From phenotypic chaos to neurobiological order

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Complex demographic and behavioral phenotypes can arise from coordinated interactions among brain systems. A single axis of co-variation spanning 'negative' and 'positive' attributes links diverse participant characteristics with specific patterns of brain connectivity.

A fundamental goal of systems neuroscience is to understand the biological mechanisms that support the diversity of human experience, ranging from discrete facets of cognition and behavior to health and disease. These observable characteristics, or phenotypes, arise through a complex web of interactions linking genetic variation, brain function and a lifetime of experiences. Comprehensive phenomic-level analyses are necessary to decipher specific roles of neurobiological processes, as well as their potential relations to behavioral syndromes and disease states. Traditional studies of circumscribed aspects of cognition and behavior have provided foundational discoveries, characterizing core aspects of brain function. However, current limits on our ability to understand many important biological phenomena suggest that we are not adequately sampling all the relevant variables and that we must broaden our phenotypic net[1]. In a report in this issue of *Nature Neuroscience*, Smith *et al.*[2] tackle this problem head-on, relating intrinsic functional brain network architecture to a host of behaviors in a single integrated analysis. In doing so, the authors identify a single mode of population co-variation spread along a 'positive-negative' axis. The identified axis links a diverse array of participant characteristics with a specific pattern of network-level connectivity, suggesting that there may be a general mode of positive brain function.

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Human neuroscience research is undergoing an extraordinary phase of development. The recent convergence of new imaging technologies, methods for online or remote behavioral collection, increased computational resources, and a cultural shift toward open access data[3,4] have provided the opportunity for data-driven discovery science. Although there is a long history of the extensive study of individuals in focused areas of cognition and behavior (for example, vision), only recently have large-scale collaborative efforts begun to generate broad phenotypic batteries that encompass brain structure and function as well as multiple domains of cognition, behavior and genetics[5–7]. In parallel, the rapid development of text-mining and machine-learning techniques have facilitated the creation of meta-analytic databases[8]. The resulting bulk consolidation and dissemination of data provide fertile ground for researchers seeking to map links across diverse neural and cognitive states.

With the deluge of open-access, phenotypically rich samples, an increased emphasis has been placed on computationally sophisticated approaches for data aggregation, dimension reduction and interpretation. In this regard, a major challenge is the heterogeneous information encompassed in these data sets, including genetics, behavior, neuroimaging and other meta-data. Methods that can jointly analyze this disparate information have the potential to provide deep insights into human brain function. For example, the human brain exhibits a network topology similar to that of other complex systems, ranging from genetic networks to the Internet. Recent work established a strong correspondence between the structure of intrinsic (resting state) and extrinsic (coactivation) networks of the human brain, suggesting that the topological characteristics of the brain at rest are closely linked to cognitive function[9]. In parallel, analyses of meta-task information, activation coordinates and resting-state data suggest that our capacity to execute diverse tasks is supported by flexible brain regions that integrate information across specialized networks[10]. Yet despite a flurry of advances, we still remain far from a mechanistic understanding of how discrete aspects of brain function might serve to influence suites of behavioral phenotypes.

As reported in this issue of *Nature Neuroscience*, Smith *et al.*[2] take an important step in this direction, conducting a single holistic analysis linking diverse demographic and behavioral phenotypes with shifts in the collective set of functional connections in the brain (functional connectome[4]). To accomplish this, Smith and *et al.*[2] capitalized on data made publicly available through the ongoing Human Connectome Project[5], employing group-independent component analyses of resting-state data to derive a functional connectome for each participant. They then separately generated participant-specific arrays of non-imaging phenomic features. To probe the existence of underlying relationships among these variable sets, the authors used canonical correlation analysis (CCA), a multivariate extension of traditional univariate correlation approaches that are widely employed in neuroscience. CCA yields optimal combinations of linear relations, or modes, across the available sets of phenomic features and patterns of functional connectivity. The resulting modes maximally co-vary across the participants' functional and phenotypic arrays (**Fig. 1**).

