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Investigating the Molecular basis of Major Depressive Disorder etiology: a Functional Convergent Genetic Approach

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Abstract

Genes play a major role in behavioral adaptation to challenging environmental stimuli, but the complexity of their contribution remains unclear. There is growing evidence linking disease phenotypes with genes on the one hand, and the genesis of stress related disorders like major depression, as a result of exposure to stressful environmental pathogens on the other. Here we illustrate the convergent role of monoaminergic genes in regulating the underlying biological mechanisms of stress and the emotions. By reviewing data that supports a role of monoaminergic and other related genes in environmental adaptation, we concluded by advocating the use of convergent approaches in examining the genetic modulation of disease phenotypes.

Keywords

Stress; Genes; Monoamines; MDD; Convergent

Introduction

Major depressive disorder (MDD) is one of the oldest, well-recognized medical disorders, having been clearly described in medical texts dating back to ancient Greece. It is a stress-related disorder that includes abnormalities of affect and mood, neurovegetative functions (such as appetite and sleep disturbances), cognition (such as inappropriate guilt and feelings of worthlessness), and psychomotor activity (such as agitation or retardation) 1. As well as being debilitating for sufferers, MDD impose enormous medical and economic burdens, making understanding the genetic mechanisms crucial 2.

The “stress-diathesis” theory of MDD predicts that multiple factors contribute to its onset and course 3. The preclinical and clinical literature strongly supports the thesis that adverse early life experiences enhance the likelihood of developing MDD later in life. This vulnerability or sensitisation may play out differently depending on the genetic constitution of the individual, the age of exposure and duration of early life adverse experience, and the availability of social buffering 3, 4. Thus the heritability (the proportion of total variance in a trait due to genetic variation) for MDD has been estimated from twin studies at 37% 5.

Even though the field of psychiatric epidemiology has identified a substantial list of putative risk factors for MDD, one difficulty has been the inability to discriminate association from causation 1, 2, 6. Four risk factors stand out in the consistency of their association with

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MDD and the level of evidence suggesting that at least some of the association is indeed causal: gender 7, 8, stressful life events 3, 9, 10, adverse childhood experiences, and certain personality traits 1.

The etiology of MDD is thus believed to be under interactive influence of genetics and environmental factors, with the estimated genetic risk being in the same range of that found for many biomedical traits such as blood pressure and serum cholesterol 1. Specifically, genes functionally responsible for the regulation of central neurotransmitters such as monoamines, corticotrophin releasing factor (CRF) and brain derived neurotrophic factor (BDNF) are likely functionally abnormal in MDD 11.

Here we will briefly review the recent advances in the characterization of genetic effects on peripheral and central neurobiochemical processes relevant for the etiology of MDD. The theoretical implications of this method will be summarized by focusing on available data on the functional relationship between monoaminergic genes and valid environmental pathogens for MDD (i.e., psychological stress) on the one hand, and gender and personality on the other.

Is the Monoamine Hypothesis Enough?

The so-called catecholamine and serotonin deficiency hypothesis have been the focus of earlier etiological theories of MDD, collectively referred to as the monoamine hypothesis of MDD 12–14. This hypothesis has been supported by clinical and preclinical evidence of a role of monoamines in the pathophysiology of stress-related disorders like MDD. Mutant mice lacking monoamine degrading enzymes such as monoamine oxidase A (*MAOA*) and catechol-methyltransferase (*COMT*) or mice lacking the serotonin transporter (*5-HTT*) display among other alterations, anxiety-like and depression-like behaviours 15–23. In line with these preclinical observations, certain kinds of personality traits appear to predispose MDD, with the best evidence available for the Neuroticism trait 24.

