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Antidepressant agomelatine attenuates behavioral deficits and concomitant pathology observed in streptozotocin-induced model of Alzheimer's disease in male rats

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ABSTRACT

Experimental findings suggest that the melatonin system has a beneficial role in models of Alzheimer's disease (ADs). The aim of the present study was to explore whether the atypical antidepressant agomelatine (Ago), which is a melatonin MT_1 and MT_2 agonist and $5\text{-}HT_{2C}$ antagonist, is effective against behavioral, biochemical and histological impairments in streptozotocin (STZ)-induced model of ADs in male rats. Male Sprague Dawley rats were treated intraperitoneally (i.p.) with Ago (40 mg/kg) for 30 days starting three months following the intracerebroventricular (icv) injection of STZ. Chronic Ago treatment reduced anxiety-like behavior of STZ-treated rats in the elevated plus maze, increased the preference to saccharine and corrected the spatial memory impairment in the eight-arm radial arm maze test. This melatonin analogue restored STZ-induced biochemical changes, including an increase of beta amyloid (Aβ) protein, and signal markers of inflammation (TNF-alpha and IL-1 beta). Ago exerted partial neuroprotection, specifically in the temporal CA3b subfield of the dorsal hippocampus and temporal piriform cortex. The ability of Ago to alleviate behavioral symptoms and concomitant neuropathological events observed in a model of sporadic ADs suggests that this melatonin alternative can be considered a promising adjuvant in this disease.

1. Introduction

Depression is considered a risk factor for Alzheimer's disease (AD) (Ownby et al., 2006). Furthermore, depression is a frequent psychiatric disorder accompanying AD leading to higher rate of mortality in the elderly. Accumulated evidence suggest common underlying mechanism in patients with depression and comorbid depression in AD, including close genetic pathways in older adults (Modrego, 2010), a hypothalamic-pituitary-adrenal (HPA) axis hyperactivity, a lack of feedback regulatory mechanism (Raadsheer et al., 1995), glutamatergic dysfunction, inflammation, cerebrovascular disease and altered synaptic plasticity (Chi et al., 2014; Dobos et al., 2012; Nihonmatsu-Kikuchi et al., 2013). Although an epidemiological link between the two diseases has been confirmed, there are few verified experimental models to support a shared pathology in both phenomena. Transgenic mouse models demonstrated that monoaminergic deficits associated with depression is a crucial condition for the development of AD (Liu et al., 2008; Romano et al., 2015). There are few recently published manuscripts describing that intracerebroventricular (icv) streptozotocin (STZ) model, which model represents 95% of sporadic AD, can provoke depressive-like behavior in animals. Thus, icv STZ-treated mice demonstrate increased immobility in tail suspension test and anhedonia (Souza et al., 2013, 2017), while rats exhibit decreased anxiety index in the elevated plus maze (EPM) test and depressive-like behavior in the forced swimming test (FST) (Navabi et al., 2018).

While commonly used antidepressants as selective serotonin re-uptake inhibitors (SSRIs) do not produce promising results as a therapeutic strategy for treatment of comorbid depression and AD, targets of inflammatory signaling (Siarkos et al., 2015), mood symptoms and cognitive impairment (Kiosses et al., 2015) or newly developed antidepressants with complex mechanism associated with effects on pre and post-synaptic monoaminergic pathway (Dale et al., 2015) can provide

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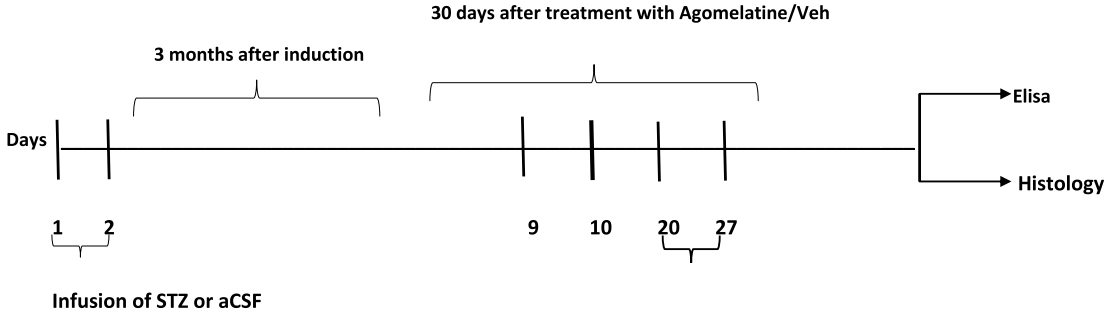

Fig. 1. Schematic illustration of the experimental protocol.

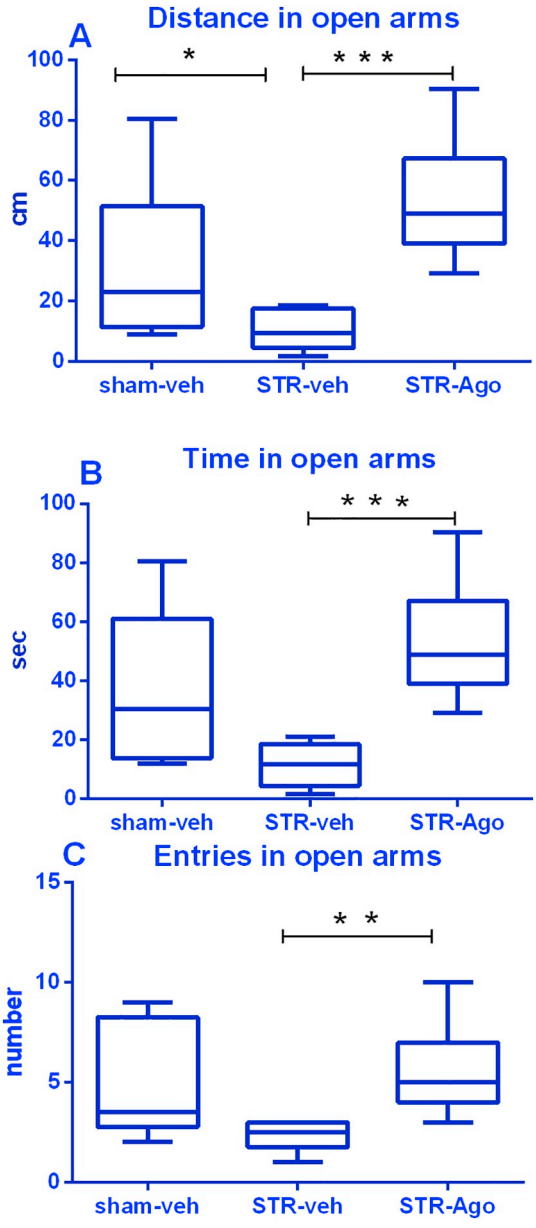

Fig. 2. Effect of chronic treatment with agomelatine (Ago) on distance (cm) (A), time (s) (B) and number of entries (C) in the open arms of the elevated plus maze test. Data is presented as mean ± S.E.M. **p < 0.01; ***p < 0.001. Sham-veh, control group treated with vehicle (n = 8), STZ-veh, icv streptozotocin-infused group and treated with vehicle, STZ-Ago, STZ-group treated with Ago.

