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STRAIN-SPECIFIC COGNITIVE DEFICITS IN ADULT MICE EXPOSED TO EARLY LIFE STRESS

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Abstract

Early life stress is a prominent risk factor for the development of adult psychopathology. Numerous studies have shown that early life stress leads to persistent changes in behavioral and endocrine responses to stress. However, despite recent findings of gene expression changes and structural abnormalities in neurons of the forebrain neocortex, little is known about specific cognitive deficits that can result from early life stress. Here we examined five cognitive functions in two inbred strains of mice, the stress-resilient strain C57Bl/6 and the stress-susceptible strain Balb/c, that were exposed to an infant maternal separation (IMS) paradigm and raised to adulthood. Between postnatal ages P60 to P90, mice underwent a series of tests examining five cognitive functions: Recognition memory, spatial working memory, associative learning, shifts of attentional sets, and reversal learning. None of these functions were impaired in IMS C57Bl/6 mice. In contrast, IMS Balb/c mice exhibited deficits in spatial working memory and extradimensional shifts of attention, i.e., functions governed primarily by the medial prefrontal cortex. Thus, like recently discovered changes in frontocortical gene expression, the emergence of specific cognitive deficits associated with the medial prefrontal cortex is also strain-specific. These findings illustrate that early life stress can indeed affect specific cognitive functions in adulthood, and they support the hypothesis that the genetic background and environmental factors are critical determinants in the development of adult cognitive deficits in subjects with a history of early life stress.

Keywords

early life stress; inbred mouse strains; recognition memory; working memory; attention set-shifting

For several heritable psychiatric disorders, the interaction between gene and environment is thought to be critical for modulating outcome or mutating genetic risk (Kendler, 2005; Kendler and Baker, 2007). This is best documented for mood disorders in subjects with distinct genetic variants in serotonin-related genes (Caspi et al., 2010). Moreover, the impact of environmental factors on the development of psychopathology depends upon the age of

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exposure. The most persuasive example, early life stress (ELS), exerts profound effects on adult emotional behavior and increases risk for depression, anxiety disorders, and substance abuse (Holmes et al., 2005).

In studies on the impact of ELS on adult psychopathology, inbred strains of mice provide a source of natural genetic variability and phenotypic differences. For example, the isogenic strains C57Bl/6J and Balb/cJ differ in their sensitivity to ELS and adult stress (Holmes et al., 2005; Millstein and Holmes, 2007), with Balb/c being more susceptible to stress-induced behavioral impairment than the stress-resilient strain C57Bl/6 (Millstein and Holmes, 2007).

ELS can provoke long-lasting effects on endocrine responses to subsequent stress (Ladd et al., 2004) and the related gene expression changes (Navailles et al., 2010). Moreover, ELS induces alterations in dendritic morphology of pyramidal neurons of the medial prefrontal cortex (mPFC; Pascual and Zamora-León, 2007) that resemble those elicited by chronic corticosteroid administration (Cerqueira et al., 2007). Moreover, age-dependent changes in the densities of calbindin- and parvalbumin-immunoreactive interneurons in subregions of the mPFC (Helmeke et al., 2008) and altered monoaminergic innervation of the mPFC (Braun et al., 2000) have been reported to result from ELS. Recently, strain-specific changes in fronto-cortical gene expression have also been found in adult mice exposed to ELS. For example, Balb/c, but not C57Bl/6, mice exhibit increased expression of *Gαq* (Bhansali et al., 2007; Schmauss et al., 2010), decreased expression of the plasticity-inducible transcription factor *egr-1* (Navailles et al., 2010), and increased expression of extensively edited mRNA isoforms encoding 5-HT_{2C} receptors with decreased G-protein-coupling efficiency (Bhansali et al., 2007; Schmauss et al., 2010). These genes can play a role in forebrain neocortex-governed cognitive functions, especially in the mPFC (working memory, set-shifting) and the orbital frontal cortex (reversal learning): signaling through Gq-coupled receptors is necessary for working memory (Runyan et al., 2005); induced *egr-1* expression can lead to long-lasting synaptic changes that influence behaviors associated with learning and memory (Cole et al., 1989); and 5-HT_{2C}-receptor signaling in the orbital frontal cortex plays a role in early phases of spatial reversal learning (Boulougouris and Robbins, 2010).

