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Behavioral Disinhibition and Reduced Anxiety-like Behaviors in Monoamine Oxidase B-Deficient Mice

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Monoamine oxidase (MAO) B catalyzes the degradation of β-phenylethylamine (PEA), a trace amine neurotransmitter implicated in mood regulation. Although several studies have shown an association between low MAO B activity in platelets and behavioral disinhibition in humans, the nature of this relation remains undefined. To investigate the impact of MAO B deficiency on the emotional responses elicited by environmental cues, we tested MAO B knockout (KO) mice in a set of behavioral assays capturing different aspects of anxiety-related manifestations, such as the elevated plus maze, defensive withdrawal, marble burying, and hole board. Furthermore, MAO B KO mice were evaluated for their exploratory patterns in response to unfamiliar objects and risk-taking behaviors. In comparison with their wild-type (WT) littermates, MAO B KO mice exhibited significantly lower anxiety-like responses and shorter latency to engage in risk-taking behaviors and exploration of unfamiliar objects. To determine the neurobiological bases of the behavioral differences between WT and MAO B KO mice, we measured the brain-regional levels of PEA in both genotypes. Although PEA levels were significantly higher in all brain regions of MAO B KO in comparison with WT mice, the most remarkable increments were observed in the striatum and prefrontal cortex, two key regions for the regulation of behavioral disinhibition. However, no significant differences in transcript levels of PEA's selective receptor, trace amine-associated receptor 1 (TAAR1), were detected in either region. Taken together, these results suggest that MAO B deficiency may lead to behavioral disinhibition and decreased anxiety-like responses partially through regional increases of PEA levels.

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INTRODUCTION

Monoamine oxidase (MAO) (EC 1.4.3.4) is the key enzyme catalyzing the oxidative deamination of monoamine neurotransmitters in the central nervous system. Although the two MAO isoenzymes, A and B, share about 70% homology and follow the same kinetic mechanism (Bortolato *et al*, 2008; Shih *et al*, 1999a), they exhibit significant differences in substrate and inhibitor specificities: whereas MAO A displays high affinity for serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE), and is inhibited by low doses of clorgyline, MAO B prefers β-phenylethylamine (PEA) and is selectively inhibited by small concentrations of *l*-deprenyl (Bortolato *et al*, 2008; Shih *et al*, 1999a). This neurochemical divergence implies that the two MAO isoenzymes likely exert different functions in the organization of brain activity and neurophysiological processes. Their specific roles in behavioral regulation, however, remain partially elusive. A powerful tool to elucidate the influence of MAO A and MAO B on behavior is afforded by mice carrying genetic knockout (KO) of either enzyme (Cases *et al*, 1995; Chen *et al*, 2004; Grimsby *et al*, 1997; Scott *et al*, 2008).

Monoamine oxidase A KO mice have been the focus of extensive phenotypic characterization (Bortolato *et al*, 2008; Shih *et al*, 1999a). Among other features, these mutants have high brain levels of 5-HT, DA, and NE and exhibit a rather complex behavioral phenotype, including impulsive aggression, low exploratory activity, and greater retention of aversive memories (Cases *et al*, 1995; Kim *et al*, 1997; Popova *et al*, 2001; Shih *et al*, 1999b). This set of behavioral abnormalities strikingly mirrors the nosographic characteristics of Brunner syndrome, a genetic disorder induced by a nonsense mutation of MAO A gene and featuring antisocial conduct and mental retardation (Brunner *et al*, 1993).

In contrast, the neurobiological and behavioral implications of MAO B deficiency remain poorly understood. MAO B KO mice display high brain levels of PEA, but not 5-HT, DA, or NE. PEA has been implicated in the regulation of emotional responses, including exploratory activity, arousal, and behavioral reinforcement (Sabelli and Javaid,

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1995). Recently, several lines of investigations have ascertained that some of the actions of PEA are mediated by the activation of trace amine-associated receptor 1 (TAAR1) (Borowsky *et al*, 2001; Lindemann and Hoener, 2005), which has been implicated in the regulation of DA signaling in the striatum (Lindemann *et al*, 2008; Wolinsky *et al*, 2007; Xie and Miller, 2009).

In keeping with these premises, MAO B KO mice exhibit decreases in behavioral parameters reflective of stress susceptibility (Bohus *et al*, 1987; Korte *et al*, 1996; Louvart *et al*, 2005), such as forced-swim immobility and locomotor habituation (Grimsby *et al*, 1997; Lee *et al*, 2004). Low MAO B platelet activity in humans has been consistently correlated with extraversion and novelty-seeking traits, yet a causal relationship between the two phenomena has not been established (Oreland, 1993).

This scenario suggests that MAO B deficiency may result in behavioral disinhibition, a temperamental tendency characterized by novelty- and sensation-seeking personality and negligence of potential or actual dangers (Iacono *et al*, 2003). To verify this possibility, in this study we analyzed the behavioral performances enacted by MAO B KO mice in a number of models exploring different facets of responsiveness to contextual stimuli, including anxiety-like responses, exploratory activity, and risk-taking behaviors.

MATERIALS AND METHODS

Animals

We used 4–5 months old, experimentally naïve male 129/Sv mice ($n = 166$; 83/genotype), weighing 30–35 g. MAO B KO mice and wild-type (WT) littermates were generated as previously described (Grimsby *et al*, 1997). Animals were housed in group cages with *ad libitum* access to food and water. The room was maintained at 22°C, on a 12 h : 12 h light/dark cycle, with lights off at 0600 hours. Before behavioral testing, all animals were found to display equivalent physical and neurological characteristics. All experimental procedures were in compliance with the National Institute of Health guidelines and approved by the University of Southern California Animal Use Committees. To avoid potential carryover effects, each animal was used only once throughout the study. Litter effects were minimized by using mice from at least three different litters in each behavioral test.

