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Research Paper

Aripiprazole and environmental enrichment independently improve functional outcome after cortical impact injury in adult male rats, but their combination does not yield additional benefits

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ABSTRACT

Typical antipsychotic drugs (APDs) with *D_2antagonistic* properties impede functional outcome after experimental traumatic brain injury (TBI) and reduce the effectiveness of environmental enrichment (EE). Here we test the hypothesis that aripiprazole (ARIP), an atypical APD with partial *$D_2$and 5-HT_{1A}receptor agonist* activities will improve recovery after TBI and when combined with EE will further enhance the benefits. Anesthetized adult male rats received either a controlled cortical impact of moderate severity or sham injury and then were randomly assigned to EE or standard (STD) housing and once daily intraperitoneal injections of ARIP (0.1 mg/kg) or vehicle (VEH; 1.0 mL/kg) beginning 24 h after injury for 19 days. Motor (beam-walking time and beam-walk score) and cognitive (acquisition of spatial learning and memory) outcomes were assessed on post-operative days 1–5 and 14–19, respectively. Cortical lesion volume was quantified on day 21. There were no statistical differences among the sham groups, regardless of housing or treatment, so the data were pooled. The SHAM group performed better than all TBI groups on motor and spatial learning ($p < 0.05$) but did not differ from either EE group on memory retention. Regarding TBI, both EE groups improved motor and cognitive outcomes vs. the VEH-treated STD group ($p < 0.05$) but did not differ from one another ($p > 0.05$). The ARIP-treated STD group performed better than the VEH-treated STD group on beam-walk score and spatial learning ($p < 0.05$), but not beam-walking time or memory retention ($p > 0.05$). Cortical lesion volume was smaller in all treated groups compared to the TBI + STD + VEH group ($p < 0.05$). The data replicate previous work and extend the findings by demonstrating that 1) ARIP promotes recovery after TBI, but combining treatments does not yield additional benefits, which is contrary to the hypothesis, and 2) unlike APDs that exhibit D_2 receptor antagonism, ARIP does not impede rehabilitation (i.e., EE).

1. Introduction

Disruptive behaviors like agitation and aggression are common after clinical TBI (Levin and Grossman, 1978; Wolf et al., 1996; Nott et al., 2010; Ciurli et al., 2011) and are typically managed with antipsychotic drugs (APDs) (Stanislav, 1997; Lombard and Zafonte, 2005; Elovic et al., 2008; Chew and Zafonte, 2009; McNett et al., 2012). However, several preclinical studies have confirmed that daily administration of the APD haloperidol (HAL), a D_2 receptor antagonist, impedes motor and cognitive recovery after TBI in both sexes (Feeney et al., 1982;

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Goldstein and Bullman, 2002; Wilson et al., 2003; Kline et al., 2007, 2008; Hoffman et al., 2008; Free et al., 2017; Folweiler et al., 2017). Moreover, the inhibition of recovery persists for months after drug withdrawal (Phelps et al., 2015). In contrast, aripiprazole (ARIP), a partial D_2 and 5-HT_{1A} receptor agonist, has been shown to enhance cognition when provided daily after a controlled cortical impact (CCI) injury of moderate severity (Phelps et al., 2017). This finding lends support for ARIP as a safer APD for alleviating behavioral disturbances after TBI.

Given the reported improvement in cognition with daily doses of ARIP (Phelps et al., 2017), it may be an ideal treatment candidate during neurorehabilitation when the opportunities for maladaptive behaviors are more likely given the increased length of time in this setting versus acute care. However, it is yet to be evaluated whether combining ARIP with environmental enrichment (EE), a preclinical model of neurorehabilitation, promotes greater recovery or reduces the efficacy of EE. This line of investigation is important as a previous study evaluating the effects of once daily HAL provided concurrently with EE revealed that the combination paradigm produced a dual effect; EE reduced the deleterious effects of HAL, but HAL in turn attenuated the effectiveness of EE (Folweiler et al., 2017).

Hence, the goal of this study was to assess motor and cognitive recovery after TBI in adult male rats administered ARIP or VEH once daily while housed in STD or enriched conditions. The hypothesis was that ARIP would enhance recovery in STD-housed controls and the benefit would be more robust when combined with EE. Furthermore, EE alone would enhance functional outcome beyond that of STD controls as previously demonstrated (for comprehensive reviews, see Bondi et al., 2014b and 2015).

2. Materials and methods

2.1. Subjects

Fifty-two adult (3 months old) male Sprague-Dawley rats (Envigo RMS, Inc., Indianapolis, IN) weighing 300–325 g on the day of surgery were paired housed in ventilated polycarbonate rat cages and maintained in a temperature (21 ± 1 °C) and light (on 7:00 a.m. to 7:00 p.m.) controlled environment with food and water available ad libitum. After one week of acclimatization, all rats underwent a single day of beam-walk training, which consisted of 3–5 successive approximation trials to traverse the beam in under 5 s with a score of 7. All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh. Every attempt was made to limit the number of rats used and to minimize suffering.

2.2. Surgery

Controlled cortical impact (CCI) injury was produced as previously described (Kline et al., 2002a,b, 2010, 2012; Bondi et al., 2014a; Radabaugh et al., 2017). Briefly, surgical anesthesia was induced and maintained with 4% and 2% concentrations of isoflurane, respectively, in 2:1 N_2O:O_2. After endotracheal intubation the rats were secured in a stereotaxic frame and ventilated mechanically. Core temperature was monitored and maintained at 37 ± 0.5 °C with a rectal thermistor and heating pad. Employing aseptic procedures, a midline scalp incision was made, the skin and fascia were reflected, and a craniectomy (6 mm in diameter) was made in the right hemisphere with a hand-held trephine. The bone flap was removed and the craniectomy was enlarged further to fit the impact tip (6 mm, flat), which was centered and lowered through the craniectomy until it touched the dura mater. Once confirmed that the impact tip was touching the dura, the rod was retracted, and the impact tip was advanced 2.8 mm farther to produce a brain injury of moderate severity (2.8 mm tissue deformation at 4 m/s). Anesthesia was discontinued immediately after the impact and the incision was promptly closed. The rats were subsequently extubated and assessed for acute neurological outcome. Except for the impact, sham rats underwent all surgical procedures.

