Neurobiology of Learning and Memory 118 (2015) 113–119

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Modulation of adenosine signaling prevents scopolamine-induced cognitive impairment in zebrafish

Josiane Woutheres Bortolotto [a,d], Gabriela Madalena de Melo [a,d], Giana de Paula Cognato [a,b], Mônica Ryff Moreira Vianna [c,d], Carla Denise Bonan [a,d,*]

[a] Laboratório de Neuroquímica e Psicofarmacologia, Departamento de Biologia Celular e Molecular, Programa de Pós-Graduação em Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil
[b] Programa de Pós-Graduação em Bioquímica e Bioprospecção, Universidade Federal de Pelotas, Campus Universitário Capão do Leão, s/n°, CEP 96010-900, Pelotas, RS, Brazil
[c] Laboratório de Biologia e Desenvolvimento do Sistema Nervoso, Departamento de Ciências Morfofisiológicas, Programa de Pós-Graduação em Biologia Celular e Molecular, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil
[d] ZebLab – Grupo de Pesquisa em modelos experimentais em zebrafish, Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Avenida Ipiranga, 6681, 90619-900 Porto Alegre, RS, Brazil

ARTICLE INFO

Article history:
Received 19 September 2014
Revised 21 November 2014
Accepted 27 November 2014
Available online 6 December 2014

Keywords:
Adenosine
Inhibitory avoidance
Memory
Scopolamine
Zebrafish

ABSTRACT

Adenosine, a purine ribonucleoside, exhibits neuromodulatory and neuroprotective effects in the brain and is involved in memory formation and cognitive function. Adenosine signaling is mediated by adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3); in turn, nucleotide and nucleoside-metabolizing enzymes and adenosine transporters regulate its levels. Scopolamine, a muscarinic cholinergic receptor antagonist, has profound amnesic effects in a variety of learning paradigms and has been used to induce cognitive deficits in animal models. This study investigated the effects of acute exposure to caffeine (a non-selective antagonist of adenosine receptors A_1 and A_{2A}), ZM 241385 (adenosine receptor A_{2A} antagonist), DPCPX (adenosine receptor A_1 antagonist), dipyridamole (inhibitor of nucleoside transporters) and EHNA (inhibitor of adenosine deaminase) in a model of pharmacological cognitive impairment induced by scopolamine in adult zebrafish. Caffeine, ZM 241385, DPCPX, dipyridamole, and EHNA were acutely administered independently via i.p. in zebrafish, followed by exposure to scopolamine dissolved in tank water (200 μM). These compounds prevented the scopolamine-induced amnesia without impacting locomotor activity or social interaction. Together, these data support the hypothesis that adenosine signaling may modulate memory processing, suggesting that these compounds present a potential preventive strategy against cognitive impairment.

1. Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized by memory loss and cognitive deficits (Selkoe, 2001). AD etiology is not yet fully understood, though hallmark neuropathological features have been identified and include the deposition of senile plaques, neurofibrillary tangles, and progressive synaptic and neuronal loss (Haass & Selkoe, 2007). Additionally, cholinergic transmission deficiencies have been implicated in the cognitive decline observed in AD patients. According to the cholinergic hypothesis, cognitive deterioration is associated with a decrease in acetylcholine (ACh) levels due to its rapid hydrolysis by acetylcholinesterase (AChE) (Bartus, Dean, Beer, & Lippa, 1982). This hypothesis was the foundation for the use of acetylcholinesterase inhibitors (AChEi) for the treatment of cognitive deficits in AD patients (Casey, Antimisiaris, & ÓBrien, 2010; Small, 2005).

Scopolamine, a muscarinic cholinergic receptor antagonist, is known to impair learning and memory formation and used to model cognitive deficits in animal models (Klinkenberg & Blokland, 2010). The zebrafish, a small tropical freshwater teleost, has emerged as a prominent animal model for studying complex behaviors including learning and memory. Several memory tasks

Abbreviations: AD, Alzheimer disease; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; DMSO, dimethylsulfoxide; EHNA, erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride; ZM 241385, 4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo-{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl) phenol.

* Corresponding author at: Avenida Ipiranga, 6681 – Prédio 12D, sala 301, Bairro Partenon – 92425-190, Porto Alegre, RS, Brazil.
E-mail address: cbonan@pucrs.br (C.D. Bonan).

http://dx.doi.org/10.1016/j.nlm.2014.11.016
1074-7427/

were established for this model organism in recent years, including the plus maze, Y-maze, and inhibitory avoidance task, which have proven to be useful tools for evaluating several behavioral and pharmacological conditions in this species (Blank, Guerim, Cordeiro, & Vianna, 2009; Cognato et al., 2012; Sison & Gerlai, 2010). The high genomic similarity to humans (Howe et al., 2013) and the identification of classical neurotransmitter systems involved in learning and memory processing, such as glutamatergic (Todd, Slatter, & Ali, 2004) and cholinergic (Clemente et al., 2004) systems, are important aspects for modeling neurological disorders in zebrafish (Best & Alderton, 2008; Stewart, Braubach, Spitsbergen, Gerlai, & Kalueff, 2014). Previous studies have demonstrated that scopolamine induces cognitive impairment and disrupts aversive and recognition memory processing in zebrafish (Cognato et al., 2012; Kim, Lee, Kim, Jung, & Lee, 2010; Richetti et al., 2011).

Adenosine, a purine ribonucleoside, plays important roles as a homeostatic regulator and neuromodulator, controlling neuronal excitability and, consequently, modulating physiological and pathological conditions of the central nervous system (CNS) (Boison, 2012; Chen, Eltzschig, & Fredholm, 2013; Dias, Rombo, Ribeiro, Henley, & Sebastião, 2013). Adenosine signaling is mediated via four G-protein coupled receptors: A_1, A_{2A}, A_{2B}, and A_3. These receptors were identified in several species including zebrafish (Boehmler et al., 2009; Capiotti et al., 2011) and are linked to a variety of transduction mechanisms (Fredholm, IJzerman, Jacobson, Linden, & Müller, 2011). Extracellular adenosine levels are dynamically controlled by the action of ectonucleotidases, involved in adenosine production by ATP degradation and equilibrative and concentrative nucleoside transporters (Bonan, 2012). In addition, adenosine may be deaminated by adenosine deaminase (ADA), a mainly cytosolic enzyme that is also found in the external cell surface already described in zebrafish (Rosemberg et al., 2007).

