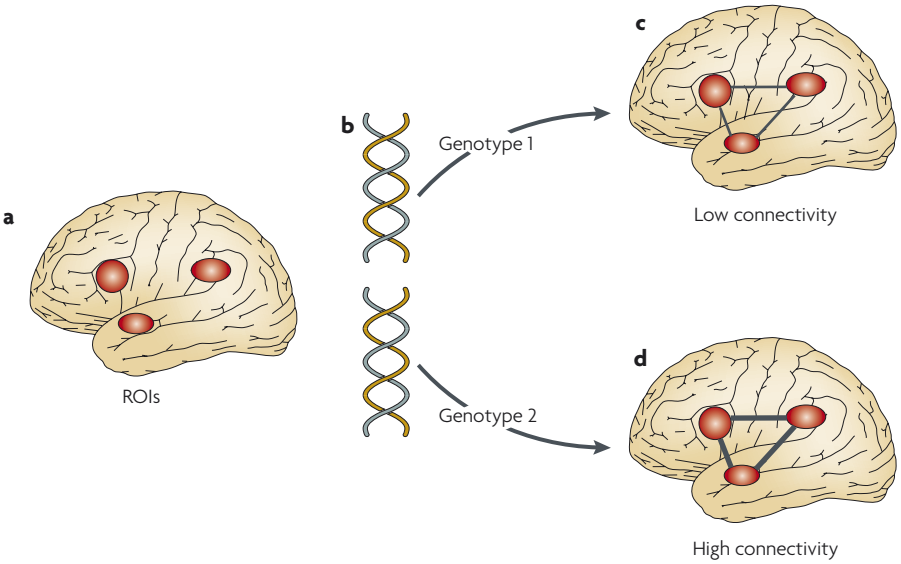

Figure 3 | **Connectivity analysis.** Analysis of connections among regions can be used to identify brain networks that are affected by genetic variation. First, regions of interest (ROIs; indicated in red in **a**) in a potential network are identified based on *a priori* hypotheses or on brain activity that has been assessed by functional MRI. Second, participants are genotyped for candidate alleles or haplotypes, yielding two or more different genotype groups to compare (**b**). Third, connectivity is assessed separately in each genotype group, by examining the strength of correlation of activity among the ROIs (**c**,**d**) or by tracing fibre tracts directly using diffusion tensor imaging. Finally, differences in connectivity between the genotype groups can be formally tested. If the genotype groups differ in connection strength (as indicated by the different line thicknesses in **c** and **d**), this indicates that the genotype influences the functioning of the network.

Pleiotropy. A systems approach should also take pleiotropy into account. Pleiotropy refers to the fact that single genes affect multiple cognitive, affective, sensory and motor processes. Although genetic research can sometimes differentiate cognitive processes by identifying specific genes that are associated with each cognitive process (as with the ANT), usually a particular gene is not associated with only one cognitive mechanism. Indeed, as many of the genes that are of interest to cognitive neuroscience code for elements of diffuse neuromodulatory systems, one should not expect them to be particularly limited in their functional relevance. For example, the dopamine, serotonin, noradrenaline and acetylcholine systems project to many different brain structures and regulate multiple aspects of cognition, affect, sensory processing and motor output. It would be a mistake therefore to think of the genes that are related to these systems as 'cognitive' or 'affective' genes, even though most research focuses on a particular class of effects. The brain processes that govern cognition and affect certainly interact, and they are likely to be thoroughly integrated at many points[62–64]. Molecular-genetic research can help to reveal which genetic contributions are shared across different cognitive and affective mechanisms, as well as which genetic variations contribute uniquely to individual mechanisms.

Thus, for example, *COMT* variation has been implicated in working-memory function and in affect, with carriers of the Met allele showing higher levels of anxiety[65–67]. This is not surprising given that many of

the brain structures that are implicated in the assessment, valuation and salience of emotion-laden stimuli are targets of dopaminergic innervation[68]. Variation in *COMT* has an additive effect with variations in *SLC6A4* in predicting brain activation in response to affectively negative pictures in the amygdala, the hippocampus, the parahippocampal gyrus and the cingulate gyrus[69]. The Met allele was associated with greater activity in these brain regions and, in a similar study[70], with greater activation of the hippocampus and the ventral PFC in response to emotional facial expressions. The Met allele also predicted increased functional connectivity between the ventral PFC and the amygdala and hippocampus[70]. Another study found that *COMT* variation was associated with increased experience of pain, heightened negative affect and reduced μ-opioid response during a saline injection pain stressor[71]. These effects on brain function might help to explain why the Met allele is associated with greater levels of anxiety and emotional dysfunction[65–67].

