and their structures solved. In each case tested, the host behaviors are re-evoked by again transforming *E. coli* with the rescued plasmid.

A diverse set of host behaviors is observed, many of which are surprising. For example, a full range of logical operations on the "inputs" are obtained, including NAND, NOR, and NOT IF, although simple inferential thinking predicts that only NOT IF would be found. As another example, GFP expression in hosts containing plasmid circuits of type 2 (fig. 4 from the Guet *et al.* report) would not be expected to be responsive to aTc, yet some are. Moreover, circuits of the same design but built of different components sometimes show very different behaviors. Thus, simple rules of logical inference have a limited ability to predict the output of the host in response to the two input signals.

Can biological systems be unpredictable? Consider the simple circuit depicted in the figure on the previous page. The circuit is composed of two repressor genes *A* and *B* that mutually inhibit each other. We will call this a "feedback dyad" (also termed a "bistable latch" in electrical circuit design). The output is denoted by *Y*. In the absence of any perturbation, the system has two stable states: one in which *A* is "high" and *B* is "low," and one in which *A* is "low" and *B* is "high." The system could be perturbed by two inhibitors, one nullifying the inhibitory influence of *A* on *B* and the other nullifying the similar inhibitory effect of *B* on *A*. These inputs are labeled X_1 and X_2. Consider the case when both X_1 and X_2 are initially high and then both inputs are lowered simultaneously, thus effectively initiating the interaction of *A* and *B*. To which of the two possible states will the system go? Either of the two states appears to be equally likely. However, if X_1 is lowered slightly earlier than X_2, then the state of the system is *A* "high" and *B* "low," and the output *Y* is "low." Conversely, if X_2 is lowered slightly earlier than X_1, then the output *Y* is "high."

Because the arrival time of these two "input" events cannot be determined exactly—especially in a system built of only a few molecules—the output *Y* cannot be predicted exactly, even when the values of the input are known. Moreover, if the input events occur simultaneously, then the entire system can enter a "metastable" state where *A*, *B*, and the output *Y* assume some intermediate value between "high" and "low," remaining there until small factors or even stochastic fluctuations resolve the entire system into one stable state or the other. Such a system can hardly be considered "predictable."

Many of the circuits constructed by the combinatorial method of Guet *et al.* incorporate a feedback dyad (see, for example, circuit types 5, 9, and 10 of their fig. 4) or another metastable element (for example, circuit type 13). Are these circuits likely to be found in nature? The answer is certain to be yes, because they are virtually the inevitable consequence of wiring pathways together. Moreover, such circuits are likely to be highly useful in a variety of ways. For example, a feedback dyad can function as a simple memory device, its state recording which of two present signals arrived first. Such "memory" may be extremely useful when, for example, a free-living microbe is sensing a complex chemical environment, or a cell exposed to a variety of soluble factors during embryogenesis is deciding its fate. The unpredictability of a metastable circuit may itself be a useful feature—in predator-prey evasion, for example, or when an organism scans its environment by random searches. Furthermore, such systems may be indeterminate at a single-cell level but deterministic in a population at large. For example, to maintain a healthy tissue, it may be advantageous to respond to signals such that some cells divide and some die, maintaining new cells without a net increase in population. Finally, circuits with metastable components may be readily modified by small genetic or biochemical perturbations that bias resolution into one state or another, and thus can be reprogrammed to perform a variety of logical operations.

