Impulsivity, Aggression, and Serotonin: A Molecular Psychobiological Perspective

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The expression of aggressiveness, which constitutes many facets of behavior, is influenced by a complex interaction of biologic, psychologic, and social variables. Even though individual differences in impulsivity and the behavioral consequences, such as aggression, addiction, and suicidality, are substantially heritable, they ultimately result from an interplay between genetic variations and environmental factors. While formation and integration of multiple neural networks is dependent on the actions of neurotransmitters, such as serotonin (5HT), converging lines of evidence indicate that genetically determined variability in serotonergic gene expression influences complex traits including that of inappropriately aggressive behavior. This contribution reviews studies of major gene effects in inbred and knockout strains of mice with increased aggression-related behavior and discusses the relevance of several serotonergic gene variations in humans which include high aggressiveness as part of the phenotype. Although special emphasis is given to the molecular psychobiology of 5HT in aggression-related behavior in rodents, nonhuman primates, and humans, relevant conceptual and methodological issues in the search for candidate genes for impulsivity and aggressiveness and for the development of mouse models of aggressive and antisocial behavior in humans are also considered. Copyright © 2000 John Wiley & Sons, Ltd.

The societal implications of aggressiveness, which results in numerous facets of aggressive behavior and ranges from the establishment of hierarchies and dominance to antisocial behavior and delinquency, have been examined by anthropologists, psychologists, and sociologists. Biologists have implicated hormones and neurotransmitters in aggressive behavior, while behavioral pharmacologists have shown that drugs of abuse such as cocaine, amphetamines, and alcohol can lower
the threshold to violent and criminal behavior (for a review see Tecott and Barondes, 1996). In both humans and animals, the term aggression comprises a variety of behaviors that are heterogeneous for clinical phenomenology and neurobiological features (Vitiello and Stoff, 1997). While the impact of complex cultural variables on behavior impedes simple extrapolation of animal subtypes to humans, clinical observation, experimental paradigms in the laboratory, and cluster/factor-analytic statistics have been used in attempts to subdivide aggression. Based on different approaches, human aggression may be differentiated into several subtypes depending on the presence or absence of causes or motivation (spontaneous/impulsive or reactive/hostile, offensive or defensive, proactive/instrumental), nature of trigger (e.g., conditioned, response to narcissistic insult), characteristics of mediators (physiologic, biochemical, gender-specific, arousal/anger/affect-related, injurious), form of manifestation (cognitive, symbolic, verbal, physical, direct versus indirect, open versus concealed), direction (outward versus inward), and function (e.g., intentional harm, injury or damage to subjects or objects, expression of an emotional–affective reaction, compensation of hypoarousal) (Archer and Browne, 1989; Berkowitz, 1962, 1974, 1988; Buss, 1961; Campbell, Sapochnik, & Muncer, 1997; Crick and Dodge, 1996; Loeb and Stouthamer–Loeber, 1998). In a recent review on qualitatively distinct subtypes of human aggression the dichotomy between an impulsive–reactive–hostile–affective subtype and a controlled–proactive–instrumental–predatory subtype has emerged as the most promising construct (Vitiello and Stoff, 1997). Finally, antisocial behavior is also a complex phenomenon that arises out of multiple causes involving biologic, psychological, and social forces, and different forms of violent antisocial behavior may each result from different biopsychosocial pathways (Scarpa and Raine, 1997).

Individual differences in the temperamental traits of impulsivity and aggressiveness, and the ultimate behavioral consequences, such as distinct types of aggression, violence, self-injurious behavior including suicidality, and addiction, are relatively enduring and continuously distributed as well as substantially heritable, and therefore are likely to result from additive or nonadditive interaction of genetic variations with environmental influences. This possibility has encouraged many investigators to apply dimensional approaches to behavioral genetics (Plomin, Owen, & McGuffin, 1994). Research in this area consistently supports the idea that there is a strong heritable component to temperament and personality, typically accounting for between 30% and 60% of the observed variance (Henderson, 1982; Loehlin, 1992; Plomin, 1990). This is convincingly illustrated by the striking similarity of identical twins adopted out and reared apart (the so-called “Minnesota Twin Study”), some of whom had not known each other prior to the study (Bouchard, 1994). On measures of interests, skills, and personality traits, these twins had correlations between 34% and 78%, whereas fraternal twins showed correlations between 7% and 39% (Tellegen et al., 1988). While systematic studies of the patterns of inheritance of impulsivity (individual tendency towards impulsive behavior) and aggressiveness (individual tendency towards aggressive behavior) indicate that these complex traits are likely to be influenced by many genes making them polygenic or “quantitative” traits, behavioral genetics convincingly documents the significance of environmental factors. However, contrary to expectation, the relevant environmental cues appear to be those that are

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not shared by relatives reared together (McGue and Bouchard, 1998). Joint genetic and environmental influences on impulsivity and aggression were recently confirmed in mono- and dizygotic male twin pairs (Seroczynski, Bergeman, & Coccaro, 1999). While the results indicate significant heritability of a nonadditive nature, additive genetic variance accounted for 47% of the individual differences.

The relative influence of genetic and environmental factors on behavioral predispositions is among the most prolonged and contentious controversies in intellectual history (Degler, 1991). Although current views emphasize the joint influence of genes and environmental sources, the complexities of gene–gene and gene–environment interactions (genotypes may respond differentially to certain environments) as well as gene–environment correlations (genotypes may be exposed differentially to environments) represent research areas in their infancy (Bouchard, 1994; Loehlin, 1992; McGue and Bouchard, 1998). Behaviors related to impulsivity and aggressiveness seem to delineate a biologically based model of dispositions to both normal and pathological functioning, with a continuum of genetic risk underlying personality and behavioral dimensions that extend from normal to abnormal (Staner and Mendlewicz, 1998). Thus, the analysis of genetic contributions to aggressive behavior is both conceptually and methodologically difficult, so that consistent findings remain sparse. The documented heterogeneity of both genetic and environmental determinants suggests the futility of searching for unitary causes. This vista has therefore increasingly encouraged the pursuit of dimensional approaches to behavioral genetics (Plomin et al., 1994) and genomic variants with a significant impact on the functionality of components of brain monoamine neurotransmission, such as the serotonin (5HT) system, are a rational starting point for this research.

