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Animal Models of Human Anxiety Disorders: Reappraisal From a Developmental Psychopathology Vantage Point

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ABSTRACT: We are witnessing a tremendous expansion of strategies and techniques that derive from basic and preclinical science to study the fine genetic, epigenetic, and proteomic regulation of behavior in the laboratory animal. In this endeavor, animal models of psychiatric illness are becoming the almost exclusive domain of basic researchers, with lesser involvement of clinician researchers in their conceptual design, and transfer into practice of new paradigms. From the side of human behavioral research, the growing interest in gene-environment interplay and the fostering of valid endophenotypes are among the few substantial innovations in the effort of linking common mental disorders to cutting-edge clinical research questions. We argue that it is time for cross-fertilization between these camps. In this article, we a) observe that the "translational divide" can—and should—be crossed by having investigators from both the basic and the clinical sides cowork on simpler, valid "endophenotypes" of neurodevelopmental relevance; b) emphasize the importance of unambiguous physiological readouts, more than behavioral equivalents of human symptoms/syndromes, for animal research; c) indicate and discuss how this could be fostered and implemented in a developmental framework of reference for some common anxiety disorders and ultimately lead to better animal models of human mental disorders. (***Pediatr Res* 69: 77R–84R, 2011**)

Psychiatric diagnoses encompass dysfunctions across multiple domains. In the widely adopted biopsychosocial approach to mental disorders, psychiatric illnesses are conceptualized as syndromes that emerge from a host of constitutional and environmental causal agents. Similarly, it is thought that multiple brain regions—and different neurochemical pathways—underpin the behavioral, emotional, and cognitive manifestations of mental illnesses.

Although diagnostic criteria currently in use for the classification of mental disorders need further refinement, empirical findings suggest that there are several phenomenological/psychometric points of rarity that distinguish psychiatric symptoms from normal functioning and one disorder from another. Similarly, distinct diagnoses (*e.g.* general anxiety disorder) within broad groups (*e.g.* internalizing disorders) can be connected to underlying causal mechanisms that are in part shared with other disorders and partially unique to each specific diagnosis (*e.g.* in adults) (1). Anxiety disorders in childhood make no exception to this regard (2,3).

Rapidly evolving research techniques and strategies yield an ever-growing corpus of findings about the environmental and genetic determinants of the complex multifactorial phenotypes of anxiety disorders (4) and about their underlying pathophysiology (5). However, we are probably only at the beginning of the process of understanding how youth deviate from normal development into maladaptation.

For instance, it is now clear that there are several genes, each accounting for a small (≤1%) proportion of variance, that account for familial aggregation of mental disorders, including the anxiety disorders. It is also likely that these do not constitute rare variants but are most often common alleles that also influence normally distributed behavioral dimensions, such as temperamental features, and whose action can be tracked at the behavioral and at the neurofunctional levels (6,7).

However, there are still many outstanding questions that pertain to the identification (8) and the action of specific genetic variants in developmental anxiety disorders. As our interests are in developmental psychopathology, we strive to address the modes and the causes whereby development deviates from adaptation into maladaptation. These include issues connected to the mediational effects of life stressors that render some—but not all—individuals vulnerable to later illness (9).

Animal models are valuable but need reinvigoration. To answer these, and further questions, suitable animal models are indispensable. Animal models have long been used in biomedical research, including child psychiatry, but to fully exploit their potentials, a process of refinement needs to be developed in parallel with technological growth. Such process encompasses continuous conceptual refinements, incremental adherence to clinical science, and optimal pertinence and salience to those biological systems that are thought of etiopathological importance for the disorder under study and are common to man and animals.

To foster such a reappraisal, investigators from both the basic and the clinical sides need to cowork more intensively than ever in a genuine effort to cross the "translational divide,"

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Abbreviation: 5HTT, serotonin; **BDNF,** brain-derived neurotrophic factor; **DA,** dopamine; **EPM,** elevated plus maze; **KO,** knock out; **OF,** open field; **PD,** panic disorder; **RGS2,** regulator of G-protein signaling 2; **SAD,** separation anxiety disorder

as the endeavor has not been sufficiently realized. We argue here that to invert this tendency, the effort has to be taken in the first place on conceptual—rather than on technological—ground. We suspect that the reasons behind insufficient cross-fertilization between clinical and basic researchers include the objective difficulty in keeping pace with advances in more than just one field at a time, and the fact that many technologies have become cheaper than scientists' time (and time is indispensable for elaborating innovative vistas).

