2013

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Behavioural Brain Research 257 (2013) 39–48

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Review

Behavioral evaluation of eight rat lines selected for high and low anxiety-related responses

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HIGHLIGHTS

- Genetic models are powerful tools to help understand anxiety disorders.
- In order to determine the extent to which multiple anxiety traits generalize we compared eight genetic lines of rats selected for single high or low emotional responses.
- We find many behavioral traits generalize across different animal lines selected for a single trait.

ARTICLE INFO

Article history:
Received 9 January 2012
Received in revised form
12 September 2013
Accepted 13 September 2013
Available online 24 September 2013

ABSTRACT

Anxiety traits can be stable and permanent characteristics of an individual across time that is less susceptible of influences by a particular situation. One way to study trait anxiety in an experimental context is through the use of rat lines, selected according to contrasting phenotypes of fear and anxiety. It is not clear whether the behavioral differences between two contrasting rat lines in one given anxiety test are also present in others paradigms of state anxiety. Here, we examine the extent to which multiple anxiety traits generalize across selected animal lines originally selected for a single anxiety trait. We review the behavioral results available in the literature of eight rat genetic models of trait anxiety – namely Maudsley Reactive and Non-reactive rats, Floripa H and L rats, Tsukuba High and Low Emotional rats, High and Low Anxiety-related rats, High and Low Ultrasonic Vocalization rats, Roman High and Low Avoidance rats, Syracuse High and Low Avoidance rats, and Carioca High and Low Conditioned Freezing rats – across 11 behavioral paradigms of innate anxiety or aversive learning frequently used in the experimental setting. We observed both convergence and divergence of behavioral responses in these selected lines across the 11 paradigms. We find that predisposition for specific anxiety traits will usually be generalized to other anxiety provoking stimuli. However this generalization is not observed across all genetic models indicating some unique trait and state interactions. Genetic models of enhanced-anxiety related responses are beginning to help define how anxiety can manifest differently depending on the underlying traits and the current environmentally induced state.

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http://dx.doi.org/10.1016/j.bbr.2013.09.028

1. Introduction

Fear and anxiety are complex concepts, and refers to a set of emotional responses that are triggered when an individual faces a perceived threatening situation. Although these reactions clearly have well-defined and adaptive functions, they might also represent a pathological condition when occurs in an exaggerated fashion. The transient condition of fear and anxiety-related behaviors, that is only observable at particular moments and varies in intensity over time, is usually named state anxiety. On the other hand the affective characteristics of an individual across time are defined as a *trait*. Trait anxiety refers to a relatively permanent and stable characteristic that is less susceptible to influences by a particular state or situation. Since exaggerated fear and anxiety-related responses play an important role in the genesis of anxiety disorders, and share several overlapping neural circuitry [1], we will consider both reactions as a prominent components of the emotionality or anxiety construct.

In his classic work, Calvin Hall [2] employed the word "emotionality" to describe a set of defensive reactions that an animal presents in a potentially dangerous situation, such as an open field. Defecation in the open field was probably one of the first measures of animal emotionality, since this response is closely related with fear and autonomic arousal. Since then, several other animal paradigms of anxiety, mostly with rodents, have been developed (for a review see [3–6]). These tests consist of exposing an animal to an aversive environment while assessing one or a set of defensive behaviors, according to the innate or learned state characteristics of the threatening situation. As in the clinical setting, the traditional view that highlighted these experimental models was that animal defensive behaviors were mediated by a single and general construct [2]. However, as new data were collected, it became clear that animal defensive responses are mediated by a complex and multidimensional construct, i.e. the different aversive paradigms may assess different forms of anxiety [7–10]. Also, several studies showed that different inbred strains of mice and rats could be extremely different in their innate response to the same anxiety test [9]. Since one given model can easily detect difference among populations, it is clear that these defensive behaviors are influenced by genetic factors. Thus, it is very likely that both state and trait anxiety are under genetic influences. For a review concerning the comparison of different rat strains and also mutant mice see [9,11].

A genetic model traditionally used in the experimental context is lines of animals artificially selected for a particular anxious phenotype. Bidirectional selective breeding of a defensive response or any other phenotypic characteristic is a technique in which animals are bred in order to modify the frequency of the genes that underlie a particular phenotype [12]. The assumption is that after several generations of selection, the phenotypic contrast between the high and low lines will be maximized based on the effects of the genes that facilitate either the high or the low phenotypes and were polymorphic within the initial founding population. The development of bidirectional lines or strains of animals with high and low levels of emotional reactions associated with a threatening situation began in the middle of the 20th century and since then, a relatively large number of different genetic models based on this strategy have been developed [13–16]. Selection only acts upon genes that vary within a given population and different genetic models were originated from populations with highly distinct genetic backgrounds. Thus, it is likely that different genetic models of emotionality developed by different laboratories do not represent exactly the same set of genetic or neurobiological components of defensive responses. This would make, consequently, each pair of selected lines a special and unique biological system (see below).