2.3. Acute neurological evaluation

Hind limb reflexive ability was assessed immediately after the termination of anesthesia by squeezing the rats' paw every 5 s and recording the time to elicit a withdrawal response. Return of the righting reflex was determined by the time required to turn from the supine to prone position on three consecutive trials.

2.4. Drug administration

After surgery and acute neurological evaluation, the rats were randomly assigned to the following groups: TBI + STD + ARIP (0.1 mg/kg; $n = 10$), TBI + STD + VEH (1 mL/kg; n = 10), TBI + EE + ARIP (0.1 mg/kg; n = 10), TBI + EE + VEH (1 mL/kg; n = 10), and respective sham controls ($n = 3$ per group). ARIP (Toronto Research Chemicals, Toronto, Canada), was prepared daily by dissolving in dimethyl sulfoxide and saline (1:1) which also served as the vehicle (VEH). The dose of ARIP was chosen based on a dose response study from our group showing it to be the most effective (Phelps et al., 2017). Treatments began 24 h after CCI injury or sham surgery and were provided intraperitoneally once daily (*after* the daily behavioral assessments to circumvent sedative effects that may confound the results) for 19 days.

2.5. Motor performance: beam-walk time and score

Motor function was assessed using a beam-walk task originally characterized by Feeney et al. (1982). Briefly, the rats were trained to traverse a long, narrow, elevated (100 cm, 2.5 cm, 90 cm, respectively) wooden beam to enter a darkened goal box to escape aversive stimuli, which consisted of white noise and a bright light directed at the start point. Performance on this beam-walk task is measured as time to traverse the entire length of the beam and quality of ambulation, which is quantified using a rating scale from 1 to 7, where a score of 1 reflects an inability of the rat to pull itself up onto the beam, 2 indicates an inability to ambulate across the beam, 3 refers to ambulation without placing the injured hind limb on the beam, 4 reflects ambulation with the injured hind limb on the beam but not aiding in forward locomotion, 5 indicates ambulation with the injured hind limb aiding in forward locomotion but slipping off the beam > 50% of the steps, 6 reflects ambulation with the injured hind limb slipping < 50% of the steps but more than twice, and 7 represents ambulation with the injured hind limb slipping two or fewer times. Rats were trained for two days prior to TBI or sham injury to traverse the beam in under 6 s and earn a score of 7. Rats making no attempt to traverse the beam were encouraged by prodding, which consisted of a gentle tap on the tail or rump with a pencil (Feeney et al., 1982; Kline et al., 1994). Baseline performance was acquired on the day of surgery. Post-surgical performance was assessed on days 1–5 and consisted of a single trial (60 s allotted time). The average daily scores for each subject were used in the statistical analyses.

2.6. Cognitive performance: spatial learning

Spatial learning was assessed using a well-established Morris water maze (MWM) task (Morris, 1984; Kline et al., 2002a,b, 2010, 2012; Olsen et al., 2012; Folweiler et al., 2017; Okigbo et al., 2019). Briefly, the maze consisted of a plastic pool (180 cm diameter; 60 cm high) filled with tap water (26 ± 1 °C) to a depth of 28 cm and was positioned in a room with prominent extra-maze cues. The platform was a clear Plexiglas stand (10 cm diameter, 26 cm high) that was positioned 26 cm from the maze wall in the southwest quadrant and held constant throughout the experiment. Acquisition of spatial learning began on

post-operative day 14 and consisted of providing a block of four daily trials for five consecutive days (14–18) to locate the escape platform when it was submerged 2 cm below the water surface. On day 19, the platform was raised 2 cm above the water surface to evaluate visible platform performance, which is incorporated as a control procedure to determine the contributions of non-spatial factors (e.g., sensory-motor function, motivation, and visual acuity) on cognitive performance. For each daily block of trials, the rats were placed in the pool facing the wall at each of the four possible start locations (north, east, south, and west) in a quasi-randomized manner. Each trial lasted until the rat climbed onto the platform or until 120 s had elapsed, whichever occurred first. The rats that failed to locate the escape platform within the allotted time were manually guided to it. All rats remained on the platform for 30 s before being placed in a heated incubator between trials (4-min inter-trial interval). The times of the 4 daily trials for each rat were averaged and used in the statistical analyses.

2.7. Cognitive performance: memory retention

One day after the final acquisition training (day 19), but before the visible platform test, all rats were given a single probe trial to measure memory retention. Briefly, the platform was removed from the pool and the rats were placed in the pool from the location point most distal to the quadrant where the platform was previously located (i.e., "target quadrant") and allowed to freely explore the pool for 30 s. The time spent in the target quadrant was recorded and used in the statistical analyses. The data were obtained using ANY-maze video tracking software.

2.8. Histology: cortical lesion volume

Twenty-one days after surgery the rats were anesthetized with Fatal-Plus® (0.3 mL, i.p.) and perfused transcardially with 200 mL 0.1 M phosphate buffered saline (pH 7.4) followed by 300 mL 4% paraformaldehyde. The heads were immersed in the perfusate and one week later the brains were extracted, post-fixed further, dehydrated with alcohols, and embedded in paraffin. Coronal sections (7 μm thick) were cut at 1-mm intervals through the lesion on a rotary microtome and mounted on Superfrost®/Plus glass microscope slides. After drying, the sections were deparaffinized in xylenes, rehydrated, and stained with Cresyl violet. An observer blinded to experimental conditions analyzed cortical lesion volumes (mm^3) by calculating the area of the lesion (mm^2), which was done by outlining the inferred area of missing cortical tissue for each section (typically 5–7) taken at 1-mm intervals as previously reported (Olsen et al., 2012; Monaco et al., 2013, 2014).

2.9. Statistical analyses

All analyses were performed using Statview 5.0.1 software (Abacus Concepts, Inc., Berkeley, CA) on data collected by blinded experimenters. The motor and cognitive analyses were conducted using repeated-measures analysis of variance (ANOVA). The acute neurological data (i.e., hind limb withdrawal reflex and righting reflex) as well as the visible platform, probe trial, swim speed, and cortical lesion volume data were analyzed using one-factor ANOVAs. When significance was revealed with the overall ANOVA, the Newman-Keuls post-hoc test was used to determine specific group differences. The results are expressed as the mean ± standard error of the mean (S.E.M.) and were considered significant when $p \leq 0.05$.