This is not to say that well-devised initiatives have not been taken in these aims [ESF-Gene-Environment Developmental Models of Emotional Disorders: Bridging Human And Animal Research, Rome (www.esf.org/activities/exploratory-workshops/workshops-list.html?year=2007&domain); EMBL-Workshop on Translating Behavior: Bridging Clinical and Animal Model Research, Heidelberg (www.embl.de/training/events/2009/conf_113/), Gordon Research Conferences–Genes & Behavior, Lucca, (www.grc.org/programs.aspx?year=2008&program=genes)] but the importance of the issues at stake deserves better and more structured effort.

Creating an animal model of a psychiatric disorder is anything but trivial, given the complexity and the heterogeneity of such pathologies. For this reason, a first fundamental step may lie in the process of dissection of the clinical phenotype into individual behavioral, physiological, neurochemical endpoints, rather than the entire syndrome (Fig. 1). The following paragraphs provide an account of some progresses in the field at the behavioral, cellular/molecular, genetic, and the environmental manipulation levels. We end this review by providing some suggestions—from a developmental psychopathology vantage point—of how endophenotypes of physiological nature could help to better integrate clinical and basic research of early manifestations of anxiety.

Behavioral Level

Behavioral tests have been key methodological tools to assess the effects of pharmacological treatments, brain lesions, environmental manipulation, and genetic mutations. The popularity and standardization of tests such as the open field (OF), elevated plus maze (EPM), light/dark boxes, and several conditioned- or unconditioned-threat response paradigms have favored rapid communication and replication of studies. However, the transferability to psychiatric research requests careful choice of paradigms and prudent interpretation of results. According to Kalueff *et al.*(10), terminology also plays a key role. Defining a protocol as a "test" or a "model" is not a mere matter of terms, but it capitalizes on the difference between measuring a response *versus* evoking a pathology.

Accordingly, Lister (11) thinks, that the elicitation of innate, general avoidant behaviors by unconditioned, "ecological" anxiogenic environmental contexts (such as those used in OF) can hardly be used to build "models" for anxiety disorders. Belzung and Griebel (12), for example, argue that what is actually tested in this type of experiments is the mere, adaptive response that represents the spontaneous and normative reaction to open and bright environments, as it would be typically expected in a rodent (12). It is reasonable to believe that while these unconditioned responses do reflect the anxiety experienced by subjects at the moment of the test, their response distribution will be spread along a continuum, reflecting individual differences. However, human simple phobias are early-onset conditions that are not caused by learning or experience (but rather by genetic factors) (13) and sometimes (*e.g.* spider and height phobia) reflect extreme expressions of otherwise adaptive and continuously distributed behaviors. Therefore, we argue that even unconditioned tests could be of value in modeling some specific human anxiety disorders in animals. This type of tests is in addition one ideal arena to investigate genetic manipulation and pharmacological challenges.

Moreover, because some tests have not been devised with a specific target in mind, they ended for being simultaneously relevant for different traits. This aspect may constitute a limitation as far as specificity is at issue, but it may also turn to be advantageous, if—for instance—the experimenter is interested in the covariation of phenotypes or in pleiotropic effects. These so-called "hybrid paradigms" are, for example, the Suok ropewalking test used to assess anxiety and balance

