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Lack of long-term behavioral alterations after early postnatal treatment with tropisetron: Implications for developmental psychobiology

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ABSTRACT

The early postnatal period represents a critical time window for brain development. Transient Cajal–Retzius cells in layer I of the cortex play an important role in cortical lamination by modulating neuronal migration and maturation. Recent data have demonstrated that the 5-HT$_3$ receptor antagonist and alpha7 nicotinic receptor partial agonist tropisetron, acting via 5-HT$_3$ receptors expressed on Cajal–Retzius cells, can disturb the formation of cortical columns at perinatal stages. This process is thought to be involved in several neuropsychiatric disorders. Here we investigated the possible long-term behavioral effects of exposure to tropisetron at early postnatal stages in mice. We found that the administration of 1 mg/kg, intraperitoneal (i.p.) tropisetron from postnatal days 2–12 (P2–P12) did not induce significant cognitive, schizophrenia-like or emotional alterations in tropisetron-treated animals as compared to controls, when tested in multiple behavioral assays. These results may be of relevance regarding the possible protracted deleterious neuropsychiatric effects of tropisetron during early life.

1. Introduction

Serotonin (5-HT) is a critical regulator of several neurodevelopmental processes such as neurogenesis, dendritogenesis, axon branching and apoptosis (Gaspar et al., 2003). Both excessive and deficient serotonin levels during development are associated with structural and behavioral abnormalities in later life. An excess of serotonin in mice lacking the serotonin transporter leads to abnormally increased dendritic branching of neurons in the prefrontal cortex (Wellman et al., 2007) and disturbed organization of the somatosensory cortex (Persico et al., 2001). Embryonic depletion of serotonin by the reversible inhibitor of serotonin synthesis DL-P-chlorophenylalanine (PCPA) from Embryonic Days E12 to E17 alters the maturation of pyramidal neurons in the somatosensory cortex (Vitalis et al., 2007). Serotonin depletion restricted to the neonatal period induces morphological abnormalities of the cortex (Durig and Hornung, 2000) and alters behavioral responses to spatial change and novelty in the adult (Hohmann et al., 2007). Genetic deletion of the transcription factor Lmx1b, required for the differentiation of 5-HT neurons causes an almost complete absence of serotonin in the mouse brain and impairs the formation of hippocampus-dependent spatial memory, together with a phenotype of reduced anxiety (Dai et al., 2008).

The action of serotonin on neurons is mediated by several serotonin receptors grouped into seven different families (5-HT$_{1-7}$). With the exception of ionotropic 5-HT$_3$ receptors, all other serotonin receptors are metabotropic G-protein coupled receptors. The 5-HT$_3$ receptors are pentameric ligand-gated ion channels, composed of five subunits (5-HT$_{3A-E}$) (Jackson and Yakel, 1995), the two main subunits being 5-HT$_{3A}$ (Maricq et al., 1991) and 5-HT$_{3B}$ (Davies et al., 1999), of which only the former is expressed in the brain (Morales and Wang, 2002). A role for 5-HT$_3$ receptors during brain development is indicated by the fact that they are expressed at high levels in the embryonic brain both in immature GABAergic interneurons and in the proliferative ventricular zone (Tecott et al., 1995). Furthermore, during the early postnatal period and in the adult, 5-HT$_3$ receptors are continuously expressed by newborn neurons migrating from the subventricular zone (Inta et al., 2008). These receptors are also present in Cajal–Retzius cells in layer I of the developing neocortex (Chameau et al., 2009). Cajal–Retzius cells represent a transient population of neurons that play a key role in the organization of the neocortex in cortical columns, acting via its secreted glycoprotein reelin (Tissir and Goffinet, 2003). Disturbances of the columnar structure of the neocortex are proposed to be associated with autism and schizophrenia (Casanova et al., 2002). Cajal–Retzius cells receive a strong serotonergic input from the brainstem early in embryonic development (Janusonis et al., 2004). Recent data have demonstrated

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that the serotonergic input via 5-HT_3 receptors represents the main excitatory drive of Cajal–Retzius cells and that blocking 5-HT_3 receptors with tropisetron induces *in vitro* a dramatic increase in the complexity of the dendritic arborization of cortical layer II/III pyramidal neurons in organotypic slices from P0 mice cultured for 6–7 days (Chameau et al., 2009). Importantly, the increased dendritic complexity induced by tropisetron is similar to that seen by application of anti-reelin antibodies and is rescued by application of reelin, indicating a tight functional interaction between reelin and 5-HT_3 receptors (Chameau et al., 2009).

Altogether these data indicate an important modulator role for tropisetron during brain development–particularly at early postnatal stages–by inducing structural alterations in cortical neurons that have been associated with severe neuropsychiatric conditions. However, possible protracted behavioral effects of treatment with tropisetron during development have yet to be investigated. Due to its common clinical use, it is important to determine whether tropisetron exerts deleterious long-term behavioral effects. Therefore, we examined possible emotional, cognitive and schizophrenia-like effects of early postnatal treatment with tropisetron in mice using multiple standardized behavioral tests during adulthood.

