Chapter 14

Temperamental Contributions to the Development of Psychological Profiles: II. Two Candidates

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This chapter summarizes the evidence gathered on two temperaments which my colleagues and I have been studying for the past 35 years. Each bias is characterized by the infant's initial reactions to unfamiliar or unexpected events.

VARIATION IN REACTIONS TO THE UNFAMILIAR

The brains of awake humans are always prepared for the event that is most likely to occur in that context in the next moment. The sudden sound of an alarm in a quiet room lies outside the envelope of expected events and provokes an automatic alert state as well as a state we might call stimulus uncertainty. An adult wearing an unusual mask and costume entering a room in which a two-year-old child is playing also lies outside the envelope of expected events. In this case, the resulting state of vigilance is called conceptual uncertainty because the person's features are unfamiliar and discrepant from the average two-year-old's acquired schema for the humans he or she has encountered. Stimulus and conceptual uncertainty activate different circuits and, therefore, are different states. The former activates the amygdala but not always the hippocampus, whereas the latter usually activates both structures. Either state can potentiate the intensity of a physically painful stimulus and might also enhance the level of distress generated by an unwanted event (Yoshida, Seymour, Koltzenburg, & Dolan, 2013).

A third source of uncertainty emerges after nine or ten months when children recognize that they have more than one response to a situation and are uncertain over the one that is most appropriate. This state, called response uncertainty, is accompanied by a third circuit. However, the evidence implies that all

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three states typically activate a circuit involving the amygdala, anterior insula, ventromedial prefrontal cortex, and central gray (Hsu et al., 2005).

It is important to differentiate between a brief state of uncertainty accompanying a sudden alarm, which is usually associated with temporary activation of the amygdala, and a chronic state associated with activation of the bed nucleus of the stria terminalis (BNST). The BNST receives input from the amygdala and, in turn, projects to the hypothalamus and brain stem sites whose activation can be followed by secretion of the corticotropin-releasing hormone and increased autonomic activity (Yassa, Hazlett, Stark, & Hoehn-Saric, 2012). It is likely that the BNST is tonically active in children who possess a temperamental bias for chronic uncertainty.

One possible, but still unproven, mechanism could explain how such a state could be established. An unexpected criticism or punishment creates conceptual and response uncertainty, accompanied by activation of the amygdala and anterior insula and an unpleasant feeling resembling "fear". The repetition of this sequence in children who possess a bias for an excitable amygdala could generate a chronic anticipation of uncertainty-evoking events. This state would be accompanied by a chronically active BNST.

Scores on a questionnaire measuring variation in intolerance of uncertainty is consistently correlated with symptoms of anxiety and depression (McEvoy & Mahoney, 2012). It is likely that this trait most commonly occurs among those who possess a chronically active BNST generating intensely uncomfortable feelings to the anticipation or appearance of unexpected and unfamiliar events or situations in which they are not sure what behavior to select. All humans are exposed to such events regularly, but only a small proportion regard the resulting state of uncertainty as particularly noxious. Hence, the origins of intolerance of uncertainty probably lie with a combination of past experiences and a biology that rendered the BNST, and probably the amygdala as well, unusually responsive to perceptible but unexpected changes in the outside world or in sensations from the body.

UNCERTAINTY AND TEMPERAMENT

Children vary in the duration of an alert state, the slope of habituation of the alerting response to repetitions of an unexpected incentive, and the physiological processes that accompany the alerting. Each of these properties can represent temperamental biases. Some children typically react to events that generate stimulus, conceptual, or response uncertainty with a prolonged caution, wariness, withdrawal, or distress. These children are called *inhibited*. The children we call uninhibited typically show a brief period of alerting to most unexpected events, followed, after a brief interval, by a state of relaxation.

My colleagues and I believe that the two infant temperamental biases called high and low reactivity, which are assessed at four months, account for a great deal of the variation in inhibited or uninhibited behaviors that are observed after the first year (Kagan & Snidman, 2004; Kagan & Fox, 2006). Similar variation in the reactions to unexpected events have been observed in fish, mice, rats, dogs, cats, cows, monkeys, and apes (Herrmann, Hare, Cissewski, & Tomasello, 2011; Kagan, 1994). It is not a coincidence that the first two factors that emerge from most personality questionnaires refer to a dour mood, worry, caution, and shyness or a happy-go-lucky attitude, extraversion, and an attraction to risk. These two types share some features with the contrast between Galen's melancholic and sanguine categories and Jung's introvert and extravert.

Behavioral observations of a large sample of identical and fraternal twins at 14, 20, 24, and 36 months revealed that a timid-inhibited or bold-uninhibited profile had modest heritability values (Kagan & Saudino, 2001; Robinson et al., 1992). The heritability values were higher when the data were restricted to the smaller number of children who displayed extreme levels of inhibited or uninhibited behaviors while playing with two unfamiliar peers (Buss, 2011; DiLalla, Kagan, & Reznick, 1994). Children who had been selected as obviously high or low on inhibition at 21 months were clearly separated at five and a half years by an index composed of eight biological measures (including morning cortisol concentration, heart rate and variability, pupillary dilation during cognitive tasks, and voice quality) that implied higher levels of arousal among the five-year-olds previously classified as inhibited (Kagan, Reznick, & Snidman, 1987).

It is important to appreciate that behavior that appears to reflect shyness need not be the product of a temperamental bias. The children who prefer to play alone who are not anxious about playing with others should be distinguished from those who avoid interactions with peers because they feel uncertain. Rubin calls the latter children reticent (Coplan, Rose-Krasnor, Weeks, Kingsbury, & Bullock, 2012; Rubin, Hastings, Stewart, Henderson, & Chen, 1997). A temperamental bias favoring a prolonged state of uncertainty, characteristic of inhibited and reticent children, increases the risk for an anxiety disorder, should an acute or chronic stressor occur later in development (Beidel & Alfano, 2011).

HIGH- AND LOW-REACTIVE INFANTS

Extensive research with animals and humans reveals the robust fact that the amygdala almost always responds to an unexpected or unfamiliar event, whether it is aversive or pleasant. Even a stimulus as innocent as an unpredictable sequence of sounds activates this structure (Herry, Bach, Esposito, Di Salle, et al., 2007). The amygdala also mediates the acquisition of classically conditioned responses, such as freezing or changes in heart rate, to an aversive unconditioned stimulus, usually electric shock (LeDoux, 1996). The amygdala contains several distinct neuronal clusters that contain receptors for several neurotransmitters and neuromodulators that modulate the amygdalar reaction and the feelings and behaviors that define a temperament (Amaral et al., 1992).

A majority of scientists currently believe, as I do, that inherited variation in amygdalar reactivity is one important reason for the variation in behavioral signs of uncertainty, fear, or anxiety. The small proportion of rhesus monkeys who displayed a large rise in cortisol and/or freezing when an unfamiliar human approached their cage and presented a facial profile without eye contact also showed increased activity in the central nucleus of the amygdala (Shackman, Fox, Oler, Shelton, Davidson, & Kalin, 2013). Comparisons of the varied sizes of the amygdala among primate species, including monkeys and apes, revealed that the species with the larger amygdalae, often monkeys, were typically less social because they adopted a vigilant, cautious posture with unfamiliar conspecifics (Lewis & Barton, 2006).

The basolateral region of the amygdala receives input from many sources, including vision and audition, and sends projections to the ventral pallidum which contributes to limb movements in animals (Rolls, 1992). The central nucleus of the amygdala, whose embryological origin differs from that of the basolateral amygdala, is the origin of pathways to diverse targets whose outputs are popular measures of fear or anxiety. One target is the central gray, which mediates escape behaviors, immobility, arching of the back, and distress vocalizations (Chan, Kyere, Davis, Shemyakin et al., 2011). Monkeys who possess an anxious temperament have a higher level of excitability in the central nucleus of the amygdala and the central gray than non-anxious animals (Fox, Oler, Shelton, Nanda, et al., 2012).

