HHS Public Access

Author manuscript

Eur Arch Psychiatry Clin Neurosci. Author manuscript; available in PMC 2024 February 01.

Published in final edited form as:

Eur Arch Psychiatry Clin Neurosci. 2023 March ; 273(2): 411–425. doi:10.1007/s00406-022-01480-y.

Elevated levels of serotonin 5-HT_{2A} receptors in the orbitofrontal cortex of antisocial individuals

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Abstract

Aims: Antisocial behavior (ASB) is characterized by frequent violations of the rights and properties of others, as well as aggressive conduct. While ample evidence points to a critical role of serotonin in the emotional modulation of social responses, the implication of this neurotransmitter in ASB is unclear. Here, we performed the first-ever postmortem analysis of serotonergic markers in the orbitofrontal cortex (OFC) of male subjects with ASB (n=9). We focused on this brain region, given its well-recognized role in ASB pathophysiology. Given that all subjects also had a diagnosis of substance use disorder (SUD), we used two age-matched control groups: SUD-only and unaffected controls.

Methods: Tissues were processed for immunoblotting analyses on eight key serotonergic targets: tryptophan hydroxylase 2 (TPH2), the rate-limiting enzyme of brain serotonin synthesis; serotonin transporter (SERT), the main carrier for serotonin uptake; monoamine oxidase A (MAOA), the primary enzyme for serotonin catabolism; and five serotonin receptors previously documented to influence social behavior: 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_4. Analyses were performed by nested t-test.

Results: We found a significant increase of 5-HT_{2A} receptor levels in the ASB+SUD group compared with SUD-only controls. Furthermore, TPH2 levels were significantly reduced in the SUD group (including SUD-only and ASB+SUD) in comparison with unaffected controls. No difference was detected in the expression of any other serotonergic target.

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AUTHOR CONTRIBUTIONS

GB performed immunoblotting experiments, and helped with the preparation of figures, tables, and the first draft of the manuscript. SS performed statistical analyses, prepared the figures, and wrote the first draft of the manuscript. MB conceptualized the project, obtained human brain samples and funding, designed the experiments, and wrote the final version of the manuscript.

CONFLICT OF INTEREST

The authors report no conflict of interest.

ETHICS STATEMENT

Tissues were obtained from the Brain Tissue Repository at the University of Pittsburgh, through the NIH NeuroBioBank program. All brain tissues were procured, stored, and distributed according to state and federal guidelines, and regulation involving consent, protection of human subjects, and donor anonymity.

Conclusions: These results are in keeping with previous evidence showing high 5-HT_{2A} receptor binding in the OFC of pathologically aggressive individuals and point to this molecule as a potential target for ASB treatment.

Keywords

Antisocial behavior; orbitofrontal cortex; serotonin; postmortem samples

INTRODUCTION

Antisocial behavior (ASB) is a persistent pattern of violation of societal norms, often entailing damage to the basic rights and properties of others as well as covert and overt hostility[1]. Multiple disorders in the DSM-5 are characterized by pervasive ASB, such as conduct disorder (CD) in children and adolescents and antisocial personality disorder (ASPD) in adults. The prevalence of CD and ASPD are respectively estimated at 9.5%[2] and 1–4%[3–4]. Both disorders are characterized by a marked male predominance, with gender ratios ranging from 3 to 7:1[5–7].

One of the major features of ASB is the impairment of self- and interpersonal functioning with specific symptoms of antagonism and disinhibition. Although ASB is not limited to aggressive manifestations, both verbal and physical aggression are common symptoms of both CD and ASPD[8,9]. Furthermore, these disorders are highly comorbid with several major mental disorders[10–12], and particularly with substance use disorders (SUD), which are estimated to be present in more than half of adolescent CD patients[13] and nearly 90% of ASPD patients[14].

The socio-economic impact of ASB is staggering, in consideration of its association with high rates of violent behavior and criminal offending[15,16]. Making matters worse, no therapies have been approved by the Food and Drug Administration (FDA) for the treatment of CD and ASPD, partially reflecting our limited knowledge of the biological and neurochemical basis of these disorders. In particular, most available evidence on the neurobiology of ASB is based on neuroimaging and genetic findings, but very little is known about the molecular foundation of antisociality. For example, to the best of our knowledge, no studies have ever been performed on postmortem samples of antisocial individuals.

Ample evidence has shown that ASB is associated with deficits in facial affect processing[17], which lead to maladaptive social responses[18–20]. The main brain area that regulates the interpretation of social cues and the enactment of appropriate emotional response is the orbitofrontal cortex (OFC)[21,22]. In fact, this region is activated by exposure to negative emotional expression, including anger, social disapproval, guilt-related emotion, shame, and embarrassment[23,24], and its lesions reduce social expression recognition and guilt experience[22,25]. In line with this evidence, several studies have shown a key implication of the OFC in prosocial responses. For example, the results of a recent prospective study have shown a significant thinning of the OFC in affected individuals with life-course-persistent ASB [26]. These data are consistent with previous reports showing that the gray matter of the OFC is reduced in relation to aggression and antisocial traits[27–29].

Previous studies have shown that the regulation of aggression and social responses by the OFC is directly shaped by the serotonergic system[30]. Indeed, serotonin dysfunctions are a major risk factor for aggressive behavior and other key components of antisocial behavior, such as punishment sensitivity and loss of behavioral control[31]. Furthermore, alterations of serotonin metabolism have been shown in ASPD[32]. Despite these important premises, only little is known about the alterations of the serotonergic system in the OFC of antisocial individuals.

Building on these premises, here we conducted the first-ever study of postmortem OFC samples from male individuals affected by ASPD, to assess the levels of key serotonin targets in this brain region. In particular, we used immunoblotting to test the levels of the following targets: 1) tryptophan hydroxylase 2 (TPH2), the enzyme catalyzing the rate-limiting step of serotonin biosynthesis; 2) serotonin transporter (SERT), the main carrier of serotonin synaptic uptake; 3) monoamine oxidase A, the main serotonin-catabolic enzyme, and 4–8) the serotonin receptors 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_4, in consideration of their extensive association with aggression and ASB-related characteristics[33]. Given the high comorbidity of ASPD with SUD, we used two sets of controls: age-matched controls with a SUD diagnosis as well as non-affected controls.

MATERIALS AND METHODS

Human Brain Collection and Donor Information.

OFC tissues were obtained from the NIH NeuroBioBank (NBB) Brain and Tissue Repository (BTR) at the University of Pittsburgh. The right hemisphere of each brain was dissected in the coronal plane, immediately frozen and stored at −80°C in accordance with the policies and procedures utilized by the BTRs participating in the NIH NBB. Consent was obtained according to legal provisions from the next of kin. DSM5 diagnoses were made by experienced research clinicians using structured interviews with family members and/or review of prior medical records. The absence of a psychiatric diagnosis was confirmed in unaffected control subjects using the same approach. The analysis was performed in post-mortem OFC tissue and associated clinical data including age, sex, brain pH and post-mortem interval (PMI) for age- and sex-matched cohorts of 9 individuals receiving SUD diagnoses, 9 individuals receiving diagnoses of ASB+SUD and 9 individuals with no history of psychiatric disease (Table 1). As evidenced in Table 2, all subjects were males, and no significant differences were found between groups with respect to either demographic characteristics (age and race distribution) or indices of tissue quality (PMI, pH, and RIN).

Immunoblotting.