Despite the findings of altered neuronal morphology and gene expression changes, the effects of ELS on frontocortical-governed cognitive functions remain to be investigated. In the present study, we tested the hypothesis that, like the persistent frontocortical gene expression changes elicited by ELS exposure, ELS-induced cognitive deficits also occur in a strain-specific manner. Therefore, we examined several cognitive functions, including recognition and working memory, associative learning, attention-set-shifting, and reversal learning, in C57Bl/6 and Balb/c mice with and without exposure to ELS.

Method

Subjects

Male and female Balb/cJ and C57Bl/6J mice were housed in a temperature-controlled ($26 \pm 2^\circ\text{C}$) barrier facility with a 12-hour light/dark schedule (lights on at 6:00 AM) and free access to food and water. All experiments involving animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committees at Columbia University and the New York State Psychiatric Institute. All efforts were made to minimize both the number of animals and the discomfort of the animals used.

Infant maternal separation (IMS)

Pups of first-time mothers were separated from their dam daily for three hours (from 1:00 to 4:00 PM) from postnatal age day 2 (P2) until P15. Control animals were standard-facility-

reared (SFR) offspring of first-time mothers and were subjected to the same housing and husbandry conditions. Only litters of 6 to 8 pups were selected for this study. For SFR and IMS mice, a total of 10 and 12 litters, respectively, were used. Because IMS mice exhibit a delayed postnatal development as indicated, for example, by a 4-day delay of eye opening and because they are still being nursed by their dams at P21, all pups were weaned at P28. This ensured a near 100% survival rate of the IMS pups, which throughout postnatal development have about a 20% reduced body weight, even as young adults (Navailles et al., 2008). Four to five animals randomly selected from four to five different litters were group-housed by sex. At P60, one cohort of IMS mice and their SFR controls were selected for behavioral tests of depression- and anxiety-like behavior (10 mice per group derived from 10 different litters) while the other cohort was selected for cognitive behavioral testing (8 mice per group selected from 8 of the 10 (SFR) or 12 (IMS) litters described above). Behavioral tests of depression and anxiety-related behaviors were conducted with male mice. The groups of mice tested in cognitive behavioral tests were composed of equal numbers of male and female mice.

Elevated Plus Maze (EPM)

Mice were exposed to an EPM apparatus purchased from Stoelting (Wood Dale, IL), which had two open and two closed arms that joined to form the center of the maze (lane width: 5 cm, arm length: 35 cm, wall height: 15 cm, elevation above ground: 55 cm). Testing (under 100 lux/m² lightening) was performed between 1 and 4 PM. Mice were placed in the center of the maze and their time spent in open arms during a 5 min test period was recorded by the investigators during the time of testing.

Forced swim test (FST)

The same mice tested in the EPM were tested in the FST one week later. A modified version of the forced swim test was used as previously described (Bhansali et al., 2007). Briefly, between 1 and 4 PM, mice were placed into plastic buckets (23 cm deep and 19 cm in diameter) filled with 25-28°C water for six min. In this modified version of the Posolt FST, mice alternate between active swimming and passive floating, but climbing is not observed. Hence, the number of passive episodes and their duration (in sec) was monitored by the investigators during the last 4 min of the test. On the following day, mice were re-exposed to the FST protocol, and their behavior was recorded as described above.

Social Recognition

This test taxes short-term recognition memory and relies on the ability of adult mice to recognize a juvenile conspecific and to remember its perceptual features (olfactory, somatosensory, visual) for a period of 2 h. Adult mice were singly housed 24 h prior to exposure of a non-familiar juvenile mouse. During a 5-min exposure, the time interacting with the juvenile (following, sniffing, licking, etc.) was recorded (T1). Then, the juvenile was removed, but placed back 2 h later for a second 5-min test (T2). In mice with intact recognition memory, the time of social interaction during the second exposure is consistently shorter (i.e., T1–T2 equals a positive number). To confirm the validity of this test as a test of short-term memory, we also exposed additional groups of mice to a different juvenile during the second exposure.

