A Common Genetic Predisposition to Stress Sensitivity and Stress-Induced Nicotine Craving

Andras Bilkei-Gorzo, Ildikó Rácz, Kerstin Michel, Martin Darvas, Raphael Maldonado, and Andreas Zimmer

Background: Clinical studies have shown that stress is one of the main causes for relapse in abstinent smokers. In this article, we have asked whether animals with a genetic predisposition to high or low stress responsivity differ in behaviors relevant to nicotine addiction, in particular stress-induced reinstatement of drug addiction.

Methods: First, we selected animals with high, low, and average stress sensitivity from the F2 generation from an intercross of high (C57BL/6J) and low (C3H/J) emotional mouse strains. Next, these animals were trained to self-administer nicotine through a chronic intravenous catheter. After extinction of the operant behavior replacing nicotine with saline, mice were stressed with a foot shock and the reinstatement of drug-seeking behaviors was evaluated.

Results: Mice with different stress reactivity showed no difference in the acquisition, extinction, or level of nicotine self-administration. We found an immediate reinstatement of drug-seeking behavior in high stress reactive mice, in contrast to low or average stress reactive animals, which showed no significantly increased activity at the active (nicotine-associated) sensor.

Conclusions: We conclude that a genetic predisposition to high stress sensitivity contributes to relapse vulnerability but not to the initiation or maintenance of nicotine consumption.

Key Words: Craving, nicotine, operant self-administration, reinstatement, risk factors, stress

It is generally thought that a genetic predisposition to drug addiction constitutes a state of vulnerability in which environmental conditions may trigger a series of events that will ultimately manifest in the disease phenotype (1,2). A genetic predisposition to drug abuse could reflect individual differences in the hedonic drug value (3), as well as a differential vulnerability to environmental insults (4). Also, for nicotine addiction, family, twin, and adoption studies demonstrated a significant contribution of genetic risk factors (susceptibility genes) in smoking behavior (5). Stress is perhaps one of the most important environmental factors that contributes to smoking relapse, together with nicotine-associated cues and priming (6). Even nonabstaining smokers often report that they smoke to reduce stress (7) and that they feel an increased craving for tobacco during stressful life events (8). Addicted smokers expect and experience a reduction of stress from smoking (9). Surprisingly, during stressful life events (8). Addicted smokers expect and experience a reduction of stress from smoking (9). Surprisingly, the physiological facts contradict the expected and reported stress-relieving effects of smoking, because nicotine increased, and did not decrease, stress hormone levels in normal and stressed individuals (10).

The effects of nicotine on anxiety-like behaviors in animals remain unclear with some studies showing anxiolytic efficacy (11,12), while others demonstrated anxiogenic effects (13–15), depending on the method and on the duration of the administration (16).

In this study, we have asked whether there is a common predisposition to stress responsiveness, nicotine reinforcement, and stress-induced reinstatement of nicotine seeking. We first evaluated the behavioral responses in animals from the F2 generation of an intercross between C57BL/6J and C3H/J mice in four stress paradigms assessing different aspects of stress reactivity: the zero-maze and the light-dark tests are approach-avoidance paradigms for state anxiety, the Porsolt forced swim test is a model of a behavioral despair situation, and the acoustic startle response test is used to evaluate nonconditioned fear (trait anxiety). The goal of these experiments was to identify those animals with an average or the highest (HS, top 5%) and lowest (LS, bottom 5%) stress responses across different behavioral paradigms. The selection of parental strains was based on their distance on the laboratory mouse family tree, thus ensuring a high degree of genetic variance. Nicotine reinforcement and seeking was assessed in these animals as well as in the parental strains using an operant self-administration paradigm (17).

Methods and Materials

Animals

Studies were carried out on C57BL/6J and C3H/J mice, as well as intercross animals of the F2 generation. The animals were 2 to 3 months old at the start of the test series. The mice were kept in groups of three to five in reversed light-dark cycle (lights on: 19:00; lights off: 9:00). They received water and food ad libitum during the experiments, except during the first conditioning phase of the operant test where access to food was restricted (see below). Animal procedures followed the guidelines of the German Animal Protection Law and were approved by a local animal care and use committee.