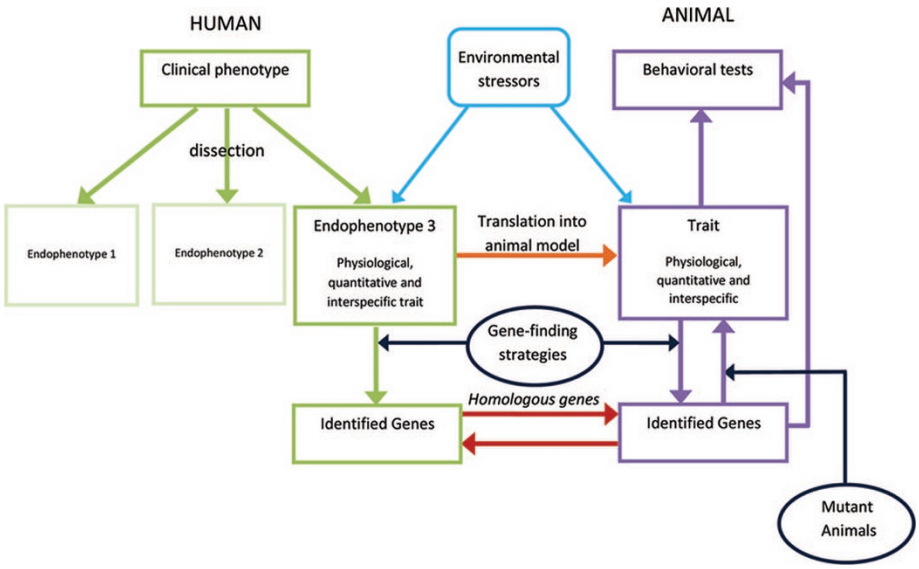

Figure 1. A human clinical phenotype may be partitioned in more than one endophenotypes that are relatively independent from each other, with physiological, quantitative, and interspecific traits being more amenable to export to animal models. By simultaneous investigations of humans and animals (*ovals*), some homologous genes may become identified and then tested for relevance in human clinical phenotypes and in animal behavioral tests.

disorders, or the T maze and Y maze, which evaluate anxiety behavior but also spatial memory (10).

In sum, while behavioral paradigms are well developed, their relevance to human anxiety—both as a normative trait and a clinical condition—is not always clear. Further conceptual exchanges between clinical and basic scientists on well streamlined, "*a priori*" hypotheses and focus on specific aspects of human disorders might help resolve part of these inconsistencies.

Genetics

Genetic factors play an unquestionable role in explaining the etiology of psychiatric disorders, numerous likely loci/genes have been identified (14–16), and many more will be soon found by Genome-Wide Association Studies (GWAS). Although quantitative genetics can measure the proportions of phenotypic variance that can be ascribed to the actions of genes and environment, the question of which specific genes are involved in the etiology of a disorder or a trait is addressed by molecular genetics techniques. Mouse models of susceptibility genes are promising for explaining the function of a gene and its contribution to abnormal manifestations at the cellular, neural, and behavioral levels.

Transgenic model organisms are widely used to this aim. Transgenic model organisms can be obtained by different techniques, and the "knock out" (KO) of a gene is nowadays commonly used. KO animals (mice most typically) undergo the complete inactivation of a target gene throughout the entire life span. This often brings about major behavioral changes or complete loss of a given behavior, which constitutes straightforward evidence of the importance of a specific gene in causing or maintaining a given behavior. For example, serotonin (5-HT) receptor 1A KO mice show clear phenotypic abnormalities in many common anxiety tests such as greater avoidance in aversive open arms of EPM, more time spent in dark compartment of the light/dark boxes, and inhibited exploration in OF (17,18).

It is well known that 5-HT is crucial during brain development, acting as a growth factor during embryogenesis, and taking part in the processes that lead to changes in brain structures for the entire developmental period (19). A number of human studies indicated that the 5-HTTLPR-S allele—which implies lower 5-HTT function—is related to increased neuroticism, trait anxiety, depression, alcohol drinking, and neural responses to a variety of stimuli (6,20–24).

In 1998, Ramboz *et al*. (17) investigated the contribution of 5-HT receptors to anxiety-like behavior in animals by using an homologous recombination to generate mice lacking specific serotonergic receptor subtypes. They demonstrated that mice without 5-HT1A receptors display decreased exploratory activity and increased fear of aversive environments, as measured by the OF and EPM paradigms. Moreover, heterozygote 5-HT1A mutants expressed approximately one-half receptor density of WT animals and displayed intermediate performances in the above-mentioned behavioral test compared with WT and homozygote KO (17).

