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BEHAVIORAL RESPONSES IN MICE SELECTIVELY BRED FOR HIGH AND LOW LEVELS OF OPEN-FIELD THIGMOTAXIS

by

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ABSTRACT

In animal psychology, the open-field (OF) test is a traditional method for studying different aspects of rodent behavior, with thigmotaxis (i.e., wall-seeking behavior) being one of the best validated OF parameters employed to measure emotionality. The main purpose of the present study was to investigate the selection response in mice selectively bred for high and low levels of OF thigmotaxis (the HOFT and LOFT lines, respectively). The mice ($N = 2048$) were selected for 23 generations, resulting in bidirectional phenotypic divergence between the two lines; that is, the HOFT mice were more thigmotactic (i.e., more emotional) than the LOFT mice across the different generations. The origin of the line difference in thigmotaxis was further investigated by using the crossfostering paradigm, with the results suggesting that the divergence between the two lines was primarily innate in origin and not influenced by differing maternal behavior. The stability of the selection trait was examined by testing the animals at different ages as well as in varying conditions. The results indicated that the line difference in thigmotaxis was not affected by age at the time of testing, and it also persisted in the different OF testing situations as well as during pregnancy and lactation. The examination of a possible coselection of other characteristics revealed that the more thigmotactic HOFT mice lived longer than the less thigmotactic LOFT mice. In addition, the HOFT mice tended to rear and explore less than the LOFT mice, supporting the general assumption that emotionality and exploration are inversely related. The two lines did not generally differ in ambulation and defecation, that is, in the traditional OF indexes of emotionality,

conforming to the suggestion that emotionality is a multidimensional construct. The effects of sex on different OF parameters were also assessed, with the results suggesting that among the HOFT and LOFT lines, the female mice were more emotional than the male mice. The examination of the temporal changes in the HOFT and LOFT lines' OF behavior revealed some contradictory findings that also partially conflicted with general assumptions. Although this study did not show prominent differences in maternal responsiveness between the HOFT and LOFT mothers, the results suggested that the line divergence in emotionality was more pronounced in the presence of a pup after parturition than during pregnancy. The present study clearly demonstrates that OF thigmotaxis is a strong characteristic for producing two diverging lines of mice. The difference in thigmotaxis between the selectively bred HOFT and LOFT mice seemed to be a stable and robust feature of these animals, and it appeared to stem from a genetic background.

Keywords: thigmotaxis, open field, HOFT and LOFT mice, selective breeding, emotionality, anxiety, exploration, maternal behavior

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LIST OF THE ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are designated by Roman numerals **I–IV**.

I Leppänen, P. K., & Ewalds-Kvist, S. B. M. (2005). Crossfostering in mice selectively bred for high and low levels of open-field thigmotaxis. *Scandinavian Journal of Psychology, 46,* 21–29.*

II Leppänen, P. K., Ewalds-Kvist, S. B. M., & Selander, R.-K. (2005). Mice selectively bred for open-field thigmotaxis: Life span and stability of the selection trait. *The Journal of General Psychology, 132,* 187–204.**

III Leppänen, P. K., Ravaja, N., & Ewalds-Kvist, S. B. M. (2006). Twenty-three generations of mice bidirectionally selected for open-field thigmotaxis: Selection response and repeated exposure to the open field. *Behavioural Processes, 72,* 23–31.***

IV Leppänen, P. K., Ravaja, N., & Ewalds-Kvist, S. B. M. (2008). Prepartum and postpartum open-field behavior and maternal responsiveness in mice bidirectionally selected for open-field thigmotaxis. *The Journal of General Psychology, 135,* 37–53.**

CONTENTS

1 INTRODUCTION

In animal psychology, selective breeding is a widely used method generally employed with mice or rats in order to examine the inheritance of various characteristics and their relationships with other behavioral or biological properties. In the past, many selection experiments in rodents have focused on behavioral characteristics measured in the traditional open-field (OF) test. The test in question enables the study of different aspects of rodent behavior, with emotionality (or anxiety or fearfulness) being most often the focus of interest in OF research. Emotionality can be measured in several different ways in the OF test, with thigmotactic (or wall-seeking) behavior being one of the most valid emotionality-related OF parameters. In the present study, mice were selectively bred for high and low levels of OF thigmotaxis for the purpose of providing new information related to mice' thigmotactic behavior and its associations with other characteristics. This information may prove useful for many disciplines focusing on anxiety research and using OF thigmotaxis as a measure of rodent emotionality because, to the knowledge of the author, mice have not previously been selected for this characteristic.

1.1 The open-field test

The OF test was originally developed by Calvin Hall in order to study the natural or spontaneous direction of rat behavior (Hall, 1934a, 1934b; Hall & Ballachey, 1932). According to Hall (Hall & Ballachey, 1932), one of the most important advantages of this method is that it enables the study of the natural direction of rodent behavior under less teleological conditions than by the usual types of apparatuses employed at that time. By a teleological condition Hall meant a condition in which the animal is forced to behave according to the aims of the experimenter (Hall & Ballachey, 1932). In Hall's OF studies, the emphasis was on rats' emotional behavior (Hall, 1934a, 1934b), and now, after more than 75 years, the OF test is still the most traditional, well validated, and perhaps also the most widely used test assessing rodent emotional behavior (Crawley et al., 1997; Ramos, Correia, Izídio, & Brüske, 2003; Weiss & Greenberg, 1998;

see also Hefner & Holmes, 2007; Malkesman et al., 2005). Although originally developed for measuring emotionality, the OF test has been used to study different aspects of rodent behavior, with activity and exploration, for example, having also been the focus of interest in OF research (Elias, Elias, & Eleftheriou, 1975; Makino, Kato, & Maes, 1991; see also Lipkind et al., 2004). Moreover, the use of the OF test has spread from animal psychology to other disciplines, such as pharmacology, neuroscience, and genetics (Choleris, Thomas, Kavaliers, & Prato, 2001; Takahashi, Kato, Makino, Shiroishi, & Koide, 2006).

As a general feature, the OF test comprises an enclosed open area in which the animal is placed and subjected to various behavioral recordings (Choleris et al., 2001; Ossenkopp, Sorenson, & Mazmanian, 1994; Walsh & Cummins, 1976). The OF apparatus provides a number of psychological stimuli in a novel environment that elicit unconditioned fear as well as multiple spontaneous behavioral responses (Castanon & Mormède, 1994; Clément, Calatayud, & Belzung, 2002; Gershenfeld & Paul, 1997; Hefner & Holmes, 2007). Because the OF test primarily measures spontaneous or unconditioned behavior, animals can exhibit a wide range of different behaviors, thus enabling the study of complex phenomena, including emotionality (Lipkind et al., 2004). Accordingly, various psychological factors as well as physiological states are included in the OF behavior of a rodent (Clément, Martin, Venault, & Chapouthier, 1995; Takahashi et al., 2006).

The OF test is based upon a rodent's contradictory tendencies to explore new areas and to stay in a protected place (Clément et al., 2002). Rodents, such as mice and rats, have a natural aversion to novelty as well as to lit and open places (Boissy, 1995; Calatayud & Belzung, 2001; Mathis, Paul, & Crawley, 1994; Steimer & Driscoll, 2003). On the other hand, novel environments also elicit exploratory behavior (Crawley, 1985; Crusio, 2001; Miyamoto, Kiyota, Nishiyama, & Nagaoka, 1992; see also Blois-Heulin & Belzung, 1995), and, consequently, the behavior of a rodent in the OF test is motivated by competition between fear and exploratory tendencies or curiosity (Crusio, 2001; Morgan & Pfaff, 2001; Palanza, Gioiosa, & Parmigiani, 2001; Steimer & Driscoll, 2003). Hence, a form of approach–avoidance conflict is apparent (Holmes, 2001; Lister, 1990).

When an animal is repeatedly exposed to the OF arena, it gradually habituates to this novel environment (Gershenfeld et al., 1997; Takahashi et al., 2006). According to Iwahara and Sakama (1972), habituation refers to response changes associated with repeated presentations of specific stimuli. Emotional responses are generally assumed to decrease with habituation (Ivinskis, 1970; Makino et al., 1991). By contrast, the parameters measuring exploratory tendencies are initially expected to increase as the level of fear diminishes, but then to wane as the field becomes more familiar to the animal (Archer, 1973; Crusio, 2001; Takahashi et al., 2006).

Usually, mice or rats are subjected to the OF test, although there has been considerable variation with regard to the shape (circular or square) and the size of the OF apparatus used. Likewise, testing conditions (e.g., the level of illumination) and procedures (e.g., the duration of the recording session and the number of testing days) have greatly varied. In the OF test, the animal is typically subjected to various behavioral recordings, with more than 30 OF parameters having been recorded over a period of 75 years. (For reviews of different OF apparatuses, procedures, and parameters used, see Choleris et al., 2001; Wahlsten, 2001; Walsh & Cummins, 1976; Weiss & Greenberg, 1998.) Behavioral recordings are nowadays seldom performed manually (e.g., Takahashi, Nishi, Ishii, Shiroishi, & Koide, 2008), more often automatically (e.g., Bolivar, Caldarone, Reilly, & Flaherty, 2000) or by means of a camera (e.g., Lamprea, Cardenas, Setem, & Morato, 2008).

Although the OF behavior of mice and rats has many similarities and at least most of the different OF parameters are thought to measure the same characteristics in both species, there are also some dissimilarities, for example, with regard to sex differences, correlations, and temporal patterns of the parameters, a factor which has to be taken into account before making any generalizations based on the study of either one of these species (for reviews, see Archer, 1973; Walsh & Cummins, 1976). In addition to differences between species, mice and rats often exhibit significant strain differences in their OF behavior, demonstrating the significance of genetic factors in determining OF behavior (see Clément et al., 1997; Takahashi et al., 2006). This also means that findings in OF studies may not always be generalizable beyond the specific strain

in question. Archer (1971) has even suggested that for many OF variables, there are wider differences across strains than between the two species. Moreover, the OF test, like many other tests for animal behavior, is highly sensitive to minor variations in testing procedures and settings as well as in general conditions, which means that the results obtained in one experiment and in one specific laboratory may not always be replicable in another experiment and in another laboratory (see Clément et al., 2002; Valentinuzzi et al., 2000; Wahlsten, 2001; Walsh & Cummins, 1976).

1.2 Emotionality

"Emotionality" is a construct that refers to the animal's fearfulness. Depending on the discipline and tradition in question, different synonyms for the term emotionality have been employed. In pharmacological studies, for example, the term anxiety is generally used (Ramos & Mormède, 1998). Other synonyms include timidity (Stead et al., 2006), emotional reactivity (Broadhurst, 1975; Brush, 2003), and, naturally, fear and fearfulness (Archer, 1973; Brush, 2003; Walsh & Cummins, 1976). Although these terms are widely used as synonyms, researchers have also suggested that qualitative differences exist between them (Boissy, 1995; Steimer & Driscoll, 2003; see also Ramos & Mormède, 1998).

The nature of the concept emotionality is much debated: Some researchers have considered emotionality as a unitary trait (Boissy, 1995; Broadhurst, 1975; Gray, 1979b), whereas others have suggested that it represents a multidimensional construct (Archer, 1973; Brush, 2003; Liebsch, Montkowski, Holsboer, & Landgraf, 1998; Ramos & Mormède, 1998). However, researchers often agree that emotionality is, at least to a certain degree, genetically determined (Boissy, 1995; Flint et al., 1995; Ramos et al., 2003; Takahashi et al., 2008; Trullas & Skolnick, 1993). On the other hand, it has also been recognized that differing mothering styles may affect rodents' emotional reactivity (Calatayud & Belzung, 2001; Clément et al., 2002; Holmes et al., 2005; Rose, Röhl, Schwegler, Hanke, & Yilmazer-Hanke, 2006), and different mice strains, for example, have often been shown to exhibit different patterns of maternal behavior (Brown, Mathieson, Stapleton, & Neumann, 1999; Carlier, Roubertoux, & Cohen-Salmon, 1982; see

also Lonstein & De Vries, 2000). In addition to diverse maternal care, a number of different pre- and postnatal environmental factors have been shown to affect a rodent's emotionality (Gray, 1987, pp. 115–136; Rose et al., 2006). In fact, a rodent's emotionality, like most traits or characteristics, is always affected by (multiple) genes and environmental factors as well as by interactions between them (Clément et al., 2002; Crawley et al., 1997; Holmes et al., 2005).

Hall (1934b) defined emotionality in the OF test as "a general upset or excited condition of the animal" elicited by the strangeness or novelty of the situation. In the OF test, emotionality is traditionally measured by recording the animal's ambulation and defecation during the testing session (Archer, 1973). More specifically, low levels of ambulation and high levels of defecation are interpreted as indicators of high levels of emotionality (DeFries, Wilson, & McClearn, 1970; Flint et al., 1995; Gervais, 1976), although the validity of ambulation and defecation as indexes of emotionality has been questioned (Lister, 1990; Ramos & Mormède, 1998). In addition to these traditional indexes, several other OF parameters have been employed to measure emotionality, such as urination, grooming, latency to move, latency to enter the wall zone, and thigmotaxis (Bronikowski et al., 2001; Markowska, Spangler, & Ingram, 1998; Poley, 1974; Roy, Belzung, Delarue, & Chapillon, 2001; Suaudeau et al., 2000). Given that researchers generally assume that emotionality and exploration are inversely related, that is, a high level of emotionality inhibits exploration and a low level facilitates it (Archer, 1973; Gray, 1987, p. 39; Holmes, 2001; Williams & Russell, 1972), the parameters measuring exploratory tendencies (e.g., rearing and the number of at-least-once-entered OF units; Crusio, 2001; Lhotellier, Perez-Diaz, & Cohen-Salmon, 1993) may also be used as measures of emotionality.

