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The aggression and behavioral abnormalities associated with monoamine oxidase A deficiency are rescued by acute inhibition of serotonin reuptake

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Abstract

The termination of serotonin (5-hydroxytryptamine, 5-HT) neurotransmission is regulated by its uptake by the 5-HT transporter (5-HTT), as well as its degradation by monoamine oxidase (MAO)-A. MAO-A deficiency results in a wide set of behavioral alterations, including perseverative behaviors and social deficits. These anomalies are likely related to 5-HTergic homeostatic imbalances; however, the role of 5-HTT in these abnormalities remains unclear. To ascertain the role of 5-HTT in the behavioral anomalies associated to MAO-A deficiency, we tested the behavioral effects of its blocker fluoxetine on perseverative, social and aggressive behaviors in transgenic animals with hypomorphic or null-allele MAO-A mutations. Acute treatment with 5-HTT blocker fluoxetine (10 mg/kg, i.p.) reduced aggressive behavior in MAO-A knockout (KO) mice and social deficits in hypomorphic MAO-A^{Neo} mice. Furthermore, this treatment also reduced perseverative responses (including marble burying and water mist-induced grooming) in both MAO-A mutant genotypes. Both MAO-A mutant lines displayed significant reductions in 5-HTT expression across the prefrontal cortex, amygdala and striatum, as quantified by immunohistochemical detection; however, the down-regulation of 5-HTT in MAO-A^{Neo} mice

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Conflict of interest. No authors declare any conflicts of interest.

was more pervasive and widespread than in their KO counterparts, possibly indicating a greater ability of the hypomorphic line to enact compensatory mechanisms with respect to 5-HT homeostasis. Collectively, these findings suggest that the behavioral deficits associated with low MAO-A activity may reflect developmental alterations of 5-HTT within 5-HTergic neurons. Furthermore, the translational implications of our results highlight 5-HT reuptake inhibition as an interesting approach for the control of aggressive outbursts in MAO-A deficient individuals.

Keywords

Serotonin; Monoamine oxidase; Serotonin transporter; animal models

STUDY OBJECTIVES AND BACKGROUND

Serotonin (5-hydroxytryptamine, 5-HT) plays a fundamental role in the orchestration of emotional reactivity and in the pathophysiology of numerous mental disorders, ranging from anxiety to pathological aggression (Hensler 2006; Merens et al. 2007; Harmer 2008). The most influential processes in the termination of 5-HT neurotransmission are its reuptake by a selective 5-HT transporter (5-HTT) and its enzymatic degradation by monoamine oxidase (MAO) A. The simultaneous inhibition of 5-HTT and MAO-A leads to higher brain 5-HT extracellular concentrations (Perry and Fuller 1992; Malagie et al. 1995; Sharp et al. 1997), as well as reduced firing of 5-HTergic neurons in the dorsal raphë nucleus (Aghajanian et al. 1970; Blier and de Montigny 1985). Although this background suggests the relevance of the interactions of 5-HTT and MAO-A in the modulation of 5-HTergic homeostasis, very little is currently known about the interplay of these two targets and its importance in the pathophysiology of psychiatric disorders.

Deficits in brain MAO-A levels have been shown to result in a higher predisposition to aggressiveness and antisocial personality (Alia-Klein et al. 2008; Soliman et al. 2011). In particular, congenital deficiency of the *MAOA* gene results in Brunner syndrome, a rare genetic X-linked disorder characterized by violent and antisocial conduct, perseverative behavioral patterns and mild cognitive deficits (Brunner et al. 1993). Recent, a new case of MAO-A deficiency due to a missense mutation has been recently described to result in autism-spectrum disorder, attention deficits and self-injurious behavior (Piton et al. 2013). In parallel, MAO-A knockout (KO) mice exhibit a number of aberrant phenotypes, including high brain concentrations of brain 5-HT and norepinephrine, dysmorphic barrel fields in the sensorimotor cortex, marked reactive aggression towards intruder conspecifics, maladaptive reactivity to environmental cues and autism-related responses (Cases et al. 1995; Vitalis et al. 1998; Godar et al. 2011; Bortolato et al. 2013a).

While the complete deficiency of MAO-A is considered as a relatively rare circumstance, genetic variants associated with a reduction of its activity are well-documented, in relation to polymorphic variants of the MAOA gene (Bortolato et al. 2008; Bortolato and Shih 2011). While low-activity allelic variations are not inherently conducive to aggressiveness, they have been associated with dysfunctional social processing and other abnormalities, which may predispose vulnerable individuals to aggressive responses in specific contexts (Caspi et al. 2002; Kim-Cohen et al. 2006). To model these variants, our group recently

characterized a novel line of hypomorphic MAO-A mutants, MAO-A^{Neo} mice (Bortolato et al. 2011). We found that this line of mice, generated by the insertion of a neomycin-resistance cassette in intron 12 of the Maoa gene, exhibits perseverative behaviors, social deficits and other subtle morphological abnormalities of the prefrontal cortex and cerebellum (Bortolato et al. 2011; Alzghoul et al. 2012); however, unlike MAO-A KO mice, these mutants do not display overt aggression.

The abnormalities of MAO-A KO and MAO-A^{Neo} mice are likely supported by alterations in 5-HTergic homeostasis. The role of 5-HTT in these anomalies, however, remains elusive. Previous research has shown that, in MAO-A KO mice, acute blockade of 5-HTT leads to a marked increase in extracellular 5-HT (significantly greater than that observed in wild-type controls) (Evrard et al. 2002). Thus, we hypothesized that, if the social deficits and perseverative responses in MAO-A-deficient mice are actually supported by the increase in 5-HT levels, inhibition of 5-HTT should lead to an exacerbation of these behavioral abnormalities. Thus, in the present study we analyzed how the behavioral responses of MAO-A KO and MAO-A^{Neo} mice may be affected by acute treatment with fluoxetine, a prototypical 5-HTT inhibitor.

MATERIALS AND METHODS

Animal husbandry

We used 3-5 month old, experimentally naïve male 129S6 mice (n=10-20 per genotype and treatment group), weighing 25-30 g. We used heterozygous MAO-A KO and MAO-A^{Neo} dams for breeding with wild-type (WT) sires to generate MAO-A KO and hypomorphic MAO-A^{Neo} animals as previously described (Bortolato et al. 2011). 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 from 8 am to 8 pm. Principles of laboratory animal care were followed and experimental procedures were in compliance with the National Institute of Health guidelines and approved by the Animal Use Committees of the Universities of Cagliari, Kansas and Southern California.

Drugs

Fluoxetine hydrochloride (Sigma Aldrich, St. Louis, MO) was dissolved in 0.9% saline solution and administered via i.p. injection at 10 mg/kg (to ensure a robust inhibition of 5-HTT), 30 min prior to testing. Control mice were treated with saline.

Behavioral testing

Additional details can be found in the supplementary materials section.

Open field—Analysis of open-field locomotor activity was performed performed using Ethovision (Noldus Instruments, Wageningen, The Netherlands) as previously described (Bortolato et al. 2011). Percent locomotor activity in the center and periphery were calculated by the distance traveled in the center or periphery over the total distance traveled. Path tortuosity was defined as the average ratio of the total distance over the summation of the beelines between the points captured every 0.16 sec (arc-chord ratio).

Marble burying—Marble burying was conducted as previously described (Bortolato et al. 2009b). Their behavior and the number of buried marbles were video recorded. A marble was considered buried if at least two-thirds of its surface area was covered in sawdust.

Water mist-induced grooming—Water mist-induced grooming was analyzed as previously detailed (Hill et al. 2007; Bortolato et al. 2011). Overall grooming duration and frequency of grooming initiations were assessed.

Social interaction—The social interaction test was performed as previously detailed (Bortolato et al. 2011). The latency, frequency and duration of exploratory social approaches of the test animal towards the novel conspecific were measured. No fighting behaviors or tail-rattling occurred during this test.