Similarly, Holmes *et al*. (18,25,26) created an animal mutant model in which the *5HTT* gene was selectively deactivated. *5-HTT* KO mice showed heightened anxiety-like behavior and inhibited exploratory locomotion in OF, EPM, light/dark, and emergency test. Moreover, blockade of the 5-HT1A receptor, *via* acute treatment with an highly selective antagonist, produced anxiolytic-like effects in 5-HTT −/− mice, but not +/+ controls, suggesting a role for the 5-HT1A receptor in mediating anxiety-related abnormalities in 5-HTT null mutant mice (26).

These results were confirmed by Hendricks *et al*. (27) who developed a mouse model with a complete depletion of central serotonin. In this model, they selectively inactivated the transcriptional program responsible to the production of serotoninergic neurons (27). The transcriptional factor Pet-1 (FEV in humans) has been shown to be a unique marker for these cells. Pet-1-deficient mice display an almost complete deprivation (85–90% reduction) of 5HT, without evident alterations to the cytoarchitecture of the brain (27). Behavioral test on the Pet-1-deficient mice showed increased aggressive behaviors compared with WT control mice and also elevated anxiety-like behaviors (27).

However, KO procedures sometimes seem to evoke complex compensatory responses (particularly during development) (28). For instance, it has been demonstrated that deleting the gene coding for dopamine (DA) transporter protein results in a cascade of compensations. These include increased synthesis of DA, reduced levels of the enzyme tyrosine hydroxylase, which rescue at a noteworthy extent the damage brought about by the deletion, and almost normal DA functioning. In some other cases, the compensatory mechanisms remain undetectable to the experimenter's eyes. Thus, caution and multilevel control are warranted when working with these models (28).

Moreover, it can be argued that the consequences of the complete silencing of a gene does not necessarily inform us about the role of that gene in influencing variance of a trait but rather about some "all-or-none" effects related to activity *versus* inactivation of that particular gene. As such, KOs may be less than optimally informative for psychopathology, a discipline inherently interested in individual variation from adaptation to maladaptation (29). Moreover, the KO procedure is not sensitive to mechanisms of genetic activation/deactivation that may take place during sensitive developmental time windows. Conditional KO (cKO) models overcome this limitation in that a gene can be selectively inactivated in a tissue-specific or time-specific fashion (30,31). This is of particular importance for the many genetic pathways that arguably modify their contribution during development.

The brain-derived neurotrophic factor (*BDNF*) gene is coding for the proteins of the neurotrophin family and has a major role in neuronal survival, differentiation proliferation, and synaptic plasticity. Several studies reported an association between the BDNF Val66Met polymorphism and anxiety traits, besides depression and suicidal behaviors (32,33). Chen *et al.* (34) generated a transgenic mouse in which BDNF met is endogenously expressed using a knock-in mouse design that yield to BDNF +/+ heterozygosis BDNF +/met and ho-

mozygous BDNF met/met. They observed that mice carrying the 66Met allele have lower hippocampal volumes, fewer dendritic arbors, and a 30% reduced activity-dependent secretion of BDNF from neurons. Moreover, the homozygous 66Met allele mice tested in anxiety paradigms like the OF or the EPM test showed more anxiety-like behavior and did not respond to the anxiolytic selective serotonin reuptake inhibitor fluoxetine. In humans, stressful life events, especially those occurring early in life (including physical or psychological childhood abuse), seem to be associated with lower levels of serum BDNF and with the onset of anxiety and mood disorders (35). Thus, how BDNF influences health and maladaptation during development is worth being studied through a multilevel approach that takes genes and environment into account.