better opportunity for treatment. The therapeutic potential of a melatonin system in AD has been reported by Rudnitskaya et al. (2015a) revealing the beneficial behavioral and neuroprotective effects of prophylactic treatment with melatonin before the development of AD-like symptoms as well as at the chronic stage of AD-like conditions in OXYS rats, which are characterized by melatonin deficit (Rudnitskaya et al., 2015b). As an antioxidant, melatonin is able to correct the pathological hallmarks of AD, tau hyperphosphorylation and Aβ generation and deposition, thereby alleviating inflammatory responses leading to neurodegeneration and cognitive impairment (reviewed in: Lin et al., 2013). Melatonin was reported to exert a potent neuroprotection against the kainic acid (KA)-induced activated forms of glia (Chung and Han, 2003) as well as Aβ-induced, increased expression of pro-inflammatory cytokines in the rat brain (Rosales-Corral et al., 2003).

Agomelatine, developed by the pharmaceutical company Servier in 2005, due to its synergism of MT_1/MT_2 melatonin receptors and antagonism of $5\text{-}HT_{2C}$ serotonin receptors possesses potent antidepressant, anxiolytic and chronobiotic activity (Guardiola-Lemaitre et al., 2014; Stahl, 2014). Recently, we have reported that administration of Ago can exert antidepressant effect in pinealectomized rats (Tchekalarova et al., 2016) as well as a strong neuroprotection in limbic brain structures during the chronic epileptic phase after the KA-induced status epilepticus (Tchekalarova et al., 2017). Ago is able to suppress the lipopolysaccharide-induced inflammatory response in the brain (Molteni et al., 2013) as well as that induced by KA in the brain and periphery in rats (Tchekalarova et al., 2018).

In the present study, based on the above findings, we hypothesized that chronic treatment with Ago could exert a beneficial effect on behavioral alterations (emotional disturbance and cognitive impairment), biochemical, histopathological modifications and neuroinflammation in the icv-STZ model of sporadic AD in male rats.

2. Materials and methods

The procedures used in this study were in agreement with the European Communities Council Directive 2010/63/EU for animal experiments. The experimental procedures were conducted in accordance with national rules on animal experiments and were approved by the Bulgarian Food Safety Agency.

2.1. Animals

Forty male Sprague Dawley rats weighing 200–230 g (eight weeks old) were obtained from the Breeding animal facility of the Institute of Neurobiology, Bulgarian Academy of Sciences. After arrival, they were housed in groups of n = 3–4 per cage and kept in an environment with a controlled light (L)/dark (D) regime, temperature and humidity (12 h/12 h L/D, temperature 20 °C, 50–60% relative humidity) with free access to standard laboratory chow and water.

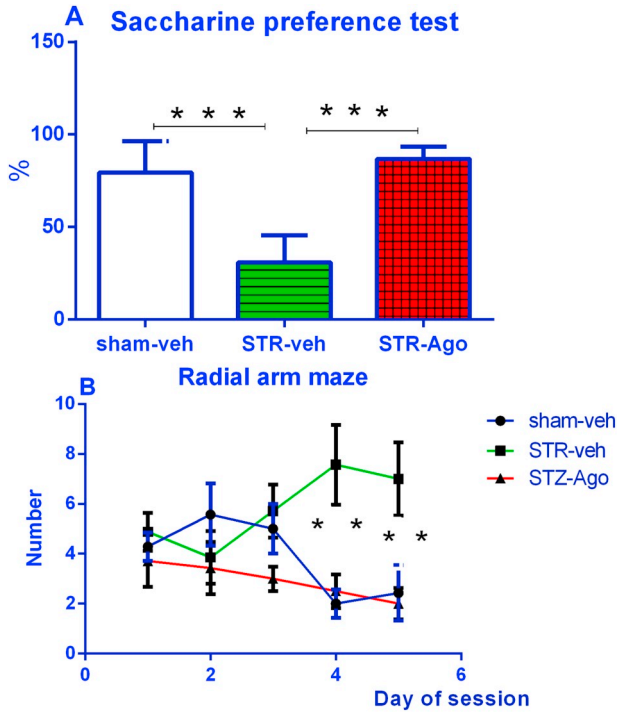

Fig. 3. Effect of chronic treatment with Ago on preference to saccharine solution (%) in the saccharine preference test (A) and working memory errors in the radial arm maze test (B). Data is presented as mean ± S.E.M. **p < 0.01; ***p < 0.001. Abbreviations in legends as in Fig. 2.

2.2. Experimental design and Ago treatment

The experimental design is shown in Fig. 1. The injection of Ago (kindly gifted by Servier Company, France) started ninety days after STZ infusion and continued for additional thirty days at a dose of 40 mg/kg, i.p., dissolved in hydroxyethyl cellulose (1%) at 4:00 p.m. (2 h before the onset of the dark phase). This dose has previously been shown to be effective in behavioral and neurobiological tests (Demir Özkay et al., 2015; Tchekalarova et al., 2016, 2017). The matched control groups received treatment in similar conditions. The following groups were used: sham-operated male rats treated with vehicle (sham-veh) (n = 12); STZ-treated male rats treated with vehicle (STZ-veh) (n = 11); STZ-treated male rats treated with Ago (STZ-Ago) (n = 13).

