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Original article

Olanzapine exposure diminishes perfusion and decreases volume of sensorimotor cortex in rats

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ABSTRACT

Background: Olanzapine is a frequently used atypical antipsychotic drug known to exert structural brain alterations in animals. This study investigated whether chronic olanzapine exposure alters regional blood brain perfusion assessed by Arterial Spin Labelling (ASL) magnetic resonance imaging (MRI) in a validated model of olanzapine-induced metabolic disturbances. An effect of acute olanzapine exposure on brain perfusion was also assessed for comparison.
Methods: Adult Sprague-Dawley female rats were treated by intramuscular depot olanzapine injections (100 mg/kg every 14 days) or vehicle for 8 weeks. ASL scanning was performed on a 9.4 T Bruker BioSpec 94/30USR scanner under isoflurane anesthesia. Serum samples were used to assay leptin and TNF-α level while brains were sliced for histology. Another group received only one non-depot intraperitoneal dose of olanzapine (7 mg/kg) during MRI scanning, thus exposing its acute effect on brain perfusion.
Results: Both acute and chronic dosing of olanzapine resulted in decreased perfusion in the sensorimotor cortex, while no effect was observed in the piriform cortex or hippocampus. Furthermore, in the chronically treated group decreased cortex volume was observed. Chronic olanzapine dosing led to increased body weight, adipose tissue mass and leptin level, confirming its expected metabolic effects.
Conclusion: This study demonstrates region-specific decreases in blood perfusion associated with olanzapine exposure present already after the first dose. These findings extend our understanding of olanzapine-induced functional and structural brain changes.

Introduction

Schizophrenia, severe mental illness, causes both structural and functional brain changes [1–3]. Long term antipsychotic treatment seems to be another contributing factor leading to brain structural changes [4]. Antipsychotic treatment has been already shown to reduce grey matter volume especially in the frontal cortex in clinical studies [3–5]. There is a robust evidence, that second generation antipsychotics are far less associated with such changes than first generation but not without any risk [6]. Higher antipsychotic dosing is associated with severe structural impairment [7]. Olanzapine (OLA) was not associated with structural brain alteration so far [3,7]. Nevertheless, this evidence may not be conclusive because it is not supported by clinical studies with OLA-treated first-episode schizophrenia patients [7]. Moreover, preclinical studies have already shown the reduction of gray matter volume caused by long-term OLA exposure in rats and macaque monkeys [8–10]. However, in clinical settings the effect of antipsychotic treatment and the psychiatric disorder itself cannot be studied separately because long-term antipsychotic treatment in healthy volunteers is not ethical. Research in experimental animals enables one to isolate the influence of the antipsychotic treatment from brain changes caused by schizophrenia animal models *per se* possibly investigated by magnetic resonance imaging (MRI) [11,12].

In preclinical studies, it has been already shown that OLA causes significant decrease of whole brain volume, frontal cerebral cortex volume and decreased cortical thickness without

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any change in total number of neurons in adult Sprague-Dawley male rats after long term OLA treatment [9,10]. These findings also correspond with the long term OLA exposure study performed in macaque monkeys [8]. Unfortunately, there is a lack of preclinical functional neuroimaging studies evaluating brain perfusion altered by antipsychotic treatment. Currently, only two reports focusing on the effect of aripiprazole treatment on rat brain perfusion are available. The first one has showed that an acute dose of aripiprazole decreases brain perfusion in the entorhinal-piriform cortex, the nucleus accumbens shell, basolateral amygdala and has no effect in the medial prefrontal cortex and parietal cortex [13], while the second study has not registered any changes induced by chronic aripiprazole treatment [14].

Interestingly, there are several Arterial Spin Labelling (ASL) MRI animal studies focused on neurodevelopmental and pharmacological models of schizophrenia showing region-dependent perfusion changes [14,15]. This represents a valuable approach to distinguish the functional brain changes caused by antipsychotic treatment from those which originate from the schizophrenia-like phenotype

It has been also proposed that functional brain hemodynamic alterations could later evolve into structural changes reported in schizophrenic patients [16,17]. Only several reports are published regarding the effect of antipsychotics treatment on brain perfusion in human. However, these findings are incoherent likely due to different imaging methods [2,18] and different treatment protocols or stage of disease [16,19].

Therefore, the aim of this study was to assess the effect of OLA treatment on brain perfusion measured by ASL in healthy rats to obtain functional neuroimaging data. We have used a previously validated model of chronic OLA-induced metabolic dysregulation in female rats, which features clinically relevant dosing and has known metabolic consequences [20–24]. Histological evaluation of brain structure was performed to verify morphological effects of chronic OLA treatment. OLA was administered also acutely in a non-depot form to measure its immediate effect on the brain perfusion. This study could shed more light on OLA-induced functional and structural brain changes.