First, we tested the stress reactivity in groups of 150 to 180 animals from the F2 generation. They were tested once weekly; each animal was left undisturbed for 7 days between two experiments. Animals from the F2 generation showing extreme average, high, or low stress reactivity based on their behavior reactivity in the four models were selected and tested in an
Elevated Zero Maze

The maze was composed of an annular white platform (outer diameter 47 cm, 5.6 cm width) elevated 40 cm above the ground. Two opposing quadrants of the device were enclosed by walls (11 cm high). Mice were placed on the brightly illuminated (550–600 lux) open part of the apparatus, and their behavior was recorded and analyzed for 5 minutes using the Videomot 2 (TSE Systems, Bad Homburg, Germany) video observation system. Time spent and motor activity in the open area were evaluated (18) and served as supplementary variables for the following principal component analysis.

Light-Dark Test

We used an animal activity monitor (Actimot, TSE Systems) equipped with two-compartment test chambers, consisting of a dark box (15 × 45 × 22 cm) and a bigger (30 × 45 × 22 cm) illuminated box (20 W white neon lamp at a 30 cm distance) connected by a 6 × 6 cm passageway. Mice were placed individually in the center of the lit box. Their movements were recorded with infrared beams (16, 2 cm high) and analyzed with the Actimot software. Time spent and horizontal activity in the open area were evaluated (19).

Startle Response Test

Animals were placed on a Plexiglas and wire mesh cage located on a vibration-sensitive platform in a ventilated, sound-attenuated chamber. Two speakers delivered the background white noise and the startle-eliciting signal (TSE Systems). The startle reactivity to a sound (12 kHz, 110 dB, 40 msec) was triggered the activation of a white control lamp for 2 seconds, which was rewarded with a food pellet (Bio-Serv, Frenchtown, New Jersey; 20 mg) using fixed ratio 1 (FR1) schedule. It was necessary to keep its head above the water (20).

Statistical Analysis and Selection of the Animals from the F2 Generation

After testing a group of animals in a stress model, we analyzed the data with Kolmogorov-Smirnov test and then transformed the original data set to approach a normal distribution. After determining the group means and standard deviations, all animals received scores on the basis of distance of the individual data from the group mean as shown in Table 1. We selected animals with a cumulative score of less than −4 or more than +4, i.e., indicating extreme low or high stress reactivity (Figure 1), as well as animals showing average sensitivity for subsequent operant studies.

Operant Responding for Food and Nicotine

First, the animals were trained in the operant procedure. In this first phase, the animals received only 80% of the amount of food that they had previously consumed. The animals were placed into the operant boxes equipped with two sensors, control lamps, and a feeder (Operant System, TSE Systems). Response on one of the two nose-poke sensors (active sensor) triggered the activation of a white control lamp for 2 seconds, which was rewarded with a food pellet (Bio-Serv, Frenchtown, New Jersey; 20 mg) using fixed ratio 1 (FR1) schedule. It was time between 40 sec and 80 sec. The amplitude of the startle response was measured and evaluated (20).

Forced Swim Test

Mice were placed in a Plexiglas cylinder (10 cm internal diameter, 50 cm high) filled with 25° ± 2°C water (20 cm height). The duration of the experiment was 6 minutes and the behavior of the animals was evaluated between the second and sixth minutes. The immobility time was measured by an observer using a stopwatch. A mouse was judged to be immobile when it remained floating in the water, making only those movements necessary to keep its head above the water (21).

### Table 1. Scoring of Animal Behaviors

<table>
<thead>
<tr>
<th></th>
<th>−3</th>
<th>−2</th>
<th>−1</th>
<th>0</th>
<th>+1</th>
<th>+2</th>
<th>+3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZM-T</td>
<td>0–.56</td>
<td>.57–3.44</td>
<td>3.45–6.33</td>
<td>6.34–12.12</td>
<td>12.13–15.01</td>
<td>15.02–17.32</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>2</td>
<td>12</td>
<td>79</td>
<td>453</td>
<td>54</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>0</td>
<td>7</td>
<td>99</td>
<td>420</td>
<td>83</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>LD-T</td>
<td>0–.95</td>
<td>.96–5.67</td>
<td>5.68–15.12</td>
<td>15.13–17.32</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n</td>
<td>16</td>
<td>55</td>
<td>479</td>
<td>72</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD-A</td>
<td>0–.57</td>
<td>.58–1.84</td>
<td>1.85–4.39</td>
<td>4.40–5.66</td>
<td>5.67–6.93</td>
<td>6.94–8.2</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>1</td>
<td>71</td>
<td>460</td>
<td>76</td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>ASR</td>
<td>1.29–1.47</td>
<td>1.10–1.28</td>
<td>.91–1.09</td>
<td>.62–.90</td>
<td>.43–.61</td>
<td>.24–.42</td>
<td>.04–.23</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>13</td>
<td>49</td>
<td>399</td>
<td>137</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>PFS</td>
<td>209.8–240.0</td>
<td>98.1–209.7</td>
<td>42.4–98</td>
<td>0–42.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>101</td>
<td>415</td>
<td>77</td>
<td>29</td>
<td></td>
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</tr>
</tbody>
</table>

