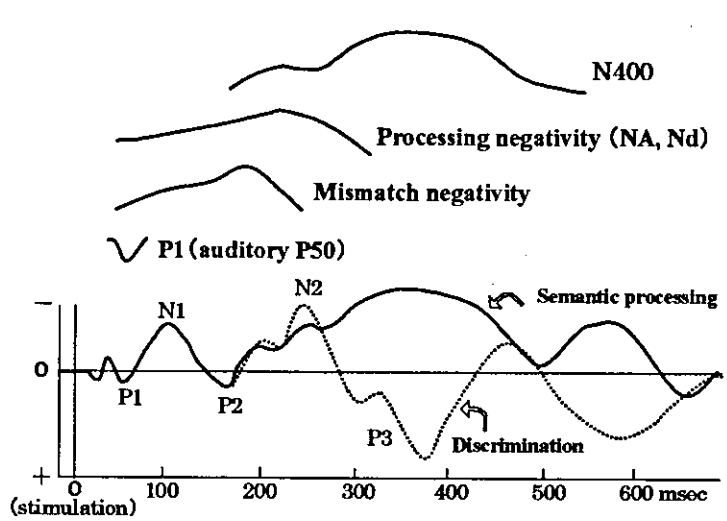


図3　各種事象関連電位の時間関係

最下段の事象関連電位は刺激弁別（discrimination）や意味処理（semantic processing）を行ったさいの原波形で、一般的にはP1、N1、P2、N2、P3で構成されるが、意味処理の場合はさらに陰性側（マイナス方向）に大きくシフトするN400が重畳する。心理課題を工夫したり波形の引算操作などによって、上に示したような特定の認知活動に対応する電位が原波形から単離できる。刺激提示時点が横軸の0ミリセカンドで、こうした事象関連電位を用いてミリセカンド単位で認知活動の時間的変化を解析できる。図は全て模式図。

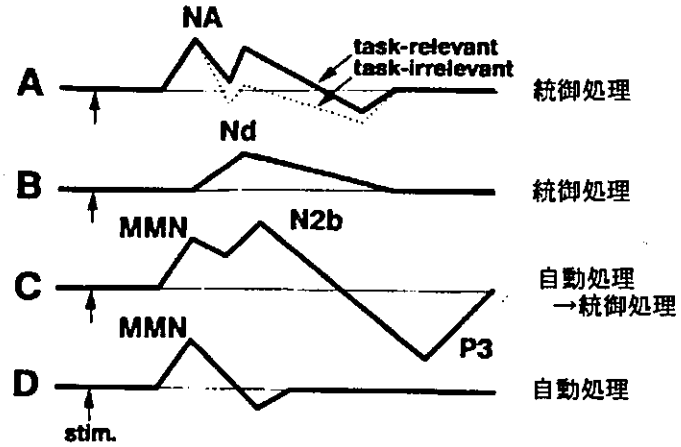
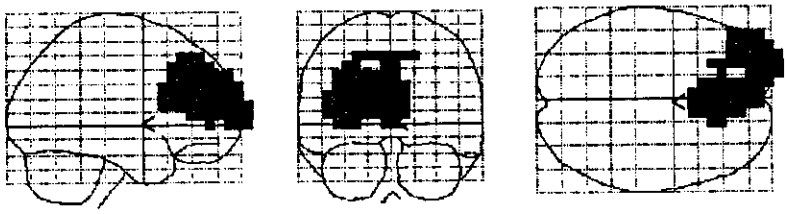


図4　早期の内因性陰性電位の相互関係（松岡[29]より引用）

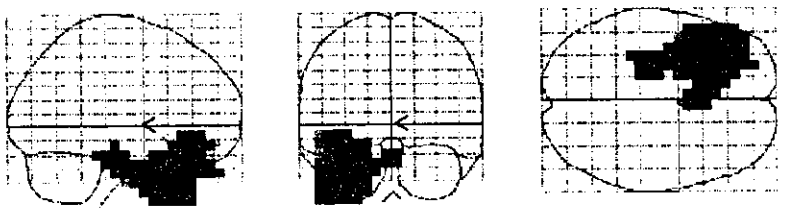
Aは二重選択課題の非標的刺激への反応から単純反応課題での反応を差し引きしたNA電位（実線は課題関連のNA、破線は課題無関連のNA）。Bは同じ二重選択課題の課題関連側非標的刺激への反応から課題無関連側非標的刺激への反応を差し引きしたNdであるが、Aの課題関連側NAから課題無関連側NAを差し引きしたものに等しい。A、Bともに注意依存的な統御処理。Cは同じ二重選択課題の課題関連側の稀な偏倚刺激への反応から標準刺激への反応を差し引きしたもので、自動処理（MMN）で検出された情報が意識化され統御処理（N2b-P3）を受けた場合の電位。Dは無視条件で稀な偏倚刺激への反応から標準刺激への反応を差し引きした電位で，自動処理のMMNのみを認める。上向きを陰性として表示。



voxel-level				x,y,z {mm}		
$p_{corrected}$	T	$(Z_=)$	$p_{uncorrected}$			
0.009	5.05	(3.94)	0.000	-4	28	14
0.039	4.20	(3.46)	0.000	-18	70	14

図5　健常群における N400 反復効果の LORETA 解析

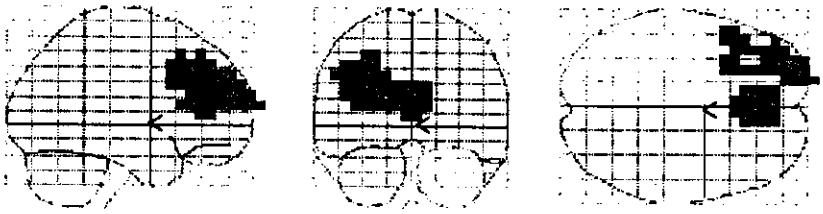
健常群（N=19）を対象に、有意味かな単語が反復して提示されたさいの N400 の変化を SPM99 で解析した。ただし、正確にはノンパラメトリックな方法である SnPM による解析が好ましい[49]。



voxel-level				x,y,z {mm}		
$p_{corrected}$	T	$(Z_=)$	$p_{uncorrected}$			
0.050	4.14	(3.43)	0.000	-18	14	-49
0.093	3.75	(3.18)	0.001	-25	-28	-21

図6　統合失調症群における N400 反復効果の LORETA 解析

統合失調症群（N=19）を対象に、有意味かな単語が反復して提示されたさいの N400 の変化を SPM99 で解析した。ただし、正確にはノンパラメトリックな方法である SnPM による解析が好ましい[49]。



voxel-level				x,y,z {mm}		
$p_{corrected}$	T	$(Z_=)$	$p_{uncorrected}$			
0.033	3.47	(3.20)	0.001	-32	21	42
0.047	3.25	(3.02)	0.001	-25	35	35
0.049	3.22	(3.00)	0.001	-18	63	28
0.037	3.41	(3.15)	0.001	-11	35	14

図7　初回提示単語に対するN400のLORETA解析

図5，図6と同一の対象で、初回提示単語に対するN400を両群間で比較した。解析方法も同一。

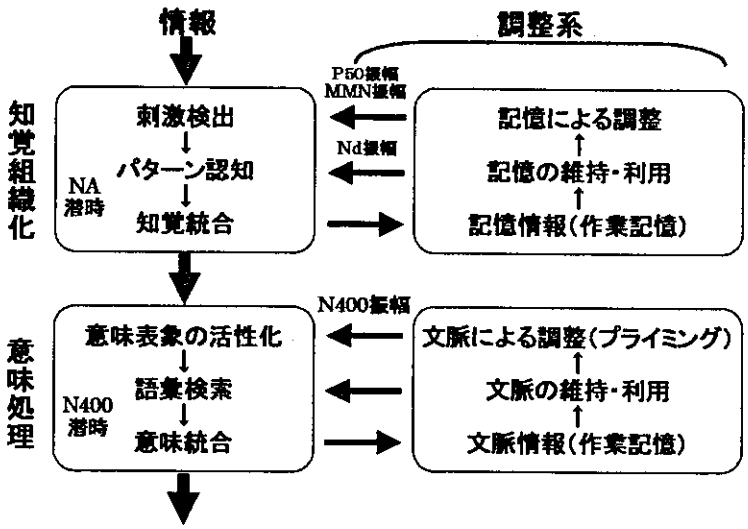


図8　認知活動と事象関連電位（松岡の論文[33]より一部改変）

感覚情報が処理（知覚組織化）され、その情報をもとにして意味処理が行われるさいの認知活動を示した。左列が能動的に行われる統御処理で、右列は統御処理を効率的に行うための調整系である。右列の処理は、不要な情報の遮断、重要な情報の取り込み、先行情報による処理の簡略化などを行っており、基本的には自動処理に相当する。付記されている事象関連電位を用いて、それぞれの活動が評価（ここでは振幅または潜時）される。