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PERSPECTIVES: NEUROSCIENCE

Windows into the Human Brain

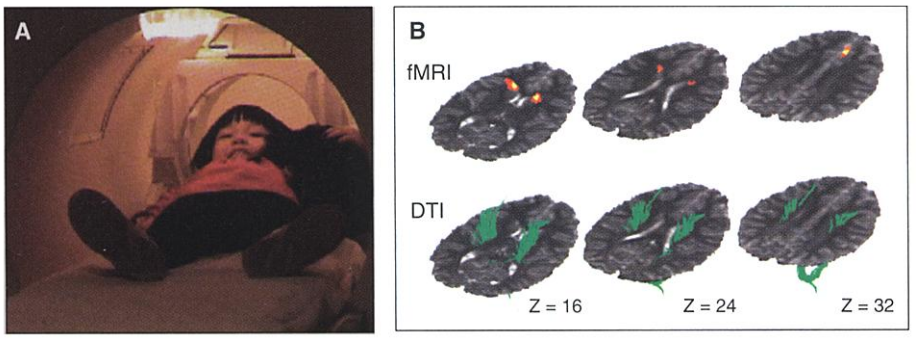
BJ Casey

The study of the developing human brain promises to move at an unprecedented rate, thanks largely to developments in magnetic resonance imaging (MRI). A decade ago, Kwong (*1*), Ogawa (*2*), and others showed that magnetic resonance is sensitive to blood oxygenation changes in the brain that may reflect changes in blood flow and neuronal activity. The insight that MRI can assess the activity of the human brain without the need for the exogenous radioactive tracers required by other methods began a new era in the study of human brain development and behavior. One key issue that could be investigated with MRI is how brain development and behavior change with growth and experience. Do children use the same cognitive and neural processes as adult humans, or do radically different neural processes underlie the superficially similar accomplishments of child and adult? This issue has been hotly debated by developmental psychologists (*3*). Schlaggar *et al.* (*4*), reporting on page 1476 of this issue, demonstrate how we may use functional MRI (fMRI) to begin to address this question. Specifically, they dissociate brain activity related to age from that related to behavioral performance in the prefrontal and extrastriate cortex of the adult and child brain during single word processing tasks.

The Schlaggar *et al.* report accompanies a surge in developmental fMRI studies (*5–9*). Differences between children and adults are typically reported in terms of the location (cerebral gyri, stereotaxic coordinates, Brodmann's areas), magnitude (percent change in MR signal), or volume (number of voxels) of brain activity (*5*). Schlaggar *et al.* examined brain activity (MR signal change) by including time as an independent variable. In this way, they could test whether the change in signal peaked at the same time and intensity for the adult and child groups. A central question is whether developmental differences in the pattern of brain activity are specific to age or to the accuracy and latency of the behavior. Typically, across tasks, children perform more poorly and variably than adults. These same concerns arise when comparing clinical and normal populations (*10*).

How can one tease apart age differences from behavioral performance differences in brain imaging studies? At least three approaches are described in the literature, including the one used by Schlaggar and colleagues. First, we can design tasks a priori that include parametric manipulations in the degree of difficulty, such that children and adults can be compared on the same or different levels of the task to equate behavioral performance. Memory or visual search tasks particularly lend themselves to this design (*11, 12*); many other tasks are not conducive to such manipulations. Second, with sufficient variability and range, we can correlate age and behavioral performance with magnitude or volume of activity, showing which brain regions are predominantly relat-

The author is at the Sackler Institute for Developmental Psychobiology, Department of Psychiatry, Weill Medical College, Cornell University, New York, NY 10021, USA. E-mail: bjc2002@med.cornell.edu



Images of the human brain. (**A**) Five-year-old child in an MRI scanner. (**B**) Recent noninvasive MRI methods measure the function and structure of a child's brain. The top row depicts patterns of brain activity indexed by fMRI in three representative axial (Z) slices. The bottom row shows corticospinal white-matter fiber tracts (green) projecting through the same three axial slices measured by DTI.

ed to maturational changes versus behavioral differences (*5, 7*). Subsequently, each of these variables can be used as a covariate to determine the degree to which these variables independently contribute to changes in brain activity. However, age and behavioral performance correlate with each other on many behavioral tasks. Finally, we can group individuals on the basis of their performance post hoc, as Schlaggar *et al.* describe. Accordingly, we can compare different age groups with similar behavioral performance or the same age groups with different performance. In the case of tasks such as single word processing, this method is effective. However, this approach is valuable only when the different age groups have overlapping distributions in response latency and accuracy.

