RESEARCH PAPER

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Vinpocetine ameliorates developmental hyperserotonemia induced behavioral and biochemical changes: role of neuronal function, inflammation, and oxidative stress

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Hyperserotonemia, during the early developmental phase, generates behavioral and biochemical phenotypes associated with autism spectrum disorder (ASD) in rats. Phosphodiesterase-1 (PDE1) inhibitors are known to provide benefits in various brain conditions. We investigated the role of a selective PDE1 inhibitor, vinpocetine on ASD-related behavioral phenotypes (social behavioral deficits, repetitive behavior, anxiety, and hyperlocomotion) in a developmental hyperserotonemia (DHS) rat model. Also, effects on biochemical markers related with neuronal function brain derived neurotrophic factor (BDNF) and phosphorylated cAMP response element binding protein (pCREB), inflammation interleukins (IL-6 and IL-10) and tumor necrosis factor–alpha (TNF-α), and oxidative stress (TBARS and GSH) were studied in important brain areas (frontal cortex, cerebellum, hippocampus, and striatum). Administration of 5-methoxytryptamine (5-MT) to rats prenatally (gestational day 12) and in early developmental stages postnatal day (PND 0 – PND 20), resulted in impaired behavior and brain biochemistry. Administration of vinpocetine daily (10 and 20 mg/kg) to 5-MT rats from PND 21 to PND 48 resulted in an improvement of behavioral deficits. Also, vinpocetine administration significantly increased the levels of BDNF, ratio of pCREB/CREB, IL-10, and GSH, and significantly decreased TNF-α, IL-6, and TBARS levels in different brain areas. Finally, our correlation analysis indicated that behavioral outcomes were significantly associated with the biochemical outcome. Vinpocetine, a selective PDE1 inhibitor, rectified important behavioral phenotypes related with ASD, possibly by improving markers of neuronal function, brain inflammation, and brain oxidative stress. Thus, PDE1 could be a potential target for pharmacological interventions and furthering our understanding of ASD pathogenesis.

Key words: autism spectrum disorder, phosphodiesterase-1, vinpocetine, brain inflammation, oxidative stress, developmental hyperserotonemia, BDNF, 5-methoxytryptamine

INTRODUCTION

Autism spectrum disorder (ASD) is not a single condition but a group of associated neurodevelopmental disorders. Characteristic features of ASD include dysfunctional social-emotional communication and social interaction, communication deficits, and occurrence of stereotypical or repetitive behavior. Several co-morbid traits, including anxiety, motor coordination/abnormalities and aggressive behavior also occur in affected individuals (Lai et al., 2014).

One finding consistent in ASD is the high serum serotonin levels and an aberrant serotonin metabolism, leading to development of ASD like phenotypes

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(McNamara et al., 2008; Yang et al., 2014; Muller et al., 2016). Serotonin is known to play a major role in neuronal development (Goeden et al., 2016) by initiating neuronal differentiation, neurogenesis and synaptogenesis (Kahne et al., 2002; Madden and Zup, 2014). Exposure to substances that increase levels of serotonin during the embryonic and early developmental phase is shown to introduce core ASD phenotypes such as presence of social behavioral deficits (Tanaka et al., 2018) and repetitive behavior (Veenstra-VanderWeele et al., 2012) in rodents. Thus, administering a non-specific serotonin receptor agonist 5-methoxytryptamine (5-MT) from gestational day (GD) 12 to postnatal day (PND) 21, during the peak serotonergic neuronal development has been shown to induce ASD related phenotypes (McNamara et al., 2008; Montgomery et al., 2018; Tanaka et al., 2018). Expression of co-morbid traits such as anxiety and hyperlocomotion are also well replicated by the DHS model (Kahne et al., 2002; McNamara et al., 2008; Altieri et al., 2015). Alterations in the levels of 5-HT during development is suggested to affect the levels of neuronal function markers such as brain-derived neurotrophic factor (BDNF) (Musumeci et al., 2015; Popova and Naumenko, 2019) and phosphorylated cyclic adenosine monophosphate response element binding protein (pCREB) (Zhang et al., 2016). Also, recent studies in other valproic acid (VPA) models of ASD from our own lab have illustrated the significance of oxidative stress in the outcome of ASD related treatments (Mirza and Sharma, 2018, 2019a).

Cyclic nucleotide phosphodiesterases (PDE) is a family comprised of 11 enzymes from PDE1 to PDE11, responsible for degradation of cyclic nucleotides viz., cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) (Francis et al., 2011). PDE1 is present heterogeneously in several brain areas (frontal cortex, striatum, cerebellum and hippocampus) (Yan et al., 1994; Medina, 2011). PDE inhibition is known to induce phosphorylation and activation of CREB, an important downstream component of the cAMP/pCREB pathway. Vinpocetine is a specific inhibitor of PDE1 enzyme (Loughney et al., 1996; Zhang et al., 2018) and has a well-established neuro- protective action (Nyakas et al., 2009). PDE1 inhibition has been shown to improve levels of pCREB in a model of fetal alcohol syndrome, resulting in a normal development of neurons (Krahe et al., 2009). Vinpocetine has also been associated with amelioration of brain inflammation, brain oxidative stress and neuronal dysfunction in rodent models of Huntington's disease and early ethanol exposure (Gupta and Sharma, 2014; Swart et al., 2017). Also, Vinpocetine has been shown to correct social behavior, repetitive behavior, anxiety, and hyperlocomotion in models of entorhinal cortex lesion, schizophrenia and fetal alcohol syndrome, respectively (Nyakas et al., 2009; Abreu-Villaça et al., 2018; Ahmed et al., 2018).

Thus, we hypothesize that the PDE1 inhibitor vinpocetine may play an important role in ameliorating DHS induced ASD-related behavioral and biochemical phenotypes. Here, 5-MT is being used as a tool to induce DHS in animals during the perinatal stages of brain development. Also, the deleterious effects of DHS on markers of neuronal function (BDNF and pCREB), brain inflammation (IL-6, IL-10 and TNF-α) and brain oxidative stress (TBARS and GSH) are reported for the first time in several brain areas. The correlation between behavioral outcomes and biochemical changes was also assessed using Pearson's correlations.