2.3. Intracerebroventricular streptozotocin-induced model of ADs

After a deep anesthesia produced by a mix of ketamine (80 mg/kg) and xylazine (20 mg/kg), injected i.p., rat was positioned in a stereotaxic device (Narishige Sci. Inst. Labs, Japan) and a midline incision over the skull was made with the periosteum removed with aseptic precautions. Two cannulas were inserted into pre-made small holes in the calvaria bilaterally above the lateral ventricles with coordinates (AP = −0.8, L = ± 1.5, H = 3.8) according to the atlas of Paxinos and Watson (2007). Freshly prepared STZ solution (3 mg/kg), dissolved in 6 µl artificial cerebrospinal fluid (ACSF) before the surgery, was bilaterally infused (3 µl/ventricle) on days 1st and 3rd, over a period of 5 min by means of 28-gauge stainless steel needle attached to a 10-µl Hamilton® syringe. The injection needle was left in place for 2 min to avoid back diffusion of the solution. The sham-veh group was treated in the same way except that it was administered with a CSF only. The scalp was then closed with a medical suture and antiseptic powder was used. The rats were treated with lactated Ringer's (2 ml/100 g of body weight/day, subcutaneously, s.c.), moistened rat chow and vitamins for a week until recovery.

2.4. Behavioral tests

The effect of chronic treatment with Ago on behavioral changes and spatial memory was assessed by using a battery of tests, carried out between 10:00 a.m. and 12:00 p.m. under artificial diffused light in a soundproof room where the animals were moved at least 30 min before each test. The behavior in the elevated plus maze (EPM) and radial arm maze (RAM) was automatically analyzed using a video tracking system (SMART PanLab software, Harvard Apparatus, USA).

2.4.1. Elevated plus maze test

Elevated plus maze test was performed as described in our previous studies (Tchekalarova et al., 2016, 2017). The animal was placed at the central area (10 × 10 cm) of the apparatus facing one of two open arms (50 × 10 cm), which were perpendicular to two enclosed arms (50 × 10 × 50 cm). The distance (cm) travelled, time spent and number of entries in open arms were measured with a cut-off time of 5 min. A greater distance, time and number spent in the aversive open arms indicated diminished anxiety.

2.4.2. Saccharine preference test

Saccharine preference test was executed as described in our previous study (Tchekalarova et al., 2016). Each rat was put in an individual cage. A pretest of two days was performed with two identical graduated bottles filled with tap water at a volume of 100 ml (1st day) and one bottle filled with tap water and another one with 1% saccharine solution (2nd day). Preference to sweet solution was expressed as a percentage of the volume of saccharine solution of the total volume of fluid consumed during 24 h.

2.4.3. Radial arm maze test

The spatial memory task was performed in a stainless steel 8-arm radial maze (RAM) (Harvard Biosci. Comp., USA) as described in our previous study (Tchekalarova et al., 2017). First, all rats were put on a diet to reduce b.w. up to 15%. Then, a pretest (shaping) for three days was performed in the maze for habituation. Sweet food pellets were placed along all the arms. Test consisted of one session per day carried out for 5 days. All arms were baited with one piece of pellet placed at the end of arm. The session was interrupted when all pellets were eaten or after a 10-min period. Re-entry into an arm from which the food pellet had already been retrieved was scored as a working memory error.

2.5. Measurement of beta amyloid, TNF-alpha, and IL-1 beta by ELISA

After decapitation of the animals, the hippocampus and frontal cortex were isolated on ice and preserved at −20 °C till the performance of the biochemical tests. The tissue samples were homogenized in 10 ml/g tissue in cold buffer containing 10 mM Tris HCl (pH 7,6), 1 mM EGTA, 50 mM NaF, 1 mM EDTA and 1 mM PMSF.

Beta amyloid was measured using an ELISA kit (AnaSpec) in pg/ml. The measurement of the TNF-alpha was performed with an ELISA kit (Invitrogen) after a centrifugation at 12,000*g*, 4 °C, for 10 min of the tissue homogenate and the concentration was expressed as pg/ml. Following the described homogenization and centrifugation procedures, the levels of IL-1 beta were assayed with an ELISA kit (Invitrogen). Amounts of IL-1beta were measured in pg/ml.

2.6. Histology

Deeply anesthetized with urethane (1500 mg/kg, i.p.) rats (n = 5 per group) were transcardially perfused by 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.3. After overnight post-fixation of isolated brains at 4° C in the same fixative, the tissue was embedded in paraffin and cut into 6 µm thick sections. Thereafter, the samples were deparaffinized with xylene, dehydrated with ethanol, and stained with