The involvement of adenosine receptors in cognitive processes in humans and rodents has been demonstrated (Gomes, Kaster, Tomé, Agostinho, & Cunha, 2011; Sebastiao, Ribeiro, & Ribeiro, 2012). *Post-mortem* analysis of frontal cortex from AD patients revealed a significant increase in A_1 and A_{2A} receptor expression in early and advanced stages of disease (Albasanz, Perez, Barrachina, Ferrer, & Martín, 2008). Epidemiological studies demonstrated that the incidence of AD was inversely associated with consumption of caffeine, which acts as a non-selective adenosine receptor antagonist (Maia & De Mendonça, 2002; Ritchie et al., 2007). Caffeine also prevented the scopolamine-induced disruption of short- and long-term memory in rodents and humans (Botton et al., 2010; Riedel et al., 1995). Studies using the A_1 and A_{2A} dual antagonist ASP5854 exhibited reversion of scopolamine-induced memory deficits in rats, whereas the specific A_{2A} antagonist KW-6002 had no such effect. Similarly, selective A_{2A} antagonist SCH58261 failed to reverse either MK-801- or scopolamine-induced amnesia in rats (Cunha et al., 2008). This evidence supports the specific involvement of A_1 receptor antagonism in cognition (Mihara et al., 2007).

Therefore, considering: (i) the role of adenosine receptors in prevention or reversion of scopolamine-induced memory deficits, (ii) the relevance of zebrafish as a powerful model for modeling neurological disorders and pharmacological studies and, (iii) the modulation of adenosine signaling exerted by nucleotide and nucleoside-metabolizing enzymes and adenosine transporters, this study aimed to investigate the effects of acute administration of caffeine, ZM 241385 (adenosine A_{2A} receptor antagonist), DPCPX (adenosine A_1 receptor antagonist), dipyridamole (inhibitor of nucleoside transporters) and EHNA (inhibitor of adenosine deaminase) in a model of pharmacological cognitive impairment induced by scopolamine in adult zebrafish.

2. Materials and methods

2.1. Animals

Adult wild type zebrafish (0.3–0.5 g) were obtained from a specialized supplier (Redfish Agroloja, RS, Brazil). Animals were kept in 30-L housing tanks with unchlorinated water at a targeted temperature of 26 ± 2 °C and continuously aerated under 14:10 h light:dark photoperiod. The fish were acclimated to the laboratory environment for at least 14 days and were fed three times a day with commercial flake food supplemented with brine shrimp. All procedures were approved by the institutional Animal Care Committee (11/00257-CEUA-PUCRS).

2.2. Pharmacological treatments

Caffeine (1,3,7-trimethylxanthine; Sigma Chemical Co., USA), ZM 241385 (4-(2-[7-amino-2-{2-furyl}{1,2,4}triazolo-{2,3-a}{1,3,5}triazin-5-yl-amino]ethyl) phenol; Tocris Cookson, USA), DPCPX (8-cyclopentyl-1,3-dipropylxanthine; Tocris Cookson, USA), EHNA (erythro-9-(2-hydroxy-3-nonyl)-adenine hydrochloride; Sigma–Aldrich, St Louis, MO) and dipyridamole (Sigma–Aldrich, St Louis, MO) were used in the study. Caffeine and EHNA were dissolved in saline (0.9% NaCl); ZM 241385, DPCPX and dipyridamole were dissolved in 1% DMSO (dimethylsulfoxide). Caffeine (10 mg/kg), ZM 241385 (10 μg/kg), DPCPX (0.5 mg/kg), dipyridamole (5 mg/kg), and EHNA (100 μg/kg) were administered via intraperitoneal (i.p.) injection in a volume of 10 μL using a 3/10-mL U100 BD Ultra-Fine™ Short Insulin Syringe 8 mm (5/16") × 31G Short Needle (Becton Dickinson and Company, New Jersey, USA) (Kinkel, Eames, Philipson, & Prince, 2010). Drug doses and administration routes were chosen and adjusted based on previous studies demonstrating effects on memory in rodents (Melani, Cipriani, Corti, & Pedata, 2010; Prediger & Takahashi, 2005). Only doses that were unable to alter locomotor activity were used to ensure the effects observed were related to memory processing. Before drug or vehicle administration, fish were anesthetized by immersion in 0.1 g/L tricaine solution (ethyl 3-aminobenzoate methanesulfonate salt; Fluka, Buchs, Switzerland).

After treatment, animals were placed in a separate tank with aerated, unchlorinated tap water to recover from the anesthesia. Caffeine, ZM 241385, DPCPX, dipyridamole, EHNA, and the appropriate vehicle (used as control group) were injected 2 h before the beginning of each experiment. One hour prior to the start of the behavioral assay, animals were transferred to a tank containing 200 μM scopolamine solution (dissolved in aerated, unchlorinated water). Animals that did not receive scopolamine were also transferred to another tank with water to control for handling effects (Kim et al., 2010).

2.3. Behavioral analysis

2.3.1. Inhibitory avoidance task

Long-term memory was evaluated using the inhibitory avoidance (IA) protocol previously described in detail (Blank et al., 2009). After treatment, zebrafish were individually trained and tested in a glass tank (18 cm L × 9 cm H × 7 cm W) divided by a sliding guillotine-type partition (9 cm × 7 cm) in two equally sized compartments, white and dark. During a training session, animals were individually placed in the white side of the tank with the partition closed. After 1 min of habituation, the partition was raised 1 cm, allowing fish to cross to the dark side of the tank. Immediately after crossing and entering the dark side, the slide partition was closed and a pulsed electric shock of 3 ± 0.2 V was administered for 5 s. Fish were then removed from the apparatus and

placed in a temporary tank; they were later returned to their housing tank. Twenty-four hours after a training session, animals were submitted to a test session. The test session repeated the training protocol except that no shock was administered. The latency to completely enter the dark compartment was measured during both sessions.

2.3.2. Exploratory assessment

After animals received the second treatment, they were placed individually in the experimental tanks (30 cm L × 15 cm H × 10 cm W) and habituated for 30 s, as previously described (Gerlai, Lahav, Guo, & Rosenthal, 2000). After the habituation period, locomotor activity was video recorded for 5 min using Logitech Quickcam PRO 9000 and quantitatively analyzed using ANY-Maze recording software (Stoelting Co. Wood Dale, IL, USA). The tank was virtually divided into equal sections with three vertical lines and one horizontal line. The behavioral patterns quantified were the number of sectional line crossings, distance traveled, mean speed, and time spent in each tank section (i.e., the bottom vs. upper levels). Because zebrafish have a natural tendency to spend more time at the bottom of a novel tank before gradually exploring higher portions of the tank over a period of minutes, time spent in each tank section can be used as a measure of anxiety (Levin, Bencan, & Cerutti, 2007). All exploratory assessment was performed between 9:00 a.m. and 1:00 p.m.

2.3.3. Social interaction

The zebrafish is a social animal. To test social interaction, animals from the same shoal were used in each experiment. Five experimental fish were placed in a small experimental aquarium (30 cm L × 15 cm H × 10 cm W). On one side of the experimental aquarium, an empty tank was placed; on the opposing side, a "stimulus tank" of identical size held 15 zebrafish. The behavior of experimental fish was recorded after 30 s of habituation time. In order to quantify any inherent preference for the "stimulus" side, the central tank was in two equal parts; the time that experimental fish spent in the virtual half adjacent to the conspecific school was measured (Gerlai, 2003).