METHODS

Animals

Adult albino Wistar rats were housed in the animal house of Amity University (Reg No. 1327/PO/ReBi/S/10/CPCSEA) at a temperature of 25 ± 2°C with relative humidity of 50 ± 5%. The animals had free access to water and a standard laboratory pellet chow diet (Ashirwad Industries, Punjab, India). Animals were exposed to the natural light and dark cycle with 12 h of light (starting at 07:00 a.m. and ending at 07:00 p.m.) followed by 12 h of dark (starting at 07:00 p.m. and ending at 07:00 a.m.). All experiments were approved by the Institutional animal ethics committee of Amity University Uttar Pradesh, Noida, U.P, India (Approval number—CPCSEA/IAEC/AIP/2019/01/22).

Drugs

We administered 5-MT (TCI chemicals, India) at dose (1.0 mg/kg, s.c.). The dose of 5-MT selected is known to alter the in utero and post-natal development of the serotonin system in rats. Also, this dose is likely to produce the reported 50% increase in the serum levels of serotonin in animals (Hough and Segal, 2016; Kahne et al., 2002; Madden and Zup, 2014; McNamara et al., 2008; Whitaker-Azmitia, 2005).Vinpocetine (Rajasthan Antibiotics Ltd., India) was suspended in 0.5% w/v carboxy-methylcellulose (CMC) and was administered by oral gavage in two doses (10 mg/kg and 20 mg/kg). All doses have been selected on the basis of literature survey, indicating sufficient brain uptake and/or have been successfully studied in several other brain conditions in rodents (Abreu-Villaça et al., 2018; Gulyás et al., 2002; Ishola et al., 2018; Shibota et al., 1982). During the behavioral assessment phase, all substances were

administered 1h prior to the beginning of the behavior paradigm (Gupta et al., 2015).

Experimental design

In total 5 groups of animals, with each group containing eight (n=8; male) animals were used in the present study. The timeline, groups involved, and behavioral and neurochemical parameters assessed in the present study are represented in Fig. 1.

Female rats were mated overnight, and fertilization was determined using a vaginal smear to confirm the presence of sperm cells. This day was considered as GD 0. To induce hyperserotonemia, dams were administered a single dose of 5-MT daily from GD 12 to the day of parturition (n=8; pregnant females) or vehicle in equal volumes (n=5; pregnant females). Pregnant female rats were housed individually until the day of parturition. Upon parturition, PND 0 litters were culled to five males and five females to maintain a standard uniform distribution of pups in each litter. Offspring of 5-MT treated females were further administered 5-MT from PND 0 – PND 20 (day of weaning). Similarly, pups of vehicle treated females were administered vehicle in equal volumes from PND 0 – PND 20. On PND 20 pups were weaned and one pup per litter from a different litter per treatment group was randomly chosen in each experiment. The animals were housed in groups of four (Kumar and Sharma, 2016b; McNamara et al., 2008). Pups not utilized further in the study were euthanized with thiopental sodium (90 mg/kg.i.p.). In neurobehavioral studies, it is important to reduce litters to a standard size in order to avoid the situation where some pups belonging from small litters receive high maternal care and others belonging to bigger litters receive less maternal care. Only male pups were used in the following treatment schedule as ASD clearly presents with a gender bias in both serotonin and VPA based models (Chakraborti et al., 2019; Melancia et al., 2018). All treatments (drug/vehicle) were administered to the offspring from PND 21 to PND 48 (4 week). Each group had n=8 (male) animals, and the groups were divided as follows: group I and II (vehicle vinpocetine per-se): offspring of vehicle treated females were administered with 0.5% w/v CMC – 10 ml/kg, p.o., or vinpocetine – 20 mg/kg daily, p.o. as per the treatment group assigned; group III (DHS): offspring of the DHS induced group were administered 0.5% w/v CMC – 10 ml/kg, p.o.; group IV and V (vinpocetine treatment): pups born to DHS females were divided further into DHS + V (10/20) groups and received vinpocetine (10/20 mg/kg daily p.o.) respectively (Ishola et al., 2018).

Behavioral assessment

During the behavioral assessment phase, all treatments were administered 1h prior to the behavior paradigm. All behaviors were conducted during the light phase (i.e., between 09:00 a.m. and 09:00 p.m. from PND 44 to PND 48). Animals were placed in the testing area

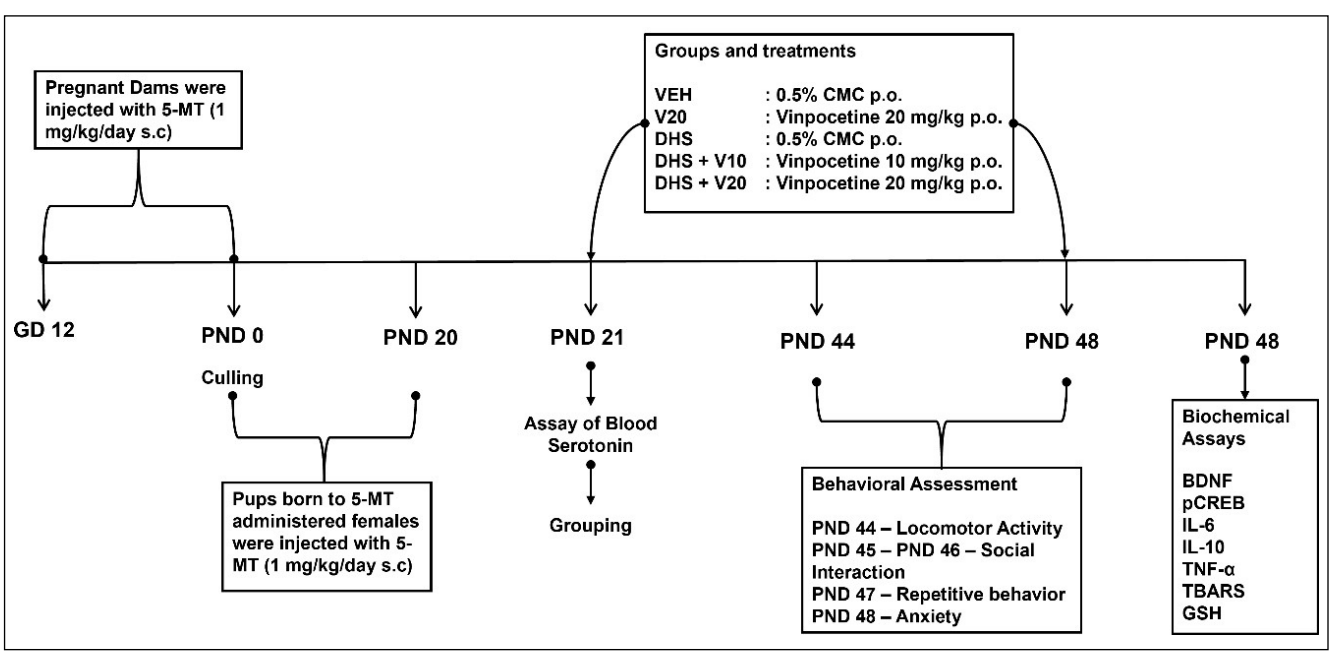

Fig. 1. Brief representation of the study timeline and key events.