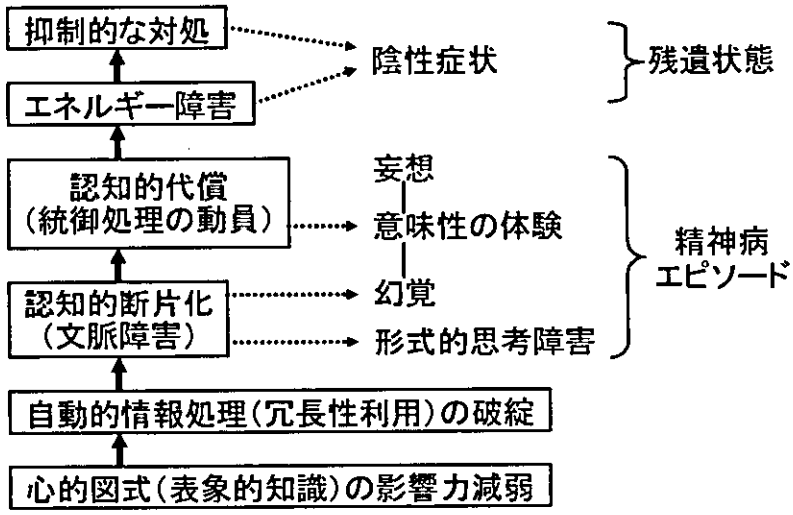


図9　認知障害と統合失調症状の関係

自動処理の破綻により統御処理に過剰な負荷がかかることで精神症状が出現するという仮説[6]

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Review

Experimental gene interaction studies with SERT mutant mice as models for human polygenic and epistatic traits and disorders

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Current evidence indicates that virtually all neuropsychiatric disorders, like many other common medical disorders, are genetically complex, with combined influences from multiple interacting genes, as well as from the environment. However, additive or epistatic gene interactions have proved quite difficult to detect and evaluate in human studies. Mouse phenotypes, including behaviors and drug responses, can provide relevant models for human disorders. Studies of gene–gene interactions in mice could thus help efforts to understand the molecular genetic bases of complex human disorders. The serotonin transporter (SERT, 5-HTT, *SLC6A4*) provides a relevant model for studying such interactions for several reasons: human variants in SERT have been associated with several neuropsychiatric and other medical disorders and quantitative traits; SERT blockers are effective treatments for a number of neuropsychiatric disorders; there is a good initial understanding of the phenotypic features of heterozygous and homozygous SERT knockout mice; and there is an expanding understanding of the interactions between variations in SERT expression and variations in the expression of a number of other genes of interest for neuropsychiatry and neuropharmacology. This paper provides examples of experimentally-obtained interactions between quantitative variations in SERT gene expression and variations in the expression of five other mouse genes: DAT, NET, MAOA, 5-HT$_{1B}$ and BDNF. In humans, all six of these genes possess polymorphisms that have been independently investigated as candidates for neuropsychiatric and other disorders in a total of > 500 reports. In the experimental studies in mice reviewed here, gene–gene interactions resulted in either synergistic, antagonistic (including 'rescue' or 'complementation') or more complex, quantitative alterations. These were identified in comparisons of the behavioral, physiological and neurochemical phenotypes of wildtype mice vs. mice with single allele or single gene targeted disruptions and mice with partial or complete disruptions of multiple genes. Several of the descriptive phenotypes could be best understood on the basis of intermediate, quantitative alterations such as brain serotonin differences. We discuss the ways in which these interactions could provide models for studies of gene–gene interactions in complex human neuropsychiatric and other disorders to which SERT may contribute, including developmental disorders, obesity, polysubstance abuse and others.

Keywords: BDNF, complex genetics, DAT, endophenotypes, knockout mice, MAO, NET, serotonin transporter

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Evidence from twin, adoption and family studies supports strong genetic contributions to most human neuropsychiatric disorders. These genetic contributions presumably arise from variations in the regulation, quantity or quality of the products of the approximately 32 000 human genes.

A small fraction of neuropsychiatric and other brain disorders result from rare single gene abnormalities that produce Mendelian patterns of inheritance in families burdened by

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these disorders. For example, Huntington's disease and the Fragile x mental retardation syndrome are well-known and comprehensively studied examples. Mouse models of the aberrant human gene products, R6/2 huntingtin and FMRP/FXRP, have helped clarify the cellular pathologic consequences of these nucleotide repeat expansion mutations (Bates 2003; Cepeda *et al.* 2003; Kooy 2003).

A far greater burden of neuropsychiatric illness is likely to arise from complex genetic underpinnings. Schizophrenia, affective disorders, substance abuse disorders, anxiety disorders and most other neuropsychiatric disorders, along with common medical disorders such as hypertension, obesity, diabetes and asthma are 'complex disorders' (Plomin *et al.* 1994). A wealth of research indicates that these complex disorders have non-Mendelian, polygenic patterns of inheritance, usually with substantial environmental contributions (Kendler 2001). Specific environmental contributions have been documented for hypertension, obesity and diabetes by demonstrations that the disorders are at least somewhat reversible by environmental changes such as diet and lifestyle.

Mouse models have proved useful in contributing to understanding some complex disorders or traits, including neurological, neuropsychiatric and behavioral disorders as well as other medical disorders (Bucan & Abel 2002; Moore & Nagle 2000; Seong *et al.* 2002; Watase & Zoghbi 2003). As one example, studies in obesity have verified genetic contributions to obesity phenotypes and quantitated the effects of gene–diet interactions on obesity phenotypes. At least five single genes (*fat, agouti, tubby, obese and diabetes*) that result in obesity phenotypes have been well-characterized and an additional group of more than 10 genes as well as about 150 additional loci have been suggested or identified by quantitative trait locus (QTL) studies or by studies of the effects of mutations in metabolic and regulatory pathways (Butler & Cone 2001; Friedman & Leibel 1992; Rankinen *et al.* 2001; Robinson *et al.* 2000). However, even in this relatively straightforward quantitative phenotype, the mechanisms that lead from single gene changes, through intermediate processes including behavior and metabolic processes, to ultimate body weight change are understood in only a few cases (Robinson *et al.* 2000). It does seem clear that at least some of the gene alterations that lead to body weight phenotypes are mediated by behavioral changes that include underactivity and increased food intake.

Mouse models have been increasingly explored as ways to dissect the complexity in other multigene disorders. Genome-wide analyses of a behavioral circling phenotype in F2 generation C57L/J × SWR/J mice revealed epistatic interactions between a single required, recessive gene on the SWR/J chromosome 14 and products of three modifying, non-recessive C57L/J genes located on chromosomes 3, 4 and 13 (Cryns *et al.* 2002). Other examples of traits which depend on multiple loci have been identified in mouse genetic studies of hypertension (Ohno *et al.* 2000), pentobarbital withdrawal seizures (Hood *et al.* 2001) and diabetes (Terauchi *et al.* 1997).

The present review describes another approach to using mouse models to study the interactive effects of variation at two or more known gene loci as a means to uncover epistatic mechanisms underlying complex brain disorders and traits. These studies are possible in the case of murine monoamine transporter and related genes because phenotypic effects of targeted disruptions of these genes have been characterized (Bengel *et al.* 1998; Cases *et al.* 1996; Ernfors *et al.* 1994; Saudou *et al.* 1994; Sora *et al.* 1998; Xu *et al.* 2000). Availability of these single gene knockout mice has now allowed us to explore gene–gene interactions by interbreeding these mice to produce animals with alterations in two or more genes. In the present review, we focus on multigene variants that have been evaluated for interactions with the serotonin transporter (SERT, *SLC6A4*) gene (Hall *et al.* 2002; Murphy *et al.* 2001; R. Ren-Patterson, L.W. Cochran, A. Holmes, S. Sherrill, S.-J. Huang, T. Tolliver, K.-P. Lesch, B. Lu & D.L. Murphy submitted; Salichon *et al.* 2001; Sora *et al.* 2001).