Polymorphic variations of these three monoaminergic genes (i.e., *5-HTT*, *COMT* and *MAOA*) have been associated with psychiatric samples, treatment response to antidepressants and temperament and antisocial behaviour, although a lack of association has also been reported 2, 25–32. Indeed, the personality domains affected by *MAOA* genetic variation (Harm Avoidance, Reward Dependence) correspond, in the Cloninger hypothesis 33, to those neurotransmitter systems (serotonin, norepinephrine) impacted by *MAOA* during neurodevelopment 34. Together, these reports strongly suggest involvement of these monoaminergic polymorphisms in a broad category of personality traits and psychiatric disorders, emphasizing that there may be no simple association between the diagnoses for mood disorders with a single genotype 2, 6, 24, 34–38.

The *5-HTT* gene, located at 17q11.1–q12, plays a central role in the regulation of the serotonin synaptic function and is considered to be a promising candidate for various psychiatric disorders 2, 6. A common functional polymorphism in the *COMT* gene on the one hand, which is the result of a G to A mutation that translates into a valine (*val*) to methionine (*met*) substitution at codon 158, has been shown to account for a fourfold decrease in enzyme activity 39, 40. Whereas homozygosity for the *met* allele is associated with low enzyme activity, homozygosity for the *val* allele on the other hand is associated with high activity, with the heterozygosity being associated with intermediate levels of activity. The functional polymorphic variation of the *MAOA* gene on the other hand, has been mapped to the short arm of the X chromosome, and a variable-number-tandem-repeat (VNTR) polymorphism in the promoter region of the *MAOA* gene has been identified 41. The polymorphism consists of 30-bp repeated sequence present in 2, 3, 3.5, 4, or 5 repeats (R), with the 3.5R or 4R, which together are called *MAOA-H* (high activity) transcribed 10

times more efficient than those with 2R, 3R or 5R also known as *MAOA-L*, (low activity) in *MAOA* functioning 42.

Although polymorphic variations of *5-HTT*, *COMT* and *MAOA* are directly implicated in the metabolism of catecholaminergic and serotonergic neurotransmitters, their role in glucocorticoid metabolism remains elusive. Given the shared involvements of monoamines and glucocorticoids in stress-related pathology, a functional relationship between the two cannot be ruled out. To our knowledge however, functional mediatory roles of *5-HTT*, *COMT* and *MAOA* on glucocorticoid metabolism remains under explored. Of interest, dysfunctional mechanisms of both monoamines and glucocorticoids have been consistently identified as valid disease endophenotypes for MDD 1–4, 6, 10, 11, 13, 43–47. In line with these reports, an involvement of these genotypes in glucocorticoid functioning has often been pointed out 45, 46, 48–52. For instance, Reul et al. showed morphological changes within the hypothalamic-pituitary-adrenocortical axis (HPA-axis) after long-term treatment with reversible *MAOA* inhibitor moclobemide 52. Lindley and colleagues demonstrated significant decreases in *MAOA* enzyme activity and *MAOB* gene expression in the liver but not medial prefrontal cortex following a one week corticosterone administration, highlighting a dissociable functional expression of monoamine genotypes on peripheral, relative to central markers 50.

Reciprocal interactions between central serotonergic system and HPA-axis are of particular relevance with regard to MDD, in which alterations of HPA-axis at the gene level impacted serotonin neurotransmission, and reciprocally, *5-HTT* knock-out affects HPA-axis dependent responses to stress in mice 49. Impairments in glucocorticoid receptor functioning was shown to affect normal adaptive responses of HPA-axis and the serotonergic system to chronic mild stress as well as altering stress-related consequences on decision-making behaviours in mice 48. Moreover, a relationship between adrenocorticotropin hormone (ACTH)/cortisol and *COMT* genotype during both pharmacological and psychological stress challenges was shown 45, 46, 51. Taken together, overwhelming evidence points to a functional relationship between monoamine metabolism and peripheral and central glucocorticoid functioning. Investigating the molecular basis of a convergent monoamine-glucocorticoid metabolism, and their implications for psychopathology, may be beneficial in the development of improved therapies for stress-related disorders like MDD.