5 days prior to the beginning of the behavioral experiments. To reduce the chances of olfaction-related cues during testing, the test arena was cleaned with 70% v/v ethyl alcohol and dried between each consecutive trial. All behaviors were assessed manually by a colleague who was blinded to the whole study.

Pup retrieval

All female rats were tested post-partum on PND4 for alteration in maternal care behavior due to 5-MT treatment during gestation. A cage was prepared, and one mother was placed opposite to the nest and the pups. The latency to retrieve the first pup and the total time taken to retrieve all pups was measured as an indicator of maternal care and 5 min was kept as the cut off time to reduce chances of hypothermia induced injury to pups (Pardon et al., 2000; Zimprich et al., 2017).

Return to dam

Only male pups were used in the return to dam paradigm. On PND17, male animals of a litter were separated from the mother using an opaque plastic sheet, which was placed in the home cage. The sheet had a small hole 2.5 × 2.5, connected with small PCV pipe of the same diameter, which only allowed the pups to crossover and did not allow the mother to pick up the pups and reach them. Pups were tested for 10 min, and time taken to reach the other side was noted for each pup. A pup was considered to have crossed to the other side when all four limbs were in a separate area (McNamara et al., 2008).

Locomotor activity

Spontaneous increases in locomotion is extensively reported as one of the important features of the DHS model. Hyper-locomotion was measured using an open field apparatus. The apparatus measured 90 cm × 90 cm with 50 cm high walls made of dark colored wood. The animals on PND 44 were introduced individually into the center of the arena for a single 10 min trial period (Mony et al., 2016). The total number of line crossings and the total number of central square entries were recorded to assess changes in locomotion of the animals.

Social behavior

Diminished and abnormal social interaction is a core phenotype of ASD, which may be induced by the DHS model (Madden and Zup, 2014; McNamara et al., 2008). Changes in social behavior were assessed using the three chambered social interaction test protocol on PND 45 and PND 46, with slight modifications (Mirza and Sharma, 2019a). The test arena measured 76 cm × 30 cm × 35 cm and was divided into three chambers with an access point between each chamber. Animals had free access to all the chambers, and each trial began with the animal being placed in the central chamber. To encourage exploration of the side chambers, all animals were habituated to the apparatus for 5 min prior to initiation of the test trial. After ending of the habituation period, the rats were tested in the sociability phase which lasted for 10 min. Animals to be placed under a wire cage were habituated to the wire cage for 30 min, 24 h prior to beginning the sociability phase. In the sociability phase, a stranger animal was placed under the wired cage in either (left or right) side chamber, while in the other chamber an empty cage would be placed. To avoid side preferences, the placement of wired cages was randomized, and the chamber with the stranger animal and the empty cage were called stranger chamber and empty chamber, respectively. Upon conclusion of the sociability phase the social preference phase was initiated 2 h after the last animal trial. In the social preference phase, each animal was allotted 10 min to explore the complete arena. During this phase, the animal earlier considered as a stranger was now rendered familiar and another novel animal was introduced into the paradigm, along with the familiar animal. Thus, the two chambers now would be familiar chamber and novel chamber. The time spent by test animals in both the side chambers was measured. The sociability index (SI) and social preference index (SPI) were calculated according to the following formula (Kumar and Sharma, 2016a).

$$SI = \frac{\text{Total time in stranger chamber}}{\text{Total time in empty chamber}};$$

$$SPI = \frac{\text{Total time in novel chamber}}{\text{Total time in familiar chamber}}$$

Repetitive behavior

One of the core diagnostic features of ASD is the presence of stereotypical or repetitive behavior, and this key clinical feature is replicated by inducing DHS (Veenstra-VanderWeele et al., 2012). The percentage of spontaneous alternation is regarded as a measure of stereotypical or repetitive behavior in animals. A y-maze apparatus was used to assess percentage of spontaneous alternations (Mirza and Sharma, 2019b). The maze makes a Y shape, with three arms of equal lengths each at an angle of 120° from the other. One of the arms was considered as the start arm and all animals were placed

at the end of this arm pointing towards the center of the maze. On PND 47, the animals were subjected to 8 min of testing in the y-maze. The exploration of three different arms in succession was considered as one alternation. Serial arm entries were observed for each animal to calculate percentage of spontaneous alternations. The percentage of spontaneous alternation was calculated with the following formula.

$$\%\text{Spontaneous alternation} = \frac{\text{Total alternations}}{(\text{Total Arm entries} - 2)} \times 100$$

Anxiety

Anxiety is the most common co-morbid trait expressed with ASD. Elevated plus maze (EPM) is a commonly used apparatus to assess anxiety-like behavior in animal models of ASD. The EPM apparatus was made up of wood with four arms at 90° to each other. Two open arms and two closed arms of 50 × 10 cm dimensions were enclosed by a 40 cm high wall. On PND 48, to facilitate exploration of the maze, all animals were placed in a pretest arena for 5 min each. Soon after, the animals were transferred to the EPM placed 50 cm high from the ground. All animals were released in the center of the maze, pointing towards the open arm and entries, and the time spent in each arm was manually recorded for 5 min (Mirza and Sharma, 2018). The basis of this test is the conflict associated with the two different parts of the maze; the open arms are aversive, bright, and unprotected, while the closed arms are covered, shadowy, and protected. To this end, the number of open and closed arm entries as well as the time spent in open and closed arms were measured. To calculate percentage of open arm entries and percentage of open arm time, we used the following formula (Degroote et al., 2018).

$$\%\text{Open arm entries} = \frac{\text{Number of entries in open arm}}{\text{Closed arm entries + open arm entries}} \times 100$$

$$\%\text{Time spent in open arm} = \frac{\text{Time spent in open arm}}{\text{Time spent in open arm + Time spent in closed arm}} \times 100$$

Biochemical assays

All chosen areas of the brain that were assessed have been previously implicated in the development of ASD and are innervated/regulated by the serotonergic system (Ciranna, 2006; Hui et al., 2018; Irifune et al., 1991; Rapanelli et al., 2017; Zhang et al., 2016). Recent studies from our lab have also implicated several brain areas to express altered brain biochemistry in other models of ASD (Mirza and Sharma, 2018; 2019a; 2019b).