Materials and methods

Animals

Thirty-four female 8-week-old albino Sprague-Dawley rats weighing 200–225 g at the beginning of the study were purchased from Charles River (Germany) and housed individually in standard polycarbonate cages. Two experiments were conducted with these subjects: A) A chronic study was performed with 26 rats: 13 OLA treated and 13 control (vehicle treated) animals. The numbers of animals used for MRI scanning were n = 7 per group, while histology was performed in groups of n = 6. B) An acute study was performed with 8 rats. At the time of sample and data collection, the rats in both studies were at the same age (16 weeks).

The animals were single-housed at the Central Animal Facility of Masaryk University, Brno, Czech Republic. Environmental conditions during the whole study were constant: relative humidity 50–60 %, temperature 23 °C ± 1 °C, normal 12 -h light-dark cycle (6 a.m. to 6 p.m. light). Standard rodent chow and water were available *ad libitum*. The rats used in the MRI experiments were transported to the Animal Facility of the Institute of Scientific Instruments of the Czech Academy of Sciences, Brno, Czech Republic and maintained under the same conditions as in the previous location. All procedures were performed in accordance with EU Directive no. 2010/63/EU and approved by the Animal Care Committee of the Faculty of Medicine, Masaryk University, Czech Republic and Czech Governmental Animal Care Committee, in compliance with Czech Animal Protection Act No. 246/1992.

Drugs and treatments

In the chronic study OLA was administered in a depot formulation for human use (olanzapini embonas monohydricus, ZypAdhera®) by an intramuscular injection at dose 100 mg/kg every 14 days for 8 weeks in the evening hours (administration on day 1, 15, 29 and 43). This mode of administration was previously verified and shown to maintain stable plasma concentrations [25]. The solvent vehicle was injected to the control group. Food was removed from the cages and all rats were subjected to overnight fasting in order to prevent weight gain differences induced by sedation in the OLA treated group as already validated and described [25]. The OLA dose is relatively higher compared to doses used in patients because this drug has a shorter half-life in rodents (2.5 h) than in humans (ca 30 h) [26,27]. Also, receptor occupancies comparable to those in humans at the dose of 7.5 mg/kg/day are required for continuous administration [27]. Rats were randomly assigned to the following groups: vehicle (VEH) treated (n = 13) and OLA treated (n = 13). All animals were weighed daily. On day 56 of the study seven rats per group were subjected to ASL MRI scanning and on day 57, were sacrificed by decapitation under short isoflurane anesthesia so that blood for serum and abdominal fat could be collected. A dissection was performed by wide laparotomy and abdominal fat tissue was collected and weighed. The serum samples were stored frozen at −80 °C until analysis. The last six rats in each group were sacrificed by the same procedure; their brains were harvested and fixed by 4% paraformaldehyde in phosphate buffer (Sigma-Aldrich, Czech Republic) for 4 h, washed by phosphate buffer and stored in a fridge at 6 °C.

In the acute study, OLA was administered during MRI measurement as an injectable formulation for human use (olanzapine, Zyprexa®) by an intraperitoneal injection at a dose of 7 mg/kg in 1.4 mL of saline delivered by an infusion pump.

Immunoassay for leptin and tumor necrosis factor-α (TNF-α)

Both analytes were assayed in serum by a multiplex system allowing parallel detection and quantification of several markers in one sample. The assay was carried out on the Bio-Plex® reader with commercially available kits (Bio-Rad®) as described earlier [20,28,29].

Histological processing and staining

The brains were washed by distilled water for 1 h and dehydrated in ascending ethanol series (30%, 50%, 70%, 95%, and 100%). In the following steps, the tissues were saturated by Xylene (Lach-Ner, Czech Republic) and subsequently by paraffin wax. The tissues were embedded into paraffin blocks and cut into 5 μm sections. For the Nissl staining the following procedure was used: The paraffin wax was removed from the sections, tissues were washed in distilled water and were stained by 1% toluidine blue solution (Sigma-Aldrich, Czech Republic) in acetate buffer (0.1 M acetic acid and 0.1 M sodium acetate; pH 5.6; Lach-Ner, Czech Republic) for 1 h. After washing by distilled water, the sections were dehydrated by ethanol, saturated by xylene and mounted by Entellan (Merck, Czech Republic). An Olympus SZX10 Stereo Microscope with Olympus U-TV0.5cx camera adapter and uEye UI-1465LE-C, SUXGA 3.3 MegaPixel Cameras were used for documentation of histological samples. The images were taken in program Quickphoto industrial 2.3; the magnification (of images is) 6.3 × .

Estimations of cortical brain area were done in MATLAB R2016a (The MathWorks Inc., Natick, MA, USA) by image analysis techniques. After transforming all images in grey-scale, contour detection and background deduction followed. Prepared images were binarized by applying a common threshold to all of them. This created binary masks of intensity with small foreign objects (caused by inhomogeneities in images). Those were eliminated by object recognition techniques and repeated image erosion / dilatation. Areas of interest were determined manually as two lines leading horizontally and vertically. The resulting fields were filled in binary mask by value 1. Final areas were simply calculated as sum of these fields (in pixels) and after calibration in squared mm.