The central nucleus is modulated by a number of factors that affect its excitability. One important modulator is a collection of GABA-ergic cells, called the intercalated islands, which lie between the basal and central nuclei of the amygdala and inhibit the latter structure when stimulated by the basolateral nucleus, the ventral tegmental area, or the medial prefrontal cortex. These cells are capable of preventing the central nucleus from innervating the autonomic or motor targets that are public signs of fear.

An infant born with a neurochemistry that produced a lower threshold of excitability in both the basolateral and central nuclei of the amygdala should display vigorous limb movements, arching of the back, and crying to unfamiliar events. The infants who display this profile, called high-reactive, should be especially vulnerable to becoming avoidant to, and distressed by, unfamiliar events later in life; that is, they should become inhibited children. By contrast, infants born with a different neurochemistry that raised the threshold of these amygdalar nuclei should display minimal motor activity and little crying and, after the first birthday, should be uninhibited.

It is important to remember that most of the amygdala's input, but not all, comes from the thalamus. The thalamus, in turn, receives postural information from the pons, which receives information about the state of the heart, lung, and gut from the medulla oblongata. Hence, inherited variation in the chemistry of any of these three subcortical structures could affect the excitability of the amygdala. This means that, theoretically, the chemistry and/or anatomy of the medulla, pons, or thalamus could contribute to the variation in the amygdala's response to novelty. Readers should appreciate that humans can experience a form of fear despite severely compromised amygdalae (Feinstein, Buzza, Hurlemann, Follmer, et al. 2013).

My colleagues and I administered a battery of unfamiliar and unexpected but non-aversive, visual, auditory, and olfactory stimuli to a large number of healthy, Caucasian, middle-class, four-month-old infants born at term and healthy to two-parent families (Kagan, 1994). The selection of four months was based on evidence suggesting that most infants display limb activity and/or crying to discrepant events by this age (Melinder, Forbes, Tronick, Fikke, & Gredeback, 2010). The decision to restrict the sample to European-Caucasians was based on the fact that the prevalence of a number of genes that could affect infant behaviors differs among individuals with a European, Asian, or African pedigree (Polimanti, Piacentini, Manfellotto, & Fuciarelli, 2012; Yaar & Park, 2012). In addition, published as well as unpublished data from my own and other laboratories suggest that the infants from these three populations vary in their behavioral responses to unfamiliar events. For example, four-month-old Caucasian infants observed in the still-face procedure displayed more frequent smiling and babbling than Chinese-American infants. This difference was especially clear for infants classified as low-reactive (unpublished data from Tronick-Snidman laboratory).

The 20% of the sample of Caucasian infants who combined vigorous limb movements, arching of the back, and frequent crying to these sources of uncertainty were classified as "high-reactive". The 40% who showed minimal limb activity and arching, without any distress, were classified as "low-reactive". The remaining 40% of the sample belonged to two other temperamental categories (frequent limb activity but no distress or minimal limb movement but frequent fretting or crying). Fox and his colleagues at the University of Maryland have found similar proportions in Caucasian infants (Calkins & Fox, 1992). It is an interesting coincidence that among rhesus monkeys a similar proportion displays fearful behavior to novelty (Suomi, 2011).

The different behavioral patterns of high- and low-reactives provide a clue to their distinctive brain physiologies. Although high-reactives moved their arms and pumped their legs far more frequently than low-reactives, a small proportion of the latter group did occasionally make the same movements, albeit with less vigor. I noted that these acts are mediated by a circuit from the basolateral nucleus of the amygdala to the ventral pallidum via the nucleus accumbens (Zorilla & Koob, 2013). But high-reactives were the only infants who combined limb movements with frequent arching of the back and crying. The latter two behaviors are mediated by a circuit from the central nucleus of the amygdala to the central gray. These facts imply that only the high-reactives possessed a state of hyper-excitability in both the basolateral and central nuclei. It is of interest that infants whose mothers had higher concentrations of salivary cortisol during the last trimester of their pregnancy were most likely to be classified as high-reactive at four months (Werner, Zhao, Evans, Kinsella, Kurzius, Altincatal, McDonough, & Monk, 2012).

Crying and arching of the back rarely occurred to initial presentations of a stimulus. Rather, most high-reactives pumped their legs and arms for several trials, building up increasingly higher levels of arousal until a threshold was passed, after which arching and crying occurred. This phenomenon could be the result of a central nucleus that was inherently excitable or one that was poorly regulated. In either case, the central nucleus would eventually reach a level that exceeded the inhibitory forces acting on it, resulting in projections to the central gray. The low-reactives never came close to that threshold.

A variety of factors could create a hyper-excitable amygdala. One popular hypothesis holds that the excitability of the central nucleus could be the result of a failure of the medial prefrontal cortex to suppress the basolateral nucleus and/or the intercalated cell islands. A second hypothesis attributes the lack of modulation of the central nucleus to the chemistry of the intercalated cells. That failure could be the result of different conditions because the intercalated cells have receptors for a large number of receptors, including GABA A and B, the D1 receptor for dopamine, and mu-opioids. An abnormality in any one of these receptor systems could lead to insufficient suppression of the central nucleus and, as a result, frequent crying and arching of the back when the infant was aroused by unfamiliar events.

ASSESSMENT

Assessment in the Second Year

About 80% of this sample returned to a laboratory at 14 and 21 months, where they were exposed to a large number of different, unfamiliar events, social and nonsocial, with their mother present—16 episodes at 14 months and 15 episodes at 21 months (Kagan, 1994). None of the events was dangerous or threatening but all were unexpected and unfamiliar and therefore should have created uncertainty. The response to moderately unfamiliar or mildly threatening events is a more sensitive index of temperament than the reaction to serious threats that frighten all children (Buss, 2011). The decision to expose the child to a large number of incentives was based on the fact that each procedure had method variance associated with its features. Thus, sampling a large variety of incentives was more likely to reveal the child's temperament. Unfortunately, some psychologists expose young children to only one or two incentives (for example a stranger and/or a robot) and assume that the resulting behaviors are a sensitive sign of the child's temperament.

The film records of the episodes were scored for the display of behaviors reflecting a state of uncertainty that most would label fear. This state was defined by fretting or crying to an incentive or failing to approach a stranger, clown, or metal robot despite a friendly invitation by an adult to do so. The children who showed four or more fears at both 14 and 21 months (about one-third of the whole sample) were classified as displaying a high level of fear. One-half of the high-reactive infants displayed a high level of fear at both ages and only one high-reactive displayed a low level of fear (defined as zero or one fear at both ages). By contrast, 75% of the low-reactives were minimally fearful at both ages and only 13% were highly fearful.

It is worth noting that the high-reactives who were highly fearful at both ages had narrower faces than the low-reactives who were minimally fearful (the facial measure is the ratio of the width of the face at the bizygomatic, or cheek bones, divided by the length of the face) (Kagan, 1994). Adult males with very broad faces, a feature potentiated by higher levels of testosterone, are less anxious over being criticized for holding unpopular beliefs (Hehman, Leitner, Deegan, & Gaertner, 2013).

A temperamental bias contributes to the behaviors one-year-old infants display in the Strange Situation—high-reactives are more likely to cry intensely when the mother leaves the room and are harder to soothe when she returns because of difficulty regulating distress states (Marshall & Fox, 2005). As a result, they are likely to be classified as "insecurely attached-resistant". One-year-olds who smiled frequently to non-social events or had low and variable heart rates, traits that are characteristic of low-reactives, were most likely to be classified as Type B—securely attached (Burgess, Marshall, Rubin, & Fox, 2003; Kagan, 1974).