Assay of whole blood serotonin levels

On PND21, 24 h after the last 5–MT injection and vehicle injection to the pups. All animals selected for the final study were subjected to blood withdrawal from tail vein (<10% of the total blood volume (64 ml/kg)) (Diehl et al., 2001). Blood was obtained in a plastic tube and was suspended in 2.2 ml of sterile water. To this end, 300 µl of the internal standard and 10 µl of a 10% w/v solution of ascorbic acid in water was added. The sample was then frozen at –20°C and stored until further analysis of 5-HT. Blood samples were thawed, and then 167 µl of methanol was added to 1 ml of blood sample in order to remove the proteins. Blood samples were centrifuged at 4500 g for 10 min at 4°C. Supernatant (500 µL) of the blood sample was suspended in 4.5 ml of the mobile buffer (Nakatani et al., 2008). Twenty microliters of the blood sample were then injected into the HPLC system equipped with a quaternary pump (water system – fluorometric detector). Separation was achieved on ODS-reversed phase column. The mobile phase consisted of 0.1 M potassium phosphate buffer/methanol 97/3 (v/v) with pH adjustment at 4.05 and was delivered at a flow rate of 1 ml/min. UV detection was performed at 270 nm, and the injection volume was 20 ml. The flow rate was set at 0.22 ml/min and the column temperature was maintained at 35°C. The serotonin levels were expressed in µg/ml of blood (Kumar and Sharma, 2016a).

Tissue preparation for biochemistry

All biochemical assays were performed in the frontal cortex, cerebellum, hippocampus, and striatum. Individual rats were sacrificed using thiopentone sodium (100 mg/kg, i.p.) followed by rapid decapitation on PND 48 (30 min post behavioral paradigm). Immediately following this, the brain was isolated onto a cold plate and washed with ice cold PBS (pH 7.4). The frontal cortex, cerebellum, hippocampus, and striatum were immediately sectioned off and mixed in 1:10 w/v ratio of RIPA buffer (Thermo Fisher Scientific) containing protease inhibitor cocktail (Genetix Biotech ltd., India), followed by homogenization using a Polytron homogenizer. Post-homogenization, samples were centrifuged (11,000 × g, 20 min, 4°C) and the supernatant was col-

lected and stored for further evaluation (Elfving et al., 2010; Wu et al., 2017).

Assay of BDNF, ratio of pCREB/CREB, TNF-α, IL-6 and IL-10

Elisa based protein assays for BDNF (ELR-BDNF), pCREB/CREB (PEL-CREB- S133-T), TNF-α (ELR-TNFa), IL-6 (ELR-IL6) and IL-10 (ELR-IL10) were carried out using commercial ELISA kits obtained from RayBio®, USA. All kits were based on sandwich *in-vitro* ELISA principle. The optical density of the samples was measured using a microplate reader at 450 nm. The concentrations for BDNF, IL-6 and IL-10 were expressed as pg/ml, TNF-α and pCREB/CREB were presented as ng/ml respectively.

Assay of thiobarbituric acid reactive substance and glutathione

The levels of TBARS were estimated using a microplate reader at 532 nm with slight modifications (Kumar et al., 2015). Isolated supernatant (100 µl) was mixed with equal volumes of 8.1% of sodium dodecyl sulphate, 250 µl of 1:1 mix of 30% acetic acid (pH 3.5) and 0.8% thiobarbituric acid was added, and this mix was incubated at 95°C for 45-60 min. This sample was cooled and centrifuged at 3000 rpm and 300 µl of supernatant was drawn and mixed with equal volumes of n-butanol: pyridine mixture (15:1 v/v). Samples were again centrifuged at 10,000 g for 5 min and the butanol fraction was taken for further assessment. The results are expressed as nM/mg.

For estimation of GSH levels, 10% w/v of trichloroacetic acid was added to the supernatant in a 1:1 ratio. This mix was centrifuged at 1000 g for 10 min, and the supernatant was then mixed with 2 ml of 0.3 M disodium hydrogen phosphate containing 0.25 ml of 0.001 M DTNB (dissolved in 1% w/v sodium citrate). The estimation for reduced glutathione was done at 412 nm. Fixed concentrations, ranging from 10–100 µM of reduced glutathione were used to plot a standard curve and the values are expressed as µM/mg of protein (Mirza and Sharma, 2018).

Assessment of brain total protein

The total brain protein was estimated in the supernatant using Coomassie plus protein assay from a commercially available kit obtained from Puregene® (Genetix), India. Bovine serum albumin was used to plot a standard curve from 1–25 µg/ml. The absorbance of samples was measured at 595 nm with a plate reader. The microplate protocol was used as given in the product information sheet to measure protein concentration.

Statistical analysis

Data is presented as mean ± standard deviation (SD). To assess the effects of DHS and target treatment on DHS induced animals, the data was analyzed with two-way ANOVA followed by Tukey's multiple comparison *post-hoc* test. For the pup retrieval test, return to dam and level of 5- HT in blood in the DHS/5-MT groups *vs.* the VEH group of animals, unpaired t-tests were used with Welch's tests to assess significance and heterogeneity of variances. Further, correlation analysis was performed to assess the relationship between behavioral and biochemical parameters. The data is represented as "r" Pearson's coefficient value. The accepted significance value was considered to be $P<0.05$. Sigma Plot version 12.5 (Systat Softwares, Inc.) was used for two-way ANOVA and Prism version 7 (GraphPad Software, Inc.) was used for t-tests and Pearson's correlations.

RESULTS

Effect of 5-MT administration on maternal behavior

Our results indicated that 5-MT administration had no significant effect on the maternal behavior as indicated by the pup retrieval test. The pup retrieval latency of vehicle-treated mothers (8.60 ± 2.07 sec) and 5-MT treated mothers (8.80 ± 1.28 sec) showed no significant difference (Fig. 2A). Similarly, the total time taken to retrieve all pups didn't show any significant changes between the vehicle treated mothers (88.80 ± 25.68 sec) and 5-MT treated mothers (99.37 ± 37.06 sec) (Fig. 2B; $P>0.05$).