Early postnatal treatment (P4–P21) with the selective serotonin reuptake inhibitor fluoxetine induces a behavioral inhibition in the adult (Ansorge et al., 2004; Karpova et al., 2009). On the other hand, fluoxetine not only increases the serotonin reuptake, but also acts as functional antagonist of 5-HT_3 receptors (Eisensamer et al., 2003). For these reasons, we analyzed, in parallel to the tropisetron-treated mice, an additional cohort of mice treated with fluoxetine from P2 to P12 and compared their action at adult stages in tests relevant for anxiety and depression, similar to previous studies (Ansorge et al., 2004; Karpova et al., 2009).

2. Materials and methods

2.1. Animals

Experiments were performed using C57BL/6N mice purchased from Charles River as breeding pairs (Sulzfeld, Germany) and their male offspring. When the female of each pair was pregnant, the male was removed and the female was monitored until delivery. The day of delivery was designated P0. Litters were culled to 5 pups and were randomly assigned to three groups. Pups of each group were injected from P2 to P12 i.p. twice daily (9:00 am and 5:00 pm), with either vehicle (0.9% NaCl, 5 ml/kg) or tropisetron (1 mg/kg, 5 ml/kg) or, in the cohort treated with fluoxetine, once daily (9:00 am) with fluoxetine (dissolved in saline, 10 mg/kg, 5 ml/kg), followed by a second daily injection with saline (5:00 pm). Tropisetron hydrochloride (Ascent) was dissolved in saline and adjusted to pH 7.35. Doses between 0.001 mg/kg and 1 mg/kg, i.p. of tropisetron are commonly used by different groups in behavioral tests (reviewed in Costall et al., 1990). Considering these data, we aimed to use the highest dose of this drug in this dose range (1 mg/kg), to better reveal possible deleterious effects of the treatment. Fluoxetine hydrochloride (Ascent) was injected at 10 mg/kg as used in previous studies (Ansorge et al., 2004; Karpova et al., 2009). Very thin needles (30 G) were used to minimize injection stress. Mice were picked up gently, by grasping the loose skin over the neck and shoulders with the thumb and index finger and held on the palm of the hand. Pups were weaned at P28 and housed in groups of 2–3/cage under standard laboratory conditions. For validation of the anxiety tests at adult stages, we analyzed in the Dark–Light-Box additionally a cohort of adult mice ($n=10$ per treatment) acutely injected with the standard anxiolytic diazepam (1 mg/kg, i.p.) or saline, given 30 min prior to the test. Only males were used for behavioral experiments. Mice were supplied with food (Ssniff, V1536) and tap water ad libitum. All experiments had been approved by were approved by the German Committee on Animal Care and Use (Regierungspräsidium Karlsruhe) and were carried out in accordance with the local Animal Welfare Act and the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Behavioral testing

Behavioral experiments were performed when treated animals reached 3 months of age. The animals were subjected to paradigms relevant to schizophrenia, partially also to autism, and to depression and anxiety, including the Open Field Test, Prepulse Inhibition of the Acoustic Startle Response (PPI of the ASR), a test of sociability (social interest), the 8-Arm Radial Maze (RAM) as a test for working memory. The emotional behavior was analyzed using established behavioral tests for anxiety, and depression, the Dark–Light-Box, the Elevated O-Maze and the Porsolt Forced Swim Test, as described earlier (Vogt et al., 2008; Fuss et al., 2010). We used two independent cohorts of mice which were subjected either to the Open Field Test, ASR and PPI, Sociability Test and RAM (tropisetron-treated $n=13$, vehicle $n=9$) or to tests relevant for emotional behavior (Dark–Light-Box, Elevated O-Maze and Forced Swim Test) (tropisetron $n=13$, vehicle $n=13$, fluoxetine $n=17$). To reduce the influence of handling, PPI was tested first in the first cohort, during the light phase with lights on at 7 pm. All other behavioral tests in both cohorts were performed during the dark phase after 2 weeks of acclimation to a reversed dark–light cycle (lights on at 7 pm). Prior to each test, mice were acclimated to the experimental room for at least 30 min. Bodyweight was weekly assessed when the cages were changed.

2.2.1. Open field test

The Open Field test examines the locomotoric and explorative characteristics of an animal placed into an unknown arena. Although the relevance of hyperlocomotion in the Open Field test to psychotic symptoms may be contentious, this behavior may serve as a useful behavioral correlate of increased dopaminergic transmission in animal models of schizophrenia (Lipska and Weinberger, 2000). Animals were observed for 30 min during their active period in a $50\times50\text{cm}^2$ arena, illuminated with 25 lx and monitored by a Video camera (Sony CCD IRIS). The resulting data were analyzed using the image processing system EthoVision 3.0 (Noldus Information Technology, Wageningen, Netherlands). For each sample, the system recorded position, object area and the status of defined events. Parameters assessed for the present study were total distance traveled, velocity, and time in center, which was defined as the area 10 cm distant from the walls.

