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Linking cognition to brain connectivity

Stephen Smith

Predicting an individual's behavior is a formidable challenge for neuroimaging. A study now finds a strong link between an individual's ability to sustain attention and an extended, but specific, set of brain connections.

The possibility of using neuroimaging data to predict an individual's behavior is of great interest because it may eventually lead to understanding how processing in the brain gives rise to cognition. Over recent decades, neuroimaging has been evolving from its original primary application of population-average brain mapping toward understanding how brain function varies across subjects and how these differences relate to the subjects' differences in behavioral performance. Moreover, whereas early neuroimaging studies concentrated largely on localizing functional areas, it is now the connectivities between these areas that are increasingly being probed. Indeed, investigating the relationship between connectivity and cognition, and how this varies across subjects, is a primary goal of major recent neuroimaging endeavors such as the Human Connectome Project[1].

In a study in this issue of *Nature Neuroscience*, Rosenberg *et al.*[2] investigated in depth one particular mind-brain relationship: whether indicators of an individual's ability to sustain attention can be found in brain connections. They found that the strengths of a specific set of brain connections can be used to predict a subject's attention ability with high accuracy. This held not just for connectivity estimates made from the attention task imaging data, but even when estimated from resting state data, collected when the subjects were not carrying out any explicit task. Rosenberg *et al.*[2] found that a large number of brain connections are involved in sustained attention and that, despite this being a highly extended network, the set of connections is specific to attention and does not successfully predict other cognitive measures, such as IQ. Furthermore, there is evidence that this extended network of connections is modulated *en masse*, with the network as a whole being weaker or stronger in subjects with different abilities to sustain attention.

To study this link between attention and connectivity, Rosenberg *et al.*[2] used a recently developed protocol[3,4] in which the subject watches a slowly varying slideshow of different scenes and is asked to respond when a particular scene type is shown. Over the course of the experiment, attention generally falls, and the average accuracy in correct reporting of scene types is found to be a sensitive indicator of sustained attention ability—one that correlates with numbers of attention-related errors in normal life, as well as level of 'mindfulness' (for example, awareness of one's present situation and thoughts). Notably, a person's level of mindfulness also correlates positively with mean reaction time in this attentional task, with slower responses relating to being more generally thoughtful.

To test whether a subject's attention score could be predicted by specific brain connections, Rosenberg *et al.*[2] started by analyzing the functional magnetic resonance imaging (fMRI) data acquired during the attention task. They parcellated the brain into 268 distinct functional regions and estimated a representative average activation time series for each region. Then they estimated the correlation between every pair of regions, generating data for ~36,000 region pairs (**Fig. 1**). Correlation is a simple measure of the functional connectivity between regions, based on the notion that functionally linked regions will co-fluctuate. These temporal fluctuations may be caused by a region altering its behavior in distinct task conditions. Alternatively, in resting state experiments, fluctuations would be spontaneous changes in the states of sensory, motor or cognitive processes. Next the authors considered the strength of each region pair's connection and tested whether this varied across subjects in the same way that the attention score varied. They found that 1,000–2,000 connections' strengths varied across subjects in a way that correlated with subjects' variations in attentional ability. These connection strengths were then averaged together within each subject, resulting in a single measure representing average connection strength, and this simplified measure of brain connectivity was found to correlate very highly with sustained attention ability.

This result still held when the approach was applied to subjects not used in this prediction-training process; such evaluations (for example, leave-one-out testing) are crucial in situations such as this, where the possibility of over-fitting the data is high. Even more convincingly, when this same set of brain connections was combined together using separate fMRI data with the subjects at rest, the connection strengths were still strongly predictive of the subjects' separately measured attention ability. This tells us that this large set of brain connections is highly related to sustained attention ability as an innate property of the individuals' brains, and not just during the explicit attention task. That is consistent with another recent study from the same authors, indicating that connectivities estimated during rest correspond closely to those estimated during a range of tasks[5]. Finally, the authors conducted a fully independent replication test, investigating whether averaging connection strength across this same set of putative attentional brain connections was predictive of attentional problems in a cohort of Chinese children with ADHD. Again, they found highly significant predictions of attentional ability, in this case relating to a