Elevated Plus Maze

The test was performed as previously described (Wall and Messier, 2000), under either dim (10 lux) or bright (300 lux) environmental light. Briefly, the apparatus was made from black Plexiglas with a light gray floor and consisted of two open (25 × 5 cm) and two closed arms (25 × 5 × 5 cm), which extended from a central platform (5 × 5 cm) at 60 cm from the ground. Mice ($n = 17$/genotype) were individually placed on the central platform facing an open arm, and their behavior was observed for 5 min by an experimenter unaware of the genotype. An arm entry was counted when all the four paws were inside the arm. Behavioral measures included: time spent and entries into each partition of the elevated plus maze, number of fecal boli.

Defensive Withdrawal

We used a variation of the protocol described by Bortolato *et al* (2006). Mice (WT = 7; MAO B KO = 10) were individually placed inside a cylindrical aluminum chamber (7 cm diameter × 11 cm length) located along one of the four walls of a dimly lit (10 lux) black Plexiglas open field (40 × 40 × 40 cm), with the open end facing the center. Mice were allowed to freely explore the environment for 15 min. Behaviors were recorded and monitored by an observer unaware of the genotype. Behavioral measures included: latency to exit the chamber; transitions between the chamber and open field; time spent in the chamber; head pokes out of the chamber; crossings (on a 4 × 4 square grid superimposed onto the video image of the open field); and velocity (ratio of crossings to time spent in the open field).

Marble Burying

Testing was performed using a modification of the methods described by Hirano *et al* (2005). Mice (WT = 20; MAO B KO = 13) were individually placed in dimly lit (10 lux) Makrolon cages (35 × 28 cm), with 5 cm of fine sawdust, for a 30-min acclimatization period. Subsequently, animals were briefly removed and 20 marbles (1 cm diameter) were placed in each cage, on top of the sawdust. Mice were then returned to the cages, and their behavior was video recorded for the following 30 min. Measures included the number of buried marbles, and the number and total duration of digging bouts. A marble was considered buried if at least two-thirds of its surface area was covered in sawdust. General activity was analyzed by counting the crossings of a grid (5 × 4 squares), as described above.

Hole Board

We used a gray Plexiglas platform (40 × 40 cm) raised to a height of 15 cm from the floor of a transparent Plexiglas box (40 × 40 × 40 cm) in a dimly lit room (10 lux). The platform consisted of 16 equivalent square compartments (12 peripheral and 4 central), each featuring a central circular hole (2.5 cm diameter). Mice (WT = 8; MAO B KO = 12) were individually placed in the center and their behavior was recorded for 6 min. Behaviors were measured diachronically in 2-min intervals, and included the number of crossings between compartments, and the time spent and number of head pokes in the peripheral and central compartments.

Novel Object Exploration

We used a modified version of the protocol described by Bortolato *et al* (2009). Mice (WT = 7, MAO B KO = 8) were individually acclimatized to a dimly lit (10 lux) gray Plexiglas cubic box (20 × 20 × 20 cm) for 15 min. Twenty-four hours later, animals were exposed to two novel black plastic cylinders (8 cm tall × 3.5 cm in diameter), affixed to the floor and symmetrically placed at 6 cm from the two nearest walls. Mice were placed in a corner, facing the center and at equal distance from the two objects. Their start position was rotated and counterbalanced for each genotype throughout the test. Behaviors were videotaped for 15 min.

Analysis included number and total duration of exploratory approaches, latency to the first exploration, and the number of crossings (measured as described in the Defensive Withdrawal section). Exploration was defined as sniffing or touching either of the two objects with the snout; sitting on the object was not considered exploration. The time spent in the central 4 squares and the object areas (defined as a 1.75 cm-wide annulus concentric to the cylinders) was also measured.

Novelty-Induced Grooming

Mice (WT = 8, MAO B KO = 7) were placed in a dimly lit (10 lux) Makrolon cage (35 × 28 cm) for 30 min. After their removal, five voluminous objects (different for size, shape, and color) were attached to the bottom of one-half of the cage (object area). Animals were returned to the empty half of the cage and left undisturbed for 20 min. Time spent in the object area and grooming bouts and duration were recorded and analyzed by an observer unaware of the genotype.

Wire-Beam Bridge

The apparatus consisted of a 30-cm high Plexiglas platform and a 50-cm high Plexiglas wall, oppositely placed at 30 cm distance. The platform was surmounted by an 8-cm deep enclosure, with a square (13 × 13 cm) opening facing the wall and placed right above the edge of the platform. Following 24 h of isolation and food deprivation, mice (WT = 6; MAO B KO = 5) were individually placed in the enclosure, under dim (10 lux) light conditions, and returned to their cage after 10 min. The edge of the platform and the wall were then connected by a horizontal, unrailed bridge (1.25 × 30 cm), made in black aluminum wire, consisting of two parallel beams (thickness: 0.01 cm) perpendicularly connected by 24 equally distanced cross-ties (1.25 cm long). The bridge was moderately flexible with a downward deflection of 1 cm per 100-g load at the center point. A circular plastic dish (6 cm in diameter) containing six food pellets (approximately 20 g) was attached onto the end of the bridge adjacent to the wall. Mice were placed in the enclosure and their behavior was recorded for 10 min. Behavioral measures included the latencies to access the bridge (with all four paws on it) and to reach the food, as well as the sniffing frequency (calculated as the ratio between the sniffing approaches to the bridge before the actual access to it and the latency to access the bridge).

PEA Level Determination

β-Phenylethylamine levels were determined as indicated by Grimsby *et al* (1997). Briefly, brain regions of WT ($n = 6$) and MAO B KO ($n = 7$) mice were identified with a stereotaxic atlas (Franklin and Paxinos, 1997), excised and homogenized in 0.5 N perchloric acid solution with [H^2]PEA as an internal standard. PEA was extracted with diethyl ether, derivatized with pentafluoropropionic anhydride and analyzed using a gas chromatograph directly interfaced with a mass-selective detector (Hewlett-Packard, Palo Alto, CA). Base peaks at 104 and 107 *m/z* were used for detection of PEA and the internal standard, respectively.