Environmental Pathogens, Gender and Genetic Susceptibility

Polymorphic variations of *5-HTT*, *COMT* and *MAOA* have been positively as well as negatively associated with mood disorders, suicide and other related psychiatric conditions 2, 4, 6, 25–27, 35, 45, 53–60. By applying a valid disease pathogen, as advocated in the intermediate phenotype approach 2, 6, 61, our group exposed individuals with varying degree of susceptibility to MDD as determined by their familial loading (i.e. healthy controls as opposed to high risk probands of major depression and age-matched major depressives) to an acute psychological and endocrine stress challenges in the laboratory 45, 46. We found functional influences of MDD susceptibility; sex; and monoaminergic allelic variations on both *catecholaminergic* and *glucocorticoid* stress responses 45, 46.

Our data provides evidence of a gender dimorphic monoaminergic gene mediation of the glucocorticoid stress response, as well as a gender dimorphic MDD susceptibility effects on HPA axis response to both psychological and endocrine challenges (Figure 1–3). Specifically, a main effect of MDD susceptibility in norepinephrine (NE) stress response was made alongside a finding of an interaction between familial risk for MDD and *COMT* regarding epinephrine (EPP) response to stress (Figure 2), whereby high MDD susceptibility

lead to more peripheral NE response and a less functional influence of *COMT* on EPP stress response respectively 46.

By applying a valid intermediate phenotype approach, we demonstrated the first convergent genetic modulation whereby only individual carriers of the *MAOA-H* gene variant showed a silencing of *COMT* function in endocrine regulation of psychosocial stress 46 (Figure 1). This dominant role of *MAOA* mediation of the glucocorticoid stress response was confirmed by our finding of the strongest influence of this genotype in mediating ACTH response to the dex-CRH challenge, in the absence of psychological stress 46 (Figure 3).

Of importance, a gender by MDD susceptibility effect was observed, with the low susceptibility individuals showing a higher acute stress response, accompanied by a marked percentage decrease in plasma ACTH levels during recovery from stress, but only in females. Conversely, the high susceptibility individuals tend to exhibit an opposite trend in the male subjects (Figure 2). Relevant for these gender dimorphic findings, we found a male dominance in HPA axis responsivity to the endocrine challenge. This sexually dimorphic nature of *MAOA* expression, whereby the dominance of endocrine stress response in low expression as compared to high expression *MAOA* variant was shown in only males, may relate to recent imaging reports of amygdala, hippocampal, and cingulate mediation of emotional memory 34, 62, 63. Both *MAOA* effects on medial prefrontal connectivity and correlations with personality traits were shown to be highly gender dimorphic, with low activity *MAOA* variation affecting medial prefrontal-amygdala connectivity in men only 34. *MAOA* being a X-chromosomal gene, it is possible that gene dosage difference could contribute to the consistent findings of gender dimorphic effects of this genotype on peripheral endocrine and neuronal responses to environmental challenges 34, 46. However, sex hormone receptors are predominantly expressed in brain regions (e.g., amygdala, cingulate and orbitofrontal cortex) where they can influence monoamine metabolism 34, suggesting an intricate role of gender in stress related pathologies 8, 24, 46, 64, 65.

Monoamines, Glucocorticoids and the Stress Response

Diathesis-stress theories of MDD predict that individuals' sensitivity to stressful events depends on their genetic makeup 4, 53, 66, 67. Behavioral genetics research supports this prediction, documenting that the risk of depression after a stressful event is elevated among people who are at high genetic risk and diminished among those at low genetic risk 38, 53. Stress and its adverse consequences are therefore accepted as a valid environmental pathogen for mental disorder 2, 4, 6, 9, 35, 61.