SERT is an especially interesting gene for consideration here in regard to murine models relevant to neuropsychiatric disorders. Differences in the expression and function of the serotonin transporter affect many human and mouse quantitative traits, including aggression, anxiety- and depression-related behaviors, locomotor activity, sleep and circadian behaviors, gut function, body weight, nociceptive responses and preferences for drugs like cocaine and alcohol (Bengel *et al.* 1998; Chen *et al.* 2001; Fabre *et al.* 2000; Gobbi *et al.* 2001; Holmes *et al.* 2002a; Holmes *et al.* 2002b; Li *et al.* 1999; Li *et al.* 2000; Li *et al.* 2003; Liu *et al.* 2002; Montanez *et al.* 2003; Murphy *et al.* 1999; Murphy *et al.* 2001; Persico *et al.* 2001; Persico *et al.* 2003; Ravary *et al.* 2001; Rioux *et al.* 1999; Salichon *et al.* 2001; Schmitt *et al.* 2003; Sora *et al.* 1998; Sora *et al.* 2001; Vogel *et al.* 2003; Wisor *et al.* 2003). Selective SERT antagonists, the serotonin-selective reuptake inhibitors (SRIs), are the most-prescribed drugs for treatment of behavioral disorders. Human SERT variants have been identified, and variants at this locus have been linked or associated with bipolar disorder, unipolar depression, suicide and obsessive-compulsive disorder (Anguelova *et al.* 2003; Bengel *et al.* 1999; McDougle *et al.* 1998; Murphy *et al.* 2000; Mynett-Johnson *et al.* 2000). Recently, in a demonstration of a major SERT gene–environment interaction, depression symptoms and diagnoses were found to occur more commonly in individuals with more life stresses who possessed the lower-expressing shorter (s) SERT 5′-flanking region length variant than those who possessed the longer (l) SERT variant (Caspi *et al.* 2003). The anxiety-related personality trait; neuroticism, which is also a risk factor for depression, was more prevalent in individuals with *s* variant in several large *n*, sibling-based studies (Greenberg *et al.* 2000; Lesch *et al.* 1996; Mazzanti *et al.* 1998).

However, small *n* studies have not yielded strong associations and the positive studies do not indicate that SERT contributes all of the genetic influences to any of the traits or disorders in which its variants may play roles (Anguelova

et al. 2003). Thus, variants in other genes could provide independent, additive and interactive contributions. A specific estimate of 8–10 other genes was made for one of these findings based on effect size of the SERT contribution to the overall genetic component of the total variance (Lesch *et al.* 1996).

We believe that it would be highly desirable to develop models in which to study such interactions between SERT variation and variation at other loci. The question of how SERT might interact with other genes in mice to yield altered phenotypes is the primary focus of this paper. However, since there are few studies of gene–gene interaction effects on behavioral phenotypes, we believe that this work provides a model for studies of gene–gene interactions in general. We thus highlight data from several types of studies that document several different kinds of gene–gene interactions.

Neurochemical changes

Epistatic interactions: brain serotonin quantitative phenotypes in SERT× DAT double mutant mice

Mice were the offspring of matings of heterozygote mice with targeted disruptions of the serotonin transporter and the dopamine transporter produced by homologous recombination in 129SvEv ES cells (Bengel *et al.* 1998; Sora *et al.* 1998). Blastocysts were obtained from C57BL/6J mice, the resulting chimeric mice were mated with C57BL/6J mice (Bengel *et al.* 1998; Sora *et al.* 1998) and backcrossing with C57BL/6J wildtype mice provided F3 – F5 DAT/SERT combined knockout mice used in these studies (Sora *et al.* 2001).

While each single knockout produced a constellation of effects on brain levels of dopamine, serotonin and norepinephrine, SERT × DAT combined knockout mice produced two interesting differences beyond those previously observed in SERT or DAT single mutant mice. First, DAT -/- × SERT +/– mice had one-third less serotonin than SERT +/– mice with wildtype, DAT +/+ alleles (Fig. 1) (Sora *et al.* 2001). Immunocytochemical studies provide evidence that

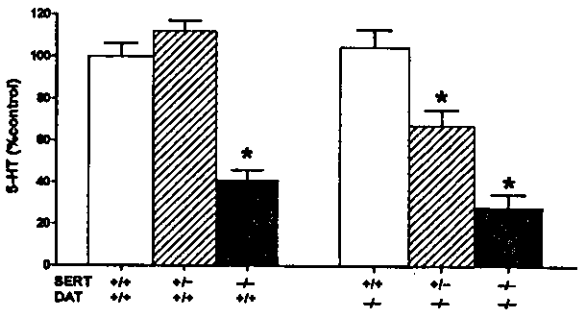

Figure 1: Striatal serotonin (5-HT) concentrations in SERT×DAT double mutant mice. Adapted graphically from data previously tabulated in Sora *et al.* (2001). *$P < 0.05$.

in SERT +/– and -/- mutant mice, but not in wildtype controls, serotonin was accumulated by dopaminergic neurons via the dopamine transporter (DAT) (Zhou *et al.* 2002). This serotonin accumulation was blocked by the selective DAT antagonist GBR12905 (Zhou *et al.* 2002). This data, and data from striatal microdialysis studies of combined DAT/SERT knockout mice (H. Shen, Y. Hagino, H. Kobayashi, K. Shinohara-Tanaka, K. Ikeda, H. Yamamoto, T. Yamamoto, K.-P. Lesch, D.L. Murphy, F.S. Hall, G.R. Uhl, I. Sora unpublished observations) are both consistent with the conclusion that heterozygote SERT +/– × DAT -/- mice are unable to maintain normal striatal tissue serotonin dynamics. Absence of such changes in DAT-/- mice with wildtype SERT +/+ alleles supports the idea that DAT uptake of serotonin provides not a normal, but a fall-back transporter function capable of substituting for the partial loss of SERT in the striatum of the heterozygote mice. These ideas fit with the lesser DAT affinity for serotonin, with evidence from studies of gastrointestinal function in SERT -/- mice (Chen *et al.* 2001) and with the results from investigations of cultured primary neurons from SERT -/- mice (Pan *et al.* 2001) indicating compensation by DAT. In addition, there are suggestions of complementary data from the hypothalamic regions of heterozygote DAT +/– × SERT -/- mice: regional dopamine concentrations are reduced to 60% of wildtype levels in these mice, reductions not found in DAT +/– × SERT +/+ mice (Sora *et al.* 2001)

Additive interactions: brain serotonin and 5-HIAA quantitative phenotypes in SERT× Brain-Derived Neurotropic Factor (BDNF) double mutant mice

Mice were offspring of matings of SERT mutant mice produced as noted above with BDNF mutant mice on C57Bl/6 × CD–1 backgrounds (Ernfors *et al.* 1994; Pozzo-Miller *et al.* 1999; Ren-Patterson *et al.* unpublished).

SERT -/- × BDNF +/+ mice exhibited 60–80% reductions in brain serotonin concentrations in striatum, hippocampus, hypothalamus and brain stem, i.e., the same amounts previously documented in comparisons of SERT -/- to wildtype littermate mice (Bengel *et al.* 1998; Ren-Patterson *et al.* unpublished). Heterozygous, BDNF +/– mice, as expected, had reductions of about 50% in BDNF mRNA in hippocampus, as previously found in whole brain or specific brain regions (Kernie *et al.* 2000; Korte *et al.* 1995; Lyons *et al.* 1999; MacQueen *et al.* 2001).

