EGTA, and 1 $MgCl_2$ (pH 7.3). The standard presynaptic pipette solution contained (in mM) 97.5, potassium gluconate, 32.5 KCl, 10 Hepes, 0.5 EGTA, 1 $MgCl_2$, 10 potassium glutamate, 2.0 Mg-ATP, 12 phosphocreatinine, and 0.5 GTP (pH 7.3). Whole-cell patch-clamp recordings were made from the calyx of Held and MNTB principal neurons, and EPSCs were evoked either by presynaptic action potentials or I_{pCa} elicited by a depolarizing pulse (1-ms duration) (*9*, *10*). For recording presynaptic Ca^{2+} currents, tetraethylammonium chloride (TEA-Cl, 10 mM) and tetrodotoxin (TTX, 1 μM) were added to the aCSF, and potassium gluconate and KCl in the presynaptic pipette solution were replaced by 110 mM CsCl, Hepes was increased to 40 mM, and 10 mM TEA-Cl was added (pH 7.3). The electrode resistance was typically 2 to 4 megohm for the postsynaptic pipette and 5 to 9 megohm for the presynaptic pipette. When EPSCs were evoked by I_{pCa}, the magnitude of the depolarizing pulse was adjusted to about 70% of the maximal size to avoid saturation in the amplitude of the EPSC against I_{pCa}. For recording I_{pCa}, the series resistance (10 to 20 megohm) was compensated by 70 to 80%. Recordings were low-pass filtered at 2.5 to 20 kHz and digitized at 5 to 48 kHz with CED 1401 interface (Cambridge Electronics Design). Leak currents were subtracted for presynaptic Ca^{2+} currents by a P/N protocol (*9*). Recordings were made at room temperature (22° to 26°C). The difference between groups was evaluated by unpaired *t* test or Kolmogorov-Smirnov test, with $P < 0.05$ taken as the level of significance. When GDPβS or GTPγS (both Li^+ salts) was included in the pipette solution, GTP was omitted. Infusion of GDPβS or GTPγS into the calyx was done as previously reported (*10*). Briefly, an Eppendorf yellow tip was heated and pulled to produce a tip diameter of 50 to 70 μm, and the pipette solution containing guanine nucleotides was back-filled into the tube and installed into a patch pipette with its tip positioned by 500 to 600 μm proximal to the tip of the patch pipette. After control responses were recorded, GDPβS or GTPγS was delivered into the patch pipette by a positive pressure manually applied through a syringe. When Lucifer Yellow (0.05%) was injected into a calyx by this method, fluorescence became detectable within 1 min after injection and reached maximal intensity within an additional 2 min. A GDPβS concentration of <0.2 mM in the whole-cell pipette did not prevent the baclofen effect (*9*).

12. The $GABA_B$ receptor ligand baclofen (10 to 20 μM) inhibited EPSCs through activating presynaptic heterotrimeric G proteins (*9*) by 72 ± 4.3% before the presynaptic infusion of GDPβS, but by only 11 ± 2.6% after GDPβS infusion ($n = 4$ cells).
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18. In some experiments GTPγS was photo-released by an ultraviolet flashlight (10 s) from a caged compound included in the presynaptic pipettes (38 μM) (*9*).
19. Recovery was complete with GTP (101 ± 2%, $n = 6$), GDPβS (98 ± 3%, $n = 6$), or with no guanine nucleotides (97 ± 2%, $n = 9$) in the presynaptic pipettes.
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Abolition and Reversal of Strain Differences in Behavioral Responses to Drugs of Abuse After a Brief Experience

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Inbred strains of mice are largely used to identify the genetic basis of normal and pathological behaviors. This report demonstrates that a moderate period of food shortage, an ecologically common experience, can reverse or abolish strain differences in behavioral responses to the abused psychostimulant amphetamine. The period of food shortage occurred when the animals were mature and was terminated before the administration of amphetamine. Strain differences in behavior appear highly dependent on environmental experiences. Consequently, to identify biological determinants of behavior, an integrated approach considering the interaction between environmental and genetic factors needs to be used.

Genetic analyses using inbred strains of mice are increasingly utilized to identify biological determinants of normal and pathological behaviors (*1*–*3*). A basic prerequisite of these investigations is the existence of consistent and reliable behavioral differences between inbred strains, which are then used to identify the genetic determinant of behavioral phenotypes (*1*, *2*, *4*). A recent report described variation in the behavior of inbred strains that can occur across laboratories (*4*). These results are particularly troubling because they are observed despite the explicit effort to maintain identical experimental settings and environmental conditions. Although the authors restrict the effects of environmental variables to phenotypes with a small genetic influence, doubt remains as to whether major gene-environment interactions might be currently overlooked.