It should be recognized that in animal psychology, emotionality cannot be directly measured; instead, researchers have to rely on behavioral parameters that are presumably associated with the emotional state (Boissy, 1995; Clément et al., 2002; Liebsch et al., 1998; Ohl, Toschi, Wigger, Henniger, & Landgraf, 2001). Moreover, the OF parameters supposed to measure emotionality are not independent from nonemotional components, such as activity and exploration (Crawley et al., 1997; File, 2001; Ramos & Mormède, 1998). An applicable

example of this is ambulation, a traditional index of emotionality, which naturally also indicates motor activity (Flint et al., 1995) and is often used to measure exploration as well (see Archer, 1973; Gray, 1982, p. 40; Ramos et al., 2003; Walsh & Cummins, 1976). In fact, ambulation (or locomotion) and exploration have even been used as synonyms in many OF studies (see Choleris et al., 2001). Furthermore, several parameters measuring different aspects of OF behavior are based on the animal's ambulation in the OF arena (e.g., thigmotaxis for measuring emotionality and the number of at-least-once-entered OF units for measuring exploration).

1.3 Thigmotaxis

Thigmotaxis is one of the most employed and best validated OF parameters measuring emotionality in mice and rats. Thigmotactic or wall-seeking behavior refers to the propensity of a rodent to stay in close contact with the OF wall, owing to the underlying tendency to avoid open, unknown, and potentially dangerous places (see Choleris et al., 2001; Russell, 1979). Thigmotaxis is considered to belong to the category of phylogenetically prepared fear reactions for the purpose of avoiding predators (Fanselow & De Oca, 1998; Grossen & Kelley, 1972; Treit & Fundytus, 1989). It is assumed that the more thigmotactic a rodent is, the more emotional it is (Clément & Chapouthier, 1998; Valle, 1970; see also Holmes, 2001). Consequently, a highly emotional rodent tends to move around the less anxiogenic OF wall, whereas a less emotional one moves more in the OF center (Lipkind et al., 2004; Ragnauth et al., 2001). This assumption is supported by observations showing that an aversive stimulus, such as a bright light (Hirsijärvi & Junnila, 1986; Valle, 1970) or a foot shock (Grossen & Kelley, 1972), increases thigmotaxis, whereas a repeated (Ossenkopp et al., 1994) or prolonged (Choleris et al., 2001) exposure to the OF decreases thigmotaxis. In pharmacological studies, thigmotaxis has been found to be a valid index of anxiety in mice because anxiolytic drugs have been proven to decrease and anxiogenic drugs to increase wall-seeking behavior (Choleris et al., 2001; Simon, Dupuis, & Costentin, 1994). Anxiolytic drugs have been shown to reduce thigmotaxis in highly emotional rats as well (Stead et al., 2006). Moreover, Treit and Fundytus

(1989) discovered that thigmotaxis in rats is suppressed by anxiolytic agents with a relative potency that is similar to their relative potency in the treatment of human anxiety. In addition, the time spent on the most protected areas of the OF apparatus has been validated as an index of emotionality on the basis of genetic correlations in rats (van der Staay, Kerbusch, & Raaijmakers, 1990).

In an early study, Hall and Ballachey (1932) paid attention to the rats' tendency to move around the walls of the OF apparatus (for an analysis of the influences the wall exerts on the mouse's path in the OF, see Horev, Benjamini, Sakov, & Golani, 2007). Since then, researchers have measured OF thigmotaxis in different ways, which include the calculation of the thigmotactic ratio (McIlwain, Merriweather, Yuva-Paylor, & Paylor, 2001; Sanberg & Ossenkopp, 1977; Valle & Bols, 1976; see 3.3.1 Open field) as well as recording the time spent in the OF center (or its proportion out of the entire session), the distance traveled in the OF center (or its proportion out of the total distance traveled), the number of entries into the OF center, and the latency to enter the center of the OF (Lipkind et al., 2004). Although thigmotaxis is generally measured in mice or rats, it has also been employed when examining the behavior of other rodents, such as Mongolian gerbils (Nauman, 1968). In addition to rodents, thigmotaxis can be measured when studying other animal species, such as American cockroaches (Creed & Miller, 1990).

1.4 Selective breeding

In animal psychology, selective breeding is a powerful tool mainly employed for studying the inheritance of a certain characteristic and for revealing correlational relationships between the selection trait and other behavioral or physiological properties (Lagerspetz & Lagerspetz, 1983; Streng, 1974). In fact, lines selected on the basis of some behavioral trait have often been found to differ in a number of other behavioral or biological characteristics as well (Boissy, 1995; Broadhurst, 1975; Castanon & Mormède, 1994; Castanon, Perez-Diaz, & Mormède, 1995; Wimer & Wimer, 1985). Furthermore, the examination of correlated responses to selection trait may provide valuable information about the mechanisms underlying a specific behavioral pattern (Wimer & Fuller,

1966). Moreover, if bidirectional selection, based on the phenotypes of the animals, is capable of producing two lines with a steady separation between the mean scores of the selection trait, there is presumptive evidence that the behavior in question is determined, at least to some extent, by the genes handed down from parents to offspring (Gray, 1987, p. 43; Hall, 1934b; Hurnik, Bailey, & Jerome, 1973; Lagerspetz & Lagerspetz, 1983; Stead et al., 2006). It should be noted, however, that selection always acts on the behavioral characteristic in question and not on the genotypes of the animals (see Clément et al., 2002). In addition, selective breeding only acts upon genes that vary within a given animal population (Ramos et al., 2003).

Generally, the aim of the selection experiments is to establish two breeding lines derived from the same strain with the maximum difference in the selection trait and the minimum difference in other behaviors and physiological parameters not primarily related to the selection trait (Liebsch et al., 1998). In the past decades, several selection experiments have been performed on mice and rats in order to study different aspects of OF behavior, and selective breeding has shown that genetic factors make a large contribution to phenotypic variation in several measures of OF behavior (Takahashi et al., 2006). Selection experiments performed on mice have been based, for example, on OF activity or ambulation (DeFries, Gervais, & Thomas, 1978; Ewalds-Kvist, Selander, & Kvist, 1999) as well as on rearing behavior (van Abeelen, van der Kroon, & Bekkers, 1973), whereas selection studies on rats have focused, for instance, on OF defecation (Broadhurst, 1975) and central locomotion (i.e., thigmotaxis; Ramos et al., 2003). However, to the knowledge of the author, no selection experiments based on mouse OF thigmotactic behavior have previously been conducted, in spite of the fact that thigmotaxis is a widely used and well validated index of rodent emotionality. For this reason, the mice in the present study were selectively bred for high and low levels of OF thigmotactic behavior (High Open-Field Thigmotaxis [HOFT] and Low Open-Field Thigmotaxis [LOFT] mice, respectively).

Possibly most of the animal models that have been developed for assessing anxiety (or emotionality) in rodents have been based on unselected populations of animals although it might be more productive to study this issue in animals that show an innate predisposition to express high or low levels of anxiety

(Brush, 2003; Landgraf & Wigger, 2002; Lister, 1990; Steimer & Driscoll, 2003). Namely, when animals are selected for their high levels of anxiety, they would exhibit increased anxiety because it is a stable characteristic of them and not only because their level of anxiety is artificially elevated by exposure to aversive and anxiogenic stimuli (Belzung & Griebel, 2001). In other words, the selectively bred animals would mainly differ in trait anxiety, which is an enduring feature of an individual and, consequently, does not vary from moment to moment, in contrast to state anxiety, which is provoked by an anxiogenic stimulus and only experienced at a particular moment in time (Belzung & Griebel, 2001; Lister, 1990).

1.5 The present dissertation

In the present study, the mice were bidirectionally selected for thigmotaxis, which is one of the best validated parameters measuring emotionality (or anxiety or fearfulness) in the traditional OF test. By means of selective breeding, the purpose of this study was to create two lines of mice that would differ greatly with regard to OF thigmotactic behavior and thus provide new and relevant basic information about this characteristic and its background (genetic vs. acquired) as well as its associations with other behavioral or biological properties. Because selective breeding for thigmotaxis enables the creation of an animal model of trait anxiety, as opposed to state anxiety, it was expected that the line difference in emotionality between the present mouse lines would be robust and based on the stable and enduring features of these animals, thus providing useful information about mice' emotionality-related behavior for animal psychology as well as for various disciplines in the field of anxiety research.

2 AIMS OF THE DISSERTATION

The present dissertation had six aims. The first aim was to investigate the selection response in mice selectively bred for high and low levels of OF thigmotaxis (the HOFT and LOFT lines, respectively) and also to examine whether the difference in thigmotaxis between the two lines originated primarily from innate or acquired sources. The second aim was to investigate the stability of the selection trait in different conditions. The third aim was to examine whether the selection on the basis of OF thigmotaxis also resulted in line differences with respect to some other characteristics, that is, whether some other properties were coselected in conjunction with thigmotactic behavior. The fourth aim was to assess possible sex differences in certain OF behaviors among the HOFT and LOFT lines. The fifth aim was to investigate the effects of repeated exposure to the OF on the HOFT and LOFT lines' OF behavior. Finally, the sixth aim of this study was to examine the OF behavior of the HOFT and LOFT female mice during pregnancy and lactation, as well as to investigate possible line differences in maternal responsiveness. These research questions were examined in four separate studies (**I–IV**). The detailed objectives of each study were as follows:

Study I. The aim of Study **I** was to examine whether the line difference in OF thigmotaxis between the selectively bred HOFT and LOFT mice originated mainly from innate or acquired sources. This was investigated in two separate experiments by using the crossfostering paradigm. In addition to thigmotaxis, the effects of line, sex, and fostering on eight different OF behaviors (ambulation, defecation, exploration, grooming, latency to move, radial latency, rearing, and urination) were examined.

Study II. The objective of Study **II** was to investigate whether the selectively bred HOFT and LOFT lines would differ in the length of their life spans or in basic metabolic characteristics, that is, in food intake and excretion. Moreover, it was asked whether the two lines would differ in OF defecation, a traditional index of emotionality, when exposed to the OF arena for 60 min. In addition, OF thigmotaxis was measured at seven different ages during the mouse's life span

to examine whether the line difference in thigmotaxis would be stable over time or, alternatively, vary as a function of age. The stability of the selection trait was further examined by investigating whether the line difference in thigmotaxis would be present in a home-cage condition as well and also by asking whether the difference in thigmotaxis between the two lines would persist when the characteristics of the OF test, that is, the shape of the OF arena and the location of the starting point were varied.

Study III. The main purpose of Study **III** was to examine the response to bidirectional selection for OF thigmotaxis in mice for 23 generations. OF ambulation and rearing scores were also registered with each generation to reveal a possible coselection of these characteristics. Namely, ambulation is a traditional, although much debated, index of emotionality, and given that exploration and emotionality are inversely related, it was expected that the lines would also differ in rearing (an index of exploration). The impact of sex on the aforementioned parameters was also examined. The second objective of this study was to investigate the effects of repeated exposure to the OF on nine OF behaviors (ambulation, defecation, exploration, grooming, latency to move, radial latency, rearing, thigmotaxis, and urination) in the HOFT and LOFT mice. This was examined by testing the animals in the OF on 5 consecutive days, with the effects of sex being investigated as well.

Study IV. The aim of Study **IV** was to examine possible line differences between the HOFT and LOFT female mice with respect to eight OF behaviors (ambulation, defecation, exploration, grooming, latency to move, rearing, thigmotaxis, and urination) during pregnancy and after parturition in the presence of a single pup in the OF arena. Moreover, behavioral changes in the OF parameters between the pre- and postpartum conditions were investigated as well. In addition, the HOFT and LOFT female mice were examined with regard to maternal responsiveness by using a retrieval test as well as by measuring the mothers' attraction to their pups in the OF arena. It was hypotesized that the less emotional LOFT mice would show stronger maternal responses than the more emotional HOFT mice.

3 METHODS

3.1 Subjects and housing conditions

The selectively bred HOFT and LOFT mice were derived from a Swiss albino outbred stock originating from Malmö, Sweden. They were bred, reared, and selected in the laboratory at the Department of Psychology at Åbo Akademi University, Finland. The selective breeding of the mice started in 1993. All of the animals were reared on a cycle of 12-hr light and 12-hr dark, the lights switching on at 7 a.m. and off at 7 p.m., in a noiseless room with a normal room temperature (approximately 20–23 ^{0}C). They were fed standard laboratory pellets, R3 (1260 kJ/100 g), from Lactamin, Sweden, and had free access to fresh tap water. The cages had a bedding of wood shavings, and they were changed for clean cages weekly. In each generation, the animals were weaned at 4 weeks of age.

Study I comprised two crossfostering experiments. In Experiment 1, the subjects consisted of a total of 219 mice (112 females and 107 males), originating from the 17th generation (S_{17}) of the HOFT (n = 104; 54 females and 50 males) and LOFT (n = 115; 58 females and 57 males) lines. After weaning, the mice were group-housed in polycarbonate cages measuring 23 × 17 × 14 cm. At the age of approximately 70 days, the animals were individually housed in aluminum cages measuring 14 × 14 × 12 cm.