Sometimes genetic dissection of anxious behaviors initiate in mouse research, to be later transferred to man. Yalcin *et al*. (36) used an inbred strain of mice to identify a quantitative locus that influences anxiety. They found that an anxiety-related quantitative trait locus (QTL) could be subdivided in three regions, one of which containing the regulator of G-protein signaling 2 (*RGS2*) gene. The RGS is a family of proteins that negatively regulate the intracellular signaling of G protein-coupled receptors. In particular, the RGS2 protein is expressed in cortical and limbic areas of the brain and regulates both the serotonergic and noradrenergic systems. By a quantitative complementation design, Yalcin *et al* (36) demonstrated that the *RGS2* is a quantitative trait gene that modulates anxiety in mice, which made the human ortholog *RGS2* gene a candidate gene for anxiety disorders in man. Human correlational studies indeed showed that polymorphisms in the *RGS2* gene were associated with various anxiety conditions: panic disorder (PD), general anxiety disorder, posttraumatic stress disorder, and anxiety-related temperament (7,37). Recently, Smoller *et al*. (7) studied the association between the *RGS2* gene and childhood anxious temperament, and anxiety-related brain functions; they found that the *RGS2* gene is significantly associated with childhood behavioral inhibition (a precursor of social anxiety disorder) and with increased limbic activation during a task of emotional expressions' recognition. However, it has to be noted that these results were recently in part disconfirmed by Fullerton *et al*. (38), who found no evidence of association between human neuroticism and the single nucleotide polymorphisms (SNPs) lying in the human regions homologous to those of mouse were found.

A mouse model of RGS2 KO mice was developed by Oliveira-Dos-Santos *et al.* (39) who targeted a mutation of RGS2. Interestingly, RGS2 mutant mice display increased anxiety responses and decreased male aggression in the absence of cognitive or motor deficits.

To sum up, targeting single genes can be a reliable approach to understand the implications of a simple deletion/insertion at the behavioral level but this is not enough to clarify the cascade of events leading to complex phenotypic variability. Because no single gene deletion—or polymorphism—is sufficient to explain a multifactorial disease or condition, the application of multiple genetic manipulations and environmental interventions are wanted. These will create closer approximations of the causal mechanisms that lead to normal variation and to pathology (40). Future studies will encompass the modeling of organisms targeted to multiple susceptibility loci and multilevel approaches (41).

Models of Environmental Manipulation

A good deal of research has been devoted to the detection of elements that may act as risk factors and/or precipitants of psychiatric conditions and to describe the clinical precursors of anxiety and depressive disorders. Early-life experiences may have long-term consequences (42–46) and have been associated to functional and morphological abnormalities in brain regions connected with emotionality such as the amygdala, the hippocampus, and the prefrontal cortex (47,48).

These studies suggest the presence of critical developmental periods during which exposure to stress makes individuals prone to develop psychiatric disorders later in life (49). Environmental insults and adversities occurring in the early stages of life, when brain seems to be more sensitive to some external influences, may lead to alterations that sometimes become irreversibly incorporated into regulatory physiological processes. The creation of animal models aimed at reproducing early life adversities may thus shed light on developmental aspects and windows of risk.

Because mammals depend heavily on caregivers at the beginning of life, maternal separation and interference with the mother-infant bond have been investigated extensively in rodents. Table 1 offers a synopsis of some of this type of paradigms, together with their behavioral and biological correlates in mothers and pups (Table 1; Ref. 50–59). Although Table 1 necessarily oversimplifies a complex research field, it depicts how—depending on length of separation and the developmental stage—the animals' behaviors and biological correlates vary widely in response to separation.

In rodents, the most popular separation paradigm implies the separation of pups from the dam for 3 or more hours per day, during the first 2 wk of life or till weaning. It has been found that such long separation produces increased anxiety-like and depression-like behaviors, such as less percentage of time spent in the center of the OF arena, more time spent in the closed arms of EPM, and longer immobility in the swim test, compared with normally reared subjects (60,61). The same separation paradigm has been found associated with heightened HPA-axis response to stress in adulthood and augmented vulnerability to alcohol and drug abuse (53,62).