Importantly, a selective breeding procedure aiming the creation of genetic models for trait anxiety attempts to modulate only one particular dimension of the anxiety construct. For example, one can modulate (through selective breeding for high and low levels) active avoidance behavior. The question that remains is if another dimension of the anxiety construct, like open field inner locomotion, will also be modulated in the same direction in the same genetic model i.e. the susceptibility for a specific anxiety-like behavior necessarily means susceptibility to other anxiety or fear provoking situations? An analysis of the phenotypic responses of genetic lines to different measures of state fear and anxiety could provide some answers to this question. In this sense, the rationale of the present study was to determine the extent to which multiple anxious or fearful traits generalize across different animal lines selected for a single anxiety trait.

Behavioral results reported in the literature of 8 rat genetic models with distinct selection criteria with regard of innate anxiety or aversive learning paradigms, across 11 animal tests of anxiety widely used in rodents, are reviewed and summarized in Table 1. Since these models were developed in different laboratories in different times, and the designation of some of them has changed, we also provided below a small historical review of how each line was initially developed and further characterized concerning its original hypothesis of emotionality.

2. Maudsley Reactive and Non-reactive rats

Broadhurst, at the Maudsley Hospital, University of London, began in 1954 the development of two lines of rats based on the procedure of Hall [2], who used the number of fecal boli excreted in the open field as a measure of emotionality in rats. The lines were named Maudsley Reactive (MR: high-defecating; i.e., high anxiety-related response) and Maudsley Non-reactive (MNR: low-defecating; i.e., low anxiety-related response). After only four generations of mating male and female rats with the highest and lowest rates of defecation in the open field, differences between MR and MNR rats were found to be consistent [17,18].

Table 1
Behavioral profile of eight genetic models (columns) across eleven animal tests of anxiety (lines). The results for rats selectively bred for high anxiety-related responses are always presented first in relation to the counterpart animals. White cells indicate that differences between the two groups are in the right direction. Cells filled with a dotted pattern indicate mixed results. Black cells indicate that the result challenged some aspect of the genetic model (i.e., motor effect, no differences between the two groups, or differences in the opposite direction). Superscript numbers indicate the bibliographic references of the behavioral result. M, male; F, female; FP, female proestrus; FD, female diestrus.

Animal model of anxiety-like behavior	Maudsley Reactive and Non-Reactive rats	Floripa High and Low rats	Tsukuba High and Low Emotional rats	High and Low Anxiety-related Behavior rats	High and Low Ultrasonic Vocalizations rats	Roman High and Low Avoidance rats	Syracuse High and Low Avoidance rats	Carioca High and Low Conditioned Freezing rats
Open field								
Defecation	Parameter employed to create the line	Floripa L = Floripa H[9] Floripa L > Floripa H[10]	THE > TLE[11]	--------	--------	RLA = RHA[32] RLA/Verh > RHA/Verh[33]	SLA/Bru < SLA/Bru[41]	--------
Ambulation	MR < MNR[1]	Parameter employed to create the line	THE < TLE[11]	HAB < LAB[19]	USV High < USV Low[23]	RLA < RHA[32] RLA/Verh < RHA/Verh[33]	SLA/Bru = SHA/Bru[41]	--------
Elevated plus maze								
% Time in Open Arms	MR < MNRA[5] MR/Har = MNRA/Har[6]	Floripa L < Floripa[9] F: Floripa L < Floripa H[10] M: Floripa L = Floripa H[10]	--------	Parameter employed to create the line	USV High < USV Low[24] USV High = Random[24]	RLA/Verh < RHA/Verh[34] RLA/I < RHA/I[35, 36] RLA/Verh > RHA/Verh[37]	--------	CHF < Control[42] CHF < CLF[43]
Closed arm entries	MR/Har > MNRA/Har[6]	Floripa L < Floripa H[9, 10]	--------	HAB = LAB[17, 18]	--------	RLA/Verh < RHA/Verh[33]	--------	CHF = Control[42] CHF = CLF[43]
Light-dark box								
Time in the light compartment	--------	Floripa L < Floripa H[9]	--------	HAB < LAB[17]	--------	RLA/Verh < RHA/Verh[38] RLA/Verh > RHA/Verh[37]	--------	--------
Locomotor activity	--------	Floripa L < Floripa H[9]	--------	HAB < LAB[17]	--------	--------	--------	--------
Social interaction								
Social activity	--------	--------	--------	HAB < LAB[17]	PF:USV High > USV Low[23] DF:USV High = USV Low[23] M:USV High = USV Low[23]	RLA/Verh = RHA/Verh[37, 38]		CHF < Control[41]
Locomotor activity	--------	--------	--------	HAB < LAB[17]		RLA/Verh = RHA/Verh[37, 38] RLA/Verh < RHA/Verh[38]		CHF = Control[41]
Ultrasonic vocalization								
Frequency	MR/N > MNRA/N[4]	--------	THE > TLE[12]	HAB > LAB[20]	Parameter employed to create the line	--------	--------	--------
Acoustic startle response								
Habituation	MR/Har > MNRA/Har[8]	--------	--------	--------	--------	--------	--------	--------
Sensitization	MR/Har < MNRA/Har[6]	--------	--------	HAB < LAB[18]	--------	RLA/Verh > RHA/Verh[25]	--------	--------
Fear-potentiated startle								
Startle amplitude	MR/Har = MNRA/Har[6]	--------	--------	--------	--------	RLA/Verh > RHA/Verh[26]	--------	--------
Active avoidance								
Two-way	MR < MNRA[7] MR/Har = MNRA/Har[6]	--------	THE < TLE[13]	--------	--------	Parameter employed to create the line	Parameter employed to create the line	--------
One-way	--------	--------	--------	--------	--------	Only *1 sec in the safe compartment:* RLA/I < RHA/I[28, 29]	--------	--------
Passive avoidance								
Step-down	--------	--------	THE > TLE[14]	--------	--------	--------	--------	--------
Step-through	--------	--------	THE = TLE[15]	--------	--------	--------	--------	--------
Conditioned emotional response								
Supression ratio	MR > MNR[2] MR/Har > MNRA/Har[3]	--------	THE = TLE[16]	--------	--------	RLA/Verh > RHA/Verh[27]	SLA/Bru > SHA/Bru[39, 40]	--------
Conditioned freezing								
Context	--------	--------	--------	HAB = LAB[18] HAB > LAB[22]	--------	RLA/Verh > RHA/Verh[26, 30] RLA/I > RHA/I[31]	--------	Parameter employed to create the line
Discrete CS	--------	--------	--------	HAB = LAB[21]	--------	RLA/Verh > RHA/Verh[28, 30]	--------	--------