Tissues were stored at −80°C until assayed. To analyze the expression levels of MAOA, SERT, TPH2 and 5-HT receptors, tissues were weighted and diluted (1 mg/10 μl) in RIPA buffer containing 20mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1mM beta-glycerophosphate, 1mM Na_3VO_4, 1 μg/ml leupeptin and protease inhibitor cocktail.

Small aliquots of the homogenate were used for protein determination using a modified Lowry protein assay method (DC protein assay, Bio-Rad Laboratories, Hercules, CA). Samples containing 30 μg of total proteins were run onto 4–15% Criterion™ TGX Stain-free™ precast gels (Bio-Rad Laboratories) and transferred to nitrocellulose membranes (Bio-Rad Laboratories). Stain-free™ gel formulation includes a trihalo compound that, when exposed to ultraviolet (UV) irradiation, generates a covalent reaction with tryptophan residues of proteins and allows them to be visualized within the gel or after transfer to a blotting membrane. Following protein transfer, the membrane was detected by UV and blot image was collected for total protein.

Primary antibodies for anti-5-HT_{2A} (rabbit polyclonal #ab66049 Abcam, Cambridge, UK; dilution 1:100), anti-MAOA (mouse monoclonal #sc271123 Santa Cruz Biotechnology, Dallas, TX; dilution 1:750); anti-SERT (rabbit polyclonal #ab272912 Abcam; dilution 1:1000); anti-5-HT_{1A} (rabbit polyclonal #ab85615 Abcam; dilution 1:1000); anti-5-HT_{1B} (rabbit polyclonal #NB56350 Novus Biologicals, Centennial, CO; dilution 1:1000); anti-5-HT_{2C} (goat polyclonal #NB1524 Novus Biologicals; dilution 1:750); anti-5-HT_4 (rabbit polyclonal #ab60359 Abcam; dilution 1:1000); anti-TPH2 (rabbit polyclonal #PA1–778 Thermo Fisher Scientific, Waltham, MA; dilution 1:750) were incubated in TBS-T containing 3% (w/v) BSA buffer overnight at 4 °C. Next, blots were washed in TBS-T, and then incubated in TBS-T containing goat anti-rabbit HRP-conjugated (#31462, Thermo Fisher Scientific; dilution 1:10000), goat anti-mouse HRP-conjugated (#31430, Thermo Fisher Scientific; dilution 1:5000) or rabbit anti-goat HRP-conjugated (#5160–2504, Bio-Rad laboratories; dilution 1:10000) secondary antibodies, for 90 minutes at room temperature. Chemiluminescence was detected with the ChemiDoc™ XRS^+ Imaging System using the Clarity Western ECL substrate (Bio-Rad Laboratories). Bands were quantified in arbitrary units and normalized using the software Image Lab (Bio-Rad Laboratories). Samples containing the same amounts of total proteins in each experimental group were run on the same immunoblots and then analyzed together.

To accurately compare western blot signal in normalization procedure and control for potential losses of gray matter in ASB brain samples, stain-free total proteins signals were used[34,35]. Indeed, normalization based on housekeeping protein (e.g. b actin) may be suboptimal in this context since reduced gray matter, surface area and thickness of prefrontal cortex have been described in individuals with ASB[26,27,29,36,37]. However, since actin is a crucial component of cellular machinery to maintain cell morphology and trafficking, membranes were stripped and re-probed with primary antibody anti-β-actin (mouse monoclonal #sc47778 Santa Cruz Biotechnology) in order to determine its expression as index of cytoarchitectural changes. No significant changes in b actin levels were found across samples ($F_{1, 25}$= 0.14, P= 0.708).

Statistical analysis.

Demographic and neurochemical data were analyzed by Nested T-Test using GraphPad Prism package (GraphPad, San Diego, CA, USA) and expressed as means ± SEM. Race and sex distribution were compared across group by chi-square statistics. Univariable regression analysis was used to test whether PMI or age modifies significantly brain proteins levels.

Whenever PMI was found to be associated with protein expression, this index was used as a covariate in multiple regression analyses to assess its effect on potential differences in protein levels across diagnostic groups. To control for multiple comparisons, Bonferroni's correction was used, and significance was set at $\alpha < 0.00625$.

RESULTS

TPH2.

We evaluated TPH2 expression since reduced TPH2 levels and function have been associated with aggression-related personality traits in humans[38] and *tph2* null mice exhibited impulsive and hyperaggressive behavior[39]. Analyses showed that subjects with a history of SUD (irrespective of ASB) exhibited a significant decrease in TPH2 levels in comparison with unaffected controls ($F_{1,25}$ = 18.26; P=0.0002) (Figs. 1A–B). However, no differences were shown between the ASB + SUD and SUD-only groups (Fig. 1A). Regression analyses showed no statistically significant association between TPH2 levels and either PMI ($F_{1,25}$=1.64; R^2=0.06, P=0.21) (Fig. 1C) or age ($F_{1,25}$=2.19; R^2=0.08, P=0.15) (Fig. 1D).

SERT.

We next measured SERT levels, since genetic polymorphisms of this carrier have been associated with phenotypes related to ASB, including negative emotionality, aggression, violent behavior, impulsivity, and disinhibition[40–43]. Our results revealed no significant differences between samples from SUD-affected subjects and unaffected controls ($F_{1,25}$ = 1.31; P=0.47) or between SUD-only and ASB +SUD groups (Fig. 2A–B). The analysis of regression showed that PMI was negatively associated with SERT levels [$F_{1,25}$=12.04; R^2=0.33, P=0.002] (Fig.2C); however, using PMI as a covariate in ANCOVA to compare for potential differences between groups confirmed the lack of significant differences in protein levels across groups (P = 0.144). Finally, no relation was found between age and SERT protein levels ($F_{1,25}$=0.24; R^2=0.009, P=0.63) (Fig. 2D).

MAOA.

MAOA is the main enzyme catalyzing the degradation of 5-HT[44] and is the best-known molecular target implicated in the biology of ASB and aggression[45,46]. Surprisingly, our analyses revealed no significant differences in MAOA protein expression levels between samples from SUD-affected subjects and unaffected controls ($F_{1,25}$ =0.71; P=0.405) or between SUD-only and ASB+SUD groups (Fig. 3A–B). Furthermore, regression analyses showed no significant associations between MAOA levels and either PMI ($F_{1,25}$=0.78; R^2=0.03, P=0.39) (Fig. 3C) or age ($F_{1,25}$=0.89; R^2=0.03, P=0.35) and MAOA levels (Fig.3D).

5-HT_{1A}.

We then measured $5HT_{1A}$ receptors, given that pharmacological activation of this receptor has been shown to reduce aggression in patients[47–49] and animal[50,51] (but also see Ref. 52 for contrasting results). No significant differences were found between samples from SUD-affected subjects and unaffected controls ($F_{1,25}$ =0.33; P=0.57) or between SUD-only

and ASB+SUD groups (Fig. 4A). Regression analyses of 5-HT1$_A$ receptors revealed no significant association of 5-HT$_{1A}$ levels with either PMI ($F_{1,25}$=0.47; R^2=0.02, P=0.49) (Fig. 4C) or age ($F_{1,25}$=2.13; R^2=0.08, P=0.16) (Fig. 4D).

5-HT$_{1B}$.

