Neurogenetics of emotional reactivity to stress in animals

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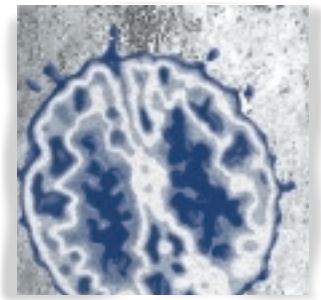

The influence of genetic factors on the nature and intensity of stress responses has been widely demonstrated in several animal species[1] and in humans.[2] This genetic component may be directly responsible for the large interindividual variation often observed for this kind of trait, or, as indicated by recent findings, it may provoke variations through interaction between genotype and environment, including postnatal environment.[3] The use of intraspecific groups of animals that differ in their genetic backgrounds and/or their responses to environmental challenges has gained more and more interest. The selection of divergent rat or mouse strains that differ in their behavioral responses to well-defined stressors, such

There is much evidence for the involvement of central monoaminergic systems, the key targets of stress, in the regulation of mood. Animal and human findings indicate that genetics play a role in the etiology of mood disorders, and so we selected divergent inbred rat strains according to their anxiety-related behaviors on exposure to novel environments. We compared these strains for psychoneuroendocrine response to stressors and/or antidepressants. Molecular genetic studies were also performed to localize the genomic regions associated with these strain-dependent anxiety profiles. We then examined human results indicating that allelic variations in the serotonin transporter (5-HTT) may play a role in the etiology of neuroticism and depression. Thus, we compared inbred rat strains for the 5-HTT, with regard to central and peripheral (platelet) protein expression and function, and the consequences of local application of a selective serotonin reuptake inhibitor (SSRI) on extracellular serotonin (5-HT) levels. Our results indicate that spontaneously hypertensive rats and Lewis rats (LEW) selectively diverge in terms of anxiety-related behaviors and that this divergence is located on chromosome 4. The use of social defeat in LEW and the analysis of its psychoneuroendocrine consequences strongly suggest that such a paradigm, which is sensitive to repeated SSRI treatment, models posttraumatic stress disorder. The Wistar-Kyoto rat may be an adequate model to study the consequences of a genetically driven hypersensitivity to stress and noradrenergic antidepressants. Our most recent findings show that the Fischer 344 and LEW strains differ in protein expression and function of hippocampal and platelet 5-HTT; the divergence in protein expression is not due to allelic variations in the gene-coding sequences and leads to marked differences in extracellular 5-HT levels under basal conditions or SSRI. These examples illustrate how the use of inbred rat strains may complement our knowledge on the genetics of behavior, in the same way as the use of transgenic mice.

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Selected abbreviations and acronyms

[³H]8-OH-DPAT	*[³H]8-hydroxy-2-(di-*n*-propylamino)tetralin*
F344	*Fischer 344 rat*
5-HIAA	*5-hydroxyindoleacetic acid*
HPA	*hypothalamo-pituitary-adrenal (axis)*
5-HT	*serotonin (5-hydroxytryptamine)*
5-HTT	*serotonin transporter*
LEW	*Lewis rat*
NA	*noradrenaline*
SHR	*spontaneously hypertensive rat*
SSRI	*selective serotonin reuptake inhibitor*
WKY	*Wistar-Kyoto rat*

as the Maudsley strains of rat,[4] provides an example of such a strategy. Interestingly, the use of divergent strains of rats to understand the physiology (including the neurochemistry) of stress responses has recently been complemented by genetic studies of quantitative trait loci, leading to a precise genomic location underlying or associated with these inherited differences in stress responses.[5]

Eight years ago, we decided to adopt a complementary approach through detection of the most divergent strains with respect to anxiety-related behaviors among commercially available inbred rat strains. Given the amount of evidence linking stress-related behaviors, and particularly anxiety, to central serotonergic systems, some of the key features of these systems in the divergent strains selected were compared under basal and stress conditions. We complemented this strategy by comparing the behavioral and neurochemical effects of psychotropic drugs, especially serotonergic and/or noradrenergic antidepressants, with and without repeated stress exposure. Finally, our most recent work, which will also be presented below, somewhat differed from our initial studies in that the inbred rat strains were selected on the basis of a neurochemical trait, ie, the serotonin transporter (5-HTT), rather than a behavioral trait.

Anxiety-related behaviors in inbred rat strains

Male and female rats were selected from six inbred strains (the Fischer 344 rat [F344], the Lewis rat [LEW], the Brown Norway rat, the Wistar-Kyoto rat [WKY], the spontaneously hypertensive rat [SHR], and the Wistar-Furth rat) and the behaviors of these animals in several stressful environments were recorded.[6] These included the open field, the elevated plus-maze, the social interaction test, and the black and white box, ie, models thought to allow a correct estimation of independent behavioral dimensions such as anxiety and locomotion.[7] A principal component (multivariate) analysis allowed us to dissect the ethological meaning of the behaviors measured in each test. In addition, our study allowed us to select two strains of rats (SHR and LEW), which differed selectively for anxiety-related behaviors in the elevated plus-maze (open arm visits), the black and white box (visits to the white compartment), and the open field (visits to the central squares), but not for locomotor-related behaviors in any test (a finding that was later confirmed by locomotion monitoring in activity cages). Thus, SHR and LEW were found to display low and high anxiety, respectively, and the difference between them was devoid of any contamination by activity-related inputs.