Note: 1, Imada [96]; 2, Singh [97]; 3, Commissaris et al. [98]; 4, Insel and Hill [99]; 5, Overstreet et al. [100]; 6, Paterson et al. [101]; 7, Broadhurst and Levine [102]; 8, Commissaris et al. [103]; 9, Ramos et al. [26]; 10, Hinojosa et al. [104]; 11, Kitaoka and Fujita [105]; 12, Naito et al. [106]; 13, Fujita and Katayama [107]; 14, Miyamoto and Fujita [108]; 15, Wada and Makino [109]; 16, Fujii et al. [110]; 17, Henniger et al. [111]; 18, Yilmazer-Hanke et al. [112]; 19, Liebsch et al. [31]; 20, Wigger et al. [113]; 21, Muigg et al. [74]; 22, Frank et al. [114]; 23, Zimmerberg et al. [115]; 24, Ditcher et al. [116]; 25, Schwegler et al. [117]; 26, López-Aumatell et al. [118]; 27, Ferré et al. [119]; 28, Morón et al. [120]; 29, Torres et al. [45]; 30, Aguilar et al. [7]; 31, Escorihuela et al. [121]; 32, Broadhurst and Bignami [35]; 33, Gentsch et al. [122]; 34, Meyza et al. [123]; 35, Driscoll et al. [124]; 36, Escorihuela et al. [37]; 37, Chaouloff et al. [125]; 38, Steimer and Driscoll [73]; 39, Brush et al. [126]; 40, Gupta and Brush [127]; 41, Brush et al. [128]; 42, Dias et al. [129], 43, Hassan et al. [130].

In the early 1960s, Broadhurst distributed these lines to investigators in North America, such as Sudak and Maas [19] at the National Institutes of Health (NIH; sublines designated MR/N and MNR/N) and Harrington [20–22] at the University of Northern Iowa. The latter actually received one reactive (designed MR/Har) and two separate non-reactive (designed MNR/Har and MNRA/Har) sublines from Broadhurst. The Harrington colony was later relocated to Lafayette Clinic, Detroit. From this stock, the Maudsley sublines were sent to Blizard [23] at Wake Forest University and Satinder [24] at Lakehead University in Canada (sublines designated MR/Har/Lu and MNR/Har/Lu). In 1987, the original MR and MNR lines developed in London were terminated but were reimported later with the MR/Har and MRNA/Har lines and have been employed in numerous studies [25].

3. Floripa High and Low rats

In 2003, Ramos and colleagues reported the development of two new rat lines selectively bred for high and low locomotion in the central aversive area of the open field [26]. Initially, they produced a highly heterogeneous population through an intercross of three rat strains (i.e., Wistar, Hooded, and Lewis) and then initiated selective breeding of male and female rats for the lowest and highest scores of central open field ambulation. These lines were named Floripa[1] Low (L: low locomotion in the central area; i.e., high anxiety-related response) and High (H: high locomotion in the central area; i.e., low anxiety-related response) rat lines. After four generations of selection, a difference between the Floripa L and H rat lines in locomotor activity within the center of an open field was observed. As expected, the L line consistently displayed lower locomotion in the central area of the open field than rats of the H line. Floripa L lines also exhibited lower locomotion in the periphery of the open field (i.e., where the animal concentrates most of its activity) compared with the H line.