Neither SERT +/+ × BDNF -/- nor SERT -/- × BDNF -/- mice survive to adulthood. SERT -/- × BDNF +/– mice, which do survive, display greater serotonin reductions in each brain region investigated than those found in SERT -/- × BDNF +/+ mice (Fig. 2). Thus, while both SERT and BDNF are well known to have pleiotropic effects, these additive effects of genetic changes, most likely during development, are logically predicted from previous neurobiological observations. *In vitro* applications of BDNF enhance the differentiation of serotonergic phenotypes in neuronal cell cultures (Eaton *et al.* 1995; Galter & Unsicker 2000), while *in vivo* BDNF administration can increase serotonin

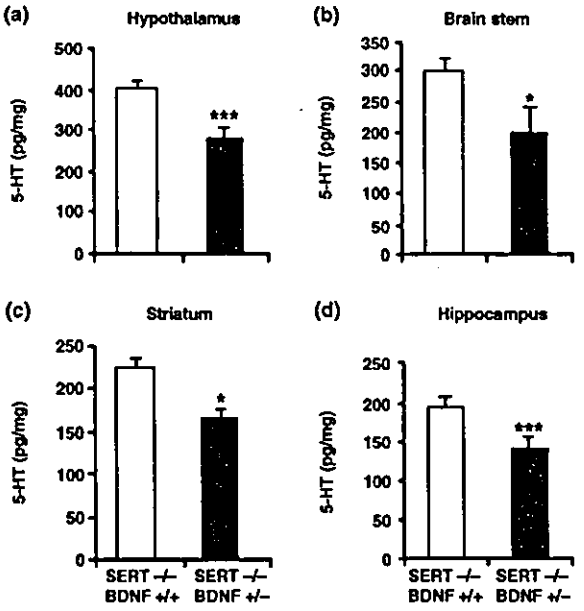

Figure 2: Brain serotonin concentrations in SERT×BDNF double mutant mice (Ren-Patterson *et al.* unpublished). $^{*}P<0.05$, $^{***}P<0.01$.

axon densities and brain serotonin concentrations (Mamounas *et al.* 1995; Pelleymounter *et al.* 1995; Siuciak *et al.* 1996). Partial reductions in the availability of BDNF in the SERT -/- × BDNF +/- mice could thus reduce the usual trophic consequences of BDNF upon the development of the entire brain serotonergic system, including SERT function and serotonin content.

Such differences in BDNF and serotonin may contribute to behavioral phenotypes. BDNF +/- mice display reduced cocaine place preference reward behavior and reduced locomotor activity responses to cocaine, two responses that could be mediated by deficits in serotonin and/or dopamine function (see below) (Dluzen *et al.* 1999; Dluzen *et al.* 2001; Hall *et al.* 2003b). Human variants at both the SERT and BDNF gene loci have been implicated in affective disorders, obsessive-compulsive disorder and polysubstance abuse liability (Caspi *et al.* 2003; Hall *et al.* 2003a; Neves-Pereira *et al.* 2002; Ozaki *et al.* in press; Sklar *et al.* 2002; Uhl *et al.* 2001). Interactions between these influences, possibly even additive interactions, could now be hypothesized and tested in human studies of locus–locus interactions.

Complementation, reversal and rescue: neurochemical and anatomical phenotypes in SERT×MAO and SERT×MAO×5-HT$_{1B}$ multiple mutant mice

Mice were generated by mating the previously described SERT mutant mice on the C57Bl/6J background (Bengel *et al.* 1998) which had been backcrossed on a C3H/HeJ background for four generations with mice with a targeted disruption of the monoamine oxidase type A gene (MAOA), which were on a mixed C3H/HeJ, 129/SvPas and C57Bl/6ByJ background. These mice were subsequently mated to mice with a targeted disruption of the serotonin 1B (5-HT$_{1B}$) receptor gene which were on a 129/Sv background and then backcrossed for 10 generations onto a C3H/He background. Subsequent matings yielded all genotypes (Salichon *et al.* 2001).

Neurochemistry

Loss of a major route of 5-HT metabolism in mice lacking MAOA led to marked, sixfold increases in brain serotonin content (Fig. 3a). These changes were partially reversed, complemented, or rescued in double mutant mice lacking both MAOA and SERT, which displayed only twofold differences from wildtype values (Salichon *et al.* 2001). Levels of the major serotonin metabolite, 5-hydroxyindolacetic acid (5-HIAA), exhibited a different pattern. 5-HIAA levels were reduced 90% in mice lacking MAOA, and were further reduced in the SERT × MAOA double mutant mice (Salichon *et al.* 2001) (Fig. 3b). Thus, ratios between 5-HT and 5-HIAA were markedly altered in mice lacking MAOA (55 vs. 1.2 in controls). This 5-HT:5-HIAA ratio was unchanged (54) in the SERT × MAOA double mutant mice. High 5-HT:5-HIAA ratios (>10) can also be produced by pharmacologic blockade of MAOA (Felner & Waldmeier 1979). Neither 5-HT, 5-HIAA nor the 5-HT:5-HIAA ratio displayed differences between MAO × 5-HT$_{1B}$ double mutant mice vs. single MAOA knockout mice (Fig. 3c,d). Further studies of these multiple-mutant mice are needed, especially of brain extracellular 5-HT concentrations, as both diminished or

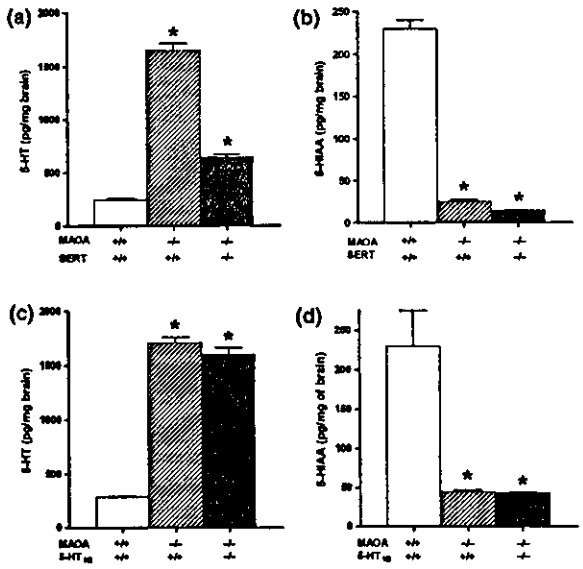

Figure 3: Brain 5-HT concentrations in SERT×MAO×5-HT$_{1B}$ mutant mice. Adapted from Salichon *et al.* (2001). $^{*}P<0.05$.

absent SERT or absent MAOA alone lead to elevated extracellular brain 5-HT and, in the case of SERT mutant mice, diminished 5-HT clearance. However, similar extracellular 5-HT data are not yet available in these double and triple mutant mice (Evrard *et al.* 2002; Mathews *et al.* 2000; Montanez *et al.* 2003).

Neuroanatomy

These multiple mutations also exert influences on the neuroanatomic development of cerebral cortical somatosensory and visual pathways (Cases *et al.* 1996; Persico *et al.* 2001; Rhoades *et al.* 1994; Upton *et al.* 1999; Upton *et al.* 2002; Vitalis *et al.* 1998). There is a striking loss of the organization of cortical barrel fields in either single mutant MAOA or single mutant SERT mice (Cases *et al.* 1996; Persico *et al.* 2001). These abnormalities in somatosensory cortical barrel fields were also observed in SERT x MAOA double mutant mice (Salichon *et al.* 2001). However, they were essentially completely rescued or complemented by deletion of the 5-HT_{1B} receptor in SERT x MAOA x 5-HT_{1B} mutant mice (Salichon *et al.* 2001) (see also Box 1). A similar rescue occurs for DAT knockout mice whereby 5-HT_{1B} +/−, but not 5-HT -/-, normalizes DAT knockout induced hyperactivity and restores cocaine-induced locomotion (F.S. Hall, X.-F. Li, S. Axelrad, I.H. Hoggatt, M. Goeb, I. Sora, R. Hen & G.R. Uhl submitted).

Such genetic evidence of complementation in this triple mutant is consistent with pharmacologic evidence using a 5-HT_{1B} agonist, indicating that it is indeed excess 5-HT acting on 5-HT_{1B} receptors that is responsible for this specific cytoarchitectonic barrelfield abnormality in SERT -/- and MAOA -/- mice (Rhoades *et al.* 1994; Salichon *et al.* 2001). When these findings are considered together, these lines of evidence support a logical and predictable relationship between genetic complementation and known biochemical pathways.