Resident-intruder test—Aggression was tested in the resident-intruder task for 10 min as previously described (Bortolato et a. 2011). Measures included the latency to attack and the number and duration of fighting behavior.

Immunohistochemistry

To verify whether the behavioral responsiveness to 5-HTT blockade in MAO-A^{Neo} and KO mice may reflect specific brain-regional changes in 5-HTT expression, we studied the immunoreactivity of this molecule in a behaviorally naïve cohort of animals across key brain regions for emotional regulation, including the prefrontal cortex, amygdala and hippocampus.

Immunofluorescent 5-HTT staining—Immunofluorescent 5-HTT staining was conducted as previously described (Bortolato et al. 2009a). Adult male behaviorally naïve mice were transcardially perfused, brains were rapidly removed and post-fixed for 6 h. After repeated washing, brains were cryoprotected in 30% sucrose solution for 48 h. Coronal sections (40 m thick) were prepared with a cryostat at levels containing the selected brain areas, and immunostaining was performed on free-floating sections. The following brain regions were defined by a stereotaxic atlas (Paxinos and Franklin 2001) and analyzed: cingulate cortex areas 1 and 3 (Cg1 and Cg3) (AP+2.20), caudate-putamen (DC) (AP+0.6), NAc core and shell (AP+1.10), basolateral amygdaloid nuclei (BLA) (AP-1.34), and hippocampus (CA1, CA3 and dentate gyrus) (AP-2.70). Tissue sections were incubated for 1 hour at 20 °C in working solution o f mouse immunoglobulin-blocking reagent, prepared as specified by the manufacturer (Vector Laboratories, Burlingame, CA). Following washing, pre-blocking of tissue sections was performed with 10% normal goat serum, 1 % bovine serum albumin (Rebsam et al., 2005) and 0.2% Triton X-100 in PBS for 1 h at room temperature. Sections were incubated for 24 h at 4 °C with mouse monoclonal anti-5-HTT (1:1000; Chemicon, Temecula, CA, USA). Sections were washed and then incubated with Alexa Fluor® 488-labeled goat anti-mouse (1:250, Molecular Probes, Eugene, OR, USA) for 1 h in the dark at room temperature. The tissue sections were rinsed and mounted onto Superfrost glass slides with anti-fading solution.

Imaging and quantitative analysis of 5-HTT immunofluorescence staining—All observations were made using an Olympus IX 61 (Olympus, Hamburg, Germany) microscope equipped with 2.0, 4, 10, 20 and 60× planapochromatic oil immersion objectives. Images were taken with a 12-bit cooled F View II camera (Olympus). After capture to the computer, images were analyzed using the Cell P AnalySIS® software module and positively stained fibers were detected. Thereafter, the percentage area of each image occupied by fibers was estimated.

Statistical analyses—Throughout the study, WT littermates of MAO-A^{Neo} and MAO-A KO mice were combined, since both groups displayed statistically similar behavioral and morphological characteristics. Normality and homoscedasticity of data distribution were verified by using the Kolmogorov-Smirnov and Bartlett's test. Parametric analyses were performed with one- or two-way ANOVAs, as appropriate, followed by Newman-Keuls test for *post-hoc* comparisons of the means. Significance threshold was set at 0.05.

RESULTS

Behavioral testing

A summary of behavioral results are shown in Table 1.

Open field—In parallel with previous findings, MAO-A^{Neo} mice displayed aberrant locomotor patterns in the novel open field; these disturbances, however, were corrected by acute fluoxetine administration (Fig. 1A).

The analysis of the distance travelled in the open field did not reveal any significant main effect for either genotype [$F(2, 85) = 2.86$; NS] or treatment [$F(1, 85) = 2.65$; NS]; however, we found a significant genotype × treatment interaction between groups (Fig. 1B) [$F(2, 85) = 4.72$; $P<0.05$]. *Post-hoc* testing revealed that saline-treated MAO-A mutants exhibited lower locomotor activity than their WT counterparts ($P<0.05$ for both genotypes), however, fluoxetine reduced locomotion only in WT mice ($P<0.05$).

MAO-A^{Neo} mice exhibited increased tortuosity [$F(2, 85) = 3.39$; $P<0.05$], in comparison with both WT and MAO-A KO counterparts ($P<0.05$). While no significant main effect was detected for treatment [$F(1, 85) = 1.89$; NS], ANOVA revealed a significant genotype x treatment interaction (Fig. 1C) [$F(2, 85) = 5.37$; $P<0.01$]. *Post-hoc* scrutiny of this result revealed that saline-treated MAO-A^{Neo} mice displayed higher levels of tortuosity ($P<0.01$) in comparison with both saline-treated WT ($P<0.05$) and MAO-A KO ($P<0.05$) littermates. Furthermore, this response was counteracted by fluoxetine in MAO-A^{Neo} mice ($P<0.05$).

MAO-A^{Neo} mice displayed a significant reduction in percent locomotor activity in the center (Fig. 1D) [genotype: $F(2, 85) = 3.60$; $P<0.05$], as well as a significant increase in the same parameter calculated on the peripheral zone of the arena (Fig. 1E) [genotype: [$F(2, 85) = 6.93$; $P<0.01$], in comparison with both WT ($Ps<0.01$ for both measures) and MAO-A KO ($Ps<0.05$ for both measures) mice. Furthermore, we detected a significant genotype × treatment interaction for both indices [Center: $F(2, 85) = 4.21$; $P<0.05$ and Periphery: $F(2, 85) = 5.23$; $P<0.01$]. Specifically, saline-treated MAO-A^{Neo} mice showed a marked decrease

in percent locomotor activity in the center and an increase in the periphery compared to both saline-treated WT (Center: P<0.01 and Periphery: P<0.001) and MAO-A KO (Center: P<0.01 and Periphery: P<0.05) animals. Pretreatment with fluoxetine countered both abnormal responses (Ps<0.05) in MAO-A^{Neo} mutants.

Marble burying—MAO-A^{Neo} and MAO A KO mice buried significantly more marbles [genotype: F(2, 66) = 4.28; P<0.05] than WT animals (P<0.01 for both MAO mutant lines). ANOVA also detected a significant genotype × treatment interaction (Fig. 2A) [F(2, 66) = 3.24; P<0.05] in marble-burying activity. *Post-hoc* testing showed that saline-treated MAO-A^{Neo} (P<0.01) and MAO-A KO (P<0.01) mice displayed markedly higher marble-burying responses than saline-treated WT mice; furthermore, fluoxetine attenuated marble burying in both MAO mutant lines (Ps<0.001).

Water mist-induced grooming—No main effect for genotype was found in either the analysis of grooming frequency [F(2, 62) = 2.65; NS] or duration [F(2, 62) = 1.09; NS]. Conversely, fluoxetine elicited a significant reduction in both indices [frequency: F(1, 62) = 19.72; P<0.001 and duration: F(1, 62) = 10.16; P<0.01]. A marked genotype × treatment interaction was found for grooming bouts (Fig. 2B) [F(2, 62) = 9.40; P<0.001] and overall grooming duration (Fig. 2C) [F(2, 64) = 24.14: P<0.001]. *Post-hoc* analyses showed that saline-treated MAO-A^{Neo} mice exhibited a higher number of grooming episodes than saline-treated WT (P<0.001) and MAO-A KO (P<0.05) mice. In addition, fluoxetine significantly decreased the number of grooming episodes in MAO-A^{Neo} (P<0.001) and MAO-A KO (P<0.05) mice, but had no effect on WT animals.

Grooming duration was increased in both MAO-A KO (P<0.01) and MAO-A^{Neo} (P<0.001) mice, and this effect was significantly reduced by fluoxetine (Ps<0.001 for both MAO mutants). In contrast, this drug increased grooming duration in WT mice (P<0.01).