These facts are congruent with the observation that newborns who show the largest increase in sucking rate when the water they were ingesting unexpectedly turned sweet behave in the Strange Situation at 18 months like insecure-resistant children and unlike the most securely attached children (La Gasse, Gruber, & Lipsitt, 1989). The increased sucking rate is mediated by the amygdala's response to the unexpected change in taste sensation and projection to neurons that mediate sucking. Hence, newborns with the largest increase in sucking who became maximally distressed in the Strange Situation, probably possessed the most excitable amygdala.

Assessment at Four and a Half Years

Most four-year-old children have gained control over the display of extreme distress to unfamiliarity and it is therefore difficult to provoke unambiguous signs of fear without violating ethical standards. However, two behaviors that might index these biases are infrequent talking and smiling when interacting with an unfamiliar child or adult. If we assume that becoming quiet in an unfamiliar social setting is analogous to an animal's freezing in an unfamiliar context, children who had been high-reactive should talk and smile less often with an unfamiliar adult than those who were low-reactive. Each four-and-a-half-year-old interacted with an unfamiliar female examiner during a 60-minute battery that included cognitive tests and measurements of autonomic function. The high-reactives made fewer spontaneous comments and smiled less often than low-reactives.

Several weeks after the laboratory session, each child returned for a play session with two other unfamiliar peers of the same sex and age while their three parents sat on a coach, in a playroom containing age-appropriate toys. At the end of a half-hour of play, an adult dressed in a gorilla costume carrying toys entered the room and, after a minute, invited the children to play with the toys. The entire corpus was coded for whether the child was sociable and spontaneous with the peers and rarely stayed close to the mother (one half of the sample behaved this way and were called "uninhibited") or reticent, quiet, and spent much time proximal to the mother (the one-quarter of the group who behaved this way was called "inhibited"). The remaining 25% belonged to neither category. Forty-six percent of the high-reactives but only 10% of the low-reactives were inhibited; two-thirds of low-reactives but only one quarter of high-reactives were uninhibited (Kagan, Snidman, & Arcus, 1998).

However, only a small proportion of high-reactives showed a high level of fear at both 14 and 21 months and, in addition, were inhibited with peers at four and a half years. This select group may have had more protective parents because preservation of an inhibited profile is more likely when mothers protect their shy children from opportunities to extinguish their anxieties (Hudson, Dodd, Lyneham, & Bovopoulous, 2011). The fact that not all high-reactives who had been fearful in the second year were inhibited at four and a half years implies the continuing influence of experience on the social behaviors that have a temperamental foundation.

Rimm-Kaufman visited the kindergarten classrooms of 14 high- and 17 low-reactives four times between early September and January and observed the children's behaviors in this natural setting (Rimm-Kaufman, Rosenstock & Arcus, 1996). The high-reactives were less likely to talk, volunteer, or break a classroom rule governing proper behavior, compared with the low-reactives.

Assessment at Seven and a Half Years

The mothers of the seven-and-a-half-year olds were interviewed for the presence of fears, such as the dark, sleeping at a friend's house, large animals, or insects. If the mother described her child as highly fearful, the teacher was asked to rank the child with respect to all children of the same sex in that classroom for shyness and fearfulness. If both mother and teacher agreed that the child displayed behavioral signs of anxiety, which was true for one-quarter of the entire sample, we categorized the child as "anxious." Many more high-than low-reactives were classified as anxious (45 versus 15%). Not surprisingly, high-reactives are usually more accurate on tasks requiring reflection and the regulation of an impulsive response because they are more anxious than low-reactives over making errors that might incur adult criticism.

However, the fact that one half of the high-reactive seven-year-olds were not classified as anxious indicates again that some had learned to regulate the behavioral display of their uncertainties. Less than 15% of middle-class, mainly white, children growing up in the New York metropolitan area preserved signs of an anxiety disorder from three to six years (Bufferd, Dougherty, Carlson, Rose, and Klein, 2012). Personas often change during the preschool years even though less public aspects of a temperament are preserved.

Assessment at Eleven Years

Many of these children returned to our laboratory at 11 years of age for an evaluation that included quantification of a large number of biological variables under the direct or indirect influence of the amygdala, as well as their tendency to talk and smile while interacting with a female interviewer. As anticipated, more high- than low-reactives were quiet and non-smiling during the assessment. Forty percent of high-reactives, compared with only 10% of low-reactives, displayed inhibited behavior during the three evaluations at four, seven, and 11 years. The frequency of spontaneous smiles was the most sensitive behavioral sign of a high- or low-reactive temperament at every age (Kagan & Snidman, 2004). This observation is in accord with the fact that the amygdala sends projections to the central gray whose activation is often accompanied by suppression of smiling as well as speech (Schulz, Varga, Jeffires, Ludlow, & Braun, 2005). Although equal numbers of infant girls and boys were high-reactive, more girls than boys displayed inhibited behavior at every age from 21 months to 11 years.

Biological Measures

The selection of biological measures was guided by the hypothesis that the two groups differed in amygdalar excitability. We measured asymmetry of activation in the frontal lobes, where less power in the alpha band of the EEG in the frontal region of one hemisphere was treated as an index of activation in that hemisphere. This EEG index of hemispheric asymmetry has modest heritability and stability over intervals of one to three years (Anokhin, Heath, & Myers, 2006; Vuga et al., 2006).

The left frontal lobe is usually, but not always, more active than the right (less alpha power on the left) when individuals are relaxed; whereas the right hemisphere is more active than the left when individuals are in a state of uncertainty, tension, or unpleasant emotional arousal (Cole, Zapp, Katherine, & Perez-Edgar, 2012; Davidson, 2003; Fox, 1994; Hewig, Scholtz, Gerhards, Breitenstein, Lurken,& Naumann, 2008; Papousek, Schulter, Weiss, Samson, Freudenthaler, & Lackner, 2012; Schmidt, 2008; Tullett, Harmon-Jones, & Inzlicht, 2012). Children and adults who show right frontal activation are a little more likely to develop dysphoria and/or anxiety than those with left frontal activation who show the complementary pattern of a relaxed, happier mood (Schmidt & Fox, 1994; Schmidt, 2008). Most newborns show greater metabolic activity in the right hemisphere and are more often in a state of distress than one of joy (Lin, Roche-Labarbe, Dehaes, Fenoglio, Grant, & Franceschini, 2013). However, the specific incentive for uncertainty is important. Ten-month-olds who were right frontal active showed more obvious behavioral signs of fear to masks of people and animals, but were not significantly more fearful to an approaching stranger or a toy spider (Diaz & Bell, 2012).

There is no agreement on the biological causes of a frontal asymmetry in alpha power because different conditions can generate an asymmetry favoring one hemisphere (Coan & Allen, 2004). Healthy adults listening to the first movement of Beethoven's Fifth Symphony, who were aroused but neither anxious nor uncertain, showed increased activation of the right frontal lobe (Mikuta, Altorfer, Strik, & Koenig, 2012). However, the evidence is moderately supportive of the idea that the right hemisphere makes a more important contribution to varied states of emotional arousal and to sympathetic dominance of the heart. By contrast, the left hemisphere makes a more important contribution to relaxed states and parasympathetic control of the heart (Wittling & Pfluger, 1990; Wittling & Roschmann, 1993; Wittling, Block, Genzel, & Schweiger, 1998; Wittling, Block, Schweiger, & Genzel, 1998).

