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Brain distribution of genes related to changes in locomotor activity

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ABSTRACT

The relationship between genes and behavior, and particularly the hyperactive behavior, is clearly not linear nor monotonic. To address this problem, a database of the locomotor behavior obtained from thousands of mutant mice has been previously retrieved from the literature. Data showed that the percent of genes in the genome related to locomotor hyperactivity is probably more than 1.56%. These genes do not belong to a single neurotransmitter system or biochemical pathway. Indeed, they are probably required for the correct development of a specific neuronal network necessary to decrease locomotor activity. The present paper analyzes the brain expression pattern of the genes whose deletion is accompanied by changes in locomotor behavior. Using literature data concerning knockout mice, 46 genes whose deletion was accompanied by increased locomotor behavior, 24 genes related to decreased locomotor behavior and 23 genes not involved in locomotor behavior (but important for other brain functions) have been identified.

These three groups of genes belonged to overlapping neurotransmitter systems or cellular functions. Therefore, we postulated that a better predictor of the locomotor behavior resulting from gene deletion might be the brain expression pattern. To this aim we correlated the brain expression of the genes and the locomotor activity resulting from the deletion of the same genes, using two databases (Allen Brain Atlas and SymAtlas). The results showed that the deletion of genes with higher expression level in the brain had higher probability to be accompanied by increased behavioral activity. Moreover the genes that were accompanied by locomotor hyperactivity when deleted, were more expressed in the cerebral cortex, amygdala and hippocampus compared to the genes unrelated to locomotor activity. Therefore, the prediction of the behavioral effect of a gene should take into consideration its brain distribution. Moreover, data confirmed that genes highly expressed in the brain are more likely to induce hyperactivity when deleted. Finally, it is suggested that gene mutations linked to specific behavioral abnormalities (e.g. inattention) might probably be associated to hyperactivity if the same gene has elevated brain expression.

1. Introduction

The current tendency of biological psychiatry to focus on the genetic substrates of mental health and disease has driven much research to find gene mutations in different mental diseases, hoping to predict the risk to develop a mental illness or to find new pharmacological targets [1,2] (see the work by C. Lombroso in the last century for a more historical perspective about this approach [3,4]).

The recent technology of transgenic mice allowed to apply this paradigm through the study of the behavioral effects of deletions, insertions or mutations of single genes in mice. The main conclusion drawn from the large amount of data now available is that the relationship between behavioral tendency and gene mutation/deletion is not linear nor monotonic, because the effects of multiple genetic changes may be completely different from the sum of the effects induced by individual genes, and therefore the results of multiple genetic modifications are often unpredictable [5,6].

Indeed, the number of transgenic mice produced up to now is very large: an Internet source lists at least 5283 different transgenic animals including animals with genes deletions (knockout mice; see http://www.informatics.jax.org/imsr/IMSRSearchForm.jsp).

Interestingly, most of these animals have been behaviorally tested, but a database describing/comparing the behavioral pattern of each transgenic mouse is not yet available (however, see ref. [7] and the database of references describing individual knockout mice available at http://www.bioscience.org/knockout/knochome.htm). Such a database would allow to study the relationship between genes and behavior, and the identification of the animals that show a specific behavioral deficit

Abbreviations: CB, cerebellum; CTX, cerebral cortex; HIP, hippocampus; HPF, hippocampal formation; HY, hypothalamus; LSX, lateral septal complex; MB, midbrain; MY, medulla; OLF, olfactory regions; P, Pons; PAL, Pallidum; RHP, retrohippocampal region; STR, striatum; TH, thalamus; AMY, striatum-like amygdala nuclei; SN, substantia nigra; FC, frontal cortex; MOB, medial olfactory epithelium; SC, spinal cord.

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within the entire set of transgenic models (metanalytical approach; see also Mouse Genome Informatics (MGI) projects [8] and the web site http://www.informatics.jax.org/phenotypes.shtml). The comparison of behavioral features of transgenic animals reported in different scientific articles should consider several problems, such as the differences in behavioral testing procedures (see e.g. [9]), the age and sex of the animals (see e.g. ref. [10,11]), sample size, rearing conditions such as number of animals per cage (see e.g. [12]) and the genetic background. All these parameters may affect the results and the reproducibility of data among laboratories [5].

A previous report classifying the behavioral activity of mutant mice based on numerous articles about transgenic mice, concluded that the number of genes linked to the increase of locomotor activity might be very high [13]. Specifically, data pertaining all transgenic mice with increased or decreased locomotor activity were retrieved and filtered to limit the effect of confounding variables. Unexpectedly, the results identified a very large number of genes involved in hyperactivity after exposure to a novel environment, which have been estimated to be at least 1.56% of the genes in the genome [13]. It is unclear why the mutation of so many genes can increase locomotor activity. Moreover, it was not possible to group all these genes in just one or few neurotransmitter systems or biochemical pathways [13]. Therefore, a working hypothesis suggested that all these genes were required for the correct development/functioning of a common neuronal network necessary to decrease a spontaneously occurring hyperactivity [13]. The present article tests this hypothesis through the analysis of brain expression of genes related to changes (increase/decrease) in locomotor behavior. To this aim a database of knockout mice tested for locomotor activity in a novel environment has been realized from published studies, filtered on the basis of inclusion/exclusion criteria. The knockout mice have been divided in hyperactive, hypoactive and normoactive groups on the basis of their locomotor behavior. Finally, the basal brain distribution of the genes knocked out in these groups using two databases available in Internet (Allen Brain Atlas and SymAtlas) has been studied.