The brainstem raphe 5HT system is the most widely distributed neurotransmitter system in the brain; serotonergic raphe neurons diffusely project to a variety of brain regions (e.g., cortex, amygdala, hippocampus). In addition to its role as a neurotransmitter, 5HT is an important regulator of morphogenetic activities during early brain development as well as during adult neurogenesis and plasticity, including cell proliferation, migration, differentiation, and synaptogenesis (Azmitia and Whitaker-Azmitia, 1997; Gould, 1999; Lauder, 1990, 1993). In humans, nonhuman primates, and other mammals, preclinical and clinical studies have accumulated an overwhelming body of evidence that 5HT signaling is a major modulator of emotional behavior including anxiety and impulsivity as well as aggression (Westenberg, Murphy, & Den Boer, 1996) and integrates complex brain functions such as cognition, sensory processing, and motor activity. The diversity of these functions is due to the fact that 5HT orchestrates the activity and interaction of several other transmitter systems. While 5HT may be viewed as a master control neurotransmitter within this highly complex system of neural communication mediated by at least 14 pre- and postsynaptic receptor subtypes with a multitude of isoforms (e.g., functionally relevant splice variants) and subunits, 5HT’s action as a chemical messenger is also regulated by 5HT synthesizing and metabolizing enzymes, and the 5HT transporter.

Influenced by genotypes and environmental factors, 5HT-mediated behaviors may be diversely expressed and range from minor personality accentuations (characterized by impulsivity, hostility, irritability, psychopathic deviance or violence or by more clear-cut personality dysfunction such as antisocial, borderline, nar-
cissistic and histrionic personality traits or disorders) to major psychiatric disturbances (suicidal behavior, overt aggressive behavior, intermittent explosive disorder, pathological gambling, pyromania, bulimia, and some types of substance or alcohol abuse) (Staner and Mendlewicz, 1998). Consistent with this view, the brain 5HT system is also the initial site of action of antidepressant and antianxiety drugs and drugs with antiaggressive potential in the rodent model (the so-called serenics) (Olivier and Mos, 1992; Olivier, Mos, Raghoebar, de Koning, & Mak, 1994).

Mouse strains that have been selectively bred to display a phenotype of interest are currently being used to identify genetic loci that contribute to behavioral traits. This quantitative trait locus (QTL) approach has been applied with some success to a trait in mice called “emotionality” (Flint et al., 1995). However, such linkage analyses provide only a rough chromosomal localization, whereas the next step, identifying the relevant genes by positional cloning, remains a challenging task (Tecott and Barondes, 1996). Since mice and humans share many orthologous genes mapped to synthentic chromosomal regions, it is conceivable that individual genes identified for one or more types of murine aggressive behavior may be developed as animal models for human aggression. Following chromosomal mapping of polymorphic genes and evaluation of gene function using knockout mutants, behavioral parameters, including the type of aggression, measure of aggression, test situation, and opponent type are investigated (Maxson, 1996). Thus, the combination of elaborate genetic and behavioral analyses results in the identification of many genes with effects on variation and development of one or more forms of murine aggressive behavior.

Based on converging lines of evidence that the 5HT and serotonergic gene expression are involved in a myriad of processes during brain development as well as synaptic plasticity in adulthood, temperament predispositions and complex behavior, including impulsivity, aggression, and hostility, are likely to be influenced by genetically driven variability of 5HT function (Lesch, Greenberg, Higley, & Murphy, 2000). This review describes pertinent aspects of serotonergic gene regulation and its relevance for central 5HT system plasticity. Conceptual and methodological issues in the search for candidate genes for aggression and for the development of mouse models of human aggression are also considered. Based on the evidence that genetically driven variability of expression of proteins that regulate the central 5HT system (e.g., receptors, enzymes and transporters) is associated with complex behavioral traits, emphasis is given to the molecular psychobiological perspective of 5HT in impulsivity and aggression-related behaviors in rodents, nonhuman primates, and humans.

SEROTONIN RECEPTOR SUBTYPES

Several 5HT receptor subtypes have been implicated in impulsivity and aggression-related behavior and various models of rodent agonistic behavior which differentiate between offensive aggression and defensive/flight models have been described (Olivier and Mos, 1992; Olivier, Mos, van Oorschot, & Hen, 1995) (Table 1). Pharmacological classification based on ligand binding experiments and on the study of functional responses to agonists/antagonists were initially
utilized to define four 5HT receptor subfamilies, 5HT1–4. Molecular biology has subsequently both confirmed this classification and also revealed the existence of novel 5HT receptor subtypes for which little pharmacological or functional data exists (5HT1E, 5HT1F, 5HT5A, 5HT5B, 5HT6, and 5HT7) (Hoyer and Martin, 1997). As an indicator that this is still work in progress, an additional subunit B of the 5HT3 receptor has recently been discovered (Davies et al., 1999). Current research is also focusing on participating constituents and regulatory mechanisms of gene transcription and messenger RNA (mRNA) processing and translation, as well as intracellular trafficking and post-translational modification of proteins relevant to synaptic and postreceptor signaling. Crucial information is derived from the analysis of transcriptional control systems of 5HT receptor genes as well as from modeling complex behavior and novel therapeutic strategies in transgenic animals. In the following section some of the conceptual paradigms that have been applied to the interpretation of aggression-related phenotypes in knockout mice and in humans with variations in candidate genes will be discussed.

Table 1. Serotonergic genes implicated in impulsivity and aggression-related behaviors

<table>
<thead>
<tr>
<th>Rodents</th>
<th>Humans</th>
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<tr>
<td>Pharmacologic studies (rats/mice)</td>
<td>Knockout mice</td>
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<tr>
<td>5HT receptors</td>
<td></td>
</tr>
<tr>
<td>1A</td>
<td>↑(^{21})</td>
</tr>
<tr>
<td>1B</td>
<td>↓(^{1,12})</td>
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<td>2A</td>
<td>↓(^{12})</td>
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<td>3</td>
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<tr>
<td>7</td>
<td>nd</td>
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<tr>
<td>Tryptophan hydroxylase</td>
<td>nd</td>
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<tr>
<td>Monoamine oxidase A</td>
<td>–</td>
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<tr>
<td>5HT transporter</td>
<td>–</td>
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</tbody>
</table>

↑/↓ increase/decrease in aggression and/or related behavior.

\(^{1}\)Agonist-induced increase.

\(^{2}\)Antagonist-induced decrease.

+ association or linkage with impulsivity, aggression, and/or related behavior.

– no effect.

nd, not determined.