Social interaction—The latency to the first social approach was not significantly different across any combination of genotypes and treatments (Fig. 3A). In addition, no main effects were detected for the number [genotype: F(2, 54) = 1.58; NS and treatment: F(1, 54 = 1.90; NS] or duration [genotype: F(2, 53) = 0.32 and treatment: F(2, 53) = 2.83; NS)] od the exploratory approaches. Nevertheless, significant genotype × treatment interactions were found for both measures (Fig. 3B and 3C) [approaches: F(2, 54) = 6.06; P<0.01 and duration: F(2, 53) = 6.93; P<0.01]. *Post-hoc* analyses revealed that fluoxetine significantly increased the duration of social interaction in MAO-A^{Neo} mice (P<0.05).

Resident-intruder test—Similarly to the previous results, the latency to the first attack was not significantly different across any combination of genotypes and treatments. MAO-A KO mice engaged in a significantly higher number (Fig. 3E) [genotype: F(2, 47) = 3.41; P<0.01] and duration (Fig. 3F) [genotype: F(2, 47) = 3.75; P<0.05] of attacks, compared to both WT and MAO-A^{Neo} mice (Ps<0.05, for both measures). Moreover, fluoxetine profoundly reduced aggression frequency [F(1, 47) = 29.27; P<0.001] and duration [F(1, 47) = 37.64; P<0.001]. A statistical trend was found for a genotype × treatment interaction in relation to the number of fighting episodes [F(2, 47) = 3.12; P<0.06]. Additionally, we detected a marked change in overall fighting duration [F(2, 47) = 4.22; P<0.05]. *Post-hoc*

analysis revealed that MAO-A KO mice displayed a longer fighting duration than WT ($P<0.01$) and MAO-A^{Neo} mice ($P<0.01$); furthermore, fluoxetine reduced fighting duration in both MAO-A^{Neo} ($P<0.05$) and MAO-A KO ($P<0.001$) mice.

Immunohistochemistry

MAO-A KO mice featured a significant reduction in 5-HTT immunoreactivity (Fig. 4) in the cingulate area 3 ($P<0.05$), basolateral amygdala ($P<0.01$), and nucleus accumbens core ($P<0.001$) compared to their WT counterparts. In comparison to WT and MAO-A KO animals, MAO-A^{Neo} mice showed a significant reduction in 5-HTT immunoreactivity in the CG1 ($P<0.001$ and $P<0.01$) and CG3 ($P<0.001$) areas of the medial prefrontal cortex, in the basolateral amygdala ($P<0.001$) and in the nucleus accumbens core ($P<0.001$), shell ($P<0.01$ and $P<0.05$) and dorsal caudate ($P<0.001$) subdivisions of the striatum. Conversely, no aspecific labeling was observed when the antibody (or the appropriate secondary antibodies) was omitted from incubation (data not shown).

DISCUSSION

The results of the present study documented that acute administration of the prototypical 5-HTT blocker fluoxetine corrected several behavioral alterations observed in MAO-A^{Neo} and KO mice, including open-field tortuosity, social deficits and aggression, as well as perseverative digging, marble-burying and grooming. Given that 5-HTT inhibition should increase 5-HT synaptic levels, these results dispel the notion that hyperserotonemia in adulthood may be ultimately responsible for the aggression in MAO-A KO and the behavioral alterations in MAO-A^{Neo} mice; in fact, if these behavioral changes reflected high brain 5-HT concentrations, they should have been predictably *exacerbated*, rather than countered, by acute fluoxetine.

The aggression phenotype in MAO-A KO mice may reflect alternative disturbances in 5-HT homeostatic mechanisms. Indeed, these animals display a ~40% reduction in spontaneous firing of 5-HT neurons in the dorsal raphë (Evrard et al. 2002). In line with this concept, reduced presynaptic corticolimbic 5-HT activity has been posited as a vulnerability factor for aggressive behaviors (Siever, 2008; Coccaro et al. 2011; Rylands et al. 2012; Coccaro, 2012). It is possible that high 5-HT levels during early developmental stages in MAO-A-deficient animals may produce a compensatory decrease in 5-HT neuronal activity, as well as a desensitization of postsynaptic receptors. This hypothesis, however, is partially challenged by our recent finding that inhibition of 5-HT synthesis during the first week after birth failed to modify the aggression in adult MAO-A KO mice, and increased the proclivity to fight in WT littermates (Bortolato et al. 2013b). The early developmental link between MAO A deficiency and aggression may be dependent on the activation of specific 5-HT receptors, or reflect elevated concentrations in other monoamines, such as NE or DA.

The anti-aggressive effects of fluoxetine in MAO-A mutants also parallel clinical findings showing that fluoxetine and other 5-HTT blockers attenuate aggressive responses in susceptible individuals (Coccaro and Kavoussi, 1997; New et al. 2004; Berman et al. 2009; Coccaro et al. 2009a). Although the exact mechanism is still unclear, 5-HTT inhibitors may function to increase top-down prefrontal cortical inhibition of aggressive impulses (New et

al. 2004; Siever, 2008; Coccaro et al. 2011). Taken together, our results point to other neurochemical substrates, such as catecholamines or specific 5-HT receptors, such as 5-HT_{2A} receptors, rather than overall synaptic 5-HT levels, as major factors driving the aggressive and antisocial behaviors in MAO-A-deficient mice (Coccaro et al. 2003; Rosell et al. 2010).

Previous studies by Evrard and colleagues found that acute 5-HTT blockade induced a marked increase of extracellular 5-HT levels in MAO-A KO mice, which was accompanied by a decrease in 5-HT neuronal firing in the dorsal raphe. Thus, a *prima facie* scrutiny of our results may lead to an apparent conundrum, since 5-HTT blockade would seemingly exacerbate, rather than attenuate, the elevation in extracellular 5-HT concentrations observed in MAO-A^{Neo} and KO mice, yet attenuate the behavioral disturbances (Cases et al. 1995; Scott et al. 2008; Bortolato et al. 2011). A possible explanation for this problem may lie in the dynamic alterations of 5-HT levels induced by acute fluoxetine treatment. Specifically, a rapid increase in extracellular 5-HT due to reuptake inhibition may contrast with the tonic elevation of 5-HT levels and overcome the desensitization of several subpopulations of receptors in MAO-A KO mice, such as the somatodendritic 5-HT_{1A} (Evrard et al. 2002). Although the dynamic regulation of 5-HT release is still poorly understood, several authors have posited that the contrast between tonic and phasic 5-HT activity may be instrumental to attune behavioral responsiveness to environmental cues (Daw et al. 2002). Building on this perspective, the persistent state of tonic activation of 5-HTergic synapses resulting from constitutively low MAO-A activity may blunt the ability of 5-HTergic neurons to convey differential patterns of signals to their corticolimbic projections, and contribute to the emotional impairments associated with MAO-A deficiency. Accordingly, we previously showed that MAO-A KO mice display poor defensive responsiveness to potentially threatening stimuli and manifest overt alterations in the processing of environmental information and salience (Godar et al. 2011). Indeed, higher rates of reactive aggression have been associated with cognitive/emotional biases in informational processing towards hostile attributes (Coccaro et al. 2009b; Murray-Close et al. 2010). It is possible that the maladaptive informational processing in MAO-A KO animals may facilitate contextually inappropriate violent outbursts towards conspecifics.

Irrespective of the mechanism, however, these data suggest that the behavioral abnormalities in MAO-A-deficient mice are not simply reflective of the tonic elevation of 5-HT brain levels, but likely result from disturbances of the complex mechanisms subserving the regulation of 5-HTergic homeostasis. Specifically, the aggression, social deficits and behavioral inflexibility of MAO-A KO mice may be contributed by the lack of functional contrasts between tonic and phasic activity of 5-HT neurons. This theory may help account for the apparent discrepancy between the aggression in MAO-A KO mice and the commonly postulated association between hostile traits and low (rather than high) levels of brain 5-HT (Young and Leyton 2002).

