Human Molecular Genetics, 2006, Vol. 15, No. 18 ***2721–2731***
doi:10.1093/hmg/ddl206
Advance Access published on August 7, 2006

Monoamine oxidase A knockout mice exhibit impaired nicotine preference but normal responses to novel stimuli

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Received June 15, 2006; Revised and Accepted July 28, 2006

Nicotine is thought to act on brain monoamine systems that normally mediate diverse motivational behaviors. How monoamine-related genes contribute to behavioral traits (e.g. responses to novel stimuli) comorbid with the susceptibility to nicotine addiction is still poorly understood. We examined the impact of constitutive monoamine oxidase A (MAOA) deficiency in mice on nicotine reward and responses to novel stimuli. Age-matched, male *Maoa*-knockout (KO) mice and wild-type (WT) littermates were tested for nicotine-induced conditioned place preference (CPP); voluntary oral nicotine preference/intake; spontaneous locomotor activity in a novel, inescapable open field; and novelty place preference. Nicotine preference in WT mice was reduced in *Maoa*-KO mice in the CPP and oral preference/intake tests. Control experiments showed that these phenotypes were not due to abnormalities in nicotine metabolism, fluid intake or response to taste. In contrast, *Maoa*-KO mice were normal in their behavioral response to a novel, inescapable open field and in their preference for a novel place. The observed phenotypes suggest that a constitutive deficiency of MAOA reduces the rewarding effects of nicotine without altering behavioral responses to novel stimuli in mice. Constitutive MAOA activity levels are likely to contribute to the vulnerability or resiliency to nicotine addiction by altering the rewarding effects of nicotine.

INTRODUCTION

Individual vulnerability is a critical determinant of addiction. Only a subpopulation of those who try addictive substances, including nicotine in the form of cigarette smoking, go on to develop addiction (1,2). Interestingly, those who develop addiction often exhibit pre-existing behavioral traits. Among these is a cluster of highly correlated traits labeled 'novelty seeking' and 'impulsive sensation seeking' (3) defined, respectively, as a heritable tendency towards intense exhilaration or excitement in response to novel stimuli (4) and a trait by which an individual seeks novel sensations and experiences without considering the potential for negative consequences (5). These motivational traits exist before the onset of smoking and nicotine addiction (6–9).

Addiction is, in essence, a dysfunctional motivational behavior, as it is characterized by an uncontrollable, compulsive use of a substance despite its negative consequences. The inherently altered motivational trait might normally manifest as an altered behavioral response to novel stimuli, but might be expressed as a heightened susceptibility to addiction upon exposure to an addictive substance (2). How the brain is prewired, in addition to how an addictive substance alters the brain, can be considered a critical determinant of the development of addiction.

It is thought that genetic variations affect the likelihood of developing nicotine addiction. Genetic variations might either concomitantly or separately influence susceptibility to addiction and comorbid traits, but the mode by which genes exert these effects is likely to be complex (2,10–12), and

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the specific genes underlying inherent differences in motivational traits, including addictive behavior, are still poorly understood (13). However, both human and animal studies have implicated monoamines in novelty responses (14–16) and nicotine addiction (17–21). Monoamine oxidase A (MAOA), an isozyme of MAO that catalyzes the oxidative deamination of monoamines, is one candidate for a gene that is responsible for interindividual differences in the susceptibility to nicotine addiction and comorbid traits. MAOA is localized in brain regions that have been implicated in nicotine addiction and the behavioral response to novel stimuli (22–24). Moreover, evidence suggests that its activity levels vary widely among individuals (25,26). In post-mortem tissues taken from the human frontal cortex, up to 7-fold differences in MAOA activity have been reported (27).

Although there are rare cases of a complete deficiency of MAOA because of a point mutation in exon 8 (28), no single alleles so far identified or their combinations (i.e. haplotype) fully account for the large interindividual differences in basal MAOA activity in the general population (26,27,29–31). The T-allele at position 1460 in exon 14 and either 3.5 or 4 repeats at the variable number tandem repeat (VNTR) in the promoter region of *MAOA* have been reported to be associated with slightly elevated levels of MAOA activity (26,29–31). Some studies have shown a positive correlation between an increased risk of smoking and the T-allele (32,33), the 4-repeat VNTR in males (34), or their haplotype (33), but others have failed to confirm the positive correlation with the T-allele (35,36) and the 4-repeat VNTR (33). There are many procedural differences in these studies that make direct comparison difficult, including sample population, gender, age and the definition of addiction. These inconsistent results might also be due to the weak impact of the polymorphisms on enzyme activity in the brain (27). Although monoamine oxidases have been suggested to contribute to responses to novelty, this weak allelic effect might also be a reason for the reported lack of association between novelty seeking and *MAOA* polymorphisms (37–42).

How constitutively altered MAOA levels affect nicotine addiction has not been fully explored under experimental conditions. Fowler *et al*. (43) demonstrated that MAOA activity was lower in the brains of smokers. Because this may be largely due to MAOA inhibitors in tobacco smoke (44–46), it remains unclear how constitutively reduced MAOA levels affect susceptibility to nicotine addiction. Pharmacological inhibition of MAOA does not recapitulate the constitutive alteration of MAOA throughout development and MAO inhibitors have many more actions than the inhibition of MAOA and/or MAOB (47–54).

Because some studies show a positive correlation between smoking and high-activity alleles of MAOA (32–34), we hypothesized that nicotine induces higher levels of rewarding effects in WT mice than in *Maoa*-KO mice. We have now examined how a constitutively altered level of MAOA affects preferences for nicotine and behavioral responses toward novel stimuli using congenic *Maoa*-KO mice. Our findings show that a constitutive deficiency of MAOA affects nicotine-induced conditioned place preference (CPP) and nicotine preference/intake, but not behavioral responses to a novel environment.