Just as *COMT* is often considered to be a cognitive gene, but also influences emotional processes, so the effects of *SLC6A4,* which is often considered to be an affective gene, go beyond affect[72]. *SLC6A4* variation has such pervasive effects that it even influences the neural response to undefined or ambiguous stimuli, such as a fixation cross, which is often used as a neutral baseline condition for neuroimaging[72,73]. This suggests that such stimuli are not as neutral as is usually supposed: they are processed cognitively, and this processing differs by genotype. Thus, it might be useful to control for *SLC6A4* genotype in fMRI studies that use a neutral baseline.

Polygenicity. Considering the complexity of the neurobiological substrate for any cognitive process, there will be many genes in which functional variation can affect a single cognitive process. For example, along with *SLC6A4*, variations in other genes in the serotonin system and in other neurotransmitter systems are likely to have effects on many of the same brain processes. Variation in the tryptophan hydroxylase 2 (*TPH2*) gene, which produces the rate-limiting enzyme for the synthesis of serotonin, affects amygdala reactivity[74,75], as does variation in the acetylcholine transporter gene (*CHT1*)[76].

Not only are many genes likely to contribute additively to variation in a particular cognitive process, there is also likely to be gene–gene interaction (epistasis), in which the effect of one gene is modified by another gene. One example of epistasis is between *SLC6A4* and brain-derived neurotrophic factor (*BDNF*) in their relation to the brain structures that are involved in negative affect. A study that used structural MRI showed that the Met allele of the *BDNF* Val66Met polymorphism counteracted the effects of the 5-HTTLPR short allele: in carriers of the *BDNF* Val allele, the short allele of 5-HTTLPR was associated with reduced volume in the pACC and with reduced structural connectivity between the pACC and the amygdala, whereas in carriers of the *BDNF* Met allele these associations were not present[77].

Molecular-genetic constraints

To understand how polymorphisms are implicated in cognitive functioning (as assessed in neuroimaging and behavioural studies), we must first establish the molecular consequences of these polymorphisms — in other words, how variation in a gene affects the expression of that gene and the functioning of the gene product (FIG. 1a). Thus, molecular–genetic data are essential for understanding how a genotype is linked to a (intermediate) phenotype. Without these data, associations between genotypes and (intermediate) phenotypes are often merely descriptive, and can even be misleading.

A cautionary example is the case of the Taq1A polymorphism. Neuroimaging studies reported and replicated reports of an association between Taq1A variation and functional activity in attention-related brain areas in the medial PFC[25,78,79], as well as an association between Taq1A variation and activation of the nucleus accumbens, the orbitofrontal cortex and the amygdala in response to reward[80,81]. These effects were putatively attributed to expression of the dopamine receptor D2, because Taq1A was thought to lie in the protein-coding region of *DRD2*. However, further investigations revealed that the Taq1A polymorphism actually lies downstream of *DRD2*, in the coding region of a neighbouring gene, *ANKK1* (REFS 25,82). Although recent studies have shown some degree of linkage disequilibrium between Taq1A–*ANKK1* and functional polymorphisms in *DRD2* (REFS 83,84), the likelihood of Taq1A directly influencing dopaminergic brain function is diminished, especially because *ANKK1* expression has not been detected in the mammalian brain[82]. Molecular–genetic data that do not support neuroimaging–behaviour associations provide an important mechanism for progress in cognitive neurogenetics: developing the molecular–genetic description of candidate genes will be essential for weeding out spuriously or imprecisely nominated candidate genes.