2.2.2. Sensorimotor gating measured by PPI

PPI is a measure of sensorimotor gating both in humans and animals, such that a weak pre-stimulus (prepulse) attenuates the startle response to a loud noise presented immediately after (Powell et al., 2009). Deficient PPI is a measure of the loss of sensorimotor gating, leading to sensory flooding and cognitive fragmentation in schizophrenia patients (Braff et al., 1992). Startle and PPI testing occurred in a startle chamber (SR-LAB; San Diego Instruments, San Diego, USA), in which a loudspeaker inside the box produced a continuous background noise of 60 dB of sound pressure level (SPL) as well as the acoustic startle pulses. A white noise pulse was used as the startle stimulus, which had an intensity of 115 dB SPL and a duration of 40 ms; four different white noise intensities (65, 70, 75 and 80 dB SPL, duration 20 ms) were used as prepulses as described previously (Schneider and Spanagel, 2008). An acclimatization time of 5 min, during which the mice received no stimulus except the background noise, was followed by the presentation of 5 initial startle stimuli. After this habituation program the test program was started with six different trial types presented in a pseudorandom order: 1) pulse alone (assessing ASR), 2) control (no stimulus), 3) pulse with preceding prepulse (prepulse 65 dB, 100 ms before pulse), 4) pulse with preceding prepulse (prepulse 70 dB, 100 ms

before pulse), 5) pulse with preceding prepulse (75 dB, 100 ms before pulse) and 6) pulse with preceding prepulse (prepulse 80 dB, 100 ms before pulse). A total of 10 presentations of each trial type were given with an interstimulus interval randomized between 10 s and 20 s.

PPI was calculated as the percent decrease of the ASR magnitude in trials when the startle stimulus was preceded by a prepulse [100×(mean ASR amplitude on pulse alone trials − mean ASR amplitude on prepulse–pulse trials)/mean ASR amplitude on pulse alone trials].

2.2.3. Sociability test

The sociability test in mice is an established method to measure abnormal social interaction that represents one of the behavioral hallmarks in autistic disorders and schizophrenia (Crawley, 2007; Lipska and Weinberger, 2000). 'Sociability' measured in this test represents the propensity to spend time with another mouse, as compared to time spent in an identical but empty chamber (Moy et al., 2004). In this test each mouse was scored on measures of exploration in a central habituated area, a side chamber containing an unfamiliar conspecific ("stranger 1") in a wire cage, or an empty side chamber. The social testing arena was a rectangular, three-chambered box. Each chamber was $20\times30\times30$ cm^3 in size. Dividing walls were made of clear Plexiglas, with rectangular openings (5×6cm^2) allowing access into each chamber. The test mouse was first placed in the middle chamber and allowed to explore. The openings into the two side chambers were obstructed by Plexiglas panels during this first habituation phase. After 5 min, the doors were opened and the animal was allowed to explore the 2 outer compartments (equipped with one empty wire cage in each compartment, size $7\times7\times8$ cm^3). After the habituation period, an unfamiliar C57BL/6N male mouse ("stranger 1") that had no prior contact with the subject mouse was placed in one of the side chambers. The location of stranger 1 in the left vs. right side chamber was systematically alternated between trials. The stranger mouse was enclosed in a small wire cage that allowed nose contact through the bars but prevented fighting. The animals serving as strangers had previously been habituated to the small cages. A new identical empty wire cage was placed in the opposite chamber. Openings to the side chambers were then unblocked and the subject mouse was allowed to explore the entire social test arena for a 10 min session. The amount of time spent in each chamber and the number of entries into each chamber were measured. An entry was defined as all four paws in one chamber. The percentage of time in each side chamber in comparison to the time spent in the both chambers was analyzed.

2.2.4. 8-Arm radial maze (RAM)

The RAM is a classical test of spatial working memory in rodents, assessing the ability to remember a series of specific locations, particularly which arms had already been visited within one session (Olton, 1987; Dudchenko, 2004). The RAM test has become a widely used method for assessing working memory in rodents (Levin, 1988), as well as cognitive deficits in animal models of schizophrenia (Lipska and Weinberger, 2000). The RAM consisted of a central platform (20 cm in diameter) connected to 8 arms (50 cm long, 8 cm wide) elevated 50 cm and covered with Plexiglas tunnels to permit visual orientation. Each mouse was tested once per day for 10 consecutive days. Mice were food deprived for 3 days before and during the whole training period (80% of the initial bodyweight to keep motivation high). Mice were allowed to habituate for 1 trial before regular testing, to reduce the anxiety on the maze. At the beginning of a trial, each of the 8 arms was baited with one millet seed in food cups placed at the end of each arm. Each mouse was placed in the center of the maze and was observed for the sequence of arm entries and consumption of the food. Sessions were terminated after a maximum time of 10 min or after a mouse ate all baits. The following parameters were analyzed: time on maze (determined as time to fulfill the task), speed, correct choices and working memory errors. A working memory error was scored whenever a mouse re-entered an arm formerly baited.

