Differential Expression of HTR2A and MAOA Genes in the Prefrontal Cortex and Hypothalamus of Suicide Victims from a Mexican Population

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Title Page

Differential Expression of *HTR2A* and *MAOA* Genes in the Prefrontal Cortex and Hypothalamus of Suicide Victims from a Mexican Population

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Abstract

Background: Suicide is a major public health concern that has been associated with several neurobiological abnormalities, including dysfunction of the serotonin (5-HT) neurotransmission system. The serotonin 2A receptor (5-HT_{2A}) and the monoamine oxidase A enzyme (MAO-A), which is responsible for degrading 5-HT, are encoded by the *HTR2A* and *MAOA* genes, respectively. These genes have been associated with several psychiatric disorders and an increased risk for suicide.

Methods: Our study examined the expression levels of *HTR2A* and *MAOA* genes in the postmortem prefrontal cortex (Brodmann area 8/9) and hypothalamus (ventromedial nucleus) tissues from 15 suicide victims and 15 control subjects from a Mexican population. Gene-expression profile quantification was carried out by qPCR and determined by the $2^{-\Delta\Delta Ct}$ method.

Results: In suicide victims, the expression levels of the *HTR2A* gene were significantly higher in the prefrontal cortex. In contrast, the expression of the *MAOA* gene in the hypothalamus of the suicide victims was significantly higher than in the control subjects. When comparing adult controls against adult suicidal victims (25-59 year-old age group), a significant decrease in *HTR2A* expression in the hypothalamus was observed. These results were consistent regardless of age, sex, postmortem interval, or pH of brain tissue.

Conclusions: The evidence suggests that the pattern of differential expression of the *HTR2A* and *MAOA* genes in the brain may be involved in suicide, providing a possible molecular basis for the brain abnormalities in suicide victims.

Keywords: Suicide, *HTR2A*, *MAOA*, Gene Expression, Postmortem brain, Mexican Population.

Background

Suicide is a serious global public health concern and is the third leading cause of death in 15-19-year-olds. Globally, 800,000 individuals die by suicide each year [1]. In Mexico, from 2000 to 2015, the suicide rate increased from 5.95 to 8.50 in men (per 100,000 inhabitants) and from 1.06 to 2.00 in women [2]. Neurobiological abnormalities link serotonin (5-HT) dysfunction to suicidal tendencies [3]. There is accumulating evidence that highlights a significant decrease in the primary metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid of depressed patients who had attempted suicide [4, 5], and postmortem brain tissue of suicide victims [6], which supports the serotonin hypothesis of suicide [7]. Several studies have shown abnormal expression of serotonin-related genes in suicide and suicidal behavior [8]. The *HTR2A* and *MAOA* genes have been considered as candidates for suicide risk [9, 10].

The *HTR2A* gene is located on chromosome 13q14.2 and encodes the serotonin-2A receptor (5-$\mathrm{HT}_{2\mathrm{A}}$). This receptor is widespread throughout the frontal lobes and many structures of the limbic system [11]. Several studies [12-14] have suggested that there is an increase in the binding and density of this receptor in the prefrontal cortex of suicide victims, as well an increase in the *HTR2A* expression levels in the frontal cortex and hippocampus of teenage suicides [15].

The *MAOA* gene is located on chromosome Xp21-p11 and encodes the monoamine oxidase A enzyme (MAO-A), which is involved in the degradation process for various monoamines, including 5-HT [16]. The higher activity alleles of the MAO-A gene are associated with vulnerability to suicide [17, 18]. An increase in the activity of this enzyme was observed in the hypothalamic region of suicide victims [19].

MAOA gene KO mice have revealed that have increased concentrations of monoamines, including 5-HT, increasing the aggression levels [20]; similar findings have been reported in human males [21]. Furthermore, the attenuation of the 5-HT_{2A}receptor leads to the onset of depression and anxiety-related behaviors [22]. It has been suggested that the frequency of suicidal behavior and the severity of suicidal ideation in depressed patients is often related to aggressive traits [23].

Most of the studies have been exclusively performed in the Caucasian population. There are no reports of the expression patterns of *HTR2A* and *MAOA* genes in the brain of suicide victims from the Latino population.

Methods

The aim of the present study was to investigate whether the expression levels of the *HTR2A* and *MAOA* genes are altered in the postmortem brain of suicide victims from a Mexican population.