Other examples indicate somewhat more promise for the aim of building bridges from gene to cognitive phenotype. One relatively well-characterized case is that of *COMT* (FIG. 4). *COMT* encodes the COMT enzyme,

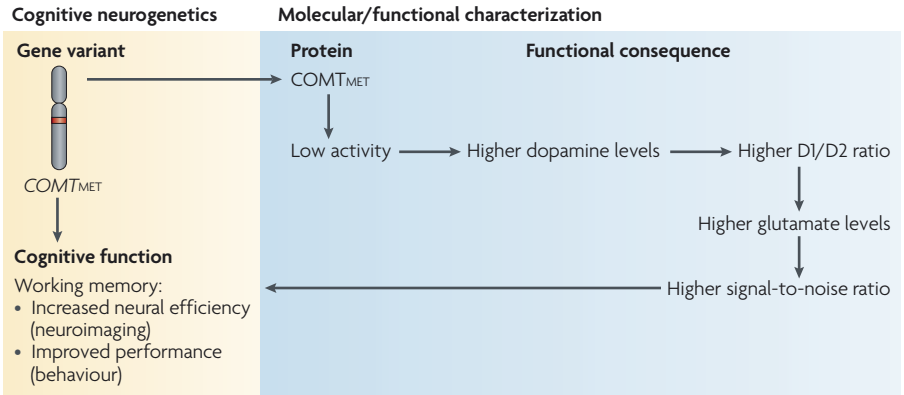

Figure 4 | **Molecular characterization of gene variations.** A growing body of research, pioneered by Weinberger and colleagues (for reviews see REFS 2,18), indicates that carriers of the catechol-O-methyltransferase (*COMT*) Met allele show better performance and greater neural efficiency during working-memory tasks. To understand how the *COMT* Met variant contributes to these effects, the molecular and functional consequences of the allele have to be established. Compared with carriers of the Val allele, carriers of the Met allele have relatively low COMT enzyme activity and presumably have higher prefrontal cortex (PFC) dopamine availability as a result. This is thought to result in a higher dopamine D1 receptor/dopamine D2 receptor binding ratio in the PFC in Met carriers (relative to Val carriers). A higher D1/D2 binding ratio is believed to affect the signal-to-noise ratio in the PFC, by modulating the excitatory release of glutamate from pyramidal cells. This in turn results in a better inhibition of noise in the surround. The increased signal-to-noise ratio in the PFC could contribute to the effects of the *COMT* Met allele on working memory.

which catabolically terminates the activity of dopamine in all dopaminergic synapses. COMT activity is thought to be a major determinant of dopamine availability in the PFC, because the dopamine re-uptake transporter, which is responsible for removing synaptically released dopamine in most brain regions, is sparsely expressed in the PFC[85–87]. Dopamine availability affects PFC functioning; for example, it is a determinant of working-memory ability[88]. Much working-memory-related investigation has therefore targeted the *COMT* Val158Met polymorphism, which is known to influence the enzymatic activity of COMT. Carriers of the Met allele have relatively low COMT activity and presumably have higher dopamine availability as a result, whereas carriers of the Val allele have relatively high COMT activity and lower dopamine availability[89]. An association of the *COMT* Met allele with better working-memory performance has been reported[48,90], but the behavioural data have been inconsistent[91–93]. Clearer insight into the effects of this polymorphism on the operation of working-memory networks has been gained through an analysis of brain-based intermediate phenotypes, especially brain activity as assessed by neuroimaging. The Met allele was associated with indices of greater neural efficiency during working-memory tasks (that is, with less recruitment of working-memory-associated brain areas for equal or better behavioural performance)[48,94,95]. This example shows the potential of the brain-based intermediate-phenotype approach for investigating the effects of genetic variability that might be obscured at the behavioural level by compensatory factors such as motivation. The history of psychiatric genetics suggests that it would be prudent to exercise continued caution regarding effect sizes reported in the first studies of a genetic association. Nonetheless, the effect of the Val158Met polymorphism on brain-based intermediate phenotypes of working memory is emerging as one of the best-replicated findings in the cognitive-neurogenetics literature[2,21,48,91,96–103].