Next, we investigated the inheritance of a number of anxiety-related behaviors in SHR and LEW.[8] To do so, breeding and crossbreeding experiments were conducted to obtain, for each sex, the parental strains and the F1 and F2 generations derived from SHR/LEW and LEW/SHR matings. Thereafter, all 267 individuals were tested in the elevated plus-maze and the open field, and inheritance calculations made to determine the origins of the behavioral strain differences. It was found that the most heritable difference between strains was the anxiety-related number of visits to the center of the open field. This was due to a direct effect of the genes, rather than to indirect maternal and grandmaternal effects. The use of microsatellites covering the whole genome confirmed this by revealing a quantitative trait locus in the F2 population that explained half of the variance associated with the visits to the center of the open field.[9] Interestingly, this locus was located in the same region of chromosome 4 where the genes encoding the substance P receptor (*Tac1r*) and neuropeptide Y (*Npy*) have been located. Additional experiments suggested that neuropeptide Y may be excluded, leaving open the possibility that an allelic variation in the gene encoding the substance P receptor participates in this behavioral difference found between SHR and LEW.

Central serotonergic systems in SHR and LEW under basal and stress conditions

Anatomical, behavioral, and pharmacological data support the hypothesis that central serotonin (5-HT) plays a role in the etiology of anxiety. As an illustration, 5-HT has

been suggested to stimulate unconditioned anxiety, whereas both stimulatory and inhibitory influences of 5-HT on conditioned anxiety have been advanced.[10-12] In 1996, ie, at a time when only 5-HT_{1B} receptor knockout mice had been engineered, we took advantage of the most recent pharmacological findings indicating that 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors played some role in anxiety to check for strain differences regarding these and other determinants of 5-HT activity.[13] We found that in vitro central tryptophan hydroxylase activity was higher in LEW than in SHR; however, ex vivo studies in midbrain and hippocampus revealed that the synthesis of 5-HT and the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) did not differ between strains. [^{3}H]8-Hydroxy-2-(di-*n*-propylamino)tetralin ([^{3}H]8-OH-DPAT) binding at midbrain 5-HT_{1A} autoreceptors and hippocampal 5-HT_{1A} postsynaptic receptors, [^{3}H]ketanserin binding at cortical and striatal 5-HT_{2A} receptors, and [^{3}H]citalopram binding at midbrain and hippocampal 5-HT transporters (5-HTT) did not vary between strains. The inhibition of 5-HT synthesis by 5-HT_{1A} autoreceptor stimulation was similar in the two strains, but forepaw treading was higher and flat body posture after 5-HT_{1A} postsynaptic receptor stimulation lower in SHR than in LEW. Finally, head shakes elicited by 1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane and quipazine (a 5-HT_{2A} receptor–mediated response) were increased in the SHR strain compared with the LEW strain; on the other hand, 1-(3-chlorophenyl)piperazine triggered similar 5-$HT_{2B/2C}$ receptor–mediated decreases in motor activity in the two strains.

This study thus showed that, although the low-anxiety strains (SHR) and high-anxiety strains (LEW) vary in terms of some aspects of 5-HT function, key anxiety-related components of central serotonergic systems (such as the 5-HT_{1A} autoreceptors) were no different. Of course, this result could be explained by the fact that the tools used at that time were insufficient or not sensitive enough to thoroughly explore central serotonergic activity. However, we should not dismiss the possibility that the basal conditions under which we performed our study were not the most adequate to reveal strain differences, if any, in central serotonergic systems. In keeping with such a hypothesis, we then explored these systems under stimulated conditions.

Social stress by repeated defeat has been shown to be endowed with neuroendocrine and behavioral effects that render this stress model useful to identify adaptive mechanisms.[14,15] Among these mechanisms, those related to central serotonergic systems (eg, hippocampal 5-HT_{1A} and cortical 5-HT_{2A} receptors)[16] have been particularly underlined. Nonetheless (i) how the neuroendocrine and behavioral effects of social stress are affected by the genetic status of the animal, and (ii) how this status affects the relationships between central serotonergic systems and adaptive processes, have not been studied. We thus analyzed the effects of repeated defeat by Long-Evans resident rats (30 min of social defeat followed by 14-18 h of sensory contact with the aggressor daily for 7 days) upon the psychoneuroendocrine profile of SHR and LEW.[17] Repeated defeat time-dependently decreased body weight growth and food intake in both strains, but these decreases were more severe and longer-lasting in the LEW strain. This strain-dependent difference could not be accounted for by differences in physical contacts with the resident rats because the number of attacks and their latency throughout the stress period were similar for the two strains. When exposed to an elevated plus-maze test of anxiety, the unstressed LEW entered the open arms less than their SHR counterparts, thus confirming above findings. This difference was amplified by social stress, which increased anxiety-related behaviors in LEW only. In the forced swimming test, LEW spent more time immobile than SHR, with stress increasing immobility in a strain-independent manner.

