GENETICS OF ANXIETY IN GENETICALLY HETEROGENEOUS N/NIH AND INBRED ROMAN RATS: DIFFERENTIAL GENE EXPRESSION IN TARGET BRAIN AREAS

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1. Abstract /Summary

BACKGROUND
The identification of genes, or causal variants, influencing phenotypic variation of quantitative phenotypes is expected to provide insights into the etiology of complex traits, including psychological/behavioral (normal and abnormal) traits. However, the study of the genetic mechanisms underlying emotional responses such as anxiety, fear, frustration, or depression, still remains a big scientific challenge.

OBJECTIVE and METHODS
The main and overall aim of the present project was to investigate possible genetic mechanisms of (mainly, though not only) anxiety/fear and frustration by using the following methodological/technical approaches: 1) studies with the Roman (RHA and RLA) rat strains, genetically selected for their extreme differences in anxiety-related traits; 2) studies on the genetically heterogeneous N/Nih-HS rat stock; 3) phenotyping studies with these 3 rat strains/stocks, as concerns their respective anxiety/fear and frustration responses, stress responses (both endocrine and behavioral), impulsivity and novelty seeking profiles and vulnerability to drug (ethanol) preference; 4) genetic studies, after that phenotyping work, by microarray gene expression profiling and fine mapping of QTLs (quantitative trait loci).

RESULTS and CONCLUSIONS
We found that, besides being a very good model of anxiety/fear and stress susceptibility, the RHA and RLA rats are an excellent model of vulnerability to frustration. The N/Nih-HS rats are also very anxious and susceptible to stress. Genetic expression (microarray) studies show that differences in anxiety and frustration responses are associated with differential expression (between high anxious and low anxious rats) of several genes in the hippocampus and amygdala. Moreover, QTL fine mapping studies (in 1500 N/Nih-HS phenotyped rats) revealed a large number of significant QTLs that contain candidate quantitative genes (or causal variants), some of them having influence on anxiety-related (and on other neurobiological)
phenotypes. The present project produced relevant results related to novel (putative) genetic mechanisms which could be linked to anxiety/fear and frustration responses/processes or (other psycho-neurobiological) traits.

2. Results (main results of the project)

The results obtained in the present project can be summarized as follows:

1.- The Roman rat strains (RHA and RLA) show important behavioral differences in measures of frustration (successive negative contrast, extinction, ERPC, PREE), of unconditioned anxiety (successive alley, elevated zero-maze), of conditioned anxiety/fear (context-conditioned freezing, fear-potentiated startle), of depressive symptoms (forced swim test) or stress-induced hormonal responses, of novelty-seeking (hole-board, emergence "Y" test) or impulsivity, of preference for abused drugs (i.e. ethanol).

2.- The genetically heterogeneous N/Nih-HS rats show similar anxiety/fear responses to those of the (high anxious) RLA rats. Still more relevant is the fact that N/Nih-HS rats display stress-induced hormonal (ACTH, corticosterone, prolactin) responses and behavioral “depressive” symptoms (i.e. immobility in the forced swimming test) which are even higher than those of the (highly anxious) RLA rat strain. In summary, the heterogeneous N/Nih-HS rats (which are maintained only at our laboratory and at the Medical College of Wisconsin, USA) have for the first time been phenotyped behaviorally at our laboratory, as well as for their hormonal stress responses. We have reported that N/Nih-HS rats display (as a population) particularly elevated stress and anxiety/fear responses, as well as a passive coping style when facing conflict/stressing/inescapable situations.

3.- The Roman rats show differences in baseline (whole) brain expression of the CAMKK2, EPXH2, PRL, CRHBP and HOMER3 genes.
4.- When submitted to a subtle and “frustrating” incentive-loss experience (successive negative contrast), the Roman rats displayed differences in hippocampal expression of the TAAR2, THGAP1, PKD2L1 and NANOS genes.

