Inhibited and Uninhibited Infants "Grown Up": Adult Amygdalar Response to Novelty

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Infants with an inhibited temperament tend to develop into children who avoid people, objects, and situations that are novel or unfamiliar, whereas uninhibited children spontaneously approach novel persons, objects, and situations. Behavioral and physiological features of these two temperamental categories are moderately stable from infancy into early adolescence and have been hypothesized to be due, in part, to variation in amygdalar responses to novelty. We found that adults who had been categorized in the second year of life as inhibited, compared with those previously categorized as uninhibited, showed greater functional MRI signal response within the amygdala to novel versus familiar faces.

The term temperament refers to the stable moods and behavior profiles observed in infancy and early childhood. Although the notion of temperament is at least 2000 years old, dating back to the Greeks (*1*), the empirical studies of Chess and Thomas (*2*) sparked a renaissance of interest in infant temperaments. Two of the most extensively studied temperamental constructs are related to the behavioral dimension of approach and withdrawal (*2*), which refers to the child's typical response to unfamiliar people, objects, and situations. The extremes of this dimension define two categories of children called behaviorally inhibited and uninhibited (*1*, *3*, *4*). Children with an inhibited temperament tend to be timid with people, objects, and situations that are novel or unfamiliar, whereas uninhibited children spontaneously approach novel persons, objects, and situations. These behavioral differences in young children were accompanied by distinctive physiological differences, including differences in heart rate and heart rate variability, pupillary dilation during cognitive tasks, vocal cord tension when speaking under moderate stress, and salivary cortisol levels (*5*, *6*).

The footprint of these early individual temperamental differences is discernible in later childhood (*7*–*11*) and early adolescence (*12*–*14*). Furthermore, the two temperamental types are at risk for developing different symptom profiles. An uninhibited temperament in early childhood is associated, given particular rearing environments, with externalizing behavior at adolescence (*13*), which ranges from display of a hot temper and stubbornness, to impulsive, aggressive, and antisocial behavior. In contrast, an inhibited temperament in early childhood is a risk factor for the development of one of the anxiety disorders in both children (*15*, *16*) and adolescents (*14*), especially generalized social phobia (alternatively termed social anxiety disorder) (*14*, *17*).

The demonstration that these temperamental categories were heritable fueled interest in the basic brain properties that might mediate the temperamental biases observed in infancy (*18*, *19*). It has been suggested that the complex behavioral and physiological profiles of these two temperamental categories might be the result of differing responses to novelty in the amygdala (*5*, *6*).

We tested this hypothesis with functional magnetic resonance imaging (fMRI) by measuring the response of the amygdala to novel versus familiar faces in 22 adults (mean age 21.8 years) who had been categorized in the second year of life as inhibited ($n = 13$) or uninhibited ($n = 9$) (*20*).

The protocol (*20*) was divided into two portions: a familiarization phase and a test phase consisting of alternating blocks of either novel (N) or familiar (F) faces (Fig. 1A). The 96-s familiarization phase consisted of 16 presentations of six faces in pseudorandom order (balanced for gender and age). Each subject viewed four novel blocks; each block consisted of 24 different identities completely unique to that block, shown once, and never repeated, and four familiar blocks consisting of repeated presentation of the same six identities that had been presented repeatedly during the familiarization phase.

A repeated-measures analysis of variance (ANOVA) was performed (*20*) on the functional imaging data from a six-voxel region in the right amygdala and a three-voxel region in the left amygdala (Fig. 1B), and yielded a significant temperament × face-type interaction [$F(1,20) = 4.21$, $P = 0.05$]. The adult subjects categorized as inhibited in the second year of life showed a significantly greater response in both the right and left amygdalae to novel faces (versus fixation), compared with those subjects who had been categorized as uninhibited [Fig. 1C; $t(20) = 2.40$, $P = 0.01$]. By contrast, there was no difference between the two temperamental types in the amygdala signal when they viewed familiar faces (versus fixation). Furthermore, inhibit-