In Experiment 2, the subjects included a total of 221 mice (124 females and 97 males), originating from the 22nd generation (S_{22}) of the HOFT (n = 92; 54 females and 38 males) and LOFT (n = 129; 70 females and 59 males) lines. After weaning, the animals were group-housed in polycarbonate cages measuring 23 × 17 × 14 cm.

Study II consisted of two experiments. In Experiment 1, the subjects were 51 mice (28 females and 23 males), originating from the 13th generation (S_{13}) of the HOFT (n = 27; 14 females and 13 males) and LOFT (n = 24; 14 females and 10 males) lines. With the exception of the life-span experiment, only the recordings

from the males were reported. After weaning, the mice were individually housed in aluminum cages measuring 14 × 14 × 12 cm until the age of approximately 3.5 months, at which time some of the animals were selected to continue the selection lines. Subsequently, the animals were group-housed in polycarbonate cages measuring 38 × 22 × 15 cm. Before each OF-thigmotaxis measurement, the mice participating in the experiment were individually housed for 4 days. Of the HOFT males, 6–13 mice were randomly chosen for different recordings, and for the LOFT males, the number of subjects in each experiment varied from 6 to 10. The measurements performed on the HOFT and LOFT male mice are presented in Table 1, which also shows the number of subjects in each recording and the age at the time of testing. All of the 51 mice participated in the life-span experiment.

In Experiment 2, the subjects comprised 18 male mice, originating from the 21st generation (S_{21}) of the HOFT ($n = 9$) and LOFT ($n = 9$) lines. After weaning, the animals were individually housed in polycarbonate cages measuring 20.5 × 10.5 × 13.0 cm until the age of approximately 19 weeks, at which time they were mated to continue the selection lines. After this, the mice were group-housed in polycarbonate cages measuring 38 × 22 × 15 cm. The LOFT males were tested at the age of 5.6 months and the HOFT males at the age of 5.9 months.

Table 1

Measurements performed on the HOFT (High Open-Field Thigmotaxis) and LOFT (Low Open-Field Thigmotaxis) male mice of Generation 13

	HOFT		LOFT	
Measurement	***n***	**Age (days)**	***n***	**Age (days)**
Open-field thigmotaxis				
1st	13	65	10	64
2nd	7	96	8	95
3rd	7	126	9	133
4th	11	208	8	201
5th	13	271	8	269
6th	12	488	6	481
7th	8	541	6	534
Open-field defecation	8	303	8	296
Home-cage thigmotaxis	6	313	7	310
Metabolism	8	473	6	466

Study III included the selection experiment and the repeated OF-exposure experiment. In the selection experiment, a total of 2048 mice (1065 females and 983 males) were used. In addition to 19 mice of the Swiss albino parental strain (10 females and 9 males), the subjects consisted of 995 HOFT (528 females and 467 males) and 1034 LOFT (527 females and 507 males) mice. Table 2 shows the number of mice in each generation. Some characteristics of the housing conditions (i.e., the type of cage and individual vs. group housing) varied between the generations, depending, for example, on the specific experiments carried out using a particular generation.

In the repeated OF-exposure experiment, the subjects consisted of 44 mice (22 females and 22 males), originating from the 23rd generation (S_{23}) of the HOFT (n = 21; 11 females and 10 males) and LOFT (n = 23; 11 females and 12 males) lines. After weaning, the animals were individually housed in aluminum cages measuring 14 × 14 × 12 cm. The mice were tested at the age of approximately 14 weeks.

Table 2

The number of mice tested in each generation during bidirectional selection for open-field thigmotaxis

	HOFT		LOFT	
Generation	♀, ***n***	♂, ***n***	♀, ***n***	♂, ***n***
1	13	7	14	9
2	16	14	16	9
3	11	17	14	20
4	37	26	15	15
5	14	19	11	12
6	32	23	26	21
7	22	24	19	25
8	23	21	13	13
9	29	23	31	28
10	34	28	19	29
11	26	15	25	32
12	15	14	21	13
13	18	18	21	14
14	12	25	12	13
15	26	24	25	30
16	19	19	20	18
17	53	50	58	57
18	17	13	16	20
19	9	12	18	12
20	18	6	14	15
21	20	21	38	32
22	54	38	70	58
23	10	10	11	12

Note. HOFT = High Open-Field Thigmotaxis; LOFT = Low Open-Field Thigmotaxis.

Study IV comprised three experiments: the prepartum OF test, the postpartum OF test, and the pup-retrieval test. The subjects included 40 nulliparous female mice, originating from the 16th generation (S_{16}) of the HOFT (n = 20) and LOFT (n = 20) lines. After weaning, the animals were individually housed in polycarbonate cages measuring 20.5 × 10.5 × 13.0 cm until the age of approximately 2 months. At that time, the mice were tested in the OF for perpetuating the selection lines, and these 40 female mice were selected to continue the HOFT and LOFT lines. The female mice were then housed and mated in polycarbonate cages measuring 23 × 17 × 14 cm. The adult male mouse was removed when the female was confirmed as gestate. The HOFT females gave birth on average to 6.2 pups (*SD* = 2.2), whereas the LOFT females gave birth on average to 6.6 pups (*SD* = 2.3). In addition to adult female mice, pups aged 0–5 days were used in this study.

3.2 Apparatuses

3.2.1 Circular open field (I–IV)

The OF apparatus consisted of a circular, flat, white, wooden arena (40 cm in diameter) with a 20-cm-high wall made of flat, white, metallic plate (see Figure 1). The field was marked with thin black lines to delineate three concentric circles. The outer circle was further divided into 12 partitions and the middle circle (24 cm in diameter) into 6 partitions. The center circle (8 cm in diameter) was not divided. That is, the floor of the arena was subdivided into a total of 19 partitions to assist in recording the ambulation of the animal, thereby providing raw scores for computation. The area of each partition was 67 cm², with the exception of the center circle (50 cm²). The center circle and the 6 partitions of the middle circle were defined as 7 inner units and the 12 partitions of the peripheral circle as outer units. The floor of the arena was cleaned between successive recordings.

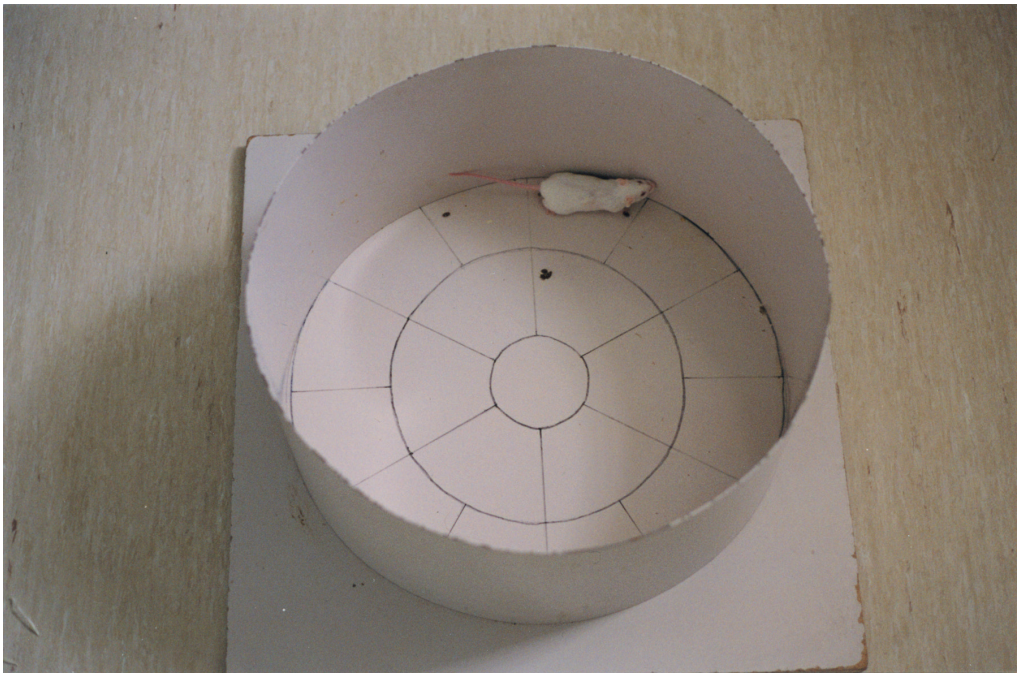

Figure 1. The open-field apparatus.

3.2.2 Square open field (II)

The square-shaped OF consisted of a flat, white, wooden arena measuring 35.5 × 35.5 cm. It was surrounded by a 23.5-cm-high wall of flat, white, wooden plate. The field was divided by thin black lines into 25 squares to assist in recording the ambulation of the animal, thereby providing raw scores for computation. The floor of the arena was cleaned between successive recordings.

3.3 Parameters

Independent and dependent variables employed in different experiments in Studies **I–IV** are shown in Table 3.

Table 3

*Independent and dependent variables employed in different experiments in Studies **I–IV***

Experiment	**Independent variables**	**Dependent variables**
Study 1		
Crossfosterings 1 and 2	Fostering, line, sex	Ambulation, defecation, exploration, grooming, latency to move, radial latency, rearing, thigmotactic ratio, urination
Study 2		
Life span	Line, sex	Number of days alive
Open-field thigmotaxis at seven different ages	Line	Inner ambulation, outer ambulation, thigmotactic ratio
Open-field defecation for 60 min	Line	Defecation
Home-cage thigmotaxis	Line	Inner ambulation, outer ambulation, thigmotactic ratio
Metabolism	Line	Excretion, food intake, weight
Open-field thigmotaxis under four conditions with varying starting points and shapes of the arena	Line	Inner ambulation, outer ambulation, thigmotactic ratio

(Table continues)

Table 3 (continued)

Experiment	Independent variables	Dependent variables
Study 3		
Selection response	Line, sex	Ambulation, rearing, thigmotactic ratio
Repeated open-field exposure	Day, line, sex	Ambulation, defecation, exploration, grooming, latency to move, radial latency, rearing, thigmotactic ratio, urination
Study 4		
Prepartum open field	Line, parturition[a]	Ambulation, defecation, exploration, grooming, latency to move, rearing, thigmotactic ratio, urination
Postpartum open field	Line, parturition[a]	Ambulation, defecation, exploration, grooming, latency to body contact with the pup, latency to move, pullus index, rearing, thigmotactic ratio, urination
Pup retrieval	Line	Latency to leave the nest, latency to grab the pup, latency to re-enter the nest with the pup

[a]Prepartum versus postpartum condition

3.3.1 Open field

Ambulation (**I, III, IV**) denotes unprovoked motor activity in the form of spontaneous whole-body movements from one OF unit to another during a 2-min period. The center starting unit was excluded from the animal's ambulation scores.

Defecation (**I–IV**) was recorded by counting the animal's fecal boli after a 2-min OF test (in Study **II**, after a 60-min exposure to the OF).

Exploration (**I**, **III**, **IV**) was registered by counting the number of at-least-once-entered OF units (Lhotellier et al., 1993), that is, the area covered by the animal during a 2-min period. The exploration scores ranged from 0 to 19.

Grooming (**I**, **III**, **IV**) was recorded by counting the number of bouts of cleaning or body scratching in the OF during a 2-min period.

Inner ambulation (**II**) was registered by counting the number of inner units entered during a 2-min OF test.

Latency to body contact with the pup (**IV**) denotes the time in seconds until the mother mouse had her first body contact with her pup. If the mother did not have body contact with her pup during the 120-s recording, it was given the maximum value.

Latency to move (**I**, **III**, **IV**) signifies the time in seconds until the mouse left the OF center (starting) unit. The minimum latency to move was 1 s. If the animal did not leave the starting unit during the 120-s recording, it was given the maximum value.

Outer ambulation (**II**) was recorded by counting the number of outer units entered during a 2-min OF test.

The ***pullus index*** (**IV**) signifies the mother mouse's attraction to her pup (Ewalds-Kvist & Selander, 1997). The pullus index was calculated by dividing the number of times the female mouse entered the OF unit where her pup was located or the adjacent one by the sum of units visited. Hence, the greater the ratio, the more prone the mother was to keeping in close proximity to her pup. The pullus index ranged from 0.00 to 1.00.

Radial latency (**I**, **III**) denotes the time in seconds until the mouse reached its first OF outer unit. The minimum radial latency was 2 s. If the animal did not reach an outer unit during the 120-s recording, it was given the maximum value.

Rearing (**I**, **III**, **IV**) was registered by counting the number of times the animal rose onto its hind legs with the front limbs either against the wall or freely in the air during a 2-min recording (Streng, 1974).

The ***thigmotactic ratio*** (**I–IV**) signifies the animal's orientation towards the OF peripheral units but does not necessarily imply bodily contact with the wall. The thigmotactic ratio was calculated by dividing the number of inner partitions entered by the total sum of units visited by the mouse (McIlwain et al., 2001; Sanberg & Ossenkopp, 1977; Valle & Bols, 1976). Hence, the smaller the ratio, the more prone the mouse was to keeping close to the OF wall. The thigmotactic ratio ranged from 0.00 to 1.00 (in fact, the center starting point caused the mathematical value to exceed 0.00 because the animal received at least one inner-unit ambulatory score before it reached the periphery).

Urination (**I**, **III**, **IV**) was recorded by counting the number of urinary spots voided in the OF during a 2-min period.