The scoring ranges were determined by first calculating from the normalized data the means ± SD, which corresponds to the 0 group. Subsequently, 1 SD was subtracted or respectively added, until the minimum value or the cutoff value was reached.


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followed by a 10-second time-out period, which was signaled with a yellow lamp. Activation of the active sensor during this period was registered but not rewarded. Response on the other nose-poke sensor (passive sensor) was not rewarded. The duration of a session was 30 minutes. The sessions were repeated daily until the number of responses on the active sensor was significantly higher than on the passive sensor and until the response stabilized, i.e., there was less than 30% variation in the number of responses in 3 consecutive days. Animals that reached this criterion were then moved into the second phase.

We first implanted a cannula into the right jugular vein. This cannula was flushed daily with a mixture of streptokinase (100 IU/mL), Augmentin (150 mg/kg), and heparin (2 UI/mL) for the entire duration of the experiment, thus verifying the patency of the cannula. The animals were allowed to recover from the surgery for 3 days. When the animals were then placed into the operant chamber, they received an injection of 20 μL nicotine solution containing 1.07 μg nicotine hydrogen tartrate (corresponding to .75 μg free base) on activation of the active nose-poke sensor using the same FR1 schedule, time-out period, and cues as in the previous session. Because nicotine has aversive effects at higher doses, we had to limit the maximum amount of daily nicotine. Therefore, the trials lasted either for 30 minutes or until the animals received 25 injections (whichever happened first). Animals reaching the criteria of stable self-administration, as described above, stepped into the third phase.

Here, nicotine was replaced with saline, thus, activation of the sensor associated with nicotine was no longer rewarded but signaled with a white lamp as previously described. This extinction phase lasted until there was no further significant difference in the number of responses on the active and passive sensors and until the variation in the responses was less than 30% on 3 consecutive days. Animals reaching this criterion were exposed to a single foot-shock stressor (five shocks, each .5 mA, duration 100 msec, separated with 60 sec) in a small chamber of a startle apparatus (TSE Systems). They were then immediately placed into the operant chamber and their activity on the sensors was registered. They still did not receive a nicotine reward, but activation of the active sensor yielded the same cue and intravenous (IV) saline injection through the cannula as in the extinction session. Data were analyzed using one-way analysis of variance (ANOVA), except the analysis of the effect of stress where we used two-way ANOVA (main factors: stress reactivity and stress) followed by Student-Newman-Keuls (SNK) test.

Results

Selection of Animals with Low and High Stress Responses

The distribution of the behavioral responses after normalization is shown in Figure 1. We applied square-root transformation for the normalization of zero-maze and light-dark test parameters. Logarithmic transformation of the startle response data resulted in a near normal distribution, while in the forced swim test, we found a high number of animals showing an immobility time close to the cutoff limit (Figure 1), thus making data normalization impossible. We therefore used the original data for scoring. A score was assigned to each animal for each test based on the distance (in standard deviations) from the group means as outlined in Table 1. A positive score reflected an increased stress resistance, while a negative score reflected an increase in stress sensitivity relative to the group means.