Subjects

This study was approved by the Ethics Committee of the General Hospital of Durango. Postmortem brain tissue was obtained from the Forensic Medical Services of the General Fiscally of the State of Durango, Mexico. Brain samples were free of neuropathological abnormalities as well as human immunodeficiency virus and hepatitis C virus antibodies. The written informed consent forms were signed by first-degree relatives. The studies were performed in the prefrontal cortex (Brodmann area 8/9) and hypothalamus (ventromedial nucleus) obtained from 15 suicide victims and 15 normal subjects (death by causes not associated with impulsive or reckless behaviors, such as workplace accident, motor vehicle accident, myocardial infarction, drowning, etc.), here in referred to as control subjects. Psychiatric diagnoses were characterized based on data obtained

from a clinical interview with first-degree relatives, which contained information about (I) medical records (II) psychiatric records, (III) declarations from the police and witnesses, (IV) demographic information, (V) acute and chronic stressful life situations, and (VI) substance abuse records. Exclusion criteria for brain samples included the presence of significant structural brain pathology on postmortem examination and history of central nervous system disease. The postmortem interval (PMI) represents the time interval between the estimated time of death and the sample collection, which was established as less than 16 hours. The subjects included in this work had a common ethnic origin.

The prefrontal cortex was defined as the gray matter obtained from the right hemisphere. The samples were dissected during the autopsy and kept at –80°C in RNAlater (Invitrogen, CA, USA).

RNA extraction and determination of expression pattern

Total RNA was isolated from 30 mg of homogenized tissue in Trizol (Life Technologies, Carlsbad, CA, USA) using the Direct-zol RNA Miniprep kit (Zymo Research Corporation, Irvine, CA, USA) according to manufacturer protocol. The RNA concentration and purity were measured by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Germering, Germany). Total RNA was confirmed by 1% agarose gel electrophoresis. The first-strand cDNA was then generated from 1 µg of template RNA using a SureCycler 8800 Thermal Cycler (Agilent, Santa Clara, CA, USA) and High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) in a 20 µL reaction, according to the instructions. Priming was performed at 25°C for 10 min, reverse transcription for 120 min at 37°C, and the final step at 85°C for 5 min using a mixture of oligo-dT primers and random primers. The cDNA was then stored at -20°C for subsequent analysis. Quantitative PCR was performed with a QuantStudio 3 Real-Time PCR System (Applied Biosystems,

Foster City, CA), using the TaqMan Gene Expression Assays; *HTR2A* (Hs01033524_m1), and *MAOA* (Hs00165140_m1). The following conditions were used: 95°C for 10 min, 40 cycles of 95°C for 15 s, and 60°C for 1 min. The relative gene expression was calculated with the $2^{-\Delta\Delta Ct}$method, using *B2M* (Hs00187842_m1) as the housekeeping gene. The experiment was performed with technical triplicates.

Statistical analyses

Data were analyzed with statistical software (SPSS 22.0; SPSS, Chicago, Ill, USA). All values are reported as mean ± SD. A Chi-Square test was used to test the equality of proportions. The normality of data was assessed by the Shapiro-Wilk test. The differences in gene expression levels and age, sex, pH of brain tissue and PMI between groups were analyzed using the independent-sample *t*-test in normal distribution data and the Mann–Whitney *U* test in non-normal distributions. The Pearson correlation coefficient was used to measure the strength between two continuous variables. Levene's test was used to determine the variance between the two groups, and *p*- value of < 0.05 was considered statistically significant.

Results

A matched case-control design was used to minimize the variability among the groups. The groups were matched by age, sex, pH of brain tissue, and PMI. The main characteristics of the subjects are provided in Table 1. Based on the classification used by Stenbacka [24], our suicide sample was mostly violent suicides such as hanging and gunshot wounds (86.67 %). There were no significant differences in age ($t = 0.153$, $p = 0.879$), pH ($t = 1.035$, $p = 0.309$), or PMI ($t = 1.383$, $p = 0.178$).

***HTR2A* expression pattern**

Significant differences in *HTR2A* expression in the prefrontal cortex between suicide victims and control subjects (t = 2.860, p = 0.008) were observed. *HTR2A* had higher expression levels in suicide victims than controls (0.8341 ± 0.30083 vs 0.4999 ± 0.32871) (Figure 1-A). Furthermore, we found a significant trend in the hypothalamus region (p = 0.067) (Figure 1-B).