One plausible hypothesis for the mechanism by which *COMT* and other dopamine-system-related genes influence the functioning of the brain networks that are involved in working memory centres around the signal-to-noise ratio of dopaminergic signalling in the PFC[18,21,91,97,102] (FIG. 4). The hypothesis posits that binding at dopamine D1 and D2 receptors affects the signal-to-noise ratio in the PFC by altering the excitatory release of glutamate from

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pyramidal cells (probably by modulating Na^+ channel opening[104] and possibly by modulating the inhibitory release of GABA from interneurons[18]). In short, the higher the D1/D2 activation ratio, the stronger the excitatory signal and the better the inhibition of noise in the surround. This is clearly important for working memory, which requires the maintenance of an informational signal over time and also requires the inhibition of noise. At levels of dopamine availability that facilitate optimal working-memory function, D1 binding in the PFC is high relative to D2 binding. However, when dopamine availability is decreased — for example, in Val carriers relative to Met carriers — the D1/D2 activation ratio also decreases. Thus, the *COMT* Val allele plausibly contributes to causing a diminished signal-to-noise ratio in the PFC and consequently to less-efficient working-memory function. This proposed mechanistic account is supported by the finding that the *COMT* Val allele is associated with less-efficient activation and reduced functional connectivity in a network of brain regions that are thought to underlie working memory, especially in the dorsolateral PFC[2,21,48,96–103,105].

Molecular characterizations of other gene polymorphisms that are associated with cognitive phenotypes have also been fairly well developed. The Val66Met polymorphism in the *BDNF* gene has been linked to fMRI and behavioural indices of long-term memory. The polymorphism affects cellular trafficking and secretion of BDNF, which might ultimately influence white-matter connectivity, long-term potentiation, and hippocampal volume and activity[100,106–110]. The link between the short allele of the 5-HTTLPR region of *SLC6A4* and trait negative affect[52–54] (discussed earlier) has been supported by findings of reduced transporter density and altered serotonergic transmission[49,50], as well as increased amygdala activity in response to negatively valenced stimuli presented across multiple modalities[55–57]. The growing list of cognitive phenotypes for which the candidacy of proposed associated genes has been supported by molecular–genetic evidence also includes executive attention[13,111] and, to a lesser extent, language function[112].

The availability of candidate genes with relatively well-described molecular–genetic characteristics is important for grounding brain-based investigations: targeting these genes is good practice for neuroimaging and behavioural association studies. In addition, screening individual genotypic differences can be a valuable, non-invasive way to identify new candidate genes that can be better characterized at the molecular level in order to inform our understanding of cognitive function. The value of both approaches has been demonstrated by investigations of long-term memory[113].

Targeting a cluster of ‘signalling-cascade’ genes, the products of which are known to play a part in memory formation at the cellular level, De Quervain and Papassotiropoulos[113] showed that better associative memory was linked to several polymorphisms located in the targeted cluster. Specifically, the authors found that a variable representing the combined genotype for the polymorphisms of the targeted cluster predicted individual differences in hippocampal and medial temporal activation among fMRI participants and also predicted individual differences in memory among a larger population. Another study[114] used a DNA-microarray analysis to screen for polymorphisms that were predictive of memory performance among a large group of subjects. They identified a T–C substitution in the *KIBRA* gene that was subsequently shown to influence the efficiency of memory-related hippocampal activation. This effect was putatively attributed to an effect of KIBRA, a cytoplasmic protein, on synaptic plasticity in the hippocampus[114], but further molecular–genetic characterization of *KIBRA* will show in more detail how it contributes to memory function. The methods that were used in this genome-wide scan demonstrate the potential for statistically rigorous investigations that proceed from the starting point of a cognitive phenotype to that phenotype’s underlying genetic factors. Although interpretive caution is always essential, DNA-microarray analysis is a powerful tool for identifying candidate polymorphisms in broad swathes of the genome, and is likely to have a great impact on future cognitive-neurogenetic investigations.

A close molecular–genetic characterization of candidate polymorphisms is of fundamental importance for successfully incorporating genetic data into cognitive neuroscience. The ultimate directive is to integrate genes and their products with brain-based intermediate phenotypes and behavioural (cognitive) phenotypes. Examining whether reported associations between genes and (intermediate) phenotypes actually fit molecular–genetic constraints is essential for moving towards this integrative goal.