The authors identified a single CCA mode relating the functional connectomes of each participant with their non-imaging phenomic features. When considered in the context of available demographic and behavioral phenotypes, a single, positive-negative axis of variation became evident (**Fig. 1**). Resembling classic descriptions of a general intelligence g factor[11], the axis identified by Smith *et al.*[2]

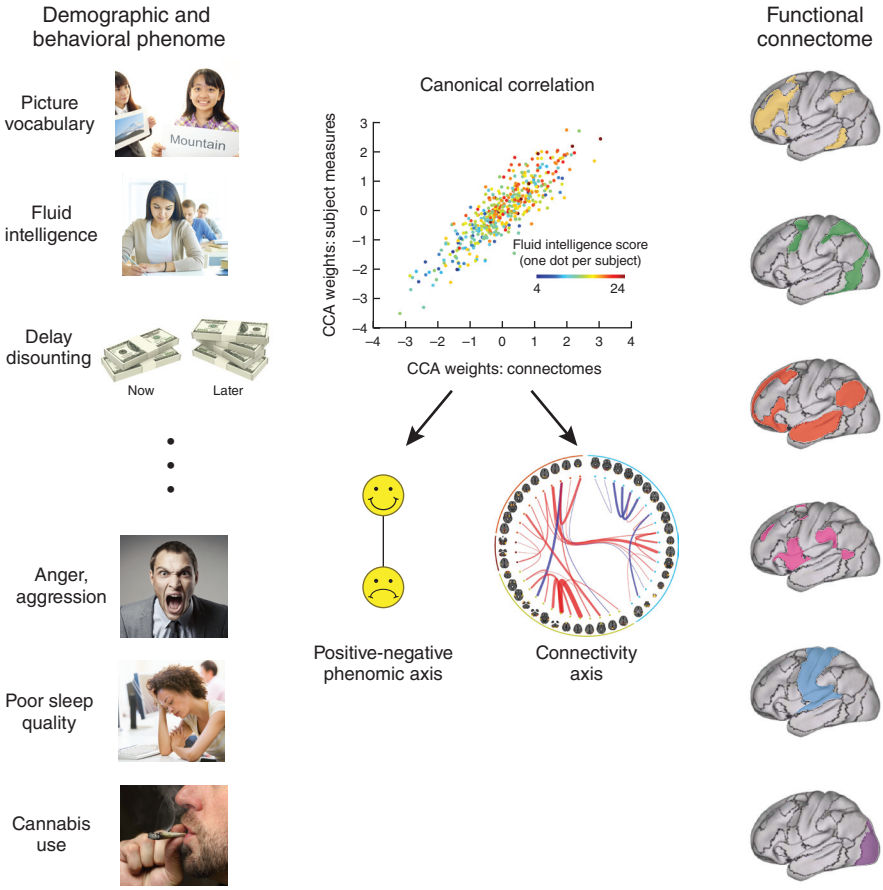

Figure 1 A single positive-negative mode of covariation links diverse demographic and behavioral phenotypes with the collective set of functional connections in the human brain. Left column illustrates some of the diverse demographic and behavioral phenotypes from the Human Connectome Project. Right column depicts the full set of functional connectivity as measured by resting-state functional magnetic resonance imaging from the HCP. Smith *et al.*[2], using CCA, found a single positive-negative axis of strong co-variation (middle top) between demographic and behavioral phenotypes (middle column, bottom left) and connectivity (middle column, bottom right). Higher scores along this axis appeared to uniformly reflect positive personal qualities or indicators (such as fluid intelligence and life satisfaction), whereas lower scores were associated with prototypical negative traits (such as substance use and anger). More positive participant scores were associated with increased connectivity of association cortex, especially the default network. The observed results suggest the potential for a general mode of positive brain function associated with a specific pattern of network-level connectivity. Top and bottom right panels of middle column reproduced from ref. 2, Nature Publishing Group. Right column adapted from ref. 15, American Physiological Society.

encompasses varied participant characteristics, including intelligence, education, estimates of life satisfaction and substance use. Strikingly, higher scores along this axis appeared to uniformly reflect positive personal qualities or indicators (such as high performance on memory and cognitive tests, life satisfaction, years of education, and income), whereas lower scores were associated with prototypical negative traits (such as substance use, rule-breaking behavior and anger).