3.3.2 Metabolism (II)

Food intake and excretion parameters proportionate to the weight of the mouse and the duration of the experiment were derived by dividing the following

original measures by mouse weight and experiment duration (72 hr = 3 days). These measures were used in the statistical analyses.

Food intake denotes the difference between the preweighed food and the remaining food after a period of 72 hr (Ewalds-Kvist & Selander, 1996).

Excretion (i.e., defecation and urination) was calculated by comparing the weight of the bedding in the cage before and after the 72-hr test period (Ewalds-Kvist & Selander, 1996).

3.3.3 Pup retrieval (IV)

Latency to leave the nest signifies the time in seconds until the adult female mouse left the nest to retrieve the pup (measured for each of the 3 pups). The minimum latency was 1 s.

Latency to grab the pup denotes the time in seconds until the female mouse grabbed the pup with her mouth minus the latency to leave the nest (measured for each of the 3 pups).

Latency to re-enter the nest with the pup signifies the time in seconds until the female mouse re-entered the nest with the pup minus the latencies to leave the nest and grab the pup (measured for each of the 3 pups).

Pup 1 total signifies the total time in seconds that the dam spent in retrieving the 1st pup (i.e., the sum of latency to leave the nest + latency to grab the pup + latency to re-enter the nest with the pup).

Pup 2 total signifies the total time in seconds that the dam spent in retrieving the 2nd pup.

Pup 3 total signifies the total time in seconds that the dam spent in retrieving the 3rd pup.

Total retrieval denotes the total time in seconds that the dam spent in retrieving all 3 pups (i.e., the sum of latency the leave the nest + latency to grab the pup + latency to re-enter the nest with the pup for all of the 3 pups).

3.4 Procedures

3.4.1 Open field (I–IV)

The OF parameters were manually recorded through direct visual observation. The latency times were measured with a stopwatch. In the beginning of the OF test, the mouse was placed in the OF center unit.

3.4.2 Crossfostering (I)

Within each line, the mice were randomly assigned to three different foster conditions: (a) unfostered (i.e., the pups were fostered by their own mother), (b) infostered (i.e., the pups were fostered by an alien mother of their own line), and (c) crossfostered (i.e., the pups were fostered by a mother from the other line). All the cages were coded to cover the animals' identity, and two experimenters blind-tested the mice in a windowless, artificially lit (with normal office fluorescent tubes) experimental room near the breeding room. One or two days before the experimental procedures took place, the mice were exposed to the OF apparatus for approximately 2 min.

In Experiment 1, the pups were assigned to different foster conditions at the age of 5–8 days: 76 pups were unfostered, 65 pups were infostered, and 78 pups were crossfostered. After weaning, the mice were separated according to sex and group-housed with their siblings. In order to avoid the effects of social isolation interfering with later recordings (see Lassalle, Bulman-Fleming, & Wahlsten, 1991; Takahashi et al., 2008), mice without siblings were discarded. At the age of approximately 70 days, the animals were individually housed for about 40 days. After this period, at the age of approximately 110 days, the mice were tested in the OF.

It was thought, however, that the pups in Experiment 1 might have been too old at the time of adoption to be influenced by differing maternal care. For this

reason, the pups in Experiment 2 were assigned to different foster conditions within 24 hr after birth: 50 pups were unfostered, 104 pups were infostered, and 67 pups were crossfostered. After weaning, the mice were separated according to sex and group-housed with their siblings for 1 week. After this, they were group-housed in same-sex groups comprising 4 to 5 mice from the same line and identical foster condition. The mice were tested in the OF at the age of approximately 140 days.

3.4.3 Life span (II)

The animals were inspected approximately three times a week during their entire life span to determine whether they were dead or alive.

3.4.4 Open-field thigmotaxis at seven different ages (II)

Thigmotaxis in the OF was recorded at seven different ages during the males' life spans (see Table 1). Before each recording, the animals were exposed to the OF apparatus for at least 2-min periods on 4 consecutive days.

3.4.5 Open-field defecation for 60 min (II)

The floor of the OF arena was covered with a 1-mm-thick white filter paper. The mouse was placed in the OF for 60 min, after which the animal's fecal boli were counted (Selander & Kvist, 1991).

3.4.6 Home-cage thigmotaxis (II)

Each mouse resided undisturbed for a period of 5 days in an OF arena in which the floor was covered with approximately 250 g of wood shavings (Kvist & Selander, 1990). Food and water were freely available, and the mouse was prevented from leaving the OF by a wire-mesh ceiling. From the 6th to the 10th day, the mouse was tested once a day with respect to thigmotaxis, with the recordings from the 10th day being reported. Given that the floor of the OF arena

(and the partitions drawn on it) was covered by wood shavings, the wire-mesh ceiling was replaced by a transparent plastic film that was divided by thin black lines into 19 partitions identical to those on the floor of the circular OF arena (see 3.2.1 Circular open field), thereby providing raw scores for computation. Thus, the ambulation of the animal was recorded by monitoring the animal's movements through the plastic film.

3.4.7 Metabolism (II)

In the beginning of the metabolism experiment, the animals were weighed and deprived of food pellets for 12 hr, but were given free access to fresh tap water. After that, the cages were replaced with clean ones containing preweighed bedding. The mice were then given access to preweighed food for a period of 72 hr, after which the remaining food and bedding were reweighed (Ewalds-Kvist & Selander, 1996).

3.4.8 Open-field thigmotaxis under four conditions with varying starting points and shapes of the arena (II)

Before the experiment started, the mice were exposed to the OF apparatus for at least 2 min on each of 4 consecutive days. On the 5th day, the mice of both lines were tested in the circular OF, using the center starting point at approximately 7 a.m. and the wall starting point at approximately 5 p.m. (cf., Kvist & Selander, 1992). On the 6th day, the same procedure was repeated in the square-shaped OF (cf., Kvist & Selander, 1992). In the center starting, the mouse was placed in the center of the OF arena, and in the wall starting, it was placed near the wall (in the square-shaped OF, in a corner).

3.4.9 Selection response (III)

In the beginning of the selection experiment, 19 Swiss albino mice (10 females and 9 males) were tested in the OF with respect to thigmotaxis (i.e., the thigmotactic ratio), and 16 mice were selected as parentals for the HOFT and

LOFT lines (4 females and 4 males for both lines). With each generation, the animals were weaned and separated by sex at 4 weeks of age. As a general rule, the mice were tested in the OF at the age of approximately 2 months, on the basis of which the individuals receiving the highest and lowest values with regard to the thigmotactic ratio were selected to continue the selection lines. The number of the selected mice mainly depended on how many mice were needed for the specific experiments performed with the next generation (for ethical reasons, the unnecessary production of mice was avoided), thereby varying between the generations; that is, the selections were not based on any fixed percentage or absolute number of selected mice. Moreover, there were no absolute criteria regarding the thigmotactic ratio that the mice had to meet in order to be selected as parents for the next generation.

Prior to the testing day, the mice were exposed to the OF apparatus for approximately 2 min on each of 4 consecutive days. In other words, the selection was based on the scores registered on the 5th day. This was because (a) the scores from the first day(s) in the OF may not provide very meaningful information (see Tachibana, 1982; van der Staay et al., 1990; Whimbey & Denenberg, 1967); (b) the physical properties of the environment (i.e., light and openness) are sufficient to make it aversive (Calatayud & Belzung, 2001; Russell, 1979); (c) the OF is, nevertheless, a relatively novel environment after four 2-min exposures, compared with the animal's home cage; and (d) the aim of this selection experiment was to create two lines of mice, the divergence in emotionality being based on the more stable and enduring features of these animals, rather than only on an initial response to novelty (see Belzung & Griebel, 2001; Lister, 1990).

As a general rule, the animals were mated at the age of approximately 2.5 months. In a few cases, the same male was mated with two different females. Accordingly to the generally used procedure, inbreeding (i.e., brother–sister matings) was avoided in order to maximize genetic variability as well as fitness and fertility (Ramos et al., 2003; Stead et al., 2006; Suaudeau et al., 2000; Viggiano, Vallone, Welzl, & Sadile, 2002; see also Green, 1966). Some generations differed from the standard procedure, for example, with regard to the testing age. In addition to the deviations explained in the original paper, the data for Generation 16 were obtained from OF testing at the age of approximately 9

months after the experimental procedures with the female mice (**IV**); that is, the data did not include all of the mice in the 16th generation.

3.4.10 Repeated open-field exposure (III)

The mice were tested in the OF for 2 min on 5 consecutive days. All the cages were coded to cover the animals' identity, and two experimenters blind-tested the mice in a windowless, artificially lit (with normal office fluorescent tubes) experimental room near the breeding room. The mice were individually brought to the experimental room, where they were tested immediately. The tests were conducted between 1:00 p.m. and 5:15 p.m.

3.4.11 Prepartum open field (IV)

A total of 26 mice (13 from each line) were tested in the prepartum condition. The OF test was performed from 1 to 7 days (for 20 mice, 1–2 days) prior to parturition because of the variability of the parturition day. The lines were not significantly differentiated by the number of days between parturition and the OF test, $t(24) = 1.49$, $p = .149$. One day prior to the testing, the mice were exposed to the OF apparatus for approximately 2 min.

3.4.12 Postpartum open field (IV)

A total of 34 mice (17 from each line) were tested in the postpartum condition. The OF test was performed from 0 to 5 days (for 28 mice, 1–2 days) after parturition. The lines were not differentiated by the number of days between parturition and the OF test, $t(32) = 0.00$, $p = 1$. At the beginning of the recording, 1 pup was placed in an outer OF unit and its mother in the OF center unit. The pup was placed in the OF arena because it has been suggested that the presence of pups may enhance parturition-related anxiolysis in their mothers (Ferreira, Hansen, Nielsen, Archer, & Minor, 1989; Hård & Hansen, 1985). Moreover, two of the OF parameters (i.e., latency to body contact with the pup and the pullus index) required the presence of a pup in the OF arena.

3.4.13 Pup retrieval (IV)

A total of 34 mice (17 from each line) were tested in the retrieval test. The test was performed from 1 to 5 days (for 30 mice, 3–4 days) after parturition. The lines were not differentiated by the number of days between parturition and the retrieval test, $t(23.7) = 0.00$, $p = 1$. If the adult female mouse had fewer than 3 pups, it was excluded from the experiment. On the other hand, if the mother had more than 3 pups, the additional pups were temporarily transferred to another cage. Thus, at the beginning of the experiment, 3 of the mother's pups were placed in the corner of the home cage opposite to the nest. The parameters were measured with a stopwatch. If the mother did not manage to complete the test in 20 min (i.e., the time limit to perform the test), it was discarded from the statistical analyses.

3.5 Statistical analyses

Study I. In both crossfostering experiments, the effects of line, sex, and fostering on the OF parameters were examined by a three-way (Line × Sex × Fostering) analysis of variance (the General Linear Model [GLM] Univariate procedure in SPSS). Group differences were evaluated by using 95% confidence intervals (CIs) for estimated marginal means.

Study II. In Experiment 1, the effects of line and sex on the length of the mice' life span were examined by a two-way (Line × Sex) analysis of variance (the GLM Univariate procedure in SPSS). Given the rather small number of subjects, the data for OF thigmotaxis at seven different ages, OF defecation for 60 min, home-cage thigmotaxis, and metabolism were analyzed by using both an independent-samples *t* test and a Mann-Whitney *U* test. Likewise, in Experiment 2, the data for OF thigmotaxis under four conditions with varying starting points and shapes of the arena were examined by using an independent-samples *t* test as well as a Mann-Whitney *U* test.

Study III. In the selection experiment, the line differences in the thigmotactic ratio, ambulation, and rearing for each generation were evaluated by using 95% CIs of the means. In addition, a two-way (Line × Sex) analysis of variance (the GLM Univariate procedure in SPSS) was performed to examine potential line and sex differences across generations. The presented mean values in the GLM analyses are estimated marginal means. In the repeated OF-exposure experiment, the OF parameters were analyzed by the GLM Repeated Measures procedure in SPSS, with line and sex as between-subjects factors. The analyses included one within-subjects factor, that is, day (five levels: 1st, 2nd, 3rd, 4th, and 5th testing days). The within-subjects factor was included because the levels of OF parameters may change across time and the potential line differences may not be initially present. The multivariate analyses were used for the repeated-measures data. Linear and quadratic trend components within these analyses were used to delineate effects across the levels of the day within-subjects factor. The mean values for line and sex differences are estimated marginal means. The line differences in thigmotaxis on each testing day were also examined by an independent-samples *t* test and a Mann-Whitney *U* test. The nonparametric test was performed because the data did not totally meet a normal distribution and could not be successfully transformed.

Study IV. The OF parameters were recorded from 26 mice in the prepartum condition and 34 mice in the postpartum condition. However, the data were available from both conditions for only 20 mice (10 from each line). Therefore, the line differences in the OF parameters in the prepartum and postpartum conditions were examined by means of a *t* test. However, when examining the main effects for time and Time × Line interactions in predicting the OF parameters, the GLM Repeated Measures procedure in SPSS was used. These analyses included one within-subjects factor (i.e., time, at two levels: prepartum, postpartum) and one between-subjects factor (i.e., line, at two levels: HOFT, LOFT). The line differences in the parameters of the retrieval test were examined by a *t* test. Before the analyses, the following parameters were log-transformed to normalize the distribution: grooming, latency to body contact with the pup, rearing, the thigmotactic ratio, and the parameters of the retrieval test.

4 RESULTS

Significant main effects for line and sex in predicting different parameters in Studies **I–IV** are presented in Table 4.