The 5HT1B receptor was the first subtype to have its gene inactivated by classical homologous recombination (Saudou et al., 1994). 5HT1B receptors are expressed in the basal ganglia, central gray, hippocampus, amygdala, and raphe nuclei. They are located predominantly at presynaptic terminals where they can inhibit release of 5HT and, as heteroceptors, of other neurotransmitters. Selective agonists and antagonists for 5HT1B receptors are lacking, but indirect pharmacological evidence suggests that 5HT1B activation influences food intake, sexual activity, locomotion, and aggression. Mice with a targeted disruption of the 5HT1B gene therefore facilitated investigation of the concept of 5HT-related impulsivity in the context of aggressive behavior. Two of the behaviors, locomotion and aggression, postulated to be modulated by 5HT1B receptors were analyzed (Ramboz et al., 1996). Wild-type and homozygous null mutant (5HT1B/−/−)
mice were found to display similar levels of locomotor activity in an open field. Impulsivity and aggression-related behavior of 5HT1B−/− male mice was assessed by isolation and subsequent exposure to a non-isolated male wild-type intruder mouse. The latency and number of attacks displayed by the knockout mice were used as indices of aggression. The 5HT1B−/− mice, when compared with wild-type mice, showed more rapid, more intense, and more frequent attacks. In addition to increased aggression, knockout mice acquire cocaine self-administration faster and ingest more ethanol than controls (Brunner and Hen, 1997). Thus, the 5HT1B receptor modulates not only motor impulsivity and aggression but also addictive behavior.

Based on the relationship of aggression, suicide, and drug abuse in clinical samples, the association of psychopathology with 5HT1B receptor gene and post-mortem human brain 5HT1B receptor binding was recently studied (Huang, Grailhe, Arango, Hen, & Mann, 1999). Two common polymorphisms were identified in the 5HT1B receptor gene, a silent C to T substitution at nucleotide 129 and a silent G to C substitution at nucleotide 861 of the coding region. While the C129 or G861 allele had 20% fewer 5HT1B receptors compared to the 129T or 861C allele, no association between suicide, major depression, alcoholism, or pathological aggression and 5HT1B receptor binding indices or genotype was identified. Lappalainen et al. (1998) have investigated whether the 5HT1B gene (the HTR1B G861C polymorphism and the short-tandem repeat locus D6S284) is linked to impulsive and aggressive behavior in two patient populations, Finnish sibling pairs and a large multigenerational family derived from a Southwestern American Indian tribe, with antisocial personality disorder and intermittent explosive disorder comorbid with alcoholism. While Finnish antisocial alcoholics had a significantly higher HTR1B-861C allele frequency than the other Finns, significant sib pair linkage of antisocial alcoholism to HTR1B-G861C to D6S284 was observed, thus indicating that a locus predisposing to antisocial behavior and aggressiveness associated with alcoholism may be linked to HTR1B at 6q13-15.

Although several lines of pharmacologic evidence in both rodent and humans have implicated other 5HT receptor subtypes in the modulation of aggression (de Boer, Lesourd, Mocaer, & Koolhaas, 1999; Moeller et al., 1998; Olivier and Mos, 1992; Rudissaar, Pruus, Skrebuhhova, Allikmets, & Matto, 1999), few studies employed genetic approaches such as selective breeding and gene targeting. In contrast to 5HT1B knockout mice, 5HT1A knockouts are less reactive and possibly less aggressive but show more anxiety-related behavior than control mice (for reviews see Lesch and Mössner, 1999; Zhuang et al., 1999), although both 5HT1A and 5HT1B receptors control the tone of the serotonergic system and mediate some of the postsynaptic 5HT effects.

Assessment of 5HT receptor expression in male mice selected for high and low offensive aggression showed that high-aggression mice, characterized by a short attack latency, decreased plasma corticosterone concentration and increased levels of 5HT1A mRNA in the dorsal hippocampus (dentate gyrus and CA1) compared to low-aggression mice that had long attack latency and high plasma corticosterone levels (Korte et al., 1996). Increased postsynaptic 5HT1A receptor radioligand binding was found in dentate gyrus, CA1, lateral septum, and frontal cortex, whereas no difference in ligand binding was found for the 5HT1A autoreceptor on cell bodies in the dorsal raphe nucleus. These results suggest that high offensive
aggression is associated with reduced (circadian peak) plasma corticosterone and increased postsynaptic 5HT1A receptor availability in limbic and cortical regions.

Pesonen et al. (1998) have recently reported that a Pro279Leu amino acid substitution in the 5HT7 receptor gene may be a predisposing allele in a subgroup of Finnish alcoholic offenders with multiple behavioral problems. The present challenge, however, is to further characterize the physiological relevance of the large variety of 5HT receptor gene products, establish their function as endogenous receptors, find selective ligands, and determine potential therapeutic application of these compounds. However, as many of these receptors are remarkably similar in their ligand-binding domains, it has as yet been difficult to design pharmacological compounds that will specifically interact with a single subtype. The new insights into neural plasticity and complexity of gene regulation in 5HT subsystems will eventually provide the means for novel approaches of studying 5HT receptor subtype-related behaviors at the molecular level.

Finally, signaling through 5HT receptors involves different transduction pathways, and each receptor subtype modulates distinct, though frequently interacting, second messenger systems and multiple effectors. The gene of the effector enzyme calcium-calmodulin kinase II (CamKII), which participates in some intracellular responses to 5HT receptor activation, has also been implicated in aggressive behavior by a knockout experiment (Chen, Rainnie, Greene, & Tonegawa, 1994). While CamKII null mutants showed global behavioral impairment, male mice heterozygous for the inactivated CamKII gene had a greater tendency to fight with each other when housed together. Specifically, they showed enhanced offensive aggression, normal defensive aggression, decreased fear-related responses, and decreased copulation. The discovery of so many hyperaggressive mutant strains in the course of gene knockout experiments highlights the extraordinary diversity of genes involved in the genetic influence on impulsivity and aggression (Tecott and Barondes, 1996).

**TRYPTOPHAN HYDROXYLASE**

Central nervous system (CNS) serotonergic activity correlates inversely with human aggressive behavior and individual differences in aggressive disposition are influenced by genetic factors. The first step of 5HT biosynthesis in 5HT neurons is catalyzed by the rate-limiting enzyme tryptophan hydroxylase (TPH). A role of L-tryptophan availability and of TPH activity in impulsivity, aggressiveness, and associated suicidality has been reported by several studies of psychiatric patients or offender populations (Dougherty, Moeller, Bjork, & Marsh, 1999; LeMarquand, Benkelfat, Pihl, Palmour & Young, 1999; LeMarquand et al., 1998; Moeller et al., 1996) (Table 1). For example, an increase in aggressive responses on a free-operant laboratory measure of aggression following experimental tryptophan depletion in healthy males was recently shown, supporting the hypothesis that low plasma tryptophan concentration and associated decrease in brain 5HT facilitates aggression-related behavior.

The human TPH gene located on chromosome 11p is a member of the aromatic amino acid hydroxylase family, spans a region of 29 kb, and contains at least 11 exons (transcribed DNA sequence after splicing) (Boularand, Darmon, & Mallet,
An unusual splicing complexity in the 5′-untranslated region (5′-UTR) results in at least four TPH mRNA species transcribed from a single transcriptional start site. Although a detailed analysis of the gene’s transcriptional control region is still lacking, DNA elements important for serotonergic neuron-specific expression of TPH appear to be contained in 6.1 kb of 5′-flanking transcriptional control region of the mouse TPH gene (Huh, Park, Cho, Joh, & Son, 1994; Son et al., 1996). Unfortunately, a mouse model with a targeted disruption of the TPH gene is not yet available for an assessment of the effect of 5HT deficiency on aggressive behavior.