MAOA disruption in humans due to either a point mutation or a chromosomal microdeletion produces changes in monoamines and in 5-HIAA similar to those found in MAOA knockout mice and is associated with mental retardation, aggression and other behavioral abnormalities (Brunner *et al.* 1993; Collins *et al.* 1992; Lenders *et al.* 1998; Murphy *et al.* 1990; Sims *et al.* 1989). Of note is the hMAOA point mutation, although it is associated with a striking behavioral phenotype, has only been observed in one extended family, although it was reported a decade ago (Brunner *et al.* 1993). More subtle changes are apparently produced by the more common, functional promoter region polymorphisms present in human MAOA and SERT genes that have both been associated with neuropsychiatric disorders, personality traits and endocrine response measures (Caspi *et al.* 2002; Caspi *et al.* 2003; Deckert *et al.* 1999; Hahn & Blakely 2002; Lesch *et al.* 1996; Manuck *et al.* 2000; Samochowiec *et al.* 1999). The current murine findings may predict interactions in humans between these gene variants when two-locus results are examined.

Physiological changes

Complementation and additive interactions: body weight changes in SERT× DAT and SERT× BDNF double mutant mice

DAT knockout mice are dwarfs, with marked growth retardation and pituitary and other endocrine abnormalities (Bosse *et al.* 1997; Giros *et al.* 1996). Initial observations suggested body weight increases in both SERT and BDNF mutant mice (Lyons *et al.* 1999; Rios *et al.* 2001; Sora *et al.* 1998). SERT deletion appears to complement the reduced body weights found in DAT knockout mice. At eight weeks of age, SERT -/- x DAT -/- mice weighed significantly more than SERT +/+ x DAT -/- mice (Sora *et al.* 2001). By 10–12 weeks of age, there is essentially complete normalization of the reduced body weight found in DAT -/- mice in the double mutant SERT -/- x DAT -/- mice (Fig. 4).

By contrast, effects of BDNF and SERT knockout appear to be more additive in nature. Neither deletion of one BDNF allele nor two SERT alleles led to any changes in body weight in SERT x BDNF mice studied at three months of age. However, the double mutant SERT -/- x BDNF +/− mice exhibited additive or supra-additive increases in body weight (Fig. 5) (Ren-Patterson *et al.* unpublished). Of interest, SERT-/- mice develop significant obesity later in life (A. Holmes, *et al.* in preparation), and thus the effect of BDNF here could also be interpreted as a change in the age of onset of this disorder.

Since pituitary and hypothalamic changes were suggested as mechanisms whereby DAT and BDNF knockouts alter weight, endocrinologic studies provide one route to understanding the obesity in these mutant mice (Nakagawa *et al.* 2002; Ren-Patterson *et al.* unpublished; Rios *et al.* 2001). Administration of BDNF, serotonin receptor agonists and serotonin releasing drugs each have pharmacologic antiobesity effects in mice with normal hypothalami (Halpern &

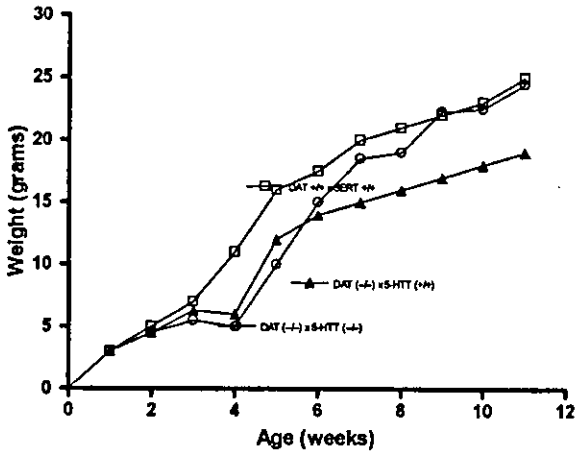

Figure 4: Body weight in SERT x DAT double mutant mice.

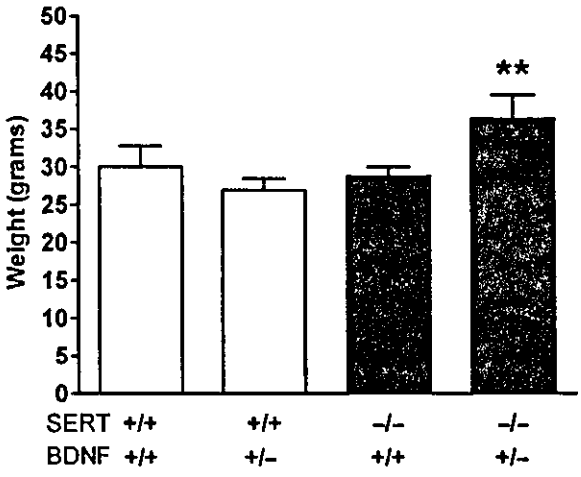

Figure 5: Body weight in SERT×BDNF double mutant mice. $^*P < 0.05$.

Mancini 2003; Heisler *et al.* 2002; Nakagawa *et al.* 2002; Nakagawa *et al.* 2003). Further, human DAT VNTR polymorphisms that provide markers for 3′ variation in this gene have been linked to a fivefold elevation in the likelihood of obesity (Epstein *et al.* 2002; Mill *et al.* 2002), although no such association has been made with the human SERT promoter region polymorphism and body weight (Hinney *et al.* 1997). Studies of interactions between a broader range of DAT, SERT and BDNF variants and weights in humans would be of obvious interest.

Behavioral changes

Epistatic interactions: reduced cocaine conditioned place preference (CPP) in SERT× DAT double mutant mice

Conditioned place preference behavior provides an index of the rewarding properties of cocaine and was studied in the same groups of SERT × DAT mice described above in some of the first behavioral studies of multiple gene knockout mice (Sora *et al.* 2001). The studies of multigene knockout mice followed those of single gene knockout mice, none of which demonstrated any reduction in cocaine place preference. Loss of either one or two DAT alleles in DAT -/- or DAT +/– mice did not lead to any change in cocaine CPP (Fig. 6) (Sora *et al.* 1998). By contrast, loss of both SERT alleles in SERT -/- mice led to enhanced cocaine CPP (Sora *et al.* 2001), an effect that was almost identical to that originally observed in SERT +/– and -/- mice (Sora *et al.* 1998) (Fig. 6).

These results from multigene knockout mice were remarkably different from data from single-gene knockout mice. The most striking changes in cocaine CPP were observed in mice lacking DAT and either one or two SERT alleles (Fig. 6c,d). Neither DAT -/- × SERT +/– nor DAT -/- × SERT -/- mice demonstrated any

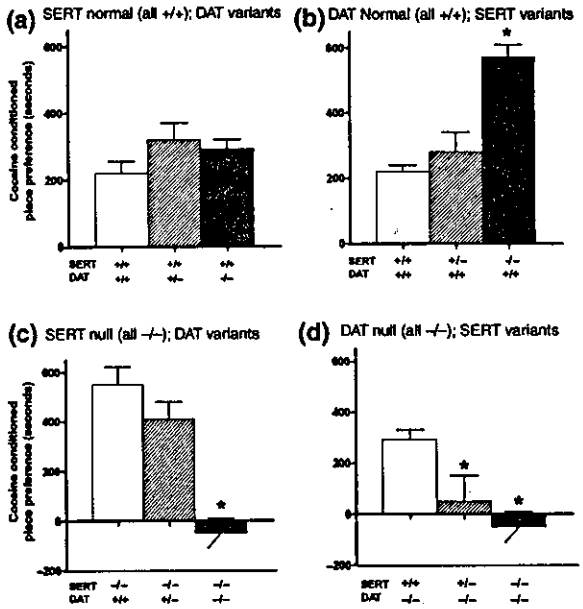

Figure 6: Cocaine conditioned place preference behavior in SERT×DAT double mutant mice. Adapted from Sora *et al.* (2001). $^*P < 0.05$.

significant preference for places where they had previously received cocaine. Demonstrating that this was not a simple quantitative change in drug responsiveness, doubling the dose of cocaine also yielded absence of cocaine CPP in these mice.