We studied the effects of food shortage on strain differences in behavioral phenotypes related to drug abuse, a behavioral pathology considered to have strong genetic influences (*5*). Food shortage was chosen for three reasons. First, it is a common and ecologically relevant environmental experience very likely to occur during the life-span of animals living in the wild. Second, it is often used in laboratory settings because it accompanies learning tests based on positive reinforcers including drugs of abuse (*6*). Third, food shortage increases the activity of biological systems mediating behavioral responses to drugs of abuse, such as the mesencephalic dopaminergic transmission and glucocorticoid secretion (*7*). Mice from the C57BL/6JIco and DBA/2JIco inbred strains were studied (*8*). C57BL/6J and DBA/2J are among the oldest and most studied inbred strains (*3*), and the recombinant inbred strains derived from them are largely used for quantitative trait loci (QTL) analysis (*1*). Two behaviors induced by the abused psychostimulant amphetamine were studied: locomotion and place conditioning. Drug-induced locomotion is the test most often used for evaluating the motor-stimulating effect of psychostimulant drugs (*9*). It represents a simple unconditioned response where genetic influences have been well characterized (*4*, *10*). In place conditioning, aversive or rewarding effects of drugs are inferred from measuring, in a drug-free state, preference

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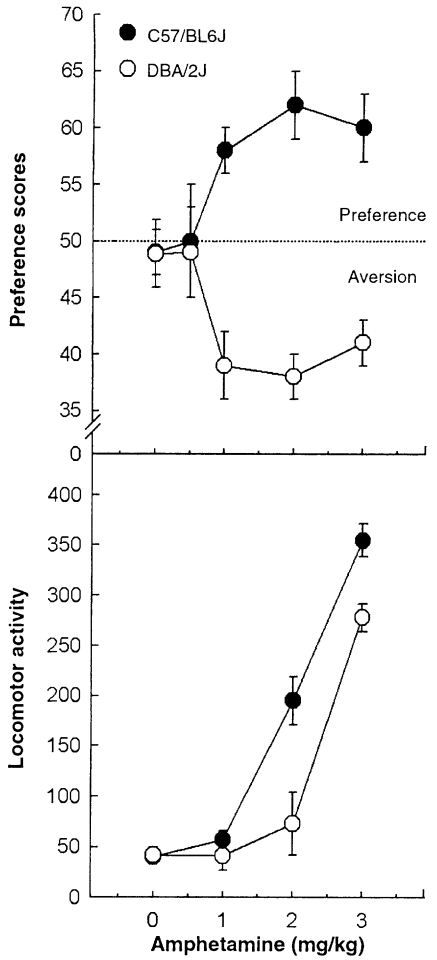

Fig. 1. Differences between C57BL/6J and DBA/2J mice in amphetamine-induced place conditioning (top, n = 11 per dose per strain) and locomotion (bottom, n = 6 per dose per strain) when food availability was unlimited. Amphetamine-induced place conditioning engendered opposite responses in C57BL/6J and DBA/2J mice [strain effects: $F(1,4) = 34.13$, $P < 0.0001$], and this difference was dose-dependent [strain × dose interaction: $F(4,110) = 5.89$, $P < 0.0005$]. C57BL/6J and DBA/2J mice did not differ in response to vehicle (0.0 mg/kg) or 0.5 mg/kg of amphetamine. In contrast, at all the other doses of amphetamine, C57BL/6J mice showed place preference [$F(4, 54) = 3.57$, $P < 0.02$] and DBA/2J mice showed place aversion [$F(4,56) = 2.73$, $P < 0.03$]. Within strains, the effects of 1, 2, and 3 mg/kg of amphetamine did not significantly differ. C57BL/6J mice were also more sensitive to the stimulant effects of amphetamine than DBA/2J mice [strain × dose interaction: $F(1,50) = 6.32$, $P < 0.002$]. The two strains did not differ for their locomotor response to vehicle (0.0 mg/kg) and 1 mg/kg of amphetamine. However, for all the other doses, locomotion was higher in C57BL/6J than DBA/2J mice.

for or avoidance of a compartment that has been repeatedly paired with a drug injection (*11, 12*).

First, we compared the effects of amphetamine in C57BL/6J and DBA/2J mice (*5*) when food availability was unlimited (ad libitum).

