Genetic association between helpless trait and depression-related phenotypes: evidence from crossbreeding studies with H/Rouen and NH/Rouen mice

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Abstract

Genetic factors are believed to be involved in the aetiology of unipolar depressive disorders. We have previously described a model built up by selective breeding of mice with different responses in the tail suspension test, a screening test for potential antidepressants. In this model, helpless H/Rouen mice are essentially immobile in this test, as well as in the Porsolt forced-swim test, whereas non-helpless NH/Rouen mice show the opposite behaviour, i.e. very low immobility. However, it is unclear whether or not the other phenotypic differences (forced swim test, locomotor activity, sucrose test, sleep patterns, effect of fluoxetine) observed between H/Rouen and the NH/Rouen mice may be attributed to a genetic drift phenomenon during the selection step, rather than being related to the trait of selection. In this study we used reciprocal crossbreeding between H/Rouen and NH/Rouen mice and obtained a segregating F2 population in order to determine whether phenotypic differences between the two lines co-segregate with the trait of selection. In this study we used reciprocal crossbreeding between H/Rouen and NH/Rouen mice and obtained a segregating F2 population in order to determine whether phenotypic differences between the two lines co-segregate with the trait of selection. In the segregating F2 population, we found significant and strong genetic correlations between helplessness in the tail suspension test and some phenotypical features associated with depressive disorders such as ‘alterations of sleep patterns’, behavioural response to fluoxetine, immobility duration in the forced swim test, and anhedonia. Our results converge with clinical observations in depressed humans. These results strengthen the validity of the H/Rouen mouse as a model of depression, notably for preclinical studies with antidepressants. In addition, this model should open the way to identifying genes related to depression-like behaviours.

Introduction

The prevalence of depression worldwide is such that this disorder represents a major health problem. It is estimated that about 10% of men and 20% of women in Western Europe will suffer from a major depressive episode at some time in their life. WHO forecasts show that global morbidity statistics will class depressive illness in first position for women and second position (after cardiovascular illnesses) for men in Europe by 2020. Although antidepressants have been used in therapeutics for 50 yr, the physiopathology of depressive disorders remains poorly understood. In addition, the onset of antidepressant action is too long and treatment-resistant major depressive disorder continues to

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be very common. These facts emphasize the need of more potent and well tolerated antidepressants based on novel experimental grounds (Insel & Charney, 2003). The monoamine hypothesis of depression suggests that one of the biological bases of affective disorders is a deficiency in the neurotransmitter serotonin (5-HT). The selective serotonin reuptake inhibitors (SSRIs), in particular, have allowed important progress towards understanding the role of 5-HT in depression. Moreover, recent data point to alterations of different 5-HT receptors in the brain of depressed patients, especially 5-HTT receptors (Lemonde et al. 2004; Parsey et al. 2006; Rausch et al. 2006), 5-HT receptors (Svenningsson et al. 2006) and 5-HT receptors (Gurevich et al. 2002).

While the search for the biological basis of depression is intensifying, novel strategies for better therapies are eagerly requested. The demonstration of the existence of inherited inter-individual variations in vulnerability to depression (Fava & Kendler, 2000; Kendler et al. 1994; Levinson, 2006; Sham et al. 2000; Sullivan et al. 2000) has led to the development of selectively bred rat models (El Yacoubi & Vaugeois, 2007; Overstreet, 2002; Willner, 1990). Using a selective breeding strategy based on the divergence of behaviour in the tail suspension test (TST), we have previously described the behavioural, neurochemical and electrophysiological characteristics of a mouse model of depression (El Yacoubi et al. 2003). Duration of immobility is found to be markedly reduced in mice administered antidepressants, showing predictive validity in the TST (Cryan et al. 2005). Robust strain differences in baseline TST have been observed, although strain rankings varied across studies (Liu & Gershenfeld, 2001; Ripoll et al. 2003; Trullas et al. 1989; van der Heyden et al. 1987). Such data point to an underlying genetic basis for immobility and are potentially useful in investigating genes that are accountable for the helpless phenotype. The discovery of genes that confer vulnerability to unipolar disorder may be a critical step towards identification of biomarkers, elucidation of underlying pathophysiological mechanisms, and development of new therapeutic strategies.