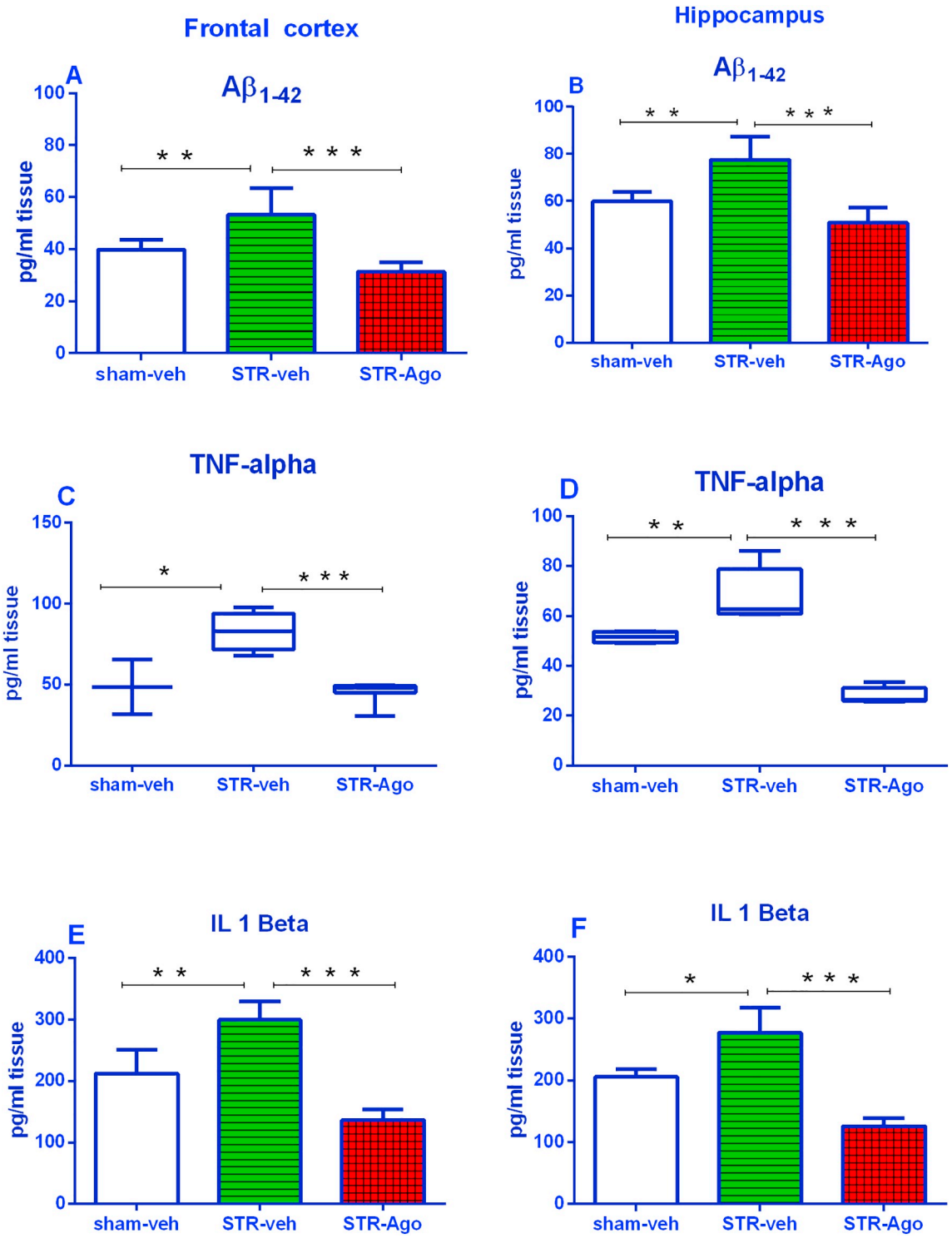

Fig. 4. Effect of chronic treatment with Ago on $A\beta_{1-42}$ (A, B), TNF-alpha (C, D) and IL-beta (E, F) (pg/mg tissue) in the frontal cortex and the hippocampus, respectively. Data is presented as mean ± S.E.M. **p < 0.01; ***p < 0.001. Abbreviations in legends as in Fig. 2.

hematoxylin and eosin. The sections were counted and analyzed as was previously described (Tchekalarova et al., 2017). The cell density was measured by Nikon's NIS Elements Digital Imaging software. The relative cell densities of the selected brain areas were quantified by determining the percentage of the measurement grid occupied by stained cells. The resulting values provide a relative index of the number of stained cells in the selected brain areas. The selected sections were comparable among rats. Cells were identified by the staining of the nuclei and distinguished by their morphology.

2.7. Statistical analysis

Statistical analysis was performed using SigmaStat® 11.0. The significant difference between groups was tested by one-way ANOVA followed by post hoc Bonferroni test. Two-way ANOVA was used for the data of the RAM test. If data was not normally distributed, ANOVA for non-parametric data (Kruskal-Wallis on ranks) followed by the Mann-Whitney *U* test was used. Results were presented as mean ± SEM. Statistically significant differences were accepted at $p < 0.05$. The

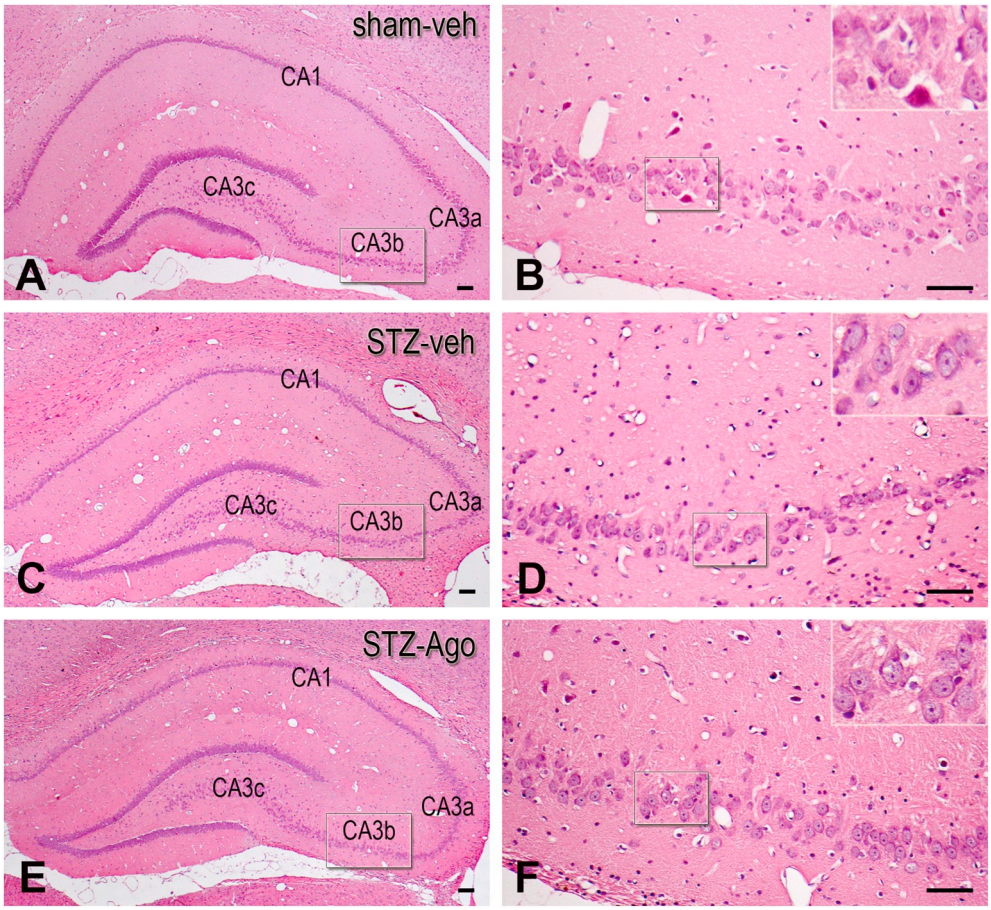

Fig. 5. Morphological effects of Ago on the hippocampal neuronal population in rats. (A) Overview of the hippocampus in the sham-operated vehicle-treated rat showing the intensely H&E-stained pyramidal neurons in all the hippocampal subfields. (B) Higher magnifications of the *CA3b* field. The insets demonstrate the morphology of the neuronal population in the respective hippocampal region shown in the small rectangles. (C) Low-power microphotograph of a H&E-stained section from the dorsal hippocampus in STZ rats treated with vehicle. (D) Hippocampal *CA3b* field at higher magnifications (insets). (E) Photomicrograph of the hippocampal formation in STZ rat treated with Ago. Details from the boxed areas in (F) reveal a partial restoration of pyramidal neurons in CA3b subfield of the hippocampus. Scale bars = 200 μm in A, C, E; 50 μm in B, D, F in higher magnification insets.