2.2.5. Dark–Light-Box

In the Dark–Light-Box animals are investigated for their exploration of an aversive light compartment, representing a measure of anxiety-like behavior (Crawley, 1985). The Dark–Light-Box consisted of two plastic chambers, connected by a small tunnel. The dark chamber measured $20\times15\times30$ cm^3 and was covered by a lid. The adjacent chamber, measuring $30\times15\times30$ cm^3, was white and illuminated from above by 600 lx. Mice were placed into the dark compartment and latency to first exit, number of exits and total time in the light compartment was recorded for 5 min.

2.2.6. Elevated O-Maze

The Elevated O-Maze imposes an approach-avoidance conflict in animals, measuring anxiety by their aversion to enter elevated, exposed sections of a round maze (Crawley, 1985). The maze consisted of a gray plastic circular runway (width 6 cm, outer diameter 46 cm, 50 cm above ground level) covered with black cardboard paper to prevent mice from slipping off the maze. Two opposing 90° sectors were protected by inner and outer walls of gray polyvinyl (height 10 cm). Animals were placed in one of the protected sectors and observed for 5 min. The maze was illuminated by 25 lx. The following parameters were analyzed: latency to first exit, number of exits and total time spent on the open sections.

2.2.7. Porsolt Forced Swim Test

The Porsolt Forced Swim Test represents a paradigm for the assessment of despair behavior by analyzing immobility scores in an inescapable aversive situation (Porsolt et al., 1977). Mice were placed into a glass cylinder (23 cm high, 13 cm diameter), which was filled with water (21 °C) up to a height of 12 cm. A testing period of 6 min was used to determine the onset and the percentage of time spent immobile. The test was repeated after 24 h under the same conditions (21 °C water temperature, red light) to determine habituation effects. Mice were monitored by a video camera (Sony CCD IRIS) from sideward. The resulting data were analyzed using the image processing system EthoVision 3.0 (Noldus Information Technology, Wageningen, The Netherlands). For each sample, the system recorded position, object area and the status of defined events. Parameters assessed were latency to start floating and total immobility time, where immobility was defined as percentage change lower than 11.5% in the object area between samples.

2.3. Statistical analyses

All statistical analyses were performed using PASW 18 for Windows. Inter-group comparisons were calculated by either Student's t-tests or by using 1-way factorial ANOVA design with treatment (tropisetron, fluoxetine or vehicle) as between subject factor. Where appropriate, the model was complemented by within subject factors to explore the dependence of treatment effects on time (e. g. bodyweight development, data of Open Field, and RAM and of Porsolt Forced Swim Test). Post-hoc analyses were performed with Bonferroni post-hoc tests. The significance threshold was set at $p<0.05$.

3. Results

3.1. General observations

Mice treated with tropisetron between P2 and P12 and analyzed 3 months later neither displayed altered home cage behavior nor bodyweight differences in comparison to vehicle-treated mice; all mice gained weight during the testing phases (repeated measurement ANOVA factor time: $F(2,80)=48.584$ $p=0.000$). In contrast, postnatal

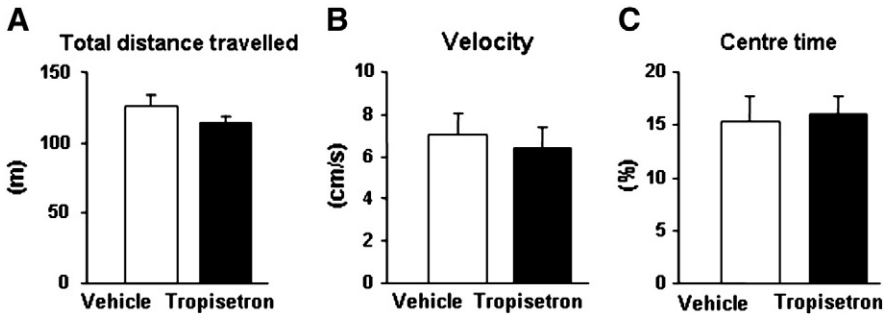

Fig. 1. Effect of postnatal treatment with tropisetron on locomotion in the Open Field Test. Both groups show similar locomotor activity, as demonstrated by comparable scores in (A) total distance traveled (m), (B) velocity measurements (cm/s) and (C) time spent (%) in the center of the arena. All data are means ± SEM.

fluoxetine treatment reduced body weight in adulthood (repeated measurement ANOVA factor treatment: $F(2,40) = 3.635$ $p = 0{,}035$ Bonferroni post-hoc (vehicle vs. fluoxetine $p = 0.051$)).

3.2. Tropisetron-treated mice display regular locomotor behavior in the open field test

We found no significant difference between tropisetron-treated mice and controls in total distance traveled (Fig. 1A), velocity (Fig. 1B) and time spent in the center of the Open Field (Fig. 1C). Moreover, we did not observe differences in any of these parameters between treated and untreated animals in the first, second or third 10-min bin (data not shown). These data allow also analyzing habituation in the arena. All mice showed a significant time effect regarding total distance traveled (repeated measurement ANOVA factor time: $F(2,40) = 34.279$ $p = 0.000$) and velocity (repeated measurement ANOVA factor time: $F(2,40) = 34.227$ $p = 0.000$) but not in terms of distance to walls and center time.

