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Review

Alcohol dependence and gene x environment interaction in emotion regulation: Is serotonin the link?☆

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Abstract

Alcohol dependence is characterized by frequent, compulsive and uncontrolled consumption of alcohol associated with behavior of maladaption and destruction. It is an etiologically and clinically heterogeneous syndrome, moderately to highly heritable, and caused by interaction of genes and environment. Alcohol dependence is related to other psychiatric diseases by common neurobiological pathways, including those that modulate reward, behavioral control as well as anxiety and stress response. Alcohol induces adaptive changes in brain function providing the basis for tolerance, craving, withdrawal, and emotional disturbance. The differentiation of psychobiological traits of addictive behavior reflecting neurobiological processes is therefore of particular importance for the dissection of the complex genetic susceptibility to alcohol dependence. A central serotonin (5-HT) deficit is thought to be involved in the pathogenesis of alcohol dependence by modulating motivational behavior, neuroadaptive processes, and resulting emotional disturbance. 5-HT-related impulsive, aggressive, and suicidal behavior has been linked to a primordial personality that is susceptible to alcohol dependence. Although variations in many of the genes that encode receptors, enzymes, and transporters of the 5-HT system have been tested as risk factors for alcohol dependence, genetic analyses of 5-HT signaling in alcohol dependence have mainly been focused on the 5-HT transporter (*5-HTT*) gene. Due to its central role in the fine-tuning serotonergic neurotransmission, a regulatory variant of the 5-HTT, which is associated with anxiety related traits, is not only a key player in the neurobiological mechanism of gene x environment interaction in the etiology of depression, but also contributes to the risk to develop alcohol dependence with antisocial behavior and suicidality. Evidence for a modulatory effect of allelic variation of 5-HTT function on limbic circuit responses to emotional stimuli suggests that genotype–endophenotype correlations may be accessible to molecular functional imaging of the brain. These new developments have broad implications for our understanding how genetic vulnerability to alcohol dependence is manifested in the brain's response to emotional stimuli.

Keywords: Serotonin transporter; Genetics; Alcohol dependence; Addiction; Environment; Plasticity; Imaging

Contents

☆ This article is dedicate to Dr. Jobst Böning, Professor at the Department of Psychiatry and Psychotherapy, University of Würzburg on the occasion of his academic farewell after several decades of research focussed on alcohol dependence.

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1. Introduction

Alcohol dependence is a chronic relapsing and remitting disease characterized by the development of tolerance, withdrawal symptoms, and craving for alcohol that is frequently unrecognized and difficult to treat. The remarkable ineffectiveness of strategies for prevention and treatment, and variation in clinical course and side effects, represent a challenge to elucidate the neurobiology of addiction. Although it is a complex disorder, with multiple subtypes and clinical phenotypes. Defining features of alcohol dependence are the persistent, compulsive, and uncontrolled use of ethanol, often in the face of negative social and psychological consequences. Both positive and negative reinforcement are thought to be critically involved in the transition from casual alcohol use to alcohol-seeking behavior. While the positive reinforcing effects of alcohol are essential to the initiation and early maintenance of intake, the negative reinforcement of alcohol-seeking behavior related to alleviation of symptoms during abstinence may even be more fundamental in maintaining abuse. Therefore, risk factors for alcohol dependence not only implicate systems involved in alcohol reward but those activated following alcohol exposure, during acute withdrawal and abstinence.

Alcohol dependence has major genetic and environmental components displaying a complex mode of interaction. The considerable heritability implies the existence of inherited functional variants of genes that alter the mechanisms of reward, cognition, stress coping, emotion regulation, and neuronal plasticity (Oroszi and Goldman, 2004). Each of these neurobiological constructs has been identified as a critical domain in alcohol dependence. Functional alleles that alter alcohol dependence-related syndromes and co-morbid disorders include common variants in enzymes that catalyse consecutive steps in alcohol metabolism, alcohol dehydrogenase 1B (ADH1B) and aldehyde dehydrogenase 2 (ALDH2), that cause an aversive flushing reaction, the dopamine receptor D4 (DRD4) coding region repeat length variation, the catechol-*O*-methyltransferase (*COMT*) Val158Met structural variation as well as regulatory variants of the serotonin transporter (*5-HTT*) and monoamine oxidase A (*MAO-A*) genes leading to differences in several aspects of neurobiology including cognitive-executive function, stress response, anxiety/negative affect, and impulsivity/aggression which are factors relevant to initial vulnerability, the process leading to addiction and relapse.

A central serotonin (5-HT) deficit reflected by lower concentrations of the major 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid and low platelet 5-HT content is thought to be involved in the pathogenesis of alcohol preference and dependence by modulating motivational behavior and neuroadaptive processes, and resulting emotional dysregulation in both humans and nonhuman primates (Higley et al., 1996a,b; Virkkunen and Linnoila, 1997). 5-HT-related impulsive, aggressive, and suicidal behavior has therefore been linked to a primordial personality that is susceptible to alcohol dependence, suggesting a link between alcohol-seeking behavior and low central 5-HT. Although variations in many of the genes that encode receptors, enzymes, and transporters of the 5-HT system have been tested as risk factors for alcohol dependence, genetic analyses of 5-HT signalling in alcohol dependence have mainly been focused on the 5-HT transporter (*5-HTT*, *SERT*, *SLC6A4*) gene due to its central role in the fine-tuning serotonergic neurotransmission (Feinn et al., 2005; Gorwood et al., 2004).