2.4. Statistical analysis

Inhibitory avoidance memory data are presented as mean ± S.E.M. Training and test latencies for each group were compared by Wilcoxon matched pairs test. Latencies of multiple groups were compared using Kruskal–Wallis and Mann–Whitney U tests. Exploratory assessment and social interaction data were analyzed via one-way analysis of variance (ANOVA) followed by post hoc comparisons using Tukey's HSD test and were expressed as the mean ± S.E.M. For all comparisons, $p < 0.05$ was considered significant.

3. Results

3.1. Caffeine, ZM 241385, DPCPX, dipyridamole, and EHNA prevent scopolamine-induced memory deficits

We first analyzed the influence of pretreatment with adenosine receptor antagonists on scopolamine-induced memory impairment using an inhibitory avoidance task. We independently evaluated the effects of caffeine (10 mg/kg), ZM 241385 (10 μg/kg), and DPCPX (0.5 mg/kg) i.p treatment, followed by pre-training scopolamine (200 μM) exposure (Fig. 1). Saline was used as vehicle for caffeine and 1% DMSO was used as vehicle for both ZM 241385 and DPCPX. Vehicle-exposed animals followed by water treatment demonstrated robust retention of memory during the test session

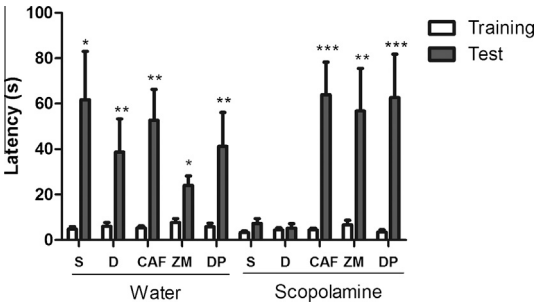

Fig. 1. Pretreatment with adenosine receptor antagonists caffeine (CAF), ZM 241385 (ZM), and DPCPX (DP) prevented scopolamine-induced memory impairment in the inhibitory avoidance task. Animals received a single i.p. injection of saline (S), DMSO (D), caffeine (10 mg/kg), ZM 241385 (10 μg/kg) or DPCPX (0.5 mg/kg) 2 h before the training session. The i.p. treatment was followed by a 1 h exposure to water or scopolamine prior to testing. Effects of scopolamine or water on the latency to enter the dark compartment during training and test sessions in the inhibitory avoidance task were evaluated. Data are presented as mean ± SEM ($n = 12$ per group) $^{*}p < 0.05$ $^{**}p < 0.001$ $^{***}p < 0.0001$ indicate differences between training and test session for each group compared by Wilcoxon matched pair test. No differences were found between training performances among all groups as evaluated by Kruskal–Wallis test.

performed 24 h after training ($p < 0.05$). Pretreatment of caffeine ($p < 0.001$), ZM 241385 ($p < 0.05$) or DPCPX ($p < 0.001$), followed by water treatment, resulted in significant differences between zebrafish training and test sessions, thus suggesting effective learning of the task. However, vehicle-exposed animals subsequently treated with scopolamine did not exhibit memory retention during the test session performed 24 h after training. Interestingly, treatment with either caffeine, ZM 241385 or DPCPX prevented the memory impairment induced by pre-training scopolamine exposure, as observed by the difference in latencies between training and test sessions for each treatment ($p < 0.0001$, $p < 0.001$, and $p < 0.0001$, respectively) (Fig. 1). In addition, there were no significant differences in the latencies for test sessions between the groups pretreated with adenosine receptor antagonists (Caffeine, ZM241385, or DPCPX) followed by water or scopolamine treatment. These results suggest that adenosine A_1 and A_{2A} receptor antagonists ameliorated scopolamine-induced memory impairment.

To elucidate the roles of nucleoside transporters and nucleoside-metabolizing enzymes in scopolamine-induced memory deficit, respectively, we tested an inhibitor of nucleoside transporters, dipyridamole, and an inhibitor of adenosine deaminase, EHNA (Fig. 2). Animals treated with 1% DMSO or dipyridamole (5 mg/kg) followed by water exposure presented robust memory retention, exhibiting significant differences between their training and test sessions ($p < 0.05$ for each group analyzed separately) (Fig. 2A). However, pre-training scopolamine exposure disrupted memory formation in 1% DMSO-treated animals. In contrast, dipyridamole prevented scopolamine-induced impairment, as evident in the significant difference between training and test session latencies ($p < 0.05$) (Fig. 2A). Similarly, pretreatment with EHNA prevented scopolamine-induced memory deficits ($p < 0.001$; Fig. 2B). Taken together, these results suggest that acute treatment of either dipyridamole or EHNA before scopolamine treatment prevents the reliably induced memory impairment that would follow.

3.2. Effects of scopolamine and purinergic modulation on exploratory assessment

Zebrafish exploratory activity was evaluated after i.p. injections of caffeine, ZM 241385, DPCPX, dipyridamole, and EHNA, combined with subsequent water or scopolamine treatment. No significant difference in either distance travelled or mean speed were found in animals that received any of the treatments when

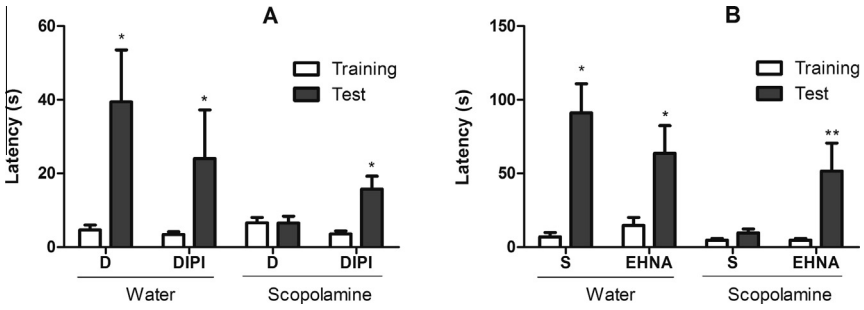

Fig. 2. Pretreatment with dipyridamole (DIPI) or EHNA prevented scopolamine-induced memory impairment during the inhibitory avoidance task. (A) Animals received a single i.p. injection of DMSO (D) or DIPI (5 mg/kg). (B) Animals received a single i.p. injection of saline (S) or EHNA (100 μg/kg). The i.p. treatment, administered 2 h before the training sessions, was followed by a 1 h exposure of either water or scopolamine prior to testing. The effects of scopolamine and water on the latency to enter the dark compartment in training and test sessions in the inhibitory avoidance task were evaluated. Data are presented as mean ± S.E.M ($n = 12$ per group); $^{*}p < 0.05$ $^{**}p < 0.001$ indicate the differences between training and test sessions for each group compared via Wilcoxon matched pair test. No differences were found between training performance among all groups as evaluated by Kruskal–Wallis test.

compared to the control group (saline or 1% DMSO; Fig. 3 and 4). Swimming coordination was also evaluated; none of the treatments altered the absolute body turn angle during swimming (Figs. 3C and 4C). Time spent in the upper and lower portions of the tank was measured to evaluate anxiety; no significant differences were found in treated animals when compared to their respective control group (saline or 1% DMSO; Figs. 3D and E and 4D and E).