5.- RLA-I rats show the partial reinforcement extinction effect (PREE), which is not present in RHA-I rats. These differences are paralleled by divergences in hippocampal gene expression of genes related with molecular-cellular communication processes (e.g. GH1, SH3RF1, F10, TLR3, ZEB2, PRKCD, IL22RA2, KL, CHRNA3, SHOX2, PRRX2, LECT1, SOX17, ADIPOQ), sensorial perception (Pgr35), stress (GH), addiction (Vcsa2, Phkg1, SNCG, Gpr35), anxiety/fear (EPXH2, SNCG), learning and memory (Kcnj13, EPHX2), and neuropsychiatric disorders (C1qtnf3, Ms4a7, SNCG, Birc6, Gpr35, PCDHGA10, SNCG).

6.- Gene expression (microarray) results in amygdala and hippocampus from N/Nih-HS rats selected for their extremely “high” or “low” anxiety profiles reveal differential expression of many genes having CNS (central nervous system) functions. The papers by Díaz-Morán et al. (see “Publications” below in this report) and her doctoral dissertation show several hundreds of genes differentially expressed in amygdala and hippocampus of N/Nih-HS rats as a function of their anxiety trait levels. There are 61 differentially expressed genes (with CNS functions) in amygdala, and another 24 CNS active (differentially-expressed) genes in the hippocampus, between anxiety-divergent N/Nih-HS groups. Of notice are the Prl5a2 and Prl4a1 genes, which regulate the functions of prolactin, a hormone which in turn has been genetically related to conditioned anxiety in the shuttle-box (i.e. in two way active avoidance acquisition) and to stress responses. From these studies we proposed several genes as candidates for anxiety regulation in amygdala, i.e. Avpr1b, Arr3, Prl4a1, Ucn3, Tacr3, Oxt and H2-M9, which are related to neuroendocrine and anxiety/fear/stress-related functions. The genes Avpr1b, Accn3, Cd74, Ltb, Nrg2, Oprdl1, Slc10a4 and Slc5a7 are differentially expressed in the hippocampus as a function of divergent anxiety/fear levels in N/Nih-HS rats.
7.- We performed a QTL fine mapping study (see Baud et al. Nature Genetics 45(7): 767-775) in 1500 phenotyped N/Nih-HS rats, as well as the complete genome sequencing of their 8 founder strains, and more than 300 QTL were identified for the over 150 measured phenotypes (behavioral, anxiety, fear, and many biological disease-related phenotypes). Over a dozen of these QTLs significantly influence anxiety-related responses. Examples are: 1) QTLs for several shuttle-box acquisition measures in chromosome 1, containing genes related with “arginine-vasopressin”. 2) QTLs containing “MAP-kinase” genes, in chromosome 5, related to conditioned fear and response latency in the shuttle-box. 3) A potent QTL for shuttle-box acquisition (anxiety) in chromosome 2, containing the “catenin delta-2” gene. 4) Several QTLs in chromosome 11, containing dopamine receptor-related genes, which are related to shuttle-box avoidance acquisition. “Merge analysis”, i.e. the combined analysis of QTL fine mapping and genome sequencing (of the 8 founder strains) results (see Baud et al. Nature Genetics, 2013, below), allowed us to propose various candidate quantitative trait genes for several complex traits (of those measured in the sample of 1500 N/Nih-HS rats), including some anxiety-related traits. In particular, the “Catenin delta-2” gene is proposed as a candidate gene for anxiety/fear-related responses (as well as for glucose tolerance). Analysis of results from that study is still under way for many traits/phenotypes and QTLs, and for possible candidate quantitative genes (within the European consortium EURATRANS). This has led to the discovery of several quantitative genes for various biological phenotypes measured in the same sample of 1500 N/Nih-HS rats (as can be seen below in the Publications section, see Tuncel et al. 2014, and Alam et al. 2014). This continuing effort will surely make it possible to establish other “candidate quantitative genes” for anxiety/fear-related responses, as well as for many other phenotypes of those measured in our large N/Nih-HS rat simple.