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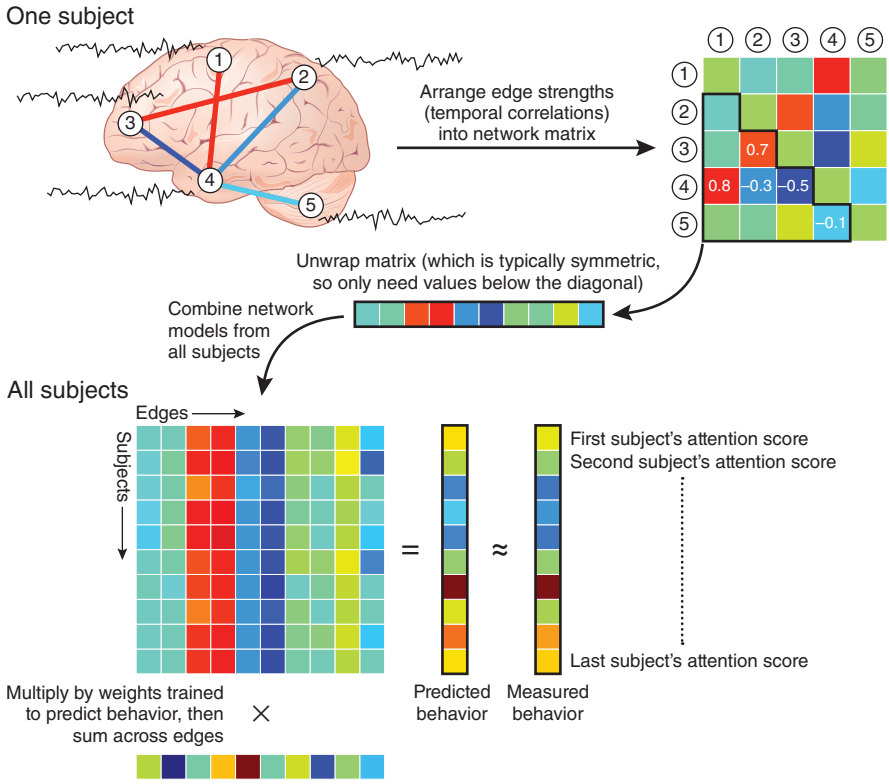

Figure 1 Overview of general analysis strategy for multivariate co-modeling of brain connectivity and behavior. First, multiple brain regions are identified; these are the network nodes. Next, representative (for example, average) fMRI activity-level time series (node time series) are estimated for each region for a given subject. These are then used to model connectivity (network edges) between the regions, for example, through temporal correlation. The nodes × nodes set of connectivity estimates can be represented by a matrix of estimated connection strengths; this can contain negative values, where two brain regions are functionally anti-correlated. The connection-strength values from the matrix are then reshaped (unwrapped) into a row vector, before being combined with the equivalent connectivity vectors from all other subjects. This results in a subjects × edges matrix representing all connection strengths from all subjects. A multivariate analysis then uses this matrix, along with the measures being predicted (for example, attention scores for each subject) to train a set of weights; these trained weights are combined with the connection strengths matrix to generate a single prediction (one value per subject) of the behavioral measure. To avoid over-fitting, this learning process is normally performed with one subset of the subjects and then applied to a second subset to evaluate the prediction accuracy.

clinical attention condition and not just to cross-subject variability among neurotypical subjects.