Building on previous results, we verified that MAO-A^{Neo} mice display several elements of perseverative and repetitive behaviors. In a novel inescapable open field, MAO-A^{Neo} mice exhibited a significant increase in thigmotaxis, as highlighted by the higher percent

locomotor activity in the center and tortuosity compared to both WT and MAO-A KO counterparts.

5-HTT blockade specifically attenuated these behavioral abnormalities in MAO-A^{Neo} mice without affecting their overall locomotor activity. High levels of thigmotaxis have been suggested to represent an increase in anxiety-related behaviors (Treit and Fundytus 1988; Simon et al. 1994). The possibility that the high thigmotaxis in MAOA^{Neo} mice may signify anxiety, however, is tempered by our previous findings in other well-established paradigms, such as the elevated plus-maze and the light-dark box, in which MAO-A^{Neo} mice failed to exhibit anxiety-related responses (Bortolato et al. 2011). Alternatively, the high tortuosity and thigmotaxis of MAO-A^{Neo} mice may signify an increase in perseverative behavioral responses (McGrath et al. 1999; Ralph et al. 2001) Indeed, previous findings, as well as our current results, show that MAO-A^{Neo} mice exhibit perseverative behaviors in several paradigms (Bortolato et al. 2011). This is particularly intriguing in view of the well-known role of 5-HTT in compulsive traits (Reimold et al. 2007; Matsumoto et al. 2010; Abudy et al. 2011). While multiple studies have found that chronic 5-HTT blockade reduces perseverative behaviors, acute fluoxetine administration has also been shown to reduce repetitive responses in different paradigms (Ichimaru et al. 1995; Boulougouris et al. 2009).

MAO-A^{Neo} and MAO-A KO mice also exhibited a significant increase in repetitive behaviors in the marble burying and water mist-induced grooming tasks, two well-validated tests of compulsive-related behaviors (Hill et al. 2007; Thomas et al. 2009; Bortolato et al. 2011). Moreover, fluoxetine administration attenuated these responses in both mutant lines. These data are paralleled by previous studies documenting acute effects of fluoxetine in behavioral rigidity (Brigman et al. 2010). As opposed to the transgenic lines, the decreased marble burying activity in fluoxetine-treated WT mice, albeit non-significant, may be partially ascribed to the 5-HTT-induced reduction of locomotor activity as shown in the open field (Li et al. 2006). Similarly, the anxiogenic effects of acute fluoxetine administration may have contributed to the increased water mist-induced grooming behavior in WT mice.

Fluoxetine treatment also significantly increased social exploration in MAO-A^{Neo}, but not MAO-A KO mice. Although previous reports have shown that both mutant lines exhibit a reduction in social interactions with a novel conspecific, the lack of a significant effect in the present data may reflect the statistical design (two-way ANOVA with 3 independent factors) which requires a higher overall number. Alternatively, the absence of any effect may be due to an overall lower sample number as MAO-A^{Neo} mice displayed a trend in the time spent in social exploration that did not quite reach statistical significance. Likewise, no genotype-specific changes were detected for the latency to first attack in the resident-intruder paradigm. This discrepancy with our previous reports (Bortolato et al. 2011) in which MAO-A-deficient mice exhibit a markedly lower latency to first attack, may be a result of the stressful impact of the saline injection on WT mice (Puglisi-Allegra and Oliverio 1981). Nevertheless, MAO-A KO animals showed a marked increase in both the number and duration of fighting bouts compared to other genotypes.

Fluoxetine elicited a robust decrease in the fighting duration of both MAO-A mutant lines. These data are in substantial agreement with previous research showing that blockade of 5-HTT reduces marble-burying and other compulsive features, as well as attenuates social impairments and aggressiveness (Ichimaru et al. 1995; Joel et al. 2004; Berman et al. 2009; Schilman et al. 2010; Chadman 2011; Gould et al. 2011). The overall improvement of social functioning, in particular has been posited to reflect the effects of 5-HTT blockade on behavioral control and social information processing (Kolevzon et al. 2006; Phan et al. 2013). These results are particularly interesting, given the clinical effectiveness of 5-HTT inhibitors in reducing perseverative symptoms in patients (Hollander and Pallanti 2002).

Our immunohistochemical analyses revealed that MAO-A KO mice displayed marked reductions in 5-HTT expression across several key brain areas for emotional regulation, such as amygdala, nucleus accumbens core and CG3 region of the prefrontal cortex. The slight, yet significant reduction in 5-HTT brain expression in MAO-A KO mice is in line with previous binding results (Evrard et al., 2002). MAO-A^{Neo} animals showed even lower 5-HTT expression than their MAO-A KO counterparts and this effect encompassed most brain areas. Lower 5-HTT binding was documented in patients with obsessive-compulsive disorder, suggesting that reduced 5-HTT levels may contribute to compulsive traits (Matsumoto et al. 2010). Although several clinical studies have found genetic variations in 5-HTT in obsessive-compulsive disorder patients (Lin 2007; Bloch et al. 2008), our findings suggest that the reduction in 5-HTT binding may also be dependent from alterations in MAO-A activity. This intriguing possibility should be evaluated by future clinical studies in OCD patients. In this perspective, it is worth noting that, while 5-HTT is conventionally employed as a reliable quantitative marker for 5-HT neurons and fibers, our results appear to advocate caution with respect to this interpretation, given that alterations in this index may be also reflective of other abnormalities in expression and/or activity of MAO-A or other 5-HTergic molecules.

Given that MAO-A KO mice exhibit more pervasive behavioral deficits than those observed in MAO-A^{Neo} counterparts, and in view of the ameliorative effects of 5-HTT blockade on behavioral impairments, it is possible that the phenotypic differences between the two lines may reflect their different degrees of 5-HTT down-regulation. The reduced expression of 5-HTT in MAO-A^{Neo} mice, however, is unlikely to represent an impoverishment in 5-HT fibers in this transgenic line; indeed, WT, MAO-A^{Neo} and MAO-A KO mice display equivalent levels of tryptophan hydroxylase 2 immunoreactivity (as quantified by immunohistochemistry with polyclonal antibodies) in the dorsal raphe nucleus (Rick Lin; personal communication). Rather, the lower 5-HTT levels in MAOA^{Neo} mice may reflect a greater ability to enact compensatory processes to functionally modulate 5-HTergic neurons (due to the presence of low, yet functional, MAO-A protein). Alternatively, the marked decrease in 5-HTT compared to their KO counterparts may be due to variations in the penetrance and expressivity of the hypomorphic mutation, which may depend on numerous factors, including developmental and environmental components: for example, the mutated *Maoa-neo* chimeric transcript (or protein), albeit non-functional, may still serve other modulatory functions by interacting with other molecular targets, and modify the phenotypical outcomes of MAO-A deficiency.

