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Neuropil Alteration of the Habenula Nucleus in the Experimental Model of Schizophrenia Induced by Ketamine

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ABSTRACT

Background and aim: Neuropil is a densely packed network of glial processes, neuronal processes, extracellular matrix, and microvascular in the central nervous system. The habenula nucleus is one of the brain regions contributing actively to emotional processing and regulating negatively motivated behaviors. This study aimed to examine the neuropil alteration of the habenula nucleus in the experimental model of schizophrenia.

Material and methods: Twenty adult Wistar rats were randomly divided into two groups. The experimental group received ketamine at a dose of 10mg/kg intraperitoneally for one week. The control group was treated with saline. At the end of the experiment, animals were deeply anesthetized, the brains were removed, and Paraffin-embedded sections of 10µm thickness were cut on microtome. The randomized sections were stained with H&E. The position of the HB was recognized, and the neuropil surface area was measured according to the stereology method.

Results: The surface area of the right (9841±1355µm2) and the left (9110±1390.5 µm2) habenula nucleus showed a meaningful difference in comparison with the right (1134±272µm2) and the left (1247±348 µm2) habenula nucleus of the control group (p=0.000). The number of astrocytes in the right HB of the experimental group (440±96.2) and the left HB of the experimental group (422±103.2) showed a meaningful difference in comparison to those of the control group (RHB: 97±31.1 and LHB: 88±9.08) (p=0.000).

Conclusions: The results of this study showed that an experimental model of schizophrenia leads to neuropil expansion in the habenula nucleus.

1. Introduction

The neuropil is the most basic tissue organization within the central nervous system. Structurally, the neuropil comprises dendrites, axons, synapses, glial processes, and microvascular. It has been proposed that the plastic nature of synaptic geometry, which is essential for neural plasticity, occurs within the densely packed forest of the CNS neuropils.[1, 2] Besides, recent findings have shown that neuropils have critical roles in cortical gyrification and functional specification of the cortical area.[3] Furthermore, compelling evidence suggests the involvement of the neuropils in a decline in cognitive performance in neurodegenerative and psychiatric disorders.[4] Schizophrenia is one of the most debilitating psychiatric disorders characterized by psychosis, hallucination, thought disorder, lack of functions such as lack of motivation, social withdrawal, and progressive cognitive deficits.[5] Postmortem and experimental studies have yielded evidence that indicates the pathological change in the CNS at both macroscopic and microscopic levels.[6, 7] For instance, It has been shown that schizophrenia is associated with neuropil contraction and synaptic pruning.[6] Among the various brain regions, the Habenula nucleus (HB) has drawn the attention of researchers owing to its pivotal role in regulating negatively motivated behaviors and its implication in psychiatric disorders. This tiny structure is part of the epithalamus, receives afferents from the diverse limbic systems and basal ganglia structures, and targets essentially all midbrain neuromodulatory systems. Due to its unique anatomic position, it is proposed to play a pivotal role in integrating sensory and experience-dependent information to regulate various motivational, cognitive, and motor processes. Dysfunction of the HB is thought to contribute to the pathophysiology of several psychiatric disorders.[8] Given the role of the HB in psychiatric disorders and the importance of the neuropils in cognitive processing, this study aimed to investigate the possible alteration of the neuropils in the HB. So, an experimental model of schizophrenia was induced by ketamine, and the neuropils of the HB were examined with stereological analysis.

2. Material and methods

Twenty male Wistar rats (8 weeks old) (200±20gr) were obtained from the animal house and transferred to the local animal house of the Educational Research Lab of Neuroscience (Ethical code: 96.P.1005). The animals were

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housed for one week to adapt to the new environment, then divided into two groups (N=10) as experimental and control. All procedures and experiments were conducted during the light period of the day cycle. The experimental group received ketamine at a dose of 10 mg/kg intraperitoneally for one week.[9] The control group was treated with saline. At the end of the experiment, the animals were deeply anesthetized with chloroform and transcardially perfused with 100 ml buffered formalin and heparin.[10] Subsequently, the animals were kept at 4°C, and the day after the experiment, the skulls were opened, and the brains were removed. The harvested brains were fixed in the same fixative. Paraffin-embedded sections of 10 µm thickness were cut on the microtome. The randomized sections were stained with H&E. The position of the HB was recognized according to the Paxinos atlas. Pictures were taken by Olympus microscope equipped with CellSens software and analyzed. We quantified the neuropil surface area of the HB using H&E-stained histological sections according to a modified method by Spocter et al.[4] This method measures the fraction of the projected profile of the section that is darkly stained, including cell bodies of neurons, glia, and endothelial cells, versus the unstained space, composed of dendrites, axons, synapses, and microvasculature.