The identification of vulnerability genes for alcohol dependence may enhance the power to explain the molecular dimension of personality and behavior at risk, lay out strategies to identify physiologic pathways and mechanisms that lead to substance abuse, provide tools to dissect the interactive effects of genes and environment in the development of addiction, and hold the potential to predict response to anticraving pharmacotherapy and other treatments. The future then would be improved premorbid risk assessment, preventive strategies, and treatment individualization. Here, I focus – from the *developmental perspective* – on the nature of an inborn variability in central 5-HT system function that might predispose an individual emotional dysregulation and to alcohol dependence. The relevance of *gene x environment interaction* in the psychobiology of alcohol dependence is also highlighted. Finally, an appraisal of recent findings regarding *molecular functional imaging* of emotionality and its potential for alcohol dependence risk assessment is provided.

2. Neurobiological constructs and endophenotypes

Since alcohol dependence is an etiologically and clinically heterogeneous syndrome caused by complex interaction of genetic and environmental factors, the differentiation of psychobiological traits of addictive behavior is of particular importance for the dissection of the complex genetic susceptibility of alcohol dependence. Various typologies of alcohol dependence have been proposed: Impulsive–aggressive behavior and early-onset defines a subgroup of alcoholics (type-2) with strong genetic predisposition to alcohol dependence (Cloninger, 1987; Gilligan et al., 1987). A substantial proportion of the genetic vulnerability to alcohol

dependence appears to be explained by an individuals' level of response to alcohol (Schuckit, 2000; Schuckit et al., 2004). Both cross-sectional and prospective studies highlight the relationship between low level of response to alcohol and the vulnerability toward alcohol dependence, and both twin and family studies have established a relatively high level of heritability.

Variation in the clinical features of alcohol dependence and response to therapeutic intervention suggests interindividual differences in vulnerability and pharmacogenetic mechanisms. Regardless of thorough definition of subgroups based on clinical phenotypes, only inadequate progress has been made in characterizing the neurobiological complexities of susceptibility and treatment response. One approach that is likely to increase the power to identify the etiological factors in neurobehavioral diseases is to use dimensionally or qualitatively delineated intermediate phenotypes, also called endophenotypes, that reflect mediating factors in behavior and are likely to be influenced by variation of distinct, though overlapping, sets of genes (Oroszi and Goldman, 2004). Endophenotypes thus identify clinical subgroups that share common neurobiological characteristics, common genetic vulnerability, or pharmacogenetic strategies.

A prototypical example of the effectiveness of endophenotypes is the observation of the facial flushing syndrome in response to alcohol which discourages alcohol consumption. This observation led to the finding that variants of ADH2 (Arg47His) and ALDH2 (Glu487Lys) confer partial protection against alcohol dependence in nearly 50% of Southeast Asians. The definition of endophenotypes relevant to brain function may facilitate our understanding of premorbid alcohol-seeking behavior, acute responses to alcohol consumption, long-term tolerance and craving, and response to pharmacotherapy. These endophenotypes include low subjective and physiologic responsivity to alcohol, impaired prefrontal cognitive–executive function resulting in attentional deficits or disinhibition, and high novelty seeking, anxiety-related and stress-reactive personality traits reflected by high neuroticism and harm avoidance as well as emotional dysregulation (Fig. 1).

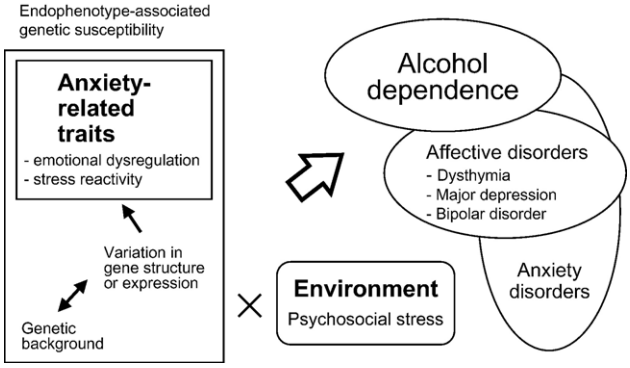

Fig. 1. Causal model of emotional dysregulation as a candidate endophenotype of alcohol dependence.

3. Serotonergic system in emotion regulation

A neural circuit composed of several regions of the ventromedial prefrontal cortex, anterior cingulate cortex, amygdala, hippocampus, ventral striatum, hypothalamus, and several other interconnected structures and involving multiple neurotransmitter systems have been implicated in emotion regulation (for review see Lesch et al., 2003). While both genetic and environmental factors contribute to the formation and function of this circuitry, the amygdala is central to processes of learning to associate stimuli with events that are either punishing or rewarding.

In humans, non-human primates, and other mammals, substantial evidence has been accumulated that the serotonergic signaling pathway integrates elementary brain functions of cognition, sensory processing, and motor activity. Serotonergic neurons of the raphe complex diffusely project to all brain regions implicated in emotional behavior. During development 5-HT shapes various brain systems, above all the emotion-regulating limbic system circuitry. The diversity of these functions is due to the capacity of 5-HT to orchestrate the activity and interaction of several other neurotransmitter systems, particularly activity-, cognition-, and reward-related dopaminergic pathways. Given these links, the 5-HT system constitutes the major modulator of emotional behavior including anxiety and stress response, as well as impulsivity and aggression, and considerable evidence links serotonergic dysfunction to depression and co-morbid conditions such as alcohol dependence.