MAOA expression pattern

The expression of the *MAOA* gene showed no significant differences in the prefrontal cortex between the 15 suicide victims and 15 control subjects (t = -1.067, p = 0.295) (Figure 2-A). However, the expression of *MAOA* was significantly increased in the hypothalamus (t = 2.464, p = 0.020) in suicide victims, as compared with that in control subjects (Figure 2-B).

Subgroup analysis

The effect of sex on the expression of *HTR2A* and *MAOA* genes was evaluated. In men, which is the majority group in both suicides (n = 13) and controls (n = 13), significant differences were maintained (*HTR2A* in prefrontal cortex: t = 3.234, p = 0.004; *MAOA* in hypothalamus: t = 2.025, p = 0.054). Further, no significant changes were observed when comparing the expression profile of violent and non-violent methods of suicide, (*HTR2A* in prefrontal cortex: t = 3.479, p = 0.021; *MAOA* in hypothalamus: t = 2.457, p = 0.054). Also, the expression pattern was evaluated across age groups, based on the classification established by the WHO: youth as the 15-24-year age group and adult as the 25-59 year age group [25]. Interestingly, in the adult subgroup (suicide subjects n = 10; control subjects n = 10) the only difference observed in the pattern of *HTR2A* expression gene in the prefrontal cortex was maintained (p = 0.017), and a significant decrease in *HTR2A* expression in the hypothalamic area was observed (p = 0.011) (Figure 3).

Effects of potential confounding variables

The Pearson correlation coefficient was used to measure the effect of PMI and pH on the expression levels of *HTR2A* and *MAOA*. None had any significant effects (data not shown).

Discussion

Most studies exclusively report the expression levels of *HTR2A* and *MAOA* in the Caucasian population except for the study by Pandey et al. [15], which included different racial origins. To our knowledge, this is the first study that has investigated the expression pattern of the 5-HT_{2A} receptor and the enzyme MAO-A in the postmortem brain of suicide victims from a Mexican population. Our results show that *HTR2A* and *MAOA* expression levels were increased in the prefrontal cortex and hypothalamus of suicide victims, respectively. Also, we observed a significant decrease in *HTR2A* expression in the hypothalamus of the adult subgroup of suicidal victims. The results shown here support previous studies that suggest that suicide may be associated with serotonin abnormalities mediated by the serotonin 2A receptor and the MAO-A enzyme.

Previous studies provided evidence of an increase in 5-HT_{2A} receptor protein levels in the frontal cortices of suicide victims compared with controls by receptor binding assays [12-15]. Cortical 5-HT_{2A} receptors have mainly been observed on layer V pyramidal cells, and it has been hypothesized that activation of this metabotropic receptor may be responsible for mediating the depolarization of GABAergic cortical interneurons [26]. There is evidence that 5-HT and DOI (2,5-dimethoxy-4-iodoamphetamine) a selective 5-HT_{2A} receptor antagonist, may reversibly reduce GABA-activated currents [27]. Interestingly, low GABRG2 expression [28], and alpha1, alpha3, alpha4, and delta GABA(A) receptor subunits [29] have been reported in the frontal cortex of

suicide victims. Thus, it is possible that the attenuated activity of GABAergic interneurons mediated by the 5-HT_{2A} receptors located on the pyramidal neurons can lead to a dysregulation of the mesocortical dopaminergic system. Morphological and pharmacological evidence suggests that there is a reciprocal interaction between the dopaminergic and serotonergic systems [30, 31], strengthening the previous hypothesis. Further, it has been reported that the dopamine circuitry is likely involved in mediating depressive-like behaviors and social withdrawal [32], impulsive aggression [33], and anxiety [34], which are traits that might contribute to the risk of suicidal behavior [3].

Another important observation of our study is the significant increase in the expression of the *MAOA* gene in the hypothalamus of suicide subjects. This result is consistent with a previous report of a significant elevation in MAO-A activity in the hypothalamic region of suicide victims [19] and with the reduction of 5-HT in the hypothalamus of this group [35]. Despite the mechanisms by which MAO-A can be associated with suicide being unclear, we hypothesized that the increase of *MAOA* expression in the hypothalamus of suicide victims could affect the activation of the hypothalamic-pituitary-adrenal (HPA) axis. The study by Mehlhem et al., [36] suggest that a blunted HPA axis activity increases the risk for suicide, possibly by a reduced ability to respond adaptively to stressors. Likewise, low cortisol levels have been associated with suicidal behavior [37] [38]. 5-HT can regulate the HPA axis via serotonin 2C receptor stimulation in the paraventricular nucleus of the hypothalamus (PVH) [39]. This may imply that the blunted HPA axis functioning may be a result of low circulating levels of 5-HT due to the increased expression of *MAOA*. Future research is needed to examine this theory.