In addition to the metabolic changes described above, the activity of the hypothalamo-pituitary-adrenal (HPA) axis was slightly stimulated in a strain-independent manner by the stressor, as indicated by increased corticosterone levels and adrenal weights, and decreased thymus weights. In LEW, but not in SHR, midbrain 5-HT metabolism was increased by stress and this difference was associated with an increased B_{max} (maximum binding capacity) value for cortical [^{3}H]ketanserin binding at the 5-HT_{2A} receptors. On the other hand, the B_{max} value for hippocampal [^{3}H]8-OH-DPAT binding at the 5-HT_{1A} receptors was decreased by stress, and this reduction was amplified in SHR compared with LEW. This study illustrates how genetics may impact the psychoneuroendocrine response to stress, and the use of socially stressed SHR and LEW may be an important paradigm in the study of adaptive processes.

This possibility was explored by measuring the impact of a 3-week period of treatment with the selective serotonin reuptake inhibitor (SSRI) fluoxetine (7.5 mg/kg/day) on the psychoneuroendocrine profiles of stressed LEW (the

SHR strain was not included in this study due to the amount of effort required for a thorough analysis of a single strain).[18] In this series of experiments, social stress consisted of a single overnight exposure to the resident rat (because the study described above revealed that the first exposure caused marked behavioral impacts). A single social defeat triggered hypophagia and body weight loss, and increased anxiety in the elevated plus-maze. It did not affect baseline plasma adrenocorticotropic hormone levels or renin activity, but decreased plasma corticosterone levels. On the other hand, the responses of these variables to subsequent acute forced swim stress were blunted (corticosterone) or amplified (adrenocorticotropic hormone, renin activity) by prior defeat. The density of hippocampal 5-HTTs, but not that of hippocampal 5-HT_{1A} and cortical 5-HT_{2A} receptors, was decreased by a single social defeat; in addition, tryptophan availability, 5-HT synthesis and metabolism, and 5-HT_{1A} autoreceptor–mediated functions (inhibition of 5-HT synthesis and hyperphagia) were unaffected. However, it was of note that fluoxetine pretreatment diminished social defeat–induced hypophagia, body weight loss, and anxiety without affecting these variables in control animals. This pretreatment increased plasma corticosterone levels in resting and acutely stressed rats, but abolished social defeat–elicited corticosterone hyporesponsiveness to acute forced swim stress. Except for a decrease in midbrain 5-HTT density, fluoxetine did not affect the other serotonergic indices analyzed.

Taken together, our results show that a single social defeat in LEW produces behavioral and endocrine alterations that may model some aspects of human anxiety disorders, especially posttraumatic stress disorder[19]; furthermore, our finding that repeated SSRI pretreatment has protective effects on some of the negative consequences of social stress opens future possibilities for determination of the precise mechanisms responsible for these consequences.

Genetic variability in the psychoneuroendocrine responses to antidepressant treatment

While the above experiments were in progress, we came across findings indicating that the SHR and the WKY strains markedly differed in their behavioral sensitivities to tricyclic antidepressants (desipramine and imipramine) on exposure to the forced swimming test. These agents decreased the duration of immobility in SHR, but proved ineffective in WKY.[20,21] Moreover, human data indicate that the efficacy of antidepressants has a strong genetic substrate, partly through the allelic variation in the activity of drug-metabolizing enzymes such as the cytochrome P450.[22] Our preliminary observation that SHR and WKY differ in both their anxiety profile (these strains display low and high anxiety scores, respectively) and their activity profile (these strains display high and low activity scores, respectively) led us to analyze their psychoneuroendocrine responses to several antidepressants. Thus, in one study, repeated fluoxetine treatments (5 or 10 mg/kg intraperitoneally [IP] daily, for 3 weeks) were administered to control SHR and WKY, whereas, in another study, repeated fluoxetine treatments were compared with imipramine and desipramine treatments (all 10 mg/kg orally daily, for 4 weeks). Both these studies were carried out in control and repeatedly stressed SHR and WKY (2 h of restraint daily throughout the 4th week).

Repeated fluoxetine treatment in control SHR and WKY

Two days after the last fluoxetine injection in the control experiments,[23] the two strains had undetectable plasma levels of fluoxetine, but detectable and similar levels of its metabolite, norfluoxetine. The elevated plus-maze test (29-30 h after the 13th administration of fluoxetine) and an open field test (48 h after the last injection of fluoxetine) were used to show that fluoxetine pretreatment did not produce anxiolysis; hence, some, but not all, behaviors were indicative of anxiety and hypolocomotion (as assessed through principal component analyses and acute diazepam studies). In both strains, the 10 mg/kg dose of fluoxetine decreased hypothalamus 5-HT and 5-HIAA levels, and reduced midbrain and/or hippocampus [^{3}H]citalopram binding at 5-HTTs, but did not affect [^{3}H]8-OH-DPAT binding at hippocampal 5-HT_{1A} receptors. However, the fluoxetine-elicited reduction in hippocampal 5-HTT binding, which was unlikely to be due to residual norfluoxetine, was much greater in WKY than in SHR, and this strain-dependent effect in WKY was associated with a reduction in cortical [^{3}H]ketanserin binding at the 5-HT_{2A} receptors. Finally, in WKY, repeated fluoxetine administration increased adrenal weights and the plasma corticosterone response to open field exposure, but did not affect the binding capacities of hippocampal mineralocorticoid and glucocorticoid recep-

tors. Beside the complex neurochemical results that are beyond the scope of the present survey, our study mainly illustrates how key psychoneuroendocrine responses to repeated fluoxetine administration may be strain-dependent. On the other hand, our data contradicted previous neurochemical and behavioral findings, as illustrated by our failure to detect anxiolysis after repeated SSRI administration.[24]