3.3. Early postnatal tropisetron treatment does not alter sensorimotor gating

Tropisetron vs. vehicle treated mice exhibited no difference between the amplitude of the ASR (Fig. 2A). Regarding the PPI, although a slight tendency toward a reduced inhibition in tropisetron treated animals was observed at all prepulse intensities applied, this did not reach statistical significance in comparison to vehicle treated mice (Fig. 2B).

3.4. No alteration in sociability after postnatal treatment with tropisetron

Tropisetron-treated mice showed no difference in sociability, preferring to a comparable extent as controls the contact with an unfamiliar mouse ("stranger 1") in comparison to an empty wire cage (Fig. 2C) (paired Student's *t*-test "stranger 1" vs. empty cage: tropisetron: $p = 0.001$, vehicle: $p = 0.000$).

3.5. Tropisetron-treated animals show no learning and memory deficits in the RAM test

Tropisetron-treated mice showed in the parameters "time spent on the maze" (Fig. 3A) or "speed" (Fig. 3B) no differences in comparison to vehicle-treated mice. Similarly, there was no difference between the groups regarding the number of working memory errors (Fig. 3C), correct choices during the first 8 visits (Fig. 3D) or procedural errors (data not shown). A repeated measurement ANOVA revealed significant effects of time in the following parameters: time spent on maze ($F(4,80) = 14.013$ $p = 0.000$), speed ($F(4,80) = 45.040$ $p = 0.000$), working memory errors ($F(4,80) = 3.249$ $p = 0.016$), and procedural errors ($F(4,80) = 3.277$ $p = 0.015$).

3.6. Administration of tropisetron or fluoxetine does not influence anxiety-related behavior in Dark–Light-Box and Elevated O-Maze

Animals treated with tropisetron showed normal anxiety-related behavior in the Elevated O-Maze, showing similar latencies to enter (Fig. 4A), a comparable number of exits (Fig. 4B) and spent a similar amount of time on the unsheltered parts of the arena (Fig. 4C). Animals treated with tropisetron displayed no alteration in comparison to controls in the Dark–Light-Box concerning the latency to enter the bright compartment (Fig. 4D), the total number of exits (Fig. 4E) and the total time in the bright compartment (Fig. 4F). Animals treated during the same time window with fluoxetine did not show as well alterations in any of the two anxiety tests (Fig. 4A–F). A one-way ANOVA with factor treatment did not reveal any statistical significant alterations between mice of all three treatment groups.

3.7. Administration of diazepam induces an anxiolytic phenotype as assessed in the Dark–Light-Box

To confirm the sensitivity of the anxiety tests, we used, in a separate cohort of adult mice the standard anxiolytic diazepam. Animals treated with diazepam showed in the Dark–Light-Box test a significant

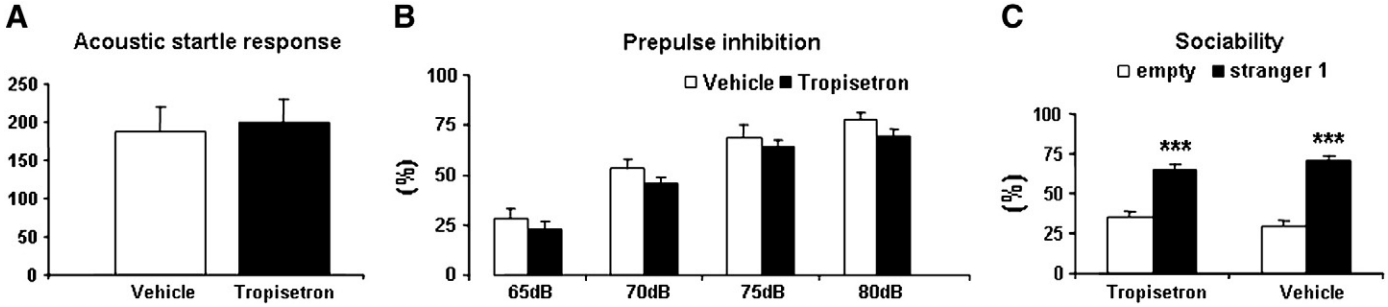

Fig. 2. Influence of early postnatal treatment with tropisetron on acoustic startle response, PPI and sociability in the adult. No significant differences between tropisetron-treated and vehicle-treated mice in (A) amplitude of the ASR, or (B) PPI for the 4 different prepulse intensities (65, 70, 75, and 80 dB) were found. Chronic postnatal tropisetron treatment had no significant effect on sociability (C). All bars are means ± SEM. ***paired Student's *t*-test $p<0.001$.