RESULTS

Maoa-KO mice are impaired in nicotine CPP

Time spent in the nicotine-paired and saline-paired compartments of the CPP apparatus was analyzed using a three-way ANOVA, including genotype (WT versus *Maoa*-KO), dose (0, 0.1, 0.2, 0.4 and 0.8 mg/kg) and compartment (nicotine-paired and saline-paired sides, repeated measure) (Fig. 1). Although overall genotype and dose effects were not significant [genotype, $F(1,74) = 0.003$, n.s.; compartment, $F(4,74) = 1.59$, n.s.], interaction was significant between genotype and dose [$F(4,74) = 2.85$, $P < 0.05$] and among genotype, dose and compartment [$F(4,74) = 4.49$, $P < 0.01$]. Newman–Keuls *post hoc* tests showed that, at 0.2 mg/kg, WT mice showed CPP and *Maoa*-KO mice showed conditioned place aversion (CPA). No significant effect was found at other doses.

Maoa-KO mice show normal levels of blood nicotine and its metabolite cotinine

In order to rule out the possibility that this behavioral phenotype reflects a difference in nicotine metabolism, we determined blood concentrations of nicotine and its metabolite cotinine following an acute injection of 0.2 mg/kg nicotine. WT and *Maoa*-KO mice showed indistinguishable levels of blood nicotine and cotinine [genotype, $F(1,14) = 0.51$, n.s.] (Table 1). The levels of nicotine and cotinine did not differ [$F(1,12) = 2.00$, n.s.], and no interaction was found [$F(1,12) = 1.58$, n.s.]. Newman–Keuls *post hoc* tests showed no difference in either nicotine or cotinine levels between WT and *Maoa*-KO mice.

Maoa-KO mice show reduced preference for oral nicotine

Nicotine preference/aversion ratios were analyzed using a three-way ANOVA, including genotype (WT versus *Maoa*-KO), concentration (0–25 μg/ml) and day (Days 4, 7, 10 and 13, repeated measure) (Fig. 2A). *Maoa*-KO mice showed less overall preference for nicotine than WT mice [genotype, $F(1,81) = 8.32$, $P < 0.01$]. Both groups showed a preference at low concentrations and an aversion at the highest concentration [concentration, $F(4,81) = 22.76$, $P < 0.01$]. Preference/aversion was stable across days [day, $F(3,243) = 2.03$, n.s.]; however, the interaction between concentration and day was significant [$F(12,243) = 2.71$, $P < 0.01$]. No other interaction was significant. Newman–Keuls *post hoc* tests showed that at 12.5 μg/ml, WT mice tended to slightly increase nicotine preference over days, whereas *Maoa*-KO mice did not (Fig. 2A and C).

Maoa-KO mice show reduced oral nicotine consumption

The amount of nicotine intake, as expressed in milligram/kilogram for each 3-day period, was analyzed by a three-way ANOVA, including genotype (WT versus *Maoa*-KO), nicotine concentration (3.125–25 μg/ml), and day (Days 4, 7, 10 and 13, repeated measure) (Fig. 2B and D). Because the homogeneity of variance was found to be violated (Hartley's $F_{max} = 223.36$, $P < 0.01$), data were analyzed following square-root transformation. Mice drank more nicotine at

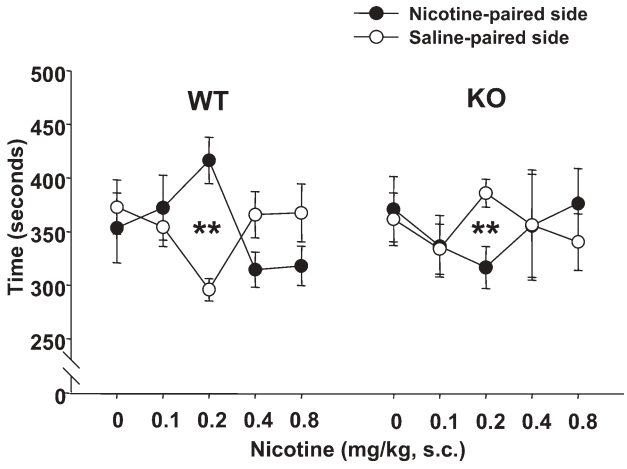

Figure 1. Nicotine-induced CPP in WT littermates and CPA in *Maoa*-KO mice. Time spent in a nicotine-paired compartment and a saline-paired compartment is plotted against nicotine dose. Data are expressed as mean ± SEM. Asterisks indicate a statistically significant difference in time between the two compartments at 1% (**), as determined with the Newman–Keuls *post hoc* test. 0 mg/kg: WT, $n = 8$, KO, $n = 6$; 0.1 mg/kg: WT, $n = 10$, KO, $n = 6$; 0.2 mg/kg: WT, $n = 15$, KO, $n = 6$; 0.4 mg/kg: WT, $n = 10$, KO, $n = 8$; 0.8 mg/kg: WT, $n = 10$, KO, $n = 5$.

Table 1. Blood nicotine and cotinine levels

	WT	*Maoa*-KO	
Nicotine (ng/ml)	28.4 (0.743)	23.6 (1.60)	n.s.
Cotinine (ng/ml)	28.8 (2.35)	29.9 (3.47)	n.s.

Normal levels of blood nicotine and cotinine in *Maoa*-KO mice. Data are expressed as mean (±SEM). Mice were injected with nicotine (0.2 mg/kg, s.c.), and blood samples were obtained 15 min later. WT, $n = 6$; *Maoa*-KO, $n = 8$. Statistical significance was determined with Newman–Keuls *post hoc* tests. n.s., non-significant.

higher concentrations [$F(3,66) = 61.08$, $P < 0.01$] and there was a daily fluctuation [$F(3,198) = 4.05$, $P < 0.01$]. Although an overall genotype effect failed to reach significance [$F(1,66) = 0.97$, n.s.], genotype had a significant interaction with concentration and day [$F(9,198) = 2.18$, $P < 0.05$]. Newman–Keuls *post hoc* tests showed that at 12.5 μg/ml, WT mice tended to slightly increase nicotine intake over days, whereas *Maoa*-KO mice did not (Fig. 2B and D).