Although the results of this control study provide some positive information, we felt that it was far too limited in its design: a single antidepressant was administered IP (instead of orally as in clinical psychiatry) to unstressed control rats, ie, very different conditions from those required to assess the potential psychoneuroendocrine benefit of the psychotropic agent. This is why we performed a second series of experiments with imipramine and desipramine that took into account the limits of the first paradigm.[25]

Repeated fluoxetine treatment in SHR and WKY receiving imipramine and desipramine

In the imipramine and desipramine experiments, it was observed that following a 24-h wash-out period, WKY displayed higher plasma antidepressant and antidepressant metabolite levels than SHR. Fluoxetine pretreatment decreased [^{3}H]citalopram binding at midbrain 5-HTTs, whereas tricyclic antidepressants and fluoxetine decreased [^{3}H]ketanserin binding at cortical 5-HT_{2A} receptors, [^{3}H]CGP-12177 binding at cortical β-adrenoceptors, and [^{3}H]nisoxetine binding at midbrain noradrenaline (NA) transporters in both strains. None of the antidepressants affected [^{3}H]8-OH-DPAT binding at hippocampal 5-HT_{1A} receptors. It was notable that repeated restraint triggered a desipramine-sensitive 140% increase in hypothalamus [^{3}H]nisoxetine binding in WKY, but not in SHR; moreover, plasma adrenocorticotropin-releasing hormone responses to a 5-min open field test were amplified by prior repeated restraint in both strains, but desipramine prevented such an amplification in WKY only. However, the elevated plus-maze and open field behaviors of SHR and WKY were unaffected by desipramine pretreatment.

A simple conclusion of these experiments is that they clearly show that the SHR and WKY strains may be useful in understanding how genetic differences in noradrenergic responses to stress and desipramine treatment impact on adaptive processes.

Genetic variability in the rat 5-HTT

The experiments described above all focused on one main question: do individuals that differ in their behavioral responses to novel environments also differ with respect to key central monoaminergic responses to stressors and/or antidepressants? The results underline how comparisons between rat strains may allow the detection of models of some value for understanding the basis of the interindividual variability in fear responses, whether these are linked to behavior (eg, the socially stressed LEW) or neurochemistry (eg, the restrained WKY).

The quest for the mechanisms explaining such strain-dependent characteristics undoubtedly require intense effort, at least effort that is beyond the scientific and human capacities of our research group. Accordingly, we recently decided to change our scientific goals by trying to focus on the genetics of central serotonergic systems, leaving behavioral and neuroendocrine research topics aside, at least in the preliminary stages of research. In other words, we decided to explore whether we could find strains of rats that differ with respect to one candidate target, ie, the 5-HTT. If this is successful, it will be followed by an intense investigation on the psychoneuroendocrine consequences of such a genetic difference.

Considering that 5-HTT knockout mice already exist,[26-28] why is our investigation in rats so important? The following points encouraged us to follow our line of research:

- Constitutive knockout models may be seen as all-or-nothing paradigms, which impede any quest on the consequences of subtle (ie, <50%) differences in gene expression.
- Adaptive processes and features linked to the genetic background of the mouse strains are trivial limits of these models.
- The use of individuals that differ in terms of the gene of interest, and also in other genes (as found using different strains), may help further define the regulatory links between the gene of interest and other unexpected genes.
- Some knockout models may be difficult to use due to their limited availability and/or the structures needed to breed them.

In humans, the 5-HTT gene is highly polymorphic, as illustrated by allelic variations in the second intron and the upstream promoter region.[29,30] Data regarding the promoter region suggest that polymorphisms in that region lead to differences in the 5-HT reuptake function of the

5-HTT.[30] Thus, the insertion/deletion of 14 and 16 copies of a 20- to 23-basepair repeat element leads to two promoter variants, the short and the long variants. When studied by means of reporter gene constructs and human lymphoblasts, the short variant was found to trigger, in a dominant manner, reductions in 5-HTT transcription and 5-HT reuptake, compared with the long variant.[30] When examined ex vivo, however, peripheral and central 5-HTT densities and/or 5-HT reuptake did not always obey allelic variation.[31] The initial finding that the short variant was associated with neuroticism[30] substantiated the hypothesis that the 5-HTT plays a role in the susceptibility to mood disorders. Subsequent studies both confirmed and refuted this initial finding; the diverging results could be accounted for by the low weight of the 5-HTT gene in some personality trait differences.[32]