4. Tsukuba High and Low Emotional rats

In 1975, Fujita reported the development of two new lines of animals with high and low emotional reactivity [27,28]. Similar to the Floripa H and L animals, locomotion was also employed as the selection criterion. However, a different perspective was adopted. In its natural habitat, the rat which easily emerges from its burrow and explores its surroundings might be less anxious or emotionally reactive than another animal that prefers its burrow. An apparatus that simulates this situation in a laboratory setting and used for the bidirectional selection of these two lines consisted of a dark starting box (7 cm × 7 cm) with a small exit to a bright straight runway (120 cm long × 20 cm wide × 45 cm high). According to this procedure, each animal is placed in the dark starting box, and 30 s later the door is opened so that the animal has access to the runway. Each test lasts for 5 min, and animals are tested for 3 consecutive days. Male and female rats with the lowest and highest ambulatory activity scores in the runway are then mated. After 34 generations of inbreeding (brother and sister mating), two strains with significant differences in activity in the runway test were defined as Tsukuba High Emotional (THE: low ambulatory activity in the runway; i.e., high anxiety-related response) and Tsukuba Low Emotional (TLE: high ambulatory activity in the runway; i.e., low anxiety-related response). It was found sex effects and sex-line interaction. As expected, THE rats showed higher latencies in leaving the start box, taking more time to arrive at the end of the runway. Most initial research with these strains has been performed in the strain's country of origin, Japan, and a review with a large amount of physiological and behavioral data was published by Fujita et al. [29].

5. High and Low Anxiety-related Behavior rats

In 1998, Landgraf and colleagues [30,31], reported the creation of two lines of Wistar rats based on open arm entries in the elevated plus maze. The percentage of time spent on the open arms was employed as the main criterion for bidirectional selection. Other open-arm parameters were also employed in the following rank order: percentage of entries into the open arms > number of full open arm entries > latency to first open arm entry. Only animals with average activity scores (distance traveled) were selected. Beginning in 1993, male and female rats with the lowest and highest proportion of open arm scores were mated together to establish the two lines now termed High Anxiety-related Behavior (HAB: low proportion of open arm scores; i.e., high anxiety-related response) and Low Anxiety-related Behavior (LAB: high proportion of open arm scores; i.e., low anxiety-related response). No sex effects were observed in the elevated plus maze.

6. High and Low Ultrasonic Vocalization rats

To investigate generational and developmental variables associated with anxiety, Brunelli et al. [32,33] reported the creation of two lines of rats selected for different rates of USVs in response to isolation. Rat pups were screened at 10 ± 1 days of age in a 2 min isolation test. Male and female pups with the highest and lowest rates of USV were selected for later breeding. After only one generation, the High line presented more USVs than the Low line. After three generations, the Low and High lines diverged significantly from each other in their USV responses rates and from control animals that were mated randomly. This selection program was the first successful study that attempted to selectively breed a neonatal phenotype among rats and has been termed USV High (high neonatal isolation-induced USV; i.e., high anxiety-related response) and USV Low (low neonatal isolation-induced USV; i.e., low anxiety-related response).

7. Roman High and Low Avoidance rats

In 1961, Bignami started a selective breeding program with Wistar rats for low and high rates of two-way avoidance. The animals were subjected to five daily sessions of 50 trials, with 30 s between trials. Each trial consisted of a light CS that preceded the onset of a footshock US. The occurrence of a crossing response from one side to the other side of a shuttle box during the CS terminated the CS and avoided the US. If the response occurred after the onset of the US, then both the CS and US were terminated. Male and female rats with the lowest and highest rates of avoidance were selected and mated together while avoiding inbreeding. After five generations, the two selected lines differed markedly (at least threefold differences) in the number of avoidance responses, with no sex differences [34]. The lines were named Roman Low Avoidance (RLA: low rates of two-way avoidance; i.e., high anxiety-related response) and Roman High Avoidance (RHA: high rates of two-way avoidance; i.e., low anxiety-related response). In 1964, these two were transferred to England, from which they were distributed to various laboratories [35]. One of the most well-known colonies was established in 1972 at the Institut für Verhaltenswissenschaft, Zürich, Switzerland. The two sublines were named RLA/Verh and RHA/Verh and have been continuously bred since then, initially by Bättig, and later by Driscoll [36]. In parallel with the RLA/Verh and RHA/Verg sublines, an inbreeding program was initiated in 1993,

[1] Floripa is the short name for the city of Florianópolis.

leading to the creation of the RLA/Verh (RLA I) and RHA/Verh (RLA/I) inbred lines [37].