It should be noted that our findings at the mRNA level are consistent with previous reports at the protein level in different populations [12-15, 19]. Interestingly, the 25-59 year-old age group showed a significant decrease in *HTR2A* expression in the hypothalamic area of suicide victims (p = 0.011). In accordance to Turecki and Brent (2016) impulsive aggression, conduct disorder, and antisocial behavior are more salient in youth suicide, whereas mood disorders are usually observed with increasing age. We hypothesized that a decrease of *HTR2A* expression may be related to the increased expression of the *MAOA* gene observed in the hypothalamus of suicide victims. It has been argued that the PVH nucleus participates in mood modulation, this is the primary driver of HPA axis responses, and interestingly, the 5-HT_{2A} receptors are present on neuroendocrine cells of this limbic nucleus [40]. The evidence suggests that the HPA axis dysfunction and maladaptive responses to stress may be associated with the pathophysiology of mood disorders [41] and suicidal behavior [36]. Future studies that directly asses the $5-\mathrm{HT}_{2\mathrm{A}}$ receptors in PVH may clarify whether alterations in 5-HT signaling may result in a HPA axis imbalance in suicide victims.

There are several limitations to our study. The psychiatric diagnosis of subjects may be a source of heterogeneity. Because our study is based on a case-control design, temporality could not be inferred with certainty. Another limitation present in this study is the lack of genotype data for subjects, since gene variants may influence the expression of the *HTR2A* gene [42-44] and the *MAOA* gene [45, 46]. In this study, it was not possible to obtain the toxicological data due to legal issues and information privacy.

Conclusion

In conclusion, our study suggests that the differential expression pattern of the *HTR2A* gene in the prefrontal cortex and hypothalamus, as well as the *MAOA* gene in the hypothalamus are involved in suicide. We propose that both molecules may serve as important vulnerability factors to suicide.

List of abbreviations

5-HT, 5-hydroxytryptamine; 5-HIAA, 5-Hydroxyindoleacetic acid; 5-HT_{2A}, 5-hydroxytryptamine receptor 2A; *B2M,* β2 microglobulin gene; DOI, 2,5-dimethoxy-4-iodoamphetamine; GABA, gamma-Aminobutyric acid; *HTR2A*, 5-hydroxytryptamine receptor 2A gene; HPA, hypothalamic, pituitary, adrenal; *MAOA*, Monoamine oxidase A gene; MAO-A, Monoamine oxidase A enzyme; KO, Knockout; PMI, postmortem interval; PVH, paraventricular nucleus of the hypothalamus; WHO, World Health Organization.

Ethics approval and consent to participate

All participants signed the informed consent. The study adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the General Hospital of Durango (CEI 2016/33).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

DRR and MBS conceived the study, participated in its design, and draft the manuscript. DRR, EMMH, helped to perform the statistical analysis and to draft the manuscript. JSP, NUE and ACSL helped with data integration and analysis. All authors read and approved the final manuscript.

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Figure 1. Expression levels of *HTR2A* in the *postmortem* brain. (A) Relative *HTR2A expression* in the prefrontal cortex (BA 8/9). (B) Relative *HTR2A expression* in the hypothalamus. The data are shown as fold change in mRNA levels and fold change was calculated based on $\mathbf{2^{-\Delta\Delta Ct}}$ values. Error bars indicate 95% confidence intervals.

Figure 2. Expression levels of *MAOA* in the *postmortem* brain. (A) Relative *MAOA expression* in the prefrontal cortex (BA 8/9). (B) Relative *MAOA expression* in the hypothalamus. The data are shown as fold change in mRNA levels and fold change was calculated based on $2^{-\Delta\Delta Ct}$ values. Error bars indicate 95% confidence intervals.

Figure 3. Expression levels of *HTR2A*in the hypothalamus of adult sample (25-59 year-old age group). The data are shown as fold change in mRNA levels and fold change was calculated based on $2^{-\Delta\Delta Ct}$ values. Error bars indicate 95% confidence intervals.