3. Results

There were no surgical or health issues (e.g., consistent weight loss or visual deficits as indicated by all rats being able to find the visible platform) throughout the study and thus the statistical analyses included the data from all 52 rats. No differences were observed in any behavioral measure among the sham controls (p's > 0.05), so their data were pooled into one group designated as SHAM.

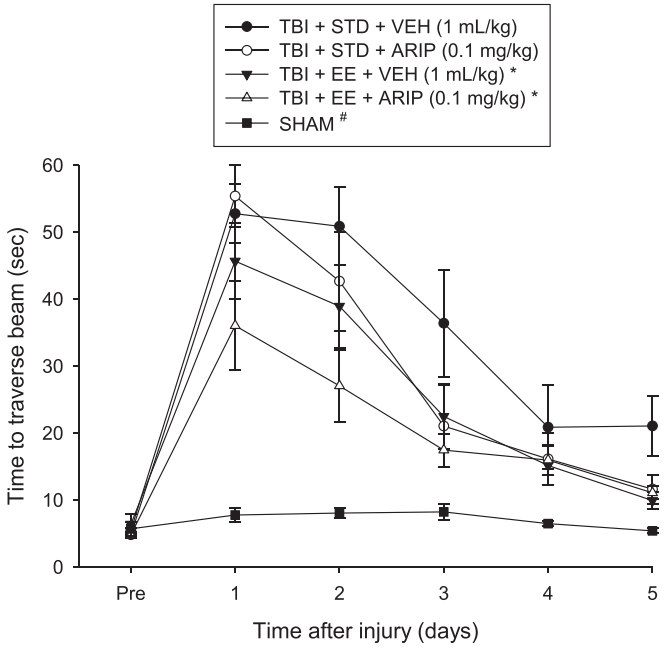

Fig. 1. Mean (± S.E.M.) time (*sec*) to traverse an elevated narrow beam prior to, and after, TBI or SHAM injury. No differences were observed among the groups prior to surgery ($p > 0.05$). After TBI, all groups differed from the SHAM controls ($^{\#}p < 0.05$). $^{*}p < 0.05$ vs. TBI + STD + VEH. No other comparisons were significant ($p > 0.05$). $n = 10$ for each TBI group and 12 for the SHAM controls.

3.1. Acute neurological evaluation

No differences were observed among the TBI groups in hind limb withdrawal reflex after a brief paw pinch [left range = 148.9 ± 4.8 s to 162.9 ± 5.7 s, $p > 0.05$; right range = 144.7 ± 4.8 s to 158.4 ± 6.0 s, $p > 0.05$] or for righting reflex [range 326.1 ± 14.6 s to 369.8 ± 23.7 s, $p > 0.05$] following the cessation of anesthesia. The lack of significant differences with these acute neurological indices suggests that all TBI groups experienced equivalent levels of injury and anesthesia. SHAM rats had reflexes that were significantly quicker than TBI rats: paw pinch [left = 19.1 ± 1.1 s; right = 14.4 ± 1.2 s] and righting reflex [126.5 ± 5.6 s].

3.2. Motor performance: beam-walk time and score

Prior to surgery, each rat traversed the beam and entered the goal box in under 6 s (Fig. 1) with no > 2 slips of the affected hind limb (Fig. 2). After CCI injury, there was a significant delay in time to traverse the beam as indicated by the ANOVA, which revealed significant Group [$F_{4,47} = 14.728$, $p < 0.0001$] and Day [$F_{5,235} = 74.830$, $p < 0.0001$] differences, as well as a significant Group x Day interaction [$F_{20,235} = 5.924$, $p < 0.0001$]. There was also a corresponding increase in affected hind limb foot slips as the ANOVA again revealed significant Group [$F_{4,47} = 34.564$, $p < 0.0001$] and Day [$F_{5,235} = 93.205$, $p < 0.0001$] differences, as well as a significant Group x Day interaction [$F_{20,235} = 6.885$, $p < 0.0001$]. According to the post-hoc analysis, all TBI groups, regardless of housing or treatment, performed significantly worse than the SHAM controls on time to traverse the beam and beam score [$p < 0.05$; Figs. 1 and 2, respectively]. The TBI + EE + VEH and TBI + EE + ARIP groups did not differ from one another on either motor assessment [$p > 0.05$], but both performed better on time to traverse the beam and foot slips

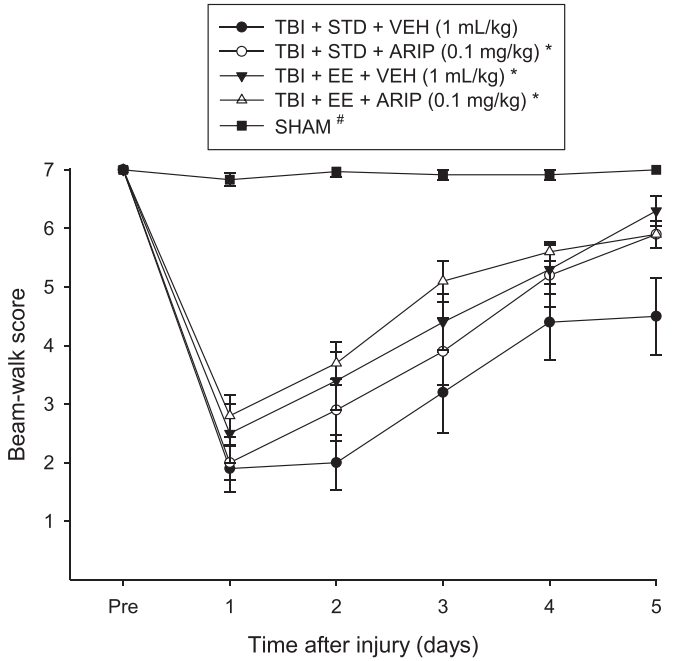

Fig. 2. Mean (± S.E.M.) beam-walk rating score while traversing an elevated narrow beam prior to, and after, TBI or SHAM injury. No differences were observed among the groups prior to surgery ($p > 0.05$). After TBI, all groups differed from the SHAM controls ($^{\#}p < 0.05$). $^{*}p < 0.05$ vs. TBI + STD + VEH. No other comparisons were significant ($p > 0.05$). n = 10 for each TBI group and 12 for the SHAM controls.