Ample evidence points to a direct implication of 5-HT$_{1B}$ receptors in the neurobiology of aggression and ASB[53–56]. Similar to what observed for 5-HT$_{1A}$ receptors, no significant differences were found between samples from SUD-affected subjects and unaffected controls ($F_{1,25}$ =0.008; P=0.93) or between SUD-only and ASB+SUD groups (Fig. 5A–B). 5-HT$_{1B}$ levels were not associated with either PMI ($F_{1,25}$=0.68; R^2=0.0265, P=0.42) (Fig. 5C) or age ($F_{1,25}$=0.97; R^2=0.0374, P=0.333) (Fig. 5D).

5-HT$_{2A}$.

Previous studies have shown that 5-HT$_{2A}$ receptors availability in OFC is increased in individuals with aggressive personality[57]. Consistently with these data, we found a significant elevation of 5-HT$_{2A}$ receptor in the ASB+SUD group compared with SUD-only controls (χ^2 =8.43; P=0.004); in contrast, no significant differences between SUD-affected individuals and unaffected controls ($F_{1,25}$ =0.006; P=0.95) were found (Fig.6A-B). No association was observed between 5-HT$_{2A}$ levels and either PMI ($F_{1,25}$=0.34; R^2=0.01, P=0.56) (Fig. 6C) or age ($F_{1,25}$=0.76; R^2=0.03, P=0.40; Fig.6D). These results suggest that enhanced 5-HT$_{2A}$ receptor expression in the OFC may be a marker of ASB.

5-HT$_{2C}$.

Selective 5-HT$_{2C}$ activation reduces impulsive aggression[58], and its polymorphism rs3618 has been associated with criminal behavior[59]. These premises notwithstanding, no significant differences were found between samples from SUD-affected subjects and unaffected controls ($F_{1,25}$ =1.01; P=0.32) or between SUD-only and ASB+SUD groups (Fig. 7A–B). Finally, 5-HT$_{2C}$ levels were not significantly associated to either PMI ($F_{1,25}$=0.04; R^2=0.001, P=0.85) (Fig. 7C) or age ($F_{1,25}$=1.87; R^2=0.07, P=0.18) (Fig. 7D).

5HT$_4$.

Finally, we evaluated 5-HT$_4$ expression levels, since neuroimaging studies reported an association between 5-HT$_4$ receptor binding and trait aggression in the whole brain, in a sex- and age-dependent fashion[60]. However, no significant changes were found in 5-HT$_4$ levels between SUD-affected and unaffected controls ($F_{1,25}$ = 0.36; P=0.0002) and between the ASB + SUD and SUD-only groups (Fig. 8A–B). The regression analysis of 5-HT$_4$ levels in relation to PMI (Fig. 8C) or age (Fig. 8D) revealed no statistically significant effects [PMI: $F_{1,25}$=0.37; R^2=0.01, P=0.54; age: $F_{1,25}$=0.007; R^2=0.0001, P=0.93].

DISCUSSION

The main results of this study show that, in postmortem OFC samples of subjects with a diagnosis of SUD in combination with either CD or ASPD, the protein levels of 5-HT$_{2A}$ receptors were significantly elevated in comparison with age-matched controls with SUD only. Conversely, no differences were observed in the OFC expression of other serotonergic

molecules, including the biosynthetic and catalytic enzymes TPH2 and MAOA, the reuptake carrier SERT, and key serotonin receptors implicated in the modulation of aggression.

These findings are in substantial evidence with previous neuroimaging data indicating high binding density of 5-HT_{2A} receptors in the OFC of aggressive individuals[57]; furthermore, previous reports documented that the expression of these receptors was increased in the OFC of young suicide victims (who typically manifest high levels of aggression)[61] and was correlated with lifetime aggression scores[62]. Finally, impulsive aggression levels were found to be correlated with 5-HT_{2A} platelet binding[63], even though this index is not representative of 5-HT_{2A} binding in the cortex[64]. As mentioned above, aggression is a frequent pathognomonic characteristic of CD and ASPD, and, particularly in early life, it serves as a robust predictor of ASB[65], generally suggesting that 5-HT_{2A} receptor in the OFC may also be an important biomarker of antisociality. In partial support of this conclusion, genetic data have also shown associations between 5-HT_{2A} genetic polymorphisms and aggression in the general population[66–68] as well as in antisocial individuals[69].

As previously mentioned, the OFC attaches emotional valence to social and environmental cues, and codes the motivational attributes of responses to these stimuli by regulating the activation of the amygdala[70,71]. For example, several studies have shown that the OFC integrates facial affective information of others[21,72], and this role is likely due to an inhibitory control over amygdaloid activation[73]. From this perspective, it is worth noting that 5-HT_{2A} receptors play a critical role in processing emotionally salient information[74] by modulating the connectivity between OFC and amygdala. For example, inter-individual variations in 5-HT_{2A} receptor density in the prefrontal cortex are inversely correlated with the activation of the amygdala by angry or fearful faces[75], and the OFC response to these facial expressions is reduced by antagonism of these receptors[76].

The function of 5-HT_{2A} receptors in the neural processing of emotionally salient facial expression is in line with previous evidence on the role of serotonin in this function[77,78]. These data suggest that the upregulation of 5-HT_{2A} receptors in the OFC may alter the connectivity of this region with the amygdala and impair the recognition of facial affect, thereby predisposing to aggression and ASB.

The results of our analyses did not reveal any other difference in the levels of any other serotonergic molecule with respect to the comparison between SUD + ASB and SUD-only subjects. These findings are surprising, given the strong implication of serotonin in the function of the OFC in the neural processing of emotional salient facial expressions[77,78]. Serotonin is also involved in the ontogeny of ASB[79] and aggression[33, 80–82]. Particularly striking is the lack of differences in MAOA levels, considering the critical role of this enzyme in the ontogeny of ASB (for a comprehensive review, see Ref. 46). An ample body of evidence has particularly indicated that low activity of MAOA in the OFC and other brain region is a critical predictor of trait aggression[83]. In contrast with these results, our present findings documented no differences in enzyme expression between different antisocial individuals and their controls. Notably, the best-documented involvement of MAOA in ASB is related to the interaction of low-activity *MAOA* genotypes and child maltreatment[84,85]. We recently reproduced this gene x environment interaction in mice and found that this

construct leads to increased aggression via an upregulation of 5-HT_{2A} receptors in the OFC and other portions of the prefrontal cortex[86]. These data point to the possibility that, even though MAOA may be only one of the mechanisms underlying ASB, the upregulation of 5-HT_{2A} receptors in the OFC is the key downstream mechanism whereby low MAOA predisposes to ASB.

We found a significant reduction in TPH2 levels in the OFC samples from SUD-affected individuals, in comparison with age-matched healthy controls. Given that TPH2 is the enzyme catalyzing the rate-limiting step of serotonin synthesis in the brain[87,88], these data suggest that the OFC in SUD may exhibit low levels of serotonin. Previous findings showed that TPH2 genetic variants predict serotonin synthesis in human OFC, again pointing to the critical influence of this enzyme in serotonin synthesis in this region[89]. In correspondence of this idea, a reduction in serotonin synthesis has been shown to predispose to some components of impulsivity[90,91], a key trait in SUD predisposition. In line with this concept, serotonin stimulation in the OFC increases the patience for a reward in mice[92]. These data complement recent reports on the critical role of serotonin in the modulation of the role of the OFC in decision-making related to drug seeking[93,94]. Nevertheless, it should be noted that our analysis was not specifically focused on one type of SUD but used this diagnostic category only as an internal control for ASB, given the high comorbidity of CD and ASPD with drug misuse. Furthermore, our study did not specifically address any of the heterogeneous constructs encompassed by SUD, such as abuse, addiction, or abstinence. Thus, extreme caution should be advocated on drawing conclusions from the present findings on the lower expression of TPH2 in SUD-affected individuals. Even so, these studies highlight the need for future analyses on TPH2 expression in the OFC of individuals affected by alcohol use disorder as well as other SUDs.