TAAR1 Receptor mRNA Level Determination

RNA was extracted from the striatum and prefrontal cortex of WT and MAO B KO mice ($n = 4$/genotype) using Trizol (Invitrogen, Carlsbad, CA). Two micrograms of total RNA were used in first-strand cDNA synthesis with M-MLV reverse transcriptase (Promega, Madison, WI) according the manufacturers instructions. The transcript was amplified with specific primers (TAAR1-F: 5′-ATGCATCTTTGCC ACGCTATC-3′, TAAR1-R: 5′-TCAAGGCTCTTCTGAACC-3′) by using iQTM SYBR Green Supermix (Bio-Rad, Hercules, CA). PCRs were performed using 1 μl of 20 × diluted cDNA with one cycle of 94°C for 3 min, followed by 40 cycles of amplification at 94°C for 30 s, 62°C (annealing temperature) for 30 s, and 72°C for 30 s. TAAR1 mRNA expression was normalized to 18S rRNA levels (Boda *et al* 2009). Relative comparison of gene expression between WT and MAO B KO mice was determined as described by Rieu and Powers (2009).

Statistical Analysis

Statistical analyses on behavioral parameters were performed with one-way or two-way ANOVAs, as appropriate, followed by Tukey's test with Spjøtvoll-Stoline correction for *post hoc* comparisons. Normality and homoscedasticity of data distribution were verified using the Kolmogorov–Smirnov and Bartlett's test. Nonparametric comparisons were carried out by Mann–Whitney test. Significance threshold was set at $P = 0.05$.

RESULTS

Elevated Plus Maze

In earlier reports, we documented that MAO B KO and WT mice do not display significant differences in anxiety-related parameters in the elevated plus-maze (such as the number of entries and the time spent in the open arms), under light conditions analogous to those kept in the housing room (300 lux) (Grimsby *et al*, 1997). Nevertheless, cogent evidence has shown that high levels of environmental illuminance exacerbate the anxiogenic properties of the open arms of the elevated plus maze (Dawson and Tricklebank, 1995). These premises prompted us to speculate that dim light conditions (10 lux) may be more appropriate to unravel subtle behavioral alterations displayed by MAO B KO mice in this paradigm. Indeed, under these conditions, MAO B KO mice did exhibit significantly more open arm entries (Figure 1a) ($F(1,18) = 4.48$, $P < 0.05$) and spent a longer time in the open arms (Figure 1b) ($F(1,18) = 4.69$, $P < 0.05$) in comparison with their WT counterparts. MAO B KO mice also spent a significantly shorter time on the center platform (Figure 1d) ($F(1,18) = 4.89$, $P < 0.05$). In contrast, the two genotypes exhibited comparable entries in the center (Figure 1c) ($F(1,18) = 1.63$, NS), as well as both entries ($F(1,18) = 0.51$, NS) and time spent ($F(1,18) = 0.08$, NS) in the closed arms (Figure 1e and f). The reduction in anxiety-like responses in MAO B KO mice was also confirmed by their lower number of fecal boli excreted during the testing session (Figure 1g) ($F(1,18) = 7.24$, $P < 0.05$). The behavioral variability between

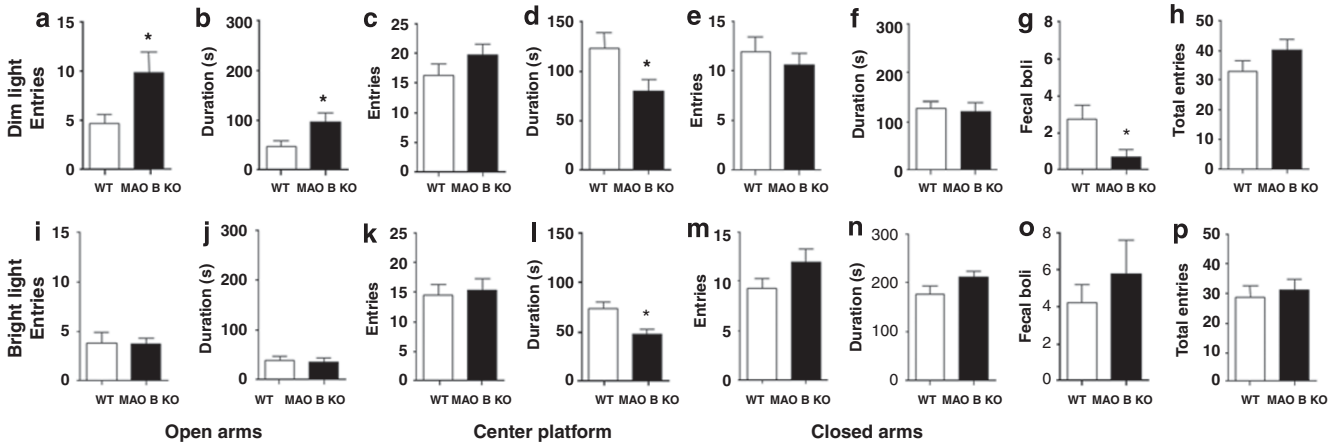

Figure 1 MAO B KO mice exhibit significant reductions in anxiety-like behaviors in the elevated plus maze under dim (a–h), but not bright (i–p) light conditions. All values are represented as means ± SEM. *$P<0.05$, compared with wild-type (WT) controls. For more details, see Results section.

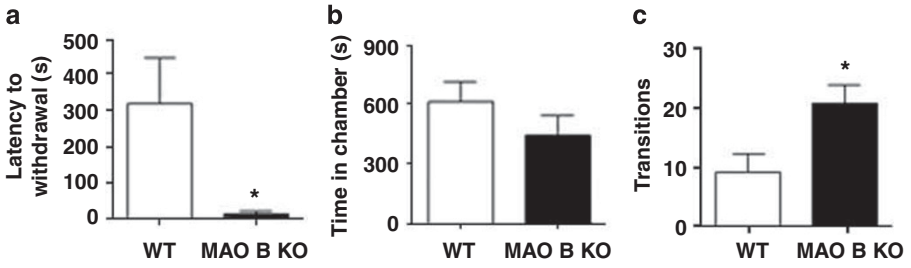

Figure 2 MAO B KO mice display decreased anxiety-like behaviors in the defensive withdrawal paradigm. All values are represented as means ± SEM. *$P<0.05$, compared with WT mice. For more details, see Results section.

genotypes did not reflect differences in activity, as shown by the equivalent number of total entries ($F(1,18)=1.89$, NS) (Figure 1h).