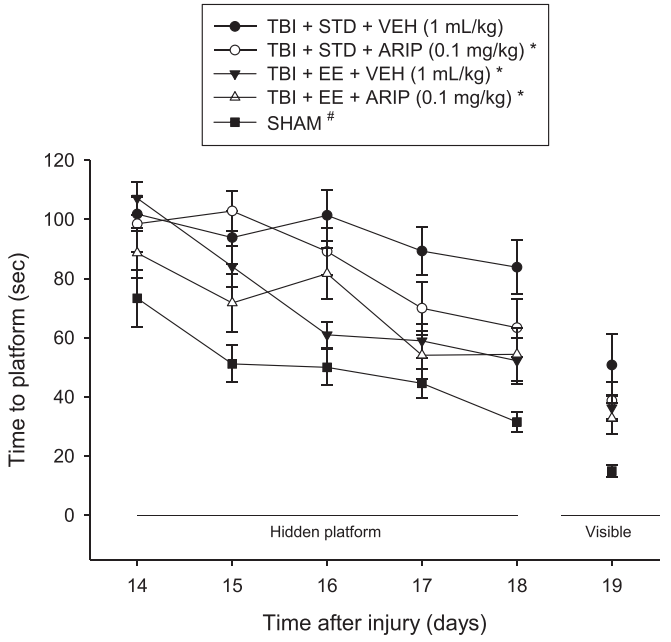

Fig. 3. Mean (± S.E.M.) time (sec) to locate a hidden platform in the MWM. * $p < 0.05$ vs. TBI + STD + VEH. $^{\#}p < 0.05$ vs. all TBI groups, regardless of treatments. The SHAM group located the visible platform faster than all TBI groups ($p < 0.05$). There were no differences in visible platform performance among the TBI groups ($p > 0.05$). n = 10 for each TBI group and 12 for the SHAM controls.

relative to the TBI + STD + VEH group [$p < 0.05$]. The TBI + STD + ARIP group exhibited a better beam-walk score relative to the TBI + STD + VEH group [$p < 0.05$].

3.3. Cognitive performance: acquisition of spatial learning, visible platform, and swim speed

The ANOVA for spatial learning revealed significant Group [$F_{4,47} = 8.532$, $p < 0.0001$] and Day [$F_{4,188} = 15.802$, $p < 0.0001$] differences, as well as a significant Group x Day interaction [$F_{16,188} = 1.897$, $p = 0.0229$]. The post-hoc analysis showed that all TBI groups required more time to find the escape platform relative to the SHAM controls [$p < 0.05$; Fig. 3]. The TBI + EE + VEH, TBI + EE + ARIP, and TBI + STD + ARIP groups performed better than the TBI + STD + VEH group [$p < 0.05$] and did not differ from one another [$p > 0.05$]. There was no difference among the TBI groups in the time to locate the visible platform [$p > 0.05$], but they all required more time than the SHAM group [$p < 0.05$]. Swim speed did not differ among the groups (range = 26.1 ± 1.8 cm/s to 30.5 ± 1.2 cm/s; $p > 0.05$).

3.4. Cognitive performance: memory retention

Analysis of the probe data revealed a significant difference among the groups [$F_{4,47} = 5.643$, $p = 0.0009$]. The post-hoc analysis revealed that memory retention, as demonstrated by a greater percentage of the 30 s allotted time spent in the target quadrant, was improved in the TBI + EE + VEH and TBI + EE + ARIP relative to the TBI + STD + VEH group (33.6 ± 1.9%, 34.7 ± 1.5%, and 26.0 ± 2.4%, respectively [$p < 0.05$]. The SHAM group (39.3 ± 1.1%) did not differ from the two EE groups, but was better than the TBI + STD + VEH and TBI + STD + ARIP (29.4 ± 3.5%) groups (Fig. 4). No other comparisons were significant.

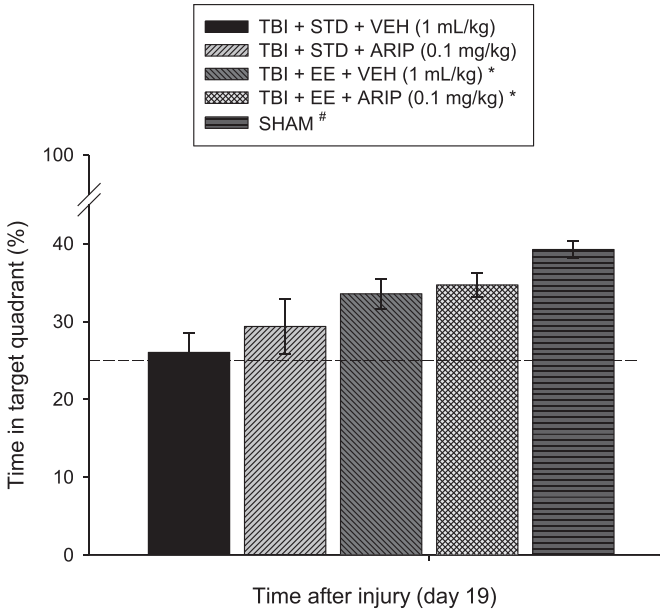

Fig. 4. Mean (± S.E.M.) percent time spent in the target quadrant. The bar graph shows the % time that each group spent in the target quadrant and the horizontal dashed line depicts exploration at chance (25%) level. $^{*,\#}p < 0.05$ vs. TBI + STD + VEH and TBI + STD + ARIP. No differences were observed among the SHAM, TBI + EE + VEH, and TBI + EE + ARIP groups ($p > 0.05$). n = 10 for each TBI group and 12 for the SHAM controls.