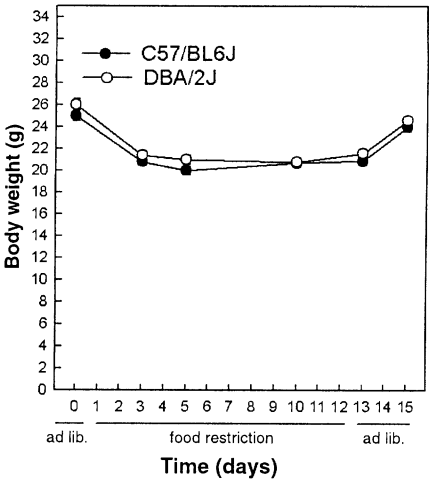

Fig. 2. Time course of weight loss and recovery during periods of food shortage and ad libitum feeding (n = 14 per strain). Weight loss and recovery did not differ in the two strains [strain × day interaction: $F(1,5) = 0.11$, $P > 0.73$].

Qualitative differences were revealed by place conditioning. Amphetamine had opposite effects in the two strains, consistently inducing conditioned preference in C57BL/6J and conditioned aversion in DBA/2J mice (Fig. 1, top). The two strains did not differ for their sensitivity to amphetamine, because place conditioning, although engendering opposite responses, was observed within the same range of doses. Quantitative differences were revealed by amphetamine-induced locomotion. A significant increase in locomotion was observed at lower doses in C57BL/6J than in DBA/2J mice (Fig. 1, bottom). These results provide an example of strain differences in the behavioral response to drugs of abuse and support the general idea (*2*) that inbred strains can provide models of genetic susceptibility to addiction.

Second, we compared the effects of amphetamine in C57BL/6J and DBA/2J mice after a period of food shortage. The amount of food that was available daily was progressively reduced. When animals lost 20% of their weight (Fig. 2), the alimentary regimen was adjusted to maintain this loss. After 12 days, food was again available ad libitum. The administration of amphetamine started 2 days after the animals reached their body weight before restriction. Independent groups of ad libitum–fed C57BL/6J and DBA/2J mice were used as controls. As in the first experiment, ad libitum–fed C57BL/6J and DBA/2J mice showed amphetamine-induced place preference and aversion, respectively. However, after food shortage, the original amphetamine-induced place aversion of DBA/2J mice was changed into a preference, and the two strains no longer differed for this phenotype (Fig. 3, top). Food shortage also modified the stimulant effects of amphetamine. When food was available ad libitum, DBA/2J mice were less sensitive to amphetamine than C57BL/6J animals (Fig. 1, bottom). In contrast, after food shortage, DBA/2J mice became more sensitive than C57BL/6J animals to amphetamine-induced locomotion and showed strong locomotor activation at a dose of 1 mg per kilogram of body weight (mg/kg). This dose was still ineffective in C57BL/6J mice (Fig. 3, bottom).

These results demonstrate the major influence of environmental variables on strain differences in behavior. When food availability is unlimited, DBA/2J and C57BL/6J mice constitute an ideal model of resistance and vulnerability to drug abuse (*2, 9*). DBA/2J mice are hyposensitive to the stimulant effects of amphetamine and show consistent aversion for a place paired with this psychostimulant, whereas the opposite is true for C57BL/6J mice. However, after a temporary reduction in food availability, DBA/2J mice show a strong amphetamine-induced place preference and become more sensitive than C57BL/6J mice to the stimulant effect of the drug. The animals tested in these experiments were adults when food shortage occurred, and they had already fully regained their preshortage body weight before receiving amphetamine. Thus, in mature animals, the past experience of a common and ecologically meaningful event can eliminate or change profound strain differences in behavior.

Glucocorticoid hormones and the mesencephalic dopaminergic projection to the nucleus accumbens are likely to mediate the different effects of food shortage in C57BL/6J and DBA/2J mice. The activity of these two biological factors is increased by food shortage and profoundly influences behavioral responses to drugs of abuse (*7*). Glucocorticoid secretion and dopamine release differ in C57BL/6J and DBA/2J mice both in basal conditions and in response to environmental challenges (*13*).