Helpless H/Rouen mice are essentially immobile in both the TST and the Porsolt forced swim test (FST). They show anhedonia and exhibit sleep-wakfulness alterations resembling those classically observed in depressed patients (Benca, 2000; Lustberg & Reynolds, 2000). Furthermore, H/Rouen mice exhibit a decrease in brain serotonergic tone, which evokes that associated with endogenous depression in humans. Finally, both behavioural impairments and serotonergic dysfunction could be improved by chronic treatment with the antidepressant fluoxetine (El Yacoubi et al. 2003). The H/Rouen line of mice may thus provide an opportunity to approach genes influencing susceptibility to depression and to investigate neurophysiological and neurochemical substrates underlying antidepressant effects. Yet, a single pair of lines selected for high and low expression of a trait was used to study new behavioural characters thought to be associated with the selected trait. Nevertheless it is highly probable that genetic drift phenomena (Henderson, 1997) have occurred during the creation of the selected lines. Comparisons between extreme phenotypes ('high' and 'low' lines) selected on the basis of a single character ('helplessness') may frequently produce significant line differences even when there is no true association between the selected trait and the trait being studied. Since the level of inbreeding in selected H/Rouen and NH/Rouen lines is substantial, drift effects are greater, resulting in the likelihood of more false-positive associations (Henderson, 1997). Here, we have examined this hypothesis by performing reciprocal crossbreedings between H/Rouen and NH/Rouen mice in the production of a segregating F2 population. Our aim was to determine whether several phenotypic differences observed between the two parental lines have a genetic relationship to the trait of selection. First, we selected extreme phenotypes concerning immobility in the TST in the segregating F2 population: H F2 (helpless) and NH F2 (non-helpless). Second, we tested these mice in different paradigms which appeared to give significantly different phenotypes in the parental lines (El Yacoubi et al. 2003) such as sleep patterns, immobility in the FST, anhedonia, motor activity and behavioural response to the antidepressant fluoxetine. Any significant difference would indicate the presence of a genetic correlation between helplessness and the tested phenotype. A genetic drift would be inferred in the case of an insignificant difference between H F2 and NH F2 mice as opposed to significant differences observed in parental lines.

Method

Animals

Mice selectively bred in the animal facilities at the Faculty of Medicine and Pharmacy (Rouen, France) for high or low spontaneous ‘helplessness’ in the TST were derived from an original stock of outbred Swiss albino CD1 mice (Charles River, France). They were kept on a 12-h light/dark cycle (lights on 07:00 hours)
with food and water available ad libitum. For breeding, male and female mice were housed together in pairs. When not under experimentation, they were kept in same-sex groups. Testing was performed between 09:00 and 17:00 hours and was in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC).

Selection and breeding
As for parental lines (El Yacoubi et al. 2003), the selection criteria for the F2 generation after crossbreeding between H/Rouen and NH/Rouen mice were a high immobility score (>115 s) for helpless (H F2) and a low immobility score (<35 s) for non-helpless (NH F2) in the TST. The mice displaying intermediate scores in the TST were discarded from the study. Each mouse was tested three times at weekly intervals before any supplementary test.

For the segregating procedure, at approximately age 3 months, two cohorts of mice from the parental lines from generations S16–S19 were used after completion of the initial TST screenings. H/Rouen females were cross-mated with NH/Rouen males (H.NH F1 generation, 13 breeders), and vice versa NH/Rouen females were cross-mated with H/Rouen males (NH.H F1 generation, 12 breeders). Further, for the production of a segregating F2 generation, mice from the F1 generation were mated (105 breeders) at random. After weaning, F1 and F2 offspring were housed as described above.

Behavioural assessments

TST

The TST, which is the selection criterion, was performed with a computerized device (ITEM-LABO, France) (Steru et al. 1987). Mice were suspended by the tail with adhesive tape to a hook connected to a strain gauge. The strain gauge transmitted movements to a computer that calculated the total duration of immobility during a 6-min test. Mice that climbed up their tails during the test session were withdrawn from the study.

Other phenotypes tested in the F2 segregated population are given below.