The level of 5-HT in the synaptic cleft (and extrasynaptic space) is restricted by the synchronized action of at least three components. Firing of raphe 5-HT neurons is controlled by 5-HT_{1A} autoreceptors located in the somatodendritic section of neurons. Release of 5-HT at the terminal fields is regulated by the 5-HT_{1B} receptor. Once released, 5-HT is taken up by the 5-HTT located at the terminals (as well as the somatodendritic fraction) of 5-HT neurons, where it is eventually metabolized by monoamine oxidase A (MAO-A). The action of 5-HT as a messenger is tightly regulated by its synthesizing and metabolizing enzymes, and, more directly by the 5-HTT. It is therefore likely that genetically driven variation in 5-HTT function dramatically affects extracellular levels of 5-HT.

4. Alcohol dependence and serotonin transporter gene

Due to its central role in the fine-tuning serotonergic neurotransmission, genetic analyses of 5-HT signaling in alcohol dependence have mainly been focused on the 5-HTT. A length variation of a repetitive sequence in the 5'-flanking regulatory region of the *5-HTT* gene (*5-HTT* gene-linked polymorphic region; *5-HTTLPR*) comprise of a predominantly short (s) low-activity and long (l) high-activity variant resulting in differential *5-HTT* expression. Allelic variation in 5-HTT function has extensively been studied for association with anxiety- and aggression-related traits as well as in various psychopathological dimensions (Lesch, 2003; Lesch et al., 1996; Lesch and Mössner, 1998; Schinka et al., 2004; Sen et al.,

2004). An association of the *5-HTTLPR* and affective illness including depression and bipolar disorder was first reported by Collier and coworkers in 1996 and subsequently confirmed in a large number studies (Cho et al., 2005; Collier et al., 1996; Lotrich and Pollock, 2004).

Aimed at the assessment of the role of allelic variation of 5-HTT function in the genetic load for alcohol dependence, initial studies showed an excess of the homozygous genotype of short *5-HTTLPR* in patients with alcohol dependence ($n=315$, $p<0.05$; odds ratio, OR=1.31) in comparison to controls ($n=216$). When the overall sample was subdivided in patients with type-2 alcoholism, patients with a history of withdrawal syndromes including seizures an/or delirium ($n=103$; $p<0.05$) the gene effect was more pronounced with ORs between 1.4 and 2.3 (Sander et al., 1998, 1997). These findings provided first evidence that genetically driven low 5-HTT function confers susceptibility to severe alcohol dependence.

The interaction between *5-HTTLPR* genotype and alcohol dependence has subsequently been reassessed in a remarkable number of studies (Edenberg et al., 1998; Gelernter et al., 1997; Gorwood et al., 2000; Hallikainen et al., 1999; Hammoumi et al., 1999; Herman et al., 2005; Hu et al., 2005; Ishiguro et al., 1999; Konishi et al., 2004a,b; Kranzler et al., 2002; Kweon et al., 2005; Lichtermann et al., 2000; Mannelli et al., 2005; Matsushita et al., 2001; Munafo et al., 2005; Nellissery et al., 2003; Nilsson et al., 2005; Olsson et al., 2005; Preuss et al., 2001; Preuss et al., 2000; Schuckit et al., 1999; Thompson et al., 2000; Turker et al., 1998; Twitchell et al., 2001; Wiesbeck et al., 2004). Population-based studies have had variable, but also negative results. Interpretation of these studies is complicated by their frequent use of relatively small samples, which greatly increases the risk of type II error, of heterogeneous subject populations, and the lack of within-family designs to control for population stratification artifacts. Given that the magnitude of *5-HTTLPR*-alcohol dependence association is expected to be small, it appears to be critical that attempts to replicate that finding use the same phenotypic definitions. As discussed previously, utilizing categorically defined diagnoses does not guarantee biological homogeneity. Alcohol dependence comprises a high degree of heterogeneity (e.g., early age-at-onset, high familial load), considerable variability in severity (e.g., withdrawal syndromes, delirium, seizures), comorbidity (e.g., anxiety, depression, and suicidality), and other complications (e.g., antisocial and/or aggressive behavior, delinquency). Therefore, studies based on diagnostic criteria may furnish biased results based on a different rate of subgroups populations. In line with this view, several recent reports show, in addition to severe withdrawal syndromes, an association between the low-activity *5-HTTLPR* s variant and violent behavior, high alcohol tolerance, as well as anxiety, depression and severe suicidal behavior, but not with alcohol dependence itself (Gorwood et al., 2000; Hallikainen et al., 1999; Nellissery et al., 2003; Olsson et al., 2005; Preuss et al., 2001, 2000; Schuckit et al., 1999; Twitchell, 2001 #683; Twitchell et al., 2001; Wiesbeck et al., 2004). The concept of low level of response with negligible ataxia and sedation following alcohol intake early in an individual's drinking career is a predictor for the development of alcohol dependence and is widely accepted genetic risk factor independent of prior history of heavy alcohol consumption (Schuckit, 2000) was supported, such that the *5-HTTLPR* s variant was associated with a low level of response to alcohol in patients with alcohol dependence (Turker et al., 1998).