Effect of 5-MT administration on pup behavior

When tested for return to dam (maternal bonding behavior), pups exposed to 5-MT perinatally took significantly more time (302.33 ± 27.32 sec) to reach the dams in comparison to their vehicle treated counterparts (124.56 ± 14.78 sec). This indicates a lack of maternal bonding in 5-MT treated pups (Fig. 2C; $P<0.05$).

Effect on peripheral serotonin levels upon 5-MT administration

Our results indicated that, upon 5-MT administration, the level of serotonin in whole blood was significantly higher in the 5-MT treated pups (0.65 ±

0.06 µg/ml, n=40) as compared to the pups only treated with vehicle (0.30 ± 0.06 µg/ml, n=25) (Fig. 2D; P<0.05). This increase in peripheral serotonin could indicate hyperserotonemia.

Effect on locomotion

When compared with vehicle-treated animals, DHS animals showed a significant increase in the number of line crossings (Fig. 3A and B; left axis) and central square entries (Fig. 3A and B; right axis) in open field, during both the 0–5 min and 5–10 min time periods (P<0.05; n=8), indicating increased spontaneous locomotor activity.

Administration of vinpocetine (10 mg/kg and 20 mg/kg) to DHS group of animals resulted in a significant reduction in the number of line crossings and number of central square entries (p<0.05; n=8), indicating a reduction in locomotion.

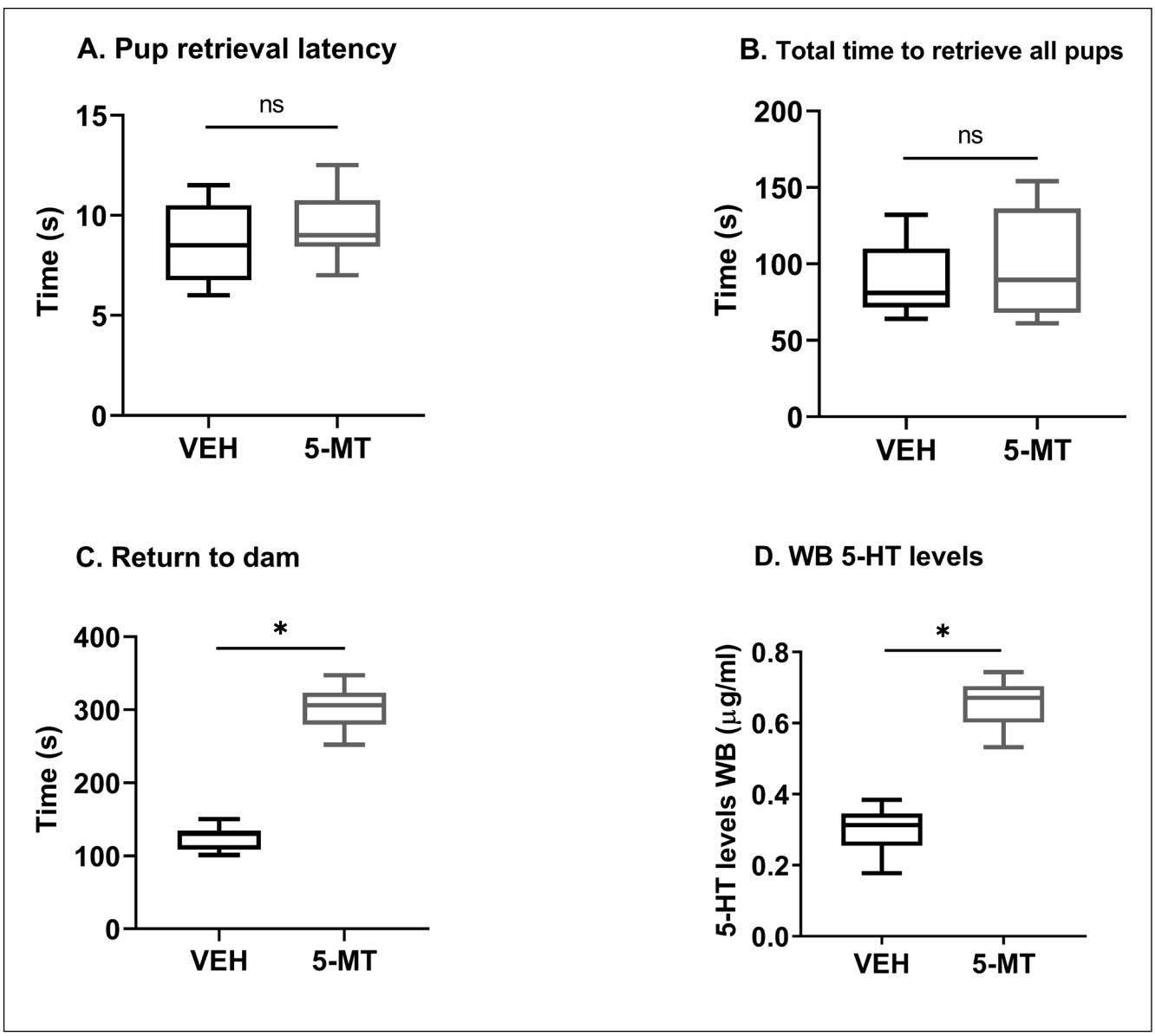

Fig. 2. Effects of 5-MT treatment on mothers and pups. The pup retrieval test indicated that maternal care (A and B; VEH (n)=5 females; 5-MT (n)=8 females) was not significantly altered, but the return to dam (C) indicated reduced maternal bonding in pups treated with 5-MT in comparison to vehicle treated pups. Similarly, high whole blood serotonin levels (D) were observed in pups on PND 21 in comparison to vehicle treated pups (VEH (n)=25 male pups; 5-MT (n)=40 male pups). (A) Pup retrieval latency – $t_{7.35}$=0.7455 (P>0.05); (B) Total time to retrieve all pups – $t_{10.77}$=0.6069 (n.s.); (C) Return to dam – $t_{61.99}$=33.96 (P<0.05); (D) Blood 5-HT levels: $t_{31.75}$=18.86 (P<0.05). Data represented as mean ± SD with significance (*P<0.05) tested using unpaired t-test with Welch's correction. 5-MT – 5-methoxytryptamine; n – number of animals; PND – postnatal day; sec – seconds; VEH – Vehicle.