As shown in Figure 2, we found a positive correlation in 9 out of 15 possible combinations of the different behavioral parameters. However, the correlation coefficients were generally low, with the exception of the time and activity measurements in the zero-maze and in the light-dark tests, thus indicating that the paradigms used in this study addressed relatively independent aspects of stress-related behaviors. We next calculated cumulative scores for each animal. Although scores between −4 and +14 were theoretically possible, the most extreme scores that we found were −7 and +8 (Figure 1). Finally, we decided to select animals with a score above 4 (HS) and below −4 (LS) for further analysis and obtained 28 HS and 25 LS mice.
of the individual behavioral responses of these animals showed that most of them had consistently lower or higher stress responses compared with the group means in several tests (data not shown); only 4 of the selected 53 animals reached a score higher than 3 in one model. We also tested the stress reactivity of 10 male and female mice from the parental strains and scored them using the same procedure as for mice from the F2 generation. For comparison, the average score values of the parental strains were 1.40 ± .39 in C57BL/6J and 2.95 ± .33 in C3H/J (p < .05 according to Mann-Whitney test). We also selected 24 F2 mice representing animals with an average stress (AS) reactivity of the selected animals. The first and second factors (mostly from the zero-maze and light-dark tests, see Table 2) accounted for 30.1% and 21.9%, respectively. The third factor contributed 16.6% to the data variation and was mostly driven by the startle response. The fourth factor (mostly from the forced swim test) contributed 16.2%, and finally, the fifth and sixth mostly motility-related factors accounted for 8.0% and 7.2% of the data variation.

Operant Nicotine Self-Administration

In the first phase of the experiment, we trained the animals to respond on a sensor and to discriminate between the active and passive sensors using food as reward. The speed of learning, i.e., the time necessary for the stabilization of the operant behavior, did not differ in the groups \( F(2,73) = .757; p > .05 \), Figure 3A). The number of responses of the active sensor during the plateau phase (maximal performance) was also not different \( F(2,73) = 1.274; p > .05 \), Figure 3B). These results indicate that the groups were similarly motivated to work for food and that they did not differ in their ability to learn the operant behavior. We excluded six animals, since they did not learn the operant task.

In the second phase of the test, we replaced the food reward with an intravenously administered nicotine reward. Each group maintained their operant behavior, indicating that nicotine was rewarding for them. Indeed, there was no difference between the groups in the number of trials until the nicotine self-administration experiment behavior stabilized \( F(2,38) = 2.335; p > .05 \), Figure 4A) or in the number of responses on the active sensor for nicotine \( F(2,38) = .894; p > .05 \), Figure 4B). A considerable percentage of the responses in the LS (38.3 ± 4.5%), AS (43.47 ± 2.97%), and HS (51.5 ± 3.5%) groups was in the time-out period; thus, the amount of nicotine delivered after the stabilization of the behavior was in one session (10.6 ± 1.2 μg in the HS group, 15.9 ± 6 μg in the AS group, and 13.8 ± 1.2 μg in the LS group). In this phase, we again lost a number of animals from

Table 2. Contribution of Measured Parameters (in Percent) to the Principal Components (Factors) Based on the Correlation Values

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
<th>Factor 4</th>
<th>Factor 5</th>
<th>Factor 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZM-T</td>
<td>23.267</td>
<td>23.275</td>
<td>.004</td>
<td>6.966</td>
<td>11.617</td>
<td>34.870</td>
</tr>
<tr>
<td>ZM-A</td>
<td>25.822</td>
<td>23.471</td>
<td>.123</td>
<td>.042</td>
<td>11.276</td>
<td>39.266</td>
</tr>
<tr>
<td>LD-T</td>
<td>22.689</td>
<td>25.789</td>
<td>1.676</td>
<td>.103</td>
<td>37.991</td>
<td>11.753</td>
</tr>
<tr>
<td>LD-A</td>
<td>22.922</td>
<td>25.342</td>
<td>.685</td>
<td>1.196</td>
<td>38.836</td>
<td>11.019</td>
</tr>
<tr>
<td>ASR</td>
<td>.061</td>
<td>2.016</td>
<td>91.386</td>
<td>6.275</td>
<td>.077</td>
<td>.185</td>
</tr>
<tr>
<td>PFS</td>
<td>5.238</td>
<td>.108</td>
<td>6.126</td>
<td>85.419</td>
<td>.203</td>
<td>2.906</td>
</tr>
</tbody>
</table>

the groups (11 from the LS group, 8 from the AS group, and 14 from the HS group), mostly due to problems with the intravenous catheter.

In the third phase of the test, we replaced nicotine with saline. This resulted first in a transient increase in the number of responses on both sensors, followed by a continuous decrease of sensor activations until there was no difference in the number of responses on the previously active (previously nicotine associated) and nonrewarded (passive) sensor was also not significantly different between the groups. HS, high stress reactive; LS, low stress reactive; AS, average stress reactive.