Several common gene variations have been described in the 5′-flanking regulatory region (T-7180G, C-7065T, A-6526G, and G-5806T), designated as nucleotides upstream of the translation start site) and in intron 7 (A218C and C779A) (intron = transcribed DNA sequence which is removed from a transcript by splicing) (Rotondo et al., 1999), while functional variants have not been reported in the coding sequence of this gene (Han et al., 1999). In a landmark study Nielsen et al. (1994) reported that the TPH A779C polymorphism influences 5-hydroxyindoleacetic acid concentrations (5HIAA), the major metabolite of 5HT, in cerebrospinal fluid (CSF), and may predispose to suicidality, a pathophysiological mechanism that may involve impaired impulse control. This finding was subsequently replicated by the same group using a family-based design in an extended sample of Finnish alcoholic offenders (Nielsen et al., 1998).

Additional investigations indicate that the intronic polymorphism may be associated with aggression and anger-related traits of personality and CNS 5HT activity assessed by pharmacologic challenge (prolactin response to fenfluramine) in healthy volunteers (Manuck et al., 1999). Similarly, in a population of male personality disorder patients, individual differences in aggressive disposition but not prolactin response to fenfluramine was associated with the intronic TPH genotypes (New et al., 1998). In Finnish offenders, previously studied for the TPH intron7 C779A polymorphism, a significant association was observed between the TPH promoter polymorphism A-6526G and suicidality (Rotondo et al., 1999). Although not consistently replicated in other populations (Abbar et al., 1995; Furlong et al., 1998; Kunugi et al., 1999), these findings in conjunction with results from association studies in various psychiatric disorders including bipolar disorder and alcoholism (Bellivier et al., 1998; Manuck et al., 1999) further support the notion that functional variant(s) in or close to the TPH gene may predispose individuals to suicidality or externally directed aggressiveness, and underscore the relevance of synthesis-dependent 5HT homeostasis in the expression of other behaviors thought to be influenced by 5HT.

**MONOAMINE OXIDASE A**

Alterations in monoamine oxidase A (MAOA) activity have been implicated in a wide range of behavioral traits and disorders (Table 1). MAOA is a mitochondrial enzyme that oxidizes 5HT and norepinephrine as well as dopamine, and is expressed in a cell-type-selective manner. MAOA-deficient mice were generated accidentally by the replacement of exons 2 and 3 of the MAOA gene with an interferon transgene (gene to be transferred) (Cases et al., 1995). Mice with a targeted
Impulsivity, aggression and serotonin display elevated brain levels of 5HT, norepinephrine, and dopamine, increased reactivity to stress, hyperactive startle responses, violent motions during sleep and abnormal posture, and aggressive behavior. Enhanced male aggressiveness was demonstrated by resident–intruder tests and by increased injury between male cage-mates. The increased aggressiveness of the MAOA mutant mice was indicated by the large percentage of mutant males that become wounded under standard group housing conditions and confirmed by enhanced offensive aggression by mutants in the resident–intruder assay (Seif and De Maeyer, 1999). The MAOA mutants also displayed increased copulatory behavior of males with non-receptive female mice. Since these phenotypical alterations are restrained by 5HT synthesis inhibition but not by catecholamine synthesis suppression, the observed behavioral abnormalities are likely to be specifically the result of attenuated 5HT degradation. Since MAOA-deficient mice also show disrupted formation of sensory maps in the visual and somatosensory systems (e.g., cortical barrelfields) (Cases et al., 1996; Salichon et al., manuscript submitted), thus underscoring the role of 5HT as a morphoregulator of brain development, it remains to be elucidated whether some of the behavioral abnormalities are influenced by structural abnormalities.

The aggressive phenotype of MAOA-deficient mice appears to complement the behavior consequences of a mutation in the coding region of the human MAOA gene. This X-linked hemizygous chain termination mutation has been linked to mild mental retardation and occasional episodes of impulsive aggression, arson, and hypersexual behavior, such as attempted rape and exhibitionism, in affected males from a single large family (Brunner, Nelen, Breakefield, Ropers, & van Oost, 1993). Affected males exhibit markedly disturbed monoamine metabolism and an absence of MAOA enzymatic activity in cultured fibroblasts. A non-conservative point mutation was found in all affected males and all carrier females; the mutation introduces a stop codon (base triplet, e.g., TAA, that serves as a signal for termination of transcription) at position 296. Although inhibition of MAOA in adults leads to antidepressant effects but not aggression-related behavior, the deviate behavior in MAOA-deficient men may be due to structural or compensatory changes resulting from altered monoamine metabolism during neurodevelopment. However, screening of volunteers in the general population and from putative high-risk groups for possible MAO deficiency states suggests that marked MAO deficiency states are very rare (Schuback et al., 1999). The fact that humans with an inactive MAOA gene also show increased impulsive aggression and sexual aggressiveness demonstrates the potential relevance of mutant mouse models to human behavior, although the rarity of the human mutation indicates that other genetic and/or nongenetic influences that contribute to these forms of misconduct (Tecott and Barondes, 1996).

The human MAOA gene is localized on chromosome Xp-11.23, extends over 70 kb, and is composed of 15 exons (Shih et al., 1993; Zhu et al., 1992). Two species of MAOA mRNA, 2.1 kb and 5.0 kb, are generated by the use of two alternative polyadenylation sites. While there is considerable controversy regarding the site where mRNA synthesis is initiated, tissue-selective length variability of the 5'-UTR with multiple transcription start sites clustered primarily around an initiator element which may also act as a negative regulatory element have been reported (Denney, Sharma, Dave, & Waguespack, 1994; Zhu, Chen, & Shih, 1996).
1994). The core promoter region contains two 90 bp repeat sequences, which are further divided into four imperfect tandem repeats, each containing an Sp1 binding site in reversed orientation (Zhu and Shih, 1997).