The only currently available rodent models of 5-HTT gene alterations are:

- Mice bearing a 100% constitutive invalidation of their 5-HTT gene.[26-28]
- Rats injected in their dorsal raphe with recombinant plasmids containing the sequence (overexpression) or a partial antisense (underexpression) sequence of the 5-HTT gene.[33]
- Rat sublines differing in terms of the platelet 5-HTT gene and protein expression as well as platelet 5-HT reuptake.[34]

The most relevant model of invalidated mice for the study of the functional consequences of human 5-HTT polymorphisms is the comparison between control and heterozygote mice; however, heterozygote mice do not display any difference in 5-HT reuptake compared with controls, although, logically, 5-HTT densities are reduced by 50%.[26] With regard to transgenic rats, the observation that gene transfers were performed after development only had transient consequences on 5-HTT and 5-HT reuptake underlines the limits of that particular model.[33] Finally, autoradiographic experiments conducted with the rat sublines differing for platelet 5-HTT protein expression and function suggest that these two sublines may not differ with regard to central 5-HTT protein expression.[34]

Detection of strain differences in 5-HTT: behavioral response

Taking into account these observations, we performed two series of experiments. The first series of experiments took advantage of the finding that WKY do not respond acutely to the tricyclics imipramine and desipramine when examined in the forced swimming test. Thus, one hypothesis could be that 5-HTT and/or NA transporters are hyposensitive to the 5-HT reuptake (imipramine) and NA reuptake (imipramine and desipramine) inhibitory properties of these antidepressants. Accordingly, we used in vitro, in vivo, and ex vivo methods to examine the 5-HTT in WKY, SHR, and LEW.[35] Acute administration of the SSRI citalopram (1-10 mg/kg, IP 1 h before an elevated plus-maze test) to SHR, LEW, and WKY promoted anxiety and/or hypoactivity in SHR and LEW, but not in WKY. This initially reinforced the hypothesis that WKY 5-HTTs are hyposensitive to drugs endowed with 5-HT reuptake properties. However, the pretreatment with citalopram increased central 5-HT levels and/or decreased 5-HIAA levels in all strains. Hippocampal, but not midbrain or striatal, [^{3}H]citalopram binding at 5-HTTs was lower in WKY than in SHR, whereas the [^{3}H]5-HT reuptake kinetics and the potencies of citalopram (1-1000 nM) needed to inhibit [^{3}H]5-HT reuptake into hippocampal and striatal synaptosomes did not differ between strains. This was confirmed in vivo by means of microdialysis in the hippocampus of freely moving rats. Thus, although LEW displayed a three- to fourfold higher baseline level of extracellular 5-HT in the hippocampus, compared with SHR and WKY, local perfusion with 1 µM citalopram promoted relative increases in extracellular 5-HT levels over baseline that were similar in all strains. Acute IP administration of 3.3 mg/kg citalopram (1 h beforehand) decreased [^{3}H]5-HT reuptake into hippocampal synaptosomes to a similar extent in SHR and WKY, thereby indicating that the systemic administration of the SSRI has strain-independent effects at hippocampal 5-HT nerve terminals. This study thus failed to detect strain differences in the 5-HTT or in its sensitivity to an SSRI, further indicating that genetic differences in the behavioral responses to SSRIs may involve 5-HTT–independent mechanisms.

Detection of strain differences in 5-HTT: 5-HT reuptake

With this negative finding in mind, we returned to a comparison of the six inbred rat strains mentioned at the beginning of this report (F344, LEW, Brown Norway rat, WKY, SHR, and Wistar-Furth rat), to which were added two standard rat strains, namely the Wistar and the Sprague-Dawley strains. This time hippocampal 5-HT

reuptake, instead of anxiety-related behavior, was taken as the criterion of selection. We observed that F344 rats displayed the highest rates of reuptake, while LEW were among those with the lowest. An analysis of various elements of central serotonergic systems in female F344 and LEW had previously indicated that 5-HTT mRNA was more abundant in the dorsal raphe nucleus of F344, compared with LEW.[36] This suggests that differences in mRNA expression underlie our observation of strain differences in protein function. We therefore performed a complete study of the central and peripheral 5-HTT in both sexes of both strains (manuscript submitted for publication). Indeed, midbrain and hippocampal [^{3}H]paroxetine binding at the 5-HTT and hippocampal [^{3}H]5-HT reuptake were increased in male and female F344, compared with their LEW counterparts, and these strain differences were observed both in rats of commercial origin and in laboratory-bred rats (thus excluding strain differences linked to late environment changes).[3] Moreover, in laboratory-bred rats, it was found that these strain differences extended to blood platelet 5-HTT protein expression and function. Saturation studies of midbrain and hippocampal [^{3}H]paroxetine binding at 5-HTT, and hippocampal and blood platelet [^{3}H]5-HT reuptake, also revealed slight, but significant, strain differences in B_{max} and V_{max} (maximal velocity) values. Although F344 and LEW differ in terms of the activity of the HPA axis,[37,38] experiments conducted in male rats that had been adrenalectomized or treated with corticosterone revealed that the strain differences in hippocampal [^{3}H]paroxetine binding at 5-HTTs and [^{3}H]5-HT reuptake were not accounted for by the HPA axis. Systemic administration of the SSRI citalopram decreased midbrain and hippocampal 5-HT turnover rates, the amplitudes of which varied in a strain-independent manner. Conversely, hippocampal extracellular 5-HT levels were reduced in F344, compared with LEW, but the magnitude of the increase in extracellular 5-HT elicited by local administration of citalopram was larger in F344. Finally, at the molecular level, no strain differences were found in the respective coding sequences of the 5-HTT gene, thus suggesting that genetic differences, if any, lie in the promoter region (note that, as opposed to mice and humans, the rat 5-HTT gene promotor has not yet been cloned).