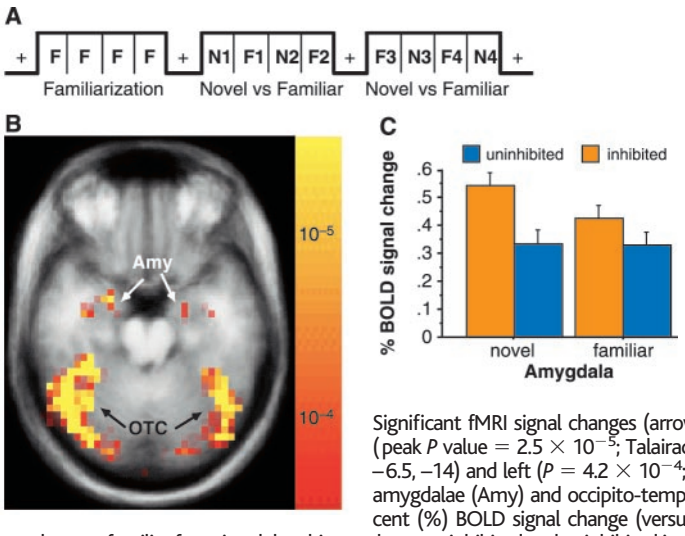

Fig. 1. (**A**) The presentation of stimuli was divided into two phases: a familiarization phase and a test phase that consisted of alternating 24-s blocks of either novel (N) or familiar (F) faces with neutral expression. Subjects viewed a fixation cross (+) during 24-s fixation blocks. (**B**) Colorized group statistical map superimposed on coronal group-averaged T1 structural image in Talairach space. Significant fMRI signal changes (arrows) are shown in the right (peak P value $= 2.5 \times 10^{-5}$; Talairach coordinates $x, y, z = 21, -6.5, -14$) and left ($P = 4.2 \times 10^{-4}$; $x, y, z = -21.5, -6.7, -18$) amygdalae (Amy) and occipito-temporal cortex (OTC). (**C**) Percent (%) BOLD signal change (versus fixation) in amygdala to novel versus familiar faces in adult subjects who were inhibited and uninhibited in the second year of life. One standard error of the mean is indicated.

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ed subjects showed significant signal increases in both the right and left amygdalae to novel versus familiar faces [Fig. 1C; $t(12) = 3.13$, $P = 0.004$], whereas adult subjects categorized as uninhibited in the second year of life did not show a significant change in BOLD signal to novel versus familiar faces. The repeated-measures ANOVA affirmed that the responses in the right and left amygdalae were similar (*21–23*).

These findings support the hypothesis (*5*, *6*) that inhibited and uninhibited infants are characterized by different amygdalar responses to novelty and suggest that some brain properties relating to temperament are preserved from infancy into early adulthood. Only longitudinal studies can demonstrate developmental continuities from early childhood to adulthood and can affirm the persistent impact of a temperamental profile in adults. New developments in brain imaging technology will be required to probe directly for temperamental differences in amygdalar responses in infants.

An inhibited temperament is a risk factor for the development of generalized social phobia (*14*, *17*), a psychiatric disorder characterized by persistent and pervasive fear of interaction with unfamiliar people and avoidance of situations where such interactions are anticipated. Two subjects in the present study, both categorized as inhibited in the second year of life, were diagnosed with generalized social phobia and showed signal changes comparable to the other inhibited subjects. We eliminated the possibility that the present results might be due to these two subjects by repeating the analyses without them. This analysis did not change the findings. These results imply that discovery of a difference in brain activity between subjects with a psychiatric diagnosis and a control group should not always be regarded as a specific marker of the disorder. The difference may reflect instead a temperamental risk factor, or diathesis, for the diagnostic category under study. Thus, the findings from cross-sectional neuroimaging studies that describe differential amygdalar responses in subjects with social phobia (*24–28*) may be influenced by, or even due to, temperamental factors persisting from early in childhood. This fact suggests the need to study further the influence of temperamental biases persisting from childhood on adult neuroimaging data.

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Reversal and Stabilization of Synaptic Modifications in a Developing Visual System

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Persistent synaptic modifications are essential for experience-dependent refinement of developing circuits. However, in the developing *Xenopus* retinotectal system, activity-induced synaptic modifications were quickly reversed either by subsequent spontaneous activity in the tectum or by exposure to random visual inputs. This reversal depended on the burst spiking and activation of the *N*-methyl-D-aspartate subtype of glutamate receptors. Stabilization of synaptic modifications can be achieved by an appropriately spaced pattern of induction stimuli. These findings underscore the vulnerable nature of activity-induced synaptic modifications in vivo and suggest a temporal constraint on the pattern of visual inputs for effective induction of stable synaptic modifications.

Synaptic modifications induced by patterned neuronal activity are essential for experience-dependent refinement of developing circuits (*1–5*) as well as learning and memory (*6–9*) in the adult brain. However, how these modifications become stabilized in the constant presence of neuronal activity in vivo remains largely unclear. We addressed this question using a specific in vivo preparation (*10*, *11*). Synaptic connections in the developing tectum of *Xenopus* are highly susceptible to modification by activity in the visual pathway. Long-term potentiation (LTP) and long-term depression (LTD) can be readily induced at retinotectal synapses by correlated spiking of retinal ganglion cells (RGCs) and tectal neurons (*12*) and by repetitive visual stimuli (*13*). In these studies, the postsynaptic tectal neurons were usually voltage-clamped at a constant membrane potential, a condition that prevents neuronal spiking. Under natural conditions, however, postsynaptic neurons are likely to spike spontaneously, as a result of random sensory inputs due to ambient illumination (*14*) or inputs from other brain regions (*15*, *16*).

We first examined whether spontaneous activity of tectal neurons affects the persistence of LTP and LTD at retinotectal synapses induced by correlated pre- and postsynaptic activity. Repetitive suprathreshold stimulation of an RGC input (at 1 Hz for 100 s) resulted in an immediate increase in the amplitude of excitatory postsynaptic currents (EPSCs), without affecting the unstimulated control input that converged onto the same tectal neuron (Fig. 1A). This potentiation was long-lasting when the

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