FST

For the FST, mice were plunged individually into a vertical Plexiglas cylinder (25 cm high, 10 cm diameter) filled with water to a depth of 9 cm (21–23 °C). After the 6-min test period the mice were removed and allowed to dry. Immobility (i.e. making only minimal movements to keep the head above water or floating) was measured for two 3-min periods by an observer blind to the condition of the mouse.

Automated locomotor activity test

Exploratory motor activity was monitored in the 20 × 20-cm arena of a Digiscan actimeter (Omnitech Electronics Inc., USA) during 45 min (three continuous 15-min sessions) (El Yacoubi et al. 2000).

Sucrose consumption test

Voluntary sucrose consumption by individually housed mice was determined using a two-bottle sucrose preference test. Consumption of a 2% sucrose solution in water in one bottle and of water in the other bottle was measured (over an 8-d period) after a 6-d period of adaptation with switches between water and sucrose solution as the only fluid. Results are expressed as sucrose intake (g/kg of body weight) and as the percentage of sucrose consumed out of the total fluid drunk.

Sleep and wakefulness analysis

Electrodes for polygraphic sleep monitoring were implanted under general anaesthesia as described in detail elsewhere (Popa et al. 2005, 2006). Recordings were performed in the home cage, after allowing at least 10 d recovery, during which time they were habituated to the recording conditions (12-h light/dark cycle, lights on 07:00 hours, one animal per cage, connection to the recording devices by means of a cable and a swivel allowing free movement in the cage). Polygraphic recordings were performed using an Embla® system and Somnologica® software (Iceland). Spontaneous sleep-wakefulness cycles were recorded during 24 h for both males and females, beginning at 19:00 hours, i.e. at the onset of the dark period. On the second day at 10:00 hours, mice were gently awakened and left to sleep again, in order to evaluate the rapid eye movement (REM) sleep latency, defined as the time interval between sleep onset and the first REM sleep episode.

The EEG signal was processed for power spectra analysis (Franken et al. 1998; Lena et al. 2004; Popa et al. 2006). EEC spectrograms and power spectra for each frequency band were compared between the H F2 and NH F2 segregating population.

Polygraphic tracings were scored every 15 s as wakefulness, slow-wave sleep (light: SWS1, or deep: SWS2), and REM sleep (Popa et al. 2006) using Somnologica software.
For analysis of the spontaneous sleep-wakefulness patterns, the amounts as well as the mean duration and number of single episodes of vigilance states for each animal were determined for every hour throughout 24 h, and averaged over 2-h or 12-h cycles. Sleep fragmentation was assessed by calculating the mean duration of SWS episodes as well as the number of wakeful bouts (Dugovic et al. 1999).

**Antidepressant treatment**

Three-month-old mice from the F2 segregating population were treated daily at 10:00 hours with fluoxetine HCl (10 mg/kg i.p., free base dose; Lilly, USA) or saline for 21 d. Solutions of fluoxetine were prepared daily. At selected times during the treatment: at days 2, 8, 15 and 22 (one day after the end of the treatment schedule), mice were subjected to the TST between 13:00 and 16:00 hours.

**Statistical analyses**

Results are expressed as mean ± S.E.M. For sleep studies, the mean values are expressed as min ± S.E.M. Statistical analyses of differences between groups were performed using ANOVA (with one or two factors and with or without repeated measures where appropriate). Where F ratios were significant, statistical analyses were extended using Newman–Keuls multiple comparison tests or Student’s t test for comparison of means. Significance levels were set at p < 0.05. Sex and reciprocal breeding effects were performed using a one-way or a two-way ANOVA. When a sex effect was found, male and female data were analysed and presented separately.