Taking stock

Although brain-based phenotypes are in some respects a new breed that go beyond more traditional clinical and psychometric phenotypes, in other ways they are not special. Our intention in this section is to emphasize that a growing consensus on standards of evidence for genetic-association studies[115] should apply to all phenotypes, including brain-based phenotypes. We briefly consider the implications of using inferential statistics: how can we be confident that a particular association is not spurious? Without circumspection, we risk ignoring the consensus regarding standards of evidence that was gained from two decades of psychiatric and behavioural-genetic research. These fields initially embraced emerging molecular technologies but did not also adopt the level of stringency that has since proved to be necessary.

The risk of overestimating associations. History suggests that caution is warranted regarding several specific issues. Consider, for example, that the first study to report an association between the *DRD2* (now *ANKK1*) Taq1A polymorphism and risk of alcoholism indicated an odds ratio of approximately 8 associated with the risk allele[116]. Similarly, the first study to report an association between the short allele of the 5-HTTLPR region of *SLC6A4* and anxiety-related traits suggested that this single locus accounted for up to 9% of the inherited phenotypic variation in these traits[117]. Subsequent large-scale primary studies and meta-analyses have indicated that these findings were gross overestimations — in some cases, meaningful effects of the polymorphisms were ruled out altogether[118,119]. Even recent studies that set out to replicate previous findings have demonstrated that the strength of the evidence of particular gene–phenotype associations declines as more data become available[120,121]. This might be because early estimates were often biased towards an inflated value and lacked sufficient statistical power. Several factors are likely to introduce overestimations of gene–phenotype associations into the literature. For example, results that do not achieve statistical significance are often not published or take a longer time to be published[122], resulting in publication bias[123]. Furthermore, if a study does not have a statistically significant outcome, researchers often perform post-hoc studies that analyze subgroups of the original study sample, because such analyses are more likely to achieve nominal statistical significance[124]. These factors are reflected in decreasing estimate of effect sizes[125], in the poor predictive value of initial reports of genetic association[126], and in the excess of results that fall just below the 0.05 alpha level[127]. Indeed, we have recently shown that factors such as the geographical region in which a study takes place[128] are associated with the degree to which the study overestimates the true effect size. There is no reason to believe that cognitive-neurogenetics studies would be immune from such considerations solely because they use a brain-derived phenotype.

Greater genetic effects on brain-based intermediate phenotypes than on behaviours? The interest in the brain as a provider of intermediate phenotypes stems from two main considerations. The first consideration we regard as being relatively uncontroversial: that intermediate phenotypes will allow us to better understand the mechanisms that give rise to complex behaviours, and the role of genetic variation in influencing these behaviours through these mechanisms. This is probably the main motivation for cognitive-neurogenetics research. The second consideration is more subtle: because brain-based phenotypes are considered to be more 'proximal' than behavioural phenotypes to genetic variation, the genetic variations might correspondingly have a greater effect on brain-based phenotypes than on behavioural phenotypes. There is some support for this in instances in which there are sufficient comparable studies to allow synthesis[51], although even in these cases the effect-size estimate (d ~0.5) for a genetic variant and a brain-based phenotype is, in our view, not dramatically greater than the effects size for the same genetic variant and psychometrically measured personality trait (d ~0.2)[129]. However, it is also not certain that genetic effects will be more penetrant at the level of the brain in all cases — individual genetic effects might subtly influence multiple brain systems which then converge to influence a more complex, distal phenotype (for example, behaviour). In this hypothetical case it might be argued that we would expect stronger (that is, aggregated)

Glossary

Candidate gene
A candidate gene is a gene with a function that suggests that it might be involved in the variation observed for a particular trait. Polymorphisms in a gene that are known to alter its function (for example, through alterations in its expression) are used in candidate-gene association studies.

Intermediate phenotype
A heritable trait or characteristic that is not a direct symptom of the condition under investigation but that has been shown to be associated with the condition. It might reflect an intermediate step in the pathway between gene and psychological function (or dysfunction). In a brain-based intermediate-phenotype approach, brain function is assayed (for example, through neuroimaging technologies) in order to measure intermediate mechanisms at a systems level.