In a second set of experiments carried out under bright light conditions (300 lux) with a different set of animals, we confirmed the lack of substantial behavioral differences between WT and MAO B KO mice (Figure 1i–p). However, the latter spent a shorter time on the center platform (Figure 1l) ($F(1,14)=8.66$, $P<0.05$), in a manner similar to their homogenotypic counterparts tested under dim light.

Defensive Withdrawal

To further characterize the ethological significance of the behavioral alterations displayed by MAO B KO mice in the elevated plus maze, we tested them in another well-validated model of anxiety, the defensive withdrawal paradigm. This test harnesses the conflict between the natural drive of rodents to explore a novel open arena and their tendency to retreat into an enclosed chamber, based on their degree of perceived threat (Takahashi *et al*, 1989). MAO B KO mice showed a significantly reduced latency to withdraw from the chamber (Figure 2a) ($U(7,10)=10$, $P<0.05$) and a marked reduction, albeit not significant, in time spent inside the chamber (Figure 2b) ($F(1,15)=3.17$, $P<0.10$). The number of transitions between the chamber and the open arena was also higher for MAO B KO mice (Figure 2c) ($F(1,15)=7.4$, $P<0.05$). However, no differences between MAO B KO and WT mice were detected in head pokes ((WT = 23.6 ± 3.6; MAO B KO = 22.3 ± 7.1) ($F(1,15)=0.03$, NS)), velocity in the open field (defined as the ratio between the number of crossings and the time spent in the arena) ((WT = 0.15 ± 0.06; MAO B KO = 0.22 ± 0.03) ($F(1,15)=1.49$, NS)), and fecal boli ((WT = 3.1 ± 1.0; MAO B KO = 1.6 ± 0.5) ($F(1,15)=1.81$, NS)).

Marble Burying

To further substantiate the hypothesis that MAO B KO mice show decreased anxiety-like behaviors, we used the marble burying paradigm. This model has been recently shown to capture aspects of environmental reactivity different from those assessed in the conflict-based assays (Thomas *et al*, 2009). Unlike their WT counterparts, MAO B KO mice buried a remarkably low number of marbles (Figure 3a) ($U(20,13)=72$, $P<0.05$), but displayed equivalent activity (Figure 3b) ($F(1,24)=0.27$, NS). The reduction in buried marbles was paralleled by a significant decrease in digging bouts (Figure 3c) ($F(1,26)=7.63$, $P<0.05$) and duration (Figure 3d) ($U(19,10)=34$, $P<0.01$). In spite of their dramatic reduction in digging behaviors, MAO B KO mice approached and actively explored the marbles, with an average frequency of 4.38 ± 0.01 exploratory bouts/min. This parameter, however, could not be efficiently compared across genotypes, as WT mice engaged in significantly more marble-burying behavior.

Hole-Board Test

Following the detection of reduced anxiety-related manifestations in MAO B KO mice, we assessed the potential impact of these alterations on the exploratory activity by testing the animals in the hole-board paradigm (Casarrubea *et al*, 2008). The total number of head dips was comparable between MAO B KO and WT (Figure 4a) ($F(1,18) = 0.28$, NS). Nevertheless, MAO B KO mice performed significantly more head dips in the center than WT mice (Figure 4b) ($F(1,17) = 4.52$, $P < 0.05$), a behavior that has been interpreted as reflective of anxiolysis (Hranilovic *et al*, 2005). Peripheral head dips were comparable between genotypes (Figure 4c) ($F(1,18) = 2.07$, NS). No other significant differences were found in either locomotor activity ($F(1,16) = 0.27$, NS) or time spent in the central quadrants (($WT = 29.2 \pm 6.6$; MAO B KO $= 76.5 \pm 29.8$) ($F(1,16) = 1.11$, NS)). Although the latency to initial head dip was equivalent across genotypes, (Figure 4d) ($F(1,18) = 0.06$, NS) MAO B KO mice exhibited a significantly decreased latency to the first head dip in one of the central holes (Figure 4e) ($U(8,12) = 17$, $P < 0.05$). Latency to the first peripheral head dip was statistically equivalent in WT and MAO B KO mice (Figure 4f) ($U(8,11) = 36$, NS).

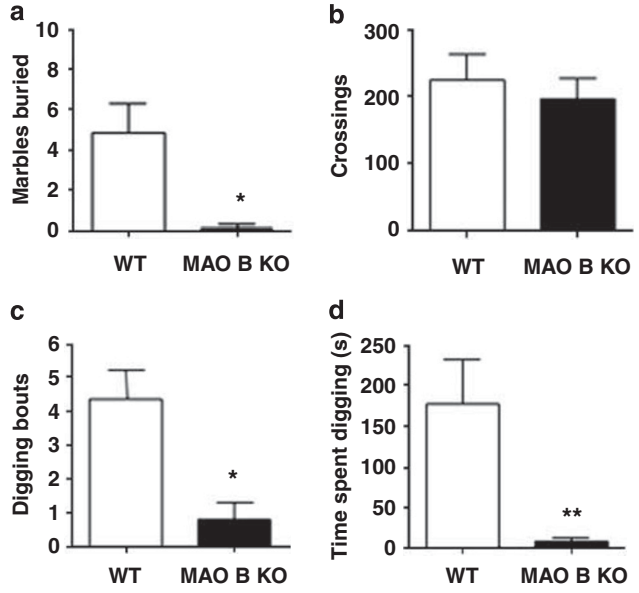

Figure 3 MAO B KO mice exhibit a reduction in marble-burying and digging behaviors. All values are represented as means ± SEM. *$P < 0.05$, **$P < 0.01$ compared with WT controls. For more details, see Results section.