Because an altered general fluid intake and body weight could affect the intake data (55), we also analyzed these two parameters (see Supplementary Material, Tables S1–S3). This analysis showed that the reduced nicotine preference and intake at 12.5 μg/ml in *Maoa*-KO mice was not due to an alteration in these parameters.

Maoa-KO mice show normal avoidance of quinine and preference for saccharin

We did not include saccharin or other sweeteners to mask the bitter taste of nicotine, because preference of saccharin itself could also be affected by gene deletion (see cf. 56). Moreover, the robust rewarding effects of saccharin or any natural sweetener overwhelm the subtle rewarding effects of nicotine,

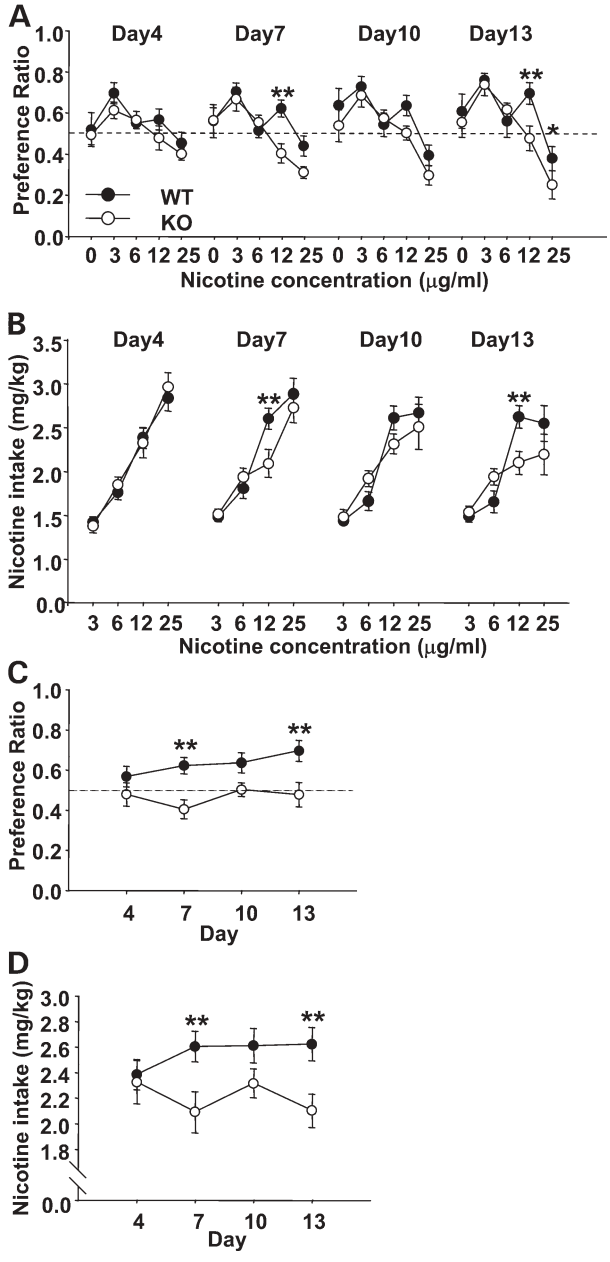

Figure 2. Attenuated nicotine preference (**A**) and intake (**B**) in *Maoa*-KO mice. (A) The ratio was calculated by dividing the amount of nicotine solution intake by the total fluid intake (water and nicotine solution) for each recording period. (B) The amount of nicotine intake, expressed as square-root values of milligram/kilogram over each 3-day period. Daily change in nicotine preference (**C**) and intake (**D**) at 12.5 μg/ml. Data are expressed as mean ± SEM. Ratios higher and lower than 0.5 in (A) and (C) indicate that mice preferred and avoided nicotine solution, respectively, as compared with water. Asterisks indicate a statistically significant difference between WT littermates and *Maoa*-KO mice at 5% (*) and 1% (**), as determined by Newman–Keuls *post hoc* tests. 0.0 μg/ml: WT, $n = 9$ and KO, $n = 8$; 3.125 μg/ml: WT, $n = 7$, KO, $n = 6$; 6.25 μg/ml: WT, $n = 9$, KO, $n = 10$; 12.5 μg/ml: WT, $n = 10$, KO, $n = 10$; 25.0 μg/ml: WT, $n = 12$, KO, $n = 10$.

and inclusion of a sweetener does not increase nicotine preference (57–59). Our procedural modification left open the possibility that *Maoa*-KO mice had a stronger aversion to the bitter taste of the nicotine solution than WT mice,

independent of the rewarding effects of nicotine. We therefore assessed the animals' taste responses to a bitter taste and a sweet taste by presenting quinine and saccharin, respectively, in a two-bottle choice test (Fig. 3). WT and *Maoa*-KO mice equally avoided quinine [genotype, $F(1,28) = 0.04$, n.s.; concentration, $F(1,28) = 48.93$, $P < 0.01$] and preferred saccharin [genotype, $F(1,27) = 0.13$, n.s.; concentration, $F(1,27) = 18.06$, $P < 0.01$] in a concentration-dependent manner.

Maoa-KO mice are normal in initial hyperactivity but exhibit delayed locomotor habituation in an inescapable open field

Locomotor activity was examined as a behavioral response in a novel, inescapable open field. Data were analyzed using a three-way ANOVA, including genotype (WT versus *Maoa*-KO), day (Days 1–3, repeated measure), and time interval (5–30 min, repeated measure). *Maoa*-KO mice showed higher levels of locomotor activity overall than WT mice [$F(1,17) = 6.77$, $P < 0.05$] (Fig. 4A). Locomotor activity decreased significantly across days [$F(2,34) = 9.02$, $P < 0.01$] and across time intervals [$F(5,85) = 18.58$, $P < 0.01$]. Interaction was significant between day and time only [$F(10,170) = 7.89$, $P < 0.01$]. The 3-way interaction was not significant [$F(1,170) = 0.61$, n.s.]. Newman–Keuls *post hoc* tests showed that WT and *Maoa*-KO mice had equal levels of locomotor activity for the first 5 min after they were placed in the open field each day, but *Maoa*-KO mice showed delayed habituation.