3.3. Effects of scopolamine, caffeine, ZM 241385, DPCPX, dipyridamole, and EHNA on social interaction

Social interaction was evaluated after i.p. injections of caffeine, ZM 241385, DPCPX, dipyridamole, and EHNA, followed by water or scopolamine (Fig. 5A and B). There were no significant changes between experimental groups in the time spent near the stimulus tank, indicating that social interaction was not altered by either scopolamine or modulators of adenosine signaling.

4. Discussion

In this study, we evaluated the preventive role of adenosine signaling modulation on scopolamine-induced memory deficits in zebrafish. Previous studies using adult zebrafish showed that scopolamine triggered robust impairment of memory during an inhibitory avoidance task (Kim et al., 2010; Richetti et al., 2011). Our data demonstrated that acute i.p pretreatments with non-selective adenosine receptor antagonist caffeine, and the selective antagonists of A_{2A} (ZM 241385) and A_1 (DPCPX) receptors all prevented the scopolamine-induced memory deficits observed in the inhibitory avoidance task. This study also demonstrates that the inhibition of either nucleoside transporters or nucleoside-metabolizing enzyme adenosine deaminase using dipyridamole and EHNA, respectively, prevents scopolamine-induced memory impairment.

Studies have also demonstrated the prevention of scopolamine-induced cognitive impairment in mice using acute pretreatment with caffeine (10 mg/kg) (Botton et al., 2010). Other studies

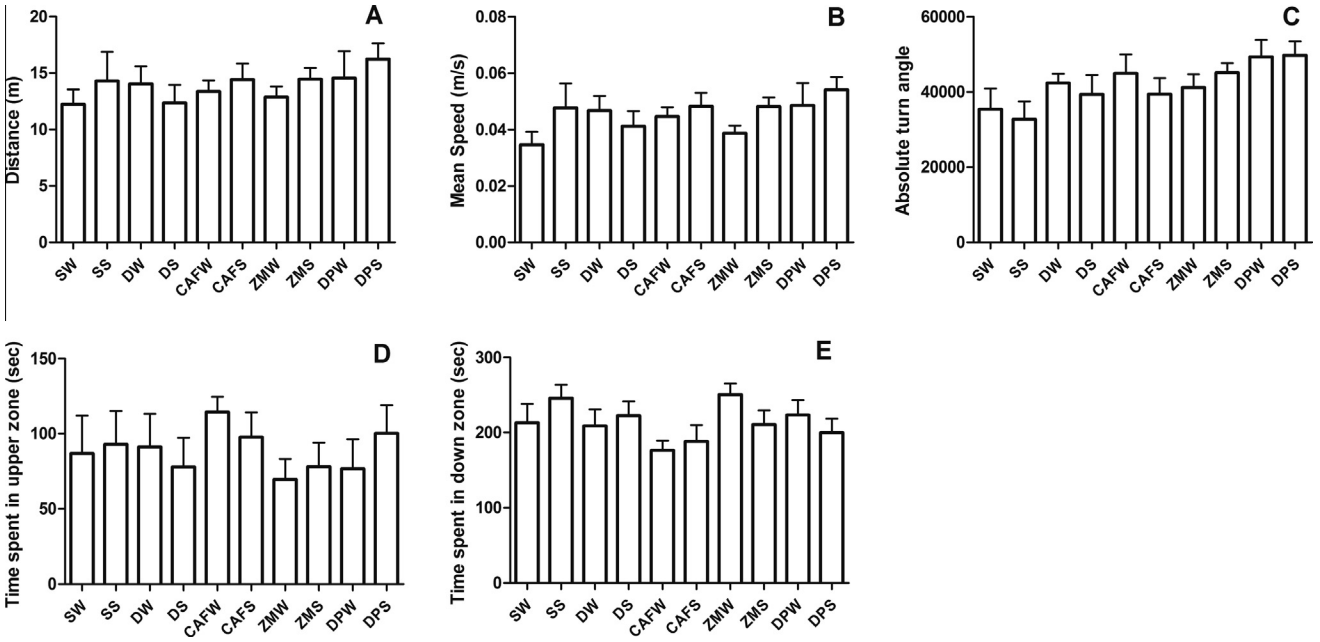

Fig. 3. Locomotor activity was evaluated after an i.p. injection of adenosine receptor antagonist caffeine (CAF), ZM 241385 (ZM) or DPCPX (DP) followed by scopolamine (S) or water (W) exposure. Metrics measured include distance traveled (A), mean speed (B), absolute turn angle (C), time spent in upper zone (D), and time spent in lower zone in (E). No differences were found between groups. Data are expressed as mean ± S.E.M. of 12 different animals for each group and were analyzed by one-way ANOVA test. SW, saline + water; SS, saline + scopolamine; DW, DMSO + water; DS, DMSO + scopolamine; CAFW, caffeine + water; CAFS, caffeine + scopolamine; ZMW, ZM 241385 + water; ZMS, ZM 241385 + scopolamine; DPW, DPCPX + water; DPS, DPCPX + scopolamine.