Attention-set-shifting task (ASST)

A day after completion of the Social Recognition test, mice were food-restricted such that they gradually (over the period of 5 to 7 days) lost 10% of their free-feeding body weight. Once mice reached this 10% reduction in body weight, they were tested in the ASST. The rodent ASST, developed by Birrell and Brown (2000), uses two stimulus dimensions, odor

and texture, that are appropriate for this species. The test apparatus was made of Plexiglas that resembled the housing cage (dimensions: 32 × 27 × 15 cm). A sliding door separated one third of the apparatus (holding box) from the remaining two thirds (test area).

On the first day, mice were habituated to the test apparatus. During this time, they learned to retrieve a food reward deeply buried in unscented terra cotta pots filled with familiar bedding media. The habituation period ended when mice retrieved the food reward in 6 consecutive trials. On the next day, mice performed the entire ASST in a single session. Briefly, they were first trained on two simple discriminations (SD) between odor (scented terra cotta pots) or texture (different digging media). Only one stimulus dimension indicated the location of the food pellet, and both dimensions were used in a randomized order. The next task was a compound discrimination (CD), in which another stimulus dimension was introduced that was not a reliable predictor of food reward, and the same positive stimulus (a particular odor or texture) used in the initial SD still guided correct response selection. In the following intradimensional shift phase (IDS), both relevant and irrelevant stimuli were changed, but the relevant stimulus dimension used in the SD and CD (odor or medium texture) remained the same. Then, the formerly irrelevant dimension became relevant and required an extradimensional shift of attention (EDS). Finally, the previously positive stimulus of the EDS became a negative one, and the previously irrelevant stimulus indicated food reward (reversal learning; EDS-Rev). In all test phases, animals had to reach a criterion of six consecutive correct trials. The number of trials to criterion is the response accuracy, and the time between stimulus onset and response selection is referred to as response latency. Details about the stimulus properties used in the test are described in Glickstein et al. (2005).

Spatial Working Memory (WM)

We employed a widely used delayed alternation task performed in a T-maze as previously described (Glickstein et al., 2002). The maze was made of 0.6 cm-thick Plexiglas. Its main alley (58 cm long) was connected to two side arms (30cm long). Alley and side arms were 11 cm wide. All walls were 18.5 cm high.

One day after completion of the ASST, mice began their training for alternate arm entries in the T-maze. The training period (lasting on average 10 to 14 days) ended when mice reached at least 70% correct arm entries (in 10 trials per day) on two consecutive days with no inter-trial delay (referred to as 5 s delay, i.e., the minimum handling time). Then, mice performed the test with three inter-trial delays (15, 20, and 30 sec). Only correct arm entries resulted in food reward, and their percentage of the total number of trials was taken as a measure of response accuracy. We have previously shown that, in this test, mice can hold spatial information in working memory for up to 20 s, but exhibit chance performance (i.e., only 50% correct arm entries) at 30 s delay (Glickstein et al., 2002).

Statistical Analysis

The FST, the EPM, and the Social Recognition test data were analyzed by factorial analysis of variance (ANOVA; effect of strain and effect of treatment (SFR or IMS)), and statistical differences were resolved *post hoc* using Bonferroni Multiple Comparisons tests. The data obtained from the ASST and the WM tests were first analyzed by Repeated Measures ANOVA followed *post hoc* by Tukey-Kramer Multiple Comparisons tests to assess the performances of SFR and IMS mice of each strain in the individual test phases (ASST) or delay periods (WM test). In addition, factorial ANOVA was used to test for differences between strains and treatment. For all cognitive test results, two-tailed Student's *t* tests were used to compare results between groups of males and females. The statistical analyses were carried out using Graph Pad InStat Version 3.0 (GraphPad Software, San Diego, CA).

Results

Emotive behavior of adult IMS Balb/c and C57Bl/6 mice

We first evaluated the impact of early life stress exposure on adult emotive behavior by comparing the behavioral responses of SFR and IMS Balb/c and C57Bl/6 mice to FST and EPM exposure.