Data were further analyzed for locomotor activity in the center and the margin areas of the open field. *Maoa*-KO mice showed higher levels of activity in the center area than WT mice [genotype, ($F(1,17) = 5.89$, $P < 0.05$] (Fig. 4B). Locomotor activity fluctuated across time within a day [$F(5,85) = 2.89$, $P < 0.05$], but not across days [$F(2,34) = 2.33$, n.s.]. Genotype had no interaction with other factors. Interaction was found between day and time only [$F(10,170) = 4.41$, $P < 0.01$]. Newman–Keuls *post hoc* tests showed that the genotype effect mainly reflected higher locomotor activity in *Maoa*-KO mice at 10 and 30 min on Day 1 and at 30 min on Day 2.

WT mice and *Maoa*-KO mice showed indistinguishable levels of locomotor activity in the margin area [$F(1,17) = 1.77$, n.s.] (Fig. 4C). Locomotor activity declined across days [$F(2,34) = 5.69$, $P < 0.01$] and across time [$F(5,85) = 46.96$, $P < 0.01$]. Genotype had a significant interaction with time [$F(5,85) = 2.56$, $P < 0.05$] and with day and time [$F(10,170) = 3.11$, $P < 0.01$]. Newman–Keuls *post hoc* tests showed that at 20 min on Day 1, *Maoa*-KO mice traveled more than WT mice (Fig. 4C). Otherwise, no significant difference was observed between *Maoa*-KO mice and WT mice.

These analyses showed that the phenotypic difference observed in total distance traveled in the entire open field depended on the difference in locomotor activity in the center area more than in the margin area although both genotypes equally traveled two to three times more in the margin than in the center. Given that the center and margin areas are of almost the same size (324 versus 352 cm^2), their avoidance of the center area is evident.

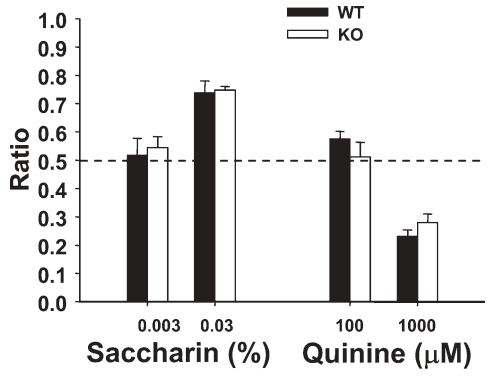

Figure 3. Normal saccharin preference and quinine avoidance in *Maoa*-KO mice. Data are expressed as mean ± SEM. Saccharin, 0.003% (30 μg/ml): WT, $n = 10$, KO, $n = 9$; 0.03% (300 μg/ml): WT, $n = 6$, KO, $n = 6$; quinine, 100 μM: WT, $n = 10$, KO, $n = 10$; 1000 μM: WT, $n = 6$, KO, $n = 6$. Ratios higher and lower than 0.5 indicate preference and avoidance, respectively.

Maoa-KO mice show normal preference for novelty

Time spent in a novel home cage versus a habituated home cage was used as an index of approach behavior towards a novel environment. Data were analyzed using a two-way ANOVA, including genotype (WT versus *Maoa*-KO) and cage (novel cage versus familiar cage, repeated measure). WT mice and *Maoa*-KO mice both preferred the novel cage to the familiar cage [cage, $F(1,37) = 13.16$, $P < 0.01$], and the levels of preference for the novel cage did not differ between the two genotypes [genotype, $F(1,37) = 0.90$, n.s.] (Fig. 5). No interaction was found between genotype and cage [$F(1,37) = 0.34$, n.s.]. Newman–Keuls *post hoc* tests showed that both WT and *Maoa*-KO mice showed a significant preference for a novel compartment.

DISCUSSION

The present study shows that a constitutive deficiency of MAOA is associated with a reversal from nicotine preference to aversion in the CPP and an attenuation of oral nicotine preference. These behavioral effects were not due to abnormalities in the metabolism of nicotine, taste sensation, fluid intake or body weight. Concomitant with the altered behavioral effects of nicotine, *Maoa*-KO mice showed delayed habituation in locomotor activity in an inescapable open field, but were normal in initial responses to novel stimuli in the open field and in a choice preference for a novel environment. Together with our previous observation that a constitutive deficiency of MAOB, the other monoamine oxidase isoenzyme, does not affect oral nicotine intake (60), the present study suggests a selective role for MAOA in nicotine reward.

Our congenic *Maoa*-KO mice and WT littermates have the genetic background of C3H/HeNTac and, to a much lesser extent, of C3H/HeOuJ (see Materials and Methods, Animals). Although both C3H/He inbred mouse lines are homozygous for a recessive gene for retinal degeneration (61), which affects behavior guided solely by pattern vision (62), these mice nevertheless respond to light and show