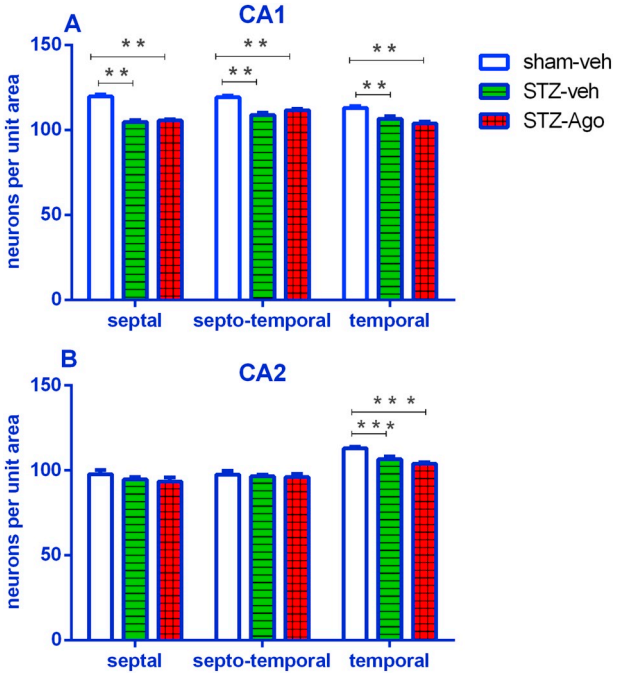

Fig. 6. Effect of chronic treatment with Ago on cell number per unit area in the CA1 and CA2 subfields of the dorsal hippocampus. Data are presented as mean ± SEM. **p < 0.01; ***p < 0.001. Abbreviations in legends as in Fig. 2.

magnitude of the differences found was expressed as effect size (eta squared for ANOVA and Cohen's *d* for pair-wise comparisons) in symmetrically distributed data.

3. Results

3.1. Effect of Ago treatment on emotional and cognitive deficit

3.1.1. Elevated plus maze test

Compared to matched controls, STZ-veh rats showed enhanced anxiety behavior at a decreased distance (Kruskal-Wallis test: H = 12.7, p = 0.002 η_p^2 = 0.53), time (one-way ANOVA: $F_{2,21}$ = 9.5, p = 0.002, Cohen's d = 0.9 η_p^2 = 0.33) and number of entries (one-way ANOVA: $F_{2,21}$ = 3.9, p = 0.039, Cohen's d = 0.9 η_p^2 = 0.33), respectively, in the aversive open arms (Fig. 2A, B, C). Chronic Ago treatment significantly attenuated STZ-induced behavioral changes on anxiety level, including distance (p < 0.001), time (p < 0.001; d = 2.1) and number of entries (p = 0.003; d = 1.4) compared to STZ-veh rats.

3.1.2. Saccharine preference test

Preference to sweet solutions was affected by icv STZ infusion (one-way ANOVA: $F_{2,22}$ = 40.3, p < 0.001) (Fig. 3A). The treatment with the melatonin analogue Ago significantly alleviated the STZ-induced decreased preference compared to STZ-veh rats (p < 0.001; d = 8.4).

3.1.3. Radial arm maze test

Two-way ANOVA demonstrated that the rate of working memory errors was affected by the group ($F_{2,104}$ = 10.9, p < 0.001). Moreover, a significant group x time interaction ($F_{8,104}$ = 2.6, p < 0.012) indicated that icv STZ infusion significantly impaired hippocampus-dependent spatial memory. Post hoc test showed that the STZ-veh group needed a longer time to complete the task compared to both sham-veh group (4th and 5th session: p < 0.001 and p = 0.009, respectively)

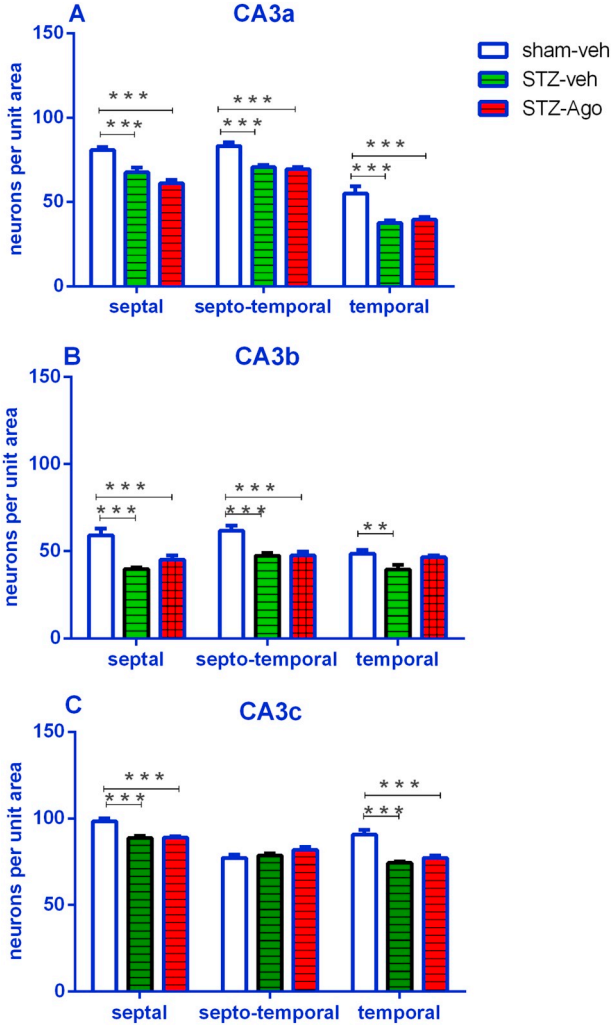

Fig. 7. Effect of chronic treatment with Ago on cell number per unit area in the CA3a, CA3b and CA3c subfields of the dorsal hippocampus. Data is presented as mean ± SEM. **p < 0.01; ***p < 0.001. Abbreviations in legends as in Fig. 2.

and STZ-Ago group (4th and 5th session: $p < 0.001$ and $p = 0.006$, respectively $\eta_p^2 = 0.51$) (Fig. 3B).