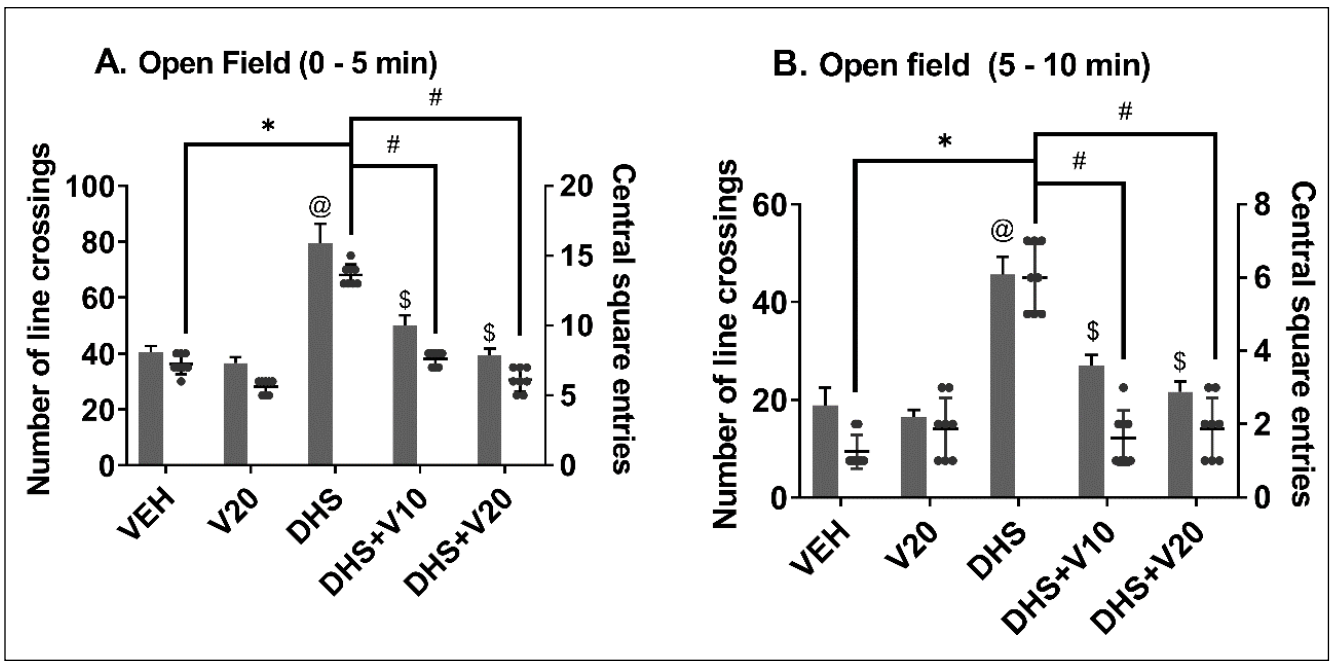

Fig. 3. Effect of PDE1 inhibition on an open field. In the open field paradigm significant changes were observed between DHS and control animals, as well as between DHS and vinpocetine treated animals, in both the 0–5 min (A) and 5–10 min (B) time periods. Open field (0–5 min) – Number of line crossings (primary axis); @$F_{1,36}$=39.779; \$$F_{2,36}$=0.7752; Central square entries (secondary axis); *$F_{1,36}$=40.022; #$F_{2,36}$=40.968. Open field (5–10 min). Number of line crossings (primary axis); @$F_{1,36}$=61.067; \$$F_{2,36}$=24.423; Central square entries (secondary axis); *$F_{1,36}$=24.521; #$F_{2,36}$=14.406 Data represented as the mean ± S.D (n=8, males), two-way ANOVA followed by Tukey's test. For (A), (B) (*, @) P<0.05 *versus* control group; (#, \$) P<0.05 *versus* DHS group. VEH – vehicle control group; DHS – Developmental hyperserotonemia group; V10/20 – Vinpocetine 10/20 mg/kg.

Effect on social behavior

Sociability phase

In the sociability phase, control animals spent significantly more time in the stranger chamber when compared to the empty chamber, while the animals in the DHS group exhibited significant increases in time spent in the empty chamber along with significant reductions in the time spent in the stranger chamber. This suggests impairment of sociability in DHS animals when compared with control animals. Administration of vinpocetine (10 mg/kg and 20 mg/kg) to the DHS group of animals significantly reduced time spent in empty chamber and increased time spent in stranger chamber, as well as an increase in the sociability index to (P<0.05; n=8) (Fig. 4A and B). This indicates an improvement in sociability following administration of vinpocetine.

Social preference phase

In the social preference phase, control animals spent increasingly more time in the novel chamber than the familiar chamber, while DHS resulted in a significant increase in time spent in the familiar chamber along with a significant reduction in time spent in the novel chamber and social preference index (P<0.05; n=8) (Fig. 4D). This suggests impairment in social preference in DHS-induced animals when compared with control animals. Administration of vinpocetine (10 mg/kg and 20 mg/kg) to the DHS group of animals significantly reduced time spent in the familiar chamber along with a significant increase in time spent in the novel chamber and social preference index to DHS animals (P<0.05; n=8) (Fig. 4C and D). This indicates an improvement in the social preference by vinpocetine.

Effect on anxiety

The percentage of time spent in open arm (Fig. 5A; left axis) and percentage of open arm entries (Fig. 5A; right axis) were significantly decreased in the DHS group (P<0.05; n=8), when compared with the control group. Thus, this reflects and anxiety-like state in the animals in the DHS group when compared to the animals in the VEH group. In contrast, treatment with vinpocetine (10 mg/kg and 20 mg/kg) resulted in a significant increase in the percentage of time spent in open arm (P<0.05; n=8) and percentage of open arm entries (P<0.05; n=8) when compared to the DHS