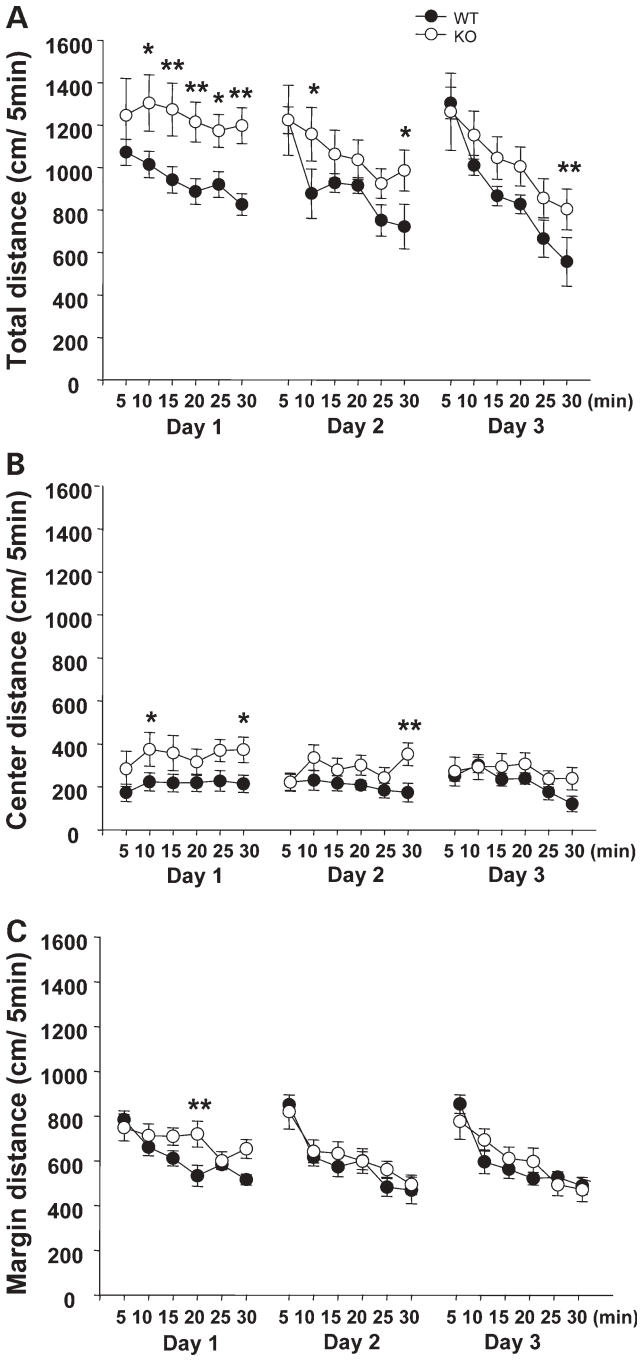

Figure 4. Delayed habituation in an inescapable open field in *Maoa*-KO mice. Distance traveled in the entire 676 cm^2 field (**A**), in the center area (**B**) and in the margin area (**C**) is shown. Data are expressed as mean ± SEM. * and ** indicate $P < 0.05$ and $P < 0.01$ (Newman–Keuls *post hoc* test). WT, $n = 10$; *Maoa*-KO, $n = 9$.

light-guided behaviors (63). It is unlikely that this sensory defect caused the phenotypic differences between WT and *Maoa*-KO mice, because WT and *Maoa-KO* mice are equally affected by this sensory defect. WT and *Maoa*-KO mice showed CPP and CPA, respectively, probably because our CPP apparatus also included tactile cues (see Materials and Methods). Similarly, WT and KO mice probably

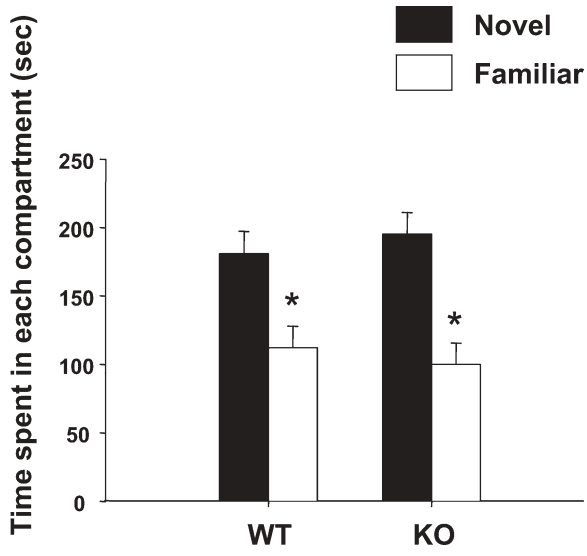

Figure 5. Normal preference for a novel home cage over a habituated home cage in *Maoa*-KO mice. Data are expressed as mean ± SEM. Asterisks indicate a statistically significant difference in time spent in the novel and habituated (i.e. familiar) home cages, as determined with the Newman–Keuls *post hoc* test. WT littermates, $n = 21$; *Maoa*-KO, $n = 18$.

developed preference/aversion to nicotine solution, a novel cage and the margin area of the open field primarily using sensory cues other than vision.

Maoa and nicotine reward

An acute systemic injection of nicotine (0.2 mg/kg, s.c., 15 min) yielded blood concentrations of 28.4 and 23.6 ng/ml in WT and *Maoa*-KO mice, respectively. WT and *Maoa*-KO mice consumed up to 8 mg/kg/3 days (i.e. 2.7 mg/kg/day). In mice drinking 60 mg/kg/day of nicotine, nicotine concentrations are maintained around 114 ng/ml in the blood and 300 ng/g in the brain (64,65). It is thus estimated that WT and *Maoa*-KO mice maintained ~5 ng/ml blood nicotine during oral intake. These blood nicotine concentrations are within the range seen in smokers (66–68).

Nicotine induced a CPP in WT mice and a CPA in *Maoa*-KO mice at a single dose (i.e. 0.2 mg/kg), but not at lower or higher doses. This is consistent with other studies that demonstrated that nicotine induces CPPs within an extremely narrow dose range in mice. The effective free-base doses of nicotine in mice are reported to range from 0.175 to 0.35 mg/kg in most studies; lower or higher doses are generally ineffective (69–73). The effective mouse doses also fall into the effective dose range for rats of 0.1–1.0 mg/kg (74). A similarly narrow and shallow dose–response curve has also been reported for intravenous nicotine self-administration in rats (75). Consistent with these data, WT mice showed oral nicotine preference within a narrow concentration range (i.e. 3–12.5 μg/ml, see Fig. 2A). This narrow effective dose seems to mimic the phenomenon in which smokers try to maintain a certain narrow target nicotine concentration (76).

MAOA deficiency due to a premature stop codon in exon 8 is associated with borderline mental retardation in men (28), and a learning deficit could have impaired the formation of CPP in

Maoa-KO mice. However, MAOA deficiency has no effect on motor learning and actually increases several types of conditioned fear behaviors in mice (77). Because *Maoa*-KO mice did show a robust CPA, it is unlikely that the absence of CPP in *Maoa*-KO mice reflects a generalized learning deficit.