The results obtained from studies on the effects of genetic manipulations on behavioral phenotypes recently raised the question about the importance of background variables. Behavioral analyses of knockout and transgenic mice have shown that the genetic background hosting a mutation profoundly influences its phenotype (*14*). This observation is troubling because it casts a doubt about the generalization of results produced in the laboratory. In fact, the gene-phenotype relation observable in specific laboratory-selected genetic backgrounds may not be generalized to all the highly variable genomes found in the world. Our results add a new level of complexity to this issue by showing that major strain differences in drug abuse–related phenotypes are controlled by an ecologically relevant environmental variable. This implies that the attempt to identify "genetic" or "environmental" causality as independent main effects is probably logical-

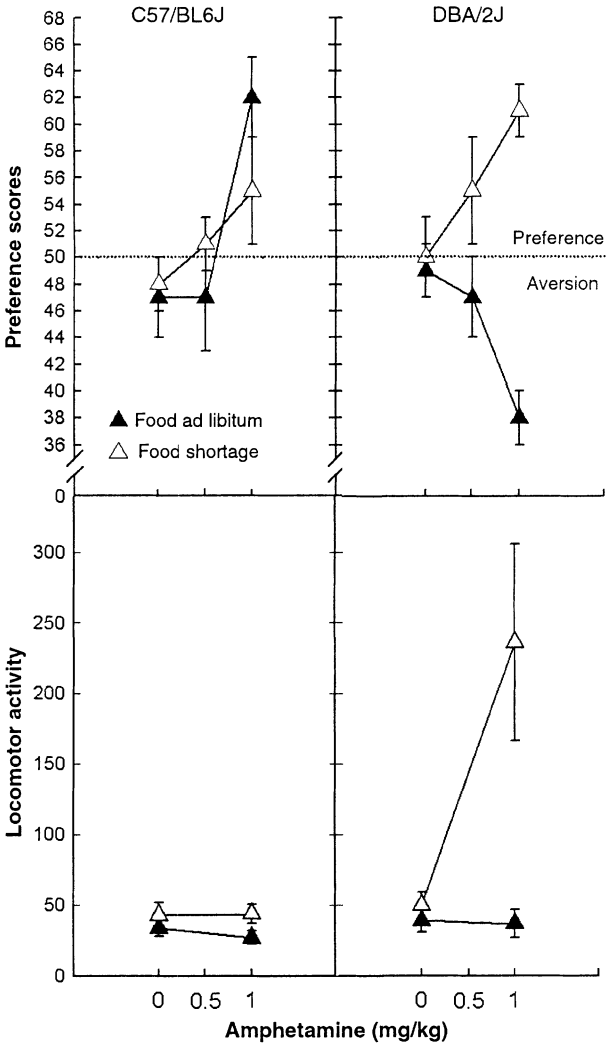

Fig. 3. Amphetamine-induced place conditioning (top, n = 8 per dose per strain) and locomotion (bottom, n = 6 per dose per strain) in C57BL/6J and DBA/2J mice when food availability was either unlimited or reduced. Food shortage had a different effect on amphetamine-induced place conditioning in C57BL/6J and DBA/2J mice [food availability × strain × dose interaction: $F(2,105) = 3.142$, $P < 0.05$]. Food shortage did not significantly modify the behavior of C57BL/6J mice [food availability × dose interaction: $F(1,51) = 0.96$, $P > 0.33$]. In DBA/2J mice, it changed place aversion to place preference [food availability × dose interaction: $F(2,48) = 5.36$, $P < 0.01$]. Food shortage also had a different effect on amphetamine-induced locomotion in C57BL/6J and DBA/2J mice [food availability × strain × dose interaction: $F(1,56) = 16.53$, $P < 0.0002$)]. After food shortage, the locomotor response to amphetamine did not change in C57BL/6J mice [food availability × dose interaction: $F(1,28) = 0.03$, $P > 0.85$]; however, it increased in DBA/2J mice [food availability × dose interaction $F(1,28) = 17.36$, $P < 0.001$].

ly and procedurally flawed. Consequently, the evaluation of genetic effects on behavioral phenotypes should consider interactions among genes, as well as interactions between genes and environment.

One of the ultimate goals of genetic research in animals is to identify gene products that can contribute to the appearance of behavioral pathologies. The obligatory starting point of many of these investigations is a stable difference between strains in the behavioral phenotype of interest. These differences are then used for genetic analyses to identify the gene products influencing the targeted behavior. The observation that a common environmental experience can modify the behavioral differences of inbred strains has a major implication: Genetic analyses performed in different environmental settings may erroneously lead to attributing an identical phenotype to different genes. Clinical studies indicate that there are gene-environment interactions in the etiology of most if not all psychopathologies (*15*). Thus, to accelerate our understanding of the pathophysiological bases of diseases and to speed the development of appropriate therapeutic strategies, we must develop experimental models that take into consideration the interaction between the environment and the genome.

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