Novel Object Exploration

During both the acclimation phase and the exposure to novel objects, MAO B KO and WT mice exhibited similar magnitude and patterns of locomotor activity (data not shown). MAO B KO mice approached the objects with a significantly reduced latency (Figure 5a) ($U(7,8) = 6$, $P < 0.01$) and explored them for a significantly longer time (Figure 5b) ($F(1,13) = 5.17$, $P < 0.05$). In contrast, the number of exploratory approaches was analogous between genotypes (($WT = 80.9 \pm 19.3$; MAO B KO $= 95.6 \pm 8.3$) ($F(1,13) = 0.96$, NS)). Statistical analysis also showed a trend for MAO B KO mice to spend longer time in object areas (($WT = 161.1 \pm 28.5$; MAO B KO $= 263.6 \pm 36.9$) ($F(1,13) = 3.97$, $P < 0.10$)). These results suggest that MAO B KO mice may be characterized by a stronger drive (or a reduced timidity) to explore unfamiliar objects.

Novelty-Induced Grooming

Earlier studies (Kalueff and Tuohimaa, 2004) and preliminary observations obtained by our group have shown that 129/Sv mice display poor spontaneous grooming activity in response to novel environmental stimuli. Thus,

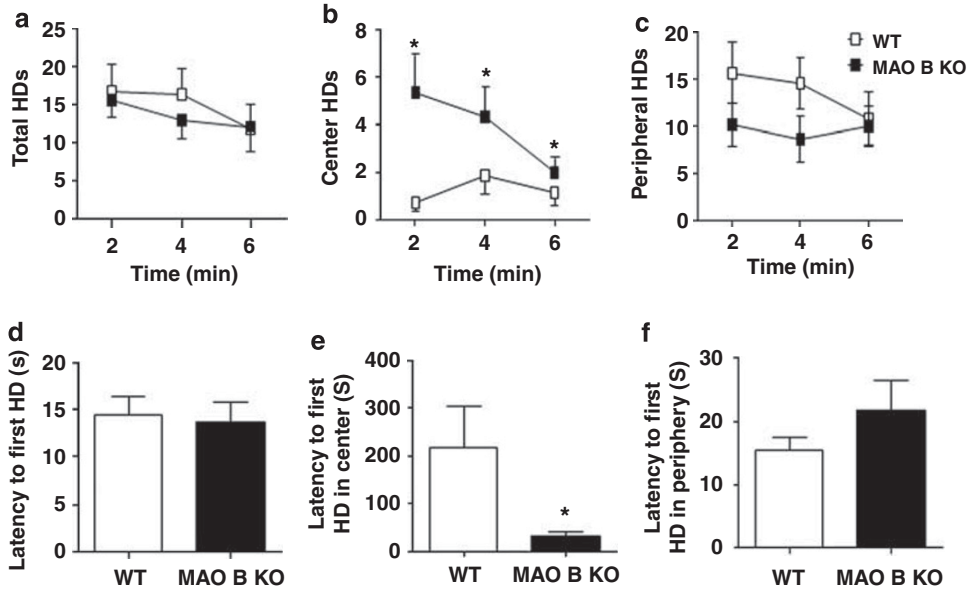

Figure 4 MAO B KO mice explore central holes with longer duration and shorter latency in the hole-board test. All values are represented as means ± SEM. *$P < 0.05$, compared with WT controls. For more details, see Results section.

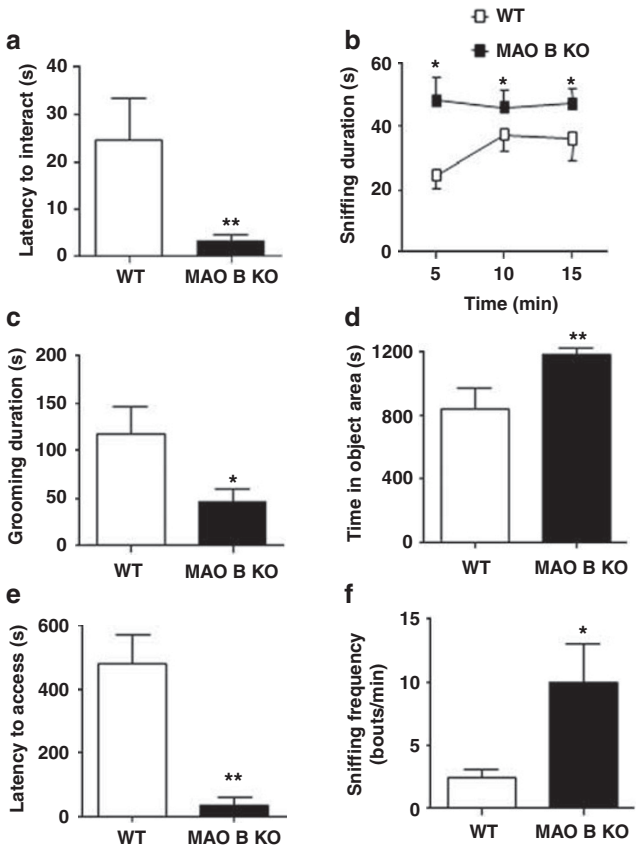

Figure 5 MAO B KO mice display higher levels of exploration targeting novel objects and risk-taking behavior in the wire-beam bridge test. All values are represented as means ± SEM. $^{*}P<0.05$, $^{**}P<0.01$ compared with WT littermates. For more details, see Results section.

we optimized our protocol to detect novelty-induced grooming and avoidance by placing five voluminous objects in one-half of the cage. MAO B KO mice spent significantly less time grooming (Figure 5c) ($F(1,12)=4.93$, $P<0.05$), but engaged in a similar number of grooming bouts (($WT=7.71\pm1.69$; MAO B $KO=5.71\pm1.26$) ($F(1,12)=0.78$, NS)) in comparison with their WT counterparts. Furthermore, MAO B KO mice spent a significantly longer time in the object area (Figure 5d) ($U(8, 7)=6$, $P<0.01$). These results confirm that MAO B KO mice display less environmental and object-related neophobia than WT mice.