Our analyses on the potential influence of PMI and age on the expression of serotonin receptors pointed to a significant inverse association between this index and SERT levels, which was not paralleled by similar findings on any other serotonergic target. While our analyses showed that this effect did not interfere with the comparisons across different diagnostic groups, these results are interesting, as they appear to suggest a specific sensitivity of the serotonin carrier to post-mortem degradative processes, at least in the OFC. The degradation of SERT in the brain is a relatively fast process[95] requiring PKC-dependent phosphorylation and internalization[96,97]. Thus, our findings may be in line with previous data showing that PMI has a major impact on the levels of proteins targeted by phosphorylation, as detected by western blotting[98]. Alternatively, the observed decrease in SERT levels may reflect a PMI-dependent degradation in serotonergic axon (given the presynaptic nature of SERT); however, this possibility is challenged by the fact that, in our dataset, only SERT, and not other (mainly) presynaptic targets were affected by PMI (such as 5- HT_{1B} receptors). Furthermore, no correlation was found between PMI and the length of SERT-immunoreactive of the OFC[99].

Several limitations should be acknowledged. First, our analysis only encompassed a limited number of samples, and our diagnostic criteria were not accompanied by any data on specific psychological domains related to ASB, such as aggression, psychopathy, etc. Second, while we studied total protein levels, it is possible that changes in receptor and

enzyme concentrations may not correspond to changes in binding and signaling. Third, due to the specific predominance of ASB in males and in SUD-affected subjects, we could not verify the specific impact of these targets in antisocial women and/or without a comorbid SUD diagnosis. Even with these important limitations, this is the first study performed on postmortem brain samples of individuals with ASB. As mentioned above, these data are strikingly aligned with previous evidence from our group on the upregulation of 5-HT_{2A} in the OFC and other areas of the prefrontal cortex of mouse models reproducing gene x environment interactions in ASB[86]. Furthermore, we and others have documented that 5-HT_{2A} receptor blockade prevents and blocks aggressive behaviors in several rodent models of aggression[86, 100–103]. Taken together, our data and this background strongly suggest that the upregulation of 5-HT_{2A} receptors in the OFC may be a biomarker of antisociality and that selective 5-HT_{2A} receptor antagonists approved for clinical use - such as the recently approved drug pimavanserin - may have therapeutic potential in the treatment of CD and ASPD. Future studies are warranted to fully evaluate these intriguing possibilities.

ACKNOWLEDGMENTS

This work was partially supported by National Institute of Mental Health (NIH R01 MH104603) and by a Skaggs Foundation Grant (to M.B.). Post-mortem human samples were obtained through the NIH NeuroBioBank from the University of Pittsburgh Brain Tissue Repository. The authors would like to thank Jill Glausier, Karen Odeh, and Roberto Cadeddu for their help in obtaining the brain samples and editorial suggestions.

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Key points:

- We performed the first-ever postmortem analysis of serotonergic brain markers in antisocial individuals
- Comparisons were drawn with age-matched subjects with similar substance use disorder diagnoses
- The orbitofrontal cortex of antisocial individuals exhibits increased 5-HT_{2A} receptor level

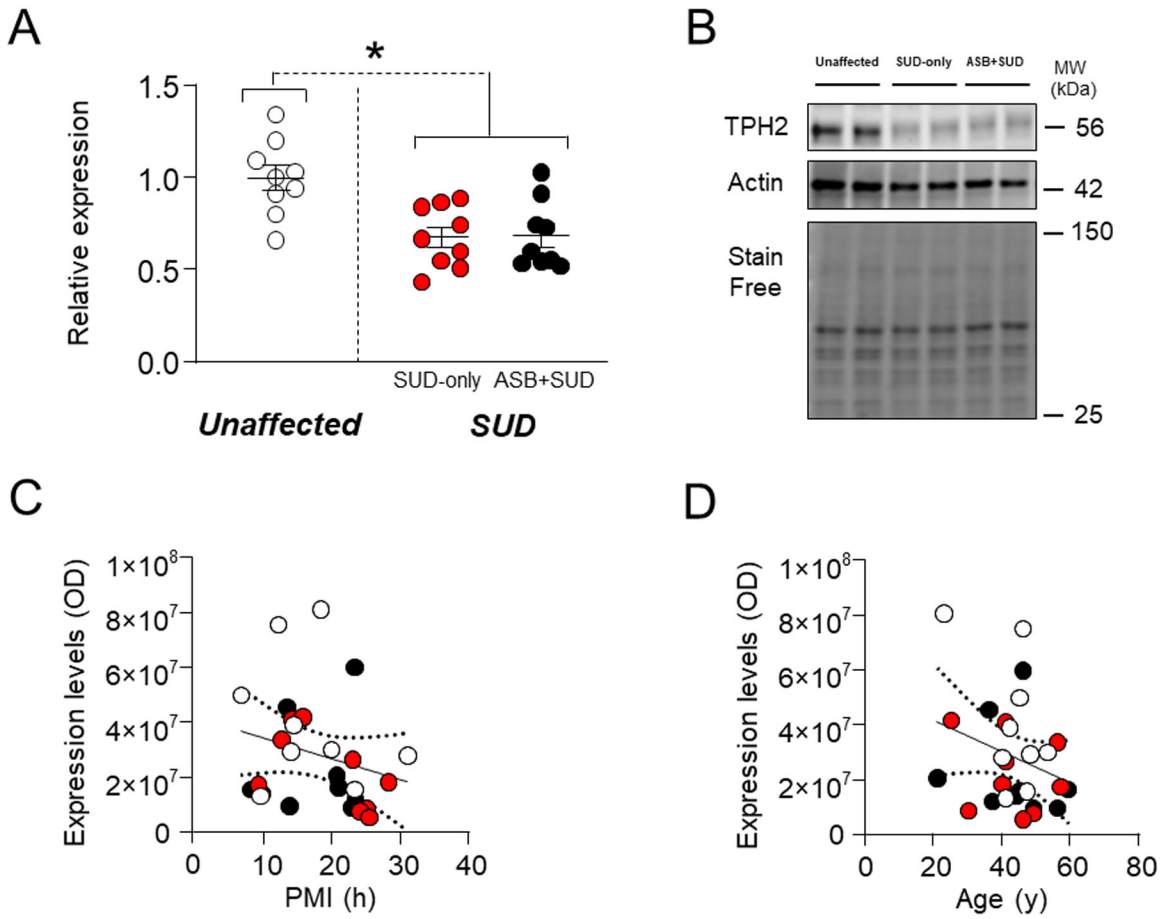

Fig. 1. Analysis of TPH2 expression levels in the OFC of antisocial individuals.