Although the MAOA gene is a potential candidate for affective illness, none of several previously described gene variants (Black, Chen, Craig, & Powell, 1991; Hinds, Hendriks, Craig, & Chen, 1992; Hotamisligil and Breakefield, 1991) are consistently associated with disease (Craddock et al., 1995; Kawada, Hattori, Dai, & Nanko, 1995; Lim et al., 1995; Nöthen et al., 1995; Parsian and Todd, 1997; Rubinsztein et al., 1996). A functional 30 bp repeat polymorphism was identified in the promoter region of the human MAOA gene that differentially modulates gene transcription (Deckert et al., 1999; Sabol, Hu, & Hamer, 1998) as well as enzyme activity in fibroblasts. A corresponding functional repeat polymorphism was recently found in rhesus monkeys (Syagailo and Lesch, manuscript in preparation). Variation in the number of repeats (three to five) of this MAOA gene-linked polymorphic region (MAOA-LPR) had different transcriptional efficiency when fused to a luciferase reporter gene and transfected into cell lines. The transcriptional efficiency of the three-repeat allele was two-fold lower than those with longer repeats and enzyme activity is correlated with repeat length (Denney, Koch, & Craig, 1999). Preliminary evidence indicates that length variation of the MAOA-LPR confers vulnerability to antisocial behavior in alcohol-dependent males (Samochowiec et al., 1999) is linked to impulsivity, hostility and lifetime aggression history as well as CNS serotonergic function in a community sample of men (Manuck, Flory, Ferrell, Mann, & Muldoon, 2000), may be associated with aggression-related behavior in rhesus monkeys (Bennett, Lesch, & Higley, unpublished results), and appears to be a risk factor for panic disorder and unipolar depression in female patients (Deckert et al., 1999; Schulze et al., 2000) but not for other psychiatric disorders (Furlong, Rubinsztein, Walsh, Paykel, & Rubinsztein, 1999; Syagailo et al., manuscript submitted).

**SEROTONIN TRANSPORTER**

While 5HT controls a highly complex system of neural communication mediated by multiple pre- and postsynaptic 5HT receptor subtypes, high-affinity 5HT transport into the presynaptic neuron is mediated by a single protein. The 5HT transporter (5HTT) removes 5HT from the synaptic cleft and determines the magnitude and duration of postsynaptic receptor-mediated signaling, thus playing a pivotal role in the fine-tuning of 5HT neurotransmission (for a review see Lesch, 1997). The 5HTT is also the initial target for several antidepressant drugs (e.g., clomipramine, fluoxetine) which also display antiaggressive properties. There has been progress in the elucidation of the 5HTT gene’s organization and regulation. A polymorphism in the 5’-flanking transcriptional control region of the 5HTT gene that results in allelic variation in functional 5HTT expression is associated with anxiety, depression, and aggression-related personality traits (Lesch et al., 1996). Advances in 5HTT gene knockout studies are also changing views of the relevance of adaptive 5HT uptake function and 5HT homeostasis in brain development and plasticity as well as processes underlying drug dependence and neurodegeneration (Bengel et al., 1998; Sora et al., 1998). Despite a growing body
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of evidence for a potential role of the 5HTT in the integration of synaptic connections in the mammalian brain during development, adult life, and old age, detailed knowledge of the molecular mechanisms involved in this fine-tuning process is just beginning to emerge. For this reason we will draw heavily upon examples and issues that have emerged from our and collaborating groups’ work on the 5HTT gene to illustrate our points.

Functionality of Gene Variants

The human and murine 5HTT genes are located on chromosome 17q12.2 and chromosome 11, respectively. Both genes are composed of 14 exons spanning 35 kb and with conservation of the exon/intron organization and, to a lesser extent, the 5’-flanking non-coding as well as regulatory sequences (Bengel et al., 1996; Lesch et al., 1994). The role of these transcription factor motifs in the regulation of human and murine 5HTT gene transcription was studied by transfection studies using reporter gene fusion constructs of 5’-flanking sequences (Heils et al., 1998). In humans, transcriptional activity of the 5HTT gene is modulated by a polymorphic repetitive element (5HTT gene-linked polymorphic region, 5HTTLPR) located upstream of the transcription start site. Additional variations have been described in the 5’ untranslated region (5’UTR) due to alternative splicing of exon 1B (Bradley and Blakely, 1997), in intron 2 (variable number of a 16/17 base pair tandem repeat, VNTR-17) (Lesch et al., 1994), and 3’UTR (Battersby et al., 1999). Comparison of different mammalian species confirmed the presence of the 5HTTLPR in hominoids (great apes) and cercopithecoids (old world and new world monkeys) but not in prosimian primates and other mammals (Lesch et al., 1997). The unique structure of the 5HTTLPR gives rise to the formation of a DNA secondary structure (e.g., cation-dependent tetrastrands aggregation) that has the potential to regulate the transcriptional activity of the associated 5HTT gene promoter. The secondary structure of the 5HTTLPR is also likely to precipitate a 381 bp somatic deletion in the 5HTT gene’s promoter region (del(17)(q11.2)), observed in 20–60% of genomic DNA isolated from human brain and mononuclear cells (Lesch et al., 1999).

When fused to a reporter gene and transfected into human 5HTT expressing cell lines, the short (s) and long (l) 5HTTLPR variants differentially modulate transcriptional activity of the 5HTT gene promoter (Lesch et al., 1996). The effect of 5HTTLPR length variability on 5HTT function was determined by studying the relationship between 5HTTLPR genotype, 5HTT gene transcription, and 5HT uptake activity in human lymphoblastoid cell lines. Cells homozygous for the l variant of the 5HTTLPR produced higher concentrations of 5HTT mRNA than cells containing one or two copies of the s form. Membrane preparations from l/l lymphoblasts showed higher inhibitor binding than did s/s cells. Furthermore, the rate of specific 5HT uptake was more than two-fold higher in cells homozygous for the l form of the 5HTTLPR than in cells carrying one or two copies of the s variant of the promoter. Further evidence from studies of 5HTT promoter activity in other cell lines, mRNA concentrations in the raphe complex of human postmortem brain, platelet 5HT uptake and content, and in vivo SPECT imaging of human brain 5HTT confirmed that the s form is associ-
ated with lower 5HTT expression and function (for a review see Lesch et al., 2000).

**Temperament, Personality, and Behavioral Traits Related to Impulsivity and Aggressiveness**

Following systematic attempts to characterize genetically driven variation in 5HT uptake function, the 5HTT has assumed importance as a piece in the mosaic-like texture of personality traits such as anxiety, negative emotionality, impulsivity, and aggressiveness (Table 1). The contribution of 5HTTLPR variability to individual phenotypic differences in temperament, personality, and behavior was explored in two independent population/family genetic studies. In our initial study, we found population and within-family associations between the low-expressing s allele and Neuroticism, a trait related to anxiety, hostility, and depression, on the NEO Personality Inventory—revised (NEO-PI-R), a self-report inventory based on the five-factor model of personality (“Big Five”) (Costa and McCrae, 1992), in a primarily male population (n = 505), and that the s allele was dominant (Lesch et al., 1996). Individuals with either one or two copies of the short 5HTTLPR variant (group S) had significantly greater levels of Neuroticism, defined as proneness to negative emotionality, including anxiety, hostility, and depression, than those homozygous for the long genotype (group L) in the sample as a whole and also within sibships. Individuals with 5HTTLPR S genotypes also had significantly decreased Agreeableness, a dimension reflecting expression of a spectrum of traits ranging from cooperativeness to aggressiveness. Recently, this association was reassessed in a new sample (n = 397, 84% female, primarily sib-pairs). The findings robustly replicated the 5HTTLPR–Neuroticism association, and the dominance of the s allele (Greenberg et al., 1999). Combined data from the two studies (n = 902) gave a highly significant association between the s allele and higher NEO Neuroticism both across individuals and within families, reflecting a genuine genetic influence rather than an artifact of ethnic admixture.