Table 4

*Significant main effects for line and sex in predicting different parameters recorded from the HOFT (High Open-Field Thigmotaxis) and LOFT (Low Open-Field Thigmotaxis) mice in Studies **I–IV***

Experiment	Significant line differences	Significant sex differences
Study 1		
Crossfostering 1	Exploration: LOFT > HOFT Grooming: LOFT > HOFT Radial latency: HOFT > LOFT Rearing: LOFT > HOFT Thigmotactic ratio: LOFT > HOFT Urination: HOFT > LOFT	Defecation: females > males Rearing: males > females
Crossfostering 2	Exploration: LOFT > HOFT Latency to move: LOFT > HOFT Rearing: LOFT > HOFT Thigmotactic ratio: LOFT > HOFT	Ambulation: males > females Defecation: females > males Exploration: males > females Rearing: males > females Urination: females > males
Study 2		
Life span	Number of days alive: HOFT > LOFT	

(Table continues)

Table 4 (continued)

Experiment	Significant line differences	Significant sex differences
Open-field thigmotaxis at seven different ages	Inner ambulation: LOFT > HOFT (at Recordings 1, 4, 5, and 6) Outer ambulation: HOFT > LOFT (at Recordings 2, 3, and 4) Thigmotactic ratio: LOFT > HOFT (at Recordings 1, 3, 4, 5, 6, and 7)	
Open-field defecation for 60 min	Defecation: HOFT > LOFT	
Home-cage thigmotaxis	Outer ambulation: HOFT > LOFT Thigmotactic ratio: LOFT > HOFT	
Metabolism	Weight: LOFT > HOFT	

(Table continues)

Table 4 (continued)

Experiment	Significant line differences	Significant sex differences
Open-field thigmotaxis under four conditions with varying starting points and shapes of the arena	Inner ambulation: LOFT > HOFT (in each situation) Thigmotactic ratio: LOFT > HOFT (in each situation)	
Study 3		
Selection response	Rearing: LOFT > HOFT Thigmotactic ratio: LOFT > HOFT	Ambulation: males > females Rearing: males > females Thigmotactic ratio: males > females
Repeated open-field exposure	Ambulation: LOFT > HOFT Exploration: LOFT > HOFT Grooming: LOFT > HOFT Rearing: LOFT > HOFT Thigmotactic ratio: LOFT > HOFT (on the 5th day)	Ambulation: males > females Exploration: males > females

(Table continues)

Table 4 (continued)

Experiment	Significant line differences	Significant sex differences
Study 4		
Prepartum open field	Exploration: LOFT > HOFT Rearing: LOFT > HOFT Thigmotactic ratio: LOFT > HOFT	
Postpartum open field	Ambulation: LOFT > HOFT Defecation: HOFT > LOFT Exploration: LOFT > HOFT Pullus index: LOFT > HOFT Rearing: LOFT > HOFT Thigmotactic ratio: LOFT > HOFT	
Pup retrieval	Latency to leave the nest for the 1st pup: LOFT > HOFT Latency to re-enter the nest with the 2nd pup: LOFT > HOFT	

4.1 Crossfostering (I)

Detailed results for the two crossfostering experiments are presented in the original paper. Only those findings are shown here that were replicated in both experiments. Due to the small procedural differences between the two experiments (i.e., housing and the testing age), it was not justified to assess the effects of the adoption age by comparing the results of the two crossfostering experiments.

In Experiments 1 and 2, the GLM revealed significant main effects for line in predicting the thigmotactic ratio, $F(1, 206) = 14.42$, $p < .001$ and $F(1, 208) = 9.37$, $p = .002$, respectively, exploration, $F(1, 206) = 24.06$, $p < .001$ and $F(1, 208) = 11.25$, $p = .001$, respectively, and rearing, $F(1, 206) = 41.96$, $p < .001$ and $F(1, 208) = 58.31$, $p < .001$, respectively. That is, in Experiments 1 and 2, the LOFT mice were less thigmotactic (*M*s = .18 and .25, respectively) but explored (*M*s = 15.0 and 16.0, respectively) and reared (*M*s = 5.5 and 7.7, respectively) more than the HOFT mice (for the thigmotactic ratio in Experiments 1 and 2, *M*s = .12 and .20, respectively; for exploration, *M*s = 12.6 and 14.4, respectively; for rearing, *M*s = 1.9 and 2.5, respectively). In Experiments 1 and 2, there were also significant main effects for sex in predicting rearing, $F(1, 206) = 5.05$, $p = .026$ and $F(1, 208) = 10.49$, $p = .001$, respectively, and defecation, $F(1, 206) = 19.19$, $p < .001$ and $F(1, 208) = 41.10$, $p < .001$, respectively. That is, in Experiments 1 and 2, the male mice reared more (*M*s = 4.3 and 6.2, respectively) but defecated less (*M*s = 0.6 and 0.7, respectively) than the female mice (for rearing in Experiments 1 and 2, *M*s = 3.1 and 4.0; for defecation, *M*s = 1.4 and 2.1, respectively).

When predicting the thigmotactic ratio, there was no significant main effect for, or interactions involving, fostering in either Experiment 1 or 2.

4.2 Life span and stability of the selection trait (II)

For Experiments 1 and 2, detailed results for all of the parameters of the different recordings are presented in the original paper.

In Experiment 1, the GLM revealed a significant line difference in life span; that is, the HOFT mice exhibited longer life spans (*M* = 691.2 days, *SD* =

127.2) than the LOFT mice (M = 600.1 days, SD = 214.3), $F(1, 47) = 4.71$, $p = .035$. There was no statistically significant sex-related difference or Line × Sex interaction. Both the t test and the Mann-Whitney U test showed that the LOFT males were significantly heavier (M = 33.6 g, SD = 2.0) than the HOFT males (M = 28.9 g, SD = 1.2), $t(12) = 5.39$, $p < .001$; $U = 0.0$, $p = .002$. By contrast, the lines did not differ in food intake or excretion.

OF thigmotactic behavior was recorded seven times at different ages during the males' life spans (for ages at the time of testing, see Table 1). With the exception of the second recording at the age of approximately 95 days, the HOFT males always exhibited significantly lower thigmotactic ratios (i.e., were more thigmotactic) than the LOFT males (for both the t tests and the Mann-Whitney U tests, ps < .05; see Figure 2). Both tests showed that the line difference in inner ambulation was statistically significant at Recordings 1, 4, 5, and 6 (ps < .05), with the LOFT males entering more inner OF units than the HOFT males. Both tests also showed that the lines were significantly differentiated by outer ambulation, with the HOFT males entering more outer OF units than the LOFT males, at Recordings 2, 3, and 4 (ps < .05).

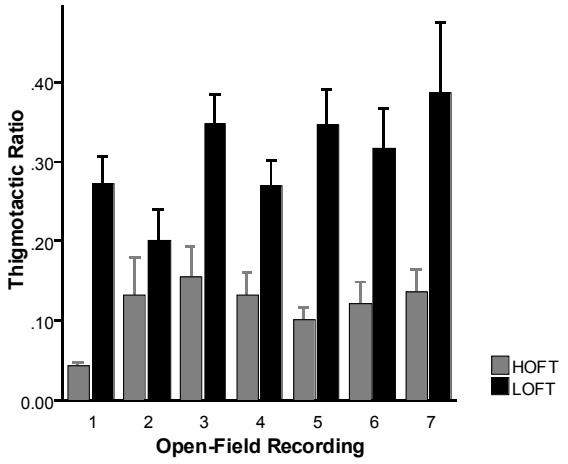

Figure 2. Mean thigmotactic ratio in the open field recorded at seven different ages during the HOFT (S_{13}) and LOFT (S_{13}) male mice' life spans. With the exception of the second measurement, the line difference was statistically significant ($p < .05$) at all ages. Error bars represent the standard error. HOFT = High Open-Field Thigmotaxis; LOFT = Low Open-Field Thigmotaxis.

Both the *t* test and the Mann-Whitney *U* test showed that the HOFT males defecated more fecal boli ($M = 14.8$, $SD = 2.3$) than the LOFT males ($M = 11.1$, $SD = 2.5$), $t(14) = 3.06$, $p = .008$; $U = 8.0$, $p = .011$, during the 60-min exposure to the OF. Moreover, the *t* test showed that the HOFT males exhibited significantly lower thigmotactic ratios ($M = .16$, $SD = .16$), compared with the LOFT males ($M = .35$, $SD = .14$), $t(11) = 2.26$, $p = .045$, in the home-cage condition. However, the Mann-Whitney *U* test narrowly failed to reach statistical significance, $U = 7.5$, $p = .053$. In addition, both tests showed that in the home-cage condition, the HOFT males entered significantly more outer OF units ($M = 22.2$, $SD = 5.5$) than the LOFT males ($M = 12.1$, $SD = 5.6$), $t(11) = 3.25$, $p = .008$; $U = 3.5$, $p = .012$. The line difference in inner ambulation was not statistically significant.

In Experiment 2, independently of the location (center or wall) of the starting point and the shape (circular or square) of the OF arena, the HOFT males always exhibited significantly lower thigmotactic ratios (for both the *t* tests and the Mann-Whitney *U* tests, ps $\leq .001$) and entered fewer OF inner units (for both tests, ps $\leq .012$) than the LOFT males. By contrast, the line difference in outer ambulation was not statistically significant in any of the four situations.

4.3 Selection response and repeated exposure to the open field (III)

Figure 3 shows the means and 95% CIs for the thigmotactic ratio in 23 generations of the HOFT and LOFT mice. With the exception of Generations 1, 2, and 12, the line difference in thigmotaxis was statistically significant (i.e., the CIs did not overlap) in each generation; that is, the HOFT mice were significantly more thigmotactic (i.e., had lower thigmotactic ratios) than the LOFT mice in almost each generation of the selective-breeding experiment.

Especially after the 10th generation, the LOFT mice tended to rear more than the HOFT mice, although the line difference was not evident in each generation (see Figure 4). The inspection of CIs shows that the difference in rearing between the HOFT and LOFT lines was statistically significant in Generations 2, 11, 12, 13, 15, 17, 20, 22, and 23.

With the exception of Generations 9 and 15, the HOFT and LOFT lines were not differentiated by ambulation in the different generations (see Figure 5).

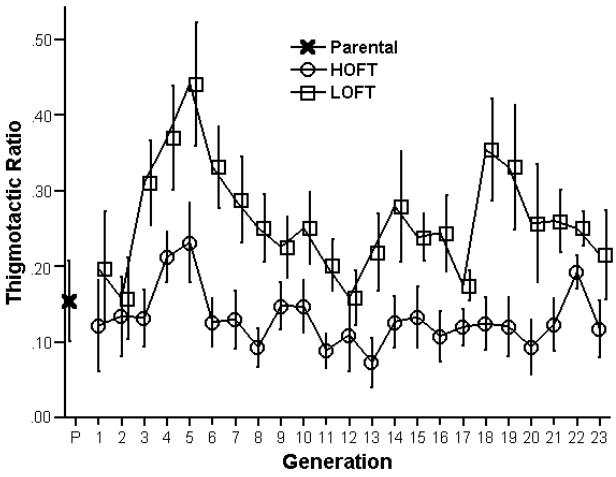

Figure 3. Mean thigmotactic ratio and 95% confidence intervals for the HOFT (High Open-Field Thigmotaxis) and LOFT (Low Open-Field Thigmotaxis) mice as a function of bidirectional selection for high and low thigmotactic ratios.

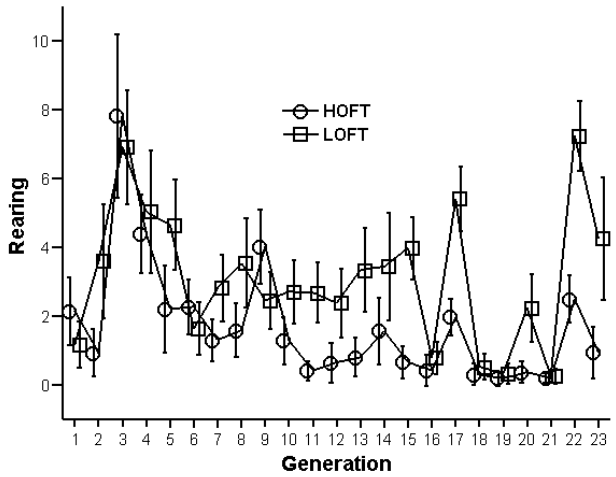

Figure 4. Mean number of rearings and 95% confidence intervals for the HOFT (High Open-Field Thigmotaxis) and LOFT (Low Open-Field Thigmotaxis) mice as a function of bidirectional selection for high and low thigmotactic ratios.

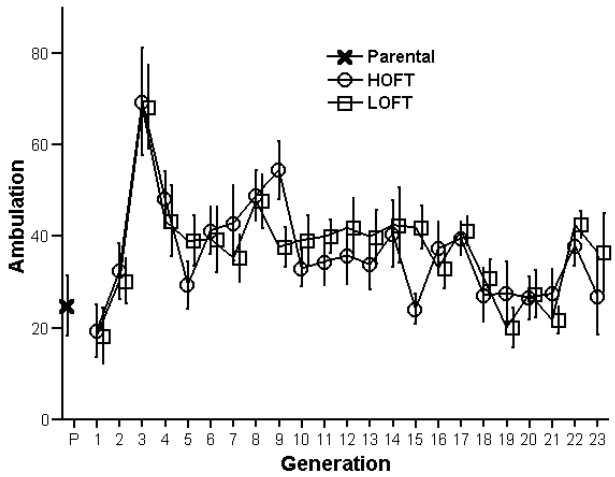

Figure 5. Mean ambulation scores and 95% confidence intervals for the HOFT (High Open-Field Thigmotaxis) and LOFT (Low Open-Field Thigmotaxis) mice as a function of bidirectional selection for high and low thigmotactic ratios.