We recently reported a gene-gene interaction between *COMT* and *MAOA* in HPA-axis response to stress 46. The majority of findings implicating *COMT* in the pathogenesis of affective disorders has been associative in nature 27, 56; although the relationship between a related candidate genotype (GABAR6 polymorphism) and HPA-axis response to psychological stress have been examined once 68. To our knowledge, only a single study into the role of *COMT* in plasma endocrine response to a pharmacological challenge has been reported, showing a relationship between *met* allelic loading and higher HPA-axis response to a Naloxone challenge in healthy individuals 51.

To our knowledge, our studies were first to examine the influence of *5-HTT*, *COMT* and *MAOA* allelic variation on the endocrine stress response 45, 46. Earlier, *COMT* has been implicated in moderating psychological and neurophysiological correlates of pain (a valid physical stressor) processing 69–71. Indeed, *COMT* has also been implicated in cognitive processes relevant for psychopathology, especially for schizophrenia 72, 73, and in cortical responses to negative emotional affect 74. These findings of an involvement of *COMT* in the cortical response to negative emotional affect are relevant for the integrative approach of

central and peripheral systems measures we are advocating here. Recently, the insula and anterior cingulate cortex has been shown to be functionally involved in experience of negative emotional response to psychological stress 75, indicating the existence of a functional convergent zone, in this case the insula, that mediates both physiological (pain) and psychological stress. Indeed, *COMT* was implicated earlier in the modulation of neural response to negative emotions 74, and in both neural and behavioural responses to noxious stimuli 71. These findings are particularly relevant in the context of *COMT* involvement in the aetiology of MDD, because this disorder is strongly associated with both negative emotionality and somatic symptoms 76. Moreover, decades of cross-sectional surveys have shown that chronic pain, MDD, and anxiety often coexist 77. Thus, the convergence of our own findings with previously mentioned reports of a moderating role of *COMT* in central and peripheral response to physical (pain) as well as emotional and psychological stress, strongly suggests this genotype to be part of an interface that modulates physiological and emotional stress responses 46· 70· 78· 79. In this light, examining the role of *COMT* on insula functioning during stress may shed more light into the genesis of stress related pathologies such as MDD.

Activation of the HPA axis is an important adaptive mechanism that enables the human body to return to homeostasis in response to physiological and psychological stressors 80. Additionally, reciprocal reverberatory neural connections exist between the CRH and noradrenergic neurons of the central stress system, with CRH and NE stimulating each other primarily through CRH1 and α_1 -noradrenergic receptors, respectively 43· 80–85. It has been shown that both CRH and the noradrenergic neurons receive stimulatory innervations from serotonergic and cholinergic systems 86· 87, and inhibitory input from γ -aminobutyric acid (GABA)-benzodiazepine (BZD) and opioid peptide neuronal systems of the brain 81· 83· 88, as well as from the end-product of the HPA axis, the glucocorticoids 43· 81–83. Of relevance to the findings reviewed here, patients suffering from MDD have hypersecretion of CRH, as evidenced by the elevated 24-hour urinary cortisol excretion, blunted ACTH responses to exogenous CRH administration, and an elevated CRH concentration in the cerebrospinal fluid (CSF) 43.

Glucocorticoids play an important role in the regulation of basal activity of the HPA axis, as well as in the termination of the stress response by acting at the extra-hypothalamic centres, the hypothalamus, and pituitary gland 43. Thus, the negative feedback of glucocorticoids on the secretion of CRH and ACTH serves to limit the duration of the total tissue exposure of the organism to glucocorticoids, minimizing catabolic, lipogenic, antireproductive, and immunosuppressive effects of these hormones 89· 90. Notably, our reported findings regarding the modulatory roles of *5-HTT*, *COMT* and *MAOA* in determining the HPA-axis endocrine stress response and not the catecholaminergic responses to these stressors may underscore the importance of normal HPA axis functioning in acute bodily responses to environmental challenges.