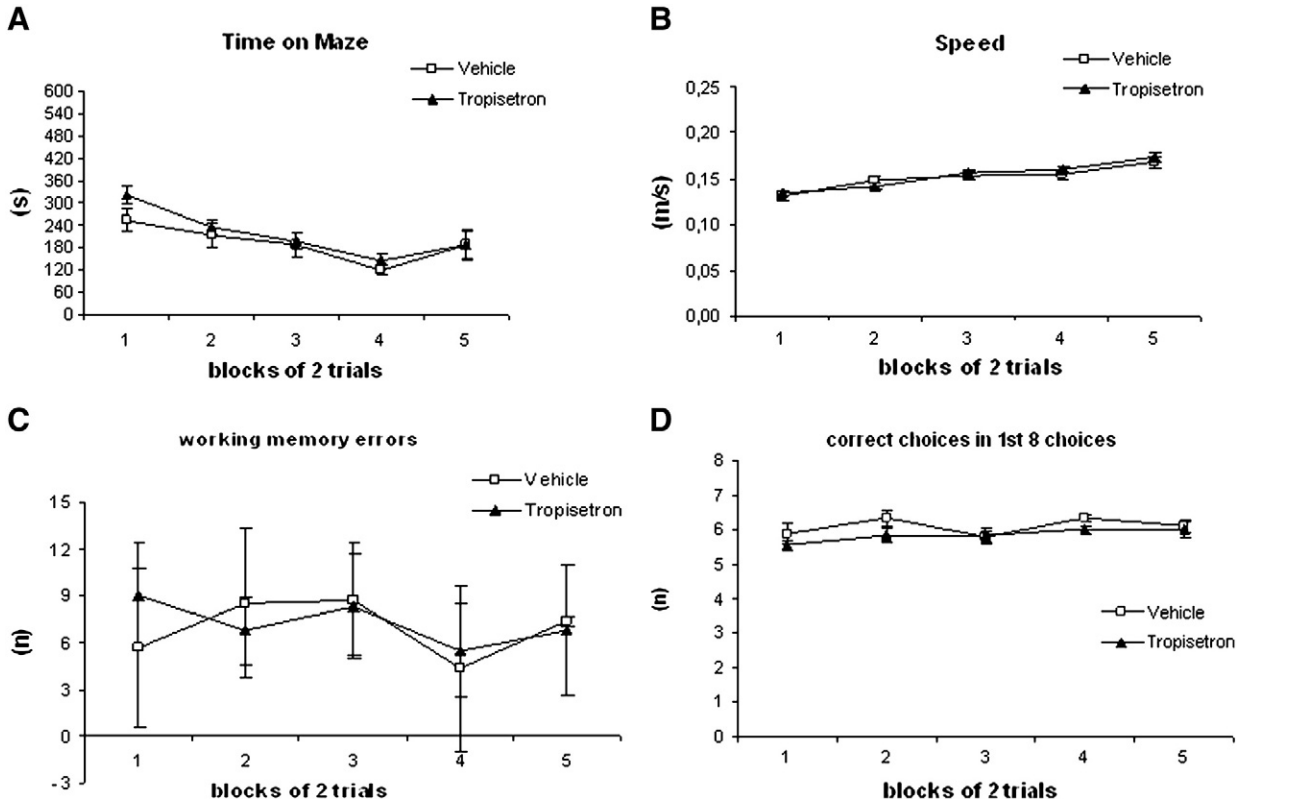

Fig. 3. Postnatal tropisetron treatment did not alter learning in RAM. No differences were observed in tropisetron-treated and vehicle-treated animals with respect to (A) time spent on the maze (time to fulfill the task), (B) speed in the maze, (C) the number of working memory errors, (n) or (D) correct choices during the first eight visits of each trial. All data are means ± SEM.

anxiolytic behavior compared to control mice treated acutely with saline. Diazepam treated mice performed more than 2 times more exits ($p = 0{,}025$) and stayed twice as much time in the bright compartment ($p = 0{,}026$) as control mice (data not shown).

3.8. Administration of tropisetron or fluoxetine does not influence despair behavior in Porsolt Forced Swim Test

A repeated measurement ANOVA revealed no significant effect of time (i.e. of the testing day) for the latency (Fig. 5A), but for the total immobility time (factor time: $F(1,40) = 28.598$ $p = 0.000$), resulting in higher total immobility time at the second day but similarly for all groups investigated (Fig. 5B). Therefore, the treatment with either tropisetron or fluoxetine did not change the floating behavior of the mice concerning latency or total immobility time.