8. Syracuse High and Low Avoidance rats

In 1965, Brush started a selective breeding program with Long–Evans hooded rats, also based on low and high rates of two-way avoidance [38]. Similar to Bignami's Roman lines, Brush's animals were required to cross from one side to the other side of a shuttle box to avoid an electrical footshock. However, Brush's procedure was slightly different from Bigami's and had only a single test session composed of 10 pretest trials, in which the CS was presented alone with an intertrial interval of 120 s. Immediately after the 10 pretest trials were 60 training trails, in which the CS was followed by the US. The warning CS was a compound auditory and visual stimulus that lasted for 5 s, whereas the US was a low-intensity footshock (0.25 mA). Male and female rats with the lowest and the highest avoidance responses during the 60 trials and that met the pretest criteria (response latencies less than 5 s on fewer than five of the 10 pretest trials and on fewer than three of the last five pretest trials) were selected and mated together.

In 1979, Brush and colleagues reported the results of 25 consecutive generations [39]. Similar to the study by Bignami [34], the two selected strains differed markedly in the number of two-way avoidance responses after five generations, with no sex differences. These strains were named Syracuse Low Avoidance (SLA/Bru: low rates of two-way avoidance; i.e., high anxiety-related response) and Syracuse High Avoidance (SHA/Bru: high rates of two-way avoidance; i.e., low anxiety-related response).

9. Carioca High and Low Conditioned Freezing rats

Some studies have indicated that conditioned freezing is a highly heritable response that can be rapidly selected [40]. Recently, Ponder and colleagues [41,42] succeeded in producing two mouse lines exhibiting high and low levels of conditioned freezing after a single generation of selective breeding. Indeed, in order to create a genetic model of enhanced fear learning, conditioned freezing in response to contextual cues previously associated with footshocks was used as the phenotype criteria for the development of the "Carioca[2]" rat lines. The breeding program began in 2006 by Landeira-Fernandez, at the Pontifícia Universidade Católica do Rio de Janeiro, Rio de Janeiro, Brazil. The basic protocol consisted of mating male and female albino Wistar rats with the highest and lowest conditioned freezing in response to the contextual cues of the experimental chamber where animals were exposed to three unsignaled electric footshocks on the previous day. Gomes and Landeira-Fernandez [43] found that after three generations, reliable differences between these two lines were already present, indicating a strong heritable component of this type of learning. Males consistently exhibit more conditioned freezing in response to contextual cues than females. The lines were named Carioca High Conditioned Freezing (CHF: high level of contextual freezing; i.e., high anxiety-related response) and Carioca Low Conditioned Freezing (CLF: low level of contextual freezing; i.e., low anxiety-related response).

10. The multidimensional aspect of anxiety

Based on the view that anxiety does not reflect a single or unitary process emphasizes the importance of developing different genetic models with distinct phenotype criteria. In this sense, the main goal of the present paper was to investigate whether a genetic model of a particular anxiety-like response would display similar results in other experimental paradigms that also require the expression of a different defensive response, including phenotypic comparisons with the recently developed CHF and CLF rats.

Studies employing multivariate statistics have been consistently employed to investigate whether different animal models of anxiety measure the same underlying latent factor [44]. These factor analysis studies indicated that different animal tests might assess different forms of anxiety. For example, File [4] showed that indices of anxiety derived from the elevated plus maze (i.e., number of entries into and time spent on the open arms), Vogel test (i.e., frequency of punished drinking), and social interaction test (i.e., time spent engaged in social interaction), loaded on three independent factors, suggested the existence of different forms of anxiety generated by each of these paradigms. Similarly, Belzung and Le Pape [8] found a weak correlation between the measures of anxiety in the elevated plus maze and in light–dark box. More details about similarities and differences between the elevated plus maze, light–dark box, and open field can be found in Ramos [44].

The same phenomenon occurs with the selected lines. As shown in Table 1 The hypothesized behavioral measure was not presented in the same direction among all genetic models. For example, divergent results were detected in the habituation, sensitization, and fear-potentiation of the acoustic startle response in the Maudsley rats. The Floripa lines also had inconsistent results with regard to open field defecation and the open arm parameters of the elevated plus maze. Tsukuba animals also presented opposite results in the acquisition of passive step-through avoidance and the suppression ratio of the conditioned emotional response. Results that corroborate to the multidimensional construct hypothesis were also found in HAB and LAB lines, when tested for fear sensitization of the acoustic startle response and conditioned freezing in response to contextual cues and a CS previously associated with footshock. The same happen with USV strains, in the open arm parameter of the elevated plus maze and social interaction test. The Roman strains also presented diverging results in anxiety tests. Finally, Syracuse rats also presented opposite results with regard to ambulation in the open field. These results clearly argue against the early conceptualization of emotional reactivity as a unitary construct and reinforce the approach that proposes that anxiety is a complex, multidimensional, and dynamic phenomenon [45,9,10].