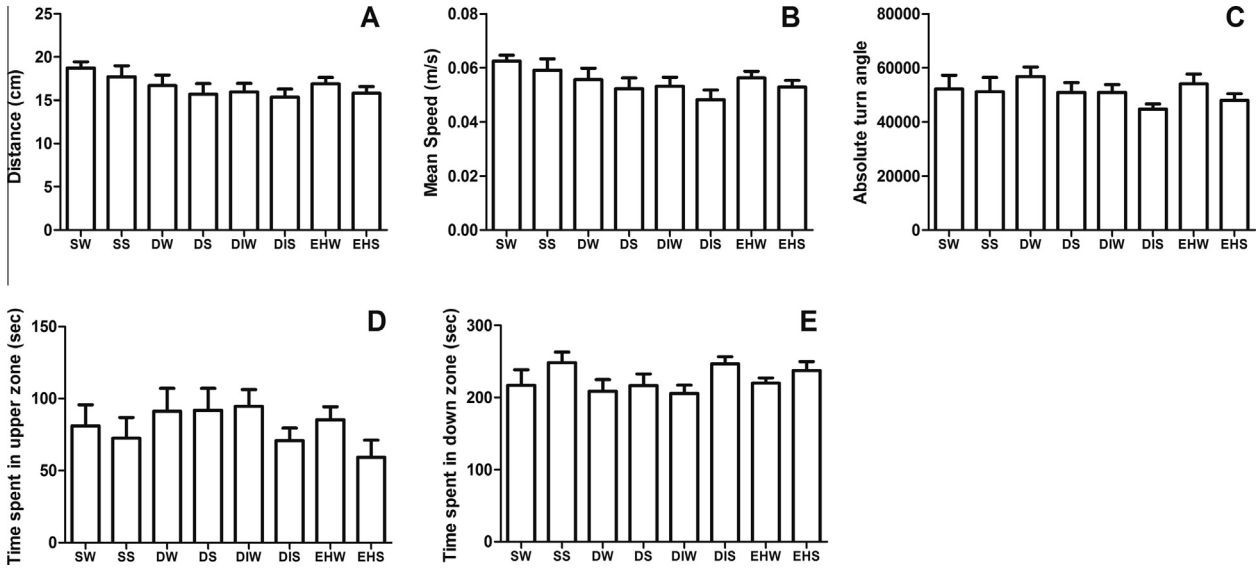

Fig. 4. Locomotor activity was evaluated after an i.p. injection of dipyridamole (DI) or EHNA (EH), followed by scopolamine (S) or water (W) exposure. Analyzed metrics include distance traveled (A), mean speed (B), absolute turn angle (C), time spent in upper zone (D), and time spent in lower zone in (E). No differences were found between groups. Data are expressed as mean ± S.E.M. of 12 different animals for each group and were analyzed by one-way ANOVA test. SW, saline + water; SS, saline + scopolamine; DW, DMSO + water; DS, DMSO + scopolamine; DIW, dipyridamole + water; DIS, dipyridamole + scopolamine; EHW, EHNA + water; EHS, EHNA + scopolamine.

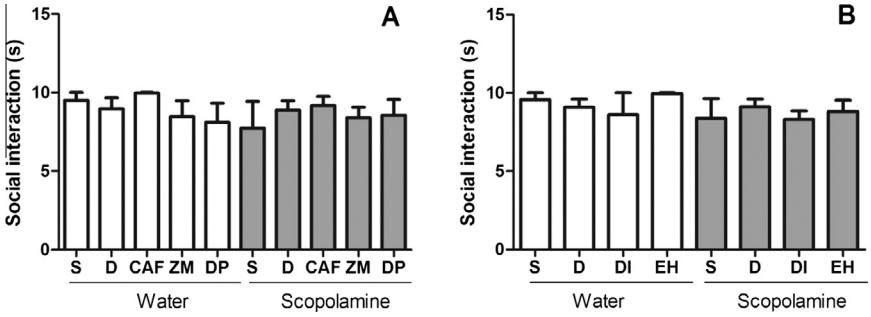

Fig. 5. Social interaction was evaluated after the i.p. injection of adenosine receptor antagonist caffeine (CAF), ZM 241385 (ZM), DPCPX (DP; A) or dipyridamole (DI), EHNA (EH; B), followed by scopolamine or water treatment. Saline (S) or saline plus DMSO (D) was used as a control. No differences were found between groups. Data are expressed as mean ± S.E.M. of 6 different groups and were analyzed via one-way ANOVA.

revealed similar beneficial effects of caffeine in mice with respect to memory deficits induced by a variety of factors such as alcohol (Spinetta et al., 2008) and beta-amyloid (Dall'Igna et al., 2007). Furthermore, chronic intake of caffeine in a transgenic mouse model of AD improved cognition in mice (Arendash et al., 2006) and reduced the deposition of senile plaques (Arendash et al., 2009; Cao et al., 2009). Short and long-term memories formed during an inhibitory avoidance task were improved in middle-aged mice chronically treated with caffeine (Sallaberry et al., 2013). In humans, a placebo-controlled blind crossover study showed that three cups of coffee per day attenuated the scopolamine-induced impairment of several parameters in a word learning task, including free recall from short- and long-term memory, retrieval quality and speed (Riedel et al., 1995). Other studies performed in humans corroborate the potential of caffeine with respect to reducing cognitive decline (Ritchie et al., 2007; van Gelder et al., 2007). Our results are in agreement with previous studies, demonstrating that acute caffeine treatment prevents the memory disruption caused by scopolamine in zebrafish.

The psychostimulant effect of caffeine on the central nervous system (CNS) is primarily due to the antagonism of adenosine receptors A_1 and A_{2A} (Ferré, 2008; Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). We have shown that selective antagonism of adenosine A_{2A} or A_1 receptors by ZM 241385 and DPCPX, respectively, prevented scopolamine-induced memory deficits during an inhibitory avoidance task in zebrafish. Other studies have also described the beneficial effects of A_{2A} antagonism on the synaptic mechanisms of learning and memory in AD animal models and patients (Cunha & Agostinho, 2010; Takahashi, Pamplona, & Prediger, 2008). Rats overexpressing A_{2A} receptors presented memory deficits (Giménez-Llort et al., 2007), while mice lacking adenosine A_{2A} receptors have improved spatial recognition memory (Wang, Ma, & van den Buuse, 2006). Interestingly, the A_{2A} receptor antagonist SCH5861 was unable to prevent the memory deficits induced by scopolamine treatment in rats during a Y-maze task (Cunha et al., 2008). The discrepancies between our work and this result can be attributed to the different memory tasks employed and/or the different methods of scopolamine administration. In addition, we did not observe alterations in locomotion after scopolamine treatment, which differs from the results observed by Cunha et al. (2008). Our results suggest that A_{2A} antagonism can improve memory performance without changes in locomotor activity.

Our results demonstrated that blockade of adenosine A_1 receptor by DPCPX prevents scopolamine-induced memory impairment in zebrafish. Accordingly, previous work demonstrated significant improvement of scopolamine-induced memory impairment using DPCPX in mice (Zhang & Ren, 2003). Another study characterized

the effects of a novel adenosine A_1 antagonist, FR94921, on passive avoidance memory. FR194921 ameliorated scopolamine-induced memory deficits in rats, suggesting this compound harbors a potential for cognitive enhancement (Maemoto et al., 2004). Similar results were observed by Pitsikas and Borsini (1997) using an antagonist of A_1 receptors, BIIP 20, which restored the memory deficits caused by scopolamine in rats (Pitsikas & Borsini, 1997).