Taken together, the results of this series of experiments indicate that the F344 and LEW strains will be useful in the study of the impact of genetics on 5-HTT and how allelic control of 5-HTT (which remains to be demonstrated) affects stress responses.

Conclusion

This short survey of our most recent experiments aimed to illustrate how the use of different inbred rat strains is a positive complementary approach to already existing transgenic models. However, such a strategy requires adequate criteria for selection, especially regarding behavioral traits. In this respect, we show that multivariate analyses can be used to remove contaminant behaviors. This strategy therefore measures the impact of stressors and/or antidepressants in animals that are genetically prone to display hypersensitivity to fear-related events. This is illustrated by our proposal that the socially stressed LEW is an appropriate model of posttraumatic stress disorder, whereas the WKY may prove important in future studies into the genetic basis of the hypersensitivity of central noradrenergic systems to stress and NA-related tricyclics. Our results in LEW also underline the need to use ethologically relevant models of stress, such as social stress, rather than aversive stressors without any clearcut relevance to humans (eg, electric shocks). The final series of experiments described above illustrate how a strategy based on an initial screening of inbred rat strains applies to key neurochemical targets, such as the 5-HTT, thereby filling a gap in the animal models currently available for the study of the consequences of human allelic variations in 5-HTT. This survey was never intended to indicate that a comparison between inbred rat strains is the most valuable strategy, but rather to show that it is a valuable complement to currently existing models, most of which involve the use of transgenic strategies in mice. ❑

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Neurogenética de la reactividad emocional al estrés en los animales

Existe bastante evidencia acerca del compromiso de los sistemas monoaminérgicos centrales –los blancos clave del estrés– en la regulación del ánimo. Los hallazgos en animales y humanos indican que la genética juega un papel en la etiología de los trastornos del ánimo, y por eso se seleccionaron cepas puras de ratas que se diferencian según sus conductas relacionadas con la ansiedad y la exposición a nuevos ambientes. Se compararon estas cepas respecto a la respuesta psiconeuroendocrina a estresores y/o antidepresivos. También se realizaron estudios de genética molecular para localizar las regiones genómicas asociadas con estos perfiles de ansiedad dependientes de la cepa. Luego se examinaron los resultados en humanos, los cuales indican que las variaciones alélicas en el transportador de serotonina (T 5-HT) pueden jugar un papel en la etiología del neuroticismo y la depresión. De este modo, se compararon cepas puras de ratas para el T 5-HT, en relación con la expresión y función de proteínas centrales y periféricas (plaquetas), y las consecuencias de la aplicación local de un inhibidor selectivo de la recaptación de serotonina (ISRS) en los niveles extracelulares de serotonina (5-HT). Nuestros resultados indican que las ratas espontáneamente hipertensas y las ratas de Lewis (LEW) difieren selectivamente respecto a las conductas relacionadas con la ansiedad y que esta diferencia se localiza en el cromosoma 4. El uso del rechazo social en las ratas LEW y el análisis de sus consecuencias psiconeuroendocrinas sugieren fuertemente que este paradigma, que es sensible a un tratamiento repetido con ISRS, constituye un modelo para el trastorno por estrés postraumático. La rata Wistar-Kyoto puede constituir un modelo adecuado para estudiar las consecuencias de una hipersensibilidad al estrés y a los antidepresivos noradrenérgicos genéticamente determinada. Nuestros hallazgos más recientes muestran que las cepas Fischer 344 y LEW se diferencian en la expresión de proteínas y en la función del transportador de serotonina en el hipocampo y las plaquetas; la diferencia en la expresión de proteínas no se debe a las variaciones alélicas en las secuencias codificadoras de genes, y conduce a diferencias marcadas en los niveles de 5-HT extracelular en condiciones basales o con ISRS. Estos ejemplos ilustran cómo el empleo de cepas puras de ratas pueden ayudar a nuestro conocimiento acerca de la genética del comportamiento, al igual que la utilización de los ratones transgénicos.

REFERENCES

1. Castanon N, Mormède P. Psychobiogenetics: adapted tools for the study of the coupling between behavioral and neuroendocrine traits of emotional reactivity. *Psychoneuroendocrinology*. 1994;19:257-282.

2. Plomin R, Owen MJ, McGuffin P. The genetic basis of complex human behaviors. *Science*. 1994;264:1733-1739.

3. Wood GK, Marcotte ER, Quirion R, Srivastava LK. Strain differences in the behavioural outcome of neonatal ventral hippocampal lesions are determined by the postnatal environment and not genetic factors. *Eur J Neurosci*. 2001;14:1030-1034.