3.5. Histology: cortical lesion volume

Analysis of the cortical lesion volume data revealed a significant Group effect [$F_{3,16} = 5.890$, $p = 0.007$]. Mean cortical lesion volumes of the TBI + STD + ARIP, TBI + EE + VEH, TBI + EE + ARIP groups were 36.6 ± 2.5 mm^3, 34.3 ± 1.2 mm^3, and 33.2 ± 0.6 mm^3, which

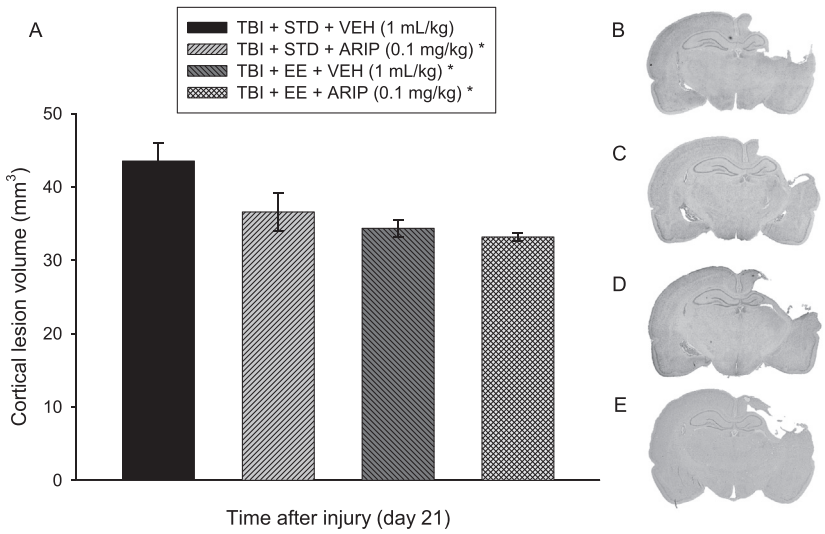

Fig. 5. A Mean (± S.E.M.) cortical lesion volume (mm^3) 3 weeks after cortical impact injury. *$p < 0.05$ vs. TBI + STD + VEH. No other comparisons were significant ($p > 0.05$). B–E depict average size lesions, at the level of the dorsal hippocampus for TBI + STD + VEH, TBI + STD + ARIP, TBI + EE + VEH, and TBI + EE + ARIP, respectively. $n = 5$ for each TBI group.

did not differ from one another [$p > 0.05$], but were significantly smaller than the 43.5 ± 2.5 mm^3 of the TBI + STD + VEH group [$p < 0.05$; Fig. 5].

4. Discussion

Preclinical models of TBI have been useful for gaining insight into the pathophysiology of brain trauma as well as elucidating numerous therapeutic approaches to enhance functional outcome (Kochanek et al., 2011; Bondi et al., 2015; Kline et al., 2016; Semple et al., 2016; Archer et al., 2018; O'Neil et al., 2018). They have also revealed that some pharmacological agents used initially for secondary effects of TBI, such as agitation, can be deleterious to the recovery process (Feeney et al., 1982; Goldstein and Bullman, 2002; Wilson et al., 2003; Kline et al., 2007, 2008; Hoffman et al., 2008; Phelps et al., 2015; Free et al., 2017; Folweiler et al., 2017). Once such class of drugs are the antipsychotics, specifically those with D_2 receptor antagonist properties, such as HAL and risperidone (Kline et al., 2007, 2008; Hoffman et al., 2008; Phelps et al., 2015). However, despite their negative effects on cognitive outcome after TBI, APDs are needed and are frequently used to control agitation, and to a lesser extent aggression (Stanislav, 1997; Lombard and Zafonte, 2005; Elovic et al., 2008; Chew and Zafonte, 2009; McNett et al., 2012).

The aim of the current study was to evaluate the effects of ARIP, an atypical APD, on motor and cognitive recovery, as well as histological protection, after CCI injury of moderate severity in adult male rats. ARIP, unlike HAL, has no D_2 antagonist effects, but instead functions as a partial D_2 and 5-HT_{1A} receptor agonist (Burris et al., 2002; Wood and Reavill, 2007; Tanahashi et al., 2012). The hypothesis was that ARIP would confer cognitive benefits when provided alone, as previously shown (Phelps et al., 2017), but the benefits would be further enhanced when combined with EE, a preclinical model of neurorehabilitation (Kline et al., 2010, 2012; Bondi et al., 2014b, 2015; Radabaugh et al., 2017).

Motor performance was significantly improved in all treatment groups. Specifically, beam traversal time was reduced in both EE groups (VEH and ARIP) relative to the STD-housed VEH controls. In the more sensitive beam-walk test, where the accuracy of limb placement is quantified and scored according to the well-established method of Feeney et al. (1982), both EE groups and the STD-housed ARIP group performed better than the STD-housed VEH group and did not differ from one another. The three treatment groups also hastened acquisition of spatial learning in the MWM in comparison to the VEH-treated STD-housed group, and again did not differ from one another. In the memory retention test, only the EE groups (VEH and ARIP) performed at the level of the SHAM controls. The three treatment groups also exhibited significantly smaller cortical lesion volumes compared to the STD-housed VEH controls and did not differ from one another. The benefits observed with ARIP alone replicate a previous report (Phelps et al., 2017). The lack of differences between the ARIP + STD and ARIP + EE groups indicates that no additive effect was achieved, which is contrary to the hypothesis. Nonetheless, the data show that ARIP does not inhibit spontaneous recovery or reduce the effectiveness of EE, but instead improved recovery to the same extent as neurorehabilitation (i.e., EE).

The rationale for combining ARIP and EE was based on the growing thought that polytherapy may be more efficacious and perhaps more translatable than single therapies after TBI. Additionally, a more robust effect was observed in motor speed and cognition when ARIP was provided to patients with schizoaffective disorder undergoing rehabilitation (Matsuda et al., 2014). Work from our laboratory has shown that combining the therapies 8-OH-DPAT and EE provides additive protection against the CCI-induced loss of choline acetyltransferase (ChAT) positive cells (Kline et al., 2010), while the combination of buspirone and EE enhances cognitive performance after pediatric TBI (Monaco et al., 2014). Additive effects with other combined therapies have also been reported (for comprehensive review, see Kline et al., 2016). However, no additive benefits of ARIP and EE were observed in the current study. It has been speculated in the past that the robust benefits consistently achieved with continuous EE provides a floor effect that limits recovery beyond a certain threshold. To remedy this potential discrepancy and to optimize EE as a viable preclinical model of neurorehabilitation, we have begun to delay the administration and limit the amount of EE provided to rats after TBI (Matter et al., 2011; de Witt et al., 2011; Radabaugh et al., 2016, 2017; Lajud et al., 2018). An interesting and informative future direction would be to evaluate ARIP combined with the optimized (i.e., more clinically relevant) EE paradigm to determine if an additive effect may emerge.