**Results**

TST scores for H/Rouen, NH/Rouen, F1 intercrosses and F2 segregating populations

H/Rouen and NH/Rouen mice of selective breeding were used to generate intercrossed F1 generations. Crossbred offspring mice were 355 males (162 from NH.H and 193 from H.NH) and 379 females (145 from NH.H and 234 from H.NH). The numbers of mice that were identified in the TST are shown in Table 1. Significant (p < 0.001) gender differences were found; the helpless phenotype appeared more frequently in females than males (Fig. 1) and, conversely, the non-helpless phenotype was more frequent in males. Indeed, for the crossbreeding NH.H F1, the non-helpless phenotype was found in 21% of males and 5% of females, and the helpless phenotype was found in 2% of males and 16% of females. Corresponding percentages for the reverse crossbreeding H.NH F1 were 12% of non-helpless males and 1% of non-helpless females, and 3% of helpless males and 22% of helpless females. Furthermore, a reciprocal breeding effect was found (p < 0.01). Thus, with respect to the line of origin of the sire or the dam, there was significant difference concerning the distribution of despair-related behaviour in the F1 intermediate offspring (Fig. 1). As expected, the data showed robust line-specific differences between H/Rouen and NH/Rouen mice in despair-related behaviour, i.e. the duration of immobility in the TST. In contrast, male as well as female NH.H and H.NH intermediates of the F1 generation (crossbred offspring) displayed intermediate immobility scores in the TST (Table 1, Fig. 1).

The distribution of phenotypes in the TST assessed as immobility times for mice with stable behaviour...
belonging to the segregating F2 generation of cross-breeding is shown in Fig. 2. The total number of tested male mice was 1315, of which 39% had a stable behaviour in the TST (helpless, non-helpless and intermediate score during the three trials) and the total number of tested female mice was 1486, of which 24% displayed a stable behaviour in this test. The study of the distribution of helpless F2 (H F2) vs. non-helpless F2 (NH F2) mouse phenotypes in the total F2 population (2801 mice) gave the following percentages: the NH F2 phenotype was found in 9% of males and 2% of females, the H F2 phenotype appeared in 2% of males and 5% of females.

**TST and FST**

We tested whether immobility scores in the FST would be in accordance with those obtained in the TST (Fig. 3). Mice that were helpless or non-helpless in the TST procedure were also helpless or non-helpless in the FST procedure. In the TST procedure, statistical analysis showed an interaction between factors helpfulness and gender in parental lines ($F_{1,50} = 5.78$, $p < 0.05$). Indeed H/Rouen female mice were more immobile than NH/Rouen mice ($p < 0.001$) like H F2 (helpless) and NH F2 (non-helpless) in the segregating F2 population ($p < 0.001$). In the FST procedure, a statistical analysis also showed a significant difference between H/Rouen and NH/Rouen mice in the parental lines ($F_{1,50} = 89.07$, $p < 0.001$) and between helpless and non-helpless in the segregating F2 population ($F_{1,126} = 22.7$, $p < 0.001$).

Immobility times observed in the TST performed in parental lines were 85-fold longer in H/Rouen female mice and 171-fold longer in H/Rouen male mice, compared to female and male NH/Rouen mice, respectively (Fig. 3a). In comparison, for the FST, immobility times were only ~3 to 4-fold longer in male and female H/Rouen mice than in NH/Rouen mice (Fig. 3c). Moreover, for females and males of the F2 segregating population tested in the TST (Fig. 3b) and the FST (Fig. 3d), immobility times were longer in helpless than in non-helpless mice, as those observed in parental lines (H/Rouen vs. NH/Rouen mice).

**Locomotor activity**

Horizontal locomotor activity (ambulation) was significantly ($p < 0.001$) reduced in male and female
H/Rouen mice compared to that recorded in NH/Rouen mice (Fig. 3e). The same pattern was observed in mice of both sexes from the segregating F2 population (p < 0.05, Fig. 3f).

The TST, locomotor activity test and FST were performed in the same animals. The correlation coefficient between TST and FST was $r=0.48$ ($p<0.001$), that between TST and locomotor activity was $r=-2.02$ ($p<0.05$), and that between FST and locomotor activity was $r=-0.13$ ($p>0.05$). No other tests were performed after the FST because of stress generated by this experiment. Sleep studies and sucrose test were performed in different groups of animals.