Table 1. Main characteristics of the subjects

	Suicide victims	Control subjects	*p*
Sex, Male/Female	13/2	13/2	1*
Age at death (years)	32.07 ± 12.40	31.4 ± 11.41	0.879+

PMI (hours)	6.26 ± 2.63	4.86 ± 2.90	0.178+
pH	6.19 ± 0.30	6.06 ± 0.37	0.309 +
Cause of death, (%)			
	Hanging (80)	Accident (multiple injuries) (86.6)	
	Poisoning (13.33)	Myocardial infarction (6.67)	
	Gunshot wound (6.67)	Drowning (6.67)	
Diagnosis, n (%)			
Alcohol abuse /dependence	1 (6.67)	1 (6.67)	1*
Drug abuse /dependence	1 (6.67)	1 (6.67)	1*
Major depression	2 (13.33)	0 (0)	0.143*

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438 Values are expressed as mean ± SD. PMI = *post-mortem* interval; * = Chi-Square test; + = independent *t*-test

Figures

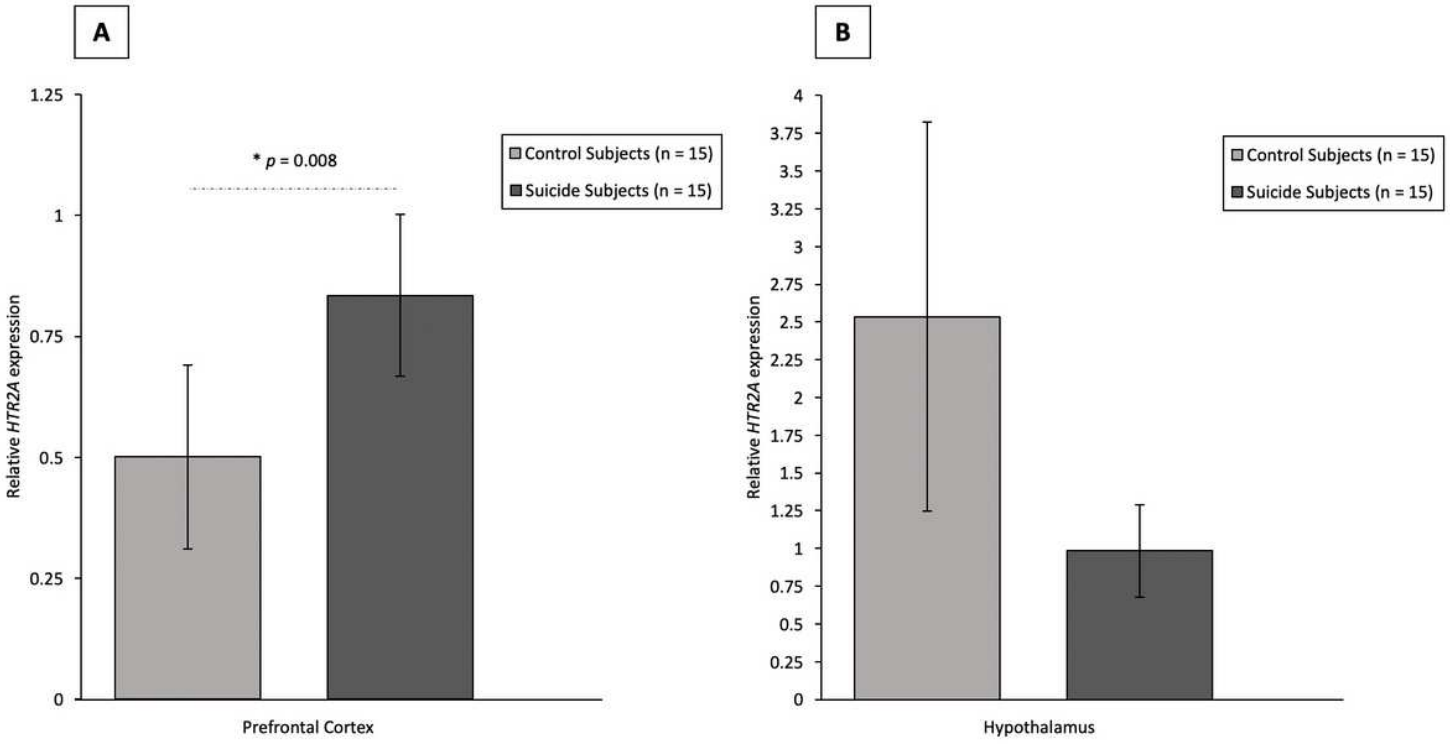

Figure 1

Expression levels of HTR2A in the postmortem brain. (A) Relative HTR2A expression in the prefrontal cortex (BA 8/9). (B) Relative HTR2A expression in the hypothalamus. The data are shown as fold change in mRNA levels and fold change was calculated based on □−ΔΔ□□ values. Error bars indicate 95% confidence intervals.