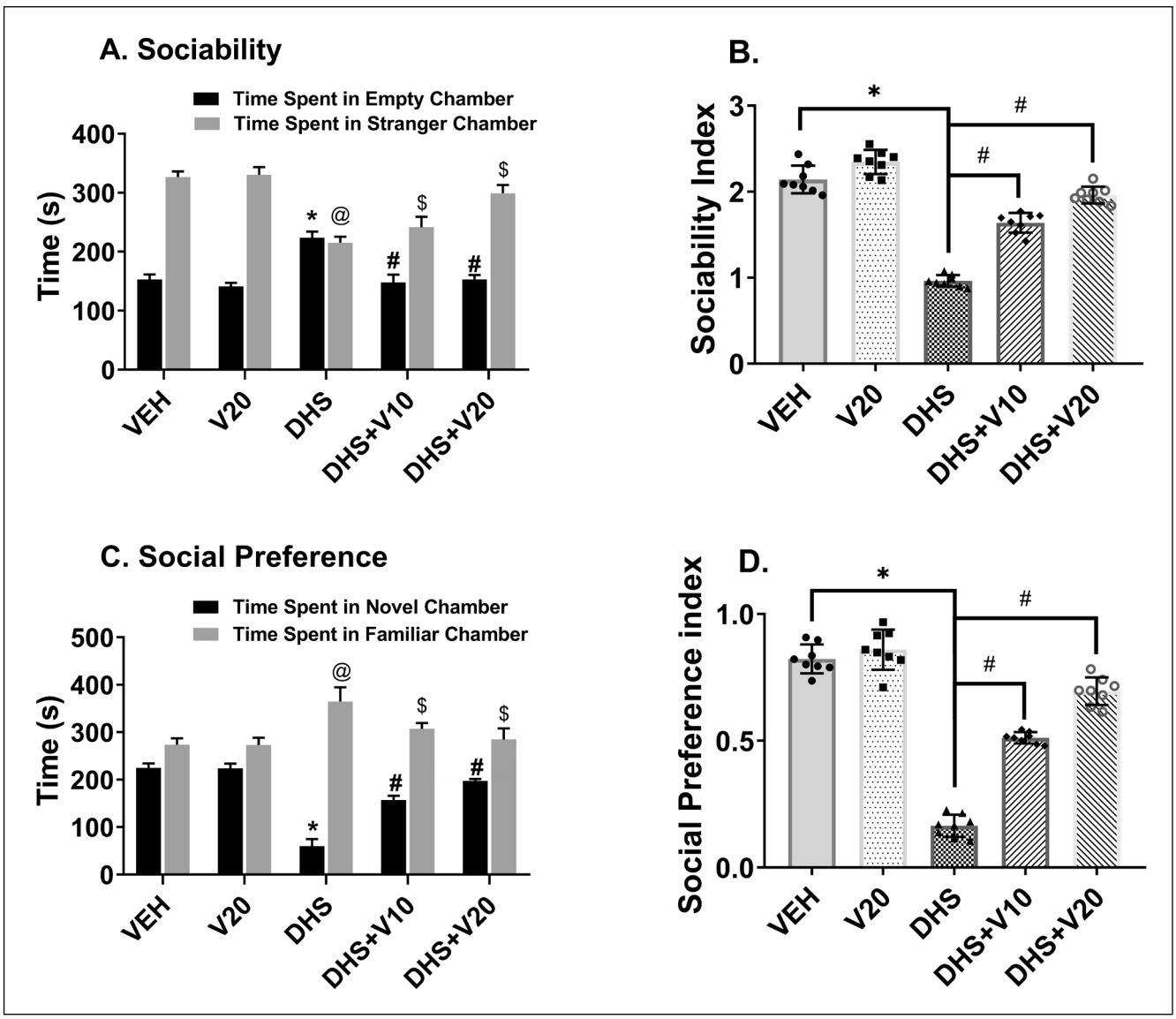

Fig. 4. PDE1 inhibition and its effect on social behavior and locomotion. Significant changes were observed in sociability index (B), also evident from the time spent in each chamber (A) in case of DHS animals against control and vinpocetine treated animals against DHS animals. Similar results were observed in the social preference (C) and social preference index (D). (A) Sociability – *$F_{1,36}$=48.069; @$F_{1,36}$=77.111; #$F_{2,36}$=39.531; \$$F_{2,36}$=16.033. (B) Sociability index – *$F_{1,36}$=99.24; #$F_{2,36}$=31.005. (C) Social preference – *$F_{1,36}$=79.487; @$F_{1,36}$=28.655; #$F_{2,36}$=23.166; \$$F_{2,36}$=9.835. (D) Social preference index – *$F_{1,36}$=82.129; #$F_{2,36}$=20.617. Results are expressed as mean ± SD (n=8); two-way ANOVA followed by Tukey's test. (*, @) P<0.05 *versus* control group; (#, \$) P<0.05 *versus* DHS group. VEH – vehicle control group; DHS – Developmental hyperserotonemia group; V10/20 – Vinpocetine 10/20 mg/kg.

group. Thus, this indicates a reduction in anxiety by the administration of vinpocetine to the DHS exposed animals.

Effect on repetitive behavior

The animals in the DHS group showed a significant decrease in percentage of spontaneous alternation when compared with the control group (P<0.05; n=8). Vinpocetine (10 mg/kg and 20 mg/kg) administration to DHS group animals (DHS+V5/10/20) significantly increased the percentage of spontaneous alternation in comparison to the DHS group animals (P<0.05; n=8) (Fig. 5B). This indicated a reduction in repetitive behavior following vinpocetine administration.

Effect on neuronal function markers

A significant decrease in the levels of BDNF (Fig. 6A) and pCREB/CREB (Fig. 6B) were observed in