**Sucrose test**
In order to validate the immobility models that may not reflect only helplessness, we next addressed the issue of anhedonia (Willner, 1997) by examining whether voluntary consumption of a sucrose solution

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**Fig. 2.** Distribution of phenotypes in the tail suspension test assessed as immobility time (mean of three trials at weekly intervals) for mice from the F2 population. The distribution of numbers of mice of the total population of females ($n=1315$, left panels) and males ($n=1486$, right panels) belonging to the F2 generation is shown. The immobility classes (observation time-frames are presented in 5-s epochs) are shown from 0 s to 305 s.
Horizontal activity, expressed as the number of beam crossings (El Yacoubi et al. interactions between factors, asterisks (***p < 0.001) depict main effect of strain. Locomotor activity in the open field of H/Rouen and NH/Rouen mice [parental lines (c)] and of the segregating F2 population (f) recorded for 6 min in the tail suspension test (trait of selection). Duration of immobility in H/Rouen and NH/Rouen mice (c) and of the segregating F2 population (d) in the FST. Numbers of animals were n = 12–14 for the parental line, and n = 12–58 for the F2 population. Error bars represent S.E.M. Statistical significance was determined by two-way ANOVAs. Since there were no interactions between factors, asterisks (***p < 0.001) depict main effect of strain. Horizontal activity, expressed as the number of beam crossings (El Yacoubi et al. 2000), was recorded for 45 min (n = 12–14 for parental lines, n = 12–58 for F2 population). Error bars represent S.E.M. *p < 0.05, **p < 0.001 compared to non-helpless mice, using two-way ANOVA. Anhedonia was assessed using a two-bottle free-choice test in parental H/Rouen and NH/Rouen mice (g) and the segregating F2 population (h). Sucrose solution preferences (%) are shown (n = 36–40 for parental line, n = 13–27 for F2 population). Error bars represent S.E.M. Statistical significance was determined by two-way ANOVAs. Since there were no interactions between factors, asterisks (***p < 0.001) depict main effect of strain.

Fig. 3. Behavioural studies. The results for parental lines (M, males; F, females) are shown in the top panels and those for the segregating F2 population in the bottom panels. Duration of immobility in H/Rouen and NH/Rouen mice [parental lines (a)] and of the segregating F2 population (b) recorded for 6 min in the tail suspension test (trait of selection). Duration of immobility in H/Rouen and NH/Rouen mice (c) and of the segregating F2 population (d) in the FST. Numbers of animals were n = 12–14 for the parental line, and n = 12–58 for the F2 population. Error bars represent S.E.M. Statistical significance was determined by two-way ANOVAs. Since there were no interactions between factors, asterisks (***p < 0.001) depict main effect of strain. Locomotor activity in the open field of H/Rouen and NH/Rouen mice [parental lines (c)] and of the segregating F2 population (f). Horizontal activity, expressed as the number of beam crossings (El Yacoubi et al. 2000), was recorded for 45 min (n = 12–14 for parental lines, n = 12–58 for F2 population). Error bars represent S.E.M. *p < 0.05, **p < 0.001 compared to non-helpless mice, using two-way ANOVA. Anhedonia was assessed using a two-bottle free-choice test in parental H/Rouen and NH/Rouen mice (g) and the segregating F2 population (h). Sucrose solution preferences (%) are shown (n = 36–40 for parental line, n = 13–27 for F2 population). Error bars represent S.E.M. Statistical significance was determined by two-way ANOVAs. Since there were no interactions between factors, asterisks (***p < 0.001) depict main effect of strain.

would differ between H/Rouen and NH/Rouen mice on the one hand, and between helpless and non-helpless mice from the F2 segregating population on the other. As illustrated in Fig. 3g, female NH/Rouen mice displayed a higher (p < 0.001) sucrose preference than female H/Rouen mice. For the F2 segregating population, both male and female helpless mice displayed a reduced (p < 0.001) preference for the sucrose solution compared to non-helpless mice (Fig. 3h). Calculation of the correlation between TST and sucrose test gave a r value of 0.29 (p < 0.01).