To better understand the role of adenosine signaling in memory impairment, we tested the effects of inhibitors of nucleoside transport (dipyridamole) and adenosine deamination (EHNA) on scopolamine-induced memory deficits in zebrafish. Nucleoside transporters were acutely blocked with dipyridamole, resulting in a significant improvement in scopolamine-induced memory impairment. Dipyridamole was previously used in a model of vascular cognitive impairment in rats caused by bilateral carotid artery occlusion (Melani et al., 2010). After one week of intravenous perfusion, it significantly restored spatial memory in the Y-maze test. Dipyridamole is commonly used to inhibit platelet aggregation and reduce thrombi formation *in vivo* (Heptinstall, Fox, Crawford, & Hawkins, 1986). However, few investigations have demonstrated any neuroprotective properties of this compound *in vivo*. *In vitro* studies, however, have revealed a neuroprotective effect of dipyridamole in neuronal cultures, most likely due to its role as an antioxidant (Blake, 2004; Farinelli, Greene, & Friedman, 1998). More recently, studies have suggested that dipyridamole can augment vessel function, restoring blood flow in cerebrovascular disease (Chakrabarti & Freedman, 2008). In blood cells, the antiplatelet mechanism of dipyridamole is due in part to the inhibition of adenosine uptake. The increased extracellular concentration of adenosine results in vasodilation (Geiger, 2001). As adenosine is known for its neuroprotective role in the CNS, it is possible that dipyridamole acts by controlling nucleoside levels and, consequently, inducing an improvement in scopolamine-induced memory deficits in zebrafish.

Adenosine deaminase catalyzes the irreversible deamination of adenosine to inosine (Franco et al., 1997). A previous study demonstrated that scopolamine increased adenosine deaminase activity in rat cerebral cortex and hippocampus synaptosomes. However, adenosine levels measured by HPLC did not change upon scopolamine treatment (Gutierres et al., 2012). Our results demonstrate that acute pretreatment with EHNA, an ADA inhibitor, protected zebrafish from the memory impairment caused by scopolamine. Adenosine has been reported as a neuromodulator, with an important role in synaptic plasticity and memory processing, and its depletion can disrupt memory formation (de Mendonça, Costenla, & Ribeiro, 2002; de Mendonça & Ribeiro, 1997). The data presented here suggests that modulation of adenosine levels via the inhibition of nucleoside transporters or adenosine metabolism can prevent scopolamine-induced effects, reinforcing the proposed involvement of adenosine signaling in cognitive function.

Scopolamine is a classical model of induced amnesia. However, some studies have been questioned the use of scopolamine for inducing cognitive impairment due to differences in data related to locomotor analyses (Klinkenberg & Blokland, 2010). As shown in Figs. 3 and 4, we did not observe changes in locomotor parameters between control and treated fish. In addition, there were no changes in the time spent in the upper or lower portions of the tank, suggesting that scopolamine did not alter anxiety parameters in zebrafish. These data are in agreement with previous studies in both zebrafish and rodents (Botton et al., 2010; Gutierres et al., 2012; Kim et al., 2010; Richetti et al., 2011). However, Cunha and coworkers (2008) observed scopolamine-induced hyperlocomotion that was not prevented by SCH58261 (Cunha et al., 2008).

Zebrafish are a social species and they prefer to swim in groups (Engeszer, Patterson, Rao, & Parichy, 2007; Miller & Gerlai, 2007). Evidence suggests that the cholinergic system participates in social recognition (Wang, Karp, Winblad, & Fratiglioni, 2002; Winslow & Camacho, 1995). Our data revealed no changes in social interaction between the control and treated groups (Fig. 5). In mice, social interaction was not altered by different doses of scopolamine; however, social memory was impaired by scopolamine in a dose-dependent manner (Riedel, Kang, Choi, & Platt, 2009). These results suggest that scopolamine promotes memory disruption without changes in social interaction.

5. Conclusion

In conclusion, our results support the idea that the antagonism of adenosine receptors or a possible increase of adenosine levels induced by inhibition of nucleoside transport or adenosine catabolism prevented scopolamine-induced memory deficits. This data corroborates the hypothesis that adenosine signaling is involved in memory processing in zebrafish and may be a target for the development of preventive strategies against cognitive impairment.

Acknowledgments

This work was supported by DECIT/SCTIE-MS through Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) (Proc. 10/0036-5 — PRONEX) and CNPq (Proc. 473275/2011-2). G.P.C, C.D.B and M.R.V. were recipients of fellowships from CNPq. J.W.B was the recipient of a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Albasanz, J. L., Perez, S., Barrachina, M., Ferrer, I., & Martín, M. (2008). Up-regulation of adenosine receptors in the frontal cortex in Alzheimer's disease. *Brain Pathology, 18*(2), 211–219.

Arendash, G. W., Mori, T., Cao, C., Mamcarz, M., Runfeldt, M., Dickson, A., et al. (2009). Caffeine reverses cognitive impairment and decreases brain amyloid-beta levels in aged Alzheimer's disease mice. *Journal of Alzheimer's Disease, 17*(3), 661–680.

Arendash, G. W., Schleif, W., Rezai-Zadeh, K., Jackson, E. K., Zacharia, L. C., Cracchiolo, J. R., et al. (2006). Caffeine protects Alzheimer's mice against cognitive impairment and reduces brain beta-amyloid production. *Neuroscience, 142*(4), 941–952.

Bartus, R. T., Dean, R. L., 3rd, Beer, B., & Lippa, A. S. (1982). The cholinergic hypothesis of geriatric memory dysfunction. *Science, 217*(4558), 408–414.

Best, J. D., & Alderton, W. K. (2008). Zebrafish: An in vivo model for the study of neurological diseases. *Neuropsychiatric Disease and Treatment, 4*(3), 567–576.

Blake, A. D. (2004). Dipyridamole is neuroprotective for cultured rat embryonic cortical neurons. *Biochemical and Biophysical Research Communications, 314*(2), 501–504.

Blank, M., Guerim, L. D., Cordeiro, R. F., & Vianna, M. R. (2009). A one-trial inhibitory avoidance task to zebrafish: Rapid acquisition of an NMDA-dependent long-term memory. *Neurobiology of Learning and Memory, 92*(4), 529–534.

Boehmler, W., Petko, J., Woll, M., Frey, C., Thisse, B., Thisse, C., et al. (2009). Identification of zebrafish A2 adenosine receptors and expression in developing embryos. *Gene Expression Patterns, 9*(3), 144–151.

Boison, D. (2012). Adenosine dysfunction in epilepsy. *Glia, 60*(8), 1234–1243.

Bonan, C. D. (2012). Ectonucleotidases and nucleotide/nucleoside transporters as pharmacological targets for neurological disorders. *CNS & Neurological Disorders Drug Targets, 11*(6), 739–750.

Botton, P. H., Costa, M. S., Ardais, A. P., Mioranzza, S., Souza, D. O., da Rocha, J. B., et al. (2010). Caffeine prevents disruption of memory consolidation in the inhibitory avoidance and novel object recognition tasks by scopolamine in adult mice. *Behavioural Brain Research, 214*(2), 254–259.

Cao, C., Cirrito, J. R., Lin, X., Wang, L., Verges, D. K., Dickson, A., et al. (2009). Caffeine suppresses amyloid-beta levels in plasma and brain of Alzheimer's disease transgenic mice. *Journal of Alzheimer's Disease, 17*(3), 681–697.