Indeed, polymorphic variations in the *COMT* gene have been shown to predict HPA axis response to a Naloxone challenge and in neuronal and subjective response to pain stressors 51· 71. Additionally, the influence of polymorphic variations of the Mu-Opioid receptor (A118G) and the T1521C single nucleotide polymorphism (SNP) in the GABA (A) α_6 receptor subunit gene (GABRA6) 68· 80 were shown to modulate HPA axis response to psychological stress. Converging with these findings, our observed dominant monoaminergic functional modulation of glucocorticoid stress response, in relation to catecholamine stress response, survived multiple comparisons (figure 1&3).

Traditionally, these genes are known to metabolize catecholamines, even though their direct/indirect involvement of glucocorticoid metabolism has not been ruled out. It remains for

future research to examine the molecular pathways through which these genes influence glucocorticoid mediated central and peripheral processes relevant for environmental adaptation.

Glucocorticoids also promote the termination of stress reactions through the complex feedback loops, mediated in part through the hippocampus and PVN, ultimately leading to the repression of target genes implicated in the stress response, such as CRH 11, 70, 77, 91, 92. Of interest to our own findings, it has been shown that heritable influences account for approximately 62% of the etiological variance in the glucocorticoid response to stress 93, 94. Together, our findings of main and interaction effects of monoaminergic genes, in line with similar preclinical and clinical reports of monoaminergic gene-mediated effect of HPA axis functioning, suggest a convincing role of these genotypes in disturbed HPA-axis regulation.

Peripheral and Central Markers

Genetic variation including those involving monoaminergic genes, may partially underlie complex personality and physiological traits—such as impulsivity, risk taking and stress responsivity—as well as a substantial proportion of vulnerability to addictive diseases 95–97. Specifically, low expression *MAOA* variation was shown to be associated with antisocial and anxious-depressive traits in alcoholic males 98.

Comorbidity is known to be common between anxiety and depressive disorders, associated with other psychiatric disorders and frequently found coexisting with long-standing chronic medical conditions such as cardiovascular disease and diabetes mellitus 99, 100. In light of these reports, one can assume a monoaminergic genetic mediation of personality, whereby underlying physiological mechanisms of these and other related genotypes during cognitive and emotional processes, can exert their effects on behavioral phenotypes relevant for the genesis of personality.

Our own findings replicate earlier reports of an existing noradrenergic and cortisol dependent stress response 108, 109. It is likely that our observed *COMT* and *MAOA* regulation of the HPA axis response to stress is mediated partly by the central noradrenergic system. Most importantly, *MAOA-L* allelic variation, associated with increased risk of violent behaviour, was recently shown to predict pronounced limbic volume reductions and hyper responsive amygdala during emotional arousal. On the other hand, a diminished reactivity of regulatory prefrontal regions during emotional arousal was found in *MAOA-L* individuals compared to high expression variant 2. Previous reports confirmed an association between *MAOA-L* and aggression as well as aberrant emotional regulation². Together, these findings and our own observations of an interaction between *COMT* and *MAOA* in HPA-axis response to psychological stress, whereby a combination of the low expression variants of both genotypes leads to elevated endocrine stress response, suggests that low expression monoaminergic gene variants in general, may mediate vulnerability factors in the pathogenesis of emotional disorders (Figure 4).

Perhaps certain genetic markers may result to endophenotypes like HPA axis dysfunction, leading to susceptibility for some of these co-occurring neuropsychiatric disorders 54, 100. Given the strong evidence that multiple genetic mediators/factors are involved in co-occurring neuropsychiatric conditions 54, 100, it might be important to investigate the roles some common underlying genetic mechanism play in homeostatic regulation, in the face of adverse environmental circumstances.

These genotypes reviewed here have been extensively reported to modulate central neuronal regulation of stressful emotional experience in imaging studies². Indeed, gene expression

profile of peripheral blood mononuclear cells (PBMCs) collected from trauma survivors, were recently shown to identify a gene expression 'signature' for post traumatic stress disorder (PTSD) 110-111. In Segman *et al*, patients were studied immediately following a traumatic event, 1 month, and 4 months later. Their results suggest that patterns of gene expression profile in PBMCs can identify survivors who either persistently manifested full criteria for acute and chronic PTSD or remained healthy at follow up. Such findings of a psychiatric correlate in peripheral blood, in convergence with our own finding are important. They show that peripheral markers can be used to determine symptom/disease outcome in psychiatry when they are meticulously analyzed during disease onset 110-111. Together, these findings may have both theoretical and pharmacological implications 110-111.