3.2. Effect of Ago treatment on Aβ levels in the frontal cortex and the hippocampus

One-way ANOVA revealed that the amount of $A\beta_{42}$ content in the frontal cortex ($F_{2,20} = 16.2$, $p < 0.001$, $\eta_p^2 = 0.42$) and the hippocampus was affected by the group ($F_{2,20} = 15.9$, $p < 0.001$, $\eta_p^2 = 0.575$). Post hoc test showed that while the STZ-veh group was characterized by an increased amount of $A\beta_{42}$ in the frontal cortex and the hippocampus compared to sham-veh ($p = 0.05$), Ago treatment reversed that increase back to control levels of $A\beta_{42}$ content in the frontal cortex and the hippocampus in the STZ group ($p < 0.001$) (Fig. 4A).

3.3. Effect of Ago treatment on signal markers of inflammation (TNF-alpha and IL-1 beta) in the frontal cortex and hippocampus

For the TNF-alpha, Kruskal-Wallis test demonstrated a significant difference among groups both in the frontal cortex ($H = 7.5$, $p = 0.023$) and in the hippocampus ($H = 13.6$, $p = 0.001$). The STZ-veh group exhibited an elevated level of TNF-alpha in these structures

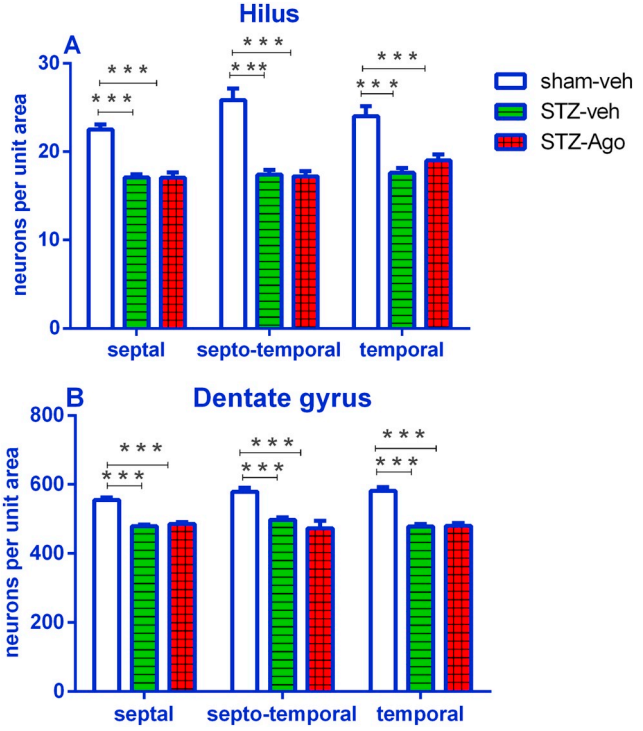

Fig. 8. Effect of chronic treatment with Ago on cell number per unit area in the hilus and dentate gyrus of the hippocampal formation. Data is presented as mean ± SEM. ***p < 0.001. Abbreviations in legends as in Fig. 2.

compared to sham-veh ($p = 0.05$). However, the treatment with Ago corrected STZ-induced changes of this pro-inflammatory marker in the frontal cortex and the hippocampus, respectively ($p < 0.001$) (Fig. 4B).

One-way ANOVA showed that the level of the other marker of inflammation, IL-1 beta, was also significantly affected by group in the frontal cortex ($F_{2,20} = 42.8$, $p < 0.001$, $\eta_p^2 = 0.92$) and the hippocampus ($F_{2,20} = 52.5$, $p < 0.001$, $\eta_p^2 = 0.88$). The icv infused STZ in the veh-treated group was characterized by a higher level of IL-1 beta compared to sham-veh in the frontal cortex ($p = 0.02$) and the hippocampus ($p < 0.001$). The STZ-Ago group was characterized by IL-1 beta levels close to the sham-veh group both in the frontal cortex and the hippocampus ($p < 0.001$) (Fig. 4C).

3.4. Effect of Ago treatment on icv STZ-induced decrease of cell number per unit area in the hippocampus

The severity of cell impairment in the dorsal hippocampus is demonstrated in the representative Fig. 5A–F. The mean number of cells per unit area did not differ between the STZ-vehicle group and STZ-Ago group in the CA1 (septal, septo-temporal and temporal), temporal CA2, CA3a and CA3c (septal, septo-temporal and temporal), CA3b (septal and septo-temporal) fields as well as in the hilus and the dentate gyrus (septal, septo-temporal and temporal) of the dorsal hippocampus (Figs. 6A, B, 7A, B, C, 8A, B). Furthermore, the rate of cell loss per unit area in the septal and septo-temporal basolateral amygdala (BL) and the piriform cortex (Pir) was similar in the STZ-veh and STZ-Ago groups (Figs. 9A–I, 10C, D). The chronic Ago treatment had neuroprotective effect specifically in the temporal CA3b field of the hippocampus (Figs. 5A–F, 7B) and the temporal piriform cortex (Figs. 9G–I, 10D).

4. Discussion

The present study revealed that chronic treatment with Ago exerted neuroprotective effect on behavioral, biochemical and histological