As nicotine exerts both rewarding and aversive effects (74), the reversal from nicotine reward to aversion in CPP could result from either enhanced aversion or a combination of reduced reward and enhanced aversion. It could be that the constitutive MAOA deficiency results in CPA in *Maoa*-KO mice by enhancing associative learning between some aversive effects of nicotine and environmental cues (77). Alternatively, the constitutive MAOA deficiency might developmentally alter a neuronal system that evaluates the affective valence of nicotine. More work is needed to determine the neural mechanisms through which CPP is reversed to CPA in *Maoa*-KO mice. Regardless of the exact mode of action, MAOA deficiency reversed the net effects of nicotine from reward to aversion in the CPP. Manipulation of other single genes or pharmacological blockade of the neuronal acetylcholine receptor results in similar reversals from CPP to CPA or vice versa at single doses of nicotine, cocaine and fluoxetine (78–80). The reduction in nicotine reward in *Maoa*-KO mice could be one of the reasons why an MAOA inhibitor facilitates smoking cessation (81).

Constitutive inactivation of single genes is likely to variably impact many distinct aspects of addiction (82–84). The CPP paradigm utilizes an association formed between environmental cues and the rewarding effects of a drug and assesses how drug-associated cues induce approach on a drug-free test day (85–87). As drug-associated cues are a potent instigator of relapse in humans (88), MAOA might contribute to behavioral relapse in nicotine addicts.

The constitutive alteration of MAOA, as seen in humans as well as our mouse model, is likely to alter the susceptibility to nicotine addiction by secondarily affecting many related molecules and systems throughout development. This action of genetic alteration is likely to be distinct from pharmacological inhibition of MAOA and MAOB during adulthood. In fact, studies have shown that simultaneous, irreversible inhibition of both MAOA and MAOB by tranylcypromine or phenelzine increased nicotine self-administration in rats (89,90). Moreover, when given together, clorgyline, an irreversible MAOA inhibitor, and selegiline, an irreversible MAOB inhibitor, enable nicotine to increase locomotor activity in mice, although neither drug alone is effective (90). Similarly, tranylcypromine prolongs nicotine-sensitized locomotor activity in rats (91). It should be noted that the selective inactivation of the *Maoa* gene and the pharmacological inhibition of MAOA by clorgyline induces many different and often opposite effects on various behaviors (92). Caution is needed in comparing the effects of constitutive genetic inactivation of *Maoa* and pharmacological inhibition of MAOA and MAOB on behavior. Although constitutive MAOA abnormalities are likely to developmentally alter many related molecules and result in compensatory alterations in humans and mice, many MAOA/B and MAOA inhibitors exert diverse actions other than MAO inhibition. The non-selective MAOA/B inhibitor tranylcypromine inhibits CYP2A6, the principle enzyme responsible for metabolizing nicotine into cotinine (54). Clorgyline and tranylcypromine inhibit monoamine uptake in various brain regions (47–50). Because nicotine-induced dopamine release in the striatum is significantly potentiated by the inhibition of dopamine uptake (93), this action might affect nicotine's behavioral effects. Clorgyline also binds to the σ opioid receptor (51–53). This effect poses an interpretative problem, as a σ opioid receptor agonist blocks the acquisition of nicotine-induced CPP (94).

Our data are consistent with human studies that demonstrate a correlation between high-activity alleles of *MAOA* and higher levels of nicotine addiction (32–34). Our previous study showed that the constitutive inactivation of MAOB did not alter oral nicotine intake or preference in mice (60). *MAOB* polymorphisms are not correlated with smoking risks in humans (95,96). These observations suggest a rather specific role for MAOA in nicotine addiction in mice and humans. Because low-activity *MAOA* alleles in humans and the absence of MAOA in mice are correlated with lower levels of smoking and nicotine preference, respectively, increased levels of serotonin or norepinephrine, which are caused by reduced MAOA activity in both humans and mice, might mediate this association. More work is needed to ascertain the neurochemical basis for the conversion of nicotine reward to aversion in *Maoa*-KO mice.

Maoa and novelty responses

The constitutive deficiency of MAOA did not affect the animals' locomotor activity for the first 5 min in a novel, inescapable open field. Moreover, *Maoa*-KO mice and WT mice had indistinguishable levels of preference for a novel compartment in a two-compartment novelty test. Together with our previous observation that *Maoa*-KO mice show normal motor activity in an open field (97), these results suggest that a constitutive MAOA deficiency does not alter an animal's reaction to novel stimuli. Consistent with this interpretation, there is no correlation between high-activity alleles of *MAOA* and novelty seeking or related traits in humans (37–42).

Because *Maoa*-KO mice and WT mice differed in the rate of decline in locomotor activity at subsequent time points on Day 1, MAOA is likely to contribute to locomotor habituation in an inescapable open field. Taken together, our results suggest that distinct genetic bases exist for an initial locomotor response and subsequent habituation in a novel environment. Delayed habituation in an inescapable open field, as well as a high level of initial locomotor response, has been correlated with a higher rate of self-administration of nicotine and other addictive substances (98,99). However, our data did not support this correlation at a single gene level: delayed habituation in an inescapable open field was correlated with *reduced* CPP and oral intake. What then are the properties reflected in high levels of locomotor activity or delayed habituation that is correlated with increased nicotine self-administration? It has been suggested that hyperactivity in an open field might be correlated with an animal's ability to acquire motor learning rather than the rewarding and reinforcing effects of drugs (100). Because CPP and oral intake are not dependent on motor learning, we might have failed to see a positive correlation between nicotine reward in our tasks and locomotor activity in an open field.