Another association encountered in the original study between the s allele and lower scores of NEO Agreeableness, including the subscales Straightforwardness, Compliance and Trust, was also replicated and was stronger in the primarily female replication sample. Gender-related differences in 5HTTLPR-personality trait associations are possible since several lines of evidence demonstrate gender-related differences in 5HT-system functioning in humans and in animals (Fink, Sumner, Rosie, Wilson, & McQueen, 1999; McQueen, Wilson, & Fink, 1997). These findings include effects of gonadal steroids on 5HTT expression in rodent brain and differences in anxiety-related behaviors in male and female 5HTT knockout mice. While such evidence provides a theoretical basis for possible gender-related differences in the 5HTTLPR–personality association, we found that the 5HTTLPR has a qualitatively similar influence in women and men. However the results of the replication sample suggest a possibly stronger association between 5HTTLPR-S genotypes and a predisposition to lower Agreeableness and related traits in women.

These findings show that the 5HTTLPR influences a constellation of Neuroticism and Agreeableness, traits of negative emotionality related to interpersonal
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hostility and aggression. Other efforts to detect associations between the 5HTTLPR and personality traits, which are discussed elsewhere (Greenberg et al., 1999; Lesch et al., 2000), have been complicated by the use of small sample sizes, heterogeneous subject populations, ethnic and sociocultural characteristics, and differing methods of personality assessment. The relationship between these two aspects of negative emotionality is not unexpected in view of the previously observed negative correlation between Angry Hostility, a subscale of Neuroticism, and Agreeableness, indicating that both dimensions assess a behavioral predisposition toward uncooperative and aggressive interpersonal behavior. The effect sizes for the 5HTTLPR–personality associations, which were comparable in the two samples, indicate that this polymorphism has a moderate influence on these behavioral predispositions of approximately .30 standard deviation units. This corresponds to 3–4% of the total variance and 7–9% of the genetic variance, based on estimates from twin studies using these and related measures which have consistently demonstrated that genetic factors contribute 40–60% of the variance in personality traits. Thus, the results are consistent with the view that the influence of a single, common polymorphism on continuously distributed traits is likely to be small in humans as well as different quantitative characteristics in other species (Plomin et al., 1994).

At first sight, association between the high-activity 5HTTLPR l allele with lower Neuroticism and related traits seemed inconsistent with the known antidepressant, antianxiety, and antiaggressive effects of 5HTT inhibitors (SRIs). Likewise Knutson et al. (1998) reported that long-term inhibition of the 5HTT by the SRI paroxetine reduced indices of Hostility through a more general decrease in negative effect, a personality dimension related to Neuroticism. The same individuals also demonstrated an increase in directly measured social cooperation after paroxetine treatment, an interesting finding in view of the replicated reciprocal association between 5HTTLPR genotype and Agreeableness. That a drug which inhibits the 5HTT lessened negative emotionality and increased social cooperation appears to conflict with findings that the 5HTTLPR long allele, which confers greater 5HTT expression, is associated with lower NEO Neuroticism and higher NEO Agreeableness. However, since both 5HT and 5HTT play critical roles in brain development that is different from its function regulating neurotransmission and neurogenesis in the adult (Azmitia and Whitaker-Azmitia, 1997; Gould, 1999), this inconsistency may be more apparent than real. In support of this notion it has recently been reported that prenatal exposure of mice to a clinically relevant dose of paroxetine produced no major behavioral alterations but increases in some anxiety-related measures in infant offspring and in aggressive behavior in adult males (Coleman, Christensen, Gonzalez, & Rayburn, 1999).

The conclusion that the 5HTT may affect personality traits via an influence on brain development is strongly supported by recent findings in rodents and non-human primates. Studies in rats confirmed that the 5HTT gene is expressed in brain regions central to emotional behavior during fetal development but not later in life (Hansson et al., 1998; Hansson, Mezey, & Hoffman, 1999), hence enduring individual differences in personality could result from 5HTTLPR-driven differential 5HTT expression during pre- and perinatal life. Transient 5HT expression in thalamocortical neurons is required for the formation of the barrel-field cortex in neonatal rodents, presumably by maintaining extracellular 5HT
concentrations. The somatosensory cortex of 5HTT−/− mice displays no 5HT-stained barrels at P7 and only very few cytochrome oxydase-stained whisker barrels both at P7 and adulthood (Persico et al., 1999; Salichon et al., manuscript submitted). Heterozygote 5HTT+/− mice develop all cortical barreelfields, but frequently present irregularly shaped barrels and septa. The confirmation that a 50% decrease of 5HTT availability and subtle changes in the dynamics of 5HT transport in 5HTT+/− mice (the model that most closely resembles the impact of the s 5HTTLPR variant on functional 5HTT expression) exerts long-term effects on cortical development and adult brain plasticity may be an important step forward in establishing a neurobiological groundwork for the neurodevelopmental hypothesis of not only Neuroticism, but also impulsivity and aggression-associated personality traits. Supportive evidence is beginning to come from studies of rhesus macaques, a higher nonhuman primate species that like humans carries a functional 5HTTLPR polymorphism (Lesch et al., 1997). Rhesus monkey infants with the low-expression rh5HTTLPR s allele displayed higher behavioral stress reactivity compared to infants homozygous for the l allele (Champoux et al., 1999). Thus, both animal models are consistent with the finding in humans that it is the low-activity s allele that is associated with increased negative emotionality. The findings are intriguing in light of recent speculation that the recent appearance of the 5HTTLPR-associated genetic variation may have helped permit more sophisticated modulation of social behaviors important to group survival and reproductive success during the evolution of higher order primates (Buss, 1991, 1995).

Gene–Environment Interaction and Behavior in a Nonhuman Primate Model

Personality defines the framework to adapt to other people as a crucial task in long-term reproductive success. Extraversion and Agreeableness are important to the formation of social structures ranging from pair-bonds to coalitions of group’s Emotional Stability and Conscientiousness are critical to the endurance of these structures, while Openness may reflect the capacity for innovation. Although studies in nonhuman primates have yielded models of aggressive behaviors, insights into the biological mechanisms that underlie these behaviors are only beginning to emerge. Since the genetic basis of present-day temperamental, personality, and behavioral traits may reflect selective forces among our remote ancestors (Loehlin, 1992), research efforts have recently been focused on rhesus macaques. In this nonhuman primate model environmental influences are probably less complex, can be more easily controlled for, and are thus less likely to confound associations between temperament and genes. All forms of aggression in rhesus monkeys—major categories are defensive and offensive aggression—appear to be modulated by environmental factors, and marked disruptions in the mother–infant relationship likely confer increased risk (Kalin, 1999).