HPA-axis involvement in the maintenance of homeostasis during stress has been reported many times. One might speculate that an interaction between *COMT* and *MAOA* on peripheral endocrine responsivity to stress may be related to a combined influence of these genotypes on central catecholaminergic functioning, that might in turn affect CRH functioning. This has been supported by our finding of a stronger *MAOA* involvement in HPA axis regulation to the dex/CRH challenge than the other genotypes under study. Although future research is needed to verify this hypothesis, our findings of an additive effect of these genotypes on peripheral ACTH response to psychological stress strongly supports this idea. Also, individuals heterozygous of the *5-HTT* gene showed exacerbated ACTH response to stress 46. Additionally, higher *met* allelic loading occurs in combination with a *MAOA-L* variation results to elevated ACTH response to psychological stress.

Perhaps these convergent genetic mediations of the endocrine stress response, whereby higher peripheral hormonal responses to acute psychological stress are consistently related to a combination of certain genotypes, are an adaptive mechanism and may therefore represent adaptive stress response and not a vulnerability factor. It remains for future research to examine if these low expression gene variants, that lead to higher availability of monoaminergic neurotransmitters resulting in pronounced central and peripheral reactivity, are resilience factors to stressful experience or not.

The integrative mechanisms reviewed here, highlights the fact that complex psychological traits like mental disorders have noisier genetic architectures like say malaria, and should therefore be treated as such 2-6-112. These gene-gene interactions portrays the existence of additive effects of two monoaminergic genes in human endocrine stress responses. Such findings underscore the complex nature of gene-environment interactions and their roles in the pathogenesis of psychiatric disorders, and may contribute in the understanding of physiological pathways to homeostatic regulation.

In light of our reported findings, a lack of interaction between *5-HTT*, in relation to *COMT* and *MAOA* may suggest a functionally unique role of the serotonergic system in the HPA axis functioning that might be strongly mediated by CRH 45-46. However, given the putative role of both *COMT* and *MAOA* in the metabolism of catecholamines, our only observed stress related gene-gene interaction between *MAOA* and *COMT* in ACTH response to stress may likely involve catecholaminergic influence on HPA axis 45-46.

Dissociation between these two systems may suggest the existence of complementary molecular pathways that play relevant roles in the regulation of the HPA axis, further suggesting a functional role of monoaminergic genes in environmental adaptation 6-46. The emergence of imaging genetics — a strategy for mapping neural structure and activity as a function of genotype in living humans — has encouraged a conceptual transformation by showing that the greater power of intermediate phenotypes lies in using genetic risk variants as tools for the discovery of the mediating neural mechanisms that bridge the gap from DNA

sequence to pathological behaviour 2. In sum, it might be beneficial to combine experimental approaches that look into the genetic mediation of intermediate phenotypes relating to peripheral endocrine, central (neuronal functioning) and behavioural processes relevant for disease phenotypes.

Conclusion

Here, we used MDD as an example to illustrate how a certain class of genes can modulate complex behavioral adaptation relevant for a disease phenotype. Although many limitations stand in the way of undertaking large scale studies designed to characterize convergent genetic influence on disease phenotypes relevant for mental health, a step towards such a direction is warranted. Such an approach may not just help in the development of new treatments, but further help us understand and appreciate the complexity of the biological system that is designed to maintain homeostatic balance in the face of environmental challenges.