Our study suggests that variation in MAOA activity is likely to alter the impact of nicotine reward and possibly the degree of nicotine addiction, providing an example of a gene affecting addiction susceptibility without influencing one of its comorbid behavioral traits (i.e. novelty response). As how genetic variations influence addiction susceptibility and comorbid motivational traits is likely to be complex (2), our finding does not rule out the possibility that other genes concomitantly contribute to both nicotine addiction and novelty responses. Nor does it rule out the possibility that MAOA also contributes to traits other than novelty responses. Because pharmacological MAOA inhibition also reduces the behavioral effects of morphine and cocaine (101,102), more work is needed to assess the general role played by MAOA in other forms of addiction and behavioral traits other than novelty responses.

MATERIALS AND METHODS

Animals

We used age-matched, male *Maoa*-KO mice and WT littermates at the age of 2–4 months. An insertional deletion in the *Maoa* locus occurred following the injection of an IFN-β minicassette into a one-cell embryo of the C3H/HeOuJ inbred strain of mice, thereby providing *Maoa* inactivation against a coisogenic genetic background (103). Exons 2 and 3 were replaced by an IFN-β transgene that is silenced by methylation in brain tissues. The mice were later backcrossed to C3H/HeNTac mice for more than 10 generations, providing a congenic C3H/HeNTac background. This congenic mouse line is expected to have few allelic differences between WT and *Maoa*-KO mice, as the flanking and nonflanking alleles were derived from C3H/HeOuJ and C3H/HeNTac, respectively, and few allelic differences are expected between C3H/He substrains (104).

The genotypes of the mice were determined using tail tissues at the age of 10 days. We used two sets of PCR primers for genotyping: (1) CTC AGA AGT CGG ATC TGA and CAG TAG ATT CAC TAC CAG and (2) GAT TCT CTC CTA TTG TCT and AAA GAC AGT TGT GAA GCC. These primers were designed to identify the presence of the inserted transgene.

Maoa-KO and WT mice were housed individually in their home cages (28 cm × 17 cm × 12 cm) at the time of weaning to prevent stress associated with the frequent fighting initiated by *Maoa*-KO mice (103). They were maintained on a 14 h light/10 h dark cycle with light from 06:00 to 20:00 and had free access to food and water unless described otherwise. All studies were carried out in accordance with the *Guide for Care and Use of Laboratory Animals* of the Albert Einstein College of Medicine.

Drugs

(−)-Nicotine hydrogen tartrate salt (Sigma, St Louis, MO, USA) was used for injection in the CPP test. (−)-Nicotine (+)-bitartrate salt (99% liquid, 1.01 g/ml, Sigma) was dissolved in water for the oral intake test. In both cases, the doses and concentrations are expressed as those of the free base.

Behavioral analysis

Conditioned place preference/aversion. The apparatus used was a rectangular Plexiglas box composed of three distinct compartments. Two large compartments (24.5 cm × 18 cm × 33 cm) had distinguishable visual and tactile cues: one compartment had black-and-white striped walls and a wire mesh floor with 2.1 mm × 2.1 mm openings and was lit at 5.6 lux; the other compartment had gray walls and a wire mesh floor with 3.7 mm × 3.7 mm openings and was lit at 3.66 lux. These two large compartments were separated by a central compartment (13 cm × 18 cm × 33 cm). Each large compartment was divided from the center compartment by a guillotine door (18 cm × 37 cm).

Experimentally naive mice (WT, $n = 8–15$ per dose; *Maoa*-KO, $n = 5–8$ per dose) were used for this test. The experiment included three sessions. During the first session (Day 1), the guillotine doors were opened 5 cm above the floor and the mice were allowed to explore the three compartments freely for 15 min. On a group basis, neither *Maoa*-KO mice nor WT littermates showed a bias to either of the two large compartments [genotype, $F(1,112) = 0.0001$, n.s.; compartment, $F(1,112) = 3.48$, n.s.], thereby establishing our procedure as an unbiased paradigm. During the second session (Day 2), two pairings were given at least 5 h apart. The guillotine doors were closed and mice were confined to either of the two large compartments for 30 min immediately following saline or nicotine administration (0, 0.1, 0.2, 0.4 or 0.8 mg/kg, s.c.); the order of nicotine and saline injections and the compartment of confinement were counterbalanced, so that the number of mice that received nicotine in each compartment in either the morning or afternoon was approximately equal. The behavioral phenotype at 0.2 mg/kg (Fig. 1) was not affected by whether nicotine was given in the morning or afternoon [$F(1,17) = 0.25$, n.s.] or whether nicotine was paired with one of the two large compartments or the other [$F(1,17) = 1.61$, n.s.]. During the third session (Day 3), the guillotine doors were opened 5 cm above the floor. Each mouse was placed in the central chamber and was allowed to move freely in the three chambers for 15 min. A rater blinded to genotype and treatment recorded the time animals spent in the previously nicotine- and saline-paired compartments as an index of CPP or CPA.

Plasma nicotine/cotinine assay. WT and *Maoa*-KO mice (WT, $n = 6$; *Maoa*-KO, $n = 8$) received a single s.c. injection of 0.2 mg/kg nicotine, the dose at which WT and *Maoa*-KO mice differed in the CPP/CPA test. We did not use oral nicotine intake for this analysis, because the time and amount of nicotine intake in relation to the time of sacrifice cannot be controlled. Blood was taken from the retro-orbital artery 15 min after injection and mixed with ethylenediaminetetraacetic acid (EDTA) (0.9 mg EDTA/0.5 ml blood, Sigma). Nicotine concentrations peak at this time point (105). The samples were then centrifuged at 3000 rpm for 10 min at room temperature, and supernatants were used as plasma samples. Capillary gas chromatography with nitrogen–phosphorus detection was used to determine the concentrations of nicotine and its major metabolite, cotinine (see 105 for details).