Irrespective of ASB, individuals with a history of SUD exhibited a significant decrease in TPH2 levels in comparison with unaffected controls. **A**. Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with TPH2 levels. **D.** Simple linear regression analysis representing the association of age with TPH2 levels. * P= 0.0002 compared to unaffected control (Nested T-Test). ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval

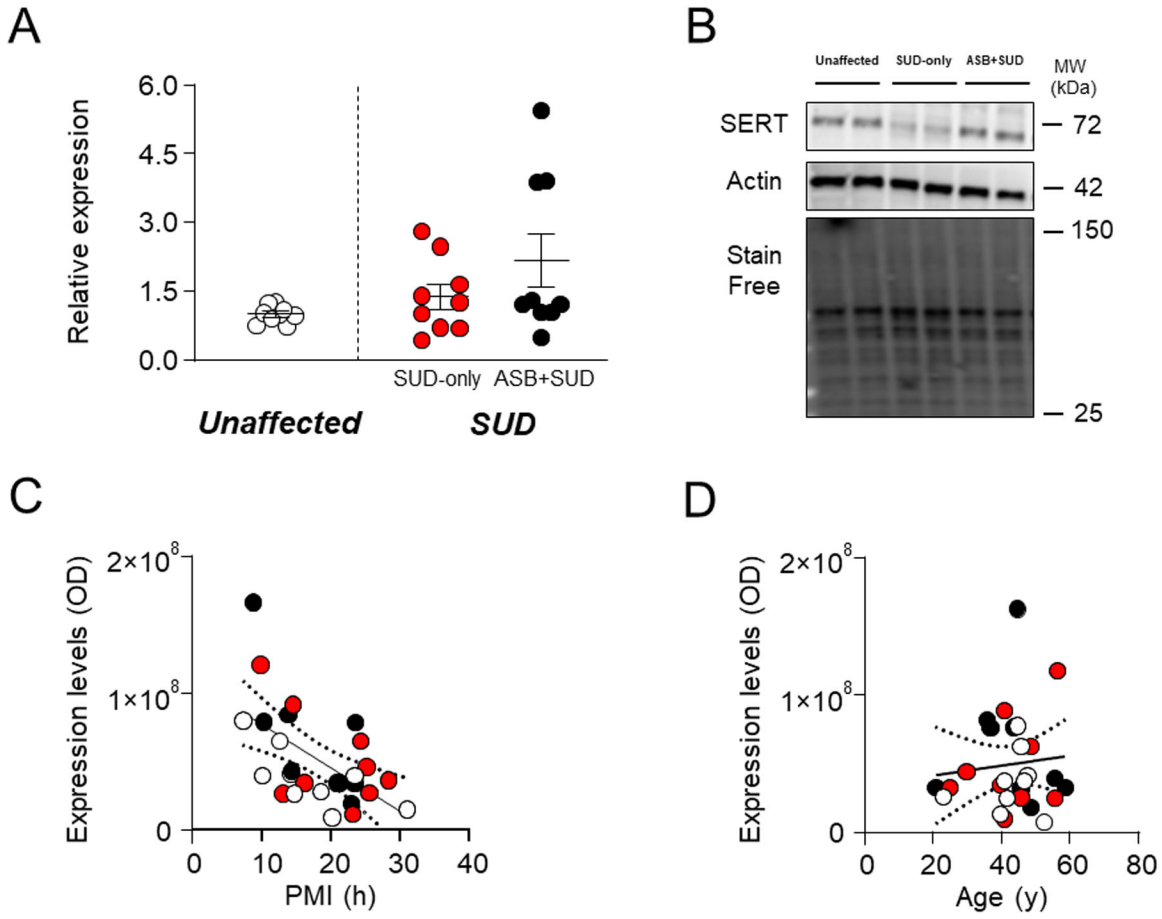

Fig. 2. Analysis of MAOA expression levels in the OFC of antisocial individuals.

No differences were detected between groups. **A**. Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with MAOA levels. **D.** Simple linear regression analysis representing the association of age with MAOA levels. ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval

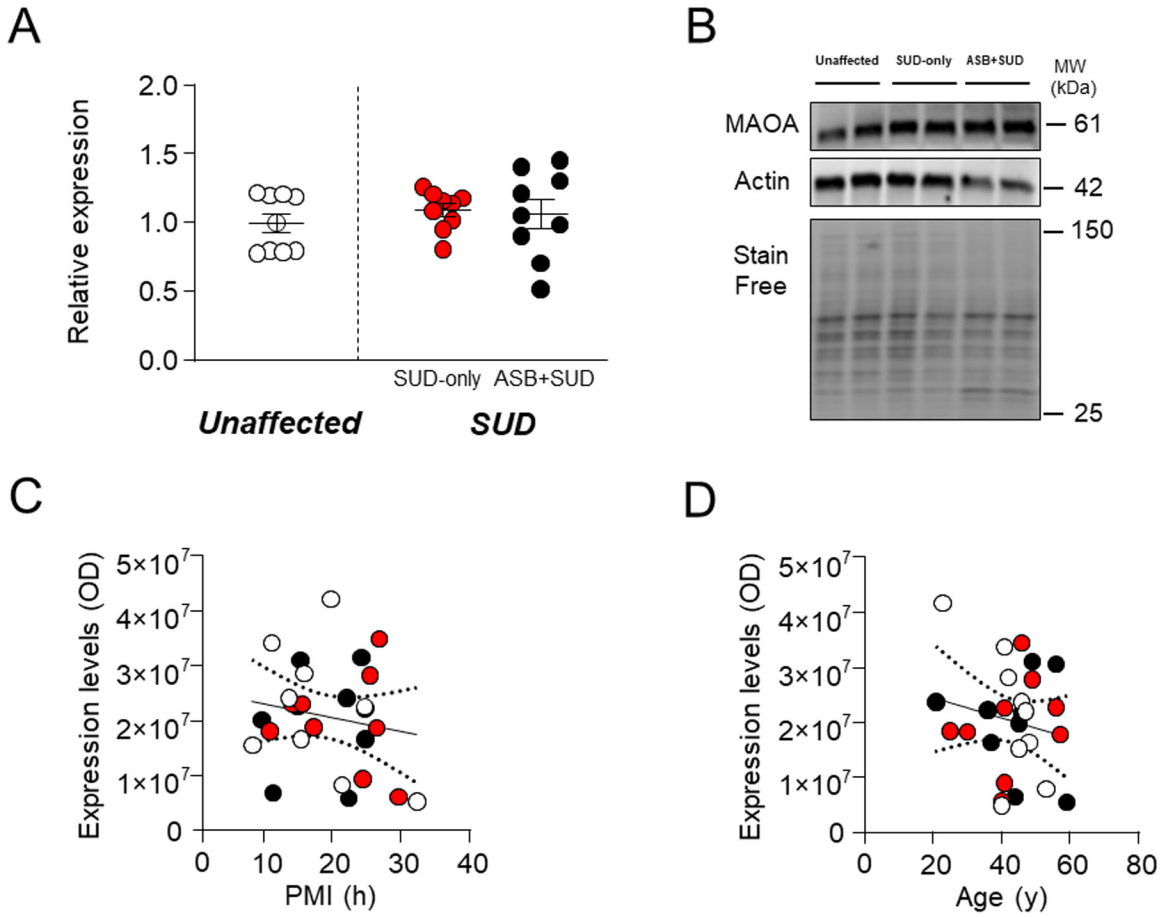

Fig. 3. Analysis of SERT expression levels in the OFC of antisocial individuals.