Two comprehensive *meta*-analyses of case-control studies confirmed that the s allele of the *5-HTTLPR* is a risk factor for phenotypes related to alcohol dependence with still unknown boundaries (Feinn et al., 2005; Gorwood et al., 2004). One family-based study showed a large excess of transmission of the *5-HTTLPR* s variant. Feinn et al. (2005) evaluated 17 population-based studies including 3489 alcoholics and 2325 controls investigating the link between *5-HTTLPR* alleles and alcohol dependence. The frequency of the *5-HTTLPR* s allele was significantly associated with alcohol dependence (OR=1.18, 95% CI=1.03–1.33). A somewhat greater association was detected among individuals with alcohol dependence complicated by either a co-morbid psychiatric condition or an early onset or more severe alcohol dependence subtype OR=1.34, 95% CI=1.11–1.63). This confirm initially reported results that allelic variation at *5-HTT* locus contributes to risk for alcohol dependence, with the greatest effect observed among individuals with a co-occurring clinical feature (Sander et al., 1998, 1997).

Of related interest, genome-wide linkage analyses identified two areas of chromosome 1 of specific interest because they are in the same general area highlighted in earlier genome scans (Oroszi and Goldman, 2004). Other regions of interest were observed on chromosome 4, 11, 18 as well as a section on chromosome 17q in the same general region as the *5-HTT*. Given both the limitation of the diagnostic approach and the limitations due to polygenicity and/or neurobiological heterogeneity future studies will require extended, homogeneous, and ethnically matched samples.

5. Variation of serotonin transporter function in a primate model

Since the genetic basis of present-day personality dimensions and behavioral traits may reflect selective forces among our remote ancestors, research efforts have recently been focussed on rhesus macaques (*Macacca mulatta*). In this nonhuman primate model environmental influences are probably less complex, can be more easily controlled for, and thus less likely to confound correlations between behavior and genes. All forms of emotionality in rhesus monkeys – major categories are anxiety and aggression – appear to be modulated by environmental factors, and marked disruptions to the mother–infant relationship likely confer increased risk (Suomi, 2003).

In rhesus monkeys, maternal separation and replacement of the mother by an inanimate surrogate mother during the first months of life results in long-term consequences for the functioning of the central 5-HT system, defects in peer interaction and social adaptation, and is associated with increases in anxiety- and depression-related behaviors like rocking and grooming (Higley et al., 1991). These studies suggest that early

environmental trauma can directly induce long term plastic changes in the brain that alter anxiety-related responses in adulthood.

One of the most replicated findings in psychobiology is the observation of lower 5-HIAA, the major metabolite of 5-HT, in the brain and cerebrospinal fluid (CSF) in impulsive aggression and suicidal behavior. In rhesus monkeys brain 5-HT turnover, as measured by cisternal CSF 5-HIAA concentrations, shows a strong heritable component and is traitlike, with demonstrated stability over an individual's lifespan (Higley et al., 1991, 1992). Early experiences have long-term consequences for the function of the central 5-HT system, as indicated by robustly altered CSF 5-HIAA levels, as well as anxiety, depression, aggression-related behavior, in rhesus monkeys deprived of their mother at birth and raised only with peers.

Comparison of different mammalian species revealed that the repetitive sequence in the 5'-flanking regulatory region of *5-HTT* (*5-HTTLPR*) is unique to humans and simian primates. In hominoids all alleles originate from variation at a single site, whereas an alternative site for a 21-bp length variation was found further upstream in the *5-HTTLPR* of rhesus monkeys (*rh5-HTTLPR* s and l variant) (Lesch et al., 1997). The fact that a predominantly bi-allelic structure the *5-HTTLPR* is preserved in hominoids and the cercopithecoids, represented by the macaques, indicates remarkable conservation of this part of the *5-HTT*'s transcriptional control region throughout the different lineages of these two superfamilies and that a progenitor *5-HTTLPR* sequence possibly representing viral DNA may have been introduced into the genome some 40 million years ago. The *5-HTTLPR* sequence was shown to be informative in the comparison of closely related species and reflects the phylogeny of the macaques, great apes, and humans (Wendland et al., in press-a,b). The independent appearance and propagation of a bi-allelic *5-HTTLPR* may be an example of a one-time event in the evolutionary history of a species with the potential for a gain-of-function. A plausible gain-of-function resulting from allelic variation of 5-HTT function might well be emotion regulation in the process of socialization. Most notably, the occurrence of two common alleles of the *rh5-HTTTLPR* in rhesus monkeys, which are orthologous to the length variation in the human *5-HTT* and result in a correspondingly altered transcriptional efficiency, renders this primate model uniquely useful to dissect the relative contribution of genes and environmental cues to central serotonergic function and related behavioral outcomes.

The model of maternal separation was used to study *gene x environment interaction* by testing for associations between central 5-HT turnover and *rh5-HTTLPR* genotype. In the two monkey populations with dramatically different social and rearing experience early in life, the interactive effects of environmental experience and the *rh5-HTTLPR* on cisternal cere 5-HIAA levels, stress hormone response, and 5-HT-related behavior as well as alcohol preference/consumption was assessed. CSF 5-HIAA concentrations were significantly influenced by genotype for peer-reared, but not for mother-reared subjects (Bennett et al., 2002). Peer-reared rhesus monkeys with the low-activity s variant *rh5-HTTLPR* had significantly lower concentrations of CSF 5-HIAA than their homozygous l/l counterparts. Low 5-HT turnover in monkeys with the s allele is congruent with studies that show reduced transcriptional efficiency of the human *5-HTT* associated with the *5-HTTLPR* s allele (Lesch et al., 1996). This suggests that the *rh5-HTTLPR* genotype is predictive of CSF 5-HIAA concentrations, but that early experiences make unique contributions to variation in later 5-HT functioning. This finding provided first evidence of an environment-dependent association between 5-HTT variation and 5-HT system function, thus revealing an interaction between rearing environment and *rh5-HTTLPR* genotype. Similar to the *5-HTTLPR*'s influence on personality traits in humans, the effect size is modest, with 4.7% of variance in CSF 5-HIAA accounted for by the *rh5-HTTLPR* x rearing environment interaction. The findings suggest that the *rh5-HTTLPR* genotype is predictive of CSF 5-HIAA concentrations, but that early experiences make unique contributions to variation in later 5-HT system function and thus provides evidence of an environment-dependent association between *5-HTT* and a direct measure of brain 5-HT function. The consequences of deleterious early experiences of maternal separation seem consistent with the notion that the *5-HTTLPR* may influence the risk for affective disorders and co-morbid conditions including alcohol dependence.