When examining line and sex differences across generations, the GLM revealed significant main effects for line and sex in predicting the thigmotactic ratio, *F*s(1, 2025) = 321.86 and 26.07, *p*s < .001, η^2s = .14 and .01, respectively. That is, the HOFT (*M* = .14) and female (*M* = .18) mice were more thigmotactic than the LOFT (*M* = .25) and male (*M* = .21) mice. Likewise, significant main effects were found for line and sex in predicting rearing, *F*s(1, 2025) = 95.09 and 28.19, *p*s < .001, η^2s = .05 and .01, respectively. That is, the LOFT (*M* = 3.5) and male (*M* = 3.1) mice reared more than the HOFT (*M* = 1.9) and female (*M* = 2.2) mice. In addition, a significant sex difference was found in predicting ambulation, with the males (*M* = 39.7) ambulating more than the females (*M* = 36.1), *F*(1, 2025) = 16.01, *p* < .001, η^2 = .01. No significant Line × Sex interactions were found.

In the repeated OF-exposure experiment, the GLM Repeated Measures procedure revealed a significant main effect for day in predicting ***ambulation***, *F*(4, 37) = 7.65, *p* < .001, η^2 = .45, and a significant quadratic trend component, *F*(1, 40) = 22.67, *p* < .001, η^2 = .36, indicated that ambulation decreased after Day 1, but increased again for Days 4 and 5. There were also significant main effects for line and sex in predicting ambulation, *F*s(1, 40) = 11.09 and 8.20, *p*s

= .002 and .007, η^2s = .22 and .17, respectively. That is, the LOFT (M = 33.2) and male (M = 32.2) mice ambulated more than the HOFT (M = 19.5) and female (M = 20.4) mice.

A significant main effect for day was found in predicting ***exploration***, $F(4, 37) = 8.91$, $p < .001$, $\eta^2 = .49$, and a significant quadratic trend component, $F(1, 40) = 34.06$, $p < .001$, $\eta^2 = .46$, indicated that, compared with Day 1, exploration was lower during Days 2 and 3, but increased again for Days 4 and 5. When predicting exploration, there were also significant main effects for line and sex, Fs(1, 40) = 14.47 and 6.42, ps < .001 and = .015, η^2s = .27 and .14, respectively. That is, the LOFT (M = 12.6) and male (M = 12.0) mice explored more than the HOFT (M = 8.7) and female (M = 9.4) mice.

There was a significant main effect for day in predicting ***grooming***, $F(4, 37) = 4.34$, $p = .006$, $\eta^2 = .32$, and a significant linear trend component, $F(1, 40) = 17.72$, $p < .001$, $\eta^2 = .31$, indicated that grooming decreased progressively across the testing days. There was also a significant main effect for line, $F(1, 40) = 5.26$, $p = .027$, $\eta^2 = .12$. That is, the LOFT mice exhibited higher grooming than the HOFT mice, Ms = 1.2 and 0.5, respectively.

When predicting ***radial latency***, there was a significant main effect for day, $F(4, 37) = 5.98$, $p = .001$, $\eta^2 = .39$, and a significant linear trend component, $F(1, 40) = 18.16$, $p < .001$, $\eta^2 = .31$, indicated that radial latency decreased linearly across the testing days. In predicting radial latency, there were no significant main effects for, or interactions involving, line or sex.

A significant main effect for day was found in predicting ***rearing***, $F(4, 37) = 5.56$, $p = .001$, $\eta^2 = .38$, and a significant quadratic trend component, $F(1, 40) = 8.51$, $p = .006$, $\eta^2 = .18$, indicated that, compared with the 1st day, rearing was lower during Days 2 and 3, but increased again for Days 4 and 5. There was also a significant main effect for line, $F(1, 40) = 7.18$, $p = .011$, $\eta^2 = .15$. That is, the LOFT mice exhibited higher rearing than the HOFT mice, Ms = 3.5 and 1.2, respectively.

There was a significant main effect for testing day in predicting the ***thigmotactic ratio***, $F(4, 35) = 2.71$, $p = .045$, $\eta^2 = .24$, and a significant linear trend component, $F(1, 38) = 8.20$, $p = .007$, $\eta^2 = .18$, indicated that the thigmotactic ratio decreased progressively over time. The main effect for line

was nonsignificant, $F < 1$. Likewise, the Day × Line interaction failed to reach statistical significance, $F(4, 35) = 1.94$, $p = .126$, $\eta^2 = .18$. However, both the *t* test and the Mann-Whitney *U* test indicated that on Day 5, the thigmotactic ratio was significantly lower among the HOFT mice ($M = .12$, $SD = .08$) than among the LOFT mice ($M = .22$, $SD = .14$), $t(41) = 2.82$, $p = .007$; $U = 122.0$, $p = .009$.

Line, sex, and day played no significant role in predicting ***defecation***, ***latency to move***, or ***urination***.

4.4 Prepartum and postpartum open-field behavior and maternal responsiveness (IV)

The data for all of the parameters of the pre- and postpartum OF tests as well as of the retrieval test are presented in the original paper. Here only the statistically significant findings are shown.

In the prepartum condition, the *t* test showed that the HOFT dams were more thigmotactic (i.e., had lower thigmotactic ratios; $M = -2.9$, $SD = 0.6$) than the LOFT dams ($M = -1.5$, $SD = 0.6$), $t(24) = 5.86$, $p < .001$. In addition, the HOFT mothers explored less ($M = 10.0$, $SD = 3.3$) than the LOFT mothers ($M = 14.3$, $SD = 4.4$), $t(24) = 2.83$, $p = .009$. Moreover, rearing was lower among the HOFT mice ($M = 0.1$, $SD = 0.3$) than among the LOFT mice ($M = 0.7$, $SD = 0.9$), $t(14.1) = 2.25$, $p = .041$ during pregnancy.

Also in the postpartum condition, the *t* test indicated that the HOFT mothers were more thigmotactic ($M = -2.9$, $SD = 0.7$), contrasted with the LOFT mothers ($M = -1.7$, $SD = 0.5$), $t(28.7) = 5.53$, $p < .001$. Likewise, the HOFT dams explored less ($M = 11.9$, $SD = 2.8$) than the LOFT dams ($M = 17.4$, $SD = 1.9$), $t(32) = 6.53$, $p < .001$. Similarly, rearing was lower among the HOFT females ($M = 1.2$, $SD = 0.8$) than among the LOFT females ($M = 1.8$, $SD = 0.8$), $t(32) = 2.16$, $p = .039$ during lactation as well. Contrary to the prepartum condition, the HOFT and LOFT lines differed with regard to ambulation in the postpartum OF test; that is, the LOFT dams ambulated more ($M = 57.9$, $SD = 20.1$) than the HOFT dams ($M = 35.1$, $SD = 16.7$), $t(32) = 3.59$, $p = .001$. Moreover, in the postpartum condition, the lines were also differentiated by defecation, with the HOFT mice defecating more ($M = 3.8$, $SD = 2.0$) than the LOFT mice ($M = 1.6$, $SD = 1.4$),

$t(32) = 3.79$, $p = .001$. In addition, the pullus index was higher among the LOFT mothers (i.e., the LOFT mothers were more attracted to their pups; $M = .38$, $SD = .12$) than among the HOFT mothers ($M = .26$, $SD = .17$), $t(32) = 2.27$, $p = .030$.

When examining the main effects for time and Time × Line interactions, the GLM revealed that there was a significant main effect for time in predicting ambulation, $F(1, 18) = 27.10$, $p < .001$, $\eta^2 = .60$, exploration, $F(1, 18) = 11.06$, $p = .004$, $\eta^2 = .38$, grooming, $F(1, 18) = 34.21$, $p < .001$, $\eta^2 = .66$, and rearing, $F(1, 18) = 63.57$, $p < .001$, $\eta^2 = .78$. That is, ambulation, exploration, grooming, and rearing increased after parturition. There was also a significant Time × Line interaction in predicting grooming, $F(1, 18) = 12.96$, $p = .002$, $\eta^2 = .42$. That is, grooming increased more conspicuously among the LOFT mice than among the HOFT mice after parturition. In addition, there was a significant Time × Line interaction in predicting defecation, $F(1, 18) = 11.00$, $p = .004$, $\eta^2 = .38$. More specifically, whereas defecation increased among the HOFT mothers after parturition, it decreased among the LOFT mothers.

In the pup-retrieval test, the *t* test showed that the latency to leave the nest to retrieve the 1st pup was shorter among the HOFT dams ($M = 0.32$, $SD = 0.34$) than among the LOFT dams ($M = 0.83$, $SD = 0.44$), $t(28) = 3.52$, $p = .002$. Similarly, the latency to re-enter the nest with the 2nd pup was shorter among the HOFT mothers ($M = 0.45$, $SD = 0.20$), compared with the LOFT mothers ($M = 0.69$, $SD = 0.38$), $t(23.3) = 2.21$, $p = .038$. No other significant line differences were found.

5 DISCUSSION

5.1 Selection response and the origin of the line difference in thigmotaxis

The selective breeding of mice for OF thigmotaxis resulted in bidirectional phenotypic divergence, with the mean differences of the two lines being always in the same direction across the different generations; that is, the HOFT mice were more thigmotactic than the LOFT mice in each generation (although for three generations, the mean difference was not statistically significant). Consequently, OF thigmotactic behavior was proven to be a strong characteristic for producing two diverging lines of mice, suggesting a genetic background of this feature (see Gray, 1987, p. 43; Hurnik et al., 1973; Lagerspetz & Lagerspetz, 1983; Stead et al., 2006). The genetic determination of thigmotaxis is also supported by the crossfostering study showing that, when tested in adulthood, the thigmotactic behavior of the HOFT and LOFT mice resembled that of their genetic line, not their foster mother's line; that is, the line difference in thigmotaxis was primarily innate in origin and not influenced by differing maternal behavior. This is in line with the crossfostering study on rats selected for high (HAB) and low (LAB) levels of anxiety-related behavior, which showed that the rats' divergence in emotionality was genetically determined, rather than postnatally acquired through maternal behavior (Landgraf & Wigger, 2002). It should be noted, however, that the crossfostering paradigm does not control for prenatal maternal influences on animal behavior (Gray, 1987, p. 43; Poley & Royce, 1970).

The results of the selection experiment as well as of the crossfostering study thus support the assumption that emotionality in rodents is highly genetically determined (Boissy, 1995; Flint et al., 1995; Ramos et al., 2003; Takahashi et al., 2008; Trullas & Skolnick, 1993). Support for a genetic explanation of individual differences in mice' thigmotactic behavior also comes from studies showing interstrain differences in this respect (Fredericson, 1953; Gershenfeld & Paul, 1997; Stoltenberg & Hirsch, 1998), although interstrain differences do not eliminate the possibility that a specific trait may also be determined by differing mothering styles (Calatayud & Belzung, 2001; Clément et al., 2002; Holmes

et al., 2005; Poley & Royce, 1970). In the present study, however, postnatal maternal factors showed no effects on the determination of the line difference in thigmotaxis. As previously mentioned, because the crossfostering study did not control for prenatal (i.e., uterine) environmental factors, the divergence between the HOFT and LOFT mice may, at least partially, also stem from prenatal maternal influences.

In the selection experiment, the LOFT mice deviated more notably from the parental values than the HOFT mice, probably because the mean thigmotactic ratio of the parental mice was relatively low and, as a result of a "floor effect", could not approach a much lower level during the course of the selective breeding. Asymmetry in the bidirectional selection response is, however, commonly observed (Brush, 2003; Ramos et al., 2003; Stead et al., 2006). It is also noteworthy that the genetic gain of the selective breeding increased only up to the 6th generation and was relatively modest in all. This may be due to the small number of mice in the parental population and, although largely outbred, the genetic homogeneity of Swiss albino mice (Staats, 1966). The nonlinear selection response across different generations is, however, frequently observed in other selection studies as well (see Ramos et al., 2003).

5.2 Stability of the selection trait

The line difference in thigmotaxis between the HOFT and LOFT mice was not affected by age at the time of testing; that is, the HOFT mice were always more thigmotactic than the LOFT mice across the different ages, although the line difference in thigmotactic ratio was not statistically significant at the age of approximately 95 days. This agrees with Elias et al. (1975) suggestion that, even if the strain differences in emotionality may be age dependent to a certain degree, when comparing strains differing greatly in this respect, strain differences can be observed at all ages.

The line difference in thigmotaxis was present during pregnancy and lactation as well, which is consonant with studies in rats showing that the differences in anxiety-related behavior between lines that were selectively bred for high and low levels of anxiety persisted throughout pregnancy and during

lactation (Neumann, 2003). Moreover, the divergence in thigmotaxis between the HOFT and LOFT mice was also preserved in the home-cage condition, in which the OF arena was highly familiar to the animals, meaning that the line difference was not provoked only by the novelty of the OF apparatus. Likewise, the line difference did not vary with the characteristics of the OF testing; that is, neither the location of the starting point (center or wall) nor the shape of the OF arena (circular or square) affected the difference in thigmotaxis between the HOFT and LOFT mice.