Fig. 1A summarizes results of the FST. There was a significant difference in the total time of immobility between SFR mice of both strains (ANOVA, $F(3,27)=6.61$, $p=0.0017$). *Post hoc* Bonferroni Multiple Comparisons tests revealed that SFR C57Bl/6 mice exhibited significantly greater immobility than Balb/c mice (Fig. 1A). Nevertheless, the FST behavior of IMS C57Bl/6 mice did not significantly differ from their SFR controls. IMS Balb/c mice, however, exhibited significantly more immobility than their SFR controls (ANOVA, $F(3,25)=7.04$, $p=0.0014$ (Fig. 1A).

For the EPM test, ANOVA revealed a significant difference between SFR mice of both strains ($F(3, 33)=9.608$, $p=0.0001$). *Post hoc* Bonferroni tests revealed that SFR Balb/c mice spent significantly less time in the open arms compared to SFR C57Bl/6 mice (Fig. 1B). Moreover, there were differences between SFR and IMS mice of both strains ($F(3,29)=12.96$, $p<0.00001$), with IMS mice having spent significantly less time in the open arms compared to their SFR controls (Fig. 1B).

In summary, while IMS mice of both strains exhibited similar anxiety-like behavior in the EPM, only IMS Balb/c mice also showed increased depression-like behavior in the FST.

Social Recognition of SFR and IMS Balb/c and C57Bl/6 mice

In the Social Recognition test, both male and female mice were used. Two-tailed Student's *t* tests revealed no differences between males and females of either group, neither in *T1 measures* (Balb/c-SFR: 131.4 ± 9.5 s (males) and 118 ± 14.5 s (females), $p=0.5$; Balb/c-IMS: 117.8 ± 15.5 s (males) and 105.3 ± 14.8 s (females), $p=0.6$; C57Bl/6-SFR: 131.9 ± 7.0 s (males) and 155.1 ± 11.5 s (females), $p=0.1$; C57Bl/6-IMS: 124.5 ± 14.0 s (males) and 146.4 ± 20.2 s (females), $p=0.57$), nor in the *T1-T2 values* for re-exposure to the same juvenile ((Balb/c-SFR: 56.8 ± 7.2 s (males) and 59.4 ± 9.9 s (females), $p=0.9$); Balb/c-IMS: 52.8 ± 8.4 s (males) and 59.4 ± 12.5 s (females), $p=0.70$; C57Bl/6-SFR: 45.0 ± 13.4 s (males) and 51.6 ± 13.5 s (females), $p=0.74$; C57Bl/6-IMS: 56.4 ± 16.9 s (males) and 46.0 ± 12.8 s (females), $p=0.57$). Thus, data from males and females were combined in each group.

SFR and IMS mice of both strains exhibited no significant differences in their total times of interaction with a conspecific juvenile when they were exposed to the same juvenile during the second exposure (ANOVA, $F(7,35)=7.393$, $p>0.05$), i.e., the time of social interaction with the juvenile was on average 50 sec less than during the first exposure (Fig. 2). Moreover, SFR and IMS mice of both strains exhibited significant differences in their interaction times when exposed to the same or a different juvenile in the second exposure (ANOVA, $F(7, 35)=7.393$; $p<0.0001$). *Post hoc* Bonferroni Multiple Comparisons tests revealed that SFR and IMS mice of both groups spent significantly more time interacting with the novel juvenile during the second exposure. In fact, the time of interaction was indistinguishable between first exposure (T1) and second exposure (T2) (Fig. 2).

Altogether, these results indicate unaltered short-term recognition memory of species-relevant sensory information (olfactory, somatosensory, etc.) in both strains of mice exposed to the IMS.