6. *Rh5-HTTLPR* in developmental neurobiology: setting the stage for emotion regulation

For investigation of the contribution of genetic and early rearing environment to the development of behavioral traits, studies were extended to the neonatal period, a time in early *development* when environmental influences are modest and least likely to confound gene-behavior associations. Rhesus macaque infants with the s variant of the *rh5-HTTLPR* displayed higher behavioral stress-reactivity compared to infants homozygous for the l variant (Champoux et al., 2002). Mother-reared and peer-reared monkeys were assessed on days 7, 14, 21, and 30 of life, on a standardized primate neurobehavioral test designed to measure orienting, motor maturity, reflex functioning, and temperament. Main effects of genotype, and, in some cases, interactions between rearing condition and genotype, were demonstrated for items indicative of orienting, attention, and temperament. In general, heterozygote animals demonstrated diminished orientation, lower attentional capabilities, and increased affective responding relative to l/l homozygotes. However, the genotype effects were more pronounced for animals raised in the neonatal nursery than for animals reared by their mothers. These results demonstrate the contributions of rearing environment and genetic background, and their interaction, in a nonhuman primate model of behavioral development.

Accumulating evidence demonstrates the complex interplay between individual differences in the central 5-HT system and social success. In monkeys, lowered 5-HT functioning, as indicated by decreased CSF 5-HIAA levels, is associated with lower rank within a social group, less competent social behavior, and greater impulsive aggression. It is well

established that, while subjects with low CSF 5-HIAA concentrations are no more likely to engage in competitive aggression than other monkeys, when the engage in aggression it frequently escalates to violent and hazardous levels. The interactive effect of *rh5-HTTLPR* genotype and early rearing environment on social play and competition-elicited aggression was also explored (Barr et al., 2003b). Infant rhesus monkeys homozygous for the l variant were more likely to engage in rough play than were l/s individuals with a significant interaction between *rh5-HTTLPR* genotype and rearing condition. Peer-reared infants carrying the s variant were less likely to play with peers than those homozygous for the l allele, whereas the *rh5-HTTLPR* genotype had no effect on the incidence of social play among mother-reared monkeys. Socially dominant mother-reared monkeys were more likely than their peer-reared counterparts to engage in aggression. In contrast, peer-reared but not mother-reared monkeys with the low-activity s allele exhibited more aggressive behaviors than their l/l counterparts. This genotype by rearing interaction for aggressive behavior indicates that peer-reared subjects with the s allele, while unlikely to win in a competitive encounter, are more inclined to persist in aggression once it begins. Moreover, high composite scores for alcohol intake and alcohol-elicited aggression are associated with the low-activity *rh5-HTTLPR* s variant in male rhesus monkeys, a potential model for type II alcoholism (Barr et al., 2003a).

Of note in this context, functional variation in the gene for the 5-HT-metabolizing enzyme MAO-A has been implicated in panic disorder (Deckert et al., 1999), and cluster B personality disorders (Jacob et al., 2005), as well as in antisocial and aggressive behavior of alcohol dependence (Samochowiec et al., 1999; Schmidt et al., 2000), particularly when associated with early adverse experiences (Caspi et al., 2002). Interestingly, an interaction between this *MAO-A* variation and the *5-HTTLPR* influencing alcohol binge drinking risk in young women was recently reported (Herman et al., 2005). Transcription of the *MAO-A* gene in rhesus monkeys is modulated by an orthologous polymorphism (*rhMAO-ALPR*) in its upstream regulatory region. In a recent study on male rhesus monkeys raised with or without their mothers were tested for competitive and social group aggression, high-and low-activity alleles of the *rhMAO-ALPR* show a genotype x environment interaction effect on aggressive behavior, such that mother-reared male monkeys with the low-activity-associated allele had higher aggression scores (Newman et al., 2005). Similar to the findings with the *5-HTT*, these results suggest that the behavioral expression of allelic variation in MAO-A activity is sensitive to social experiences early in development and that its functional outcome might depend on social context.

Taken together, these findings provide evidence of an environment-dependent association between allelic variation of 5-HTT or MAO-A expression and central 5-HT function, and illustrate the possibility that specific genetic factors play in 5-HT-mediated social competence in primates. The objective of further studies will be the elucidation of the relationship between the *rh5-HTTLPR* or *rhMAO-A-LPR* 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 gene variants of the 5-HT signalling pathway, 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.