In summary, our study shows that the genetic reduction and ablation of MAO-A activity results in perturbations in 5-HT homeostasis through changes in 5-HTT expression and function. These findings help clarify the functional impact of MAO-A with respect to 5-HTergic regulation. Moreover, our results indicate that acute 5-HTT blockade attenuates the social deficits, as well as perseverative behaviors exhibited by both MAO-A mutant lines. Future studies are warranted to study the acute effects of 5-HTT blockade on 5-HT release and 5-HT neuronal firing in MAO-A mutants. Subsequent experiments aimed at the delineation of region-specific roles of MAO-A and 5-HTT in the regulation of behavioral responses will be critical to define the involvement of 5-HT in aggressive behaviors and social impairments featured in neuropsychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

Abudy A, Juven-Wetzler A, Zohar J. Pharmacological management of treatment-resistant obsessive-compulsive disorder. CNS Drugs. 2011; 25:585–596. [PubMed: 21699270]

Aghajanian GK, Graham AW, Sheard MH. Serotonin-containing neurons in brain: depression of firing by monoamine oxidase inhibitors. Science. 1970; 169:1100–1102. [PubMed: 5449322]

Alia-Klein N, Goldstein RZ, Kriplani A, Logan J, Tomasi D, Williams B, Telang F, Shumay E, Biegon A, Craig IW, Henn F, Wang GJ, Volkow ND, Fowler JS. Brain monoamine oxidase A activity predicts trait aggression. Journal of Neuroscience. 2008; 28:5099–5104. [PubMed: 18463263]

Alzghoul L, Bortolato M, Delis F, Thanos PK, Darling RD, Godar SC, Zhang J, Grant S, Wang GJ, Simpson KL, Chen K, Volkow ND, Lin RC, Shih JC. Altered cerebellar organization and function in monoamine oxidase A hypomorphic mice. Neuropharmacology. 2012; 63:1208–1217. [PubMed: 22971542]

Berman ME, McCloskey MS, Fanning JR, Schumacher JA, Coccaro EF. Serotonin augmentation reduces response to attack in aggressive individuals. Psychological Science. 2009; 20:714–20. [PubMed: 19422623]

Blier P, de Montigny C. Serotoninergic but not noradrenergic neurons in rat central nervous system adapt to long-term treatment with monoamine oxidase inhibitors. Neuroscience. 1985; 16:949–955. [PubMed: 3005914]

Bloch MH, Landeros-Weisenberger A, Sen S, Dombrowski P, Kelmendi B, Coric V, Pittenger C, Leckman JF. Association of the serotonin transporter polymorphism and obsessive-compulsive disorder: systemic review. American Journal of Medical Genetics. Part B Neuropsychiatric Genetics. 2008; 147B:850–8.

Bortolato M, Chen K, Shih JC. Monoamine oxidase inactivation: from pathophysiology to therapeutics. Advanced Drug Delivery Reviews. 2008; 60:1527–1533. [PubMed: 18652859]

Bortolato M, Chen K, Godar SC, Chen G, Wu W, Rebrin I, Farrell MR, Scott AL, Wellman CL, Shih JC. Social deficits and perseverative behaviors, but not overt aggression, in MAO-A hypomorphic mice. Neuropsychopharmacology. 2011; 36:2674–2688. [PubMed: 21832987]

Bortolato M, Frau R, Piras AP, Luesu W, Bini V, Diaz G, Gessa G, Ennas MG, Castelli MP. Methamphetamine induces long-term alterations in reactivity to environmental stimuli: correlation with dopaminergic and serotonergic toxicity. Neurotoxicology Research. 2009a; 15:232–245.

Bortolato M, Godar SC, Davarian S, Chen K, Shih JC. Behavioral disinhibition and reduced anxiety-like behaviors in monoamine oxidase B-deficient mice. Neuropsychopharmacology. 2009b; 34:2746–2757. [PubMed: 19710633]

Bortolato M, Shih JC. Behavioral outcomes of monoamine oxidase deficiency: preclinical and clinical evidence. International Review of Neurobiology. 2011; 100:13–42. [PubMed: 21971001]

Bortolato M, Godar SC, Alzghoul L, Zhang J, Darling RD, Simpson KL, Bini V, Chen K, Wellman CL, Lin RC, Shih JC. Monoamine oxidase A and A/B knockout mice display autistic-like features. International Journal of Neuropsychopharmacology. 2013a; 16:869–888. [PubMed: 22850464]

Bortolato M, Godar SC, Tambaro S, Li FG, Devoto P, Coba MP, Chen K, Shih JC. Early postnatal inhibition of serotonin synthesis results in long-term reductions of perseverative behaviors, but not aggression in MAO-A-deficient mice. Neuropharmacology. 2013b; 75:223–32. [PubMed: 23871843]

Boulougouris V, Chamberlain SR, Robbins TW. Cross-species models of OCD spectrum disorders. Psychiatry research. 2009; 170:15–21. [PubMed: 19819024]

Brigman JL, Mathur P, Harvey-White J, Izquierdo A, Saksida LM, Bussey TJ, Fox S, Deneris E, Murphy DL, Holmes A. Pharmacological or genetic inactivation of the serotonin transporter improves reversal learning in mice. Cerebral Cortex. 2010; 20:1955–63. [PubMed: 20032063]

Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA. Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. Science. 1993; 262:578–580. [PubMed: 8211186]

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R. Role of genotype in the cycle of violence in maltreated children. Science. 2002; 297:851–4. [PubMed: 12161658]

Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC, Maeyer EA. Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science. 1995; 268:1763–1766. [PubMed: 7792602]

Chadman KK. Fluoxetine but not risperidone increases sociability in the BTBR mouse model of autism. Pharmacology, Biochemistry and Behavior. 2011; 97:586–594.

Coccaro EF, Kavoussi RJ. Fluoxetine and impulsive aggressive behavior in personality-disordered subjects. Archives of General Psychiatry. 1997; 54:1081–8. [PubMed: 9400343]

Coccaro EF, Lee R, McCloskey M. Norepinephrine function in personality disorder: plasma free MHPG correlates inversely with life history of aggression. CNS Spectrums. 2003; 8:731–6. [PubMed: 14712171]

Coccaro EF, Lee RJ, Kavoussi RJ. A double-blind, randomized, placebo-controlled trial of fluoxetine in patients with intermittent explosive disorder. Journal of Clinical Psychiatry. 2009a; 70:653–62. [PubMed: 19389333]

Coccaro EF, Noblett KL, McCloskey MS. Attributional and emotional responses to socially ambiguous cues: validation of a new assessment of social/emotional information processing in healthy adults and impulsive aggressive patients. Journal of Psychiatric Research. 2009b; 43:915–25. [PubMed: 19345371]

Coccaro EF, Sripada CS, Yanowitch RN, Phan KL. Corticolimbic function in impulsive aggressive behavior. Biological Psychiatry. 2011; 69:1153–9. [PubMed: 21531387]

Coccaro EF. What is the nature of serotonergic abnormalities in human aggression? Biological Psychiatry. 2012; 72:980–1. [PubMed: 23153521]

Daw ND, Kakade S, Dayan P. Opponent interactions between serotonin and dopamine. Neural Networks. 2002; 15:603–616. [PubMed: 12371515]

Evrard A, Malagie I, Laporte AM, Boni C, Hanoun N, Trillat AC, Seif I, De Maeyer E, Gardier A, Hamon M, Adrien J. Altered regulation of the 5-HT system in the brain of MAO-A knock-out mice. European Journal of Neuroscience. 2002; 15:841–851. [PubMed: 11906526]

Godar SC, Bortolato M, Frau R, Dousti M, Chen K, Shih JC. Maladaptive defensive behaviours in monoamine oxidase A-deficient mice. International Journal of Neuropsychopharmacology. 2011; 14:1195–1207. [PubMed: 21156093]

Gould GG, Hensler JG, Burke TF, Benno RH, Onaivi ES, Daws LC. Density and function of central serotonin (5-HT) transporters, 5-HT1A and 5-HT2A receptors, and effects of their targeting on BTBR T+tf/J mouse social behavior. Journal of Neurochemistry. 2011; 116:291–303. [PubMed: 21070242]

Harmer CJ. Serotonin and emotional processing: does it help explain antidepressant drug action? Neuropharmacology. 2008; 55:1023–1028. [PubMed: 18634807]

Hensler JG. Serotonergic modulation of the limbic system. Neuroscience Biobehavior Reviews. 2006; 30:203–214.

Hill RA, McInnes KJ, Gong EC, Jones ME, Simpson ER, Boon WC. Estrogen deficient male mice develop compulsive behavior. Biological Psychiatry. 2007; 61:359–366. [PubMed: 16566897]

Hollander, E.; Pallanti, S. Current and experimental therapeutics of OCD.. In: Davis, K.; Charney, D.; Coyle, J.; Nemeroff, C., editors. Neuropsychopharmacology: The fifth generation of progress. Lippincott Williams and Wilkins; Philadelphia: 2002. p. 1648-65.