Linkage disequilibrium
The non-random association (that is, correlation) of alleles at two or more loci, so that certain combinations of alleles occur together more frequently than would be expected by chance. This means that a true causative locus might in fact be one that is in linkage disequilibrium with the one that is under investigation in a genetic-association study.

Odds ratio
A measure of effect size, defined as the ratio of the odds of an event occurring in one group to the odds of it occurring in another group. In the context of a genetic-association study, this might be the odds of major depression occurring in one genotype group against the odds of it occurring in another genotype group.

Polymorphism
The presence of two or more variants (alleles) in a gene or other DNA sequence in a population. The most commonly investigated polymorphisms are single-nucleotide polymorphism (SNPs), in which a point mutation has occurred (for example, a C base is substituted for a T base in the DNA sequence).

Psychometrics
The design, administration and interpretation of quantitative tests for the valid and reliable measurement of psychological variables (phenotypes).

Publication bias
The greater tendency for statistically significant results to be published (relative to non-significant results). This might be due to the unwillingness of the author to submit non-significant results, to the unwillingness of the journal to accept them, or to both. Published studies might therefore not be representative of all the studies that have been conducted.

Trait
In relation to psychological variables such as mood, a trait is the dispositional level of a particular mood or emotion (for example, trait anxiety) and reflects the mean level of the mood or emotion over time. It is usually highly correlated with the current 'state' level. More generally, a trait is a dimension along which individuals can differ in behavioural dispositions.

Box 1 | **Statistical power and false positives**

The pattern of results in the psychiatric-genetics literature indicates that true effect sizes of single gene polymorphisms on psychometric or clinical phenotypes are considerably smaller than originally envisaged. In fact, it is now clear that most of the studies that have been conducted to date were grossly under-powered to detect these effects. It is only because there have been multiple attempts to replicate several gene–disease associations that we have been able to identify the true effect sizes.

Let us assume that 90% of our hypotheses are in fact null (that is, there is no association). (Empirically this might be quite conservative, particularly in the case of genetics, in which there is an enormous number of variants that we might wish to test. Nevertheless, the general conclusions below are valid regardless of this value.) Let us also say that 1000 studies are conducted, with an average of 80% statistical power (history tells us this might also be optimistic!) and a 5% alpha level. In this case we will correctly reject the null hypothesis in 80% × 100 = 80 studies, but will falsely reject the null hypothesis in 5% × 900 = 45 studies.

The ratio of true versus false findings would therefore be 80:45. This in itself is a cause for concern when interpreting 'significant' results. Most importantly, however, if we reduce statistical power (that is, sample size), then the proportion of true to false findings among studies that achieve statistical significance will necessarily decrease.

This is true in all scenarios, because typically the alpha is fixed (although a more stringent alpha will improve the situation), whereas the statistical power is highly variable across studies. The rate of false positives among studies that achieve statistical significance increases as power decreases. In an emerging field such as imaging genetics, which typically uses small sample sizes and in which fewer true replication studies are available (perhaps owing to complexities of study design or the costs that are associated with neuroimaging), this is a particular concern.

genetic effects at the distal phenotype than at the proximal phenotype.

The question of whether brain-based phenotypes will provide us with greater statistical power with which to detect genetic effects is therefore an empirical one. A few examples indicate that this can be the case, but cannot prove a general rule. Moreover, whether greater statistical power corresponds to substantial power (in absolute terms) is an important question. It is dangerous to assume that when investigating imaging phenotypes, relatively small sample sizes will suffice (compared with sample sizes for more traditional psychometric or clinical phenotypes)[130]. Qualitatively the cognitive-neurogenetics literature resembles the early psychiatric-genetics literature, with large effect sizes in early reports and smaller effect sizes over time. For example, the evidence to date for the reported association between 5-HTTLPR polymorphisms and amygdala activation in response to threat seems to suggest a relatively robust effect[131]. However, there is also evidence that the first published study provided an overestimate of this effect, and there is evidence of publication bias, consistent with the pattern in the wider psychiatric-genetic literature[131]. It was only after more than 10 years of attempted replication that we were able to discern the extent to which early psychiatric-genetic studies were overly optimistic with regard to any true effect size. Compared with typical genetic-association studies that use psychometric or clinical phenotypes, imaging-genetic studies require more financial resources and access to specialist equipment, reducing the impetus to conduct replication studies that would permit data aggregation and provide more convincing evidence than a single study. The danger of assuming that genetic effects at proximal (brain-based) phenotypes are greater than at other phenotypes is therefore twofold: it can seem to justify the use of smaller sample sizes (leading to an increased risk of false-positive results (BOX 1)) and can lead to overconfidence in initial studies (leading to fewer replication studies being conducted).