This genetic evidence for epistatic influences of SERT knockouts on the results of DAT knockouts, and vice-versa, provided the first substantive evidence that two of cocaine's actions, blockade of dopamine transporters and blockade of serotonin transporters, were both essential for its full spectrum of rewarding properties, as least those measured by the CPP assay. To our knowledge, this observation remains one of the strongest demonstrations of an epistatic effect in behavioral genetics or neuropharmacology.

Additive (supra-additive) interactions: enhanced cocaine CPP in SERT× NET double mutant mice

Norepinephrine transporter (NET) knockout mice displayed no reductions in cocaine CPP, and in fact had modestly enhanced place preference (Xu *et al.* 2000). SERT -/- × NET -/- double knockout mice displayed a striking enhancement of cocaine CPP (Fig. 7c). One suggestion for this observation is that cocaine, which binds to NET as well as SERT and DAT, possesses not only euphoriant and rewarding effects but also aversive effects (Miller *et al.* 2001; Uhl *et al.* 2002). If these latter effects were mediated in part by cocaine's actions at NET and SERT, deletion of NET and SERT might thus lead to greater reward-related behavior than those found in SERT -/- mice alone (Hall *et al.* 2002; Sora *et al.*

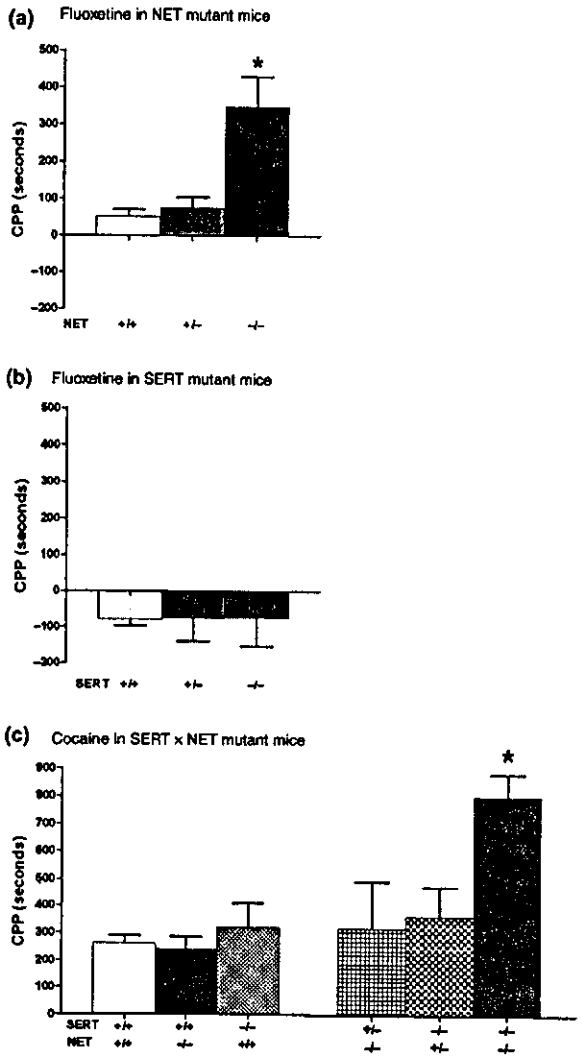

Figure 7: Conditioned place preference in NET, SERT and SERT×NET mutant mice. Adapted from Hall *et al.* (2002). $^{*}P < 0.05$.

1998). These ideas are consistent with our observations that the SERT inhibitor fluoxetine becomes rewarding in CPP studies in NET mutant mice (Fig. 7a) (Hall *et al.* 2002). These observations again suggest interactions that can be sought in human studies of the influences of DAT, NET and SERT genes on psychostimulant reward in humans, since human NET, like DAT and SERT, has multiple polymorphisms (Hahn & Blakely 2002).

Interpretation, perspectives and conclusions

Several different types of interactions were observed between the SERT mutation and mutations in two other members of the monoamine transporter family (DAT, NET) and in other genes involved in serotonin catabolism (MAOA), serotonin signaling (5-HT_{1B}) and serotonergic system development (BDNF). It seems striking to us that in studies of interactions between genetic lesions in just six genes, we have been able to clearly document many of the possible kinds of gene–gene interactions. Of course, these observations were made using mutants that were likely to provide interactions based on prior neurochemical and behavioral observations. Furthermore, they were made in mutant mice in which we have exquisite control over environmental variables. Nevertheless, the magnitude of the interactions observed, the different levels of analysis at which they have been observed and the relatively great frequency with which we have identified them point to the possibilities that these mouse model system interactions could inform human studies and contribute to the examination of a number of aspects of phenotypes that might enhance sensitivity for detecting such gene–gene interactions in human genetic investigations.

Several of the findings illustrated in SERT double mutant mice provide examples of epistasis as originally described and named by Bateson and Punnett in their study of the inheritance of chicken combs (Bateson *et al.* 1905). The rare occurrence of one type of comb, which did not fit with simple Mendelian theory, was best explained by a two-factor solution in which the rare phenotype only occurred in double homozygotes resulting from two genes segregating in a Mendelian fashion (Bateson *et al.* 1905; Bateson 1909; Phillips & Johnson 1998). Among the gene interactions in mice reviewed here, new phenotypes occurred in SERT -/- × DAT -/-, SERT +/+ × DAT -/-, SERT -/- × MAOA -/- × 5-HT_{1B} -/- mice and MAOA -/- × 5-HT_{1B} -/- mice. Non-additive gene interactions leading to quantitative phenotypes were originally termed 'epistacy' (Fisher 1918). Concepts of 'physiological epistasis' (Cheverud & Routman 1995) as differentiated from statistical epistasis (Cordell 2002) have been based on 'building block' descriptions of phenotypes that have allowed delineation of suppressor and compensatory mutations and genetic complementation (Cheverud & Routman 1995; Fisher 1918; Long *et al.* 1995; Nadeau 2001; Phillips 1996; Phillips & Johnson 1998; Stephan 1996; Tachida & Cockerham 1989; Wagner *et al.* 1998). Indeed, interactions described in this paper might be termed experimental epistasis via targeted gene mutagenesis in mice; these represent a novel approach to modeling gene × gene consequences that affect behavior.

One consideration regarding the examples reviewed here is that mice of different background strains were utilized in these studies. Thus, additional gene effects beyond the two or three of those directly manipulated as part of these experiments might be present as a consequence of possible strain-related genetic background contributions. For example, modest strain differences in brain serotonin content have been reported (Daszuta & Barrit 1982). However, the serotonin changes described here in the double and triple mutant

Box 1: An example of genetic heterogeneity and experimental complementation: gene deletion effects on somatosensory cortical development.

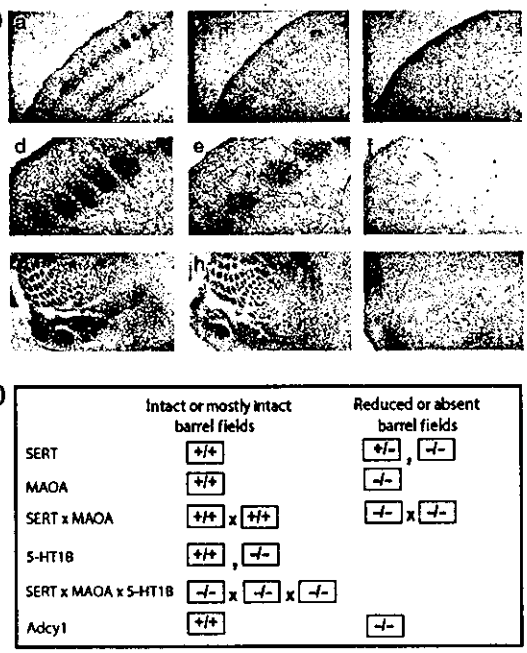

Figure 8: (a) Absence of somatosensory cortex layer 4 barrels in SERT -/- mice and intermediate phenotype (reduced barrel definitions) in SERT +/– mice (Persico *et al.* 2001). (b) Additional gene deletions besides SERT that produce essentially identical (e.g. MAOA) or highly similar (Adcy1) phenotypes.