Performance of SFR and IMS Balb/c and C57Bl/6 mice in the ASST

The various test phases of the ASST engage three specific cognitive processes: associative learning (CD), maintaining sets/set-shifting (IDS, EDS), and reversal learning (EDS-Rev). For SFR mice of both strains, Repeated Measures ANOVA revealed significant differences in the number of trials to criterion between test phases (C57Bl/6: $F(4,36)=7.01$; $p=0.0003$ and Balb/c: $F(4,20)=13.6$; $p<0.0001$). *Post hoc* analysis showed that both strains required significantly more trials to complete the EDS (i.e., the most difficult test phase) than SD, CD, or IDS phases (C57Bl/6: $p<0.01$ compared to SD and CD and $p<0.001$ compared to IDS and Balb/c: $p<0.001$ compared to SD, CD, and IDS). Moreover, whereas ANOVA factorial analyses revealed no significant differences in the number of trials to criterion in each test phase between SFR mice of both strains ($F(9,68)=4.344$, $p>0.05$), there were strain differences between IMS mice ($F(9,71)=11.89$, $p=0.0001$). *Post hoc* Bonferroni Multiple Comparisons tests resolved these differences exclusively for the EDS phase in which IMS Balb/c mice required significantly more trials to criterion than IMS C57Bl/6 mice ($p<0.001$) (Fig. 3). Moreover, while the performance of SFR and IMS C57Bl/6 mice was indistinguishable ($F(9,75)=5.68$, $p>0.05$ for all test phases), ANOVA revealed significant differences between SFR and IMS Balb/c mice ($F(9, 64)=17.889$; $p<0.0001$). *Post hoc* Bonferroni Multiple Comparisons revealed significantly impaired performance of IMS Balb/c mice in the EDS phase of the ASST ($p<0.001$) (Fig. 3). Thus, IMS Balb/c, but not IMS C57Bl/6, mice exhibit a deficit in extradimensional set-shifting.

Like in the Social Recognition test, no differences between males and females in either group were found their performance in the ASST, neither in the *CD phase* (Balb/c-SFR: 6.4 ± 0.2 (males) and 6.5 ± 0.3 (females), $p=0.8$; Balb/c-IMS: 7.8 ± 0.9 (males) and 6.3 ± 0.3 (females), $p=0.15$; C57Bl/6-SFR: 7.3 ± 0.9 (males) and 9.4 ± 1.0 (females), $p=0.2$; C57Bl/6-IMS: 7.8 ± 0.6 (males) and 8.6 ± 1.5 (females), $p=0.55$), the *IDS phase* (Balb/c-SFR: 6.2 ± 0.2 (males) and 6.8 ± 0.8 (females), $p=0.5$; Balb/c-IMS: 10.0 ± 1.4 (males) and 10.4 ± 1.7 (females), $p=0.8$; C57Bl/6-SFR: 7.8 ± 1.0 (males) and 6.8 ± 0.8 (females), $p=0.45$; C57Bl/6-IMS: 8.3 ± 0.5 (males) and 7.8 ± 0.8 (females), $p=0.6$), the *EDS phase* (Balb/c-SFR: 9.4 ± 1.2 (males) and 11.7 ± 1.8 (females), $p=0.32$; Balb/c-IMS: 21.5 ± 3.2 (males) and 22.3 ± 1.9 (females), $p=0.85$; C57Bl/6-SFR: 7.0 ± 0.7 (males) and 8.8 ± 1.7 (females), $p=0.41$; C57Bl/6-IMS: 12.5 ± 1.3 (males) and 11.0 ± 1.5 (females), $p=0.71$), nor the *EDS-Rev phase* (Balb/c-SFR: 8.8 ± 0.9 (males) and 9.0 ± 1.5 (females), $p=0.9$; Balb/c-IMS: 8.8 ± 0.8 (males) and 10.3 ± 0.8 (females), $p=0.21$; C57Bl/6-SFR: 8.8 ± 1.8 (males) and 12.3 ± 1.0 (females), $p=0.2$; C57Bl/6-IMS: 11.8 ± 1.4 (males) and 9.7 ± 0.9 (females), $p=0.3$).

Spatial working memory of SFR and IMS Balb/c and C57Bl/6 mice

Figure 4 summarizes the performance of SFR and IMS Balb/c and C57Bl/6 mice in the T-maze test. For SFR C57Bl/6 mice, Repeated Measures ANOVA revealed that the percentage of correct arm entries differed significantly between delay periods ($F(3,15)=7.18$; $p=0.0063$). *Post hoc* Tukey-Kramer Multiple Comparisons tests showed that the percentage of correct arm entries was significantly higher at the 5 and 15 s ($p<0.01$ and $p<0.05$ respectively) delays when compared to the 30 s delay (at which performance by chance was reached). A similar result was obtained for SFR Balb/c mice ($F(3,21)=5.0$, $p=0.0093$). Their percentage of correct arm entries at the 5 s delay was significantly higher compared to the corresponding percentages of correct arm entries measures at 20 and 30 s delays ($p<0.05$).