In summary, imaging phenotypes might indeed prove to be stronger than behavioural or other relatively distal phenotypes in some cases — our point is only that this cannot be assumed *a priori*. Rather, multiple replication studies (each ideally being well powered) must be conducted to assess the true magnitude of any effect and whether the effect is substantially greater than those afforded by behavioural phenotypes.

On the whole, it seems reasonable that conventions for scientific confidence should be the same for cognitive-neuroscience-based phenotypes and for any other phenotypes. This would include, for example, appropriate correction for multiple comparisons across all statistical tests, comparisons to genome-wide thresholds, and explicit information about the study's power to detect a range of effects. Replication studies should be conducted in sufficiently large samples to convincingly distinguish the proposed effect from no effect, and samples should be sufficient to enable permutation testing of statistical significance[115].

Conclusions and future directions

The study of genetic variation as a factor in determining human brain function is not only relevant for health, it also has the potential to inform models of normal cognitive function. However, simply identifying associations between variations in the genome and variations in brain-based and behavioural measures is not enough. Psychological theory will be an indispensable pillar for building an understanding of gene–brain–behaviour relationships[5], and will be needed for the rigorous development and validation of behavioural tasks. Another pillar will be the use of a systems approach that takes into account both the complexity and nonspecificity of gene effects and the interactions between and among genetic polymorphisms and brain systems. A third pillar will be detailed molecular–genetic analysis of the effects of polymorphisms on gene expression and function. Fitting associations between genes and (intermediate) phenotypes to molecular–genetic data can help weed out spurious associations and strengthen the link from cognition to molecular mechanisms that build and guide neural systems.

Optimistically speaking, genetic variation can provide a qualitatively different type of data with which to test and inform cognitive hypotheses and putative dissociations (for example, parsing components of attention on the basis of differential genetic contributions). This is similar to the way in which neural data help to inform cognitive science by providing empirical constraints that help to parse and explain individual differences in psychological constructs (for example, intelligence, personality and clinical status). Diverse cognitive-neuroscience measures are taken to be proxies for individual differences in psychological function and convey new information about cognitive processes, even in the absence of overt behavioural differences[62]. There is potential for genotypic and molecular–genetic data to provide complementary proxy measures, filling out the profile of a cognitive function at biologically detailed levels of description (for example, genotype and bio-molecular implementation).

Such potential can be glimpsed where neural systems and characteristics that support a particular cognitive function (for example, hippocampal plasticity in associative memory) have been linked to genetic

variations (for example, the *BDNF* Val66Met polymorphism) that are plausibly related to that function on the basis of molecular effects (in this case, BDNF trafficking and secretion). However, even the best-replicated cognitive-neurogenetic findings are still relatively new, and familiarity with the history of psychiatric genetics urges caution. We now know that the magnitude of single-gene effects on psychometric and clinical phenotypes is modest. Although brain-based intermediate phenotypes might reveal larger effects than behavioural phenotypes in some instances, a general advantage for brain-based phenotypes remains to be conclusively established. The value of brain-based intermediate phenotypes might instead lie in their potential to elucidate mechanisms. Crucially, replication, large sample sizes, statistical stringency and appropriate skepticism regarding early findings are all necessary if we are to obtain insights that we can be reasonably certain are true.