In both psychology and systems neuroscience, researchers have historically sought discrete cognitive and biological factors that might account for broad shifts in intelligence or other global domains of functioning. Although work in this area has been equivocal, there is emerging consensus that efficient information transfer among underlying brain regions likely facilitates adaptive functioning and high intelligence. Consistent with this conjecture, when considering the single mode emerging from their CCA analyses in the context of each participant's functional connectome, Smith *et al.*[2] report that higher participant scores on the positive-negative axis are associated with increased connectivity of association cortex, especially the default network[12]. Originally named from the observation that the associated set of interacting brain areas engages when individuals are left undisturbed to think to themselves, the default network has since been implicated in a diverse set of functions, including autobiographical memory, imagination and mental construction, and theory of mind. The default network supports myriad cognitive functions that could conceivably influence the positive-negative axis identified by Smith *et al.*[2].

As is frequently the case, while Smith *et al.*[2] establish a promising method for distilling tractable relations linking phenotypically rich imaging and behavioral data types, this work raises intriguing questions that warrant future study. First, why do the brain regions that most strongly contribute to the observed effects primarily overlap with the default network, but only weakly with attentional and executive control networks most often associated with adaptive behavioral control and intelligence? Analyses such as those reported by Smith *et al.*[2] are inherently influenced by a host of factors, including depth and breadth of the available phenotypes, the modality of the imaging data and its associated signal properties, and the extent to which selected data processing pipelines approach biological truth. Although this work suggests that coordinated interactions in the default network might contribute to a general mode of positive functioning, replication and the establishment of converging evidence from other *in vivo* imaging approaches and analytic techniques is warranted.

Second, paralleling intriguing and hotly disputed work fractionating the g factor[13], a major question would be the possible presence, or absence, of sub-axes. Diverse brain networks support the emergence of complex behavioral phenotypes, such as intelligence and substance use, when they are studied in isolation. The observed positive-negative axis, and its associated relations with default network integrity, likely belie the intricate neural circuitry supporting discrete sub-facets of behavior.

There are several important limitations to consider when interpreting the analyses reported in Smith *et al.*[2], as well as the broader push to leverage innovative computational approaches in in neuroscience. First, as noted by the authors, these approaches are correlational in nature. Although we can speculate about causal relations, the precise mechanisms that link variations in brain connectomics and individual differences in behavior remain an open question. A related limitation, pervasive in the field, arises from the generalizability of observed phenotypic relations across the broader population. Sample characteristics, including truncated ranges of variation in some measures, demographic biases in self-report, and data quality, can couple with a lifetime of environmental effects to influence results.

Critically, resting-state imaging reflects just one possible acquisition state from which

researchers could derive functional connectivity estimates. As an approach, it is not without limitations[14]. Intrinsic estimates of brain activity occur in the context of accompanying cognitive processes—for example, internally directed mental operations—and the coactivation of associated regions. Although unmeasured during 'rest', mental activity and coupled behaviors (for example, eye movement, mind wandering and attentional deployment) could systematically differ across subgroups in the population. When interpreting analyses of resting-state data, readers should consider that observed associations with behavior could arise from underlying differences in stable properties of brain organization and/or individual differences in transient task- or state-dependent factors.