Surface estimation and astrocytes number per volume

We took advantage of the unbiased stereological analysis to measure the surface area of the neuropils. For quantification of the neuropils (unstained areas), sections were selected at 20 µm intervals. A stereological frame (140 µm ×180 µm) was superimposed on each randomized field, and then the hit points or intersection neuroglial cells (phase A) Vs. Extracellular space (phase B) was counted. The number of astrocytes and degenerating neurons were counted in the randomized selected (50 fields per group) areas (each selected field: 2520 μm^2) and then estimated in volume according to Cavalieri's method as the following:[11]

$$Vol[i] = T. \sum Ai$$

Statistical analysis

The data was expressed as mean ± SD and compared using one-way analysis-of-variance (ANOVA). Any significant ANOVA was followed by a post-hoc Tukey test to detect which pairs of groups caused the significant difference. P-values <0.05 were considered statistically significant.

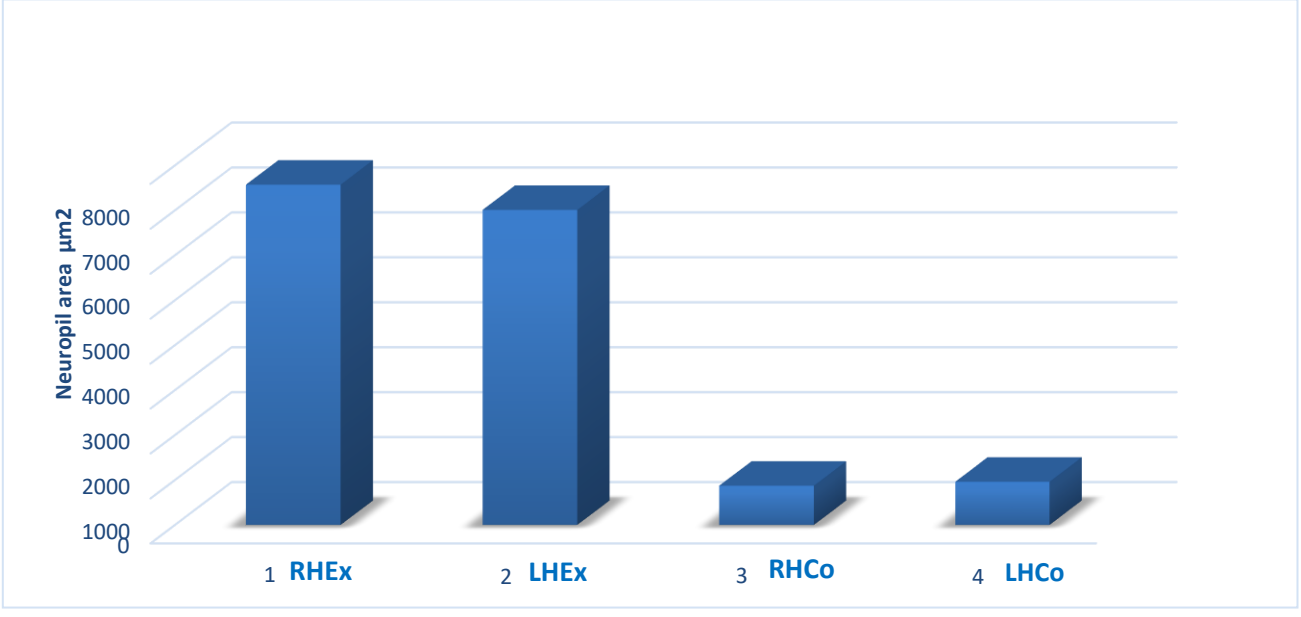
3. Results

The surface areas of the neuropils in the experiment group showed a significant difference compared to those of control animals (p=0.000). Comparison between the neuropil surface area in the right and the left HB in the experiment group showed no meaningful difference (p=.369) (Table 1 and Graph 1).

Table 1. Quantitative analysis of the habenula nucleus.