MRI

The MRI assessment was organized as follows: in the chronic part of the study OLA-treated and vehicle-treated rats were scanned once. In the acute part of the study, ASL scanning was performed in drug-naive rats three times consecutively. The first measurement was conducted before any treatment, the second started at the time of OLA administration (minute 0) and the third commenced 20 min later. The selected slice is positioned at −3.14 from Bregma [30]. Thanks to the selection of a single slice, the MRI examination requiring general anesthesia could be kept so short that induction of any brain perfusion aberration could be considered negligible [31]; the total time of MRI measurement was 30 min in the chronic administration group and one hour in the acute study. The analyzed regions of interest (ROIs) were sensorimotor and piriform cortices and hippocampus. We included both cortical regions in the selected slice because all cortical regions are known to be affected by the impaired neurodevelopment in schizophrenia [3]. Likewise, hippocampal structural and functional changes are neuroimaging hallmarks of schizophrenia, and schizophrenia-like phenotype in rodents are probably related to cognitive symptoms [32].

All MRI was performed on a 9.4 T Bruker BioSpec 94/30USR scanner with a 2 × 2 surface array rat head RF receive coil and a volume transmit RF coil. The measurement was conducted under anesthesia by 2% Isoflurane and 1000 mL/min of oxygen. This anesthetic protocol was shown to be suitable for brain perfusion measurements [33,34] though isoflurane verifiable alters CBF in rats [35]. We control for this effect by using vehicle treated group under the same condition. We laid the animals on a thermal pad and monitored their body temperature and respiratory curve during the measurement process. We used the RARE sequence with TR =3500 ms, TE =36 ms, FOV 40.3 × 30.5 mm^2, and image matrix 256 × 256 to obtain fifteen T2-weighted anatomical axial slices with the thickness of 1.25 mm. These slices covered the brain from the root of the olfactory bulbs to the cerebellum and provided the background for the selection of an axial slice suitable for the ASL measurement. ASL is a non-invasive perfusion imaging modality that uses magnetically labelled ^1H proton spins of blood water as an endogenous tracer of the cerebral blood flow (CBF). By variation of the labelling, CBF-weighted images or quantitative CBF maps can be obtained [36]. In our measurements, one axial slice with 1.25-mm thickness was obtained with FAIR-RARE sequence applied with TR =10,000 ms, TE =37.78 ms, TI stepped through 30, 50, 100, 200, 300, 500, 700, 900, 1000, 1100, 1500, 1800, 2200, 2800, 3200 ms, FOV 40.3 × 30.5 mm^2, and image matrix 128 × 96. The RARE readout excitation was preceded by a 16-ms adiabatic inversion pulse with the bandwidth of 4866.2 Hz, affecting either a 4.25-mm thick slab selectively, or the whole volume unselectively. By matching each of these two image series to a T1 relaxation model, the $T_{1,sel}$ and $T_{1,nonsel}$ maps were obtained, from which the CBF map was calculated according to the equation [37].

$$CBF = \lambda . \frac{T_{1,nonsel}}{T_{1,blood}} \left(\frac{1}{T_{1,sel}} - \frac{1}{T_{1,nonsel}} \right)$$

where CBF is the cerebral blood flow (usually expressed in mL/min in 100 g of tissue, λ is the blood-brain partition coefficient, expressing the ratio of the quantity of water per gram of tissue to the quantity of water per milliliter of blood, which is known to be 0.89 ± 0.03 mL(blood)/g(tissue) in the rat brain [38], $T_{1,nonsel}$ and $T_{1,sel}$ are the apparent longitudinal relaxation times derived from the image series applying nonselective and slice-selective inversions, respectively, and $T_{1,blood}$ is the longitudinal relaxation of capillary blood. T1 of blood was kept at the default value 2.4 s (R1 = 0.4 s), in accordance with the literature [39].

The perfusion maps were calculated in Paravision 5.1 (Bruker Biospin, Ettlingen, Germany) and further analyzed in manually drawn brain ROIs by own MATLAB R2010a code (The MathWorks Inc., Natick, MA, USA). Regions of interest were drawn manually according to the rat brain atlas [30].