The complex picture found in animal models of anxiety might reflect the clinical diversity generally found among human patients, in whom pathological anxiety is classified into several categories [46,47]. In this regard, experimental paradigms that generate behavioral inhibition caused by conflict between approach and avoidance tendencies are sensitive to some drugs as benzodiazepine compounds. These animal models also indicated that substances that decrease serotonergic activity increased anxiety, whereas those that increase serotonergic neurotransmission produced anxiogenic effects. In contrast, other animal models that require vigorous escape responses to proximal aversive stimuli appeared to be resistant to benzodiazepine drugs, whereas substances that increased serotonergic activity produced an anxiolytic effect [48].

Different neural circuitries also appear to be involved in distinct dimensions of anxiety. Gray and McNaughton [49] argued that the septo-hippocampal system contributes to the cognitive component of anxiety (worry), whereas the amygdaloid complex and its projections to the ventral portion of the periaqueductal gray are critically involved in the regulation of defensive freezing behavior in response to innate or conditioned aversive stimuli [50]. Active defensive responses to proximal stimuli, generally associated with nociception, appear to involve the dorsal portion of the periaqueductal gray and its ascending projections to forebrain structures

[2] Carioca is the name given to those born in Rio de Janeiro.

related to the sensorial processing of aversive stimuli [51]. Also, lesions studies revealed subtle differences between anxiety and fear responses. For example, lesions in the central amygdale (CeA) mitigate startle responses in models of conditioned fear but not in a light enhanced paradigm [52], whereas lesions in the bed nucleus of stria terminalis (BNST) showed opposite results [53]. These studies suggested independent circuitry for fear and anxiety responses in the startle response paradigm. For a review concerning the underlying emotional circuits of state × trait and fear × anxiety see [1].

11. The linkage of emotional traits

As shown in Table 1, none of the eight models, including the most traditional ones, such as Maudsley and Roman animals, were evaluated in all eleven paradigms. Therefore, additional experiments are necessary to further evaluate the behavioral profile of each of these pairs contrasting lines selectively bred for high and low anxiety-related behavior. However, although relatively incomplete, the behavioral results reported in the present work suggest that emotional systems share some features. For example, selection for the animals that show high defecation in an open field task (Maudsley lines) produces animals divergent for locomotion in the same test. The divergence in locomotion in the open field test was also observed for the Tsukuba, HAB and LAB, USV High and Low, and Roman lines. Some similarities were observed between Maudsley, Roman and Syracuse lines in the conditioned emotional response task, with the "anxious" lines showing a greater suppression ratio.

Also, the present review found a remarkable relationship between anxiety-like responses during early development and adulthood. The USV lines were created to produce a developmental-genetic model system. The hypothesis is that autonomic and behavioral temperamental differences in infancy might cause behavioral or autonomic nervous system dysfunction in adulthood [33]. The results appear to be encouraging because the USV High and Low lines selected for different rates of USV in response to isolation during infancy and tested during adulthood presented reliable differences in several animal models, such as the open field, social interaction test, and elevated plus maze. Moreover, MR, THE, and HAB pups consistently presented more USV isolation calls than their respective counterpart lines/strains.

The fact that differences in emotionality in adulthood might be already present early in development converges with results from clinical studies, which indicated that there is an influence of temperamental factors present in childhood on the development of anxious symptoms during adult life [54]. These results are also in agreement with the conceptual distinction between trait and state anxiety.

However, the genetic correlation between anxious and fearful behaviors are not a "all or nothing" kind of system [16]. Among all genes with effect on emotional responses, some have pleiotropic influences in several behaviors, whereas others are linked to very particular tests. In this sense, the overlap between these behaviors will depend on the genetic background of each population studied and are expected to be mostly partial only (see below). For detailed review of genes related to animal models of anxiety see [55–57].

12. Phenotype comparisons and methodological limitations

Importantly, one needs to be extremely careful when interpreting either the presence or absence of correlations/associations between two phenotypic traits (e.g., behavioral, anatomical, biochemical, etc.) in one or several pairs of selected lines. Therefore, a few genetic considerations about the selection method should be clarified. First, two pairs of rat lines that are selected in different laboratories will differ, not only with regard to the behavioral method used to select them, but also in the genetic characteristics of their initial populations. Therefore, even if the foundation rat lines have the same name (e.g., Wistar), which is obviously often not the case, because they are outbred, each sample of animals screened in the first generation (S0) of each study has different polymorphisms for different genes. Selection can only act upon the genes that vary (i.e., are polymorphic) in that specific population. Behaviors are almost always polygenic (i.e., they are influenced by myriad genes). Thus, if two genes, A and B, are equally relevant to a trait, but each of them is polymorphic only in one of the two starting populations, future differences between the lines will be related to gene A only in one line pair and related to gene B only in the other line pair. Therefore, if genes A and B act through different neurobiological mechanisms, then the two analogous genetic models (e.g., Maudsley and Roman) may display emotional similarities that are attributable to different underlying mechanisms. In conclusion, two traits that are correlated in one model and uncorrelated in another model, although they effectively share biological pathways, should not be surprising. Thus, two final lines could be equally fearful, for example, through different biological mechanisms.