Group	RHEx	LHEx	RHco	LHco
Hit of pints	78.1±10.75*	72.3±11.03*	9±2.16	9.9±2.76
Neuropil area	9841±1355µm²#	9110±1390.5 µm²#	1134±272µm²	1247±348 µm²

RH (Right Habenula), Ex(Experimental), LHEx (Left Habenula), Ex(Experimental). Co (Control). The hit of pints. The neuropil surface area in theexperimental group showed meaningful differences in comparison with those of the control. (# and * p<0.05).



Graph 1. The neuropil surface area of the experimental group showed meaningful differences compared to those of the control group(P<0.05). RH: Right Habenula. LH: Left Habenula. Ex: Experimental. Co: Control.

The microscopic examination of the HB in the experiment animals revealed deeply stained neurons with prominent dense nucleoli and scattered hyperchromatic compact dark neurons with sharp margins (Figs. 1-3). The number of astrocytes in the selected fields was estimated according to the modified stereological method; the right HB of the experimental group (440±96.2) and the left HB of the experimental group (422±103.2) showed meaningful differences in comparison to those of the control group (RHB: 97±31.1 and LHB: 88±9.08) (p=0.000).

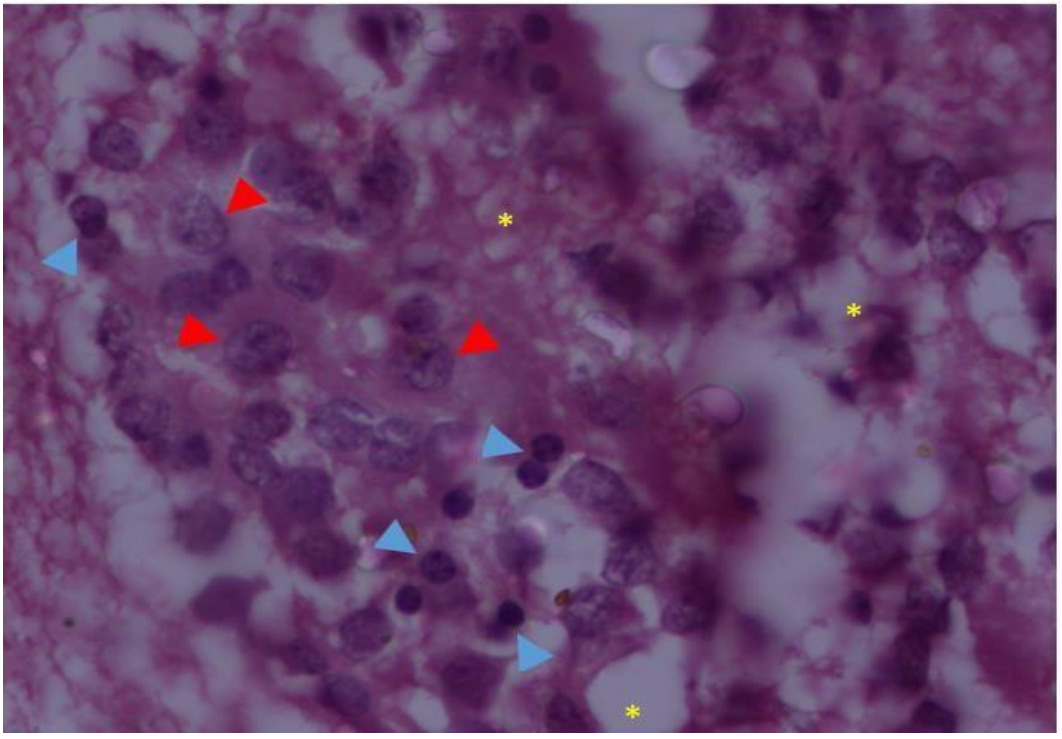


Fig. 1. HB in the experimental group. Numerous astrocytes (blue arrowheads) are seen among the neurons (Red arrowheads). The neuropils are seen as the unstained spaces (yellow stars) (X100).