No significant alteration was reported between groups. **A**. Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with SERT levels ($F_{1,25}$=12.04; R^2=0.33, p=0.002) **D.** Simple linear regression analysis representing the association of age with SERT levels. ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval

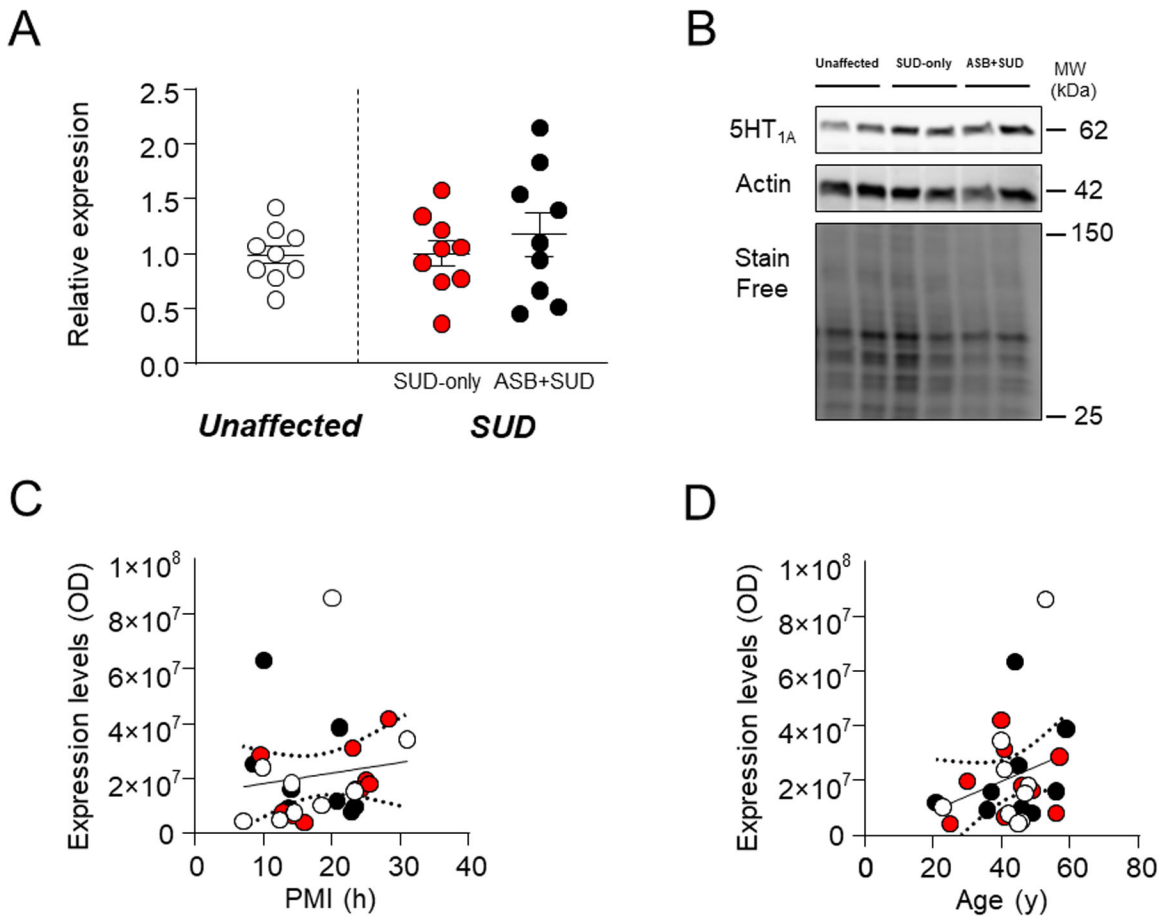

Fig. 4. Analysis of $5HT_{1A}$ receptor levels in the OFC of antisocial individuals.

No significant change was reported between groups. **A**. Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with $5HT_{1A}$ receptor levels **D.** Simple linear regression analysis representing the association of age with $5HT_{1A}$ receptor levels. ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval

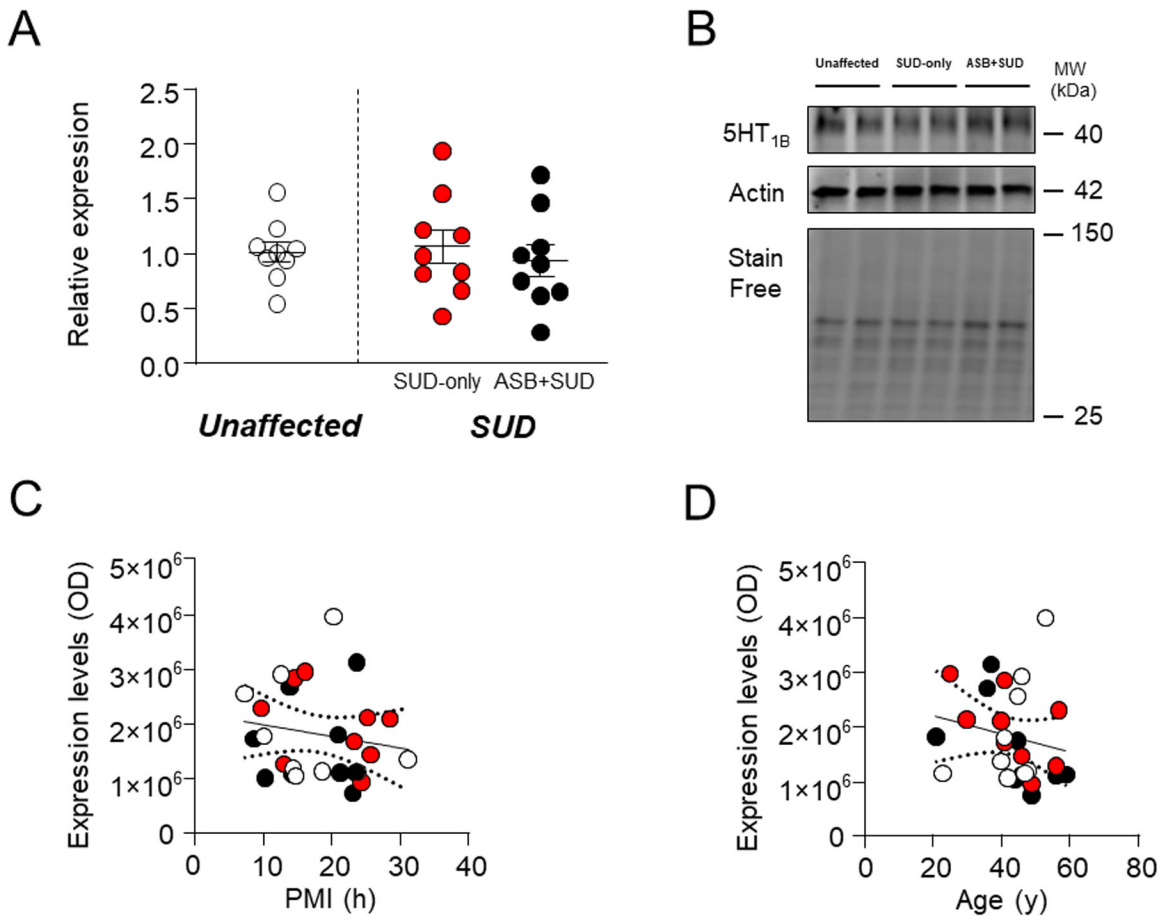

Fig. 5. Analysis of $5HT_{1B}$ receptor levels in the OFC of antisocial individuals.