The fact that the visceral input to the brain, especially to the amygdala and mid-and posterior insula, is greater to the right than to the left hemisphere implies that children with more activity in the heart, gut, and respiratory system should show less power in the alpha band, and more power in the beta or gamma bands, in the right frontal lobe, reflecting greater activation of this region (Cameron, 2002; Critchley, 2005). These facts imply that children with a great deal of visceral activity should display more labile affect and have a higher probability of displaying right frontal activation. Those with less visceral activity should find it easier to regulate strong emotion and should be more likely to show left frontal activation.

This idea is partially supported by the fact that six-year-old African-American boys from economically disadvantaged families who showed greater activation of the right frontal lobe were rated by their teachers as high on both the internalizing traits of anxiety and avoidance as well as the externalizing traits of aggression and impulsivity, compared with boys from the same ethnic group who were left frontal active (Gatzke-Kopp, Jetha, & Segalowitz, 2012). A small group of German men imprisoned for a crime provided more persuasive support for the presumed relation between left frontal activation and a personality marked by minimal anxiety. The most violent prisoners in this group displayed the most extreme levels of left frontal activation (Keune, van der Heiden, Varkuti, Konicar, Veit, & Birbaumer, 2012).

As anticipated, more high- than low-reactives showed greater activation of the right, compared with the left, prefrontal cortex, affirming earlier work by Fox and colleagues (Fox, Henderson, Rubin, Calkins & Schmidt, 2001; Hane, Fox, Henderson, & Marshall, 2008). This relation was much clearer for the high-reactives with large asymmetry values who had been highly fearful in the second year. It is relevant that depressed patients who showed right frontal activation were less likely to be helped by a therapeutic drug than patients with left frontal activation (Bruder, Sedoruk, Stewart, McGrath, Quitkin, & Tenke, 2008). In addition, a coupling in the right frontal lobe between the low frequency delta band, whose origin is in limbic sites, and the higher frequency beta band is more common among shy children as well as those with a parent who had social phobia (Miskovic, Campbell, Santesso, Van Ameringen, Mancini, & Schmidt, 2011).

The biological measure that best separated the high- from the low-reactives at age 11 was that the magnitude of the brain stem evoked potential from the inferior colliculus to a series of click sounds. The waveform generated by the inferior colliculus, called Wave 5 because it is the fifth structure in the chain from basilar membrane to auditory cortex, occurs about six milliseconds after the onset of a sound. Activity in the basolateral nucleus of the amygdala potentiates the excitability of the inferior colliculus. As a result, adults anticipating an electric shock, a state that would have excited the amygdala, displayed a potentiated Wave 5 (Baas, Milstein, Donlevy, & Grillon, 2006). A circuit involving the inferior colliculus, central gray, and amygdala appears to be a fear circuit in animals. Rats who avoid exploring unfamiliar spaces, classified as more fearful, display a larger evoked potential in the inferior colliculus to tones than rats who were less fearful because the projections from the basolateral nucleus of the amygdala to the colliculus are potentiated in the fearful animals (Nobre & Brandao, 2011).

These results imply that high-reactives, who are presumed to have a more excitable amygdala, should have a larger Wave 5. The high-reactives did display a larger Wave 5 than the low-reactives. Moreover, the high-reactives who had been most inhibited while playing with two unfamiliar children at four and a half years had larger Wave 5 values than the small number of high-reactives who had been more sociable with the peers.

The third variable separating high- from low-reactives was the magnitude of an event-related potential (ERP) between 300 and 500 milliseconds to unfamiliar, unexpected scenes. The amygdala sends projections to the locus ceruleus, ventral tegmentum, and the basal nucleus of Meynert. These structures project to cortical neurons responsible for the magnitude of the ERP waveform to unfamiliar events. Children with a more excitable amygdala should show larger P300 or N400 waveforms to unfamiliar events that are unexpected. The high-reactives showed larger N400 waveforms to ecologically invalid, but nonthreatening, scenes (e. g., a child's head on an animal's body, a car in mid-air) than low-reactives (See Rossignol, Campanella, Maurage, Heeren, Falbo, & Philippot, 2012 for a similar result).

Finally, activity in the cardiovascular system, which also reflects amygdala activity, separated the two groups. A spectral analysis of supine heart rate revealed that more high- than low-reactives had relatively more power in the lower (around .04 Hz) compared with the higher frequency band (around .10 Hz) of the cardiac spectrum. This observation implies that the high-reactives had greater vasomotor tone and less vagal tone on the heart associated with respiration than the low-reactives (Reyes del Paso, Langewitz, Mulder, van Roon, & Duschek, 2013). The combination of greater power in the lower frequency band and a higher resting heart rate characterized one of two high-reactives but only one of three low-reactives (Kagan & Snidman, 2004).

Many investigators have reported associations between a high and minimally variable heart rate and signs of behavioral inhibition and equally robust associations between a low and variable heart rate and sociability, minimal fear, and resilience to stress (Bornas, Llabres, Noguera, Lopez, Barcelo et al. 2005; Brooker & Buss, 2010; Brosschot, Van Dijk, & Thayer, 2007; Oldehinkel, Verhulst, & Ormel, 2008; Ortiz & Raine, 2004). These cardiac properties are heritable, moderately stable over time, and associated with less excitability of the neurons of the central gray. Infants and school age children with an Asian pedigree have a higher and less variable heart rate than those with a European pedigree (Eyre, Fisher, Smith, Wagenmakers, & Matyka, 2013; Kagan, 1994). I noted earlier that Chinese-American toddlers are more inhibited than the average child with a European pedigree.

Low-reactive boys had the lowest and most variable heart rates and, in addition, were the least fearful children on every assessment. One of four low-reactive boys, but not one high-reactive, were prototypic Clint Eastwood types. They were minimally anxious, matter of fact, not effusive in affect, and displayed a relaxed posture, high vagal tone, a small Wave 5, and a small N400 waveform to the invalid scenes.

Even among four-year-olds rated as shy in preschool settings, those with a low and variable heart rate were better able to control impulsive acts on tasks requiring effortful control (Sulik, Eisenberg, Silva, Spinrad, & Kupfer, 2013). It is relevant that beagles and cocker spaniels, compared with fox terriers, Shetland sheep dogs, and basenjis, were the least timid to novelty and had lower heart rates than the other three breeds (Scott & Fuller, 1965).

High-reactive eleven-year-olds did not display larger potentiation of the eye blink startle to a loud sound when they saw a light that warned of the possible delivery of an aversive air puff to the throat or an unpleasant scene. This finding is inconsistent with the popular assumption that potentiated startle to aversive events is larger in those who are chronically anxious (Bradley, Cuthbert, & Lang, 1999). But this result is supported by others (Lipp, Siddle, & Dall, 2000; Schmidt et al., 1999).

Assessment at Fifteen Years

A sample of 15-year-olds that included 59 low-reactives, 49 high-reactives, and 38 who belonged to neither group were visited at home for a lengthy interview. Close to one-half of these youths were also seen in the laboratory. The high-reactives displayed the most frequent movements of hands and legs and the greatest postural tension during the three-hour interview during which a female interviewer posed three differently worded questions at different times that asked about the adolescent's worries. The questions were: "What things make you nervous or anxious?," "What do you worry about?," and "Can you name two times when you were worried over the past few months?." The major targets of worry were: (1) performance in school or in extra-curricular activities, (2) uncertainty over meeting unfamiliar people or going to unfamiliar places, (3) social rejection and maintaining friendships, (4) physical harm, and (5) the health of a close relative.