For cognitive neuroscience, the allure of genetic investigation is that it ostensibly represents a way to engage the 'how' question of neural information processing with a set of tools that can bridge the gaps between psychological theory, biological mechanism and genome. A less obvious yet equally exciting possibility is to use genetic variation to parse cognitive and affective phenotypes. Although the promise of genetic data to inform cognitive neuroscience is still largely unproven, and although statistical and practical dangers are evident, some preliminary findings suggest proof-of-concept. Progress in the field will depend on thoughtfully applying psychological theory to behavioural tasks, engaging the complexity of genetic and neural networks, and integrating brain-based associations with molecular–genetic data.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
ANKK1 | *BDNF* | *CHT1* | *COMT* | *DARPP32* | *DRD2* | *DRD4* | *KIBRA* | *MAOA* | *SLC6A4* | *TPH2*

FURTHER INFORMATION
John Fossella's homepage: http://originsgenomeresources.net
Jeremy Gray's homepage: http:// www.yale.edu/scan/

ALL LINKS ARE ACTIVE IN THE ONLINE PDF

OPINION

Applications of real-time fMRI

R. Christopher deCharms

Abstract | For centuries people have aspired to understand and control the functions of the mind and brain. It has now become possible to image the functioning of the human brain in real time using functional MRI (fMRI), and thereby to access both sides of the mind–brain interface — subjective experience (that is, one's mind) and objective observations (that is, external, quantitative measurements of one's brain activity) — simultaneously. Developments in neuroimaging are now being translated into many new potential practical applications, including the reading of brain states, brain–computer interfaces, communicating with locked-in patients, lie detection, and learning control over brain activation to modulate cognition or even treat disease.

From the times of the ancient philosophers through to the modern pursuits of cognitive neuroscience and neurology, it has been a human passion to comprehend the physical basis of what we experience subjectively as the mind. The Western attempt to 'see' the mind in the brain has been ongoing since before Galileo looked at the planets through the telescope or Hooke looked at cells through the microscope. René Descartes famously used introspection and reason as tools to try to discover the basis of mental events[1]. In this historical context, our generation is the first to explore increasingly direct glimpses of the mind–body interface, through the science of neuroimaging. We can perceive the flow of our subjective experiences through introspection and, using neuroimaging technology, can simultaneously view a display of the physical processes that are taking place in our brain during those experiences ('introneuroimaging'). Imagine Descartes' wonder (FIG. 1) at our modern capabilities for mapping the physical substrates of our own minds.

Over the past few decades, neuroimaging has measured the patterns of brain activation that are associated with different cognitive processes, and has thereby illuminated previously hidden terrains of human brain function[2,3]. In attempts to create 'maps' of the functional roles of the many regions of the brain that are applicable to all people, neuroimaging measurements have typically been pooled and averaged across many individuals and across many repetitions of a task. Now, real-time functional MRI (rtfMRI) is exploring the possibility of watching one's own brain activation 'live'. The ability to observe one's own brain as the mind's processes unfold might allow us to become aware of and learn to control some of the most important aspects of human life: conscious experience, cognition, emotion, action, non-conscious functions, and even the breakdown of these processes in disease. On a technical level this possibility is brought about by recent advances in neuroimaging and computing[4–7]. Early experiments in this new field are just taking shape, as discussed in this Perspective: methods have been developed for reading patterns of brain activation in real time, for manipulating computerized devices using only the brain, for communicating with a patient who was thought to be in a vegetative state, for learning to control individual regions in one's own brain and thereby alter one's cognition, and potentially even for controlling disease symptoms such as chronic pain.

In the past twenty years there has been a revolution in our understanding of the human brain and the localization of processes that were largely outside the bounds of biological science a generation ago, such as executive function, mental imagery, emotion and conscious experience. As an acknowledged supporter of rtfMRI, in this Perspective article I describe where these developments — specifically the new technology of rtfMRI — have led, and I provide some conjectures regarding their possible applications in the foreseeable future. The more technical aspects of this field have been reviewed elsewhere[6–9].

rtfMRI methods and prior approaches

Compared with prior methods for measuring brain function, functional neuroimaging provides measurements of brain physiology that are highly distributed (sampling very large numbers of spatial locations, often spanning the brain) and highly parallel (providing an ongoing stream of information from each of the many measurement points). For example, MRI can currently sample from ~2^{16} spatial locations per second (FIG. 1), each location with a dimension on the order of 3x3x3mm[10–12]. The technical brilliance of MRI is that it provides a unique means by which to precisely 'address' each point in space on the basis of the physical properties of magnetic resonance, and thereby