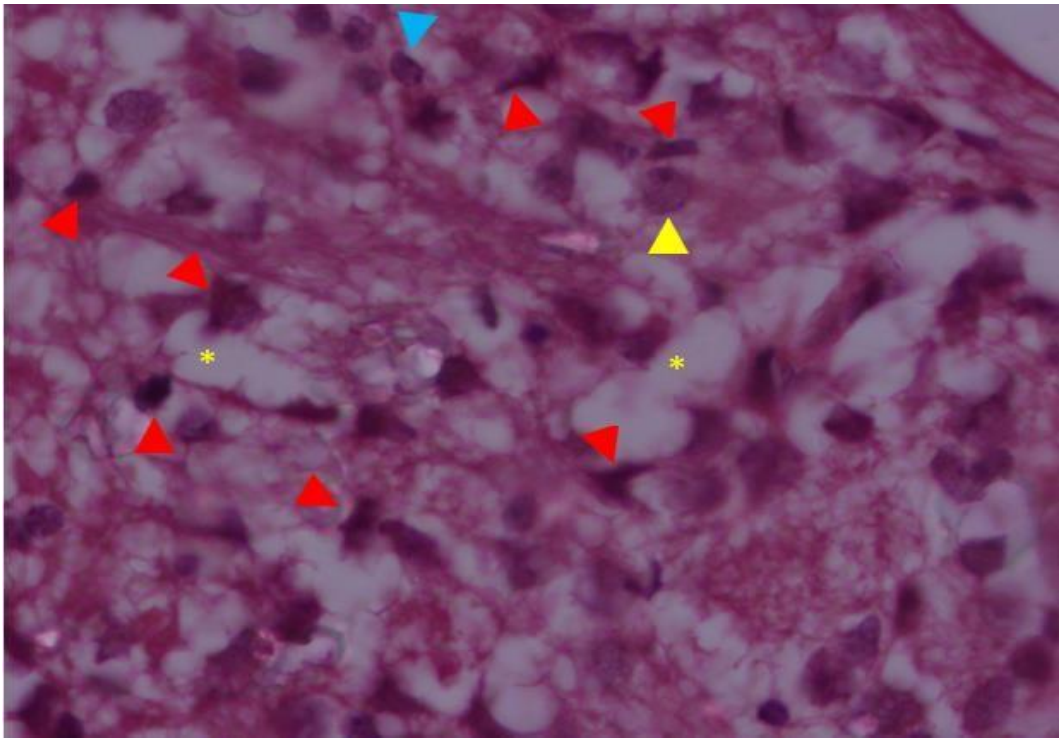


Fig. 2. HB in the experimental group. Hyperchromatic compact dark neurons with sharp margins (red arrowheads), astrocytes with round to elliptical nucleus (blue arrowheads) and normal neurons (yellow arrowheads) Neuropils (yellow stars) (X100).

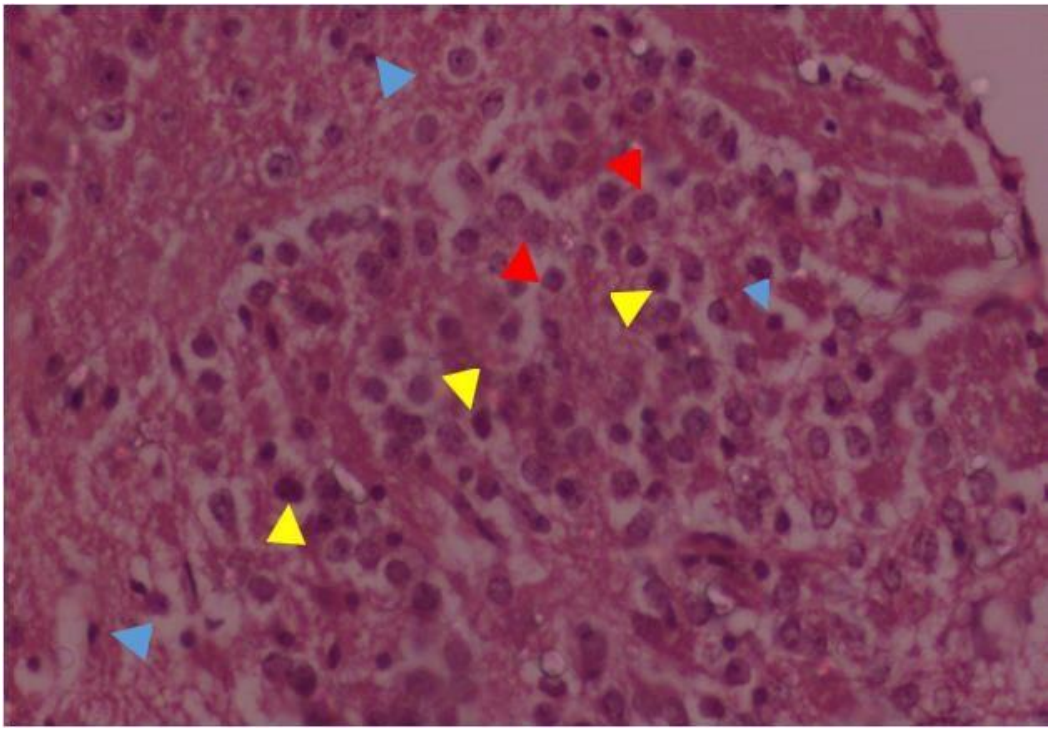


Fig. 3. HB nucleus of the Experimental group. Astrocytes (blue arrowheads) and healthy neurons (red HB nucleus of the control group. Some neurons stained deeply with prominent dense nucleoli (yellow arrowheads) are seen (X40).