4. Broadhurst PL. Reactive and nonreactive strains of rats: a survey. *Behav Genet*. 1975;5:299-319.

5. Moisan MP, Courvoisier H, Bihoreau MT, et al. A major quantitative trait locus influences hyperactivity in the WKHA rat. *Nat Genet*. 1996;14:471-473.

6. Ramos A, Berton O, Mormède P, Chaouloff F. A multiple-test study of anxiety-related behaviours in six inbred rat strains. *Behav Brain Res*. 1997;85:57-69.

7. Belzung C, Le Pape G. Comparison of different behavioral test situations used in psychopharmacology for measurement of anxiety. *Physiol Behav*. 1994;56:623-628.

8. Ramos A, Mellerin Y, Mormède P, Chaouloff F. A genetic and multifactorial analysis of anxiety-related behaviours in Lewis and SHR intercrosses. *Behav Brain Res*. 1998;96:195-205.

9. Ramos A, Moisan MP, Chaouloff F, Mormède C, Mormède P. Identification of female-specific QTLs affecting an emotionality-related behavior in rats. *Mol Psychiatry*. 1999;4:453-462.

10. Griebel G. 5-HT–interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther*. 1995;65:319-395.

11. Graeff FG. Role of 5-HT in defensive behavior and anxiety. *Rev Neurosci*. 1993;4:181-211.

12. Martin JR, Bös M, Jenck F, et al. 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. *J Pharmacol Exp Ther*. 1998;286:913-924.

13. Kulikov A, Aguerre S, Berton O, et al. Central serotonergic systems in the spontaneously hypertensive and Lewis rat strains that differ in the elevated plus-maze test of anxiety. *J Pharmacol Exp Ther*. 1997;281:775-784.

14. Fuchs E, Kramer M, Hermes B, Netter P, Hiemke C. Psychosocial stress in tree shrews: clomipramine counteracts behavioral and endocrine changes. *Pharmacol Biochem Behav*. 1996;54:219-228.

15. Kudryavtseva NN, Bakshtanovskaya IV, Koryakina LA. Social model of depression in mice of C57BL/6J strain. *Pharmacol Biochem Behav*. 1996;38:315-320.

16. McKittrick CR, Blanchard DC, Blanchard RJ, McEwen BS, Sakai RR. Serotonin receptor binding in a colony model of chronic social stress. *Biol Psychiatry*. 1995;37:383-393.

17. Berton O, Aguerre S, Sarrieau A, Mormède P, Chaouloff F. Differential effects of social stress on central serotonergic activity and emotional reactivity in Lewis and spontaneously hypertensive rats. *Neuroscience*. 1998;82:147-159.

18. Berton O, Durand M, Aguerre S, Mormède P, Chaouloff F. Behavioral, neuroendocrine and serotonergic consequences of single social defeat and repeated fluoxetine pretreatment in the Lewis rat strain. *Neuroscience*. 1999;92:327-341.

19. Shalev AY, Bonne O, Eth S. Treatment of posttraumatic stress disorder: a review. *Psychosom Med*. 1996;58:165-168.

20. Lahmame A, Armario A. Differential responsiveness of inbred strains of rats to antidepressants in the forced swimming test: are Wistar-Kyoto rats an animal model of subsensitivity to antidepressants? *Psychopharmacology*. 1996;123:191-198.

Neurogénétique de la réactivité émotionnelle au stress chez l'animal

La participation des systèmes monoaminergiques centraux, cibles-clés du stress, dans la régulation de l'humeur, est largement prouvée. Les résultats chez l'homme comme chez l'animal montrent que la génétique joue un rôle dans l'étiologie des troubles de l'humeur, ce qui nous a amenés à sélectionner des lignées pures de rat dont le comportement lié à l'anxiété diverge après exposition à de nouveaux environnements. Nous avons comparé la réponse neuro-psycho-endocrinienne de ces lignées à certains facteurs de stress et/ou antidépresseurs. Des études de génétique moléculaire ont également été conduites afin de localiser les régions génomiques associées aux profils anxieux liés à ces lignées. Nous avons examiné ensuite les résultats chez l'homme qui montrent que les variations alléliques dans le transporteur sérotoninergique (5-HTT) peuvent jouer un rôle dans l'étiologie des comportements névrotiques et de la dépression. Nous avons donc comparé des lignées pures de rats pour le 5-HTT, en ce qui concerne l'expression et la fonction des protéines périphériques (plaquettes) et centrales, et les conséquences de l'application locale d'un inhibiteur sélectif de la recapture de la sérotonine (ISRS) sur les concentrations de sérotonine extracellulaires (5-HT). Nos résultats montrent une divergence sélective entre les rats spontanément hypertendus et les rats Lewis (LEW) en termes de comportements liés à l'anxiété, cette divergence étant située sur le chromosome 4. Le comportement social des rats LEW devant l'échec et l'analyse de ses conséquences neuro-psycho-endocriniennes suggèrent fortement qu'un tel paradigme, sensible au traitement répété par ISRS, constitue un modèle pour l'état de stress posttraumatique. Le rat Wistar-Kyoto peut servir de modèle pour l'étude des conséquences d'une hypersensibilité au stress et aux antidépresseurs noradrénergiques génétiquement induite. Nos résultats les plus récents montrent que les lignées Fischer 344 et LEW diffèrent aux niveaux de l'expression et de la fonction protéiques du 5-HTT hippocampique et plaquettaire ; la divergence dans l'expression protéique n'est pas due aux variations alléliques dans les séquences codées par gènes et entraîne des différences importantes dans les concentrations extracellulaires de 5-HT en conditions basales ou sous ISRS. Ces exemples montrent que l'utilisation de lignées pures de rats peut compléter nos connaissances sur la génétique des comportements, de même que l'utilisation des souris transgéniques.

