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

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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, 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).

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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

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operant nicotine self-administration model, as well as mice from the parental C57BL/6J and C3H strains.

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 (18) were evaluated and used for the selection of the animals. Distance traveled in the closed area and the number of area changes were also registered 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 measured after 5 minutes habituation. Reaction to the startle-eliciting stimulus was measured seven times, with an intertrial 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 23° ± 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).

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

Table 1. Scoring of Animal Behaviors

	Scores						
	− 3	− 2	− 1	0	+ 1	+ 2	+ 3
ZM-T							
Range	0–.56	.57–3.44	3.45–6.33	6.34–12.12	12.13–15.01	15.02–17.32	
n	2	12	79	453	54	22	
ZM-A							
Range	0–2.08	2.09–6.18	6.19–10.28	10.29–18.49	18.50–22.54	22.55–26.64	26.65–30.74
n	0	7	99	420	83	12	1
LD-T							
Range		0–.95	.96–5.67	5.68–15.12	15.13–17.32		
n		16	55	479	72		
LD-A							
Range		0–.57	.58–1.84	1.85–4.39	4.40–5.66	5.67–6.93	6.94–8.2
n		1	71	460	76	8	6
ASR							
Range	1.29–1.47	1.10–1.28	.91–1.09	.62–.90	.43–.61	.24–.42	.04–.23
n	11	13	49	399	137	11	0
PFS							
Range			209.8–240.0	98.1–209.7	42.4–98	0–42.3	
n			101	415	77	29	

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.

ASR, acoustic startle response; LD-A, light-dark test activity in lit compartment; LD-T, light-dark test time in lit compartment; PFS, Porsolt forced swim; ZM-A, zero-maze test activity in open compartments; ZM-T, zero-maze test time in open compartments.