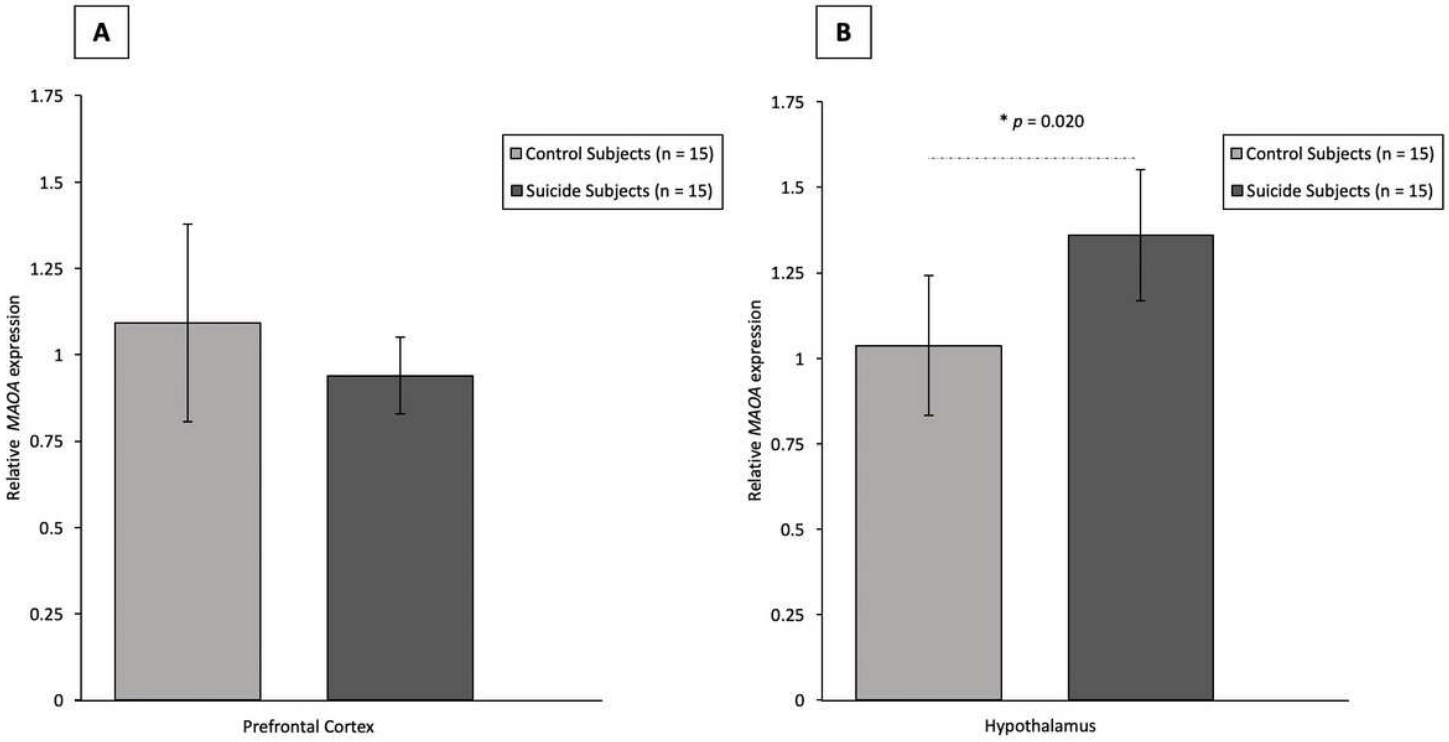

Figure 2

Expression levels of MAOA in the postmortem brain. (A) Relative MAOA expression in the prefrontal cortex (BA 8/9). (B) Relative MAOA expression in the hypothalamus. The data are shown as fold change in mRNA levels and fold change was calculated based on 2−ΔΔ!" values. Error bars indicate 95% confidence intervals.