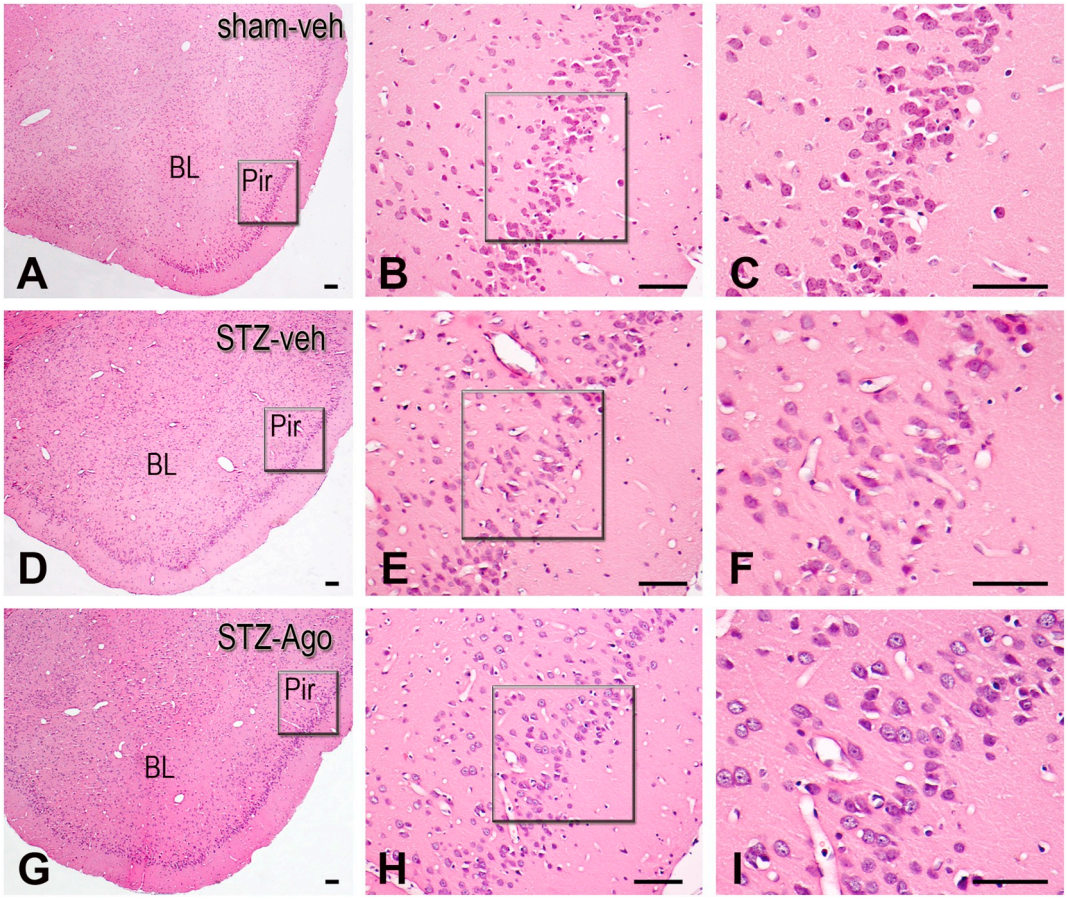

Fig. 9. Morphological effects of Ago on the basolateral amygdala (BL) and the piriform cortex (Pir) in rats. (A) Overview of the hippocampus in the sham-operated vehicle-treated rat showing the intensely H&E-stained neurons in the BL and Pir. Higher magnifications of the Pir in (B) and (C). The insets demonstrate the morphology of the neuronal population in the respective part of the Pir shown in the small rectangles. (D) Low-power microphotograph of a H&E-stained section from the BL and Pir in the STZ rats treated with vehicle. Pir at higher magnifications (insets) in (E) and (F), respectively. Photomicrograph of the BL and Pir in the STZ rat treated with Ago. Details from the boxed areas in (H) reveal a partial restoration of neurons in Pir. Scale bars = 200 μm in A, D, G; 50 μm in B, E, H; 25 μm in C, F, H.

pathological consequences in the icv STZ rat model. Four months after the initial bilateral icv infusion with STZ, the marked behavioral alterations, including anxiety, preference to sweet solutions and cognitive impairment, raised levels of $A\beta_{42}$, phosphorylated tau protein, pro-inflammatory signal molecules (TNF-alpha and IL-1 beta) in the frontal cortex and the hippocampus as well as a profound cell loss per unit area in the dorsal hippocampus, BL and Pir were detected in vehicle-treated group. The icv STZ rat model is a validated experimental model that mimic behavioral and pathological cascades of sporadic AD by causing impairment of insulin signaling pathways in brain with a diminished glucose metabolism in the cortex and the hippocampus (Hoyer and Lannert, 2008) and associated cholinergic deficit and cognitive dysfunction (Salkovic-Petrisic et al., 2014). Recently, Knezovic et al. (2015) reported that the icv STZ rat model is characterized by a time- and dose-dependent deficit in cognitive functions and concomitant formation of $A\beta_{1-42}$ plaque and hyperphosphorylated tau protein burden. Here, we confirmed previous findings (Knezovic et al., 2015; Kosaraju et al., 2013; Shingo et al., 2013) that the 3–4 months after icv STZ infusion of a high dose of 3 mg/kg is a crucial period for detection and accumulation of $A\beta_{1-42}$ in the frontal cortex and the hippocampus. In the present study, the detected neuropathological burden correlated with an impairment in the hippocampus-dependent spatial memory, measured in RAM, which is also in accordance with previous experimental (Ekuni et al., 2013; Knezovic et al., 2015; Rudnitskaya et al., 2015a, 2015b; Sheng et al., 2017) and clinical data of cognitive decline from mild cognitive impairment to progressive memory loss in AD (Sabbagh et al., 2010).

We have found that chronic treatment with Ago reduced $A\beta_{42}$ accumulation as well as pro-inflammatory signal molecules, TNF-alpha and IL-1 beta, in the frontal cortex and the hippocampus of icv STZ rats. To the best of our knowledge, this is the first study to report the effect of this melatonin analogue, which is clinically used as an antidepressant, on neuropathological consequences resembling AD symptoms as a result of STZ-induced dysregulation of insulin receptor signaling pathway. Recently, long-term oral administration of melatonin in senescence-accelerated OXYS rats, a nontransgenic model of sporadic AD, was shown to alleviate the $A\beta_{1-42}$ in the frontal cortex and the hippocampus as well as to slow down degenerative changes specifically in the CA1 field of the hippocampus (Rudnitskaya et al., 2015b). Accumulated extracellular Aβ and intracellular neurofibrillary tangles of hyperphosphorylated tau underlie plastic synaptic alterations and neuronal loss in structures related to cognitive functions, e.g. the hippocampus. In this study, we showed that Ago was able to improve hippocampus-dependent spatial memory by alleviating neuropathological concomitant burden associated with increased Aβ, as well as pro-inflammatory signal molecules (TNF-alpha and IL-1 beta). However, Ago did not exert strong neuroprotection in the vulnerable for behavioral changes brain regions. Recently, we reported that Ago prevented epilepsy associated neuronal loss in the dorsal hippocampus (CA1, CA2, CA3 field) and the hilus of the dentate gyrus without affecting decline in spatial memory in post-status epilepticus model induced by KA in rat (Tchekalarova et al., 2017) suggesting a lack of positive relationship between spatial memory and neuroprotection in the hippocampus that could underlie the efficiency of Ago in icv STZ model as well. However, the beneficial