It is particularly noteworthy that the number of brain connections found to be relevant for predicting sustained attention is relatively high (more specifically: a large set of connections is involved during successful attention, and a different large set correlates with lack of attention). This may relate to the fact that 'attention' is not so much a well-defined narrow cognitive function (such as visual working memory or mathematical processing), but rather a higher level contextual control—selecting, directing, modulating and sustaining more specific cognitive processes and even affecting the extent to which sensory inputs (such as painful stimuli) are consciously experienced. It is also noteworthy that the successful prediction of attention, based on the strength of multiple network connections, was achieved with a modeling approach that produced a predictive feature by averaging all relevant edges together. This suggests that, to a large degree, a single factor modulates a subject's entire sustained attention system (and ability). It is not the case, however, that this set of connections is a non-specific large network with unfocused cognitive relevance; for example, the authors found that this set of edges was not predictive of IQ or age. To some extent, this is in interesting contradistinction to the single general mode of population covariation in functional networks and behavior that we recently reported[6], where one set of brain connections covaries across subjects with a wide range of behavioral and demographic measures that includes not just sustained attention, but also vocabulary, fluid intelligence, life satisfaction, working memory and delay discounting. That result suggests that there also exists a modulation of many cognitive processes that is even more global than the more specific attention-related factor found by Rosenberg *et al.*[2].

There is debate about the relative merits of different methods for inferring network connections from data such as fMRI time series[7]. Correlation-based connectivity estimation, as used here, is the most common approach for resting-state fMRI data and is relatively robust and applicable to networks with large numbers of connections. However, correlation is a rather abstract measure of connectivity; one criticism is that it is not a quantitative biological measure[8], being affected by confounding factors such as noise level and amplitude of within-region fluctuations. Despite such concerns, it is intuitive and reasonable to expect that the strength of spontaneous (resting state) correlation between regions is a good indicator of their functional connectedness. However, during task experiments, this logic may be harder to conceptualize—particularly in the case of constant tasks[9], where one might even expect that the regions most highly involved in the task would be highly and nonvaryingly activated, therefore potentially reducing their fluctuation amplitude and reducing sensitivity to measuring correlation between truly connected regions. What does it mean, in such scenarios, to utilize correlated fluctuations around constant levels of activation to measure connectivity? Fortunately, in this attention study, the attentional task continuously varies over time, with different responses being requested for different types of visual scenes, so one may expect functionally relevant fluctuations in activity level. Furthermore, performance in this task changes over time[3,4]: in addition to a gradual deterioration in attention over the duration of the experiment, there is evidence of at least two distinct cognitive states in use at different times, likely with different attentional mechanisms coming into play in the different states. It is likely that fluctuations in the fMRI signal reflect these changes in attention level and strategy, and hence contribute to the measures of correlation between brain regions that are found to drive the attention predictions.

There is growing interest in studying how the active connections between brain regions relate to behavior. As this area matures, we can hope to be able to derive models of brain function at the macroscopic scale (as seen by the noninvasive neuroimaging technique used here) that can be related both to lower-level mechanistic biological models derived from cell-level invasive studies and cell-level connectivity mapping, and to higher-level large-scale epidemiological studies concentrating on between-subject differences and

population-level modeling of behavior and pathology. As a result, macroscopic-scale whole-brain neuroimaging studies, such as the work from Rosenberg *et al.*[2], have a crucial role in bridging the modeling of mechanisms of brain function and pathology to the final outcomes of externally apparent behavior and disease.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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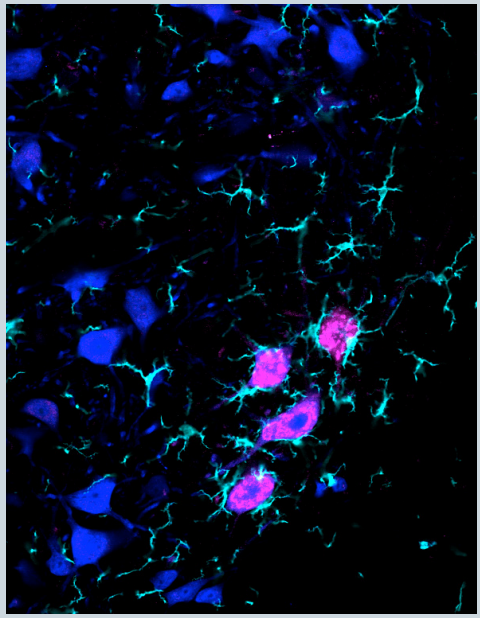
A peripheral messenger for chronic pain