Second, because of practical reasons, selection experiments in rodents can only be performed in relatively small samples of larger foundation populations. In such small samples, totally avoiding two genetic phenomena, namely genetic drift and inbreeding [58], is virtually impossible. Both of these factors can produce significant increases or decreases in allele frequency, possibly leading to fixation, differentially in either the high or low selected line (e.g., 100% of allele "A" in the high line and 100% of allele "a" in the low line), and this may occur in any gene that has absolutely no effect on the selected trait. Consequently, these lines may differ in innumerable behavioral, anatomical, and biochemical traits that have nothing to do with the desired phenotype (e.g., emotionality), similar to any random pair of unselected inbred strains. Thus, significant correlated traits may be spurious unless they are proven to appear in different independent selected studies, which was the case for several behaviors discussed above, or in different replicate lines of the same study [58].

Finally, the importance of linked genes should not be overlooked. Because genes lie on chromosomes and because the starting rat populations may not be highly outbred, two neighboring polymorphic genes, if in linkage disequilibrium, tend to pass their alleles on to the following generations as a "package" (i.e., allele "A" together with allele "B" and allele "a" together with allele "b"). If only the A/a variation is relevant to the selected phenotype, then the final high/low lines will differ also for the B/b polymorphism and all of the cascading phenotypes influenced by B/b, thus creating an additional false positive result and possibly leading the neuroscientist to believe that fearfulness somehow relates to all of these accidental phenotypic differences [59].

13. Problem of locomotor activity

One of the main potential problems of using animal models of anxiety is the interaction between the behavioral measurements of emotionality and the animal's locomotor activity. Indeed, motor effects have been found in the Maudsley (measured in the elevated plus maze), Floripa (measured in the elevated plus maze and light–dark box), HAB/LAB (measured in the light–dark box and social interaction test), and Roman (measured in the elevated plus maze) animals. In all of these cases, rats with high anxiety-related responses also exhibited a reduction in locomotor activity. These motor effects might represent an important confounding variable because the differences in emotionality among these animals might be at least partially explained by differences in locomotor activity.

Anxiety and locomotor activity are intimately associated. Most defensive reactions involve a decrease in exploratory ambulation and an increase in freezing behavior. Therefore, discarding any reasonable influence of locomotor activity on the occurrence of anxiety-like responses is almost impossible. Even in paradigms in which anxiety and motor indices are relatively well dissociated, such as in the elevated plus maze, unclear is how these performance variables may in fact interact in this animal model of anxiety. For example, hypoactivity in the elevated plus maze can overcome the detection of anxiogenic-like effects in some experimental manipulations [60] but not in others [61]. Moreover, a motor effect in the elevated plus maze can be part of the defense response to an anxiogenic compound [62].

Procedural manipulations can be made to estimate the possible modulatory effect of locomotor activity on defensive reactions. For example, Tsukuba [29], HAB/LAB [30] and Roman [63] animals did not exhibit any motor differences when measured under basal conditions in their home cage using a radiotelemetric system. Therefore, the motor effect observed in these genetic models of anxiety is not associated with general spontaneous locomotor activity but is a reaction to a possible threatening situation.

14. Sex differences

Although the main objective of the present review was not to make comparisons regarding sex effects among the selected lines, sex differences in anxiety-related behaviors are a known and well reported phenomenon, though it underlying basis are still weakly understood [64]. In fact, gender differences in emotionality have not been demonstrated in a consistent direction throughout several behavioral models. For example, in the open field males usually show less locomotor activity and higher defecation levels than females [5]. Such results are traditionally interpreted as an indicative that males are more “fearful” or “anxious” than females. Gender differences favoring males have been also observed in contextual fear conditioning [65,66] as well as in other spatial learning, such as the Morris water maze [67] and the 12-arm radial maze [68]. Tests carried in other three anxiety models (Social Interaction, Elevated Plus Maze and Vogel Conflict Test) also indicate sexual differences. Nevertheless, the differences varied throughout the tests, with females demonstrating less anxiety in the Elevated Plus Maze, and being more anxious in the Vogel Conflict Test [69]. Moreover, Blanchard and colleagues [70] showed that females are more anxious than males in situations of potential danger, such as in the presence of a cat (for a review, see [71]. In this sense, significant sex genotype interactions have been observed in several molecular and behavioral studies on emotional behaviors [72].