As mentioned, ARIP exerts numerous physiological effects through its actions as partial 5-HT_{1A} and D_2 receptor agonists. These actions can, in part, serve as potential mechanisms by which ARIP facilitated functional recovery post-TBI. Previous studies have demonstrated that 8-OH-DPAT, a 5-HT_{1A} receptor agonist, conferred neuroprotection and enhanced functional recovery when administered acutely or chronically after experimental brain trauma (for comprehensive review see Cheng et al., 2016). Additionally, beneficial cognitive and histological effects were observed following the administration of buspirone, another drug in this class (Kline et al., 2012; Olsen et al., 2012; Monaco et al., 2014).

5-HT_{1A} receptors are G-protein coupled receptors that exert several physiological effects through the Gi/o inhibition of adenylyl cyclase and are found in the neuronal populations of numerous brain regions including the hippocampus, amygdala, septum, entorhinal cortex, and hypothalamus as well as in many glial cells (Drago et al., 2008; Schatzberg and Nemeroff, 2009; Newman-Tancredi, 2011).

One effect of note is an alteration in cholinergic signaling following the activation of 5-HT_{1A} receptors. Given the secondary messenger cascades affected as well as the brain areas in question, it is rather intuitive to rely on the known effects of cholinergic transmission on cognition often discussed in neurodegenerative conditions such as Alzheimer's Disease. From studies in this neurodegenerative field, it is well understood that acetylcholine is intimately linked to learning and memory (Ahmed et al., 2017). Moreover, ChAT expression is significantly decreased after CCI injury of moderate severity (Kline et al., 2010) and treatment with 8-OH-DPAT has been reported to attenuate the TBI-induced loss of medial septal ChAT positive cells and improve cognitive performance (Kline et al., 2010). As a partial 5-HT_{1A} receptor agonist, ARIP may produce similar effects and thus protection of cholinergic integrity could be a possible mechanism for the findings.

The partial D_2 receptor agonist properties of ARIP could also be a contributing factor in behavioral and histological benefits reported in this study. Dysregulation of the DAergic system has long been implicated as a major component of many neurological disorders. A hypofunctional DAergic system after TBI could be normalized with ARIP as it has been reported to increase DA release in the prefrontal cortex and hippocampus of mice (Zocchi et al., 2005) and rats (Li et al., 2004). Several studies have reported fluctuations in DAergic signaling after brain trauma that may manifest in oxidative stress and cellular dysfunction as well as inflammation (Chen et al., 2017). The D_2 receptor agonist bromocriptine promotes cognitive recovery and reduces a marker of oxidative stress (Kline et al., 2002a, 2004). Other pharmacotherapies with D_2 receptor agonist properties such as methylphenidate (Kline et al., 2002b; Leary et al., 2017) and amantadine (Dixon et al., 1999; Okigbo et al., 2019) have also been shown to improve functional outcome after CCI injury. Although partial, the agonist properties provided by ARIP could help stabilize DAergic transmission in a system experiencing fluctuations of the endogenous agonist and lead to an amelioration of deficits.

Albeit ARIP is an APD, the current findings show that unlike those that exhibit D_2 receptor antagonism, ARIP does not impede rehabilitation (i.e., EE) and lend support for extending its use well beyond its capacity as an APD to function as a potentially efficacious therapeutic strategy for TBI-induced deficits. Cautious optimism for ARIP to improve impairments after clinical TBI is revealed in a case report where a patient who was not benefiting from various other pharmacotherapies saw improved verbal function when administered ARIP (Umene-Nakano et al., 2013).

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References

Ahmed, T., Zahid, S., Mahboob, A., Farhat, S.M., 2017. Cholinergic system and post-translational modifications: an insight on the role in Alzheimer's disease. Curr. Neuropharmacol. 15, 480–494.

Archer, D.P., McCann, S.K., Walker, A.M., Premji, Z.A., Rogan, K.J., Hutton, M.J.H., Gray, L.J., 2018. Neuroprotection by anaesthetics in rodent models of traumatic brain injury: a systematic review and network meta-analysis. Br. J. Anaesth. 121, 1272–1281.

Bondi, C.O., Cheng, J.P., Tennant, H.M., Monaco, C.M., Kline, A.E., 2014a. Old dog, new tricks: the attentional set-shifting test as a novel cognitive behavioral task after controlled cortical impact injury. J. Neurotrauma 31, 926–937.

Bondi, C.O., Klitsch, K.C., Leary, J.B., Kline, A.E., 2014b. Environmental enrichment as a viable neurorehabilitation strategy for experimental traumatic brain injury. J. Neurotrauma 31, 873–888.

Bondi, C.O., Semple, B.D., Noble-Haeusslein, L.J., Osier, N.D., Carlson, S.W., Dixon, C.E., Giza, C.C., Kline, A.E., 2015. Found in translation: understanding the biology and behavior of experimental traumatic brain injury. Neurosci. Biobehav. Rev. 58, 123–146.

Burris, K.D., Molski, T.F., Xu, C., Ryan, E., Tottori, K., Kikuchi, T., Yocca, F.D., Molinoff, P.B., 2002. Aripiprazole, a novel antipsychotic, is a high-affinity partial agonist at human dopamine D_2 receptors. J. Pharmacol. Exp. Ther. 302, 381–389.

Chen, Y.H., Huang, E.Y., Kuo, T.T., Miller, J., Chiang, Y.H., Hoffer, B.J., 2017. Impact of traumatic brain injury on dopaminergic transmission. Cell Transplant. 26, 1156–1168.

Cheng, J.P., Leary, J.B., Sembhi, A., Edwards, C.M., Bondi, C.O., Kline, A.E., 2016. 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptor agonists: a decade of empirical evidence supports their use as an efficacious therapeutic strategy for brain trauma. Brain Res. 1640, 5–14.

Chew, E., Zafonte, R.D., 2009. Pharmacological management of neurobehavioral disorders following traumatic brain injury–a state-of-the-art review. J. Rehabil. Res. Dev. 46, 851–879.

Ciurli, P., Formisano, R., Bivona, U., Cantagallo, A., Angelelli, P., 2011. Neuropsychiatric disorders in persons with severe traumatic brain injury: prevalence, phenomenology, and relationship with demographic, clinical, and functional features. J. Head Trauma Rehabil. 26, 116–126.

de Witt, B.W., Ehrenberg, K.M., McAloon, R.L., Panos, A.H., Shaw, K.E., Raghavan, P.V., Skidmore, E.R., Kline, A.E., 2011. Abbreviated environmental enrichment enhances neurobehavioral recovery comparably to continuous exposure after traumatic brain injury. Neurorehabil. Neural Repair 25, 343–350.