Sleep patterns

Sleep disorders are cardinal symptoms of depression and alterations of sleep architecture in depressed patients have been well described (Kupfer, 1976). Helpless and non-helpless mice of both genders exhibited similar EEG spectra in each state of vigilance (for data on female mice of the F2 segregating generation see Supplementary Table S1, available online) and a classical light-entrained rhythm of sleep and wakefulness with larger amounts of sleep during the light than the dark period (Fig. 4). However, clear differences in the amounts of vigilance states were observed between helpless and non-helpless mice, with two-way ANOVAs revealing no interaction between factors in conjunction with no gender effect. Helpless mice exhibited greater amounts of SWS1 (F1,143 = 14.4, p < 0.01) and REM sleep (F1,143 = 10.2, p < 0.01) than non-helpless mice, notably in females [Fig. 4, Supplementary Table S2 (online)]. SWS1 represented on the whole a smaller proportion of total sleep time in both genders of the helpless group compared to the non-helpless group (Supplementary Table S2). Finally, helpless mice exhibited a significant (F1,102 = 35.9, p < 0.001) decrease in REM sleep latency (in min) (helpless: 16.0 ± 1.8 and 14.6 ± 1.8 in females and males, respectively; non-helpless: 28.7 ± 2.2 and 28.3 ± 3.2 in females and males, respectively, n = 4–8 in each group). These modifications were accompanied by an increase in the number of wakefulness episodes.
in helpless mice (\(\sim 50\%\), \(p < 0.01\), in females), a parameter providing an index of sleep fragmentation (Popa et al. 2006). Finally, there was a significant correlation between time of immobility in the TST and amount of REM sleep during the dark period (\(r = 0.67\), \(p < 0.001\)).

**Effect of the antidepressant fluoxetine**

Under chronic treatment conditions, fluoxetine (10 mg/kg i.p. daily for 21 d) significantly reduced the immobility time in the TST of helpless male (\(p < 0.001\)) and female (\(p < 0.01\)) mice of the F2 segregating population, but not that of non-helpless mice (Fig. 5).

**Discussion**

In our previous work (El Yacoubi et al. 2003), a single pair of lines selected for high and low expression of the trait ‘immobility in the TST’ was used to study new behavioural characteristics thought to be associated with the selected trait. Hence it was probable that genetic drift phenomena may have occurred during the creation of the selected lines resulting in the likelihood of more false-positive associations. We tested this hypothesis with the help of reciprocal crossbreeding H/Rouen and NH/Rouen F2 populations. In particular, our aim was to determine whether five secondary phenotypic differences observed between the two parental lines are genetically correlated with the trait of selection. First, we selected extreme phenotypes concerning immobility in the TST in the segregating F2 population: H F2 (helpless) and NH F2 (non-helpless). Second, we tested these mice in different paradigms which appeared to give significant different phenotypes in the parental lines such as TST and FST, locomotor activity, sucrose test, sleep patterns, and effect of the antidepressant fluoxetine. The results reported herein strongly substantiate the hypothesis that helplessness in the H/Rouen line of mice is not accounted for by a genetic drift phenomenon during the selection steps (Henderson, 1997) but has a genetic relationship to the trait of selection. The great stability across weekly trials observed in both H/Rouen and NH/Rouen mice indicated that they may be accurate in drug screening procedures.

In addition to the TST, the FST is another ‘behavioural despair’ paradigm (Cryan & Holmes, 2005; Porsolt et al. 1977) used to identify antidepressants. As expected, a close correlation was observed between TST and FST since helpless mice in the TST were also helpless in the FST and this similarity was also observed for non-helpless mice in both tests. These results suggest that some genes responsible for these two depression-related behaviours may be relevant to both tests. However, the TST cannot be considered as a simple variant of the FST (Porsolt & Lene`gre, 1992),
and the present results lend further support to this belief. Indeed, the distinction between the two lines in the FST resulted mainly from the evolution of the score of NH/Rouen mice over generations. In CD1 mice, the immobility time in the FST under similar experimental conditions is approximately 220 s (Vaugeois et al. 1997). This is corroborated by the results observed in the segregating F2 populations. The two extreme populations differ in their behaviour in the FST in a homogeneous way. These findings confirm and broaden the growing evidence that overlapping but not similar behavioural strategies are at work in the two tests (Turri et al. 2001; Yoshikawa et al. 2002). Moreover, intra- and inter-strain differences in performance in the FST and TST indicate that despite a face value similarity, the neurochemical pathways involved in mediating performance in these two widely used tests are not identical (Bai et al. 2001). Thus, when searching for novel classes of antidepressants or phenotyping knockout mice, it remains interesting to perform both tests (Bourin et al. 2005; Duman et al. 2007; El Yacoubi et al. 2006; Fukui et al. 2007; Naidu et al. 2007). Selection pressure in the parental lines also had an effect on the horizontal component of motor activity in the open field, suggesting that some aspects of exploratory activity and coping strategies in an aversive situation may be under the influence of a common set of genes (Turri et al. 2001). The results obtained for exploratory motor activity of the H/Rouen line are in accordance with a decrease in motor activity already reported in other animal models of depression (Overstreet, 2002). In the F2 segregating population, H F2 mice also displayed a decrease of motor activity compared to NH F2 mice. However, in the F2 segregating population, the differences between helpless and non-helpless mice were of smaller amplitude than that in parental strains. These results substantiate the view that a weak genetic correlation exists between the two traits of motor activity and depression-related behaviour suggesting that only a few common genes responsible for these two phenotypes may be at work in both tests.