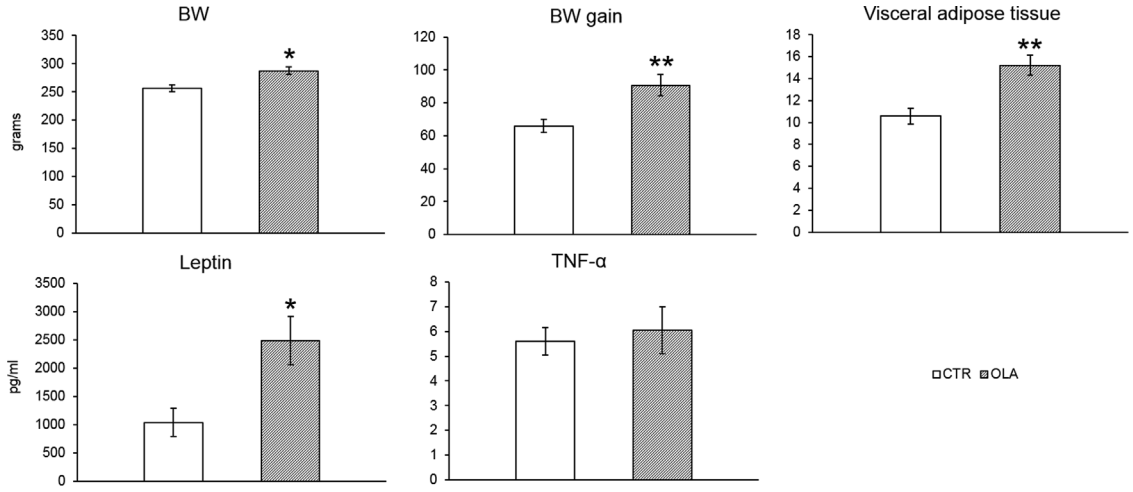

Fig. 1. Body weight, adipose tissue accumulation, leptin and TNF-α levels. The graphs show the mean ± SEM body weight (BW), body weight gain and amount of dissected adipose tissue in grams at the end of the study (day 57). The basal BW data (day 1) are not shown as the individuals' body weights were within 25 g of variability when delivered from Charles River. All BW related measures were significantly increased in the OLA treated group: BW: MWU test, $^*p = 0.018$, BW gain: *t*-test, $^{**}p = 0.010$ and adipose tissue mass: MWU test, $^{**}p = 0.003$. Leptin levels were significantly increased in the OLA treated group: *t*-test, $^*p = 0.018$ while there was no difference in TNF-α levels between the groups: *t*-test, n.s.

Statistical data analysis

Primary data were summarized using arithmetic mean and standard error of the mean estimate (±SEM). In the chronic study, the body weight data, adipose tissue mass, leptin and TNF-α levels and ASL data were compared between the groups by *t*-test when the dataset was distributed normally (Kolmogorov-Smirnov test of normality) or Mann-Whitney U (MWU) test when the normality test was significant. The ASL data from the acute study (3 time-points, i.e. basal, min 0 and min 20 measurements) were analyzed by a *t*-test for dependent variables. The analyses were calculated using Statistica 12 (StatSoft, USA). A value $p < 0.05$ was recognized as the boundary of statistical significance in all the tests applied.

Results

Metabolic markers of chronic OLA treatment

The body weight data were compared at the end of the study only because a very homogeneous group was acquired from Charles River and no body weight differences could be identified at the beginning of the study. Fig. 1 shows the data on body weight, cumulative body weight gain, adiposity, leptin and TNF-α levels. All body weights related variables were found to be significantly increased in the depot OLA treated group: body weight (MWU test, $p = 0.018$), body weight gain (*t*-test, $p = 0.010$) and adipose tissue mass (MWU test, $p = 0.003$). A significant increase of leptin was also shown in the OLA treated animals (*t*-test, $p = 0.018$) while there was found no OLA induced change in the TNF-α levels (*t*-test, $p = 0.716$, n.s.) and the absolute values in individual animals were generally below the detection limit (5 pg/mL).

ASL data after chronic OLA administration

Fig. 2 shows the anatomical images, delineation of regions of interest for the ASL analysis and perfusion map in one representative animal from each group. Fig. 3 summarizes the ASL data in mL/min per 100 g of tissue from all three regions of interest at the end of chronic OLA administration. The only significant difference was detected in the sensorimotor cortex where the treatment led to decrease of tissue perfusion (*t*-test, $p = 0.014$) while there was no difference between the groups in perfusion of hippocampus (*t*-test, $p = 0.593$, n.s.) and piriform cortex (*t*-test, $p = 0.511$, n.s.).