For IMS C57Bl/6 mice, Repeated Measures ANOVA also revealed significant differences in the percentage of correct arm entries at different delay periods ($F(3,18)=15.87$; $p=0.0001$). In these mice, the percentage of correct arm entries at 5 s delay was significantly higher than the corresponding percentages at the 20 and 30 s delays ($p<0.01$ and $p<0.001$, respectively) and the percentage of correct arm entries at 15 s was higher compared to corresponding

measures obtained at 30 s delay ($p < 0.001$). Finally, for IMS Balb/c mice, significant differences revealed by Repeated Measures ANOVA ($F(3,21)=104.6$, $p=0.0001$) were resolved *post hoc* for the percentages of correct arm entries at 5 and 15 s delays, which were significantly higher than the corresponding percentages at 20 and 30 s delays ($p < 0.001$).

A comparison of the percentages of correct arm entries at the 5, 15, 20, and 30 s delays between SFR mice of both strains revealed no significant differences (ANOVA, $F(7, 45)=3.85$, $p > 0.05$ for all delay periods). However, strain differences were revealed for IMS mice (ANOVA, $F(7,52)=27.68$, $p < 0.0001$), which were resolved *post hoc* for the 20 s delay period, at which IMS Balb/c mice achieved a significantly lower percentage of correct arm entries when compared to the corresponding percentage of IMS C57Bl/6 mice ($p < 0.01$) (Fig. 4).

In addition, there were significant differences between SFR and IMS Balb/c mice (ANOVA, $F(7, 49)=19.301$, $p < 0.0001$). *Post hoc* analysis revealed significantly lower percentages of correct arm entries for IMS mice at 20 and 30 s delays ($p < 0.01$) (Fig. 4). In contrast, there were no significant differences between SFR and IMS C57Bl/6 mice ($F(7,48)=5.211$, $p > 0.05$ for all delay periods).

Finally, for the WM test, no differences were found between males and females, neither at the 5 s delay (Balb/c-SFR: 77.5 ± 1.4 (males) and 78.8 ± 5.5 (females), $p=0.83$; Balb/c-IMS: 73.8 ± 2.4 (males) and 77.5 ± 2.5 (females), $p=0.32$; C57Bl/6-SFR: 77.1 ± 2.6 (males) and 75.0 ± 3.2 (females), $p=0.61$; C57Bl/6-IMS: 72.5 ± 0.6 (males) and 73.3 ± 3.3 (females), $p=0.85$), the 15 s delay (Balb/c-SFR: 68.3 ± 4.4 (males) and 65.0 ± 3.1 (females), $p=0.88$; Balb/c-IMS: 75.0 ± 3.5 (males) and 72.5 ± 2.5 (females), $p=0.80$; C57Bl/6-SFR: 67.5 ± 4.9 (males) and 71.0 ± 3.9 (females), $p=0.68$; C57Bl/6-IMS: 65.0 ± 2.0 (males) and 70.0 ± 2.9 (females), $p=0.85$), the 20 s delay (Balb/c-SFR: 55.0 ± 2.9 (males) and 63.8 ± 7.4 (females), $p=0.32$; Balb/c-IMS: 42.5 ± 4.7 (males) and 46.3 ± 1.3 (females), $p=0.48$; C57Bl/6-SFR: 56.7 ± 4.6 (males) and 62.5 ± 5.2 (females), $p=0.43$; C57Bl/6-IMS: 57.5 ± 1.4 (males) and 58.3 ± 1.7 (females), $p=0.72$), nor the 30 s delay (Balb/c-SFR: 61.7 ± 8.8 (males) and 58.3 ± 3.3 (females), $p=0.74$; Balb/c-IMS: 48.8 ± 1.3 (males) and 47.5 ± 3.2 (females), $p=0.73$; C57Bl/6-SFR: 52.5 ± 7.5 (males) and 42.0 ± 7.8 (females), $p=0.43$; C57Bl/6-IMS: 46.2 ± 5.9 (males) and 45.0 ± 8.7 (females), $p=0.91$).