Consequently, on the basis of these findings, the difference in thigmotaxis between the selectively bred HOFT and LOFT mice seemed to be a stable and robust feature of these animals, suggesting that the two lines differed with regard to trait anxiety rather than to state anxiety (see Belzung & Griebel, 2001; Lister, 1990).

It should be noted, however, that when the mice were repeatedly exposed to the OF apparatus, the HOFT and LOFT lines significantly differed in thigmotaxis only on the 5th day (by means of a *t* test and a Mann-Whitney *U* test), although in the crossfostering study, the line difference was repeatedly found on the 2nd day in the OF (i.e., after one habituation period). This might question the stability of the selection trait; however, because the HOFT and LOFT lines were selected on the basis of the scores obtained on the 5th day, it could be expected that the line difference in thigmotaxis would be most pronounced on that day and may not always manifest itself on other days. Compatible with the present finding, Streng (1974) discovered that the line difference between mice bidirectionally selected for activity was highest during the first 4-min period of the testing for which they had been originally selected, with the line difference progressively decreasing and almost disappearing at the end of the 20-min recording. In addition, the unexpectedly small line difference in thigmotaxis in the repeated OF-exposure experiment seems to be, at least partially, due to the low level of ambulation exhibited by the HOFT mice (recall that in contrast to the general findings, the two lines differed with regard to ambulation in this experiment). More specifically, if the HOFT mouse went directly to the wall and barely ambulated thereafter, the mouse was obviously highly thigmotactic; however, the single inner unit that the mouse had to traverse in order to reach

the wall contributed disproportionately to the thigmotactic ratio, increasing it excessively (remembering that a high ratio indicates a low level of thigmotaxis).

5.3 Coselection of other characteristics

Across the 23 generations, the HOFT and LOFT lines differed with regard to rearing in most generations after the 10th one, with the LOFT mice rearing more than the HOFT mice. In addition to the selection experiment, the line difference in rearing manifested itself in each experiment in which the number of rearings was measured. Exploration scores were not registered with each generation, but when recorded, a line difference in exploration was always found; that is, the less thigmotactic LOFT mice explored more than the more thigmotactic HOFT mice. This means that selective breeding for OF thigmotaxis also resulted in a coselection of differences in rearing and exploration. Given that rearing represents one aspect of exploratory behavior (Archer, 1973; van Abeelen, 1970; Crusio, 2001; Hoover-Plow, Skomorovska-Prokvolit, & Welsh, 2001; Steimer & Driscoll, 2003), these findings are in line with the premise in many OF studies that emotionality and exploratory tendencies are inversely related (Archer, 1973; Holmes, 2001).

As a general rule, the HOFT and LOFT lines did not differ in ambulation across the different generations. This is consonant with previous observations showing that lines differing in thigmotaxis do not always differ in ambulation (Ramos, Berton, Mormède, & Chaouloff, 1997; Ramos & Mormède, 1998). Moreover, it has been previously found that drugs affecting the level of thigmotaxis do not alter locomotor activity or alter it in an inconsistent manner (Choleris et al., 2001; Simon et al., 1994; see also Gross, Santarelli, Brunner, Zhuang, & Hen, 2000). However, in the repeated OF-exposure experiment as well as in the postpartum OF condition, the HOFT mice ambulated less than the LOFT mice. Because ambulation is a traditional index of emotionality, with low levels of ambulation indicating high levels of emotionality (Gervais, 1976; Turri, Datta, DeFries, Henderson, & Flint, 2001), this finding is in line with the view of the HOFT mice being more emotional than the LOFT mice.

As was the case with ambulation, the HOFT and LOFT lines did not

generally differ in OF defecation, which is also considered to be a traditional index of emotionality (Walsh & Cummins, 1976). This may be due to the fact that emotionality is probably a multidimensional construct (Archer, 1973; Brush, 2003; Liebsch et al., 1998; Ohl et al., 2001; Ramos & Mormède, 1998), and, consequently, different parameters of emotionality only measure different aspects of it. On the other hand, some researchers (e.g., Lister, 1990; Ramos & Mormède, 1998) have questioned the use of ambulation and defecation as indexes of emotionality in the first place. However, during the 60-min OF exposure as well as in the postpartum OF condition, a line difference in defecation was found, with the findings being consonant with the traditional view: The more emotional HOFT mice defecated more than the less emotional LOFT mice. Moreover, each time the two lines differed in grooming, the divergence was in the same direction; that is, the LOFT mice groomed more than the HOFT mice. This finding is consonant with a theory formulated for rats that grooming is related to the state of dearousal due to habituation to a stressful situation, thus correlating negatively with emotionality (File, Mabbutt, & Walker, 1988; Spruijt, van Hooff, & Gispen, 1992). Consequently, in line with this suggestion, the less emotional LOFT mice habituated sooner to a stressful situation than the more emotional HOFT mice, thus exhibiting a higher level of grooming.

In addition to the aforementioned OF behaviors, the HOFT and LOFT lines differed in the length of their life spans. More specifically, the more thigmotactic (i.e., the more emotional) HOFT mice exceeded the less thigmotactic LOFT mice in longevity. This is at variance with previous observations demonstrating that strains exhibiting high levels of emotionality in a novel environment typically have a short life span (Viveros, Fernández, Guayerbas, & De la Fuente, 2001), although evidence for a positive correlation between emotionality and the length of the life span has also been provided (Markowska et al., 1998). A mouse's life span is determined by interactions between the environment and a number of genes (Russell, 1966; Yunis et al., 1984). Moreover, it has been proposed that immunoregulatory processes are one of the principal mediating factors in controlling longevity in mice (Popp, 1982; Yunis et al., 1984).

5.4 Sex differences

Across the 23 generations, the female mice of both lines were more thigmotactic than the male mice, although in the crossfostering study as well as in the repeated OF-exposure experiment, the two sexes did not differ with regard to thigmotaxis. The female mice also ambulated and reared less than the male mice across all generations as well as in most of the other experiments. Moreover, each time a sex difference in defecation, urination, or exploration was found, the female mice defecated and urinated more but explored less than the male mice.

All of these findings suggest that the female mice of the HOFT and LOFT lines were more emotional than the male mice. This contradicts Gray's (1971, 1987, p. 93) suggestion that male rodents are more fearful than female rodents (see also Gray & Buffery, 1971). However, Gray (1971, 1979b, 1987, p. 94) has also proposed that the use of inbred or selectively bred strains may affect the typical emotionality-related sex differences in mice as well as in rats. More specifically, selective breeding (especially when accompanied by inbreeding) may lead to a reduction, disappearance, or even reversal of the usual sex differences in rodents (see Gray, 1971, 1979a, 1979b). Accordingly, although male mice may normally, for example, defecate more but ambulate, explore, and rear less than the female mice in the OF, these typical sex differences may be reversed when testing selectively bred mice (see Gray, 1971, 1979a, 1979b). Although Gray emphasized the significance of inbreeding in reducing or even reversing typical sex differences in rodents, this might explain the present findings in the selectively bred HOFT and LOFT lines in spite of the fact that inbreeding was avoided in the present selection study. More specifically, due to the small number of mice in the parental population as well as the genetic homogeneity of Swiss albino mice (Staats, 1966), the HOFT and LOFT lines were presumably genetically highly homogenous, thus resembling inbred lines, although brother–sister matings were avoided.

It is noteworthy, however, that previous studies examining laboratory rodents' sex differences in emotionality have shown highly variable results (see Ramos et al., 2003). In contrast to Gray's view, but in line with the present study, Viveros et al. (2001) found female mice from an outbred Swiss strain to

exhibit a higher level of fear when compared with male mice. Moreover, Archer (1971, 1975, 1979) has strongly questioned rodents' typical sex differences in emotionality and pointed out that with regard to OF defecation and ambulation, for example, mice may have no typical sex differences at all. In addition, Archer (1977) has demonstrated that female mice exhibited more pronounced initial emotional responses, such as defecation and restricting their movements to the OF wall, than the male mice, which finding is consonant with the present results derived from the HOFT and LOFT lines. It should be noted, however, that in Archer's study, the more pronounced emotional responses in female mice were exhibited by two inbred strains, which means that these results do not necessarily contradict Gray's suggestion. However, Archer (1977) also pointed out in his study that the findings did not support Gray's claim because the outbred strain exhibited far fewer sex differences than the two inbred strains.

5.5 Repeated exposure to the open field

When the HOFT and LOFT mice were repeatedly exposed to the OF apparatus, the mice of both lines ambulated, explored, and reared more on the 1st day than on the 2nd and 3rd days, and after the 3rd day, all of these behaviors increased again for the 4th and 5th days. This partly contradicts the general assumption that many behaviors influenced by exploratory drive are at first expected to increase as the inhibitory effects of fear decrease, but then to wane as the OF arena becomes more familiar to the animal (Archer, 1973; Crusio, 2001; Takahashi et al., 2006). It is plausible, however, that a 5-day period for 2-min exposures may not be a sufficient time span for studying a process of habituation. More specifically, the increase in the parameters measuring exploratory tendencies on 4th and 5th days may be due to a decrease in the level of fear. Moreover, if the test had been sufficiently repeated, a decrease in these behaviors might have been observed.

With regard to the high levels of ambulation, exploration, and rearing on Day 1, ambulation on the 1st day, at least in rats, may indicate an attempt to escape (Aulich, 1976; Williams & Russell, 1972), thereby correlating positively with emotionality (Gray, 1982, p. 40; Whimbey & Denenberg, 1967), with

high levels of ambulation in mice on the 1st day having also previously been reported (Blizard, 1971; Whitford & Zipf, 1975). Consequently, an attempt to escape could explain the high levels of ambulation, exploration, and rearing in the present study on Day 1. According to this view, a drop in the level of ambulation after the hyperactivity of the 1st day reflects the habituation of escape attempts, and the subsequent increase in ambulation reflects an increase in exploratory tendencies with a progressively decreasing level of fear (see Russell & Williams, 1973). Thus, different motivational factors may underlie ambulation in the OF on the 1st day, compared with subsequent days (Archer, 1973). It is noteworthy, however, that although Whimbey and Denenberg (1967) showed high ambulation on the 1st day to be indicative of high emotionality, contrasted with the subsequent days when it indicates the opposite, they also suggested that test scores from the 1st day in the OF may not provide very meaningful information at all (see also Nagy & Glaser, 1970; Tachibana, 1982; van der Staay et al., 1990).

During the course of 5 days, the repeated exposure to the OF resulted in a linear increase in thigmotaxis (i.e., a decrease in the thigmotactic ratio) in the HOFT and LOFT lines. This contradicts previous studies (Ossenkopp et al., 1994; Williams & Russell, 1972; see also Russell, 1979) as well as the general assumption that emotional responses related to fear are highest during the initial exposure to the OF and then expected to decrease with habituation (Ivinskis, 1970; Makino et al., 1991; Takahashi et al., 2006). This also contradicts the present finding that exploratory tendencies (i.e., ambulation, exploration, and rearing) were higher on the 4th and 5th days than on the 2nd and 3rd days, suggesting a decrease in the level of fear on Days 4 and 5. Consequently, it is unlikely that the linear increase in thigmotaxis during the 5 days would indicate an increased level of fear. This discrepancy may be due to the fact that the HOFT and LOFT mice were selectively bred for thigmotaxis, which may have, in turn, altered the mice' typical behavioral pattern in this respect.

The repeated exposure to the OF also resulted in a linear decrease in radial latency and grooming across the testing days. The linear decrease in radial latency may be related to the progressive increase in thigmotaxis over time. More specifically, when the mouse becomes more thigmotactic, it is more

prone to seek the OF wall, and, consequently, the latency to reach the periphery shortens.

The linear decrease in grooming, on the other hand, contradicts the assumption that grooming is an indicator of dearousal due to habituation to a stressful situation and, consequently, correlates negatively with emotionality (File et al., 1988; Spruijt et al., 1992). Instead, the progressive decrease in grooming across the testing days is in line with the more traditional view, according to which grooming is a reaction to a stressful situation and often provoked by novelty, thus correlating positively with emotionality (Clément & Chapouthier, 1998; Dunn, Guild, Kramarcy, & Ware, 1981; Steimer & Driscoll, 2003). However, in the repeated OF-exposure experiment, there was also a line difference in grooming, with the less emotional LOFT mice grooming more than the more emotional HOFT mice. Consequently, the line difference in grooming is compatible with the view that grooming is an indicator of dearousal due to habituation to a stressful situation, thus correlating negatively with emotionality. Nevertheless, this interpretation obviously contradicts the finding that grooming linearly decreased over time. More specifically, because emotional responses are supposed to decrease with habituation (Ivinskis, 1970; Makino et al., 1991; Takahashi et al., 2006), grooming should increase over time if it is related to the state of dearousal due to habituation to a stressful situation.

Thus, it should be recognized that in the repeated OF-exposure experiment, some of the findings (e.g., the linear increase in thigmotaxis across the testing days and the temporal patterns of ambulation, exploration, and rearing) seem to be contradictory and also to conflict with general assumptions as well as with previous studies. In addition, on the basis of the present results, the nature of grooming seems unclear. Contradictory results are, however, commonly found in studies examining temporal changes in OF parameters, possibly due to the large number of uncontrolled factors involved in OF testing situations (Archer, 1973). Moreover, it has been shown that the patterns of temporal changes in different OF behaviors are strain-dependent in mice as well as in rats (see Takahashi et al., 2006). Consequently, the effects of repeated testing on various OF behaviors have not yet been clearly determined.