4. Discussion

Here we showed that treatment with tropisetron during the first postnatal week did not induce delayed deleterious emotional or

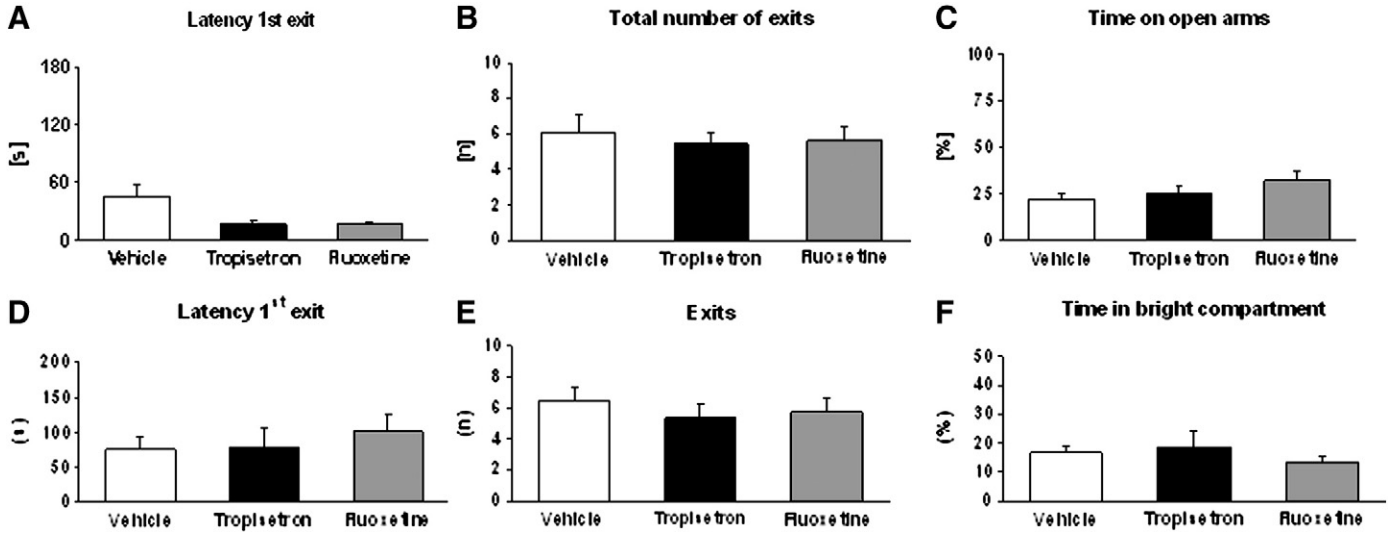

Fig. 4. No difference in anxiety-like behavior in the adult after postnatal treatment with tropisetron or fluoxetine. Both tropisetron-, fluoxetine- and vehicle-treated mice showed a similar latency to enter the bright compartment (A), number of exits (B), and time spent in the bright compartment (C) in the Elevated O-Maze. Mice of all treatment groups displayed similar anxiety-like behavior in the Dark–Light-Box, as measured by (D) the latency to enter the bright compartment, (E) the number of exits and (F) the time spent in the bright compartment. All bars are means + SEM.

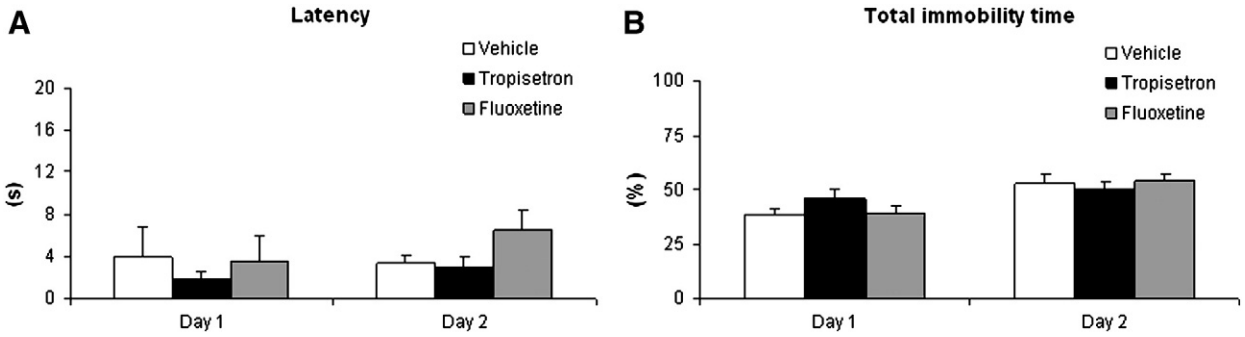

Fig. 5. Despair behavior in adult mice after postnatal treatment with tropisetron, fluoxetine or vehicle. Treatment with either substance resulted in comparable despair behavior measured by (A) the latency to be immobile and (B) the total immobility time. All bars are means + SEM.

cognitive effects in adult mice. To our knowledge, the data presented here represent the first behavioral analysis of tropisetron in rodents covering this developmental period. During this time the very dynamic brain growth spurt takes place, a period with increased vulnerability to drug-induced deleterious effects (Olney et al., 2002) and to several disturbances proposed to be associated with severe neuropsychiatric disorders, such as schizophrenia (Tseng et al., 2009; du Bois and Huang, 2007; Inta et al., 2010). Our data may be relevant with regard to the effects of tropisetron during pregnancy and early life in humans, since the period investigated here in mice (P2–P12) roughly corresponds to that during the third trimester of pregnancy and early postnatal period in humans (Hagberg et al., 2002). Significantly, antagonists of 5-HT_3 receptors are used during pregnancy in the management of nausea and vomiting (Mazzotta and Magee, 2000; Asker et al., 2005; Siu et al., 2002).