Wire-Beam Bridge Test

Low levels of platelet MAO B activity have been strongly associated with features of the behavioral disinhibition spectrum, including impulsivity, sensation-seeking, and risk-taking (Hirschfeld-Becker *et al*, 2003; Ruchkin *et al* 2005). To capture these elements, we measured the proclivity of animals to cross an unrailed flexible bridge suspended over a 30-cm deep gap, to reach a food reward. MAO B KO mice exhibited a significantly shorter latency to access the bridge (Figure 5e) ($U(6, 5)=1.5$, $P<0.01$) and latency to touch the food (($WT=483.83\pm81.64$; MAO B $KO=48.00\pm23.66$) ($U(6, 5)=1$, $P<0.01$)) to obtain the food reward in comparison with their WT counterparts. In the time before accessing the novel bridge, MAO B KO mice engaged in a significantly higher sniffing frequency towards it (Figure 5f) ($U(6, 5)=2$, $P<0.05$) compared with WT mice. These results further support that MAO B KO mice display greater impulsivity, sensation-seeking, and risk-taking behaviors than WT mice.

Table 1 MAO B KO Mice Exhibit Significantly Higher PEA Levels in all Regions Tested

Region	PEA levels	
	WT ($N=7$)	**MAO B KO** ($N=6$)
Cortex	1.68 ± 0.28	7.4 ± 0.40***
Striatum	2.24 ± 0.37	25.8 ± 3.42***
Hippocampus	3.52 ± 0.87	9.18 ± 0.87***
Thalamus	6.55 ± 0.68	12.5 ± 2.24*
Cerebellum	2.13 ± 0.56	7.81 ± 1.69**

All values are represented as means ± SEM.
$^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared with WT littermates.

PEA Brain-Regional Levels

We previously determined that MAO B KO mice feature high brain levels of PEA (Grimsby *et al*, 1997). To identify the regional determinants of the influence of PEA on the spectrum of behavioral alterations in MAO B KO mice, we examined PEA levels in several regions associated with anxiety and emotional reactivity. MAO B KO mice displayed higher PEA levels in the frontal cortex (MAO B KO/WT ratio: 4.4; $F(1,11)=145.44$, $P<0.001$), striatum (MAO B KO/WT ratio: 11.5; $F(1,11)=55.20$, $P<0.001$), hippocampus (MAO B KO/WT ratio: 2.6; $F(1,11)=20.94$, $P<0.001$), thalamus (MAO B KO/WT ratio: 1.9; $F(1,11)=7.42$, $P<0.05$), and cerebellum (MAO B KO/WT ratio: 3.7; $F(1,11)=11.67$, $P<0.01$) compared with their WT counterparts (Table 1).

TAAR1 Transcript Brain-Regional Levels

As the most dramatic increase in PEA-regional levels featured by MAO B KO mice was observed in the striatum (1150 % in comparison with WT) and frontal cortex (440% in comparison with WT), we tested the expression of the transcript of its selective receptor, TAAR1, in these regions. Striatal TAAR1 mRNA was comparable between MAO B KO and WT mice (Relative expression: WT: 1 ± 0.24; MAO B KO: 1.37 ± 0.35) ($F(1,6)=1.16$, NS). Similarly, no significant difference was identified between both genotypes in mRNA expression in the frontal cortex (relative expression: WT: 1 ± 0.32; MAO B KO: 1.54 ± 0.62) ($F(1,6)=3.03$, NS).

DISCUSSION

The major result of this study is that MAO B deficiency in mice leads to behavioral disinhibition in several models

of contextual anxiety and risk-taking behavior, as well as enhanced novelty-seeking responses with respect to unfamiliar objects. These findings are in substantial agreement with previous cross-sectional investigations, documenting that low platelet MAO B enzymatic activity is correlated with several facets of behavioral disinhibition (Buchsbaum *et al*, 1976; Oreland, 1993; von Knorring *et al*, 1984), including novelty- and sensation-seeking personality (Fowler *et al*, 1980a; Reist *et al*, 1990; Ruchkin *et al*, 2005), poor impulse control (Paaver *et al*, 2007; Skondras *et al*, 2004), and proclivity to engage in risky behaviors (Blanco *et al*, 1996).

Previous studies indicate that low platelet MAO B activity is highly heritable (Oxenstierna *et al*, 1986) and may influence behavior since the neonatal stage (Sostek *et al*, 1981), suggesting that this index may be a genetic determinant for uninhibited personality (Blanco *et al*, 1996; Oreland and Hallman, 1995). MAO B deficiency in Norrie disease patients was reported to result in no overt physical or mental alterations (Lenders *et al*, 1996). However, it should be observed that the severe degree of sensory and perceptual impairments induced by Norrie disease (early-onset blindness and progressive hearing loss) most likely masked alterations in environmental reactivity in these subjects.

The role of MAO B in emotional regulation is further supported by a host of clinical studies, showing that chronic administration of *l*-deprenyl exerts mood-enhancing and anxiolytic effects in depression (Mendlewicz and Youdim, 1980; Quitkin *et al*, 1984; Robinson *et al*, 2007) and other disorders (Goad *et al*, 1991; Tariot *et al*, 1987; Tolbert and Fuller, 1996). Interestingly, *l*-deprenyl (both in acute and chronic administration) elicits only minor or no anxiolytic-like effects in rodents (Commissaris *et al*, 1995; De Angelis and Furlan, 2000; Nowakowska *et al*, 2001). The most likely explanation for the apparent discrepancy between these reports and our results lies in the genetic nature of MAO B inactivation examined in this study, which cannot be completely recapitulated by the outcomes of chronic exposure to enzyme inhibitors (Whitaker-Azmitia *et al*, 1994).

In rodents, emotional reactivity and novelty-seeking behavior is measured as a function of the exploratory activity toward unfamiliar environments and objects (Oreland, 1993; Robinet *et al*, 1998; Fornai *et al*, 1999). Accordingly, the validity of novelty-induced tasks as animal models of anxiety (Pellow *et al*, 1985; Takahashi *et al*, 1989) is based on the opposition between exploratory drive and neophobia-derived avoidance (Dellu *et al*, 2000). This contrast can be influenced by certain environmental manipulations, such as the variation of light intensity in the experimental room (Dawson and Tricklebank, 1995).