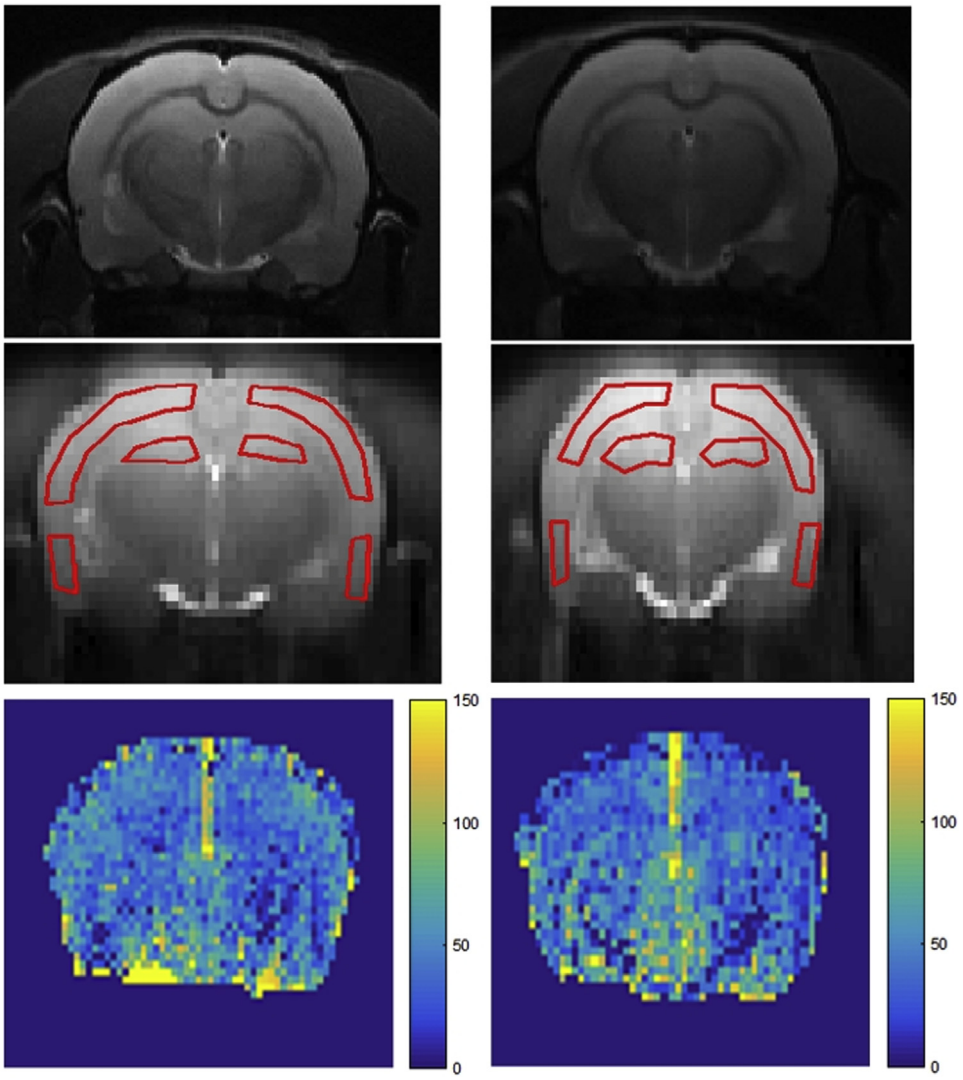

Fig. 2. ASL image. The figure shows anatomical images in the top part, the delineation of regions of interest for the ASL analysis (central part) and perfusion map in one representative animal from each group (bottom part). The vehicle treated animal in the left column and the OLA treated animal in the right column.

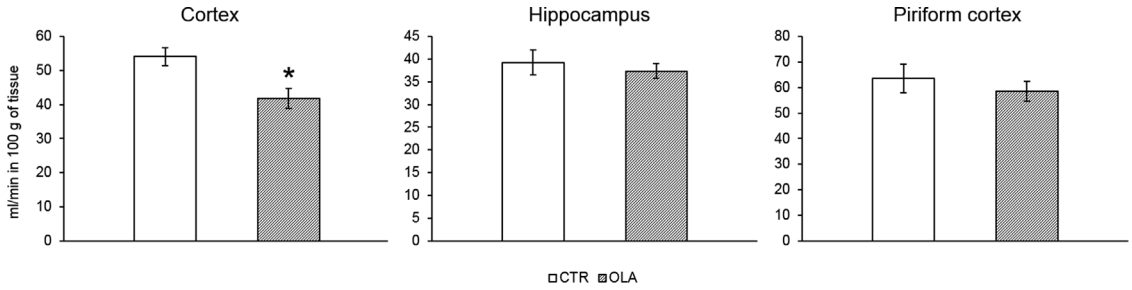

Fig. 3. ASL data after chronic OLA treatment. The bar graphs indicate the mean ± SEM of tissue perfusion in mL/min per 100 g of tissue. OLA treatment induced significant decrease of perfusion in sensorimotor cortex, *t*-test, $^{*}p = 0.014$.

Histology

The typical looks of the control and chronically OLA treated rats' brains and the delineation of the assessed ROIs are shown in Fig. 4. The brain sections from all OLA treated animals were characterized by a detachment of the ventral part of hippocampus from the rest of the brain. This appears in the figures as blank space. Fig. 5 indicates the comparison of cortical tissue mean areas. Chronic OLA administration induced a highly significant decrease of the tissue area (MWU test, $p < 0.001$).

ASL data after acute OLA administration

Fig. 6 shows the ASL data in mL/min per 100 g of tissue from all three regions of interest before and after acute OLA administration. A *t*-test for dependent samples indicated a significant decrease of perfusion in cortex20 min after OLA administration compared to data before the administration ($p = 0.041$) while there were no significant differences in the piriform cortex and hippocampus at any time-point.