No significant change was reported between groups**. A.** Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with $5HT_{1B}$ receptor levels **D.** Simple linear regression analysis representing the association of age with $5HT_{1B}$ receptor levels. ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval

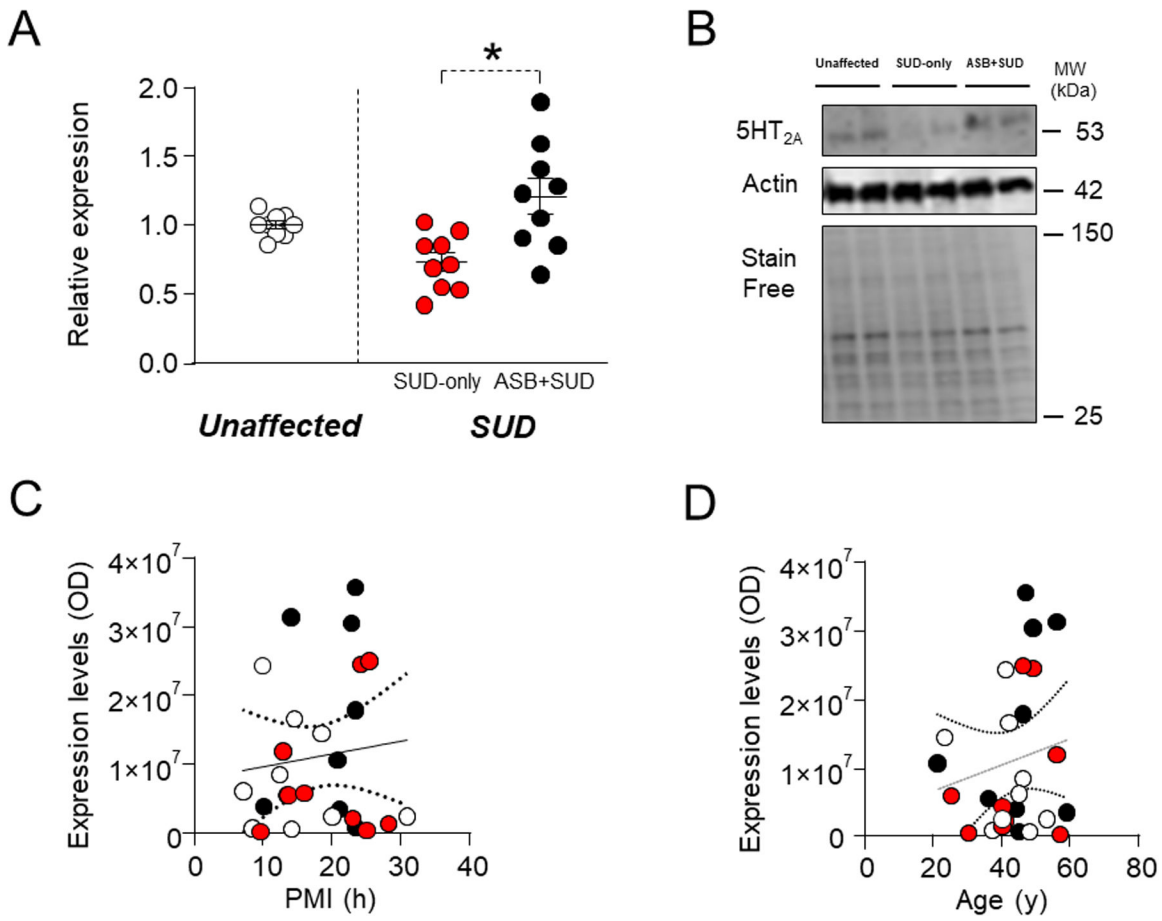

Fig. 6. Analysis of $5HT_{2A}$ receptor in the OFC of antisocial individuals.

Individuals with ASPD or CD and a history of SUD exhibited a significant increase in $5HT_{2A}$ levels in comparison with SUD-only subjects. **A.** Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with $5HT_{2A}$ receptor levels. **D.** Simple linear regression analysis representing the association of age with $5HT_{2A}$ receptor levels.

* P= 0.004 compared to SUD-only group (χ^2 =8.43). ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval

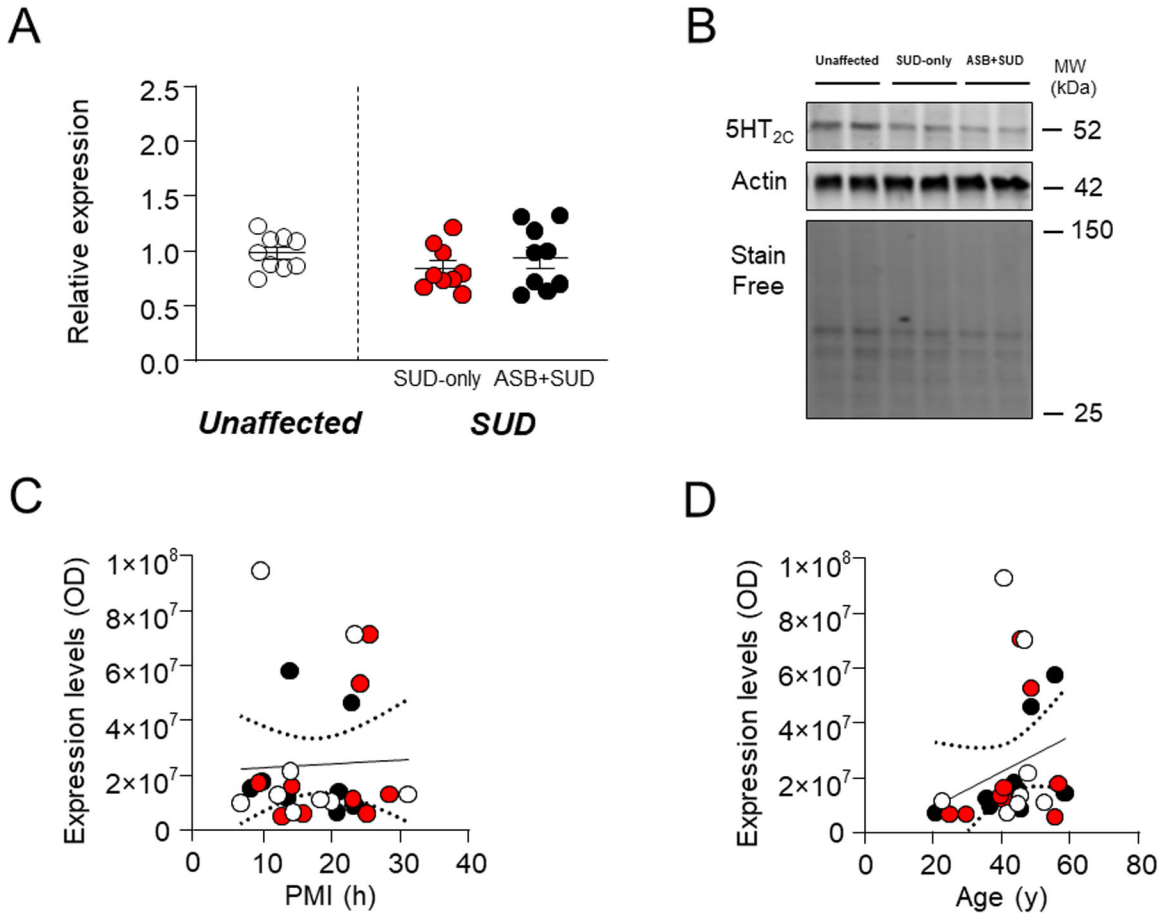

Fig. 7. Analysis of $5HT_{2C}$ receptor levels in the OFC of antisocial individuals.