2. Materials and methods

The complete list of genes described in this paper is reported in Table 1. This list has been derived from an initial set of 15,806 abstracts containing key words "locomotor", "activity", "mice", retrieved from PubMed (http://www.ncbi.nlm.nih.gov/sites/entrez/). This set was then filtered to include only the publications describing knockout mice and their locomotor behavior. These publications were then organized in a table containing: (i) the name of the gene knocked out, (ii) various parameters about the behavioral test, such as test duration, number of repetitions, size and shape of the arena, behavioral procedures, horizontal and vertical (rearing) activity, measurement system (photocells, video tracking etc), (iii) data about the sample, such as sample size, age and sex of the animals, type of control mice, genetic background, and (iv) statistical analysis (effect size expressed as % change compared to wt littermates and significance level).

Finally, the animals in this first table have been selected according to the following inclusion criteria:

(1) Age of animals from 2 to 6 months;
(2) Minimum 7 animals per experimental group, each individually tested;
(3) Experimental groups consisting of only males (no females);
(4) Behavioral observations about horizontal locomotor activity (changes in rearing frequency were not considered);
(5) Control mice consisting of wild type littermates;
(6) Genes had to be expressed in the brain (this information has been retrieved from the Allen Brain Atlas and SymAtlas, as described below).

The following exclusion criteria have been used:

(1) The behavioral data did not discriminate male from female mice as these were grouped together;
(2) Animals were not studied in a novel environment;
(3) Behavioral changes occurred only for very short intervals (less than 5 min) or after very long exposure to novelty (more than 30 min) or only after repeated exposures to novelty;
(4) Mice were prepuberal or aged;
(5) Inappropriate control groups (no wt-littermates) or small experimental groups with less than seven individuals;
(6) Genes were not expressed in the brain (this exclusion criterion was used to minimize the possibility that the alterations in locomotor behavior were due to systemic effects of the genetic deletion).

The investigations that fulfilled all these requirements were then used to categorize the locomotor activity. The animals were divided in three groups, according their locomotor activity:

(i) Increased activity or (ii) decreased activity, if locomotor activity changed with an effect size (increase or decrease respectively) greater than 20%; and (iii) normoactive animals, showing an effect size smaller than 10% compared to their wt littermate controls, which was not statistically significant (therefore animals showing no significant differences from controls but with an effect size greater than 10% were excluded too). Animals in the normoactive group satisfied the following additional criteria: 1) the targeted gene had no known "isoforms" which could replace its function, and 2) the targeted gene was involved in brain functions, since neurological/behavioral abnormalities (e.g. anxiety, memory deficit etc) were also reported.

Afterwards, the brain expression pattern of the candidate genes has been retrieved from two different databases: the Allen Brain Atlas (http://mouse.brain-map.org), a database describing the mRNA brain expression pattern of mouse genes based on the *in situ* hybridization technique and the SymAtlas (now available through the BioGPS portal http://biogps.gnf.org/) which quantifies mRNA expression pattern based on microarray technique.

Specifically, the Allen Brain Atlas has been used to retrieve two different mRNA quantifications from 17 different brain regions: the expression density and the expression level (http://mouse.brain-map.org/pdf/InformaticsDataProcessing.pdf) [14].

The expression level (L) is the product of the average pixel intensity (I) per total area of positive cells (a_g), normalized by the area of all cells (a_{max}): $L = I^*(a_g/a_{max})$.

The expression density (D) is the number of positive cells (n_g) normalized by the total number of cells (n_{max}) in the same region: $D = n_g/n_{max}$.

The expressions L and D were already quantified by Allen Brain Atlas database for the following brain regions: cerebellum (CB), cerebral cortex (CTX), hippocampal region (HIP), hippocampal formation (HPF = HIP + subiculum), hypothalamus (HY), lateral septal complex (LSX), midbrain (MB), medulla (MY), olfactory areas (OLF = olfactory bulb, anterior olfactory nuclei, and pyriform cortex), Pons (P), Pallidum (PAL), retrohippocampal region (RHP), striatum (STR), thalamus (TH), striatum-like amygdalar nuclei (AMY = central + anterior + medial amygdalar nucleus). Future studies will address possible subdivisions of these brain regions (in particular the cerebral cortex) in order to obtain spatially defined results.

The SymAtlas database has been used to retrieve the mRNA expression value from the following brain regions: CB, CTX, HIP, HY, substantia nigra (SN), OLF, STR, AMY, frontal cortex (FC), medial olfactory epithelium (MOB), preoptic nucleus (PO), and spinal cord (SC). The database used (Mouse GNF1M Gene Atlas) reported the mRNA expression from multiple probes normalized with the Robust Multi-array Analysis (gcmrna algorithm) [15,16].

Data were submitted to two-way analysis of variance group × brain region separately for the two databases. Posthoc comparisons were