Discussion

The purpose of the current study was to investigate the effect of the chronic depot OLA exposure on cerebral blood perfusion as a potential starter of structural brain changes in a validated model of OLA-induced metabolic disturbance. Considering that one adult rat month is comparable to three human years [40], the chronic treatment used in this study corresponds approximately to 6 human years. This period is likely long enough to get valuable information about the chronic treatment effects. Our data confirm that chronic depot OLA administration leads to the expected development of characteristic metabolic levels. Furthermore, there were found no changes in TNF-α level in depot OLA treated animals as we reported previously [20]. This confirms low probability of systemic inflammatory state as a potential confounding factor which may influence brain perfusion and also contribute to development of metabolic disturbances [41]. Functional and structural brain changes occurring after OLA treatment and their potential origins are explored and discussed below.

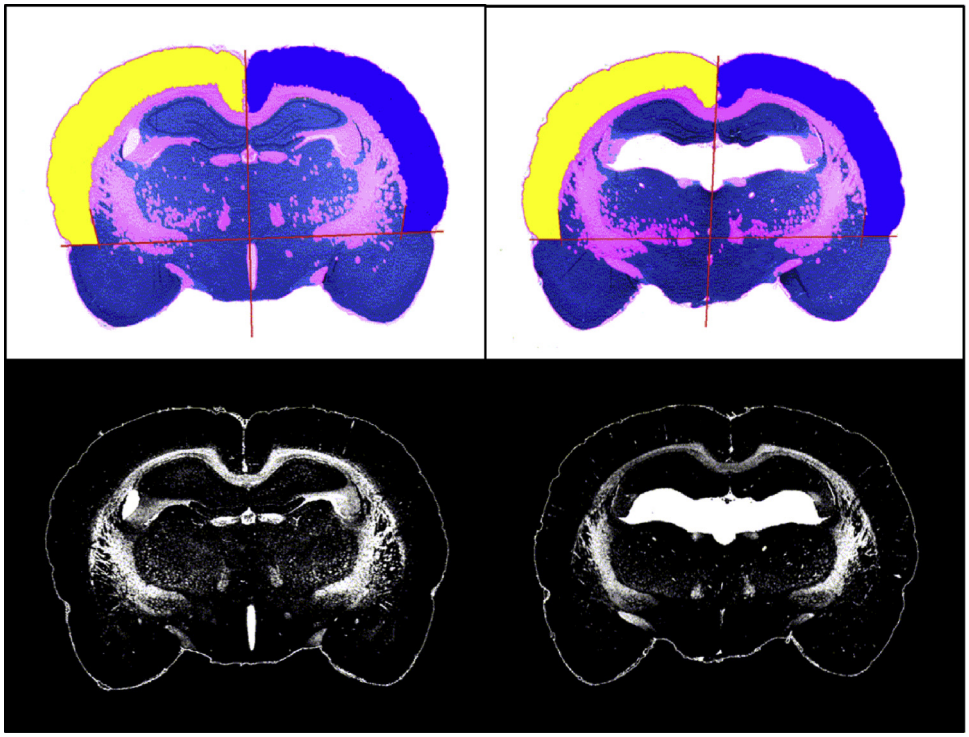

Fig. 4. Analysis of cerebral cortex of rats from the histological sections. Left panels show a typical section of the control rats' brain, right panels show an OLA treated rat. In the upper figures the red lines and pink areas determinate the border of the evaluated cerebral cortex marked by the yellow (left side) and dark blue (right side) areas. The bottom figures of the same sections of rat brains in grey-scale images show corpus callosum used for the border localization of cortex areas and computation of its size.

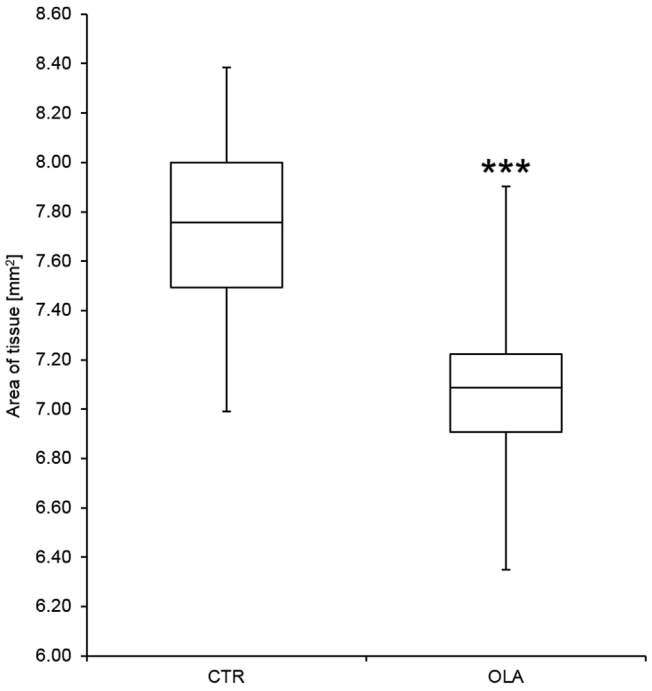

Fig. 5. Cortical volume assessed by histology. The graph shows tissue area in regions of interest of groups CTR and OLA. OLA treatment induced significant reduce of cerebral cortex area (MWU test, $p < 0.001$). Middle row represents the mean value, box shows first and third quartile and error bars indicate the minimum and maximum values.