On the contrary, a paradigm that uses shorter duration of separation (15 min per day for the first 2 wk of life, commonly referred to as "handling") can yield opposite effects, such as less fear and more explorative behavior than control mice, as indicated by the higher percentages of time spent in the open arms in EPM (63,64). Moreover, handling promotes physiological and behavioral development, improved performance in cognitive tasks, and seems to buffer the corticosterone secretion response to novelty in acute stress (52). Many authors believe that this ameliorative effects are because of the increased maternal care that is provided by dams after a short separation from litter (65,66).

Table 1. *Synopsis of the most common environmental manipulations adopted with rodents*

Environmental manipulation	Developmental stage	Paradigm	Maternal behavior	Behavior of pups in adulthood	HPA function	Body weight
Handling	PND 1–14 or until 21 weaning (50)	15 min of daily separation from mother	Increased levels of maternal care (grooming and licking) compared with nonhandled offspring (51)	Decreased emotionality in OF and EPM compared with normally reared	Decreased plasma ACTH and CORT levels in response to a stressful challenge (52)	Increased BW in handled subjects (50)
Maternal separation	PND 1–14	3 h of daily separation from mother	Decreased levels of maternal care compared with nonseparated offspring (51)	Increased emotionality in OF and EPM compared with normally reared	Increased plasma ACTH and CORT levels in response to a stressful challenge (53)	Reduction in BW at PND 21 (weaning) (54)
Cross-fostering (used usually between different inbred strain mice to dissect the effects produced by gene and environment)	PND 1	Single change of mother (usually of two different inbred strains as BALB and C57/B6)	Decreased level of maternal care of BALB dams compared with C57/B6 dams	BALB offspring adopted by C57 dams → show equal emotionality compared with BALB. C57 offspring adopted by BALB dams → show increased or equal emotionality compared with nonfostered C57 (55)	Offspring C57 raised by BALB dams show increased basal level of CORT compared with nonfostered C57 but no differences in BALB. No differences after a stressful challenge (55)	Unavailable in paper 55, but usually reported as normal in similar protocols
Communal nesting	Before parturition, till weaning (56)	Two or three mother are put in the same cage 5 d before parturition	Increased allogrooming (maternal care and sibling interactions)	Increased emotionality in OF (but not if paired with a mate) and EPM but not in a social interaction test and depression-like behavior (56)	Decreased CORT levels after acute and prolonged social stress	No differences in BW at PND 1 or 13
Double mothering	From the partum until weaning (57)	Second female with the dam and its litter in the rearing cage	Increased maternal care (nursing, grooming/licking)	No alterations in emotionality tasks compared with control	In basal condition and following restraint, levels of CORT are comparable to those of control subjects	Increased BW at PND 7 and at weaning in subjects raised by two lactating females
Sibling deprivation	PND 1 (58)	Litters were culled to one pup	Increased maternal licking/grooming during the first postnatal wk	Increased locomotor activity in OF test and exploration of open arms in EPM	Unknown	No differences in BW at weaning
Stress pregnant female	Gestation (intrauterine development) (59)	Different kind of stress for early pregnancy, second wk of pregnancy, and last wk of pregnancy	No differences in maternal behavior	Maladaptive behavioral stress responsivity, anhedonia in male (early stress)	Increased HPA axis responsivity in response to stress in male (early stress)	No differences in BW

ACHT, adrenocorticotropic hormone; BW, body weight; CORT, corticosterone; PND, postnatal day.

According to Meaney and Szyf (67) increased levels of maternal care, especially licking/grooming, lead to epigenetic modifications, which in turn cause heightened expression of a glucocorticoid receptor in the hippocampus. These differences found in DNA methylation patterns seem to be reversed by postnatal environment (68). Such forms of by-phasic, "dose-dependent" effects of separation should constitute for human researchers a hint toward better fine-grained research on separation and attachment.

D'Amato *et al.* (57) found—by a model of double mothering—that the introduction of a second lactating female in the nest, improves several hippocampal-dependent tasks, including long-term object discrimination, reactivity to spatial change, and fear conditioning response. This may be due to the "overstimulation" yielded by two instead of one caregiver. At the structural level, the same pups had increased dendritic length and spine density in the CA1 hippocampal region, demonstrating the plasticity of the immature brain structures (57).