Tapping into neurobiological substrates underlying individual differences in personality and its relevance for emotionality, in conjunction with the mediatory roles of genes on peripheral and central response to environmental challenges, future psychiatric genetic studies may provide a window into the physiological mechanisms involved in the regulation of homeostasis and the maintenance of physical and mental health.

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Figure 1.

A: gender dimorphic MAOA related ACTH stress response, showing a marked increase in *MAOA-L* influence on plasma ACTH levels but only in males. B: gender dimorphic cortisol stress response in female individuals homozygous for the short allele of the *5-HTT* gene. C & D: Percentage change in ACTH stress response, with only individuals carrying the *MAOA-L* variation showing any observable ACTH response to psychological stress. Of note, only the findings shown in figure d, e and f survived multiple comparisons at $p < 0.05$. Figures were published earlier in modified from Mol. Psychiatry, REF46. A color version of this figure can be viewed online.

Figure 2.

A. Percentage change in ACTH stress response in a gender dimorphic manner, males showing a higher ACTH response to stress as a function of degree of MDD susceptibility, while females showed an opposite trend in B. C. Percentage change in plasma NE stress response as function of MDD susceptibility, with the high risk individuals showing more plasma NE levels compared to low risk individuals. B. D & E shows the percentage change in plasma EPP stress response, with only the low risk individuals showing a met allelic load dependent EPP response to psychological stress. These findings were significant at $p < 0.01$ uncorrected for multiple comparisons. A color version of this figure can be viewed online.

Figure 3.

A. Peak response of ACTH during the dex-CRH challenge as a function of gender, with the male individuals showing more HPA axis response to the endocrine challenge. B. Peak response of cortisol during the endocrine challenge as a function of *MAOA* genotype, with the *MAOA-H* individuals showing higher cortisol response. C. Area under the curve (AUC) response of cortisol to the endocrine challenge as a function of *COMT* genotype, with the presence of a *val* allele resulting to higher cortisol response to the endocrine stress challenge. D. AUC response of cortisol during the dex-CRH challenge as a function of *MAOA* with the *MAOA-H* variation leading to higher cortisol response to the endocrine challenge. Of note, only the *MAOA*-related findings survived multiple comparison at the $p < 0.05$ level. Figures a and b from Mol. Psychiatry, REF 46.

Figure 4.

Intermediate phenotypes as tools for gene discovery versus neural mechanism characterization. Examples of two alternative approaches to the identification of genetic variants linked to psychiatric disorders are illustrated, with the relevant genes, neural systems and behavioral phenotypes highlighted in red, and arrows indicating the direction of research inference. a. the gene discovery approach, behavioral or neural systems phenotypes are used to reduce genetic complexity and increase penetrance to identify genes implicated in psychiatric disorders. For example, deficiencies in the electrophysiological response to auditory stimulation were used to identify an association of schizophrenia with the $\alpha 7$ nicotinic receptor 101. In the figure, prefrontal cortex dysfunction has been linked to catechol-*O*-methyltransferase (*COMT*) and *GRM3* genetic variations 102- 103, and emotional regulation has been linked to variation in *COMT*, *MAOA* and *5-HTT* 2- 104- 105, and so could have been hypothetically employed as a phenotype to identify these genes. b. in the neural mechanism approach, genes known to be associated with psychiatric disorders or behavioral traits are used to discover neural mechanisms mediating their complex emergent phenotype associations, implicating these mechanisms in the psychiatric disorders to which they have been linked. Examples include the use of the *COMT* Val158Met polymorphism to

characterize prefrontal function and prefrontal-midbrain interactions linked to risk for schizophrenia 102, 106, and the delineation of cingulate circuitry regulating amygdala function mediating risk for anxiety and depression through an investigation of the *MAOA* VNTR 105, 107. BA 25, Broadman's area 25; HF, Hippocampal formation; MB, midbrain; OFC, orbitofrontal cortex. Reproduced with permission from Nat. Rev. Neuroscience REF 2 (2006) © Macmillan Publishers Ltd. A color version of this figure can be viewed online.