In a dimly lit elevated plus maze, MAO B KO mice displayed a significant reduction in anxiety-like behavior, signified by an increase in open-arm time and entries, as well as a reduction of defecation frequency (Tarantino and Bucan, 2000). In contrast, a bright illuminance level (300 lux) failed to elicit significant differences between MAO B KO and WT mice in these anxiety-related parameters, possibly due to 'floor effects'.

Dim light conditions have been extensively used to capture fine modifications in anxiety-like behaviors (Bortolato *et al*, 2006; Bourin *et al*, 2001; Genn *et al*, 2003; Löw *et al*, 2000; Rubino *et al*, 2008). In this study, a low environmental luminosity provided an optimal setting to reveal the reduction in anxiety-like behaviors in MAO B KO mice, suggesting that the deficiency of this enzyme results in subtle, context-dependent changes in anxiety regulation. This contention is also supported by the observation that the behavioral abnormalities in MAO B KO mice could not be detected in their home cages (data not shown), but only in the presence of novel objects or contexts.

In the defensive withdrawal test, MAO B KO mice exhibited reductions in latency to exit the chamber and in transitions between the chamber and the open arena. Both parameters are highly dependable indices to measure defensive behaviors (Arborelius and Nemeroff, 2002), and their reduction is considered reflective of reduced fearfulness or deficits in threat detection. This interpretation is also supported by the significant decline in time spent on the elevated plus maze central platform, which has been suggested to indicate potential impairments in decision-making or impulse-control processes (Rodgers *et al*, 1992; Trullas and Skolnick, 1993).

The reduction in anxiety-related responses in MAO B KO mice was also confirmed by the nearly complete abrogation of their marble-burying behavior. This murine assay has been validated to explore different aspects of anxiety-like behaviors than conflict-based paradigms (Thomas *et al*, 2009), in a manner sensitive to anxiolytic and antidepressant drugs (Borsini *et al*, 2002; Broekkamp *et al*, 1986; Nicolas *et al*, 2006; Njung'e and Handley, 1991). Marble burying reflects digging activity, but is independent from locomotion or exploration (Gyertyan, 1995; Thomas *et al*, 2009); Accordingly, MAO B KO mice dug significantly less than WT counterparts, but displayed a comparable number of crossings.

Our findings on the patterns of exploratory activity in the hole-board test are also supportive of the reduced anxiety-like behaviors in MAO B KO mice. Although overall locomotor activity was comparable between genotypes, MAO B KO mice manifested a lower level of avoidance toward the central holes, indicating a reduction in thigmotactic behavior (Hranilovic *et al*, 2005) and novelty-related aversion (Brown and Nemes, 2008). As animals were initially placed in the center of the hole board, the increased number of central head dips may also reflect an enhancement in perseverative behavior, following exploration of the first central hole. Nevertheless, this possibility is partially tempered by the equivalent latency to the first peripheral head dip between the two genotypes, which was accompanied by a similar locomotor activity (and number of head dips in the external zone) in the first 2-min period of testing. This phenomenon most likely indicates that MAO B KO mice did not exhibit an initial tendency to neglect holes in the external zone of the hole board.

The possibility that the behavior enacted by MAO B KO mice may be reflective of low neophobia is also supported by their reaction to unfamiliar objects. Indeed, in comparison to their WT counterparts, MAO B KO mice exhibited a stronger inclination to explore novel objects (with higher duration and lower latency), as well as lower levels of novelty-induced grooming and reduced avoidance of object-laden areas. Alternatively, these responses may also

signify poor impulse control and higher drive toward risk-taking behaviors in MAO B KO mice. In rodents, impulsivity is generally studied by means of go/no-go tests, which measure the capacity to withhold behavioral reactions (Dalley *et al*, 2008). However, as these tasks are based on operant responses, they cannot be dependably used in MAO B KO mice, due to alterations in mnemonic acquisition in these mice (Bortolato *et al*, in preparation). To obviate this pitfall, we tested MAO B KO mice in the wire-beam bridge task, an assay devised to verify their inclination to engage in risk-taking behaviors (such as walking on a novel, flexible bridge placed above a 30 cm deep gap) to reach a rewarding goal. The prevalence of motivational drives over the ability to adjust behavioral responses to contextual elements is considered a key feature of impulsiveness (Jentsch and Taylor, 1999; Bechara *et al*, 2000). On exposure to the novel bridge, MAO B KO mice exhibited a significantly shorter latency to both access and cross the bridge to reach the food reward compared with WT mice. This divergence most likely signifies enhanced risk-taking behavior in MAO B KO mice, and may reflect alterations in decision-making processes and emotional regulation in this genotype (Llewellyn, 2008). Interestingly, MAO B KO mice engaged in a significantly higher frequency of sniffing bouts toward the bridge than WT mice, showing that their shorter latency to bridge access was not reflective of inadequate exploration of the bridge itself or lower risk assessment.

In a previous report, we described that MAO B KO mice exhibited lower immobility in the forced swim test than WT counterparts (Grimsby *et al*, 1997). In mutant mice, this behavior has been associated with either enhanced (Parks *et al*, 1998) or reduced anxiety-like behavior (Bale and Vale, 2003; Tschenett *et al*, 2003). Our present findings help define the conceptual framework for the interpretation of the stress response exhibited by MAO B KO mice, suggesting that their behavior in the forced swim paradigm may reflect their enhanced ability to counteract the stress induced by novel contextual factors and hazardous situations. Substantial evidence has shown that the increased mobility in forced swim test is associated with a lower vulnerability to stress-induced anhedonia and depression (Strekalova *et al*, 2004; Trzctñska *et al*, 1999), as well as decreased neophobia (Gundersen and Blendy, 2009). Accordingly, decreased grooming activity has been shown to be a dependable criterion to measure increased inclination to cope with stress (Kalueff and Tuohimaa, 2004). The higher resistance to stress in MAO B KO mice is also corroborated by their significantly lower levels of hyperthermia (Bouwknecht *et al*, 2007) induced by 2 h of physical restraint, in comparison with WT littermates (WT: ΔT: $1.09 \pm 0.13^{\circ}$C; MAO B KO: ΔT: $0.12 \pm 0.22^{\circ}$C; $P < 0.01$) (unpublished data).