Capiotti, K. M., Menezes, F. P., Nazario, L. R., Pohlmann, J. B., de Oliveira, G. M., Fazenda, L., et al. (2011). Early exposure to caffeine affects gene expression of adenosine receptors, DARPP-32 and BDNF without affecting sensibility and morphology of developing zebrafish (Danio rerio). *Neurotoxicology Teratology, 33*(6), 680–685.

Casey, D. A., Antimisiaris, D., & ÓBrien, J. (2010). Drugs for Alzheimer's disease: Are they effective? *Pharmacy and Therapeutics, 35*(4), 208–211.

Chakrabarti, S., & Freedman, J. E. (2008). Dipyridamole, cerebrovascular disease, and the vasculature. *Vascular Pharmacology, 48*(4–6), 143–149.

Chen, J. F., Eltzschig, H. K., & Fredholm, B. B. (2013). Adenosine receptors as drug targets–what are the challenges? *Nature Review Drug Discovery, 12*(4), 265–286.

Clemente, D., Porteros, A., Weruaga, E., Alonso, J. R., Arenzana, F. J., Aijón, J., et al. (2004). Cholinergic elements in the zebrafish central nervous system: Histochemical and immunohistochemical analysis. *The Journal of Comparative Neurology, 474*(1), 75–107.

Cognato, G. P., Bortolotto, J. W., Blazina, A. R., Christoff, R. R., Lara, D. R., Vianna, M. R., et al. (2012). Y-Maze memory task in zebrafish (Danio rerio): The role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. *Neurobiology of Learning and Memory, 98*, 321–328.

Cunha, R. A., & Agostinho, P. M. (2010). Chronic caffeine consumption prevents memory disturbance in different animal models of memory decline. *Journal of Alzheimer's Disease, 20*(Suppl), S95–S116.

Cunha, G. M., Canas, P. M., Melo, C. S., Hockemeyer, J., Müller, C. E., Oliveira, C. R., et al. (2008). Adenosine A2A receptor blockade prevents memory dysfunction caused by beta-amyloid peptides but not by scopolamine or MK-801. *Experimental Neurology, 210*(2), 776–781.

Dall'Igna, O. P., Fett, P., Gomes, M. W., Souza, D. O., Cunha, R. A., & Lara, D. R. (2007). Caffeine and adenosine A(2a) receptor antagonists prevent beta-amyloid (25–35)-induced cognitive deficits in mice. *Experimental Neurology, 203*(1), 241–245.

de Mendonça, A., Costenla, A. R., & Ribeiro, J. A. (2002). Persistence of the neuromodulatory effects of adenosine on synaptic transmission after long-term potentiation and long-term depression. *Brain Research, 932*(1–2), 56–60.

de Mendonça, A., & Ribeiro, J. A. (1997). Adenosine and neuronal plasticity. *Life Sciences, 60*(4–5), 245–251.

Dias, R. B., Rombo, D. M., Ribeiro, J. A., Henley, J. M., & Sebastião, A. M. (2013). Adenosine: Setting the stage for plasticity. *Trends in Neurosciences, 36*(4), 248–257.

Engeszer, R. E., Patterson, L. B., Rao, A. A., & Parichy, D. M. (2007). Zebrafish in the wild: A review of natural history and new notes from the field. *Zebrafish, 4*, 21–40.

Farinelli, S. E., Greene, L. A., & Friedman, W. J. (1998). Neuroprotective actions of dipyridamole on cultured CNS neurons. *The Journal of Neuroscience, 18*, 5112–5123.

Ferré, S. (2008). An update on the mechanisms of the psychostimulant effects of caffeine. *Journal of Neurochemistry, 105*, 1067–1079.

Franco, R., Casado, V., Ciruela, F., Saura, C., Mallol, J., Canela, E. I., et al. (1997). Cell surface adenosine deaminase: Much more than an ectoenzyme. *Progress in Neurobiology, 52*, 283–294.

Fredholm, B. B., Bättig, K., Holmén, J., Nehlig, A., & Zvartau, E. E. (1999). Actions of caffeine in the brain with special reference to factors that contribute to its widespread use. *Pharmacological Reviews, 51*, 83–133.

Fredholm, BB., IJzerman, AP., Jacobson, KA., Linden, J., & Müller, CE. (2011). International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors an update. *Pharmacological Reviews, 63*(1), 1–34.

Geiger, J. (2001). Inhibitors of platelet signal transduction as anti-aggregatory drugs. *Expert Opinion on Investigational Drugs, 10*, 865–890.

Gerlai, R. (2003). ZebraFish: An Uncharted Behavior Genetic Model. *Behavior Genetic, 33*(5), 461–468.

Gerlai, R., Lahav, M., Guo, S., & Rosenthal, A. (2000). Drinks like a fish: Zebrafish (Danio rerio) as a behavior genetic model to study alcohol effects. *Pharmacology, Biochemistry and Behavior, 67*, 773–782.

Giménez-Llort, L., Schiffmann, S. N., Shmidt, T., Canela, L., Camón, L., Wassholm, M., et al. (2007). Working memory deficits in transgenic rats overexpressing human adenosine A2A receptors in the brain. *Neurobiology of Learning and Memory, 87*(1), 42–56.

Gomes, C. V., Kaster, M. P., Tomé, A. R., Agostinho, P. M., & Cunha, R. A. (2011). Adenosine receptors and brain diseases: Neuroprotection and neurodegeneration. *Biochimica et Biophysica Acta, 1808*(5), 1380–1399.

Gutierres, J. M., Carvalho, F. B., Schetinger, M. R., Rodrigues, M. V., Schmatz, R., Pimentel, V. C., et al. (2012). Protective effects of anthocyanins on the ectonucleotidase activity in the impairment of memory induced by scopolamine in adult rats. *Life Sciences, 91*(23 24), 1221–1228.

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: Lessons from Alzheimer's amyloid beta-peptide. *Nature Reviews Molecular Cell Biology, 8*, 101–112.

Heptinstall, S., Fox, S., Crawford, J., & Hawkins, M. (1986). Inhibition of platelet aggregation in whole blood by dipyridamole and aspirin. *Thrombosis Research, 42*(2), 215–223.

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., et al. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, 496*(7446), 498–503.

Kim, Y. H., Lee, Y., Kim, D., Jung, M. W., & Lee, C. J. (2010). Scopolamine-induced learning impairment reversed by physostigmine in zebrafish. *Neuroscience Research, 67*(2), 156–161.

Kinkel, M. D., Eames, S. C., Philipson, L. H., & Prince, V. E. (2010). Intraperitoneal injection into adult zebrafish. *Journal of Visualized Experiments, 30*(42).