A question that Schlaggar *et al.* fail to answer is whether the differences in brain activity that they observed between children and adults are due to an immature central nervous system or a lack of experience with the task. This question highlights the beauty of fMRI, which can safely be repeated in the same subjects multiple times, allowing us to track changes in cortical activation after children have had extensive practice with a particular task. Such an approach may provide a more definitive test of whether developmental differences are maturation- or experience-based by assessing brain activity both before and after training. Karni *et al.* (*13*) showed rapid learning effects in primary motor areas of adults who performed motor sequence learning tasks within a single session. These effects increased even further with several weeks of training during which the cortical activity became less diffuse. This example of initial diffuse cortical activity early in learning parallels results from developmental fMRI studies showing diffuse activity in children relative to adults (*5, 14*). This is not to say that differences in brain activity between age groups are due to experience alone; even without normal stimulation, changes in neuronal connections and synaptic pruning occur during development (*15*). Rather, these findings highlight the maturation-versus-experience question and suggest a more precise test: examining brain activity before and after extended practice on a task to determine whether the immature system after extended practice engages in the same neural mechanisms as the mature system. Using fMRI to trace learning-related changes in cortical areas should be informative when investigating the impact of behavioral training interventions for developmental disorders such as dyslexia; such research is under way at the Sackler Institute and other institutions worldwide.

So what is in store for the field of developmental science with continued advances in MRI? Along with advances in fMRI, the method of diffusion tensor imaging (DTI) has arrived (see the figure). DTI more precisely measures neuroanatomical changes in white-matter fiber tracts (*16*). This technique holds promise for tracking neuroanatomical changes in the strength and number of neuronal connections and in fiber myelination with learning and development. Ultimately, we will be able to correlate DTI-based neuroanatomical measures with behavioral and neurophysiological measures of the speed of cognitive and neural processing. This will be achieved by combining DTI with fMRI and methods of higher temporal resolution (such as, evoked potential responses). Clearly, these methods will promote our understanding of how human brain development and behavior change with growth and experience.

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PERSPECTIVES: BIOCHEMISTRY

The 22nd Amino Acid

John F. Atkins and Ray Gesteland

Two complementary reports (*1*, *2*), on pages 1459 and 1462 of this issue, provide compelling evidence that the genetic code of certain Archaea and eubacteria encodes a 22nd amino acid. This nonstandard amino acid, called pyrrolysine, is encoded by the RNA nucleotide triplet UAG, a stop codon that halts translation of mRNA. Krzycki, Chan, and their colleagues (*1*, *2*) show by chemical and structural analysis of proteins from the archaeon *Methanosarcina barkeri* that pyrrolysine is present in the active site of the enzyme, methogenic methylamine methyltransferase, which catabolizes methylamines leading to the production of methane. These authors demonstrate that UAG is at the corresponding position in the mRNA encoding this enzyme, and identify special characteristics of the tRNA carrying this nonstandard amino acid.

The way in which pyrrolysine is encoded bears striking parallels to the encoding of the 21st amino acid, selenocysteine. Selenocysteine is found in Archaea, eubacteria and animals, including mammals (*3*, *4*). Both nonstandard amino acids are encoded by the RNA nucleotide triplets (codons) that signify a command to stop translation of mRNA into protein (UGA is the "stop codon" encoding selenocysteine). The notion that at least 22 amino acids are directly encoded by the nucleotide sequence of mRNA reflects the greater richness of the genetic code than is apparent from the standard textbook account.

Originally, the coding problem was defined in terms of how the 20 common amino acids could be specified by four RNA nucleotides. As the triplet nature of the genetic

The authors are in the Department of Human Genetics, University of Utah, Salt Lake City, UT 84112–5330, USA. E-mail: john.atkins@genetics.utah.edu J.F.A. is also at the Science Foundation Ireland, Dublin 2, Ireland.