One of the most replicated findings in psychobiology is the observation of lower 5HIAA in the brain and CSF of subjects with impulsive aggression and suicidal behavior (for a review see Asberg, 1997). Human and nonhuman primate behavior is similarly modified by deficits in 5HT function. In rhesus monkeys 5HT turn-
over, as measured by cisternal CSF 5HIAA concentrations, shows a strong heritable component and is traitlike, with demonstrated stability over an individual's lifespan (Higley, Suomi, & Linnoila, 1991; Higley et al., 1993; Kraemer, Ebert, Schmidt, & McKinney, 1989). Low or lower than average CSF 5HIAA concentrations have been reported in individuals who display inappropriate aggression as children, engage in frequent impulsive and violent criminal behavior, exhibit excessive alcohol abuse and dependence, and attempt suicide.

Recently, a study has assessed its generalizability across primates by making simultaneous comparisons between and within closely related species. Between-species analyses indicated higher CSF 5HIAA concentrations in pigtailed macaques (Macaca nemestrina), and higher rates of high-intensity aggression, escalated aggression, and wounds requiring medical treatment in rhesus macaques (Macaca mulatta) (Westergaard, Suomi, Higley, & Mehlman, 1999). Within-species analyses indicated that interindividual differences in CSF 5HIAA concentrations were inversely correlated with escalated aggression and positively correlated with social dominance rank, further supporting the notion that 5HT functioning plays an important role in controlling impulsivity, which regulates severe impulsive aggression and social dominance relationships in nonhuman primates, and that between-species differences in agonistic temperament can be predicted by species-typical CNS 5HT functioning.

Not unexpectedly, CSF 5HIAA concentrations are also subject to the long-lasting influence of deleterious events early in life as well as by situational stressors. Monkeys separated from their mother and reared in the absence of conspecific adults (peer reared) have altered serotonergic function and exhibit behavioral deficits throughout their lifetimes when compared to their mother-reared counterparts. Comparison of different mammalian species indicates that the 5HTTLPR is unique to humans and simian primates. In hominoids all alleles originate from variation at a single locus (polymorphic locus 1, PL1), whereas an alternative locus for a 21 bp length variation (PL2) was found in the 5HTTLPR of rhesus monkeys (rh5HTTLPR) (Lesch et al., 1997). While the intervening 5HTTLPR sequence displays intraspecies and interspecies variability, the 5HTTLPR-associated insertion/deletion event has been detected as identical positions in humans (del(17)(q11.2)) and rhesus monkeys (Lesch and Jatzke, unpublished observation). The 5HTTLPR sequence may be informative in the comparison of closely related species and reflects the phylogeny of the old world monkeys, great apes, and humans. The presence of an analogous rh5HTTLPR and resulting allelic variation of 5HT activity in rhesus monkeys provides a unique model to dissect the relative contributions of genes and environmental sources to central serotonergic function and related behavioral outcomes.

Genotype–environment interaction was recently studied by testing associations between central 5HT turnover and rh5HTTLPR genotype in rhesus monkeys with well characterized environmental histories (Higley et al., 1998). The monkeys’ rearing fell into one of the following categories: Mother reared, either reared with the biological mother or cross-fostered; or peer reared, either with a peer group of three to four monkeys or with an inanimate surrogate and daily contact with a playgroup of peers. Peer-reared monkeys were separated from their mothers, placed in the nursery at birth, and given access to peers at 30 days of age either continuously or during daily play sessions. Mother-reared and cross-fostered
monkeys remained with the mother, typically within a social group. At roughly seven months of age, mother-reared monkeys were weaned and placed together with their peer-reared cohort in large, mixed-gender social groups. Since the monkey population encompassed two groups that received dramatically different social and rearing experience early in life, the interactive effects of environmental experience and the rh5HTTLPR on cisternal CSF 5HIAA levels and 5HT-related behavior was assessed (Bennett et al., manuscript submitted). CSF 5HIAA concentrations were significantly influenced by genotype for peer-reared, but not for mother-reared subjects. Peer-reared rhesus monkeys with the low-activity rh5HTTLPR s allele had significantly lower concentrations of CSF 5HIAA than their homozygous l/l counterparts. Low 5HT turnover in monkeys with the s allele is congruent with in vitro studies that show reduced binding and transcriptional efficiency of the 5HTT gene associated with the 5HTTLPR s allele (Heils et al., 1996; Lesch et al., 1996). This suggests that the rh5HTTLPR genotype is predictive of CSF 5HIAA concentrations, but that early experiences make unique contributions to variations in later 5HT functioning. This finding is the first to provide evidence of an environment-dependent association between a polymorphism in the 5′ regulatory region of the 5HTT gene and a direct measure of 5HT functioning, cisternal CSF 5HIAA concentration, thus revealing an interaction between rearing environment and rhHTTLPR genotype. Similar to the 5HTTLPR’s influence on NEO Neuroticism in humans, however, the effect size is small, with 4.7% of variance in CSF 5HIAA accounted for by the rh5HTTLPR–rearing environment interaction.

Previous work has shown that monkeys’ early experiences have long-term consequences for the functioning of the central 5HT system, as indicated by robustly altered CSF 5HIAA levels, as well as anxiety, depression, and aggression-related behavior, in monkeys deprived of their parents at birth and raised only with peers (Higley et al., 1991, 1993; Kraemer et al., 1989). Intriguingly, the biobehavioral results of deleterious early experiences of social separation are consistent with the notion that the 5HTTLPR may influence the risk for affective spectrum disorders. Evolutionary preservation of two prevalent 5HTTLPR variants and the resulting allelic variation in 5HTT expression may be part of the genetic mechanism resulting in the emergence of temperamental traits that facilitate adaptive functioning in the complex social worlds most primates inhabit. The uniqueness of the 5HTTLPR among humans and simian nonhuman primates, but not among prosimians or other mammals, along with the role 5HT plays in complex primate sociality, form the basis for the hypothesized relationship between the 5HTT function and personality traits that mediate individual differences in social behavior. This conclusion concurs with an increasing body of evidence for a complex interaction between individual differences in the central 5HT system and social success. In monkeys, lowered 5HT functioning, as indicated by decreased CSF 5HIAA levels, is associated with lower rank within a social group, less competent social behavior, and greater impulsive aggression (Higley et al., 1996, 1992; Mehlman et al., 1994, 1995). It is well established that, while subjects with low CSF 5HIAA concentrations are no more likely to engage in competitive aggression than other monkeys, when they engage in aggression it frequently escalates to violent and hazardous levels.