7. Stress reactivity, reinforcement, and alcohol abuse

Among the systems involved both in positive reinforcement of alcohol-induced reward and negative reinforcement of alcohol withdrawal is the hypothalamic–pituitary–adrenal axis. Acute exposure to alcohol activates the hypothalamic–pituitary–adrenal cascade with increased release of corticotropin-releasing hormone (CRH), corticotropin (ACTH), and adrenal glucocorticoids, which are known to potentiate the positive reinforcing effects of drugs of abuse. A surge of CRH release also occurs in the central nucleus of the amygdala and forebrain structures during alcohol intake and withdrawal may contribute to anxiety associated with acute alcohol withdrawal. The brain 5-HT system is also involved both in reinforcement of alcohol intake and symptoms of withdrawal presumable via reciprocal interacting influences between the hypothalamic–pituitary–adrenal axis and central 5-HT activity. 5-HT is one of the key neurotransmitters to be released in response to alcohol. While 5-HT release following consumption of alcohol is involved in activation of dopaminergic reward pathways, adaptive attenuation in release following alcohol exposure can lead to pain, dysphoria, and depression. Length variation of *5-HTTLPR*, in conjunction with the presence of a glucocorticoid response element (Glatz et al., 2003), renders gene transcription differentially responsive to stress-induced levels of corticosteroids, a phenomenon that may particularly be relevant among both human and rhesus carriers of the *5-HTTLPR s* allele.

Since the *5-HTTLPR* is associated with anxiety as well as increased risk of developing depression in the face of adversity and targeted inactivation of the *5-Htt* in mice results in anxiety-like behavior with exaggerated hypothalamic–pituitary–adrenal axis responses to stress with evidence for gender specificity (Bouali et al., 2003; Holmes et al., 2003; Li et al., 2000, 1999), the impact of *rh5-HTTLPR* x rearing condition interaction on stress-elicited endocrine responses was determined in infant rhesus macaques. ACTH and cortisol levels in monkeys reared with their mothers or in peer-only groups, were determined at baseline and during separation stress at 6 months of age. Cortisol levels increased during separation, and there was a main effect of rearing condition, with decreased cortisol levels among peer-reared macaques. Monkeys with *rh5-HTTLP*R l/s genotypes had higher ACTH levels. ACTH levels increased during separation, and there was a separation x rearing condition x *rh5-HTTLPR*

interaction, such that peer-reared individuals with a l/s genotype had higher ACTH levels during separation than l/l monkeys. A confirmatory study further revealed that this interaction is sexually dichotomous and the interactive effect may underlie the increased incidence of certain stress-related disorders in women (Barr et al., 2004b). These findings confirm the data from studies in human populations and *5-Htt* knockout mice that allelic variation of 5-HTT function affects hypothalamic–pituitary–adrenal axis activity and that the influence of *rh5-HTTLPR* on hormonal responses during stress is modulated by early experience and displays sexual dichotomy.

Previous research revealed that peer-reared primates consume more alcohol as young adults and pear-reared female rhesus macaques have exaggerated hypothalamic–pituitary–adrenal axis responses to alcohol and that low CSF levels of 5-HIAA, known to be associated with alcohol-seeking behavior in humans and animal models (Higley and Linnoila, 1997), are observed among peer-reared monkeys with the *rh5-HTLPR* l/s genotype (Bennett et al., 2002). Because their environments can be controlled, use of the macaque model permits investigation of independent influences as well as potential interactions between 5-HT system-related genes, maternal deprivation, and stress in the etiology of alcohol dependence. Given that 5-HT signalling and hypothalamic–pituitary–adrenal axis hormones is involved in the reinforcement of alcohol intake and contribute to the risk for symptoms of withdrawal and relapse in alcohol dependence in a gender-specific manner, the interactive effect of *rh5-HTTLPR* genotype and early rearing environment on the patterns of preference and consumption across a 6-week alcohol consumption paradigm was examined in primates (Barr et al., 2004a). Female rhesus macaques were reared with their mothers in social groups or in peer-only groups and as young adults, they were given the choice of an 8.4% alcohol solution or vehicle for 1 h per day. Interactions between rearing condition and *rh5-HTTLPR* genotype with dramatically higher levels of ethanol preference were demonstrated l/s peer-reared female. An effect of rearing condition on alcohol consumption during the 6 weeks as well as a phase by rearing interaction, such that peer-reared animals progressively increased their levels of consumption across the course of the study was also found. This was especially evident for peer-reared females with the *rh5-HTTLPR* l/s genotype. These data confirm an interaction between 5-HT system activity and early experience in vulnerability to alcohol dependence.

In unison, these findings in rhesus macaque cohorts indicate that nonhuman primate studies are useful to identify environmental factors that either compound the vulnerability conferred by a particular genetic disposition or, conversely, act to improve the behavioral outcome associated with a distinct genetic makeup. Consequently, it is increasingly accepted that much of the impact of genes on emotionality including anxiety and depression depends on interactions between genes and the environment. Such interactions would lead to the expression of environmental effects only in the presence of a permissive genetic background. Not unexpected, a recent study by Caspi et al. (2003) robustly confirmed that individuals with one or two s versions of the *5-HTTLPR* are up to two-fold more likely to get depressed after stressful events such as bereavement, romantic disasters, illnesses, or losing their job. Moreover, childhood maltreatment significantly increased probability to develop depressive syndromes in later life in individuals with the low-activity s allele of the *5-HTTLPR*. These results further support the notion how a combination of genetic disposition and specific life events may interact to facilitate the development of mental illness. What went largely unnoticed, though, were its implications for the relevance of studying the genetics of personality. Depression is strongly associated with anxiety- and depression-related traits, the factual personality dimensions that have been linked to allelic variation of the 5-HTT. Given the high comorbidity between anxiety and depression and the evidence for their modulation by common genetic factors (Kendler, 1996), it is likely that predisposition to mood disorders will also be determined by environmental influences whose impact on the brain is under genetic control.