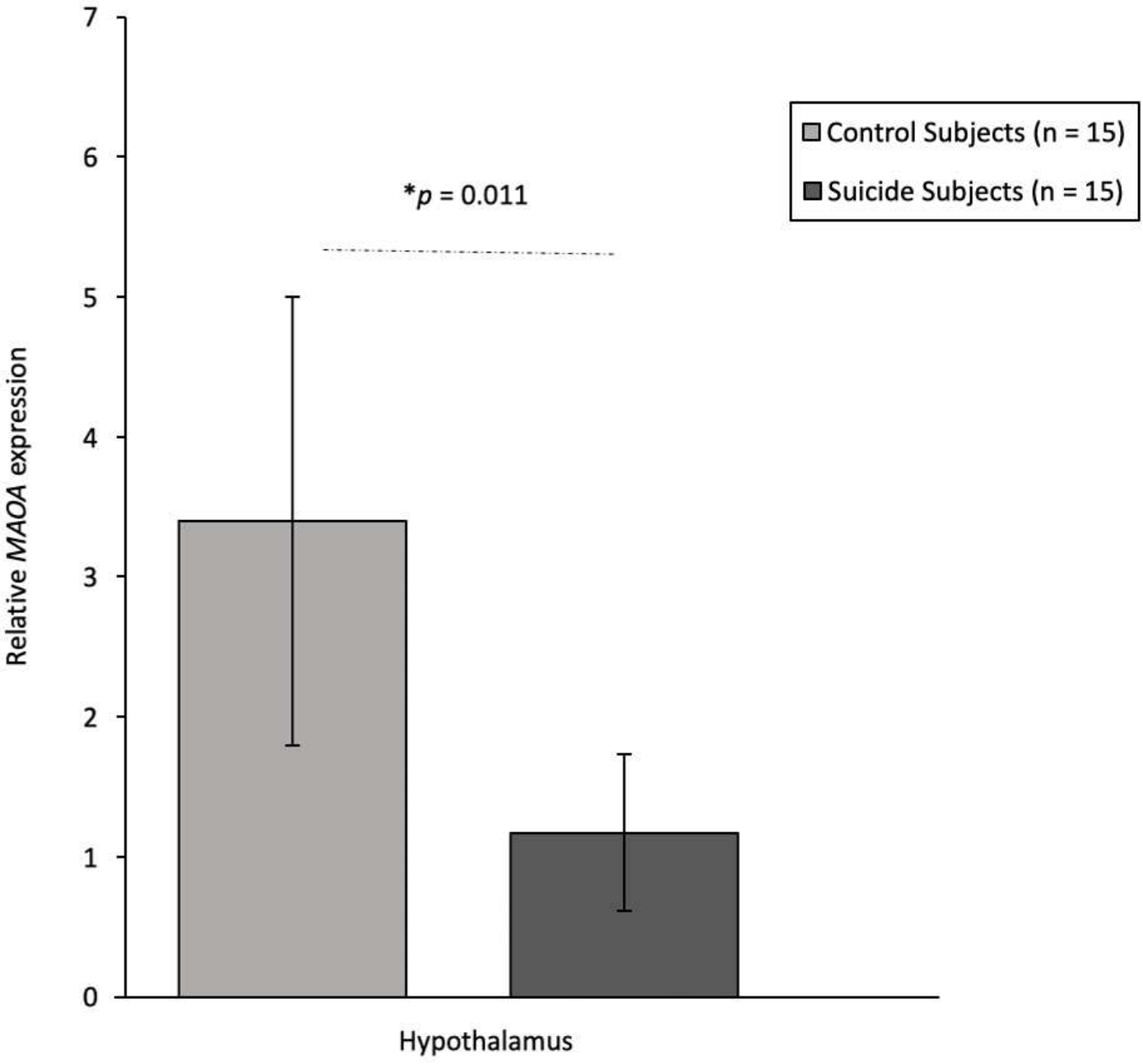

Figure 3

Expression levels of HTR2Ain the hypothalamus of adult sample (25-59 year-old age group). The data are shown as fold change in mRNA levels and fold change was calculated based on 2−ΔΔ!" values. Error bars indicate 95% confidence intervals.