Association between the rh5HTTLPR genotype and aggressive behavior was
studied by analyzing the joint effects of genotype and early rearing environment on competition-elicited aggression. Socially dominant mother-reared monkeys were more likely than their peer-reared counterparts to engage in competitive aggression. Moreover, under both rearing conditions, monkeys with the low-activity s allele exhibited more aggressive behaviors than their l/l counterparts. The lack of a genotype by rearing interaction for competitive aggression indicates that subjects with the s allele, while unlikely to win in a competitive encounter, are more inclined to persist in aggression once it begins. A role of s allele-dependent low 5HTT function in nonhuman primate aggressive behavior is in remarkable agreement with the association of NEO subscales Neuroticism (increased angry hostility) and Agreeableness (decreased compliance = increased aggressiveness and hostility) and the 5HTTLPR s genotypes.

Taken together, these findings provide evidence of an environment-dependent association between allelic variation of 5HTT expression and central 5HT function and illustrate the possibility that specific genetic factors play a role in 5HT-mediated social competence in primates. The objective of further studies will be the elucidation of the relationship between the rh5HTTLPR genotype and sociality in monkeys as this behavior is expressed with characteristic individual differences both in daily life and in response to challenge. Because rhesus monkeys exhibit temperamental and behavioral traits that parallel anxiety, depression, and aggression-related personality dimensions associated in humans with the low-activity 5HTTLPR variant, it may be possible to search for evolutionary continuity in this genetic mechanism for individual differences. Nonhuman primate studies may also be useful to help identify environmental factors that either compound the vulnerability conferred by a particular genetic makeup or, conversely, act to improve the behavioral outcome associated with that genotype.

CONCLUSION AND FUTURE DIRECTIONS

Converging lines of evidence suggest that variation in serotonergic gene regulation and in the activity of the respective gene products plays a critical role in synaptic plasticity, thus setting the stage for expression of complex traits and their associated behaviors throughout development and adult life. Moreover, genetically driven variation of 5HT system function, in conjunction with other predisposing genetic factors and with inadequate adaptive responses to environmental stressors, is also likely to contribute to impulsivity and aggression-related behavior emerging from compromised brain development and from neuroadaptive processes.

Impulsivity, aggressiveness, and associated aggressive behavior are common traits, the expression of which must be carefully modulated to ensure the success of individuals, small groups, and large societies, especially within the current framework of rapid globalization (Tecott and Barondes, 1996). While displaying multiple facets, impulsivity and aggressiveness is currently being studied from different perspectives. Some lessons can be learned from the past misconceptions of the sociobiology of aggression in particular and behavioral genetics in general. More functionally relevant polymorphisms in genes within a single neurotransmitter system, or in genes which compromise a functional unit in their concerted actions, need to be identified and assessed in both large population and
family-based association studies to avoid stratification artifacts and to elucidate complex interactions of multiple loci. Moreover, genetic influences are not the only pathway that lead to individual differences in personality dimensions, behavior, and psychopathology. Complex traits are most likely to be generated by a complicated interaction of environmental and experiential factors with a number of genes and their products. Even pivotal regulatory proteins of neurotransmission, such as receptors, transporters, and modifying enzymes, will have only a modest impact, while noise from non-genetic mechanisms may seriously obstruct identification of relevant genes. Although current methods for the detection of gene–environment interaction in behavioral genetics are largely indirect, the most relevant consequence of gene identification for personality and behavioral traits may be that it will provide the tools required to systematically clarify the effects of gene–environment interaction.

Based on the remarkable progress in technologies that allow the alteration or elimination of individual genes to create transgenic animal models, gene knockout strategies are likely to increase our knowledge about which gene products are involved in behavioral traits. However, because a missing gene might affect many developmental processes throughout ontogeny and compensatory mechanisms may be activated in knockouts, behavioral data from mice with targeted gene deletions should be interpreted with caution (Nelson and Young, 1998). It is becoming increasingly evident that many neurotransmitters and receptors are expressed at early periods of neural development, and it is likely that they participate in the structural organization of the nervous system. This is well illustrated by the cytoarchitectural abnormalities of the somatosensory cortex reported in MAOA and 5HTT-deficient mice (Cases et al., 1996; Persico et al., 1999). An additional shortcoming of current knockout experiments is the inability to provide region-specific control of the disruption. The ability to use native and exogenous promoters to control the expression of specifically targeted genes may allow region-specific and temporal control of protein expression. Systems that may prove useful include the tetracycline-responsive inducible promoter, and the LoxP-cre method of inducibly deleting sections of DNA. The development of conditional knockouts, in which a specific gene can be inactivated any time during ontogeny, should avoid these imperfections associated with behavioral data from constitutive knockouts.

Notwithstanding the confounding issues, the “classic” knockout mouse remains a powerful tool for modeling the genetic basis of behavior (Gingrich and Hen, 2000). Constitutively created mutations mimic genetic variability in the sense that they are present during the entire developmental process and that the spectrum seen in human behavior is the result of developmental adaptation. As demonstrated in knockout mice, the developmental impact of a mutation might be more prominent than the actual loss of function that occurs with its absence in adulthood. A major challenge is therefore the identification of neural mechanisms that underlie aggressiveness and attempts to unravel the genetic basis of impulsivity and aggressiveness should also reflect on the complex nature of these traits, which is expressed in many different facets. Such facets can be distinguished by specific testing procedures that identify particular categories of aggression, such as isolation-induced offensive aggression, defensive aggression, predatory aggression, shock-induced or irritability-associated aggression, and infanticide (Tecott and Barondes, 1996). Despite the usefulness of the gene knockout strategy in identi-
fying specific gene products that may be involved in aggression, this approach is limited to known candidate genes. Due to the complexity in the expression of aggressive behavior, it is impossible to predict which genes contribute to the variability of this trait in different populations. Thus, QTL analysis, although technically demanding, should ultimately prove to be an important complementary approach, because it is likely that identification of the particular alleles of the various genes that influence aggressiveness in inbred strains will facilitate elucidation of epistatic (gene–gene) interactions as well as the phenomenon of pleiotropy, the multiple and apparently independent effects of a genes on phenotypical expression (Hamer, personal communication).

The current state of the art of this field illustrates how progress in behavioral genetics might be accelerated by closer integration of neuroscience and genetic approaches and a dimensional, semi-quantitative approach to behavioral phenotypes. Further studies of the genetics of human behavioral traits using association techniques, linkage strategies, and newer methods in development, such as single nucleotide polymorphism (SNP) analysis, may be especially useful in achieving the ultimate goal of refining the conceptions of behavioral genetics.

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