Particularly interesting is that early trauma inflicted by childhood maltreatment is interacting with allelic variation of 5-HTT function is increasing the vulnerability to develop mood disorders (Caspi et al., 2003). A remarkable body of evidence suggests that emotionality and stress response can be influenced by experiences early in life and it has long been supposed that severe early life trauma may increase the risk for anxiety and affective disorders. For example, adults experiencing four out of a list of seven severe early traumatic events showed a more than four-fold increased risk for depressive symptoms and about a 12-fold increased risk for attempted suicide (Felitti et al., 1998). No direct correlation between any specific childhood trauma and a specific adult anxiety or affective disorder could be made, however, suggesting that other, possibly genetic, factors determine the precise pathology that is precipitated by the traumatic event. The observation that during early developmental stages individuals are particularly susceptible to adverse environmental influences is confirmed by animal studies that have demonstrated influential effects of the quality of maternal care on life long brain functioning, emotional behavior, and disease risk.

8. Molecular functional imaging of emotionality: prediction of risk?

Imaging techniques become increasingly elaborate in displaying the genomic influence on brain system activation in response to environmental cues, thus representing tool to bridge the gap between multiple alleles with small effects and complex behavior as well as psychopathological dimensions. Evidence for a modulatory effect of the *5-HTTLPR* on prefrontal cortex activity suggests that genotype–endophenotype correlations may be accessible to *molecular functional imaging* of the brain (Fig. 2). In two subsequent studies, Fallgatter et al. (1999, 2004) were the first to report an association between *5-HTTLPR* genotype and prefrontal cortex–limbic excitability detected by event-related potentials (ERP) with two different tasks of cognitive response control (Go–NoGo and error-processing task). Individuals with one or two s allele of the *5-HTTPLR*

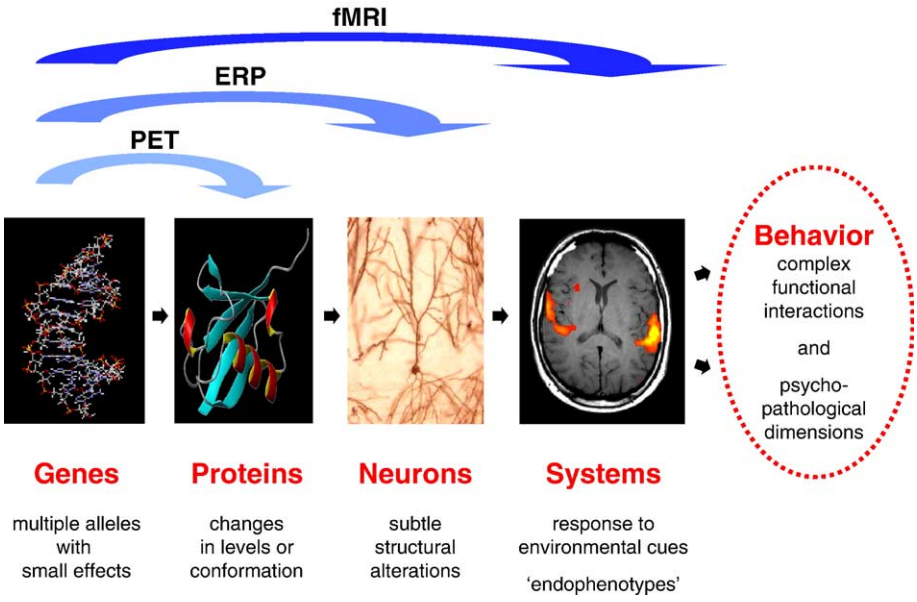

Fig. 2. Molecular functional imaging of genotype–endophenotype correlations as tool to bridge the gap between alleles with small effects and complex behavior as well as psychopathological dimensions. ERP, event-related potentials; fMRI, functional magnetic resonance imaging; PET, positron emission tomography.

showed higher prefrontal brain activity as compared to subjects homozygous for the l variant, thus indicating that the low-activity s variant is associated with enhanced responsiveness of the prefrontal cortex, particularly the anterior cingulate cortex. These findings strongly suggest a relationship between cognitive brain function and allelic variation of 5-HTT function.

Using functional magnetic resonance imaging (fMRI), Hariri et al. (2002) likewise observed that individuals with one or two copies of the low-activity s variant of the *5-HTTLPR* exhibit greater neuronal activity of the human amygdala, a brain system central emotionality and social behavior, as assessed by, in response to emotional stimuli compared with individuals homozygous for the l allele. This *5-HTTLPR*-related effects on the response bias of amygdala metabolic response to environmental threat was subsequently confirmed in a large cohort of both healthy men and women, indicating that the allelic variation of 5-HTT function may represent a classic susceptibility factor for affective disorders by biasing the functional reactivity of the amygdala in the context of stressful life experiences and/or deficient cortical regulatory input (Hariri et al., 2005).

Subsequent investigations were focused on the modifying impact of the limbic cortex in the context of *5-HTTLPR*'s role in depression risk. Although patients with depression display a decreased volume in the subgenual division of the anterior cingulate cortex, together with altered activity of the limbic circuit components involving anterior cingulate cortex and amygdala, an enduring disagreement remained whether these abnormalities predispose for the development of depression and comorbid conditions or are a consequence of the depressed state. To address this question, Pezawas et al. (2005) used a complementary fMRI approach to confirm that the low-expressing s allele of the *5-HTTLPR* is associated with depression-typical structural and functional alterations. Carriers of the s variant showed reduced gray matter volume of both the perigenual anterior cingulate cortex and the amygdala. In addition, the findings revealed *5-HTT* genotype-dependent correlation of amygdala activity with the activity of the rostral and caudal segment of the anterior cingulate cortex, indicating a genetically regulated dynamic coupling which renders the amygdala more responsive to emotional stimuli. Heinz et al. (2005) also observed of increased amygdala activation in carriers of the *5-HTTLPR* s allele which was paralleled by enhanced functional coupling with the ventromedial prefrontal cortex. Moreover, it was shown that positively valenced emotional scenes also evoked amygdala activation, consist with the general task for the amygdala in both positive and negative emotion regulation, but only the response to negative stimuli was associated with the *5-HTT* genotype.