Ichimaru Y, Egawa T, Sawa A. 5-HT1A-receptor subtype mediates the effect of fluvoxamine, a selective serotonin reuptake inhibitor, on marble-burying behavior in mice. Japanese Journal of Pharmacology. 1995; 68:65–70. [PubMed: 7494384]

Joel D, Ben-Amir E, Doljansky J, Flaisher S. 'Compulsive' lever-pressing in rats is attenuated by the serotonin re-uptake inhibitors paroxetine and fluvoxamine but not by the tricyclic antidepressant desipramine or the anxiolytic diazepam. Behavioral Pharmacology. 2004; 15:241–252.

Kim-Cohen J, Caspi A, Taylor A, Williams B, Newcombe R, Craig IW, Moffitt TE. MAOA, maltreatment, and gene-environment interaction predicting children's mental health: new evidence and a meta-analysis. Molecular Psychiatry. 2006; 11:903–13. [PubMed: 16801953]

Kolevzon A, Mathewson KA, Hollander E. Selective serotonin reuptake inhibitors in autism: a review of efficacy and tolerability. Journal of Clinical Psychiatry. 2006; 67:407–414. [PubMed: 16649827]

Lin PY. Meta-analysis of the association of serotonin transporter gene polymorphism with obsessive-compulsive disorder. Progress in Neuropsychopharmacology & Biological Psychiatry. 2007; 31:683–9.

Li X, Morrow D, Witkin JM. Decreases in nestlet shredding of mice by serotonin uptake inhibitors: comparison with marble burying. Life Sciences. 2006; 78:1933–1939. [PubMed: 16182315]

Malagie I, Trillat AC, Jacquot C, Gardier AM. Effects of acute fluoxetine on extracellular serotonin levels in the raphe: an in vivo microdialysis study. European Journal of Pharmacology. 1995; 286:213–217. [PubMed: 8605960]

Matsumoto R, Ichise M, Ito H, Ando T, Takahashi H, Ikoma Y, Kosaka J, Arakawa R, Fujimura Y, Ota M, Takano A, Fukui K, Nakayama K, Suhara T. Reduced serotonin transporter binding in the insular cortex in patients with obsessive-compulsive disorder: a [11C]DASB PET study. NeuroImage. 2010; 49:121–126. [PubMed: 19660554]

McGrath MJ, Campbell KM, Veldman MB, Burton FH. Anxiety in a transgenic mouse model of cortical-limbic neuro-potentiated compulsive behavior. Behavior Pharmacology. 1999; 10:435–443.

Merens W, Willem Van der Does AJ, Spinhoven P. The effects of serotonin manipulations on emotional information processing and mood. Journal of Affective Disorders. 2007; 103:43–62. [PubMed: 17363069]

Murray-Close D, Ostrov JM, Nelson DA, Crick NR, Coccaro EF. Proactive, reactive, and romantic relational aggression in adulthood: measurement, predictive validity, gender differences, and association with Intermittent Explosive Disorder. Journal of Psychiatric Research. 2010; 44:393–404. [PubMed: 19822329]

New AS, Buchsbaum MS, Hazlett EA, Goodman M, Koenigsberg HW, Lo J, Iskander L, Newmark R, Brand J, O'Flynn K, Siever LJ. Fluoxetine increases relative metabolic rate in prefrontal cortex in impulsive aggression. Psychopharmacology (Berl.). 2004; 176:451–8. [PubMed: 15160265]

Paxinos, GF.; Franklin, KBJ. The Mouse Brain in Stereotaxic Coordinates. Academic Press; San Diego: 2001.

Perry KW, Fuller RW. Effect of fluoxetine on serotonin and dopamine concentration in microdialysis fluid from rat striatum. Life Sciences. 1992; 50:1683–1690. [PubMed: 1375306]

Phan KL, Coccaro EF, Angstadt M, Kreger KJ, Mayberg HS, Liberzon I, Stein MB. Corticolimbic brain reactivity to social signals of threat before and after sertraline treatment in generalized social phobia. Biological Psychiatry. 2013; 73:329–36. [PubMed: 23164370]

Piton A, Poquet H, Redin C, Masurel A, Lauer J, Muller J, Thevenon J, Herenger Y, Chancenotte S, Bonnet M, Pinoit JM, Huet F, Thauvin-Robinet C, Jaeger AS, Le Gras S, Jost B, Gérard B, Peoc'h K, Launay JM, Faivre L, Mandel JL. 20 ans après:a second mutation in MAOA identified by targeted high-throughput sequencing in a family with altered behavior and cognition. European Journal of Human Genetics. in press.

Puglisi-Allegra S, Oliverio A. Naloxone potentiates shock-induced aggressive behavior in mice. Pharmacology, Biochemistry and Behavior. 1981; 15:513–4.

Ralph RJ, Paulus MP, Fumagalli F, Caron MG, Geyer MA. Prepulse inhibition deficits and perseverative motor patterns in dopamine transporter knock-out mice: differential effects of D1 and D2 receptor antagonists. Journal of Neuroscience. 2001; 21:305–313. [PubMed: 11150348]

Rebsam A, Seif I, Gaspar P. Dissociating barrel development and lesion-induced plasticity in the mouse somatosensory cortex. Journal of Neuroscience. 2005; 25:706–710. [PubMed: 15659608]

Reimold M, Smolka MN, Zimmer A, Batra A, Knobel A, Solbach C, Mundt A, Smoltczyk HU, Goldman D, Mann K, Reischl G, Machulla HJ, Bares R, Heinz A. Reduced availability of serotonin transporters in obsessive-compulsive disorder correlates with symptom severity - a [11C]DASB PET study. Journal of Neural Transmission. 2007; 114:1603–1609. [PubMed: 17713719]

Rosell DR, Thompson JL, Slifstein M, Xu X, Frankle WG, New AS, Goodman M, Weinstein SR, Laruelle M, Abi-Dargham A, Siever LJ. Increased serotonin 2A receptor availability in the orbitofrontal cortex physically aggressive personality disordered patients. Biological Psychiatry. 2010; 67:1154–62. [PubMed: 20434136]

Rylands AJ, Hinz R, Jones M, Holmes SE, Feldmann M, Brown G, McMahon AW, Talbot PS. Pre- and postsynaptic serotonergic differences in males with extreme levels of impulsive aggression without callous unemotional traits: a positron emission tomography study using (11)C-DASB and (11)C-MDL100907. Biological Psychiatry. 2012; 72:1004–11. [PubMed: 22835812]

Schilman EA, Klavir O, Winter C, Sohr R, Joel D. The role of the striatum in compulsive behavior in intact and orbitofrontal-cortex-lesioned rats: possible involvement of the serotonergic system. Neuropsychopharmacology. 2010; 35:1026–1039. [PubMed: 20072118]

Scott AL, Bortolato M, Chen K, Shih JC. Novel monoamine oxidase A knock out mice with human-like spontaneous mutation. Neuroreport. 2008; 19:739–743. [PubMed: 18418249]

Sharp T, Umbers V, Gartside SE. Effect of a selective 5-HT reuptake inhibitor in combination with 5-HT1A and 5-HT1B receptor antagonists on extracellular 5-HT in rat frontal cortex in vivo. British Journal of Pharmacology. 1997; 121:941–946. [PubMed: 9222551]

Siever LJ. Neurobiology of aggression and violence. American Journal of Psychiatry. 2008; 165:429–42. [PubMed: 18346997]

Simon P, Dupuis R, Costentin J. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behavioral Brain Research. 1994; 61:59–64.