If one steps on the pointy end of a tack, the piercing injury that ensues leads to an acute sensation of discomfort commonly referred to as pain. Pain is usually intense at the time of injury but eventually wanes as tissues heal. When the injury is more serious and tissue damage encompasses nerves, however, pain can become pathological and persist long after tissues have healed. Although nerve injuries can cause long-lasting changes in sensory processing in the periphery, maladaptive changes also occur centrally at the level of the spinal cord. Yet we know relatively little about the mechanisms that connect an injury in the periphery to maladaptive plasticity in the CNS. As reported on page 94 of this issue, Guan and colleagues have now increased our knowledge of this connection by identifying a key set of molecules mediating the influence of the injured state of sensory neurons on cells in the spinal cord.

In what was initially designed as an unbiased exploratory study, the authors performed RNA sequencing on dorsal root ganglia (DRGs) from mice that had received a peripheral nerve injury. One of the RNA species that was greatly upregulated after the induction of neuropathic pain encodes colony-stimulating factor 1, or CSF1, a cytokine involved in the differentiation and proliferation of macrophages and microglia. Given that spinal microglia play a central role in the development of neuropathic pain and that CSF1 can be secreted, this initial observation suggested the possibility that CSF1 could be a trigger for microglial activation and the induction of chronic pain. Of course, this scenario would require that CSF1 be transported to the spinal cord and that microglia have the ability to perceive it. In a subsequent series of experiments, this is exactly what the authors showed. After ligation of the dorsal root between the DRG and the spinal cord, they observed an accumulation of CFS1 at the ligature sites, suggesting that, once expressed, the protein travels along the axons of the sensory neurons toward the spinal cord. In concert, the expression of the CSF1 receptor (CSF1R) increased in microglia in the dorsal horn of the spinal cord. Concomitantly, expression of CSF1 was also upregulated in motor neurons, but only those that had been damaged by the peripheral injury. As can be seen on the accompanying image, the motor neurons (blue) that were injured expressed CSF1 (magenta), and were in close proximity with many of the processes emerging from nearby microglia, which themselves expressed CSF1R (cyan).

Thus, all the elements required for CSF1 to act as a messenger between the peripheral nerves and the spinal cord are in place. But does CSF1 actually contribute to the development of pain? To answer this question, the authors used a two-pronged approach. They showed that deletion of the *Csf1* gene from sensory neurons prevented the activation of spinal microglia that normally follows peripheral injury and the hypersensitivity to mechanical stimulation that is a hallmark of neuropathic pain. In addition, they found that intrathecal injection of CSF1 in uninjured mice was sufficient to both activate microglia and induce chronic pain.

Going a step deeper into the mechanisms, Guan *et al.* also found that DAP12, a transmembrane adaptor protein important for microglial function, was critical for CSF1-induced mechanical hypersensitivity, thus placing it downstream of CSF1. Unexpectedly, although CSF1 stimulated both the activation and proliferation of spinal microglia, DAP12 was required only for its activation. Notably, CSF1 injection could induce pain in mice lacking P2X4, an ATP receptor previously involved in microglia activation and neuropathic pain. This suggests that the CSF1–CSF1R–DAP12 pathway is a key signaling cascade in neuropathic pain but that it acts in parallel to the known ATP–P2X4 pathway.

In sum, the expression of CSF1 represents a pivotal link between peripheral nerve injury and the central mechanisms of neuropathic pain that involve microglia. Microglia are well known to react to various types of injuries in the CNS. In their study, the Basbaum laboratory has identified a key set of factors promoting this activation in the context of neuropathic pain and, in the process, potentially revealed new targets for treating chronic pain. It is tempting to speculate that CSF1 could play a similar role in other conditions that involve an initial insult, such as stroke.

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