In addition to maternal environment, early social experience, *i.e.* stimulation received by littermates, seems to play an influence (56,58,69). Li *et al*. (58) sought to dissect the role of postnatal environment in maternal and sibling contribution. They found that deprivation of littermates stimulation causes a reduction in anxiety-like behaviors (including an enhancement of locomotor activity in the OF test and exploration of open arms in the EPM) in adulthood. This paradoxical outcome was explained by the authors as the result of increased maternal care, seemingly brought about in association with littermates' deprivation. Accordingly, Weaver *et al*. (70) concluded that maternally mediated factors are much more substantial than sib-mediated factors in mice.

In sum, environmental manipulation in mouse—maternal separation most prominently—affects behavior, brain structures, and even the genome by patterns that are often consistent at several simultaneous levels of analysis. This also provides important food for thought to developmentalists. It is for instance tempting to connect these experiments to the equivalent events of childhood parental loss or separation anxiety in human. However, inasmuch as the relevance of animal anxiety-like behaviors to human developmental anxiety disorders remains unclear, prudence is in order. For instance, history of child parental loss and separation anxiety disorder (SAD) may correlate only marginally in man (46,71), which in turn brings about the issue of which human manifestation can be best recognized in these paradigms.

Endophenotype Level

Utility and use of endophenotypes. Perhaps one way ahead is offered by human endophenotypes that can be transferred to animal studies. The use of well-validated human endophenotypes that share part of the susceptibility with a corresponding diagnosis, but are at least partially independent of the clinical manifestation of the disorder, may become one "gold standard" in the field. These endophenotypes can be used as tools to investigate the mechanisms of illness while circumventing some of the limits connected with the inferential nature of behavioral testing (46,72–74).

It is along this line of reasoning that the translation of valid human endophenotypes into animal models can be promising. And even more so, when endophenotypes are of physiological and quantitative nature, rather than behavioral, given the noninferential nature of laboratory measures and their distributional features, which are more likely to approximate normality. However, to ensure that transferability from man to animal is feasible and sound, it is also important that the psychobiological system mapped by the endophenotype has close correspondence in human and the target animal. In other words, according to our proposal, the ideal endophenotype for human-animal transfer should be a physiological, quantitative, and interspecific trait. In addition, because we are interested in models that are relevant to psychopathology and development, the trait should be amenable to experimental designs that address key issues such as: continuity/discontinuity, time stability, and specificity across developmental windows of risk.

Human studies show that SAD predicts heightened risk to develop PD in adulthood (75,76) and both these conditions are associated with heightened sensitivity to CO_2 (44,77,78). In turn, CO_2 hypersensitivity shares common genetic determinants with both SAD and PD, with childhood parental loss adding a significant contribution to explain covariation (44). Because CO_2 sensitivity is an interspecific trait, it can be considered an interesting candidate endophenotype to transfer into an animal model of PD and early parental separation. Some authors studied the impact of maternal separation (3 h/d for 10 consecutive days from postnatal d 1) on respiratory responses in rats. They reported the presence of respiratory alterations to both hypoxic and hypercapnic air mixtures and opposite patterns of ventilatory responses in male and female rats (79,80). Although the fact that both the response to hypoxia and hypercapnia is altered in this paradigm, and that opposite patterns of ventilatory response to hypercapnia have been observed in male and female rats, these initial data have an interest as they suggest that maternal separation *per se* is as an event that may induce respiratory dysregulation. Moreover, they do not address the causes of such effect, which could ultimately lie in genetic control of sensitivity to the environment. Continued improvements in creation of valid animals models will depend on well-defined endophenotypes, lying between gene expression and clinical symptoms, that necessitate increased collaborations between clinical and basic scientists.

In conclusion, conceiving an animal model of a psychiatric disorder requires multiple inputs from multiple fields of expertise. Clinician researchers can help to identify endophenotypes to be translated into animal models. Basic scientists on the other side, are better placed to provide the appropriate means to transfer human data knowledge into a laboratory model. Active and constant collaboration between both sides is likely to constitute the only way toward true progress in the field.

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