5.6 Open-field behavior during pregnancy and lactation along with maternal responsiveness

The HOFT and LOFT mice exhibited their typical line differences in thigmotaxis, exploration, and rearing during pregnancy and lactation as well; that is, the HOFT mothers were more thigmotactic but explored and reared less than the LOFT mothers. However, in the postpartum condition in the presence of a single pup in the OF arena, the mothers of the two lines also differed in ambulation and defecation. More specifically, the HOFT mothers ambulated less but defecated more than the LOFT mothers. Given that ambulation and defecation are traditional indexes of emotionality, with high levels of defecation and low levels of ambulation interpreted as high levels of emotionality (Flint et al., 1995), this finding is consonant with the assumption that the HOFT mice were more emotional than the LOFT mice.

In both lines, ambulation, exploration, grooming, and rearing increased after parturition. Because all of these behavioral activities demand physical movements, this increment may be, at least partially, due to the fact that it is harder to be physically active in the later stage of pregnancy than after parturition. However, the increase in grooming was much more conspicuous among the LOFT mothers than among the HOFT mothers. Moreover, defecation increased among the HOFT mothers after parturition, whereas it decreased among the LOFT mothers. If grooming is linked to the state of dearousal due to habituation to a stressful situation and thus negatively correlated with emotionality (File et al., 1988; Spruijt et al., 1992), the more obvious increase in grooming in the LOFT mothers agrees with the finding that, unlike in the prepartum condition, the LOFT mothers ambulated more but defecated less than the HOFT mothers in the presence of a pup after parturition. This means that the difference in emotionality between the two lines may have been more pronounced in the presence of a pup after parturition than during pregnancy. Therefore, the more emotional HOFT mothers may have been more susceptible to novelty-induced anxiety generated by pups than the less emotional LOFT mothers (see Fleming & Luebke, 1981; Lonstein & De Vries, 2000). It should be recognized, however, that parturition exerted no effect on thigmotaxis, which means that

this suggestion must remain conjectural.

During lactation, the less thigmotactic LOFT mothers were more attracted to their pups in the OF (i.e., had higher pullus indexes) than the more thigmotactic HOFT mothers, although the two lines did not differ in latencies to first body contact with the pup. Lonstein and De Vries (2000) have previously suggested that virgin rodents' sex differences in parental responding may be related to sex differences on the basal levels of fear, with the less fearful sex being less susceptible to novelty-induced anxiety generated by pups and, consequently, exhibiting stronger parental responses. Thus, in line with this suggestion, it could be expected that the less thigmotactic LOFT mothers would show stronger maternal responsiveness than the more thigmotactic HOFT mothers. However, in the retrieval test, the HOFT mothers exhibited shorter latencies in two parameters, although the lines did not differ in the total time spent to retrieve either one of the pups or the 3 pups on the whole. Consequently, the hypothesis that the less emotional LOFT mothers would show stronger maternal responsiveness than the more emotional HOFT mothers was only partially confirmed.

5.7 Methodological considerations

One potential limitation in this selection study is the absence of a control line (i.e., a line that is randomly selected) in order to control for the effects of genetic drift, that is, the accumulation of random changes in a gene pool. On the other hand, although a control line has sometimes been bred in conjunction with bidirectional selection experiments (e.g., DeFries et al., 1970), it is generally bred with selections in one direction only (e.g., Bronikowski et al., 2001); that is, when the selection experiment comprises two lines selected in the opposite direction (i.e., "two-way" or "divergent" selection), an unselected control line is not necessary because each selected line acts as a control for the other and the selection response is measured in the form of the divergence between the two lines (Falconer & Mackay, 1996, p. 195).

Another potential limitation in this study is that the selections were not based on any fixed percentage or absolute number of mice; instead, the number

of selected mice in each generation varied, mostly depending on how many mice were needed for the specific experiments performed on the next generation and, naturally, on how many pups were born. Moreover, there were no absolute criteria with regard to the thigmotactic ratio that the mice had to meet in order to be selected as parents for the next generation. Consequently, these methodological issues may have affected the divergence in thigmotaxis between the two lines, possibly making it more variable across the different generations.

The genetic gain of the selective breeding increased only up to the 6th generation and was relatively modest in all. As already mentioned, this may be due to the small number of mice in the parental population as well as the genetic homogeneity of Swiss albino mice (Staats, 1966). Accordingly, to maximize the genetic pool of the Swiss albino parental mice, their number should have been greater; that is, with greater genetic heterogeneity in the parental population, there would have been more genetic material upon which the selective breeding could have acted (see Ramos et al., 2003), and this, in turn, might had increased the phenotypic divergence between the two lines. However, even with the low number of parental mice, the line difference in thigmotaxis was evident in each generation (although for three generations, it was not statistically significant).

With a few exceptions, the general procedures (e.g., housing conditions and testing age) were highly similar for different generations when measuring the selection response. However, when performing different experiments other than the measurement of the selection response, there was some variation with regard to general procedures (e.g., single vs. group housing, the type of cage, testing age, the number of habituation periods prior to testing, and the time of testing). It is well known that the OF test is highly sensitive to minor variations in testing procedures and general conditions (Walsh & Cummins, 1976; see also Wahlsten, 2001). Consequently, the general procedures in different experiments could have been more uniform. On the other hand, instead of comparing the results of different experiments, the main purpose of the present study was to compare the two lines with each other within the same experiment, the procedures being highly similar in each experiment for both lines.

In the present study, the OF parameters were manually recorded through direct visual observation (by one or two experimenters). However, it might

be more reliable to use a camera, for example, in order to make sure that the scores for different parameters are correctly registered. This would be especially important when the mouse is very active and, consequently, ambulates very fast and may also exhibit many other OF behaviors at a rapid rate.

Finally, another possible limitation in this study relates to the way the thigmotactic ratio was measured. More specifically, the mouse started from the OF center unit, and in order to reach the peripheral units, it had to traverse at least one inner unit of the middle circle in the OF arena (see Figure 1). The problem lies in situations when the mouse headed directly to the outer wall zone after leaving the center starting unit. In those cases, the mouse may not have "chosen" to walk on the middle circle, but it had to enter it to reach the periphery. In these situations, the mouse may have been highly thigmotactic, but it received one obligatory inner-unit score before it reached the wall. Although the center starting unit was not included in the ambulation scores, this obligatory inner unit was included, and if the mouse's total ambulation scores were very low, this inner-unit score had a disproportionate contribution to the thigmotactic ratio, increasing it excessively (remembering that a high ratio indicates low thigmotaxis). This methodological issue became evident in the repeated OF-exposure experiment, in which especially the more thigmotactic HOFT mice ambulated very little (significantly less than the LOFT mice) and, consequently, received disproportionately high thigmotactic ratios, at least partly, due to this fault. Accordingly, this obviously may have reduced the line difference in thigmotaxis in the repeated OF-exposure experiment (in this experiment, the two lines differed in thigmotaxis only on the 5th day). However, if the mouse's ambulation scores were relatively high, this fault may not have caused any notable effects. One way of resolving this problem would be that, instead of the center unit, the mouse would start from an inner unit located on the middle circle. In this way, the mouse could move directly to the wall zone or, alternatively, keep ambulating in the inner area of the OF arena, depending on its own choice.

5.8 Conclusions

To the knowledge of the author, the HOFT and LOFT lines are the first mice that have been bidirectionally selected for OF thigmotaxis. Consequently, they provide new information related to mice' thigmotactic behavior and also further our understanding of its associations with other characteristics, which may be useful for several disciplines using thigmotaxis as a measure of rodent emotionality. Given that anxiety disorders are so common among people, causing an enormous amount of personal suffering as well as financial costs for societies, basic research focusing on the etiology and biological basis of anxiety as well as on psychopharmacological means of relieving the symptoms is likely to be increasingly important in the future. By means of breeding animals selectively for an anxiety-related characteristic, such as thigmotaxis, it is possible to create an animal model of trait anxiety that would be based on stable and enduring differences between two animal lines, thus making the model potentially more useful for anxiety research than the animal models based on merely state anxiety.

The present study clearly demonstrates that OF thigmotaxis is a strong characteristic for producing two diverging lines of mice, suggesting a genetic background underlying this behavior. This suggestion is further supported by two crossfostering experiments showing that, when tested in adulthood, the thigmotactic behavior of the HOFT and LOFT mice resembled that of their genetic line, not their foster mother's line. The present findings also demonstrated that the line difference in this selection trait was stable over time and persisted through different conditions and testing variations, thus indicating that it was a robust feature of these animals. Consequently, it seems plausible that the divergence between the HOFT and LOFT lines was based on differences with regard to trait anxiety rather than to state anxiety.

In addition to thigmotaxis, the HOFT and LOFT lines repeatedly differed in OF exploration and rearing, conforming to the general assumption that emotionality and exploratory tendencies are inversely related. By contrast, the two lines did not generally differ in ambulation and defecation, that is, in the traditional OF indexes of emotionality. This is in line with the view that emotionality may be a multidimensional construct and different parameters

only measure different aspects of it. However, in those few experiments in which a line difference in ambulation or defecation was found, the divergence between the two lines was consonant with the traditional interpretation of these parameters as well as with the present assumption that the HOFT mice were more emotional than the LOFT mice; that is, the HOFT animals ambulated less but defecated more than the LOFT animals. Consequently, the present study suggests that although the mice selected for thigmotaxis may not always differ in the other OF indexes of emotionality (possibly due to its multidimensional nature), in certain situations, the line difference in emotionality may also manifest itself in the form of these other parameters (recall that the HOFT and LOFT lines sometimes differed in grooming as well).

Thus, on the basis of the present findings and, naturally, assuming that thigmotaxis is a valid index of emotionality, exploration and rearing seem to be strongly emotionality-related OF parameters. This is to be expected, given the generally assumed inverse relationship between emotionality and exploratory tendencies. This study also partially supported the traditional view that ambulation and defecation are linked with emotionality, although the linkage was relatively weak. However, as already mentioned, this may be due to the fact that emotionality is probably a multidimensional construct, with different parameters only measuring different aspects of it. In the present study, grooming seemed to be, at least to some extent, connected to emotionality as well, although the findings supported more readily the view of grooming being negatively correlated with emotionality, rather than the traditional view of positive correlation. By contrast, latency to move, radial latency, and urination seemed to be quite unrelated to emotionality in this study, at least as far as emotionality is defined by differences in thigmotaxis, because there was a line difference in these respects in only one experiment (i.e., in either Crossfostering 1 or 2). However, all of these suggestions regarding the OF parameters' validity for measuring emotionality must remain conjectural because on the basis of the present study, no definitive conclusions in this respect can be drawn.

The need for further investigation of the validity of the different OF parameters and their interrelationships became apparent in several findings of the present study. For example, across the 23 generations, the male mice

were less thigmotactic and ambulated as well as reared more than the female mice. Thus, in this case, ambulation seemed to measure emotionality, as traditionally assumed, because a high level of ambulation was related to a low level of thigmotaxis as well as a high level of rearing. However, with regard to the line differences across the 23 generations, the LOFT mice were naturally less thigmotactic and also reared more than the HOFT mice, but there was no line difference in ambulation. On the other hand, in the repeated OF-exposure experiment, there was no line difference in thigmotaxis (excluding the 5th day), but the lines were significantly differentiated by ambulation instead, with the LOFT mice ambulating more than the HOFT mice. Consequently, there seems to be clear evidence that ambulation is, at least to a certain extent, emotionality-related (when ever there was a line difference, the less emotional LOFT mice ambulated more than the HOFT mice), but for some reason, the lines did not generally differ in this respect. Likewise, with regard to the temporal changes in the HOFT and LOFT lines' OF behavior, the present findings are, to some extent, contradictory and highlight the need for further clarification of this issue. It should be noted, however, that inconsistent findings in OF studies may, at least partially, also stem from the fact that there are many uncontrolled factors involved in OF testing situations.

The present study suggests that highly thigmotactic mice may have longer life spans than mice with lower levels of thigmotactic behavior. On the basis of the results, it also appears that in the HOFT and LOFT lines, the female mice were more emotional than the male mice. Finally, although this study did not reveal prominent line differences in maternal responsiveness, it suggests that the difference in emotionality between the HOFT and LOFT mothers was more pronounced in the presence of a pup after parturition than during pregnancy. This refers to the possibility that, contrasted with the LOFT mothers, the more emotional HOFT mothers may have been more susceptible to novelty-induced anxiety generated by pups.

As with any behavioral characteristic, the expression of many OF behaviors in mice is, at least partially, strain-specific. For this reason, selection experiments based on mice' thigmotactic behavior should be conducted with different strains before drawing any general conclusions with regard to this selection trait. Ramos

et al. (2003) selected rats for central locomotion in the OF, a characteristic that is closely related to thigmotaxis. However, as already mentioned, the HOFT and LOFT mice are possibly the only lines so far that have been selected for mice' OF thigmotactic behavior. Furthermore, the HOFT and LOFT mice were not tested in any other emotionality tests besides the OF, which means that future studies should examine whether the mice bidirectionally selected for OF thigmotaxis would differ in other devices or paradigms supposed to measure rodents' emotionality. Finally, future researchers should also investigate whether the different ways of measuring OF thigmotaxis (see 1.3 Thigmotaxis) are compatible with each other, and if not, which method would be the most reliable and valid.

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