In this ASL study, we detected OLA-induced perfusion changes in sensorimotor cortex after both, chronic and acute drug treatment. These findings indicate that the effect observed after chronic treatment is exerted already by an acute dose and seems to persist. However, the exact mechanism responsible for these changes is unknown. The pharmacodynamic profile of OLA is complex and its efficacy in schizophrenia is supposed to be mediated through dopamine (D2) and serotonin type 2 (5-HT2) antagonism. However, OLA strongly interacts with all D receptor subtypes and also many other receptors exerting antagonistic effect on histaminergic (H1), serotonergic (5-HT3, 5-HT6), adrenergic α1 receptors and muscarinic receptors [42,43]. Some of these mechanisms are therefore likely to contribute to the perfusion changes observed in this study.

Dopamine has probably the best explored hemodynamic effects. It profoundly influences all segments of the cerebral circulation [44] and it seems to directly affect especially intraparenchymal cortical blood flow [45]. This evidence is supported by a functional MRI study which showed a close correlation between dopaminergic stimulation (both direct and indirect) and hemodynamic changes in the brain tissue [46]. Krimer and colleagues assessed the effect of direct dopamine application onto microvessels in the brain slices and observed vasoconstriction [45]. However, when serotonergic and adrenergic receptors were blocked and dopamine activated only D receptors it caused a vasodilation [44,47]. This suggests that at physiological conditions, selective activation of D receptors exerts vasodilation. Therefore, tissue perfusion noninvasively measured by ASL MRI, may indicate changes in cerebral blood flow associated with pharmacological manipulation of the dopaminergic system [48]. Based on the current evidence, we hypothesize that the decreased cortical perfusion could be due to its antagonism on D receptors potentially causing vasoconstriction. Nevertheless, the potential role of other neurotransmitter systems cannot be ruled out.

Furthermore, a study visualizing the dopaminergic neurons' terminals suggested a functional connection between dopaminergic neurotransmission and microcirculation in the cerebral cortex. This could be explained by a close contact of dopaminergic neurons with penetrating arterioles and cerebral capillaries [45,47]. Moreover, the authors suggest that central dopaminergic neurons are uniquely positioned to control the cerebral microcirculation thus neural activity may participate in the regulation of cerebral blood flow. The density of dopaminergic vascular innervation varies regionally, being greatest in dopamine-rich regions of the frontal, sensorimotor and entorhinal cortices [45,47,48] which makes these regions particularly sensitive to potential dopamine-induced perfusion changes.

It could be argued that other neurotransmitters, particularly noradrenaline and serotonin, could also significantly affect cerebral circulation. The direct vascular effects of 5-HT *in vivo* have been examined by micro-application of this amine *in situ* around individual pial arterioles in cats. In normotensive cats, topical application of 5-HT universally dilated small pial arterioles less than 70 μm resting caliber, but larger arteries with resting caliber of approximately 200 μm were constricted [44]. This is in accordance with the widely known vasoconstrictor mechanism of

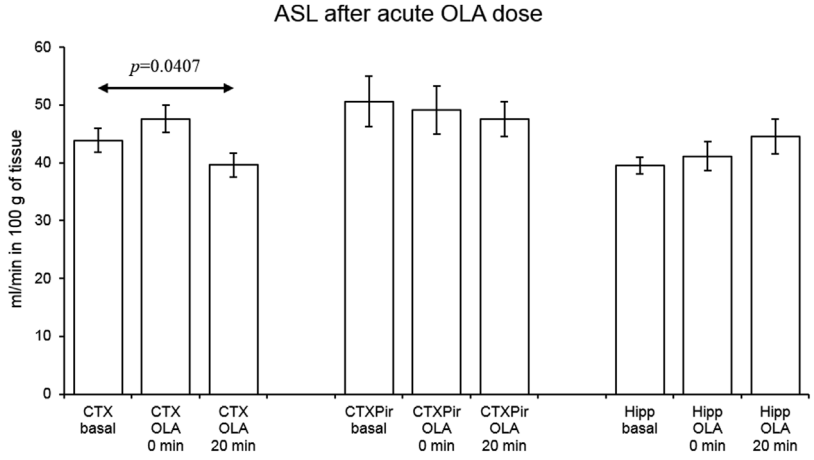

Fig. 6. ASL acute OLA. The graph shows the mean ± SEM of perfusion in mL/min per 100 g of tissue before and after acute administration of OLA at minute 0 and minute 20 in all three regions of interest: CTX – sensorimotor cortex, CTXPir – piriform cortex and Hipp – hippocampus; *t*-test for dependent samples revealed a significant decrease of perfusion in the sensorimotor cortex 20 min after OLA administration.