Our results appear at a first glance unexpected, considering the high level of expression of 5-HT3 receptors during postnatal development (Inta et al., 2008) and the morphological alterations of cortical neurons induced in organotypical slice cultures from newborn (P0) mice by tropisetron (Chameau et al., 2009). The tropisetron-induced abnormal dendritic branching of cortical pyramidal neurons (Chameau et al., 2009) may determine disorganization of the columnar structure of the neocortex. The lack of significant changes after tropisetron treatment from P2–P12 in behavioral tests relevant to schizophrenia and autism may suggest that structural effects caused by 5-HT_3 antagonism may not be paralleled by behavioral changes. However, we cannot exclude that 5-HT_3 antagonists may induce behavioral changes when given earlier or later than the period analyzed here (P2–P12) and that although the greatest loss of Cajal–Retzius cells occurs between P6 and P12 (Chowdhury et al., 2010) these cells may have different vulnerability to tropisetron at P0 compared to P2.

Antagonists of 5-HT_3 receptors have been proposed as anxiolytics, but pharmacological studies using these substances during adulthood in animal models have provided contradictory results regarding their anxiolytic capacity (Cheng et al., 1994; File and Johnston, 1989). Furthermore, genetic deletion of 5-HT_3 receptors does not induce a unitary anxiolytic phenotype either (Bhatnagar et al., 2004), as these mutants showed increased distance traveled in the open arms of the Elevated Plus Maze, but no change in exploratory behavior in the Open Field or Dark–Light-Box. In addition, these mice exhibited an unaltered basal hypothalamic-pituitary-adrenal axis (HPA) activity and decreased stress responsiveness, together with dysregulation in the level of expression of certain stress-associated hormones, such as corticotropin-releasing hormone (CRH) and vasopressin. We did not observe any difference in any of the two anxiety tests used here (Dark–Light-Box and Elevated O-Maze) after postnatal treatment with tropisetron. Therefore, we did not further investigate possible hormonal alterations putatively induced by this treatment, since they would have not been reflected by changes in anxiety.

Considering the agonistic effect of tropisetron on alpha7 nicotinic acetylcholine receptors, our results may be relevant for possible influences of chronic cholinergic activation from P2 to P12 on adult behavior. Several reports exist regarding the effect of nicotine during postnatal development, however with rather inconsistent results. Prenatal and early postnatal (till P11) nicotine treatments induce mild deficits in spatial learning in adult females, but not in males (Eppolito and Smith, 2006). Another study, however, found no cognitive impairment, but increased anxiety-like behaviors after postnatal exposure to nicotine (Huang et al., 2007). In addition, 5-HT_3 receptors may mediate inhibition of acetylcholine release in cortical tissue (Barnes et al., 1989; Ramírez et al., 1996), although, other studies showed that 5-HT_3 receptor antagonists do not influence or even inhibit acetylcholine release (Consolo et al., 1994; Crespi et al., 1997). Even if these results were obtained in adult animals, we cannot exclude a compensatory effect of an enhanced acetylcholine release (induced by 5-HT_3 blockade) on eventual deleterious effects of tropisetron, which may explain the lack of cognitive effects in tropisetron-treated animals seen in the present study. Nevertheless, further studies, using selective alpha7 nicotinic receptor agonists are needed to clarify their possible long-term behavioral effects.

Finally, we found no significant protracted emotional alterations induced by fluoxetine administered from P2–P12 as measured in the Open Field test, Elevated-O-Maze, Dark–Light-Box and Forced Swim Test. These results are in agreement with previous data (Ansorge et al., 2004; Karpova et al., 2009) showing that exposure to fluoxetine from P4–P21 had no effect on parameters reflecting anxiety- and depression-like behavior in similar tests as used here. We also observed a significant reduction in body weight induced by early postnatal treatment with fluoxetine, in line with previous findings (Ansorge et al., 2004; Karpova et al., 2009). We did not, however, found the behavioral inhibition in the adult (such as decreased total distance traveled in the Elevated Plus-Maze) reported by these studies following postnatal treatment with fluoxetine (Ansorge et al., 2004; Karpova et al., 2009). One possible explanation for this difference is that the fluoxetine treatment period used in our study was significantly shorter than in these other two studies (P2–P12 vs. P4–P21).

Taken together, the present results indicate that early postnatal treatment with tropisetron at 1 mg/kg does not affect cognitive and emotional development in rodents, suggesting that tropisetron may not induce autism-, schizophrenia-, anxiety- or depression-like behavioral abnormalities in the adult. Future investigations are necessary in order to clarify whether the use of tropisetron and similar drugs at earlier, prenatal time points, is also without effect on behavior during adulthood.

Conflict of interest

The authors declare no conflict of interest.

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