In summary, while there were no significant differences between SFR mice of both strains at either delay period, the performance of IMS Balb/c mice differed significantly from IMS C57Bl/6 mice at the 20 sec delay ($p < 0.01$) and from SFR Balb/c mice at 20 and 30 s delays. Hence, compared to SFR and IMS C57Bl/6 mice and SFR Balb/c mice, IMS Balb/c mice exhibited spatial working memory deficits.

Discussion

The present study uncovered two specific cognitive functions, spatial WM and extradimensional shifts of attention, that were impaired in adult mice exposed to early life stress. Other functions, such as short-term memory, associative learning, and reversal learning, were unaffected. Moreover, these ELS-induced deficits were detected in the stress-susceptible strain Balb/c, but not in the stress-resilient strain C57Bl/6. Thus, just as a number of persistent forebrain neocortical gene expression changes occur in this strain-specific manner (Bhansali et al., 2007; Schmauss et al., 2010; Navailles et al., 2010), cognitive deficits resulting from ELS are also strain-specific.

The finding that *both* WM and extradimensional set-shifting were affected in IMS Balb/c mice raises the question of a possibility of a common origin of their disruption. At the anatomic levels, both functions are primarily associated with the mPFC. Specifically, both

functions are sensitive to lesions of the infralimbic and prelimbic subregions of the rodent mPFC (Aggleton et al., 1995; Birrel and Brown, 2000). Moreover, after exposure to maternal separation and later social isolation, the same anatomic subregions experience changes in monoaminergic innervation, namely increased serotonergic innervation of the infralimbic and decreased dopaminergic innervation of the prelimbic subregions (Braun et al., 2000).

We also found that impaired performance in the EDS did not lead to impaired performance in the EDS-Rev. A similar separation of impairment of these two functions has previously been described for dopamine D2-receptor knockout mice that, despite normal performance in the EDS phase of the ASST, exhibited impaired performance in the EDS-Rev (DeSteno and Schmauss, 2009). Furthermore, it has been shown that these two cognitive domains of attentional control functions also segregate at the anatomic levels, i.e., in rodents, the EDS-Rev performance is sensitive to lesions of the orbital frontal cortex (McAlone and Brown, 2004).

Finally, recognition memory, a function governed by the perirhinal cortex and the hippocampus (Brown and Aggleton, 2001), was also unaffected in IMS Balb/c mice. Hence, the present finding suggests that cognitive functions governed primarily by the infra- and prelimbic subregions of the mPFC are especially sensitive to early life stress exposure.

It is noteworthy that, despite unaffected cognitive functioning in IMS C57Bl/6 mice, their adult emotive phenotype was affected by IMS exposure. In fact, just like IMS Balb/c mice, IMS C57Bl/6 mice exhibited increased anxiety-like behavior in the EPM. Yet, in contrast to IMS Balb/c mice that also exhibited depression-like behavior in the FST, the behavior of IMS C57Bl/6 mice in this test did not significantly differ from their SFR controls. It is possible that increased depression-like behavior further contributes to the cognitive deficits detected in IMS Balb/c mice. However, SFR C57Bl/6 mice exhibited significantly more immobility than SFR Balb/c mice during FST exposure. This difference between strains, and in particular the higher level of immobility of C57Bl/6 mice, could have obscured interpretation of the FST results described—it is possible that the time SFR C57Bl/6 mice spent in immobility had already reached a ceiling effect.

It is important to note that we found no evidence that the cognitive deficits detected in IMS Balb/c mice differ between males and females. This is in contrast to sex differences found for rodents in certain tests of fear- and anxiety-related behaviors (see, for example, Wigger and Neumann, 1999) that may be linked (at least in part) to different stress sensitivity of females at different stages of the estrus cycle (Romeo et al., 2003; a finding that motivated us to delimit our study of the FST and EPM behavior to male mice).