The genetic heterogeneity evident in Fig. 8(b) indicates that any one of three different genes can produce this very distinctive cytoarchitectonic cortical phenotype, some depending on excess serotonin availability during development (SERT, MAOA), others depending on alterations in a cellular second messenger system (Adcy1) or on expression of a phosphoprotein, Gap-43 (Abdel-Majid *et al.* 1998; Alvarez *et al.* 2002; Lu *et al.* 2003; Maier *et al.* 1999; Salichon *et al.* 2001). Complementation is also notable in that the 5-HT_{1B} gene is a highly potent modifier of the effects of SERT deletion, MAOA deletion and the SERT–MAOA interaction.

The gene dose relationships indicated in these mice are also interesting. Indeed, the phenotypes in SERT +/– mice provide the closest parallels to the common human 5′ SERT polymorphism consisting of short or long length variants (Lesch *et al.* 1996).These observations might have additional human counterparts. Absence of barrels in SERT knockout mice leads to marked reductions in cerebral cortical and thalamic glucose utilization (Esaki *et al.* 2002). Conjectural but potential human alterations in glucose utilization in cortex and subcortical regions might be observed as 'lesions' or anomalies in fluorodeoxyglucose PET scans in association with one or several neuropsychiatric disorders in which SERT, BDNF and/or DAT polymorphism findings have been reported including OCD, autism, ADHD, depression, bipolar disorder, schizophrenia and polysubstance abuse (Anguelova *et al.* 2003; Hall *et al.* 2003a; Hahn & Blakely 2002; Murphy *et al.* 2000; Murphy *et al.* 2001; Mynett-Johnson *et al.* 2000; Sklar *et al.* 2002; Uhl *et al.* 2001). The effects of SERT haplotypes in 5′ flanking regions could also be exacerbated by rarer haplotypes that also include missense variants in SERT protein coding sequences that in one instance of specific allelic heterogeneity appear to be associated with more severe clinical abnormalities that include OCD (Kilic *et al.* 2003; Ozaki *et al.* in press). Similar speculative scenarios might be constructed for other phenotypes affected by SERT and other interacting genes. One caveat that must be considered is that in some of these instances with SERT, and in other examples with different genes, evident phenotypes may be present only in homozygous mice or individuals. However, +/– mice have often not been fully investigated, with many comparisons restricted to -/- vs. +/+ mice only, and thus more data documenting findings in various heterozygous mice are still needed.

mice considerably exceed those previously reported to occur between any of the specific background strains used in these studies. In addition, most of these results were obtained using sufficient within-strain, multiple genotype comparisons. Thus, the major qualitative conclusions described here seem to be on firm ground.

There are additional examples of epistasis in mouse models. These include demonstration of tumor development attributable to digenic interactions of Scc4 and Scc5 in the absence of a main effect of either single locus (Frankel & Schork 1996; van Wezel *et al.* 1996). There is also evidence from neuropathologic and cytochemical studies indicating that the locomotor consequences of the *Lurcher* and *Staggerer* mutations occur in a time-dependent hierarchy in double mutant mice (Messer *et al.* 1991). Another example is the interactions among APP, presenilin 1, apolipoprotein E4 and tau overexpression in modeling Alzheimer's disease neuropathological and/or behavioral phenotypes (Dewachter *et al.* 2001). Polygenic effects on *Drosophila* behavior can demonstrate epistasis under environmental stress (Blows & Sokolowski 1995).

There are other examples of non-epistatic interactions, including additive effects and complementation. More severe

Table 1: Some additional examples of multigenic interactions affecting behaviors which have been investigated in mice

Phenotype	Mutant Mice	Outcome		
Male sexual behavior	Estrogen receptors			
(Ogawa et al. 2000)	α	Two of three behaviors intact		
	β	All three behaviors intact		
	α × β	Disrupted (all three behaviors absent)		
Circadian locomotor	Period genes			
activity rhythms	Per 1	Disrupted		
(Bae et al. 2001)	Per 2	Disrupted		
	Per 3	Minimal change		
	Per 2 × Per 3	Disrupted		
	Per 1 × Per 2	Severely, immediately disrupted		
Motor skills	Kv 3.1	Moderate, genetic background-dependent deficits		
(Espinosa et al. 2001)	Kv 3.3	No deficit		
	Kv 3.1 × Kv 3.3	Ataxia, tremor, myoclonus, hyperactivity, increased alcohol sensitivity with intermediate phenotype in heterozygotes		
Corticosterone, stress responses and	CRF receptors	Basal corticosterone	Stress responses	Anxiety-like behavior
anxiety-like behaviors (Bale et al., 2002)	CRF 1	(1)	(1)	(1)
	CRF 2	-	(2)	(2)
	CRF 1 × CRF 2	(1)	(1)	(1)

phenotypes emerge in double mutant mice involving genes coding for estrogen α and β receptors, Per 1 × Per 2 and two potassium channels, kv3.1 and kv3.3 than in single mutant mice (Table 1). Other kinds of interactions not described as clearly in SERT mutant mice can also occur. Dominant effects of one mutation over another have been identified. For example, in dopamine receptor mutant mice, DRD1 or DRD2 exploratory and locomotor behavior phenotypes predominated over DRD3-associated behaviors in DRD1 × DRD3 or DRD2 × DRD3 double mutant mice (Vallone *et al.* 2002; Wong *et al.* 2003). The CRF1 phenotype predominated in CRF1 × CRF2 mice (Bale *et al.* 2001) (Table 1).

The levels of analysis at which these interactions have been observed are also worth noting. Our observations of apparent epistatic interactions between DAT and SERT knockouts in cocaine CPP provided one of the initial behaviorally-striking epistatic interactions. However, other gene–gene interactions were identified only in studies that examined neurochemical, physiological or anatomical parameters. These observations point to the desirability of characterizing knockout animals at a number of levels of analysis. This is similar to notions of approaching neuropsychiatric disorders and other complex human disorders with a focus not only on diagnosis but also on intermediate traits or endophenotypes, some of which might directly overlap with those found in murine models, as is conceptually depicted in Fig. 9 (Murphy *et al.* 2001; Seong *et al.* 2002).

These murine results illustrate the likely necessity of studies of gene–gene interactions for understanding human complex traits. The relatively large number of epistatic and supra-additive gene–gene interactions documented here provides clear experimental evidence of the likelihood that the behavioral impact of human variation at many single loci may often be best understood in the context of information about the state of variation at other loci. Linkage and association genome scanning studies might be able to detect individual loci involved in instances of supra-additive interactions, in ways that would allow subsequent studies of the effects of locus–locus interactions. However, it appears quite possible that many loci whose behavioral influences were entirely epistatic might be missed completely by human genome scanning efforts, if even powerful effects of variation at a single locus required the presence of specific variations at other loci, unless the nature of the gene–gene interactions was understood.

These murine results thus also illustrate the challenges that face human studies of gene–gene interactions in complex traits. Seeking each of the possible interactions between human allelic variants produces a huge statistical challenge which only recently has begun to be addressed (Dupuis *et al.* 1995; Hoh & Ott 2003). Adding the imperative that a number of intermediate phenotypes should also be examined (Fig. 9) expands the challenge further. Under these circumstances, in the absence of a focusing *a priori* hypothesis, it is highly likely that most of the interactions that appear to be nominally statistically significant in human studies of any reasonable sample size may represent false-positive results of the many statistical comparisons that would be required (Hoh & Ott 2003).