Figure 3. Acquisition of operant behaviors with food pellets as reward. (A) The number of trials necessary to learn the operant food self-administration in LS ($n = 24$), AS ($n = 24$), and HS ($n = 27$) mice. Shown is the day (means ± SEM) when the number of responses on the active sensor was significantly higher than on the passive sensor and when the response variation was less than 30% on 3 consecutive days. (B) The number of responses (means ± SEM) on the rewarded (active) and nonrewarded (passive) sensor was also not significantly different between the groups. HS, high stress reactive; LS, low stress reactive; AS, average stress reactive.

Figure 4. Operant behaviors with nicotine as reward. (A) Shown is the day (means ± SEM) when the animals achieved a stable operant behavior, i.e., when the number of responses was significantly higher on the active sensor and when the response variation was less than 30% on 3 consecutive days ($n = 14$ in LS, $n = 15$ in AS, and $n = 13$ in HS). (B) Shown are the responses (means ± SEM) on the active and passive sensor of animals that had acquired a stable nicotine self-administration. (C) Number of trials (means ± SEM) necessary for the extinction of operant behaviors in the withdrawal period ($n = 11$ in LS and AS, $n = 8$ in HS). (D) Number of responses (means ± SEM) on the sensors previously associated with nicotine and saline after withdrawal. There was no difference between HS, AS, and LS mice in nicotine self-administration or in the extinction of nicotine-seeking behaviors. HS, high stress reactive; LS, low stress reactive; AS, average stress reactive.
groups \( F(2,29) = 1.1400; \ p > .05 \), Figure 4C). We had to reject five mice from the HS and LS groups and seven animals from the AS group because of health problems or problems with the catheter.

Finally, when we exposed the animals (HS, \( n = 8 \); AS, \( n = 11 \); LS, \( n = 11 \)) to a brief foot shock, we found a striking difference in the operant behavior between the groups \( F(2,27) = 4.329; \ p < .05 \). The number of responses on the active sensor increased significantly after stress in the HS group but not in the AS or LS groups (Figure 5). The increase of responses in HS mice cannot be attributed to a generally increased activity, because responses on the passive sensor did not change \( (5.00 \pm .97 \) from \( 7.43 \pm 2.08; \ p > .05 \) and there was no significant difference between the groups on the number of responses on the passive sensor. Thus, stress produced a reinstatement of drug-seeking behavior in HS mice but not in LS or AS mice.

The ratio of male and female mice was the same in the HS and AS groups \( (14 - 14 \) and \( 12 - 12 \), respectively, from both sexes) and also was not significantly different in the LS mice \( (9 \) male mice and \( 16 \) female mice). There was no difference in the operant learning ability between the sexes in any phase of the experiment, since the number of trials necessary to reach the criterion level was similar.

The operant self-administration procedure was repeated with the parental strains as described above. There was no difference between parental strains in the operant learning ability, since the acquisition of food or nicotine self-administration was not different (Supplement 1A). Interestingly, C3H/\( J \) mice responded more on the active but also on the inactive sensor when nicotine but not when food was used as reinforcer (Supplement 1D). Time needed for extinction of the nicotine self-administration behavior was again similar (Supplement 1E). Stress did not reindate the operant behavior of mice from the parental strains (Supplement 1F); thus they showed a similar reactivity as LS or AS mice from the F2 generation.

**Discussion**

Human and animal studies suggested that stress is one of the most important factors in the relapse to addictive behavior after a period of abstinence \( (6,22,23) \). The results of this study demonstrate that the relapse-terminating potential of stress on nicotine self-administration behavior in mice is strongly dependent on the genetically predisposed emotionality of the individual. Thus, nicotine-seeking behavior was reinstated by a brief stress exposure in mice selected for high stress responses but not in mice with low stress responses.