Soliman A, Bagby RM, Wilson AA, Miler L, Clark M, Rusjan P, Sacher J, Houle S, Meyer JH. Relationship of monoamine oxidase A binding to adaptive and maladaptive personality traits. Psychological Medicine. 2011; 41:1051–1060. [PubMed: 20810002]

Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor R. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology. 2009; 204:361–373. [PubMed: 19189082]

Treit D, Fundytus M. Thigmotaxis as a test for anxiolytic activity in rats. Pharmacology, Biochemistry and Behavior. 1988; 31:959–962.

Vitalis T, Cases O, Callebert J, Launay JM, Price DJ, Seif I, Gaspar P. Effects of monoamine oxidase A inhibition on barrel formation in the mouse somatosensory cortex: determination of a sensitive

developmental period. Journal of Comparative Neurology. 1998; 393:169–184. [PubMed: 9548695]

Young SN, Leyton M. The role of serotonin in human mood and social interaction. Insight from altered tryptophan levels. Pharmacology, Biochemistry and Behavior. 2002; 71:857–865.

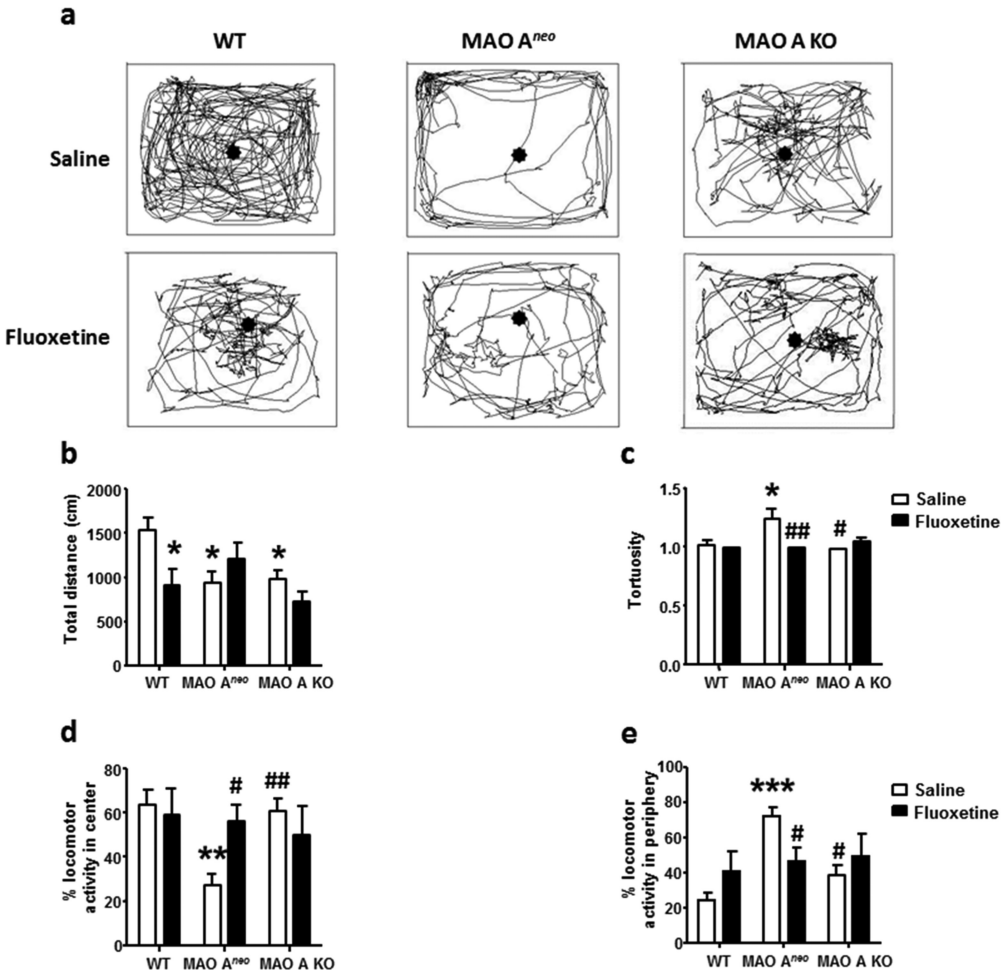

Fig. 1.

Perseverative thigmotaxis and tortuosity in MAO-A^{Neo} mice is corrected by 5-HTT blockade. (A) Typical locomotor pathways of WT, MAO-A^{Neo} and MAO-A KO mice treated with either saline or the 5-HTT inhibitor fluoxetine. (B) Saline-treated MAO-A^{Neo} and MAO-A KO mice showed a significant reduction in locomotor activity in comparison with WT counterparts. Although fluoxetine did not affect the locomotor activity in either mutant line, fluoxetine decreased the locomotor activity of WT mice. (C-E) Saline-treated MAO-A^{Neo} mice displayed a significant increase in thigmotaxis and tortuosity compared to their MAO-A KO and WT counterparts. These abnormal behaviors were prevented by pretreatment with fluoxetine. Values are displayed as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 compared to saline-treated WT mice. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared to saline-treated MAO-A^{Neo} mice. Main effects are not indicated. For more details, see text.

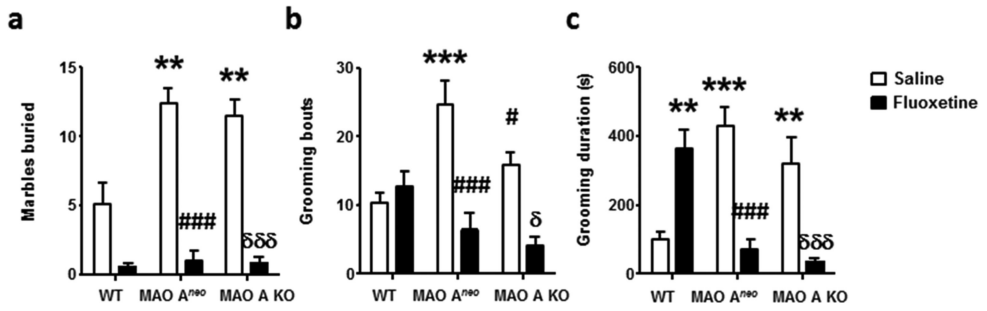

Fig. 2.

5-HTT inhibition abolishes perseverative behaviors in MAO-A^{Neo} and MAO-A KO mice. (A) Saline-treated MAO-A KO and MAO A^{Neo} mice exhibited significantly higher marble burying activity compared to saline-treated WT mice. These perseverative behaviors were countered by 5-HTT inhibition in both mutant lines. (B-C) Saline-treated MAO A^{Neo} mice displayed a marked increase in water mist-induced grooming bouts and both saline-treated mutant lines showed higher grooming duration than WT mice. Fluoxetine reduced grooming frequency in MAO-A^{Neo} mice and grooming duration in both MAO-A KO mutant lines. In contrast, fluoxetine increased the grooming duration in WT animals. Values are displayed as the mean ± SEM. **P<0.01 and ***P<0.001 compared to WT saline group. $^{\#}$P<0.05 and $^{\#\#\#}$P<0.001 compared to MAO-A^{Neo} saline treatment. $^{\delta}$P<0.05 and $^{\delta\delta\delta}$P<0.001 compared to MAO-A KO mice treated with saline. Main effects are not indicated. For more details, see text.