3. Relevance of the results

The study of the genetic mechanisms with influence on anxiety/fear, frustration or depression is still a challenge for scientific research. Loss
experiences in humans are among the main sources of stress and conflict (anxiety), leading frequently to mental disorders (anxiety, post-traumatic stress disorder, addiction, etc). Animal models of anxiety/fear, frustration, stress/depression, are essential to reproduce (assuming and knowing the limits of using laboratory animals) anxiogenic or stress-inducing traumatic life events under controlled laboratory conditions, so that genetic parameters (e.g. gene expression profiles) can be measured and related to the behavioral measures. Animal models are important tools, not only for basic science but also for applied science, as they lead to valuable knowledge on the biological/neurochemical/genetic basis of the above mentioned emotional/psychological processes, bearing in mind the conserved similarities between animals and humans with regard to many basic brain processes such as emotions, motivations, learning, memory etc. The RHA-I and RLA-I rat strains constitute a good model for neurobiological and neurogenetic studies of (normal and abnormal) behavior. Our work, within the context of the present project from La Marató de TV3, reveals that RLA/RHA rats are an excellent model of differential vulnerability to frustration (as well as to anxiety/fear and stress), and a very useful tool for research on the underlying genetic mechanisms of emotional responses through gene expression (microarray) techniques or ARNm sequencing (for instance). Using both rat strains we have shown that some hippocampal genes could be involved in the regulation of vulnerability to frustration. Moreover, from microarray and QTL fine mapping studies we have reported that several genes (some of them located at the amygdala and/or hippocampus) are related to the differential anxiety/fear responses of high and low anxious heterogeneous N/Nih-HS rats. Our work has demonstrated, in addition, that the genetically heterogeneous N/Nih-HS rat stock constitutes a unique tool for quantitative genetic (QTL) studies devoted to finding quantitative trait genes, even allowing gene-level resolution as in the case of the “catenin delta-2” gene for anxiety and various other genes for other biological phenotypes/trait (see Baud et al. 2013; Alam et al. 2014; Tuncel et al. 2014, in Publications below). That work and all QTL and genetic results are available to the international scientific community. It is to be expected that this will make it possible to test many hypotheses on
putative quantitative trait genes, besides those which could be related to anxiety/fear. We are still working on these.

In summary, the research carried out in this project produced relevant progress in the field of the putative genetic mechanisms of emotional, anxiety/fear-related traits, frustration responses, drug-seeking behavior and other complex traits.

4. Publications (ACKNOWLEDGING THE PROJECT)


Relationship between etanol preference and sensation/novelty seeking. 
*Physiology & Behavior* Accepted for publication.


Palencia M, Diaz-Moran S, Carme Mont-Cardona¹,², Toni Cañete¹, Gloria Blázquez¹, Esther Martínez-Membrives¹, Regina López-Aumatell¹,², Adolf


Other articles (acknowledging the project).-

Doctoral dissertations

Doctorand: Lidia Manzo Rodríguez
Directors: Carmen Torres Bares and José Enrique Callejas-Aguilera
Title: Factores de riesgo asociados con el consumo de alcohol en ratas Romanas de Alta (RHA-I) y Baja (RLA-I) Evitación
Classification: excellent cum laude. Awarding body: University of Jaén.
Date: November 2012

Doctorand: Marta Sabariego Almazán
Directors: Carmen Torres Bares and Ignacio Morón Henche
Title: Correlatos genéticos y conductuales de la frustración. Estudios con ratas Romanas de Alta (RHA-I) y Baja (RLA-I) Evitación
Classification: excellent cum laude (international mention). Awarding body: University of Jaén
Date: September 2013

Doctorand: Lucas Cuenya
Directors: Alba Mustaca and Carmen Torres
Title: Diferencias individuales en las respuestas ante el cambio de refuerzo
Classification: excellent cum laude (unanimous). Awarding body: National University of Córdoba (Argentina)
Date: 2012

Doctorand: Sira Díaz Morán
Director: Alberto Fernández Teruel
Title: Temerosidad en ratas heterogéneas (N/Nih-HS) y Romanas (RHA/RLA): Estudios hormonales y de expresión génica diferencial. (Tesis publicada en TDX; Servei de Publicacions de la UAB, 2012).
Classification: excellent cum laude (unanimous). Awarding body: Universidad Autónoma de Barcelona (UAB)
Date: October 2012