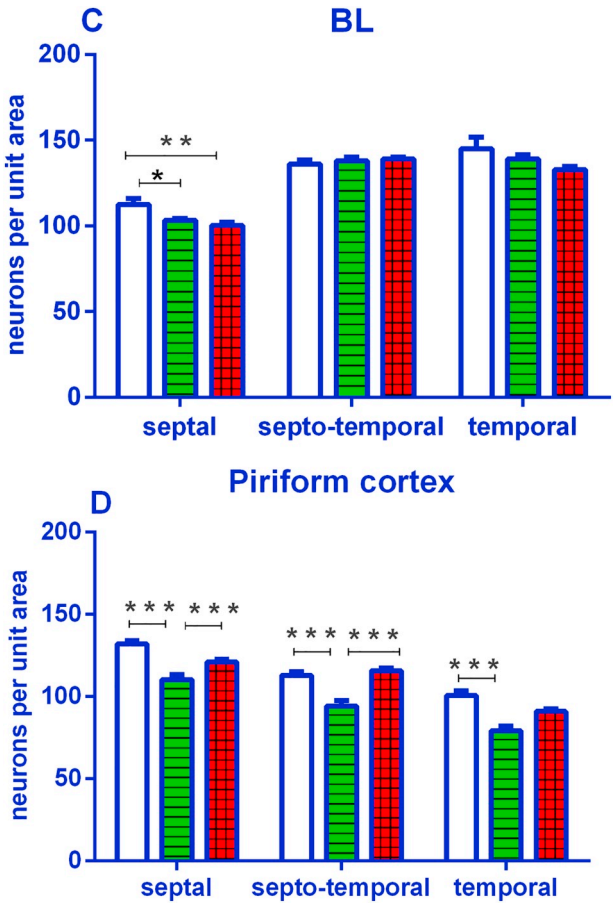

Fig. 10. Effect of chronic treatment with Ago on cell number per unit area in the basolateral amygdala (BL) and the piriform cortex (Pir). Data are presented as mean ± SEM. **p < 0.01; ***p < 0.001. Abbreviations in legends as in Fig. 2.

effect of Ago on memory impairment in the icv STZ rat model suggests that precisely the suppression of characteristic hallmarks of AD and Aβ is the required condition for its beneficial effect on cognitive function. Melatonin as well as melatonin agonist Neu-P11 were able to restore impairments in the performance of memory task in the RAM of 18-month-old OXYS rats and in the novel object recognition test of Sprague Dawley rats with intrahippocampal injection of $A\beta_{(1-42)}$ (He et al., 2013; Rudnitskaya et al., 2015b). Interestingly, while Neu-P11 was efficient both after chronic use and a single injection in the morning and afternoon, respectively, melatonin exerted a facilitation of memory performance after only a single injection in the afternoon (He et al., 2013). The time-dependent effects of melatonin are related to its chronobiotic properties and to activation of melatonin receptors while 5-HT1A/1D receptors might also be involved in the effects of Neu-P11. Both Neu-P11 and Ago demonstrated anxiolytic activity, which was not dependent on time of treatment in intact animals (Millan et al., 2005; Papp et al., 2006; Tian et al., 2010). Like melatonin, Ago is characterized by a putative chronobiotic activity (reviewed in: Tchekalarova et al., 2015). The limitation of the present study is that we did not explore the effect of age-matched control group treated with Ago to confirm that it behaves as a "disease-dependent" drug. However, previous studies indicated that Ago in control rats had no effect on any of the behavioral parameters that we have tested here (Morley-Fletcher et al., 2011; Tchekalarova et al., 2016). Here, the chronic treatment with Ago was carried out in the afternoon when it is supposed that the chronobiotic activity of this drug associated with activity on MT receptors would be fully expressed in the icv STZ rat model. Melatonin MT_1 and MT_2 receptors are suggested to have different, region-specific roles in AD. Thus, whereas receptors are decreased in the cortex and the pineal gland (Brunner et al., 2006), MT_1 receptors are increased and MT_2 receptors are lost in one of the most vulnerable structure in AD, the hippocampus (Savaskan et al., 2002, 2005). The observed receptor plasticity in AD might be related to changes in melatonin levels because the hormone was shown to autoregulate the density of MT receptors (Guerrero et al., 2000).

Anxiety and depression are comorbid psychological symptoms of AD that affect about 90% of patients (Cerejeira et al., 2012). Most experimental studies are focused on behavioral changes related to cognitive functions and a few reports consider alterations related to anxiety and depression. Transgenic mice (3xTg-AD), nontransgenic model of sporadic ADs (senescence-accelerated OXYS rats) as well as icv STZ mice and Wistar rats were reported to have increased anxiety-like symptoms in the EPM and/or open field tests (Chen et al., 2013; Gutierres et al., 2014; Pinton et al., 2011; Sterniczuk et al., 2010). In the present study, we demonstrated that Ago treatment attenuated the increased anxiety-like behavior in icv STZ rats. Recently, Rudnitskaya et al. (2015a, 2015b) reported that melatonin administration alleviated changes in anxiety level tested in the EPM of OXYS rats by increasing the number of entries and the time spent in the open arms. Fear responses are associated with a hyperactivity of the amygdala which is a neurobiological base of anxiety (Forster et al., 2012). Ago was unable to restore STZ-induced cell loss suggesting a lack of a link between anxiolytic effect of this melatonin analogue and neuronal damage in the amygdala.

Neuroinflammation is considered a crucial step in the development of the neurodegenerative process of AD (Buckwalter and Wyss-Coray, 2004). In the icv STZ model, neuroinflammation is detected as early as 1 week after the injection of low dose of STZ (Nazem et al., 2015). Solmaz et al. (2015) reported that TNF-alpha was increased in brain of rats two weeks after icv STZ. We demonstrated that TNF-alpha and IL-beta were increased four months after icv STZ and that chronic Ago treatment suppressed these inflammatory signaling molecules. Recently, we found that Ago treatment produced a strong anti-inflammatory response both in the periphery (plasma IL-1 beta) and the brain (attenuation of gliosis) in a post-status epilepticus induced model of epileptogenesis (Tchekalarova et al., 2018).

Taken together, the finding of the present study revealed that Ago treatment, started during a progression of neurodegenerative process in icv STZ rat model of AD, exerts beneficial effects on emotional disturbance and memory decline by suppressing the concomitant hallmark of AD, Aβ, pro-inflammatory signal molecules, TNF-alpha and IL-1 beta, in the frontal cortex and the hippocampus. Future experimental and clinical studies are required to ascertain the molecular mechanisms underlying the beneficial potential of this antidepressant in AD.

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Declarations of interest

None.

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