Oral nicotine intake. Separate groups of experimentally naive mice (WT, $n = 7–12$ per concentration; *Maoa*-KO,

$n = 6–10$ per concentration) were used for this analysis. We followed our standard oral administration procedure (60,105) with a slight modification: from the time the mice were separated from the parents until the behavioral analysis, a single water bottle was placed alternately on the right and left side of the cage top every 3 days to prevent the development of a position preference for drinking. Food was available *ad libitum*. At the onset of experiment, two bottles were provided in each cage, one containing (−)-nicotine (1.01 g/ml unit; final concentrations were 0, 3.125, 6.25, 12.5 or 25 μg/ml) in tap water, and the other containing tap water only. Nicotine in an alkaline medium is readily absorbed through the mucous membrane (67,106), and nicotine consumed orally accumulates in the mouse brain and exerts many physiological effects there (64,65,107–110). The nicotine bottle was always placed on the right side and the water bottle was placed on the left side. Each animal was given a single concentration of nicotine. We did not switch the nicotine bottle position or give different concentrations to the same animals, as data so collected would be confounded by the animal's ability to switch reinforced behaviors (see 60,105 for details) and the rate of sensitization or tolerance to nicotine intake and preference over days. For the 0 μg/ml concentration, mice received two bottles of water. WT and *Maoa*-KO mice showed equal preference for both sides for water drinking, confirming that pre-test switching of a single water bottle broke any position preference. The weight of each bottle, as well as body weight, was assessed and fresh nicotine solution and water were given between 10:00 AM and 11:00 AM every 3 days for a total of 13 days. Thus, data were recorded on four recording days (Days 4, 7, 10 and 13). Bottle weight has been shown to be a reliable measure of fluid intake (60,105,111). Nicotine preference or aversion was expressed as a ratio of the fluid intake from the nicotine bottle divided by the total fluid intake from the nicotine bottle and the water bottle. For the 0 μg/ml concentration, a ratio was calculated by dividing water intake from the water bottle on the right side divided by the total water intake from both sides. Nicotine consumption was expressed as nicotine intake per body weight over each 3-day period (mg/kg).

Taste preference/aversion. Experimentally naive mice received one bottle containing water and another bottle containing a solution of either saccharin (0.003%, 30 μg/ml: WT, $n = 10$; *Maoa*-KO, $n = 9$; 0.03%, 300 μg/ml: WT, $n = 6$; *Maoa*-KO, $n = 6$; Acros Organics, Fairlawn, NJ, USA) or quinine hemisulfate salt (100 μM: WT, $n = 10$; *Maoa*-KO, $n = 10$; 1000 μM: WT, $n = 6$; *Maoa*-KO, $n = 6$; Sigma). The amount of solution consumed from each bottle was measured on Day 4. As in nicotine drinking, the bottle containing saccharin or quinine was placed on the right side and a water bottle was placed on the left side. Taste preference or aversion was expressed as the ratio of fluid intake from the saccharin or quinine bottle to the total fluid intake from both the saccharin or quinine bottle and the water bottle.

Novel, inescapable open field. We tested experimentally naive WT mice ($n = 10$) and *Maoa*-KO mice ($n = 9$) in four sets of automated activity apparatuses made from transparent Plexiglas (26 cm × 26 cm × 38.5 cm, Truscan, Coulbourn Instruments, Allentown, PA, USA). This apparatus detects horizontal activity through a set of beams located 1.5 cm above the 676 cm^2 floor. Each side had 16 beams, dividing the open field into 289 squares (1.52 cm × 1.52 cm). The center of the apparatus is defined as the center area of 18 cm × 18 cm (324 cm^2). The margin of the apparatus (352 cm^2) is defined as the 4 cm-wide area between the center and the walls. The apparatus had 97 lux illumination in the center of the arena from the fluorescent light on the ceiling of the room.

Horizontal locomotor activity was measured as an index of locomotor activity for 30 min per day between 10:00 AM and 11:00 AM for 3 days. Mice were brought to a room adjacent to the test room at least 20 min prior to the beginning of testing each day. The apparatus was cleaned with 70% ethanol and rinsed with water after each session to remove any residual olfactory cues. The distance traveled was used as a measure of locomotor activity.

Novelty place preference. There is a controversy as to whether locomotor activity in a novel, inescapable open field reflects an animal's response to novel stimuli or stress/anxiety (60). To minimize the stress/anxiety factor, we used another task to measure novelty exploration. The apparatus was composed of two home cages (28 cm × 17 cm × 12 cm), connected side-by-side. An opaque partition was placed between the cages so that a mouse in one cage could not see the other cage. A removable opaque door (10 cm × 11 cm) was placed in the gate between the two cages. Each cage had regular bedding, water and food *ad libitum*.

We used experimentally naive WT mice ($n = 21$) and *Maoa*-KO mice ($n = 18$). On the first day, the mouse was placed in one of the two cages and housed there overnight. The gate was removed 24 h later and the mouse was allowed to explore the two cages. Because this paradigm includes a choice, it is likely to involve less stress than an inescapable open field (112,113). The only difference between the two cages was whether the mouse had been habituated or not, and the lack of habituation defines novelty in this paradigm. A rater blinded to genotype measured the time that each mouse spent in the habituated and novel cages for 5 min. More time spent in the novel cage relative to the habituated cage was defined as a preference for novel place.

Statistical analysis. Data were analyzed by ANOVA followed by the Newman–Keuls *post hoc* test. When the homogeneity of variance was violated, data were transformed into square-root values. The minimal threshold for significance was set at 5%. For additional multiple ANOVAs, the significance threshold was adjusted by Bonferroni's correction.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at HMG Online.

ACKNOWLEDGEMENTS

This work was supported by funds from the Department of Psychiatry and Behavioral Sciences and from the Program in

Human Genetics, Albert Einstein College of Medicine to N.H.; by funds from the Bronx Psychiatric Center to S.A. and by funds from the NIH (R37MH39085, Merit Award; RO1MH67968) and from Boyd and Elsie Welin Professorship to J.C.S. We thank Dr Neal Benowitz of the Clinical Pharmacology Laboratory at San Francisco General Hospital, University of California San Francisco for analysis of blood nicotine/cotinine and the late T. Klein for support and encouragement.

Conflict of Interest statement. None declared.

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