Dixon, C.E., Kraus, M.F., Kline, A.E., Ma, X., Yan, H.Q., Griffith, R.G., Wolfson, B.M., Marion, D.W., 1999. Amantadine improves water maze performance without affecting motor behavior following traumatic brain injury in rats. Restor. Neurol. Neurosci. 14, 285–294.

Drago, A., Ronchi, D.D., Serretti, A., 2008. 5-HT_{1A} gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. Int. J. Neuropsychopharmacol. 11, 701–721.

Elovic, E.P., Jasey, N.N., Eisenberg, M.E., 2008. The use of atypical antipsychotics after traumatic brain injury. J. Head Trauma Rehabil. 23, 132–135.

Feeney, D.M., Gonzalez, A., Law, W.A., 1982. Amphetamine, haloperidol, and experience interact to affect rate of recovery after motor cortex injury. Science 217, 855–857.

Folweiler, K.A., Bondi, C.O., Ogunsanya, E.A., LaPorte, M.J., Leary, J.B., Radabaugh, H.L., Monaco, C.M., Kline, A.E., 2017. Combining the antipsychotic drug haloperidol and environmental enrichment after traumatic brain injury is a double-edged sword. J. Neurotrauma 34, 451–458.

Free, K.E., Greene, A.M., Bondi, C.O., Lajud, N., de la Tremblaye, P.B., Kline, A.E., 2017. Comparable impediment of cognitive function in female and male rats subsequent to daily administration of haloperidol after traumatic brain injury. Exp. Neurol. 296, 62–68.

Goldstein, L.B., Bullman, S., 2002. Differential effects of haloperidol and clozapine on motor recovery after sensorimotor cortex injury in rats. Neurorehab. Neural Repair 16, 321–325.

Hoffman, A.N., Cheng, J.P., Zafonte, R.D., Kline, A.E., 2008. Administration of haloperidol and risperidone after neurobehavioral testing hinders the recovery of traumatic brain injury-induced deficits. Life Sci. 83, 602–607.

Kline, A.E., Chen, M.J., Tso-Olivas, D.Y., Feeney, D.M., 1994. Methylphenidate treatment following ablation-induced hemiplegia in rat: experience during drug action alters effects on recovery of function. Pharmacol. Biochem. Behav. 48, 773–779.

Kline, A.E., Massucci, J.L., Marion, D.W., Dixon, C.E., 2002a. Attenuation of working memory and spatial acquisition deficits after a delayed and chronic bromocriptine treatment regimen in rats subjected to traumatic brain injury by controlled cortical impact. J. Neurotrauma 19, 415–425.

Kline, A.E., Yan, H.Q., Bao, J., Marion, D.W., Dixon, C.E., 2002b. Chronic methylphenidate treatment enhances water maze performance following traumatic brain injury in rats. Neurosci. Lett. 280, 163–166.

Kline, A.E., Massucci, J.L., Ma, X., Zafonte, R.D., Dixon, C.E., 2004. Bromocriptine reduces lipid peroxidation and enhances spatial learning and hippocampal neuron survival in a rodent model of focal brain trauma. J. Neurotrauma 21, 1712–1722.

Kline, A.E., Massucci, J.L., Zafonte, R.D., Dixon, C.E., DeFeo, J.R., Rogers, E.H., 2007. Differential effects of single versus multiple administrations of haloperidol and risperidone on functional outcome after experimental brain trauma. Crit. Care Med. 35, 919–924.

Kline, A.E., Hoffman, A.N., Cheng, J.P., Zafonte, R.D., Massucci, J.L., 2008. Chronic administration of antipsychotics impede behavioral recovery after experimental traumatic brain injury. Neurosci. Lett. 448, 263–267.

Kline, A.E., McAloon, R.L., Henderson, K.A., Bansal, U.K., Ganti, B.M., Ahmed, R.H., Gibbs, R.B., Sozda, C.N., 2010. Evaluation of a combined therapeutic regimen of 8-OH-DPAT and environmental enrichment after experimental traumatic brain injury. J. Neurotrauma 27, 2021–2032.

Kline, A.E., Olsen, A.S., Sozda, C.N., Hoffman, A.N., Cheng, J.P., 2012. Evaluation of a combined treatment paradigm consisting of environmental enrichment and the 5-HT_{1A} receptor agonist buspirone after experimental traumatic brain injury. J. Neurotrauma 29, 1960–1969.

Kline, A.E., Leary, J.B., Radabaugh, H.L., Cheng, J.P., Bondi, C.O., 2016. Combination therapies for neurobehavioral and cognitive recovery after experimental traumatic brain injury: Is more better? Prog. Neurobiol. 142, 45–67.

Kochanek, P.M., Bramlett, H., Dietrich, W.D., Dixon, C.E., Hayes, R.L., Povlishock, J., Tortella, F.C., Wang, K.K., 2011. A novel multicenter preclinical drug screening and biomarker consortium for experimental traumatic brain injury: operation brain trauma therapy. J Trauma. 71 (1 Suppl) (S15-24).

Lajud, N., Díaz-Chávez, A., Radabaugh, H.L., Cheng, J.P., Rojo-Soto, G., Valdéz-Alarcón, J.J., Bondi, C.O., Kline, A.E., 2018. Delayed and abbreviated environmental enrichment after brain trauma promotes motor and cognitive recovery that is not contingent on increased neurogenesis. J. Neurotrauma. https://doi.org/10.1089/neu.2018.5866. [Epub ahead of print].

Leary, L.B., Bondi, C.O., LaPorte, M.J., Carlson, L.C., Cheng, J.P., Kline, A.E., 2017. The therapeutic efficacy of environmental enrichment and methylphenidate alone and in combination after controlled cortical impact injury. J. Neurotrauma 34, 444–450.

Levin, H.S., Grossman, R.G., 1978. Behavioral sequelae of closed head injury. A quantitative study. Arch. Neurol. 5, 720–727.

Li, Z., Ichikawa, J., Dai, J., Meltzer, H.Y., 2004. Aripiprazole, a novel antipsychotic drug, preferentially increases dopamine release in the prefrontal cortex and hippocampus in rat brain. Eur. J. Pharmacol. 493, 75–83.