Despite its obvious relevance beyond traits of emotionality and affective disorders, the assumption that the neutral baseline as the control condition does not itself produce changes in activation as a function of *5-HTT* genotype was not investigated in these studies. Recently, Canli et al. (2005) showed that allelic variation in *5-HTT* function is associated with differential activation to negative, positive, *and* neutral stimuli in limbic, striatal, and cortical regions using the Stroop, an attentional interference task. This task is sensitive to individual differences in personality and mood, and activates both the cognitive division and affective division of the anterior cingulate cortex, and amygdala, depending on whether the stimuli are neutral or affectively valenced. While increased amygdala activation to negative, relative to neutral, stimuli, in *5-HTTLPR* s allele carriers was confirmed, the differences were determined by decreased activation to neutral stimuli, rather than increased activation to negative stimuli. High-resolution structural images and automated processes also revealed *5-HTTLPR* genotype-related volumetric and gray matter density differences in frontal cortical regions, anterior cingulate cortex, and cerebellum. These findings are consistent with the notion that the anterior

cingulate cortex plays an integral role in both affective and (non-affective) cognitive processes and is heavily interconnected with a number of cortical and subcortical regions and therefore implicate 5-HT transport efficiency in a wide-ranging spectrum of brain processes that affect neural systems controlling affective, cognitive, and motor processes.

The data base of *5-HTTLPR* imaging was further extended and its relevance for premorbid risk assessment in alcohol use disorders supported by several flanking investigations. Bertolino et al. (2005) studied two groups of volunteers categorized by contrasting cognitive/personality styles characteristic of variable salience to fearful stimuli, phobic prone versus eating disorders prone subjects. The fMRI results showed that phobic prone individuals selectively recruited the amygdala to a larger extent than eating disorders prone subjects. Interestingly, amygdala activation was independently predicted by cognitive style and *5-HTTLPR* genotype suggesting that responsivity of the amygdala may represent an emergent property which is based on the association between genetic and psychological factors It is concluded that certain aspects of cognitive/personality style are rooted in physiological responses of the fear circuitry which interact with processing of environmental stimuli. Furmark et al. (2004) scanned social phobic patients using positron emission tomography (PET) of cerebral blood flow to report greater amygdala activation during a public, compared to a private, speaking task as a function of *5-HTTLPR* genotype.

Altogether, these findings strongly support the notion that allelic variation of serotonergic function contributes to the response of brain regions underlying human emotional and other behavior and indicate that differential excitability of limbic circuits to emotional stimuli may contribute to exaggerated anxiety-related responses as well as increased risk for affective spectrum disorders including alcohol dependence.

9. Conclusion and perspective

Although serotonergic dysfunction is linked alcohol dependence and related endophenotypes, etiopathogenetic mechanisms continue to be inadequately understood at the neuronal and molecular level. A complementary approach to genetic studies of alcohol dependence in humans and nonhuman primates involves investigation of adaptively regulated genes and their protein products (i.e., construction of transcriptome and proteome maps) implicated in the brain neurocircuitries of emotionality, behavioral despair as well as alcohol preference and consumption in 5Htt-deficient mice (Lesch et al., 2003; Kelai et al., 2003). Based on growing evidence for a critical role of the 5-HTT in the integration of synaptic connections in the mouse brain during critical periods of development, more in-depth knowledge of the molecular mechanisms implicated in these fine-tuning processes is presently evolving. Although current methods for the detection of role of environmental stressors in behavioral genetics are largely indirect and incomplete, the most relevant consequence of gene identification for behavioral traits related to alcohol dependence may be that it will provide the tools required to systematically dissect the effects of the interactions between genes and environment and to apply this advanced knowledge to the design of preventive therapeutic strategies as well as manifest disease.

Evidence for a modulatory effect of allelic variation of serotonergic function on prefrontal cortex and amygdala responses to emotional stimuli suggests that genotype–endophenotype correlations may be accessible to molecular functional imaging of the brain. These new developments have broad implications for our understanding how genetic vulnerability to alcohol dependence is manifested in the brain's response to emotional stimuli. Reflecting the heterogenous nature of the causes of alcohol-use disorders, prospective evaluations of individuals carrying specific types of risks might more efficiently and effectively identify prevention techniques relevant to the mechanism of high vulnerability.

Despite advances in the neuro- and psychobiology of alcohol dependence linking genotype to prevention and treatment has come a long way. Prevention strategies could focus on adolescents and young adults who are vulnerable due to variations in particular neurobiological domains, such as stress coping or emotion regulation. Individualization of therapy and the identification of new therapeutic targets are required for the individuals who are already affected with alcohol dependence and comorbid conditions. The hunt for alcohol dependence genes may eventually offer new insights into the different mechanisms which explain why some individuals are more at risk; it will also help us to evaluate and control our biological heritage as predictor for successful prevention and treatment response in an integrated multidimensional approach.

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