No significant change was reported between groups. **A**. Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with $5HT_{2C}$ receptor levels **D.** Simple linear regression analysis representing the association of age with $5HT_{2C}$ receptor levels. ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval

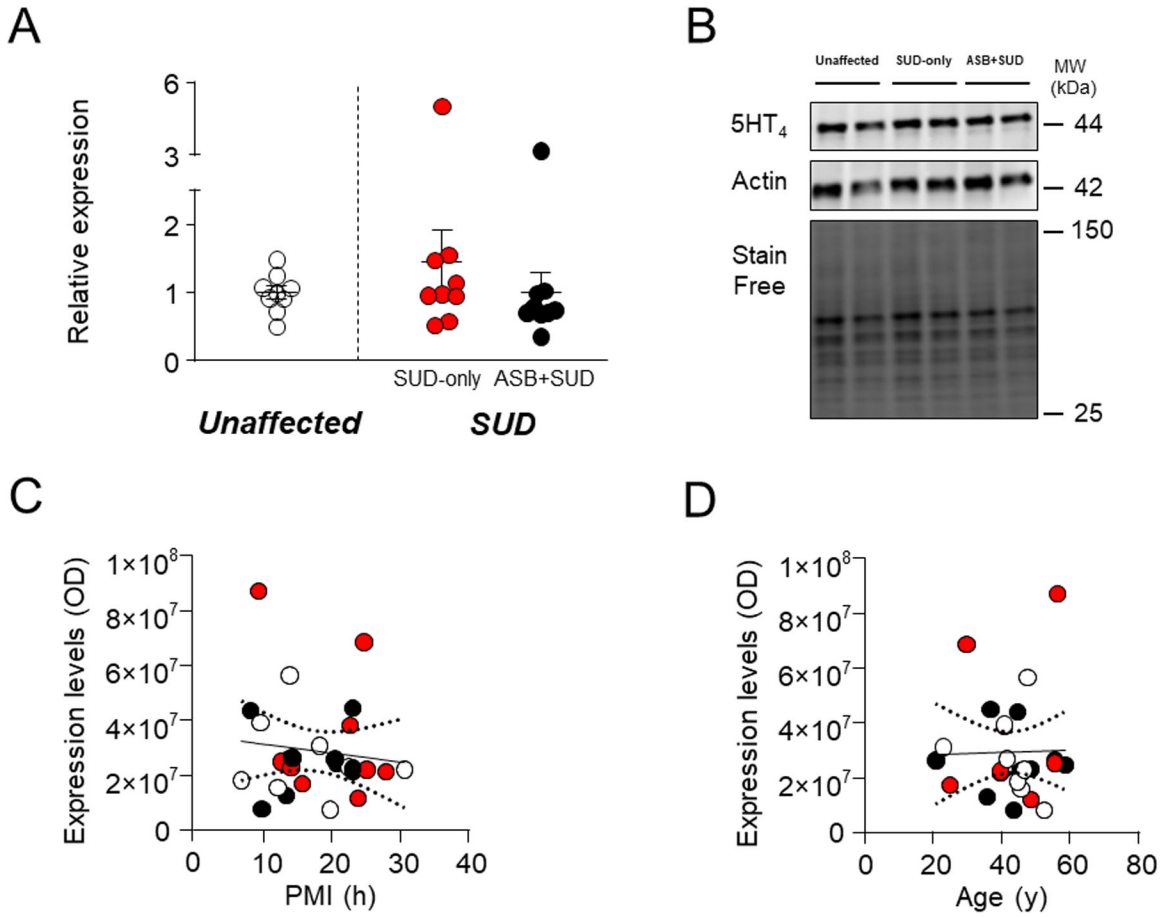

Fig. 8. Analysis of $5HT_4$ receptor levels in the OFC of antisocial individuals.

No significant change was reported between groups. **A**. Protein expression levels. **B.** Representative immunoblotting. **C.** Simple linear regression analysis representing the association of PMI with $5HT_4$ receptor levels **D.** Simple linear regression analysis representing the association of age with $5HT_4$ receptor levels. ASB: Antisocial behavior; SUD: Substance use disorder; PMI: post-mortem interval.

TABLE 1.

Demographic characteristics of subjects

Group	Manner of Death	SUD diagnoses	ASB diagnoses	Other comorbid disorders
Unaffected Controls	ACCIDENTAL	/	/	/
	NATURAL	/	/	/
	NATURAL	/	/	/
	ACCIDENTAL	/	/	/
	NATURAL	/	/	/
	ACCIDENTAL	/	/	/
	NATURAL	/	/	/
	NATURAL	/	/	/
	NATURAL	/	/	/
SUD only	NATURAL	Alcohol (S); tobacco (M); Opioids (M); Cocaine (M)	/	/
	NATURAL	Alcohol (S); Sedatives (Mi)	/	Anxiety disorder
	NATURAL	Alcohol (Mi)	/	Seizure disorder
	ACCIDENTAL	Alcohol (Mi); Cocaïne (Mi); Opioids (Mi)	/	/
	PENDING	Alcohol (Mi); Cocaine (S); Cannabis (M)	/	/
	ACCIDENTAL	Alcohol (M); Tobacco (M); opioids (Mi)	/	/
	NATURAL	Alcohol (M); Cannabis (M); Tobacco (M)	/	/
	ACCIDENTAL	Alcohol (Mi); Cocaïne (Mi); Hallucinogens (Mi)	/	/
	ACCIDENTAL	Amphetamines (M); Tobacco (M); Cannabis (Mi)	/	/
SUD+ASB	NATURAL	Alcohol (S); Opioids (M); Sedatives (M)	ASPD	/
	ACCIDENTAL	Alcohol (M); Cocaine (Mi); Cannabis (Mi)	CD (childhood onset)	Dysthymia
	ACCIDENTAL	Alcohol (S); Opioids (Mi)	CD (adolescence onset);	Intermittent explosive disorder
	NATURAL	Alcohol (S), Tobacco (S); Sedatives (M); Opioids (M); Amphetamines (Mi)	ASPD	Unspecified depressive disorder Borderline personality disorder
	ACCIDENTAL	Alcohol (S); Cocaine (S); Cannabis (M); Opioids (Mi)	ASPD	Major depressive disorder, Single episode
	ACCIDENTAL	Cannabis (M); Alcohol (Mi); Opioids (Mi); Sedatives (Mi); Cocaine (Mi)	ASPD	/

Group	Manner of Death	SUD diagnoses	ASB diagnoses	Other comorbid disorders
	SUICIDE	Cannabis (Mi)	CD (adolescence onset); ASPD;	Major depressive disorder, Single episode; learning disorders; Borderline personality disorder
	ACCIDENTAL	Tobacco, opioids (S); Cannabis (M); Alcohol (Mi)	CD (unspecified onset); ASPD	Unspecified depressive disorder; Gambling disorder; ADHD; trauma and stress-reated disorder
	ACCIDENTAL	Opioids, Tobacco (S); Alcohol (M); Cocaine (Mi)	CD (unspecified onset);	Disruptive, impulse-control disorder

S: severe; M: moderate; Mi: mild

TABLE 2.

Statistical comparisons of demographic and sample characteristics across diagnostic groups. Data are represented as means ± SEM.

	Unaffected Controls	SUD only	SUD+ASB	*p value*
Race (White:Black)	7W:2B	7W:2B	6W:3B	0.8
Sex (Male: Female)	9M:0F	9M:0F	9M:0F	1.0
Age at death	42.7 ± 2.8	42.7 ± 3.5	43.67 ± 3.8	0.9
PMI (hr)	17.5 ± 1,9	19.0 ± 2.2	17.5 ± 1.9	0.5
RIN	8.2 ± 0.4	7.3 ± 0.3	7.8 ± 0.2	0.1
pH	6.6 ± 0.1	6.4 ± 0.07	6.4 ± 0.08	0.4