triptans. However, they exert their effect mainly *via* agonism on serotonin 5-HT1B and 5-HT1D receptors. This mechanism does not correspond well with pharmacodynamic profile of OLA, which has a low affinity to all 5-HT1 receptor subtypes [49,50], and therefore it is unlikely to explain its effect on brain perfusion. Furthermore, triptans affect mainly the extraparenchymal cerebral vessels [51], i.e. they induce vasoconstriction mainly outside the blood-brain barrier with minimal effect in the cortical regions [52].

Noradrenaline is another neurotransmitter which is proposed to have an important role in controlling brain blood flow [53]. Nevertheless, the innervation of the intracortical blood vessels seems to be predominantly dopaminergic while the innervation of extraparenchymal cerebral vessels is more noradrenergic [45]. Besides, β2 receptors are probably the predominant adrenoreceptors in cerebral vessels [54,55] while OLA interacts mainly with α1 adrenoreceptors [42]. Unfortunately, there is still a lack of information about the role of noradrenaline and its adrenoceptors in the cerebral blood circulation [54] and we cannot conclude to what extent could α antagonistic effect contribute to OLA-induced brain perfusion alteration.

Furthermore, histamine has generally been viewed as a dilator of cerebral blood vessels, but the available findings suggest that the direct vasomotor effects of histamine are highly dependent on the species and cerebral artery being investigated [44]. Since analysis of the histaminergic receptors has shown that the contractile effect of histamine on the cerebral vasculature appears to be mediated *via* H1 receptors [44], we may propose that dopaminergic and histaminergic systems could affect the cortex perfusion jointly.

Of note, in our study, two different cortical regions with potentially similar density of dopaminergic terminals [45] showed a different reaction. Moreover, there was no effect of OLA treatment in the hippocampal region. In the case of the hippocampus, we can just speculate whether it is due to specific hippocampal vessels pattern [56] or the fact that dopaminergic innervation seems to be predominantly in the dorsal part of hippocampus [57]. The inconsistent findings observed in the cortices are intriguing and may suggest different reactivities to OLA effect by some unknown mechanism. However, we conclude that the this discrepancy is likely due to the small number of voxels obtained from the piriform cortex, which is a small region of interest compared to sensorimotor cortex.

Longitudinal imaging studies in schizophrenia patients treated with antipsychotic drugs including OLA demonstrate that the dose and the duration of antipsychotic treatment are related to significant reductions in grey matter volume, particularly in the frontal cortex [3,7]. However, since the examined patients were also affected by schizophrenia – considered as a neurodevelopmental disorder, the structural pathologies can be also attributed primarily to the psychiatric disorder *per se* [58,59]. Therefore, Vernon et al. (2011) performed the first longitudinal preclinical study focused on brain volume changes assessed by anatomical (T2-weighted) MRI scanning in healthy male Sprague-Dawley rats after 8 weeks of OLA administration and observed a decrease of the whole brain volume, significantly reduced cerebral cortex volume and elevated astrocyte density. A later study of the same team showed a decrease of the volume and thickness of the anterior cingulate cortex after chronic OLA treatment, while there was no effect on the total number of neurons and astrocytes proving an increased density of the tissue [9]. Our study is in accordance with these results as we registered a significant reduction of sensorimotor cortex area in chronic OLA treated female Sprague-Dawley rats. Our ASL data provide additional information by showing lower cortical CBF values already after an acute OLA dose as the proposed mechanism responsible for the structural changes occurring after chronic drug treatment. Importantly, the absolute CBF values obtained in this study may differ from other studies due to the specific ASL acquisition, the anesthetic protocols, and also because female rats, as used in this study, have lower CBF values than their male counterparts [14].

Conclusion

Taken together, our findings demonstrate region-specific functional and structural effects of OLA exposure on rat cerebral cortex. Specifically, we observed lower cortical perfusion after both acute and chronic OLA treatment, and a decreased cortical area in chronically treated rats. Therefore, we hypothesize that the decreased cortical thickness could be a result of chronic OLA-induced decrease of blood perfusion in this brain region, which appears immediately after an acute OLA dose. An important extension of this study would be the use of the OLA treatment in the animal model of schizophrenia. These models may feature hemodynamic alterations as shown in two neurodevelopmental models of schizophrenia [14,60]. Specifically, methylazoxymethanol acetate (MAM) and polyinosinic:polycytidylic acid [poly(I:C)] models feature increased perfusion of the sensorimotor cortex which may simply be normalized by OLA treatment. However, this hypothesis remains to be tested.

Conflict of interest

All authors declare no conflict of interest.

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