The comparison between the number of astrocytes in the right and the left HB was insignificant (p=.998). The number of degenerating neurons in the right HB of the experimental animals (980±62) and the left HB (890±20) showed a significant level of difference with those of the control animals(Fig. 4)(<2)(p=0.000).

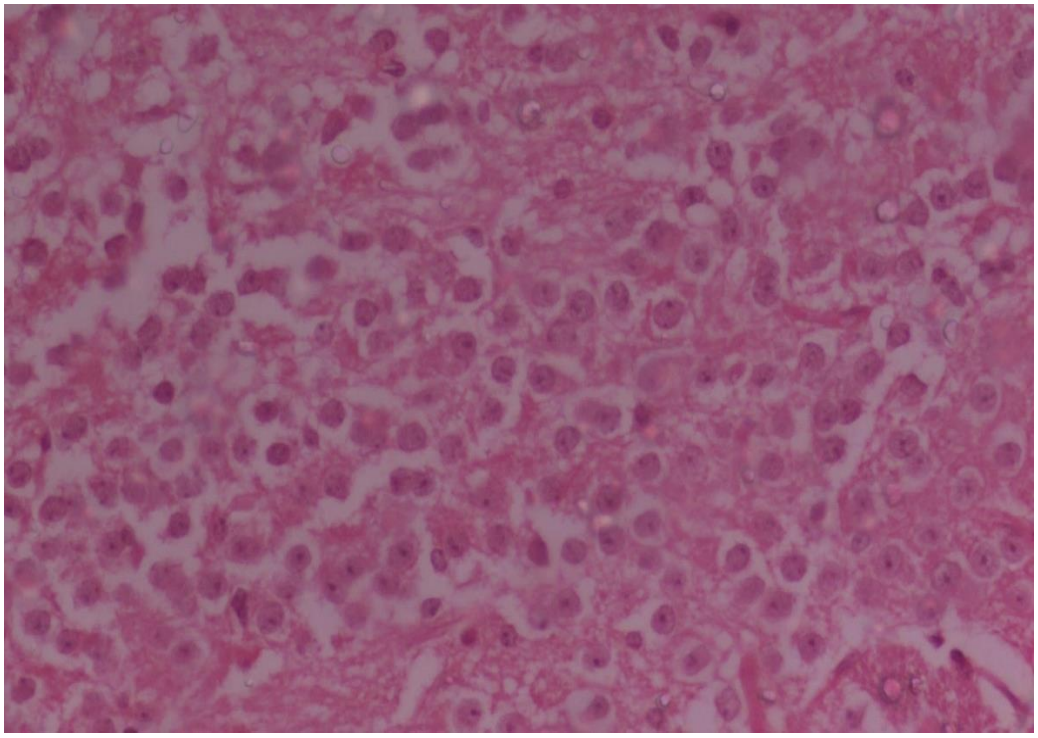


Fig. 4. HB nucleus of the control group. Neurons with prominent nucleoli are seen (blue arrowheads) (X40).