Worries over competent performances in school, on the athletic field, or on a stage were the most frequently mentioned anxieties and low-reactives were more likely than high-reactives to nominate these realistic concerns as their only sources of anxiety (61 versus 37%). This result is in accord with unpublished data from Nathan Fox's laboratory indicating that adolescent self-reports of anxiety were not predicted by the four-month classifications of high or low reactivity. This evidence supports the earlier suggestion that adolescent or adult replies to questionnaires asking about anxiety do not reflect temperamental biases.

By contrast, two-thirds of high-reactives said they worried about being in crowds, interacting with unfamiliar peers, visiting new cities, or not knowing what might happen in the future, compared with only 20% of low-reactives. Some excerpts from the interviews illustrate this concern: "In a crowd I feel isolated and left out; I don't know what to pay attention to because it is all too ambiguous;" "I worry about the future, over not knowing what will happen next;" "I like not being with others when I'm with my horses." One high-reactive adolescent who had been very inhibited in the second year told the interviewer she does not like spring because of the unpredictable changes in weather. Others did not like to answer the telephone, ask a teacher for help, fly, disagree with a friend, or try a new route to school. One fifteen-year-old told the interviewer. "I get nervous before any vacation because I don't know what will happen."

The low-reactives rarely described similar fears. One low-reactive boy forged a letter from his parents to the admissions office of a private school stating that he would not be attending the school. Low-reactives find it much easier than high-reactives to rely on the defense of denial when they think of the possible dangers that accompany risky behavior. High-reactives, by contrast, exaggerate the dangers of minimally risky actions. Each group is certain that they adopted the most adaptive life strategy. It is important to note that a fair proportion of high-reactives who confessed to unrealistic fears were not unusually shy in their social interaction with the interviewer and denied being shy when asked directly.

A survey of the fears of college students from seven different societies revealed no significant cultural or gender differences in the incidence of realistic fears of dangerous animals and disease, but significant cultural and sex differences for the less realistic fears of cockroaches, mice, crowds, or criticism (Davey et al., 1998). Unrealistic fears have higher heritability values than realistic fears (Sundet et al., 2003).

Each adolescent was given a set of 20 statements descriptive of personality and asked to rank them from most to least characteristic of the self. We created an index of a sanguine, compared with a dour, mood by averaging the ranks of four highly correlated items: "I am pretty serious;" "I think too much before deciding what to do;" "I wish I were more relaxed;" and the reverse of "I am easy-going." High scores reflected a sanguine mood; whereas low scores reflected a dour mood. Low-reactives were much more likely than high-reactives to describe themselves as sanguine.

Biological Measures

The biological assessment at age 15 was similar to the one administered at 11 years of age. The display of left or right frontal activation in the EEG was moderately stable from 11 to 15 years and more high-reactives showed greater activation of the right frontal lobe at both ages; whereas more low-reactives were left frontal active at both ages. The magnitude of the brain stem auditory-evoked potential (Wave 5) was also moderately stable. Forty percent of high-reactives but not one low-reactive had a large Wave 5 magnitude at both ages.

The high-reactives continued to maintain larger ERP waveforms and a shallower slope of habituation to continued presentations of unfamiliar scenes. The high-reactives with a shallow habituation of the N400 also had a large Wave 5 and displayed a unique behavioral profile during the interview. Two of these adolescents were reluctant to look at the interviewer and three reported atypical fears. One girl became anxious when she thought someone might touch her; a second worried about a school trip to Washington, D.C.; and a third confessed, "I feel vulnerable when I'm with people I don't know because I don't know what to do or what to say" (Kagan, Snidman, Kahn, & Towsley, 2007).

A final set of observations provides persuasive support for the suggestion that high- and low-reactives possess differentially excitable circuits connecting the amygdala and prefrontal cortex. Schwartz and colleagues recorded brain activity and structure in 135 eighteen-year-olds who had been either high- or low-reactive as infants. The adolescents first saw a set of faces with neutral expressions, repeated for many exposures, followed unexpectedly by a new set of faces, also with neutral expressions. The high-reactive 18-year-olds showed greater amygdalar activation than the low-reactives, based on the magnitude of the BOLD response to the unexpected change in faces (Schwartz, Kunwar, Greve, Kagan, Snidman, & Bloch, 2012). In a second procedure, the high-reactives displayed a larger BOLD signal to the amygdala the first time they saw an angry face (one of four classes of faces with different expressions). (See Straube, Mentzel, & Miltner, 2005 for a related result). In a final procedure, high-reactives maintained a large BOLD signal (that is a shallow slope of habituation) to the amygdala across four blocks of unfamiliar pictures, alternating with blocks of familiar scenes that they could not anticipate. Adults who reported traits that define behavioral inhibition also showed a shallow slope of habituation of the amygdala to neutral faces (Blackford, Allen, Cowan, & Avery, 2013) as did adults high in neuroticism or generalized anxiety when viewing unpleasant scenes (Schuyler, Kral, Jacquart, Burghy, Weng, et al. 2012) or cues signaling possible electric shock (Greenberg, Carlson, Cha, Hajcak, & Mujica-Parodi, 2013). These results point to the importance of examining a corpus of evidence for patterns of change over time rather than automatically looking only at the mean value across a sequence.

The shallower slope of habituation of the BOLD signal to repetitions of novel pictures implies a failure of amygdalar neurons to adapt to these events. One possible, albeit speculative, cause of this phenomenon involves a class of proteins called arrestins. These proteins attach themselves to sites on G protein-coupled receptors that have been phosphorylated and prevent the latter from activating the cell when a neuromodulator or transmitter binds to the receptor. As a result, habituation occurs. An abnormality in the arrestin proteins on the surface of amygdalar neurons could result in a failure of this structure to adapt to repeated exposures to an incentive.

A second possibility is that the high-reactives had a less dense or less effective collection of serotonin 2A receptors which mute amygdalar activity (Fisher, Meltzer, Price, Coleman, Ziolko, Becker, Moses-Kolko, Berga, & Hariri, 2009). It is worth adding that in female, but not male, rats the corticotropin-releasing factor secreted by a stressor does not activate arrestin proteins in the locus ceruleus. Hence, females remain aroused for a longer time (Valentino, Reyes, Van Bockstaele, & Bangasser, 2012). If this were true in humans it could contribute to the greater prevalence of anxiety disorders in females.

Finally, the high-reactives had a thicker cortex in a small area in the anterior ventromedial prefrontal cortex of the right hemisphere (Schwartz, Kunwar, Greve, Moran, Viner, Covino, Kagan, Stewart, Snidman, Vangel, & Wallace, 2010). The high-reactives with very large thickness values in the right hemisphere admitted to the most unrealistic fears when interviewed at age 15. Cortical thickness in this area, which attains a peak value between eight and ten years, is heritable in humans and monkeys (Lenroot et al., 2008; Lyons, Afarion, Schatzberg, Sawyer-Glover, & Mosely, 2002; Shaw et al., 2008). The low-reactive boys who had extremely thin values in this location (about 20% of this temperamental-gender group) displayed very low EEG indices of cortical arousal, left frontal activation, and unusually low sympathetic tone in the cardiovascular system.

The neurons in the anterior region of this site in the right hemisphere appear to contribute to the conscious experience of bodily feelings that could be interpreted as anxiety because these neurons send projections to the hypothalamus, amygdala, sympathetic nervous system, and central gray. There is evidence to suggest that the latter site is activated by states of uncertainty (Krueger, Moll, Zahn, Heinecke, & Grafman, 2007), including mistakes (Jenkins & Mitchell, 2010), and following the report of a change in bodily feeling (Terasawa, Fukushima, & Umeda, 2013). More relevant is the fact that a person who is uncertain about the intensity of an imminent pain stimulus exaggerates the intensity of the pain produced by the actual stimulus and shows increased blood flow to the central gray (Yoshida, Seymour, Koltzenburg, & Dolan, 2013). The neurons at this location are also active when a person is introspectively monitoring their bodily state (Price, 2005). Anxious individuals are habitually uncertain and also monitor their bodily state more frequently.