In this study, we have selected mice from the most extreme ends of the spectrum of stress-related behaviors and representative of mice having average stress reactivity. To study a possible correlation between stress responsiveness and nicotine-seeking behavior, we considered two alternative options for the selection of animals: a completely random selection of animals or selection of mice with extreme phenotypes. We decided to follow the latter, more laborious strategy because 1) we were concerned that otherwise not enough mice with extreme phenotypes would be included in our analysis, 2) we felt that it would be necessary to test a large number of animals to obtain sufficient information about the distribution and spectrum of stress-related behaviors, and 3) we were planning to utilize this data set for the future analysis of quantitative trait loci (QTL) involved in the regulation of stress-related behaviors. Because at least 20 animals from both ends of the behavioral spectrum should be included in the nicotine self-administration paradigm, we estimated that a minimum of 400 to 600 animals from the F2 generation would be necessary to reach this number, considering a certain dropout rate.

Anxiety and depression are the major groups of stress-related disorders. Thus, we selected methods assessing the stress sensitivity of animals that are commonly used in testing anxiolytic \( (24) \) and antidepressant \( (25) \) drugs and in the phenotype analysis of genetically modified mice \( (26) \). The evaluation of multiple anxiety-related parameters, in spite of the objective limitations of each of the behavioral paradigms, was essential for the selection of high and low emotional individuals. The relatively low correlation between the parameters indicated a largely independent genetic regulation of these behaviors. In agreement with this assumption, the minimum number of genes involved in the open time parameter of the zero-maze or light-dark tests calculated by the modified Castle-Wright estimator is 18 and 13, respectively, while the estimated number of genes regulating factor 1 and factor 2 is only 3. Thus, a QTL analysis should not be based on the measured values but rather on the principle components.

Importantly, mice having different stress reactivity did not show any difference in their ability to learn the operant behavior in the time needed for the stabilization of nicotine-seeking behavior or in the amount of nicotine self-administration. These findings indicate that traits of anxiety did not modify the acquisition of an operant behavior to self-administer nicotine. However, the acquisition of this behavioral response may only be correlated to the initiation of nicotine self-administration behavior. The maintenance of nicotine addiction is closely related to the high relapse potential of this drug even after long periods of abstinence. Interestingly, stress enhances nicotine consumption in humans \( (8) \) and the development of sensitization to nicotine in animals \( (27) \). Moreover, stress facilitates acquisition of cocaine \( (28) \) and amphetamine \( (29) \) self-administration. Although our animals were not intentionally stressed, the surgery, the daily procedure of washing the implanted cannula, and the daily operant trials must have been stressful. We had therefore hypothesized that HS mice might be more stressed than AS or LS mice and thus might acquire the operant behavior faster or consume more nicotine, but this was clearly not the case.

Foot-shock stress is a general method for reinstatement of drug-seeking behavior to cocaine, heroin \( (30) \), nicotine \( (17) \), and alcohol \( (31) \) in rodents. Our results indicate that trait anxiety does not affect the acquisition and consolidation of nicotine self-administration but specifically affects stress-precipitated nicotine reinstatement after a period of abstinence. In humans, psychosocial stress elicits and increases the urge to smoke \( (32) \), and abstaining smokers report stress \( (33) \) and difficulty dealing with stress \( (34) \).

We found an interaction between anxiety levels and the effects of stress on nicotine-seeking behavior, which may be driven by genetic and environmental factors. To minimize environmental influences, cages contained similar numbers of animals from the same age (in most of the cases littermates); aggression was never observed between the cagemates. The incidence of high or low stress-reactive offspring did not differ between the 24 breeding pairs, suggesting that the maternal effect did not influence significantly the emotionality of our animals. The genetic heterogeneity is thus probably the main factor that determined the stress reactivity of the mice from the F2 generation, although we cannot exclude a contribution of environmental factors as well.
Interestingly, sex of the animals did not influence the transfer of operant behavior from food to the nicotine, extinction, and its stress-induced reinstatement of the self-administration behavior, although some clinical and animals studies showed a sex difference in the vulnerability to the reinforcing effects of nicotine (35). Here we have shown that acquisition and extinction of operant behaviors for nicotine and stress-induced reinstatement of self-administration were similar in both sexes.

Nicotine relapse is the main limitation to obtaining a maintained tobacco cessation in humans. The present results show the relevance of anxiety traits in the vulnerability to stress-induced relapse to a nicotine self-administration in mice. A predisposition toward increased emotional responses affects not voluntary nicotine taking but compulsive nicotine-seeking behaviors. Our data suggest an overlap in the behavioral responses to different stressors and stress-induced reinstatement of self-administration were similar in both sexes.

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The authors declare that they have no competing financial interest.

Supplementary material cited in this article is available online.