4. Discussion

The results of our study showed that the experimental model of NMDA hypofunction of schizophrenia induced with ketamine leads to neuropils alteration, neuronal death, and astrogliosis in the HB. Quantifying results with stereology revealed that the increase in the surface area of the neuropils is associated with neuronal death and reactive astrogliosis. Reactive astrogliosis enables astrocytes to actively participate in the CNS pathological states, including remodeling of extracellular matrix (ECM) and synaptic remodeling.[12] Neuropathological studies have yielded evidence that suggests neuropil changes in schizophrenia. Prasad et al. studied the membrane phospholipid (MPL) metabolites and imaging data of the whole brain in early schizophrenia. They reported that MPL catabolite levels were increased in the thalamus in schizophrenia compared to the controls. They suggested that an elevated level of MPL reflects the neuropil contraction. Similarly, Prasad et al. reported neuropil contraction in the CA4 region of adolescent-onset and young adult-onset schizophrenia patients.[6] Neuropil represents a highly dynamic organization within the CNS and comprises neuronal cell processes, synapses, extracellular matrix, astrocytes, and microvasculature.[13] From this perspective, neuropil could be recognized as a critical zone in neuronal processing. Strictly speaking, neuropil structure reflects the balanced contribution between the neurons, glial, ECM, and microvasculature.[4] Although the detailed mechanism of the neuropil changes has not been fully understood, various factors, including inflammatory reactions and increased oxidative stress, have been thought to be involved in the neuropil alteration.[6] Recent findings suggest that ketamine exposure (chronically or acute) increases the level of NMDA receptors as a compensatory mechanism, increased calcium ion influx, higher oxidative stress level, excitotoxicity, and neuronal death.[14, 15] Additionally, increased synaptic glutamate levels could trigger oxidative stress, which induces morphological changes in the neurons.[16] Based on the results of this study, the most striking pathological changes were neuropil expansion and cytoarchitecture disruption of the HB nucleus. The presence of dark and hyperstained neurons in the HB nucleus of the experimental group was another finding that would seem to suggest ongoing neurodegeneration.[17] Furthermore, the escalating synaptic glutamate level induces astrocytes as a double-edged sword reaction. There is compelling evidence suggests the astrocytes involvement in the pathogenesis of neurodegenerative and psychiatric disorders such as schizophrenia.[18-20] The results of this study revealed that the NMDA hypofunction model of schizophrenia induced by ketamine leads to astrogliosis. These findings are in line with the previous reports. For instance, studies have also shown that schizophrenia is associated with astrogliopathy.[20] The findings of this study and previous reports collectively support the critical role of astrogliosis in the pathogenesis of schizophrenia.[20] However, what should be kept in mind is that the functional interaction between the synaptic components takes place in the neuropil. Considering the tetrapartite synapse concept, ECM, pre and post-synaptic neurons, and astrocytes.[21] It is tenable to assume that the NMDA hypofunction model of schizophrenia could lead to alterations in the synaptic geometry at the neuropil level, which in turn affect the synaptic cross-talk processing.

Interestingly, our quantified data showed that neuropil surface area increased in the HB nucleus. However, this set of findings does not support the previous research. For instance, Prasad et al.[6] reported neuropil contraction in early-onset schizophrenia. Methodological and regional differences could partially explain this discrepancy. First, Prasad et al. measured the thalamus with neuroimaging method and the level of MPL. Although the MPL assay indicates cell membrane degeneration, generalizing these findings to the neuropil geometry should be taken cautiously. We used direct microscopic examination and an unbiased stereological method to estimate the surface areas of the neuropils in the HB nucleus. Secondly, there are reports that explicitly indicate different responses of the same neuroglial elements in schizophrenia at different CNS regions. For instance, some studies have shown an increased number of astrocytes in the cingulate gyrus and a decreased number of astrocytes in other brain regions of an examined sample.[21, 22] These discrepancies may reflect the complexity of the neuroglial connectivity as a continuum and the enigmatic entity of schizophrenia. Schizophrenia is known as the most debilitating multifaceted psychiatric disorder, so it is arguable to assume the discrepancies between the different studies may reflect the different subtypes of schizophrenia.[23]

Furthermore, recent neuroimaging studies have reported conflicting results on the volume of the HB nucleus in schizophreni.[24-27] According to our results, neuropil expansion may reflect the disturbed balance between the excitatory and inhibitory synapses[28] in the NMDA hypofunction model of schizophrenia.[29] The escalating level of glutamate at the synaptic space seems to trigger excitotoxicity and an increased level of oxidative stress, leading to astrogliosis.[30] These cellular events mediate the spatial arrangement of the synapse and, subsequently, neuropil areas. To reveal the structural details and underlying mechanisms of the neuropil alteration in schizophrenia, ultrastructural and histochemistry studies, particularly in different brain regions, are recommended.

5. Conclusion

It can be concluded that experimental schizophrenia induced by ketamine leads to neuropil expansion in the HB nucleus. Additionally, experimental schizophrenia is associated with an increased number of astrocytes and neurodegeneration in the HB. The findings of this study may reflect the role of astrogliopathy and subsequent neurodegeneration in the pathogenesis of schizophrenia. Neuropil expansion in schizophrenia might be considered as the altered synaptic geometry of the HB.

Conflict of Interest

The authors declared that there is no conflict of interest.

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