The projection from the anterior site to the rostral region of the lateral central gray is of particular importance because the latter neurons mediate the arching of the back that high-reactives displayed in excess at four months. The high-reactives who displayed a very large amount of arching, compared with the high-reactives who displayed less arching, had a thicker ventromedial cortex in the right hemisphere, showed larger BOLD signals to the angry face, and shallower habituation of the BOLD signal to the unfamiliar scenes. These observations imply, but do not prove, that these high-reactives may have possessed a thicker cortex in this location when they were four months old.

Several reports imply that cortical thickness in this region is related to the traits associated with high reactivity. Healthy boys, aged seven to 17 years, who were likely to be cautious before acting had more brain tissue in the medial region of the prefrontal cortex of the right hemisphere, close to the site where high-reactive 18-year-olds had a thicker cortex (Boes, Bechara, Tranel, Anderson, Richman, & Nopoulous, 2009). The offspring of parents or grandparents who had been diagnosed as depressed also had a thicker ventromedial prefrontal cortex in the right hemisphere (Peterson et al., 2009). By contrast, German men who had committed antisocial crimes had less cortical tissue in this region (Bertsch & Grothe, 2013). Patients with lesions of this area report less depressed moods (Koenigs et al., 2008) and less intense affective responses to the contemplation of committing an aggressive act (Moretto, Ladavas, Mattioli, di Pelligrino, 2010; Young, Bechara, Tranel, Damasio, Hauser, & Damasio, 2010). Even rats with lesions of this region show less avoidance of bitter tastes (Sullivan & Gratton, 2002). Finally, this area generates a large BOLD signal when adults are trying to control a state of fear (Diekhof, Geier, Falkai, & Gruber, 2011), do not know when an unpleasant picture might appear (Somerville, Wagner, Wig, Moran, Whalen, & Kelley, 2012), or are thinking about their personal traits (Krienen, Tu, & Buckner, 2010).

Clinical Symptoms

More high- than low-reactive 18-year-olds told a clinician who was blind to their past history that they had suffered from, or were experiencing, a bout of social anxiety, depression, or generalized anxiety. (See also Clauss & Blackford, 2012). These symptoms were most prevalent among high-reactive females, a few of whom admitted to acts of self-harm. This fact may be related to some of the sex differences in brain anatomy and function that have been detected in animals during both early and later developmental stages (Cao &

Patisaul, 2013). One such difference is the fact that post- pubertal female rats show greater binding of CRF 1 receptors for corticotropin-releasing hormone in the basolateral amygdala than males, whereas males show greater binding of CRF 2 receptors. CRF 1 receptors are excitatory but CRF 2 receptors mute activity of the amygdala (Weathington & Cooke, 2012). This finding implies that the average male possesses more effective regulation of the amygdala. It is relevant that the amygdala has an unusually high density of estrogen alpha receptors which should be more active in females (Hofer, Lanzenberger, & Kasper, 2012).

A smaller number of low-reactive girls reported the same symptoms of anxiety or depression. But these girls did not possess a thicker cortex in the right ventromedial cortex nor did they show a larger BOLD signal to the amygdala to the novel neutral faces or the angry face (See Liao et al., 2011 for supporting evidence). Furthermore, 90% of the high-reactive 18-year-olds who reported depression, social phobia, or generalized anxiety had displayed a large amount of arching of the back at four months, had a very high fear score at 14 and 21 months, or showed both behavioral measures. Not one low-reactive reporting anxiety or depression displayed either behavior. The fact that high- reactives with the most frequent arching of the back at four months were most likely to possess anxious or depressive symptoms at 18 years implies that these adolescents have been living with a temperamental susceptibility to a state of uncertainty all their lives. The pattern of infant behavior that defines high-reactivity might be an endophenotype for the later development of some form of anxiety or depressive disorder.

These facts imply that current psychiatric diagnoses for depression, GAD, and social anxiety can be the result of different temperaments and life histories. This conclusion is in accord with the observation that adolescents who had been inhibited as young children are at greater risk for a bout of depression than those who became shy for the first time during adolescence (Karevold, Ystrom, Coplan, Sanson, & Mathiesen, 2012). It is likely that every DSM diagnosis has more than one etiology. Some origins can be rare, as exemplified by a 15-year-old boy whose sudden onset of obsessive-compulsive symptoms was due to an infarct of the putamen nucleus caused by an autoimmune reaction (Van Roie, Labarque, Renard, Van Geet, & Gabriels, 2013).

Cascades and Mental States

Bouts of anxiety over varied events can be the product of two cascades—one more dependent on bottom-up processes and the other more dependent on top-down events. Some individuals possess a brain chemistry and physiology, rendering them susceptible to unexpected visceral input that creates feelings with an unpleasant valence which they interpret as worry over a present or future event. High-reactives belong to this group. A second, larger group live with many realistic reasons for worry, which in turn generate brain and body changes that are experienced as an uncomfortable tension. This sequence characterizes the low-reactives who reported bouts of anxiety or depression.

Reports of anxiety and depression by adolescents or adults were medicalized during the 1970s. Before that time, American psychologists recognized that everyone was capable of becoming temporarily worried or depressed. The occasional emergence of these emotions was viewed as an inherent feature of our species rather than a property reserved for a few who inherited particular genes. It was understood that temporary bouts of anxiety or depression had to be distinguished from the hallucinations or manic-depressive swings of a smaller number who were born with a special biological vulnerability. A century earlier the term *mentally ill* applied to this rare group.

The advice given to college students who sought professional help before 1940 because they worried excessively about grades, friendships, or money often boiled down to a version of "pull up your socks" (McKinney, 1934). This advice was congruent with the social Darwinian philosophy that appealed to many successful Americans born before 1900 who favored the premise that those who were biologically fit were destined to ascend to greatness. Those who could not cope with their worries or temptations to idleness did not deserve positions of responsibility and the pleasures of wealth that hard work brought. In less than 150 years anxiety was transformed from a frequent, non-toxic state that people had to learn to cope with to a dangerous condition that must be avoided and treated at once with medicine or therapy.

Visceral Feedback

Because all adolescents meet new people and visit unfamiliar places, it is important to ask why many more high- than low-reactives mentioned these experiences as primary sources of worry. One answer is that they are more susceptible to spontaneous visceral feedback from the body to the amygdala and cortical sites which, when detected, creates a state of uncertainty and motivates a more cautious, risk-averse posture (Xue, Lu, Levin, & Bechara, 2010). But it also seems to render them more empathic towards others in need (Ernst, Northoff, Boker, Seifritz, & Grimm, 2012). The house cats who display consistent avoidance to novelty—about 15% of a typical litter—possess a hyper-excitable circuit from the medial amygdala to the ventromedial hypothalamus (Adamec & Stark-Adamec, 1986).

The brain state created by unexpected visceral input and the state that accompanies encounters with strangers or novel places both involve activation of the locus ceruleus, amygdala, insula, and association areas of cortex. Hence, the two states should became associated (Buzsaki, 2006). The persistent avoidance of strangers, however, is a product of instrumental learning. The person's experiences of reduced uncertainty when avoiding settings with strangers are required.

A fair proportion of our high-reactive 18-year-olds retained a private feeling of tension with strangers, even though they had extinguished the habits of being quiet in and avoiding unfamiliar social settings. Some even appeared to observers to be extraverts. These high-reactives were more likely than others to be deeply religious. Many said that their religious commitment helped them cope with their uncertainty. The adult offspring of a depressed parent were also at a lower risk for depression if they were religious (Miller, Wickramaratne, Gameroff, Sage, Tenke, & Weissman, 2012).