The mouse genome provides a good reflection of the gene structure found in humans, although the mouse strain-to-strain variants that have been most studied in mouse genetics do not. Engineering variants at single loci in knockout or transgenic mice may, however, provide better models for human variation. In particular, comparative studies of heterozygous and homozygous knockout mice provide models for

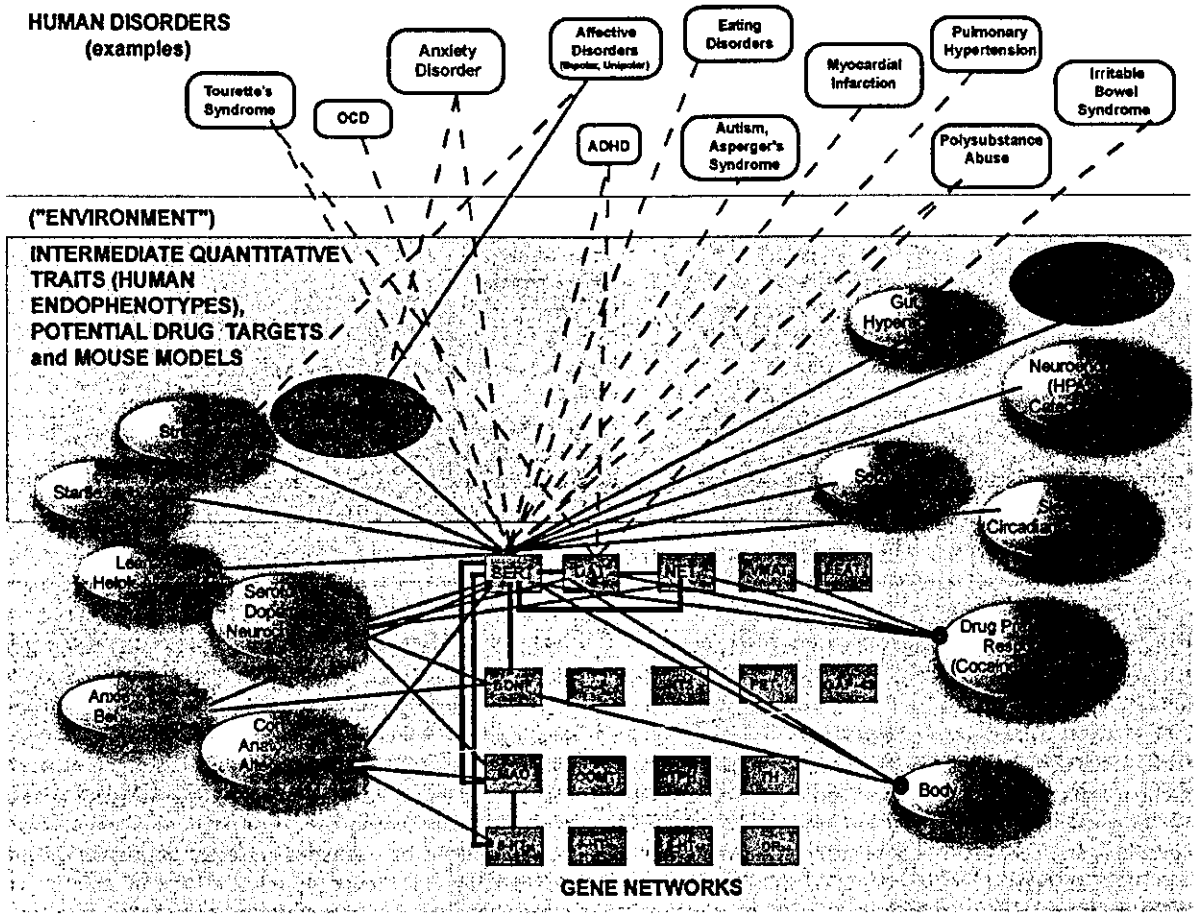

Figure 9: Hypothetical depiction of genes and interactions between genes and gene networks that lead to quantitative trait changes in mouse models. Some analogous endophenotypes that have been found associated with SERT variants are noted, as are some examples of human disorders associated with SERT variants, allowing for interactions with the environment. Some of these associations have been determined from 'top-down' human genetic studies of patients with disorders. Other still hypothetical associations are from 'bottom-up' direct studies of genes and gene interactions as reviewed in this paper (adapted from Murphy *et al.* 2001).

the level of expression variation that could model much of the human allelic variation that contributes to common complex human disorders, including behavioral disorders. Studies of interactions between such variants in mice can thus be carried out in a systematic fashion, in a laboratory setting where environmental variation does not provide much additional variance for behavioral, pharmacological and physiological investigations.

Mouse data from gene–gene interactions could thus be highly important for human studies. Expression studies in human tissues and/or using human haplotypes in *in vitro* systems can document the variations that are present in the structure, expression level and/or regulation of specific gene products. Many of these variants can be modeled in mice. Behavioral and physiological tests including imaging can be employed in mice that are as close as possible to validated models of the genetically mediated components of the human disorder. Under these circumstances, data from studies of gene–gene interactions in mice can thus generate hypotheses that are testable in a much more powerful way in human studies. A prior ability to predict both the loci at which meaningful interactive effects and also the type of interaction would occur may markedly enhance the ratio of true vs. false- positive results in this complex area.

Studies of multiple knockout mice are tedious and expensive. Newer techniques such as conditional knockouts are now being increasingly emphasized in neurobiology, although they often produce chimeric animals which are poorer genetic models of most human allelic variation. The data presented in this review provides a continuing rationale for studies of interactions between gene knockout and transgenic mice as a basis for understanding behavioral and other neurobiological

phenotypes. However, beyond this such studies may provide one of the few ways that might allow relatively secure approaches to identifying many of the gene–gene interactions likely to be important for human polygenic disorders and traits.

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LORETA

low-resolution electromagnetic tomography

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LORETA とは low-resolution electromagnetic tomography の略であり，1994 年に Pascual-Marqui[1] によって開発された新たな脳機能の三次元画像表示法である．LORETA は，隣接する神経細胞群が類似した活動をおこなう（smoothness）と仮定することで，頭皮上で測定された脳波や脳磁図データから脳の電気活動を反映する電流密度を計算する逆問題の解法であり，脳実質内に数千の正立方格子を想定し平方ラプラシアンの和を最小化して smoothness をはかることで電源推定を可能としている．同じように逆問題の解法を用いて電位発生源を推定する方法に双極子追跡法があるが，双極子追跡法のように活動点の数をあらかじめ設定する必要や，活動点以外の脳部位が電気的に静止しているといった制約条件を必要としないため，未知の活動部位や同時に複数の活動部位が存在する場合においても，その活動部位を推定することが可能であるという利点がある．また電位発生源を PET や fMRI で一般的に用いられている Talairach 標準脳アトラス上の大脳皮質と海馬のみとしており，2394 ボクセルで空間解像能 7 mm というように比較的空間解像能が高く[2]，このため脳波の利点である高い時間解像能を生かしつつ脳機能画像研究をおこなうことが可能となった．

その信頼性は，fMRI などにより従来から指摘されていた脳部位と一致した領域にその電位発生源を推定していることから，かなり高いものとされている．たとえば，視覚刺激に対して後頭葉の視覚野[3][4]，聴覚刺激に対しては側頭葉の聴覚野[5][6]，運動・視覚運動課題遂行時に視覚野と運動野[7][8]，相貌認知課題においては内側後頭側頭回[9]にそれぞれ電位発生源を fMRI 研究と同様の部位に推定している．また，てんかん焦点に関する報告では，頭皮上電極で計測した脳波の LORETA による焦点部位の解析結果は，硬膜下電極で記録されたスパイクの部位とほぼ一致していた[10]．当研究室でおこなった単語課題を遂行中の事象関連電位に対する LORETA 解析の結果では，健常者では提示語に対して両側側頭葉と左前頭前野に強い電気活動が認められるが（図 **1a**），統合失調症患者ではほぼ同部位で活動が認められるものの，その活動は減弱していた（図 **1b**）．両群の活動部位に対して SnPM (statistical nonparametric mapping) を用いて統計解析をおこなったところ，統合失調症患者では両側前頭前野を含めて広範囲にわたって脳活動が低下していることが判明した（図 **1c**）．またこのように活動部位に有意差が認められたのは刺激提示後 270～500 ms の区間であった[11]．

以上のように，LORETA はある程度の広がりをもつ神経活動や，同時に複数の部位が活動していると考えられる場合には有用な脳機能部位推定法であるといえ，また脳波や脳磁図のもつミリ秒オーダーの高い時間解像能を十分に生かしつつ脳活動の空間特性を把握するにはすぐれた技術であると考えられる．なお，開発者である Pascual-Marqui が作成した最新の LORETA 解析ソフトウェアでは，神経活動部位の経時的な変化をアニメーションで表示することが可能となっている（http://www.unizh.ch/keyinst/NewLORETA/LORETA01.htm）．

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