What causes the specific cognitive deficits in mice exposed to early life stress? It is currently believed that there are two main contributors to the strain-specific appearance of adult psychopathology after ELS exposure: (1) differences in maternal care and (2) a genetic susceptibility. The differences in maternal care between C57Bl/6 and Balb/c mice at baseline are well documented: C57Bl/6 dams exhibit significantly more arched-back nursing and licking and grooming. They also spend significantly less time ‘off the nest’ compared to Balb/c dams (Priebe et al., 2005; Millstein and Holmes, 2007). However, a daily 3-h IMS also alters the maternal behavior, and dams of both strains have been shown to spend an increased amount of time tending their pups immediately upon being reunited. In fact, the strains with lowest levels of maternal care at baseline, including Balb/c mice, exhibited the greatest responses to IMS in terms of increased time spent ‘on the nest’ (Millstein and Holmes, 2007). Thus, it is not very likely that the cognitive deficits detected in IMS Balb/c mice are solely or predominantly routed in the maternal behavior they experience during the

IMS. Thus, it will now be important to investigate the extent to which a genetic susceptibility to stress determines whether subjects exposed to ELS will develop cognitive deficits. In this regard, it is relevant to note that, in addition to anatomic evidence for altered neuronal structure and monoaminergic innervation within the mPFC, recent studies also found that IMS Balb/c mice exhibit changes in the expression of specific genes in the frontal cortex that are not found in IMS C57Bl/6 mice. Among them is the G protein subunit, which is expressed at increased levels in IMS Balb/c mice (Bhansali et al., 2007; Schmauss et al., 2010). Interestingly, signaling through Gq-coupled receptors was found to be necessary for working memory (Runyan et al., 2005), and stress-induced working memory impairments were shown to result from overactivation of PKC (Birnbaum et al., 2004), which can be activated by Gq-induced calcium increase. Thus, future studies on mechanisms underlying changes in the expression of this gene in IMS Balb/c mice could provide important information about the role of genetic or epigenetic variations that lead to altered Gαq expression following ELS in one but not the other strain of mice. Moreover, it is important to extend such studies to other genes implicated in the control of WM and attention.

Finally, the two cognitive deficits found in the stress-susceptible Balb/c mouse also serve as endophenotypes for a variety of psychiatric disorders (Park and Holzman, 1992; Erlenmeyer-Kimling et al., 2000; Marazziti et al., 2010). This is of special interest as the detection and treatment of these cognitive deficits could permit early identification and possibly onset-prevention of the deficit's associated psychiatric disorders. As such, our present findings underscore the need for a detailed assessment of the cognitive deficits of subjects exposed to early life stress, regardless of whether they already have developed clinically manifest psychopathology.

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FIGURE 1. Performance of SFR and IMS Balb/c and C57Bl/6 mice in the FST and EPM

A, immobility measured during the last 4 min of the second day of FST exposure. Data are mean $\pm$ sem of measures taken from 10 animals per group and they were compared by two-tailed Student's *t*-test. **B**, total time spent in open arms during a single 5 min exposure to the EPM. Data are mean $\pm$ sem of measures taken from the same 10 animals per group shown in **A**, and they were compared by two-tailed Student's *t*-test. IMS= infant maternal separation. SFR= standard-facility-reared controls.

FIGURE 2. Behavior of SFR and IMS Balb/c and C57Bl/6 mice in the Social Recognition test
 The difference in the total time of interaction between the first and second exposure is shown. For C57Bl/6 and Balb/c mice, data are mean $\pm$ sem of measures obtained from 8 mice per group. SFR(s) and IMS(s), re-exposure to the same juvenile. SFR(d) and IMS(d), re-exposure to a different juvenile. Significant differences revealed by ANOVA were resolved *post hoc* using Bonferroni Multiple Comparisons tests as indicated.

FIGURE 3. Performance of SFR and IMS Balb/c and C57Bl/6 mice in the ASST

The individual test phases are indicated in the order they were performed. The numbers for the trials to criterion are mean $\pm$ sem of 8 animals (4 males and 4 females) per group. Results of Repeated Measures ANOVA are summarized in the text. In this figure, results of ANOVA factorial analyses are shown that were resolved *post hoc* using Bonferroni Multiple Comparisons tests as indicated.

FIGURE 4. Performance of SFR and IMS Balb/c and C57Bl/6 mice in a test of spatial working memory

The percentages of correct arm entries at the various delay periods are mean $\pm$ sem of the 8 animals examined in Fig. 3. The line across the bars indicates performance by chance (50%). Results of Repeated Measures ANOVA are summarized in the text. In this figure, results of ANOVA factorial analyses are shown that were resolved *post hoc* using Bonferroni Multiple Comparisons tests as indicated.