Many sixteenth-century devout Christians worried more about offending God than about how others viewed them. Contemporary, agnostic adults are more likely to experience anxiety or guilt over actions that might have offended others or disrupted their social relationships. In addition, acquiring wealth, status, and friendships, not a feeling of pious virtue, are dominant motives among the current generation. These concerns and desires require the person to be preoccupied with his or her relationships with others. As a result, contemporary adults who were high-reactive infants are likely to worry about their compromised social skills and to receive a diagnosis of social anxiety. Sixteenth-century high-reactives would have been more likely to enter a monastery or convent.

DETERMINISM OR LIMITATIONS?

The important point is that some individuals inherit a biology that renders them susceptible to salient, uncomfortable feelings in unfamiliar contexts. This biology, however, does not have the power to determine the behaviors the person continues to display for many years. The feeling of vigilance upon entering a room of strangers can be a classically conditioned response that is likely to recruit the amygdala. By contrast, the avoidance of situations that evoke a vigilant state is an instrumentally conditioned response that need not require amygdalar involvement. Jung captured this distinction when he differentiated between a person's anima and persona. Temperaments affect the anima (or animus) not the persona. Most contemporary investigators measure a person's persona because it is easier to quantify.

One high-reactive adolescent boy confessed that although he feels an initial tension to signs of threat he has learned how to subdue public display of his anxiety:

"I have found that the manifestation of my anxiety can be overcome by using simple mind over matter techniques." But his temperament renders him continually vigilant. Weymar, Keil, and Hamm (2013) may have found one useful index of this vigilant state. They reported that spider phobics, compared with controls, showed a larger wave form over the occipital cortex in the EEG at about 50 milliseconds (called the C1 waveform) when detecting the one stimulus in an array of six objects that was discrepant from the other five. The amygdala sends projections to the primary visual cortex and, therefore, an excitable amygdala would potentiate the visual cortex as it potentiates the inferior colliculus to generate a larger Wave 5 in the brainstem-evoked potential.

High-reactives appear to inherit a vulnerability in cortical or subcortical structures that transmit bodily activity to the insula, amygdala, and medial and orbitofrontal cortex that renders them more likely to detect activity in the heart or gut (Kagan & Moss, 1962; Kagan, Reznick, & Snidman, 1978). Visceral sites have a much lower density of sensory receptors than skin or muscle. Hence, the signals from the cardiovascular system, lung, or gut that pierce consciousness are sufficiently ambiguous to allow a variety of interpretations. The popular terms for emotions in most languages represent subjective interpretations of an ambiguous source of information, say a rapid pulse or a tension originating in the stomach, in a particular context.

High-reactives growing up in American society will be biased to interpret some kinds of unexpected visceral feedback as implying they are worried about their ability to cope with a social challenge. The folk theory they learned implies that their feelings are a sign of anxiety and a compromise in their personality. Members of other cultures might impose different interpretations on the same visceral sensations. Cambodian refugees living in Massachusetts interpret an unexpected bout of tachycardia as implying a weak heart caused by a loss of energy that is due to a lack of sleep or a diminished appetite (Hinton & Hinton, 2002). Presumably, these adults would not acquire a social phobia. Chinese children and adults hold a more benign view of shyness and timidity than Americans. As a result, shy Chinese children who feel tense with unfamiliar peers are far less likely to be rejected by others and more likely to be perceived as competent by teachers (Chen, Chen, Li, & Wang, 2009; Chen, Wang, & Cao, 2011).

Over 75 years ago Gordon Allport argued that the concept "personality" should be defined as varied combinations of temperaments that were sculpted by different life experiences into a large number of trait patterns. I suspect that the temperamental contribution to each observed pattern is disguised and, at present, difficult to detect after the first birthday. A temperament is like the drop of black ink placed in a glass of glycerine that disappears from view after the liquid is stirred for a short while.

The incorporation of a temperamental bias into a personality trait can occur early in the first year in human as well as monkey infants (Gottlieb & Capitanio, 2012). This important fact may explain why the categories of high- or low-reactive, based on behavior at four months, were better predictors of select behavioral and biological measures at age 11, 15, and 18 years than the level of fear displayed at 14 or 21 months (Kagan & Snidman, 2004). Fearful behaviors to novelty in the second year (called behavioral inhibition) are personality traits that, in some cases, reflect both a high-reactive temperament and past experience. In others they reflect only past experience.

Perhaps the most significant implication of the temperamental biases of high- or low-reactivity is that each represents a barrier to, rather than a cause of, particular traits. The probability that a high-reactive infant will not become a consistently exuberant, sociable, fearless adolescent with a minimally excitable amygdala is very high—this prediction was correct for 90% of high-reactives. However, only 20% of the high-reactive infants became extremely shy, socially anxious adolescents with signs of an excitable amygdala. Similarly, over 90% of low-reactives were neither extremely shy or fearful, nor did they show signs of amygdala excitability, but only 40% were consistently sociable, exuberant, and in possession of a minimally excitable amygdala. Thus, limiting is a better adjective for a temperament than determining. That is why less than five percent of adolescents had four or more phobias during their adolescent years, even though it is likely that 10 to 20% of this group were high-reactive infants (Burstein, Georgiades, He, Schmitz, Feig, et al. 2012). A random sample of adults will contain a higher proportion of low-reactives who do *not* have an anxiety disorder than high-reactives who do, and a higher proportion of high-reactives who do *not* belong to criminal gangs than low-reactives who are members of such groups.

The claim that temperaments limit the phenotypes that can emerge is an instance of the more general principle that most genes set a limit on the traits, illnesses, and competences an individual can develop, rather than guarantee a particular outcome, because no one with full knowledge of an infant can know the events that will occur during the formative first two decades of development. When the relation between genes and diseases is more complete physicians will be better able to tell parents the illnesses that their newborn is unlikely to acquire than to predict the diseases that they will develop. Information typically reduces the number of alternatives without specifying one outcome.

The same principle applies to the consequences of experiences. Psychologists who knew that a large sample of American women born between 1982 and1992 grew up in homes with affluent, affectionate parents and graduated high school in the top quartile of their class would be unable to predict with great accuracy their personality traits, hobbies, vocational interests, musical preferences, romantic relationships, or politics. But they would be correct 90% of the time if they predicted that these women were not homeless, incarcerated, addicted to cocaine, or prostitutes.

CATEGORIES OR CONTINUA?

Finally, the evidence supports the suggestion that high- and low-reactivity should be regarded as genetically based categories rather than members of a continuum of behavioral reactivity. Almost all young children are initially quiet and cautious for 10 to 20 seconds when a stranger dressed in an odd costume appears unexpectedly. But only a small proportion cry and retain a timid posture over a long interval. The genes that are responsible for the brief period of caution observed in most children are unlikely to be the same genes that contribute to the small proportion who show extremely fearful reactions to novelty that persist for years.

More high than low-reactives had blue eyes, an ectomorphic body build, and a narrow face. This fact implies that some members of this category possess a qualitatively distinct set of genes. One such gene may involve alleles of the gene SOX10 whose protein products influence the differentiation of the neural crest cells in the young embryo.