Perhaps no other subject in psychology has provoked more debate and controversy than the study of human intelligence and the associated concept of a unitary factor that might broadly support behavior and cognition. Casting a wide phenotypic net and examining the associated array of behaviors in relation to the full intrinsic functional connectome, Smith *et al.*[2] identify a single mode of population covariation. Spread along a positive-negative axis, the observed results suggest the potential for a general mode of positive brain function associated with a specific pattern of network-level connectivity. There will undoubtedly be numerous questions arising from this work, ranging from the generalizability of observed effect across imaging modalities and populations to the potential existence of sub-axes. Nonetheless, these findings highlight the utility of computationally sophisticated, well-powered discovery science in the search for patterns of population covariation that link brain function with suites of demographic, cognitive and behavioral phenotypes.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Pore dilation reconsidered

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Previous experiments have suggested that many P2X family channels undergo a time-dependent process of pore dilation when activated by ATP. Li *et al.* now propose a different interpretation of the key experiments.

For most ion channels, the size of the pore appears to be fixed once the channel has opened. Some exceptions to this are clearly documented, most notably in the form of rare and transient 'subconductance' states[1]. In general, however, such states either have the same ionic selectivity as the main open state or are occupied so briefly that they are unlikely to have any physiological relevance. In this context, the apparent behavior of a group of ligand-activated cation channels is remarkable. In these channels—including P2X2 (refs. 2,3), P2X4 (refs. 2,3), P2X7 (ref. 4), TRPV1 (ref. 5) and TRPA1 (ref. 6)—the pore of the channel seems to undergo a striking increase in permeability to large molecules in a time-dependent manner. This has been referred to as pore dilation. As with almost all dynamic behavior of channels, the evidence for pore dilation has been based on inferences from electrical recordings of currents through the channels: in this case, the primary observation suggesting pore dilation is an apparent time-dependent change in the permeability ratio of large cations, such as *N*-methyl-D-glucamine (NMDG) or Tris, relative to small cations, such as sodium or potassium. In whole-cell patch-clamp recordings with an internal solution containing mainly sodium cations and an external solution containing NMDG, the reversal potential when channels are first activated by ligands is initially very negative, suggesting low permeability of $NMDG^+$ relative to Na^+, but shifts to progressively more depolarized values over seconds, suggesting an increase in $NMDG^+$ permeability. If recorded at a constant voltage in between the two reversal potentials, the current is first outward (carried by Na^+) and then inward (carried by $NMDG^+$).

Li *et al.*[7] propose a completely different explanation for this behavior: that the time-dependent change in reversal potential, although very real, is not caused by a time-dependent change in channel permeability, but rather by a dramatic change in the ion concentrations inside the cell such that, for example, intracellular Na^+ falls from 140 mM to 20 mM and intracellular $NMDG^+$ increases from 0 mM to 200 mM. These changes are especially striking given that the intracellular solution is in constant contact with an essentially infinite reservoir of solution with the original composition, exchanged through the open pipette tip of the patch-clamp pipette in whole-cell mode. The reason that they occur, according to a detailed model that Li *et al.*[7] present to support their interpretation, is that, with high enough expression of channels in the membrane, cumulative exit of sodium ions through all the channels in the cell is much faster than the ions can be replenished from the pipette, and, similarly, entry of $NMDG^+$ through channels occurs faster than $NMDG^+$ can diffuse into the pipette.

In addition to supporting their new interpretation by modeling, Li *et al.*[7] present a number of experiments most easily explained by their interpretation. For example, they found that no change in reversal potential is seen if the channels are activated for many seconds with symmetric Na^+ concentrations and then tested with the $NMDG^+_{out}/Na^+_{in}$ condition. And, perhaps most convincingly, the authors found that the change in reversal potential occurring with $NMDG^+_{out}/Na^+_{in}$ can be reversed if external $NMDG^+$ is replaced temporarily by external Na^+. They also support their modeling by making measurements of the depletion of intracellular K^+ using coexpressed potassium-selective channels.

The phenomenon of time-dependent changes in reversal potential resulting from unexpected changes in concentration of permeant ions has a long history.

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