Regarding the selection criterion of the rat lines described in the present work, it was observed sex effects only in the Floripa H and L rats (selected for ambulation in the center of the open field), Tsukuba High and Low emotional rats (selected for locomotion in a runway) and Carioca High and Low freezing rats (selected for divergent conditioned freezing responses). In the other lines, subtle differences between females within the same selected line were reported. For example, it was observed that females from “Roman High Avoidance” rats are more active and less anxious in the proestrus in comparison to females in the diestrus [73]. However, in general, the behavioral profiles of males and females from the selected lines reviewed in the present work are in agreement with most previous data from the literature, with females showing more locomotion activity than males, being apparently less anxious in some particular tests.

15. Fear extinction

Impaired extinction of conditioned fear memories is a main feature of many anxiety disorders, including PTSD and specific phobias. The proper regulation of emotional expression under changeable environmental conditions is essential for mental health. Indeed, a substantial proportion of anxiety patients do not react effectively to standard behavioral treatments and/or pharmacological.

We found fear extinction studies with the HAB/LAB, Maudsley and Roman rat lines. Muigg et al. [74] reported that HAB rats showed a considerable deficit in the ability to extinguish the conditioned freezing response to the acoustic stimulus in comparison to LAB rats. Importantly, HAB and LAB animals presented the same freezing response during the acquisition of an aversive conditioning task in response to a tone paired with an electrical footshock. Sartory and Eysenck [75] showed that MR rats required more time to step-down from a grid previously associated with footshocks than MNR animals. However, in the same study they found no differences between RHA and RLA animals in a slightly different extinction procedure, with the latency to escape into a safe compartment as a measure of conditioned fear response.

16. Conditioned fear and the interaction between two-way avoidance and freezing responses

Historically, fear conditioning was thought to be associated with one of the main causes of pathological anxiety (i.e. neurosis [76,77]). In order to investigate the genetic basis of the conditioned fear in rodents, the two-way avoidance response has been the main phenotype criteria employed for developing bidirectionally selected lines or strains based on aversive learning paradigms. That is the case for the Roman and Syracuse animals discussed in the present paper and other lines, such as Australian [78], Koltushi [79] and Hatano [80] animals. Undoubtedly, these models helped to unravel several genetic aspects of the conditioned fear and others behaviors (see above).

However, the use of the two-way avoidance response as the phenotype criteria for the development of so many genetic models of fear conditioning is curious because the learning mechanisms involved in the acquisition of this response are still unclear. Avoidance is a complex form of learning which involves the acquisition of both an operant response and associative fear [81–83]. The interaction between these two processes may interfere with the reliable measurement of emotional responses mediated by associative learning. For example, manipulations that reduce conditioned fear – such as anxiolytic drugs [84], decreases in contextual fear conditioning [85] and reduction in shock intensity [86] – enhance the acquisition of active avoidance responses. In fact, two-way avoidance learning represents one of the oldest theoretical debates in behavioral sciences (for an elegant review of this debate, see [87]).

On the other hand, the conditioned freezing response could be considered a more direct measure of aversive learning. Contextual fear conditioning is a useful paradigm for studying long-term memory in animals and has been widely shown to be a reliable behavioral index of associative fear [88]. Anatomical and electrophysiological studies have described the neural circuitry involved in both CS and contextual fear conditioning, including the entire extent of sensory inputs to endocrine, autonomic, and behavioral outputs [89–91]. Long-term potentiation in the amygdala has also been shown to mediate the formation of fear conditioning [92,93], and isomorphism appears to exist between the freezing response to contextual stimuli paired with electrical shocks and generalized anxiety disorder [94]. Recently, Ponder et al. [41] reported that

several genes in the amygdala are differentially expressed when mice were bidirectionally selected for conditioned freezing. In this regard, the CHF and CLF lines may be considered a suitable model in the understanding of the pathophysiology of fear learning in rats, hence expanding our knowledge of the human generalized anxiety disorder. Because this is a recent genetic model that is still under development, further studies are needed to evaluate the behavioral profile of these two new lines of animals.

17. Conclusions

Considering the small effects of a large set of genes influencing emotional behavior, and also the limitation of the available animal models of anxiety, we are just beginning to identify the genetic underpinnings of the anxiety disorders [95]. In this context, the use of selected lines could be particularly useful in the search of candidate genes related with several behaviors associated with these pathologies. The view that anxiety does not reflect a single or unitary construct emphasizes the importance of developing different genetic models with distinct phenotype criteria. In fact, it is our hypothesis that the proper comparison and study of several genetic models will provide a more realistic perception of emotionality than the analysis of one single model. In the present review, we showed that predisposition for a specific anxiety trait will usually be generalized to other anxiety provoking stimuli. However, this generalization is not observed across all genetic models, indicating some unique interactions between trait and state anxiety. Selectively breed anxiety trait models tested against established behavioral models of state anxiety will continue to expand our knowledge about the genetic basis of the anxiety.

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