Lombard, L.A., Zafonte, R.D., 2005. Agitation after traumatic brain injury: considerations and treatment options. Am. J. Phys. Med. Rehabil. 84, 797–812.

Matsuda, Y., Sato, S., Iwata, K., Furukawa, S., Hatsuse, N., Watanabe, Y., Anzai, N., Kishimoto, T., Ikebuchi, E., 2014. Effects of risperidone and aripiprazole on neurocognitive rehabilitation for schizophrenia. Psychiatry Clin. Neurosci. 68, 425–431.

Matter, A.M., Folweiler, K.A., Curatolo, L.M., Kline, A.E., 2011. Temporal effects of environmental enrichment-mediated functional improvement after experimental traumatic brain injury in rats. Neurorehabil. Neural Repair 25, 558–564.

McNett, M., Sarver, W., Wilczewski, P., 2012. The prevalence, treatment and outcomes of agitation among patients with brain injury admitted to acute care units. Brain Inj. 26, 1155–1162.

Monaco, C.M., Mattiola, V.V., Folweiler, K.A., Tay, J.K., Yelleswarapu, N.K., Curatolo, L.M., Matter, A.M., Cheng, J.P., Kline, A.E., 2013. Environmental enrichment promotes robust functional and histological benefits in female rats after controlled cortical impact injury. Exp. Neurol. 247, 410–418.

Monaco, C.M., Gebhardt, K.M., Chelbowski, S.M., Shaw, K.E., Cheng, J.P., Henchir, J.J., Zupa, M.F., Kline, A.E., 2014. A combined therapeutic regimen of buspirone and environmental enrichment is more efficacious than either alone in enhancing spatial learning in brain-injured pediatric rats. J. Neurotrauma 31, 1934–1941.

Morris, R., 1984. Development of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Meth. 11, 47–60.

Newman-Tancredi, A., 2011. Biased agonism at serotonin 5-HT_{1A} receptors: preferential postsynaptic activity for improved therapy of CNS disorders. Neuropsychiatry 1, 49–164.

Nott, M.T., Chapparo, C., Heard, R., Baguley, I.J., 2010. Patterns of agitated behaviour during acute brain injury rehabilitation. Brain Inj. 24, 1214–1221.

Okigbo, A.A., Helkowski, M.S., Royes, B.J., Bleimeister, I.H., Lam, T.R., Bao, G.C., Cheng, J.P., Bondi, C.O., Kline, A.E., 2019. Dose-dependent neurorestorative effects of amantadine after cortical impact injury. Neurosci. Lett. 694, 69–73.

Olsen, A.S., Sozda, C.N., Cheng, J.P., Hoffman, A.N., Kline, A.E., 2012. Traumatic brain injury-induced cognitive and histological deficits are attenuated by delayed and chronic treatment with the 5-HT_{1A} receptor agonist buspirone. J. Neurotrauma 29, 1898–1907.

O'Neil, D.A., Nicholas, M.A., Lajud, N., Kline, A.E., Bondi, C.O., 2018. Preclinical models of traumatic brain injury: emerging role of glutamate in the pathophysiology of depression. Front Pharmacol. 9, 579.

Phelps, T.I., Bondi, C.O., Ahmed, R.H., Olugbade, Y.T., Kline, A.E., 2015. Divergent long-term consequences of chronic treatment with haloperidol, risperidone, and bromocriptine on traumatic brain injury-induced cognitive deficits. J. Neurotrauma 32, 590–597.

Phelps, T.I., Bondi, C.O., Mattiola, V.V., Kline, A.E., 2017. Relative to typical antipsychotic drugs, aripiprazole is a safer alternative for alleviating behavioral disturbances after experimental brain trauma. Neurorehabil. Neural Repair. 31, 25–33.

Radabaugh, H.L., Carlson, L.J., O'Neil, D.A., LaPorte, M.J., Monaco, C.M., Cheng, J.P., de la Tremblaye, P.B., Lajud, N., Bondi, C.O., Kline, A.E., 2016. Abbreviated environmental enrichment confers neurobehavioral, cognitive, and histological benefits in brain-injured female rats. Exp. Neurol. 286, 61–68.

Radabaugh, H.L., LaPorte, M.J., Greene, A.M., Bondi, C.O., Lajud, N., Kline, A.E., 2017. Refining environmental enrichment to advance rehabilitation based research after experimental traumatic brain injury. Exp. Neurol. 294, 12–18.

Schatzberg, A.F., Nemeroff, C.B., 2009. The American Psychiatric Publishing Textbook of Psychopharmacology, 4th ed. American Psychiatric Publishing Inc.

Semple, B.D., Carlson, J., Noble-Haeusslein, L.J., 2016. Pediatric rodent models of traumatic brain injury. Methods Mol. Biol. 1462, 325–343.

Stanislav, S.W., 1997. Cognitive effects of antipsychotic agents in persons with traumatic brain injury. Brain Inj. 11, 335–341.

Tanahashi, S., Yamamura, S., Nakagawa, M., Motomura, E., Okada, M., 2012. Dopamine D_2 and serotonin 5-HT_{1A} receptors mediate the actions of aripiprazole in mesocortical and mesoaccumbens transmission. Neuropharmacology 62, 765–774.

Umene-Nakano, W., Yoshimura, R., Okamoto, T., Hori, H., Nakamura, J., 2013. Aripiprazole improves various cognitive and behavioral impairments after traumatic brain injury: a case report. Gen Hosp Psychiatry 35 (103.e7-9).

Wilson, M.S., Gibson, C.J., Hamm, R.J., 2003. Haloperidol, but not olanzapine, impairs cognitive performance after traumatic brain injury in rats. Am. J. Phys. Med. Rehabil. 82, 871–879.

Wolf, A.P., Gleckman, A.D., Cifu, D.X., Ginsburg, P.C., 1996. The prevalence of agitation and brain injury in skilled nursing facilities: a survey. Brain Inj. 10, 241–245.

Wood, M., Reavill, C., 2007. Aripiprazole acts as a selective dopamine D_2 receptor partial agonist. Expert Opin. Investig. Drugs 16, 771–775.

Zocchi, A., Fabbri, D., Heidbreder, C.A., 2005. Aripiprazole increases dopamine but not noradrenaline and serotonin levels in the mouse prefrontal cortex. Neurosci. Lett. 387, 157–161.