The association between eye color and a high-reactive bias in Caucasian children is supported by other evidence. The teachers of 133 kindergarten through Grade 3 classrooms containing mainly white children nominated the single most inhibited and least inhibited child in their classroom. Significantly more blue-eyed children were nominated as the shyest in the class; whereas more brown-eyed youngsters were nominated as minimally shy (Rosenberg & Kagan, 1987; see also Coplan, Coleman, & Rubin, 1998; Moehler, Kagan, Brunner, Wiebel, et al., 2006). Western artists seem to possess an unconscious association between blue eyes and emotional vulnerability in Caucasians. Doreen Arcus (1989) analyzed the eye color the artists working for Walt Disney studios initially gave to the main characters in seven full-length cartoon films: Snow White, Pinocchio, Cinderella, Alice in Wonderland, Peter Pan, Sleeping Beauty, and The Sword in the Stone. Of the nine major characters given blue eyes, eight had vulnerable personalities (e.g. Dopey, Pinocchio, Alice, Cinderella). Of the 24 characters who did not have blue eyes, 19 were invulnerable (e.g., the evil queen, Grumpy, Queen of Hearts, Cinderella's stepsisters). Blue eyes are often associated with a light skin color that contains less melanin than most complexions. It may not be a coincidence that trout or salmon with a large number of melanin spots display a muted HPA axis response to stress and are bolder than fish of the same species with fewer melanin spots (Kittilsen, Schjolden, Beitnes-Johansen, Shaw, et al., 2009).

Psychologists prefer to conceptualize the behavioral displays to unfamiliarity as continua rather than as categories because the statistics they use most often, usually regression and analysis of variance, are most sensitive when used with continuous variables rather than qualitative categories. Hence, it is common practice to compute correlations and ANOVAs on values that are treated as members of a continuum rather than compare individuals who fall at either extreme of a distribution (Baker, Baibazrova, Ktistaki, Shelton, & van Goozen, 2012). The latter groups usually show less behavioral variability across different settings. That is, high-reactives show less variability in cautious, timid behaviors to a dozen unfamiliar situations than the average child who typically displays timidity to only one or two incentives. Most biological measures—BOLD signal, EEG power bands, heart rate—are conceptualized as continuous variables. The increased reliance on biological measures has made it even more attractive to treat psychological processes as continuous too.

My colleagues and I believe, however, that the high- and low-reactives belong to qualitatively distinct biological categories with a pattern of brain properties that does not lie on a continuum with infants who display moderate levels of motor activity and crying. This claim is based, in part, on the fact that the predictive relations to the later behavioral and biological variables described earlier held only for the infants classified as high- or low-reactive. When we combined the four-month-old infants' crying and motor scores to create a continuous standard score that reflected overall "reactivity," there was no predictive relation to later measures. This result is affirmed by data from two independent samples of infants assessed by investigators from different laboratories. The patterns of neurochemistry that are likely to be the most frequent bases for temperaments are as qualitatively different from each other as the patterns of DNA in the virus that causes AIDS and the virus that leads to the common cold.

The fact that a measure forms a continuous distribution does not mean that each value in that distribution is due to the same causal conditions. IQ scores are continuous. However, a child with Down's syndrome and a homeless child with no parents might have the same IQs, but for very different reasons. Adults who are extremely tall or short belong to distinctive genetic categories and infants with birth weights that are two standard deviations below the expected value are at risk for psychological problems, but infants with weights 0.3 standard deviations below the norm are not at an obvious risk.

A relation between self-reported anxiety and shyness, on the one hand, and low thickness values in the medial prefrontal cortex of the *left* hemisphere and a large amygdala, on the other, held only for the adults whose self-reported anxiety scores were in the top 15% of the sample (Holmes, Lee, Hollinshead, Bakst, Roffman et al., 2012). The highest value on an index of autonomic responsiveness better differentiated the emotional states of fear and anger than the mean values (Ax, 1953). Persistent aggression, too, was characteristic of only four percent of children from a very large sample (Brody et al., 2003). It is not obvious that investigators should perform a log transformation on very high aggression scores so that an ANOVA can be implemented on values that are assumed to belong to a continuum that includes less aggressive youngsters. One reason why Max Delbruck, a physicist who decided to study the viruses that alter the DNA of bacteria, failed to make the significant discoveries he had anticipated was that he brought the physicist's premise of continuous variation in energy, charge, or velocity to his research. Hence, he made the mistake of assuming that all organisms differed from each other only quantitatively (Segre, 2011).

Nonlinear functions, characterized by qualitatively distinct phenotypes emerging at transition points, are common in nature—the formation of ice is a classic example. The behavior of a small number of ants appears random and without coherence. The apparent chaos suddenly becomes orderly when the density of the ants in the colony reaches a critical value. One biologist phrased the case for qualitative categories as follows, "The study of biological forms begins to take us in the direction of the science of qualities that is not an alternative to, but complements and extends, the science of quantities" (Goodwin, 1994, p. 198).

CONCLUDING COMMENTS

This chapter and the prior one contain two seminal suggestions to investigators studying human variation. First, almost all psychological outcomes have heterogeneous origins. Therefore, it will be useful to base constructs on patterns defined by combinations of behavioral and biological evidence; for example, behavioral signs of anxiety, such as spontaneous blinking or frequent hand and leg movements, with heart rate, EEG asymmetry, ERP waveforms, or BOLD signals.

Second, scientists must acknowledge the cascade of processes that begins with a brain profile and ends with a behavior or verbal report. They should not assume a direct relation between a measured brain state and a psychological outcome; in part because a biological state usually restricts rather than determines a particular outcome. This is certainly true for temperaments. This claim is supported by the fact that the agent's mental state—in control or a passive recipient—and interpretation of the incentive in a particular context affect the outcome because contexts select the networks that will have the greatest influence on an outcome.

The history of each of the sciences is replete with examples of the progress that occurs when previously isolated domains of inquiry are united by the probing of a common problem. We must now search for the integrative profiles that emerge when particular temperamental predispositions encounter particular family and peer environments in varied communities, and resist the aesthetic urge to simplify the problem by assuming we can arrive at a quantitative estimate of the separate contributions of temperament and life history to a personality trait or form of psychopathology because the magnitudes of those contributions vary across the variety of community and cultural settings in which a child could develop.

One reason for this claim is that there is a much larger number of possible biological states than observable psychological profiles. This means that psychological outcomes that appear to be similar can be accompanied by more than one set of physiological conditions and life circumstances. The same principle applies to diseases. Abnormally high white cell counts can be the result of a large number of different genetic anomalies.

Available methods do not permit detection of the temperaments that are elements in an adult's personality. That is why each temperament can be likened to a drop of black ink that disappears after being stirred in a vessel of glycerine. Nonetheless, a high- or low-reactive bias contributes variance to every measurement made in a setting that is unfamiliar or ambiguous. Since psychologists typically place human participants in such contexts, investigators would benefit from the development of procedures that assessed signs of these properties in their adult subjects.

This conception of temperament shares features with Carl Jung's distinction between anima and persona. Jung's intuition that observers could not know a person's anima or animus from the traits they presented to others was prescient. The task for future investigators is to chart prospectively the envelope of personas that can emerge from the temperamental biases infants reveal for a brief interval before these qualities become incorporated into the larger tapestry we call a personality profile. Each person's profile can be likened to a gray cloth woven so tightly from thin black threads of biology and thin white ones of experience it is impossible to see any black or white threads in the cloth.

Five questions about temperament have priority at this moment in the history of research on this concept. What genes and brain properties contribute to the profiles of high- and low-reactive infants; what are the other major temperaments and what procedures are likely to reveal them; what are the expected developmental courses for each of these biases; which biases represent the greatest risk for mental illnesses in particular cultures, and, finally, are there genetically based differences in the prevalence of each temperament among populations that were reproductively isolated for thousands of years before technology made the mixing of populations easier? I trust that future scientists will answer these questions and in so doing pose new ones.

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