21. Lahmame A, Del Arco C, Pazos A, Yritia M, Armario A. Are Wistar-Kyoto rats a genetic animal model of depression resistant to antidepressants? *Eur J Pharmacol*. 1997;337:115-123.

22. May DG. Genetic differences in drug disposition. *J Clin Pharmacol*. 1994;31:881-897.

23. Durand M, Berton O, Aguerre S, et al. Effects of repeated fluoxetine on anxiety-related behaviours, central serotonergic systems, and the corticotropic axis in SHR and WKY rats. *Neuropharmacology*. 1999;38:893-907.

24. Cadogan AK, Wright IK, Coombs I, Marsden CA, Kendall DA, Tulloch I. Repeated paroxetine administration in the rat produces a decrease in [^{3}H]ketanserin binding and anxiolytic profile in the elevated X-maze. *Br J Pharmacol*. 1992;107:108P.

25. Durand M, Aguerre S, Fernandez F, et al. Strain-dependent neurochemical and neuroendocrine effects of desipramine, but not fluoxetine or imipramine, in spontaneously hypertensive and Wistar-Kyoto rats. *Neuropharmacology*. 2000;39:2464-2477.

26. Bengel D, Murphy DL, Andrews AM, et al. Altered brain serotonin homeostasis and locomotor insensitivity to 3,4-methylenedioxymethamphetamine ("ecstasy") in serotonin-transporter deficient mice. *Mol Pharmacol*. 1998;53:649-655.

27. Li Q, Wichems C, Heils A, Van de Kar LD, Lesch KP, Murphy DL. Reduction of 5-hydroxytryptamine (5-HT)$_{1A}$–mediated temperature and neuroendocrine responses and 5-HT$_{1A}$ binding sites in 5-HT transporter knock out mice. *J Pharmacol Exp Ther*. 1999;291:999-1007.

28. Mannoury la Cour C, Boni C, Hanoun N, Lesch KP, Hamon M, Lanfumey L. Functional consequences of 5-HT transporter gene disruption on 5-HT$_{1A}$ receptor–mediated regulation of dorsal raphe and hippocampal cell activity. *J Neurosci*. 2001;21:2178-2185.

29. Collier DA, Arranz MJ, Sham P, et al. The serotonin transporter is a potential susceptibility factor for bipolar affective disorder. *Neuroreport*. 1996;7:1675-1679.

30. Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science*. 1996;274:1527-1531.

31. Murphy DL, Li Q, Engel S, et al. Genetic perspectives on the serotonin transporter. *Brain Res Bull*. 2001;56:487-494.

32. Greenberg BD, McMahon FJ, Murphy DL. Serotonin transporter candidate gene studies in affective disorders and personality: promises and potential pitfalls. *Mol Psychiatry*. 1998;3:186-189.

33. Fabre V, Boutrel B, Hanoun N, et al. Homeostatic regulation of serotonergic function by the serotonin transporter as revealed by nonviral gene transfer. *J Neurosci*. 2000;20:5065-5075.

34. Romero L, Jernej B, Bel N, Cicin-Sain L, Cortes R, Artigas F. Basal and stimulated extracellular serotonin concentrations in the brain of rats with altered serotonin uptake. *Synapse*. 1998;28:313-321.

35. Pollier F, Sarre S, Aguerre S, et al. Serotonin reuptake inhibition by citalopram in rat strains differing for their emotionality. *Neuropsychopharmacology*. 2000;22:64-76.

36. Burnet PWJ, Michelson D, Smith MA, Gold PW, Sternberg EM. The effect of chronic imipramine administration on the densities of 5-HT$_{1A}$ and 5-HT$_2$ receptors and the abundancies of 5-HT receptor and transporter mRNA in the cortex, hippocampus and dorsal raphe of three strains of rat. *Brain Res*. 1994;638:311-324.

37. Sternberg EM, Glowa JR, Smith MA, et al. Corticotropin releasing hormone related behavioral and neuroendocrine responses to stress in Lewis and Fischer 344 rats. *Brain Res*. 1992;570:54-60.

38. Dhabbar FS, McEwen BS, Spencer RL. Stress response, adrenal steroid receptor levels, and corticosteroid-binding globulin levels. A comparison between Sprague-Dawley, Fischer 344, and Lewis rats. *Brain Res*. 1993;616:89-98.