In order to further validate the TST as a satisfactory screening method for our model, the ability to experience pleasure was assessed in H/Rouen and NH/Rouen mice using a sweet reward in the two-bottle test procedure. A significantly defective hedonic behaviour appeared in female but not male H/Rouen vs. NH/Rouen mice. However, both male and female helpless mice from the F2 segregating population showed a reduced preference for the sweet solution. In this respect, helpless mice behaved like rodents subjected to chronic mild stress, which is now considered to be a well-validated model of depression (Strekalova et al. 2004; Willner, 1997). These results suggest that some major genes responsible for these two depression-related behaviours, helplessness and anhedonia, may be concerned in both tests.

We had shown previously that H/Rouen compared to NH/Rouen mice exhibited a lighter and more fragmented sleep and a decreased REM sleep latency (El Yacoubi et al. 2003; Popa et al. 2006) in agreement with data derived from other depression models in mice (Popa et al. 2008) and from studies in rats rendered helpless by repeated exposure to unpredictable stressful stimuli (Adrien et al. 1991). Indeed, alterations of sleep-wakefulness patterns in H/Rouen mice mimicked to some extent those observed in depressed patients such as the enhanced REM sleep ‘pressure’ and the sleep fragmentation (Kupfer, 1976; Lustberg...
Interestingly, these sleep-wakefulness patterns were also observed in helpless mice from the F2 segregating generation. Notably, enhanced REM sleep ‘pressure’ and sleep fragmentation were clearly observed in the F2 population. Thus, the different phenotypes concerning helplessness and sleep patterns have a causal relationship and do not originate from a random fixation of genes linked to ‘behavioural despair’ and sleep/wakefulness rhythms during the generation of the H/Rouen and NH/Rouen lines. These results imply a strong genetic correlation between depression-related behaviour and sleep patterns.

Finally, we examined how mice from the segregating F2 population responded to the antidepressant fluoxetine. Chronic administration of fluoxetine (10 mg/kg daily) significantly reduced the immobility in the TST of helpless mice after 3 wk treatment. These results are in accordance with previous data obtained in helpless rats, in H/Rouen mice, in mutant mice lacking the serotonin transporter, and in mice treated neonatally with a SSRI (El Yacoubi et al. 2003; Maudhuit et al. 1997; Popa et al. 2008), which represents further support for the validity of this model. The difference in drug response in the helpless and non-helpless lines, i.e. the response in helpless and the lack of response in non-helpless mice, could be due to a floor effect in non-helpless mice for which the level of immobility is already very low in the control that is probably difficult to reduce further by the drug.

In summary, the data reported herein show that a strong correlation exists between the helpless phenotype in the TST and four phenotypes observed in the parental lines such as ‘alterations of sleep patterns’, immobility in the FST, anhedonia, and the behavioural response to fluoxetine, which are relevant to depression and its treatment in humans. Thus, the present study suggests that H/Rouen mice might be of particular interest for investigating mechanisms underlying high susceptibility to helplessness. H/Rouen mice should also facilitate the study of genetic aspects of vulnerability to despair, and further, should help in the identification of genes causally related to depression in humans. In addition, the H/Rouen line should be useful for assessing the potential antidepressant action of novel therapeutic strategies targeting psychoaffective disorders.

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Statement of Interest

None.

References


Note

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