Klinkenberg, I., & Blokland, A. (2010). The validity of scopolamine as a pharmacological model for cognitive impairment: A review of animal behavioral studies. *Neuroscience and Biobehavioral Reviews, 34*(8), 1307–1350.

Levin, E. D., Bencan, Z., & Cerutti, D. T. (2007). Anxiolytic effects of nicotine in zebrafish. *Physiology and Behavior, 90*, 54–58.

Maemoto, T., Tada, M., Mihara, T., Ueyama, N., Matsuoka, H., Harada, K., et al. (2004). Pharmacological characterization of FR194921, a new potent, selective, and orally active antagonist for central adenosine A1 receptors. *Journal of Pharmacological Sciences, 96*(1), 42–52.

Maia, L., & De Mendonça, A. (2002). Does caffeine intake protect from Alzheimer's disease? *European Journal of Neurology, 9*, 377–382.

Melani, A., Cipriani, S., Corti, F., & Pedata, F. (2010). Effect of intravenous administration of dipyridamole in a rat model of chronic cerebral ischemia. *Annals of the New York Academy of Sciences, 1207*, 89–96.

Mihara, T., Mihara, K., Yarimizu, J., Mitani, Y., Matsuda, R., Yamamoto, H., et al. (2007). Pharmacological characterization of a novel, potent adenosine A1 and A2A receptor dual antagonist, 5-[5-amino-3-(4-fluorophenyl)pyrazin-2-yl]-1-isopropylpyridine-2(1H)-one (ASP5854), in models of Parkinson's disease and cognition. *The Journal of Pharmacology and Experimental Therapeutics, 323*(2), 708–719.

Miller, N., & Gerlai, R. (2007). Quantification of shoaling behavior in zebrafish (Danio rerio). *Behavioural Brain Research, 184*, 157–166.

Pitsikas, N., & Borsini, F. (1997). The adenosine A1 receptor antagonist BIIP 20 counteracts scopolamine-induced behavioral deficits in the passive avoidance task in the rat. *European Journal of Pharmacology, 328*, 19–22.

Prediger, R. D., & Takahashi, R. N. (2005). Modulation of short-term social memory in rats by adenosine A1 and A(2A) receptors. *Neuroscience Letters, 376*(3), 160–165.

Richetti, S. K., Blank, M., Capiotti, K. M., Piato, A. L., Bogo, M. R., Vianna, M. R., et al. (2011). Quercetin and rutin prevent scopolamine-induced memory impairment in zebrafish. *Behavioural Brain Research, 217*(1), 10–15.

Riedel, W., Hogervorst, E., Leboux, R., Verhey, F., van Praag, H., & Jolles, J. (1995). Caffeine attenuates scopolamine-induced memory impairment in humans. *Psychopharmacology (Berl), 122*(2), 158–168.

Riedel, G., Kang, S. H., Choi, D. Y., & Platt, B. (2009). Scopolamine-induced deficits in social memory in mice: Reversal by donepezil. *Behavioural Brain Research, 204*(1), 217–225.

Ritchie, K., Carrière, I., de Mendonca, A., Portet, F., Dartigues, J. F., Rouaud, O., et al. (2007). The neuroprotective effects of caffeine: A prospective population study (the Three City Study). *Neurology, 69*(6), 536–545.

Rosemberg, D. B., Rico, E. P., Guidoti, M. R., Dias, R. D., Souza, D. O., Bonan, C. D., et al. (2007). Adenosine deaminase-related genes: Molecular identification, tissue expression pattern and truncated alternative splice isoform in adult zebrafish (Danio rerio). *Life Sciences, 81*(21–22), 1526–1534.

Sallaberry, C., Nunes, F., Costa, M. S., Fioreze, G. T., Ardais, A. P., Botton, P. H., et al. (2013). Chronic caffeine prevents changes in inhibitory avoidance memory and hippocampal BDNF immunocontent in middle-aged rats. *Neuropharmacology, 64*, 153–159.

Sebastiao, A. M., Ribeiro, F. F., & Ribeiro, J. A. (2012). From A1 to A3 enpassant through A2A receptors in the hippocampus: Pharmacological implications. *CNS Neurological Disorders Drug Targets, 11*, 652–663.

Selkoe, D. J. (2001). Alzheimer's disease: Genes, proteins and therapy. *Physiological Reviews, 81*, 741–766.

Sison, M., & Gerlai, R. (2010). Associative learning in zebrafish (Danio rerio) in the plus maze. *Behavioural Brain Research, 207*(1), 99–104.

Small, D. H. (2005). Acetylcholinesterase inhibitors for the treatment of dementia in Alzheimer's disease: Do we need new inhibitors. *Expert Opinion on Emerging Drugs, 10*, 817–825.

Spinetta, M. J., Woodlee, M. T., Feinberg, L. M., Stroud, C., Schallert, K., Cormack, L. K., et al. (2008). Alcohol-induced retrograde memory impairment in rats: Prevention by caffeine. *Psychopharmacology (Berl), 201*(3), 361–371.

Stewart, A. M., Braubach, O., Spitsbergen, J., Gerlai, R., & Kalueff, A. V. (2014). Zebrafish models for translational neuroscience research: From tank to bedside. *Trends in Neuroscience, 37*(5), 264–278.

Takahashi, R. N., Pamplona, F. A., & Prediger, R. D. (2008). Adenosine receptor antagonists for cognitive dysfunction: A review of animal studies. *Frontiers in Bioscience, 13*, 2614–2632.

Todd, K. J., Slatter, C. A., & Ali, D. W. (2004). Activation of ionotropic glutamate receptors on peripheral axons of primary motoneurons mediates transmitter release at the zebrafish NMJ. *Journal of Neurophysiology, 91*(2), 828–840.

van Gelder, B., Buijsse, B., Tijhuis, M., Kalmijn, S., Giampaoli, S., Nissinen, A., et al. (2007). Coffee consumption is inversely associated with cognitive decline in elderly European men: The FINE Study. *European Journal of Clinical Nutrition, 61*(2), 226–232.

Wang, H. X., Karp, A., Winblad, B., & Fratiglioni, L. (2002). Late-life engagement in social and leisure activities is associated with a decreased risk of dementia: A longitudinal study from the Kungsholmen project. *American Journal of Epidemiology, 155*(12), 1081–1087.

Wang, J. H., Ma, Y. Y., & van den Buuse, M. (2006). Improved spatial recognition memory in mice lacking adenosine A2A receptors. *Experimental Neurology, 199*(2), 438–445.

Winslow, J. T., & Camacho, F. (1995). Cholinergic modulation of a decrement in social investigation following repeated contacts between mice. *Psychopharmacology (Berl), 121*(2), 164–172.

Zhang, D. S., & Ren, L. M. (2003). Effect of adenosine A1 receptor antagonist on learning and memory and analysis of its mechanism. *Yao Xue Xue Bao, 38*, 416–419.