Monoamine oxidase B KO mice feature a significant elevation in whole-brain levels of PEA, but not other monoamines (Grimsby *et al*, 1997). This premise suggests that this trace amine may have a key role in the behavioral alterations induced by MAO B genetic deficiency. Indeed, PEA has been shown to enhance mood and sensory functions in joint administration with MAO B inhibitors (which prevent its degradation) (Sabelli *et al*, 1994; Sabelli and Javaid, 1995). Furthermore, the synthetic PEA analog amphetamine is known to increase novelty-seeking behaviors and reduce impulse control in both rodents and humans (Evenden and Ryan, 1996; Leyton *et al*, 2002; Williamson *et al*, 1997).

In apparent contrast with our findings, acute administration of PEA has been shown to induce anxiety in rodents (Lapin, 1990, 1993). However, congenital, chronic exposure to high PEA levels may reduce anxiety-spectrum responses in MAO B KO mice, probably by the progressive recruitment of processes opposing the anxiogenic effects of this trace amine. Similarly, MAO B KO mice also fail to exhibit other alterations reminiscent of the effects induced by acute PEA administration, such as hyperlocomotion (Mantegazza and Riva, 1963), stereotyped behavior (Moja *et al*, 1976), and anorexia (Dourish and Boulton, 1981). Final verification of the involvement of PEA in the behavioral performance of MAO B KO mice would require pharmacological manipulations to reduce their PEA levels, such as the inhibition of its synthesis. The accomplishment of this objective, however, is hindered by the lack of substrate specificity of the only PEA-synthesizing enzyme as yet characterized in mice, aromatic *l*-amino acid decarboxylase (EC4.1.1.28) (Kubovcakova *et al*, 2004; Zucchi *et al*, 2006). This enzyme catalyzes key reactions in the synthesis of all the other major neurotransmitter systems, such as DA, NE, and 5-HT (Allen *et al*, 2009), and its inhibition results in a number of nonspecific effects on several brain functions (Fisher *et al*, 2000).

To partially circumvent these limitations, in this study we have examined the differential expression of PEA levels in several brain regions associated with emotional reactivity. Although the increase in PEA brain levels involves several brain regions of MAO B KO mice, the most marked enhancements in PEA levels were observed in the striatum and prefrontal cortex, two regions extensively implicated in behavioral disinhibition (Johansson and Hansen, 2000; Winstanley *et al*, 2006).

The involvement of striatum and prefrontal cortex in behavioral disinhibition has been linked to the functional activity of DAergic system (Pattij *et al*, 2007), suggesting that DA may be implicated in the behavioral alterations in MAO B KO mice. This possibility is supported by a host of studies underscoring the key role of DA in behavioral disinhibition (Black *et al*, 2002; Megens *et al*, 1992; van Gaalen *et al*, 2006) and anxiolysis (Shabanov *et al*, 2005; Picazo *et al*, 2009). Previous studies have shown that PEA induces modification of the DA signaling, which may have a role in the behavioral responses mediated by this trace amine (Kuroki *et al*, 1990; Sotnikova *et al*, 2004). Interestingly, although MAO B KO mice do not feature alterations of striatal DA synthesis, uptake, and release, they do exhibit alterations in DA receptors in this region (Chen *et al*, 1999). Furthermore, structural changes in other monoamine systems (such as 5-HT) may also be induced by MAO B deficiency (Whitaker-Azmitia *et al*, 1994; Oreland *et al*, 2007).

Emerging evidence points to a role of TAAR1 receptor in the PEA-mediated modulation of DAergic signaling (Xie and Miller, 2009; Lindemann *et al*, 2008). Although no apparent compensatory changes in TAAR1 receptor expression were detected in either striatum or frontal cortex, the present data cannot allow any final conclusion on the

functional contribution of this receptor to the behavioral spectrum of MAO B KO mice. Although pharmacological studies with TAAR1 receptor antagonists may help elucidate this issue, these agents are currently unavailable (Sotnikova *et al*, 2009).

In summary, this study documents that MAO B deficiency in mice results in behavioral disinhibition and reduced neophobia. Our results complement previous findings on the correlation between low MAO B platelet activity and novelty-seeking personality, suggesting a potential causal link between the two phenomena. Nevertheless, both the interpretation of the behavioral phenotype in MAO B KO mice and its translational validity should be considered with caution, in view of several limiting considerations. First, although all the observed abnormalities in MAO B KO mice are evocative of behavioral disinhibition, some of them—such as the increased exploration of novel objects or the decreased marble burying—may also reflect other disturbances in perceptual, attentional, emotional, and cognitive regulation. Further investigations on the impact of MAO B deficiency in these behavioral domains are necessary to elucidate this possibility and further refine our understanding of the complex phenotype exhibited by MAO B KO mice. Second, the results observed in MAO B KO mice may not be directly applicable to clinical manifestations, in view of the predominance of this isoenzyme in the human brain (Fowler *et al*, 1980b; Oreland and Gottfries, 1986; Kalaria *et al*, 1988), which contrasts with its relatively poor expression in rodents (Saura *et al*, 1996). Third, although dopamine is degraded by MAO A in mice (Cases *et al*, 1995; Fornai *et al*, 1999), it is mainly metabolized by MAO B in primates (Garrick and Murphy, 1980), suggesting that the reported alterations in murine phenotype may only partially reproduce the behavioral outcomes of MAO B deficiency in humans. Irrespective of these considerations, these findings strongly support the role of MAO B in the modulation of the neural pathways underlying behavioral disinhibition and emotional reactivity toward contextual stimuli, and warrant further investigations on the function of this enzyme in the regulation of anxiety-related endophenotypes.

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DISCLOSURE

All authors report no biomedical financial interests or potential conflicts of interest.

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