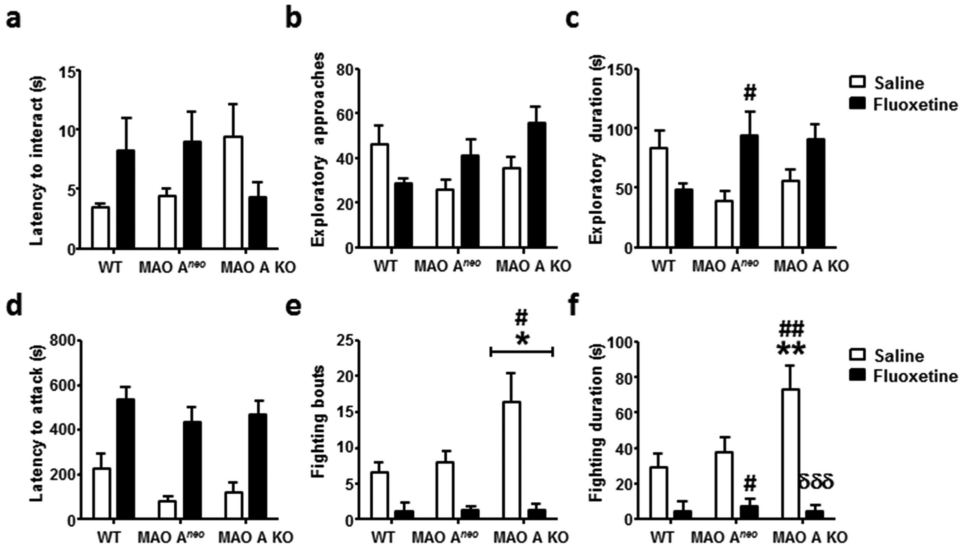

Fig. 3.

5-HTT blockade elicits diverse effects on aggressive and social behaviors in MAO-A^{Neo} and MAO-A KO mice. (A-B) No significant differences were detected in the latency to interact with a novel conspecific or the number of social approaches in any genotype. (C) 5-HTT inhibition significantly increased the duration of social behavior of MAO-A^{Neo} mice. (D) Fluoxetine nonspecifically increased the latency to attack and (E) reduced fighting behaviors in all three genotypes. MAO-A KO mice showed an increase in fighting bouts compared to their MAO-A^{Neo} and WT counterparts. (F) The high levels of aggression in MAO-A KO mice were significantly decreased by fluoxetine treatment. Fluoxetine also significantly decreased fighting duration in MAO-A^{Neo} mutants. Values are displayed as the mean ± SEM. *P< 0.05 and **P<0.01 compared to WT mice treated with saline. $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared to saline-treated MAO-A^{Neo} mice. $^{\delta\delta\delta}$P<0.001 compared to MAO-A KO mice treated with saline. Main effects are not indicated. For more details, see text.

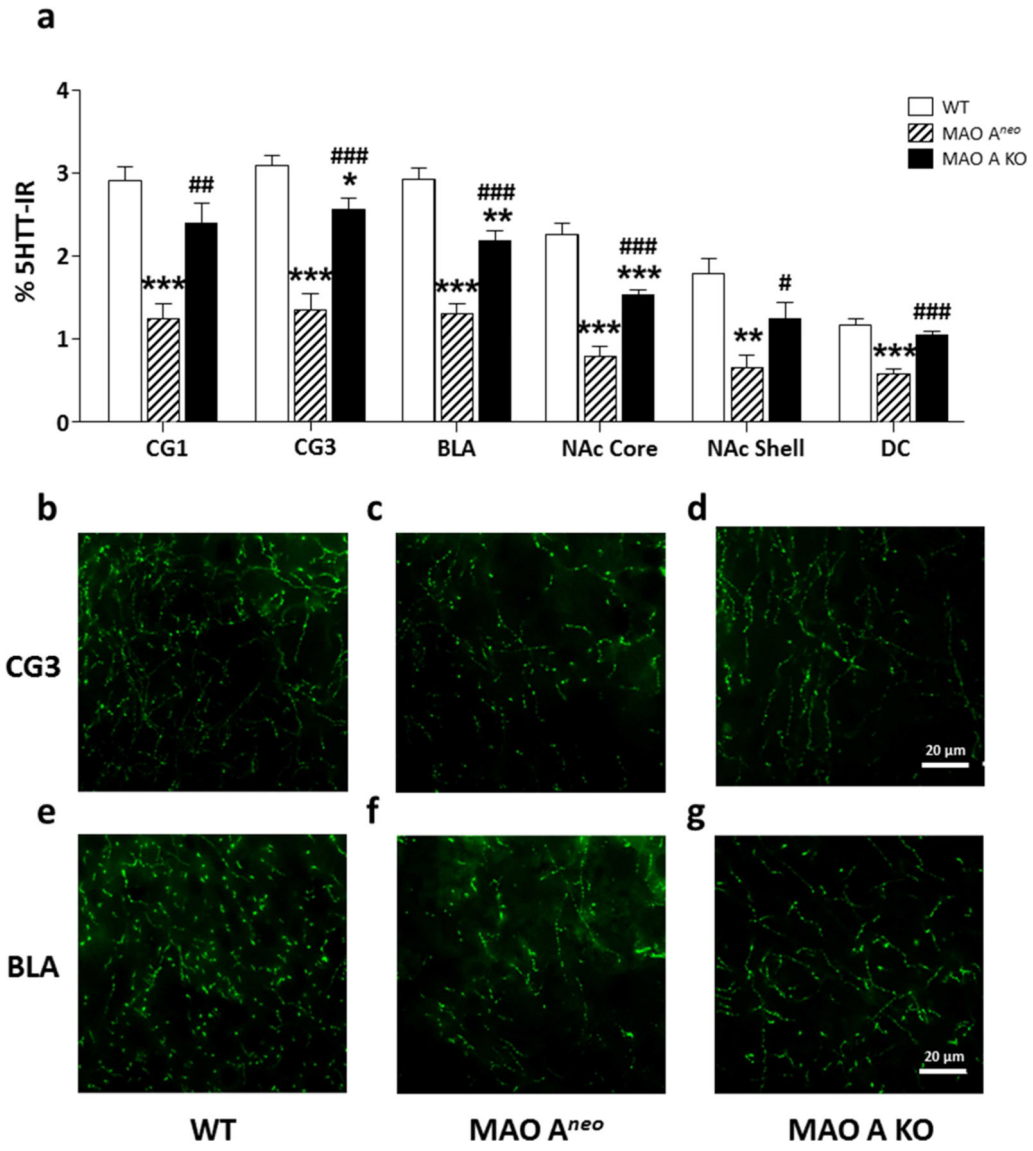

Fig. 4.

Hypomorphic MAO-A^{Neo} mice exhibit a profound reduction in 5-HTT immunoreactivity (IR) in several brain regions. (A) MAO-A^{Neo} mice show a significant reduction in % 5-HTT IR compared to both WT and MAO-A KO mice in cingulate area 1 (CG1), cingulate area 3 (CG3), basolateral amygdala (BLA), nucleus accumbens (NAc) core and shell and dorsal caudate (DC). MAO-A KO mice display a reduction of 5-HTT immunoreactivity in the CG3, BLA and NAc core regions in comparison to WT counterparts. (B-D) Representative images of 5-HTT immunofluorescent patterns in CG3 across genotypes. (E-G) Representative images of 5-HTT immunofluorescent patterns in BLA across genotypes. Values are displayed as the mean ± SEM. *P<0.05, **P<0.01, and ***P<0.001 compared to WT mice. $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 compared to MAO-A^{Neo} mice. IR, immunoreactivity; 5-HTT, Serotonin transporter; CG3, Cingulate area 3; CG1, Cingulate area 1; BLA, Basolateral Amygdala; NAc, Nucleus Accumbens; DC, Dorsal Caudate.

Table 1

Paradigm	Behavior	WT	MAO A^{Neo}	MAO A KO
Open field	Distance traveled	↓*	Ø	Ø
	Tortuosity	Ø	↓*	↑
	% Activity in center	Ø	↑**	Ø
	%Activity in periphery	Ø	↓**	Ø
Marble burying	Marbles buried	Ø	↓***	↓***
Water mist-induced grooming	Grooming episodes	Ø	↓***	↓***
	Grooming duration	↑***	↓***	↓**
Social interaction	Latency to first approach	Ø	0	Ø
	Social approaches	Ø	↑*	↑*
	Social duration	Ø	↑*	Ø
Resident intruder	Latency to first attack	↑**	↑**	↑**
	Fighting episodes	↓*	↓**	↓**
	Fighting duration	↓*	↓**	↓***