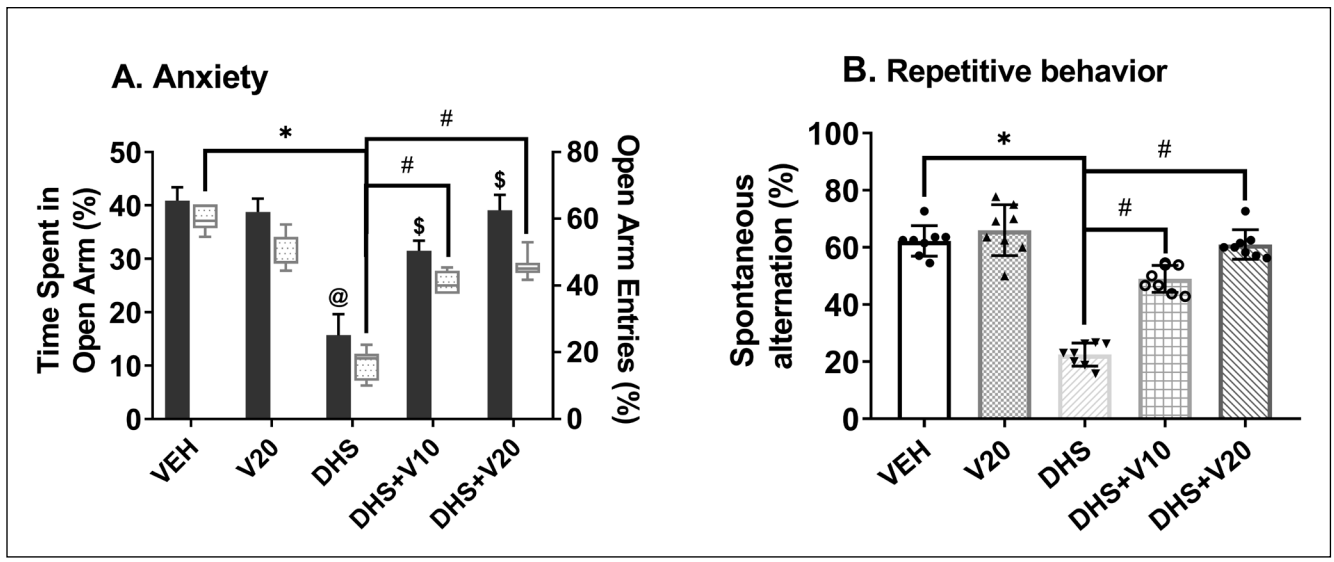

Fig. 5. Effect of PDE1 inhibition on EPM and repetitive behavior. EPM was used as a measure of anxiety. A significant decrease in percentage of TSOA (A – primary left axis) and percentage of OAE (A – secondary right axis) respectively, indicated increased anxiety in DHS group *vs.* VEH group which was amended by administration of vinpocetine. The percentage of spontaneous alternation (B) was used to measure repetitive behavior. A sharp decline in percentage of spontaneous alternation indicated presence of repetitive behavior in the DHS group *vs.* VEH group. Treatment with vinpocetine amended the dysfunction. (A) Percentage of TSOA (Primary axis) – $*F_{1,36}$=28.137; $\#F_{2,36}$=11.269; percentage of OAE (Secondary axis) – $*F_{1,36}$=53.271; $\#F_{2,36}$=7.583. (B) Percentage of spontaneous alternation – $*F_{1,36}$=39.642; $\#F_{2,36}$=19.108. Data represented as the mean ± SD (n=8, males), two-way ANOVA followed by Tukey's test. For (A) (*, @) p<0.05 *versus* control group; (#, $) P<0.05 *versus* DHS group; For (B) *P<0.05 *versus* control group; #P<0.05 *versus* DHS group. VEH – vehicle control group; DHS – developmental hyperserotonemia group; V10/20 – vinpocetine 10/20 mg/kg; TSOA – time spent in open arm; OAE – open arm entries.

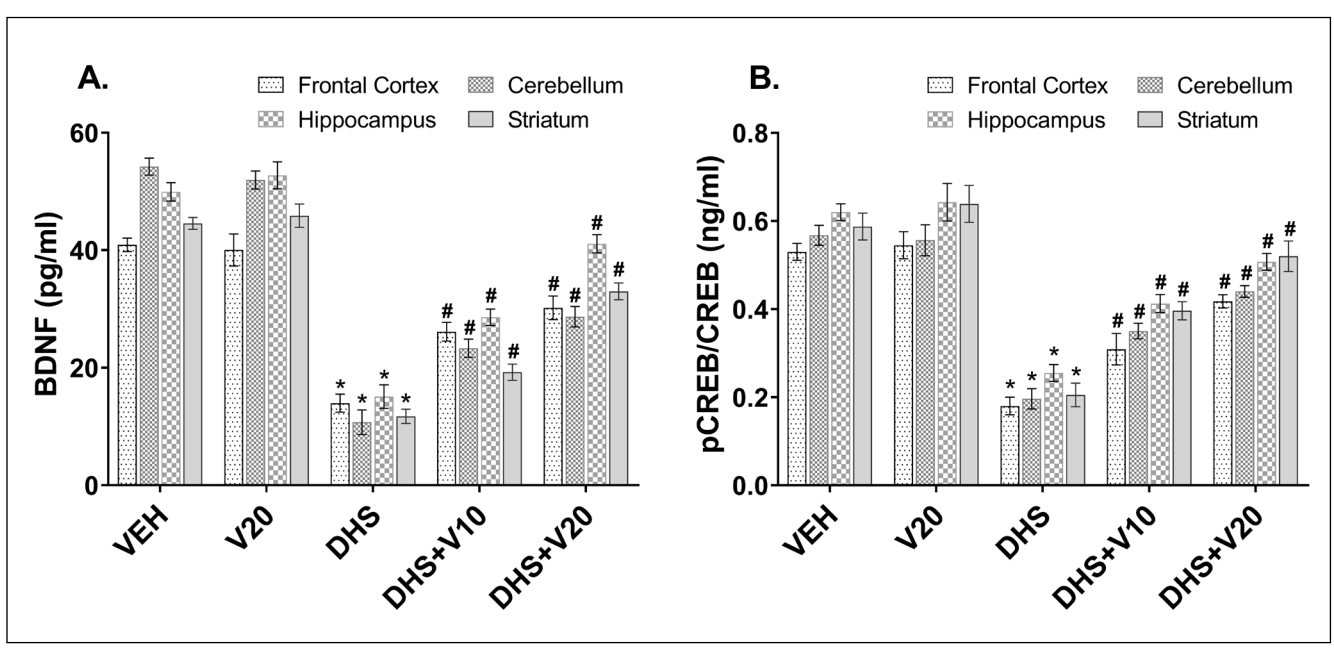

Fig. 6. Effect of PDE1 inhibition on neuronal function markers. The graphs represent gross protein values of the indicated protein in four different brain areas. Results indicate an increase in levels of BDNF and pCREB in the treatment groups when compared to the DHS group. Data represented as the mean ± SD (n=8, males), two-way ANOVA followed by Tukey's test. *P<0.05 *versus* control group; #P<0.05 *versus* DHS group. (A) BDNF levels – fc – $*F_{1,36}$=137.626; $\#F_{2,36}$=14.156; crb – $*F_{1,36}$=349.854; $\#F_{2,36}$=11.018; hp – $*F_{1,36}$=131.052; $\#F_{2,36}$=25.169; st – $*F_{1,36}$=173.340; $\#F_{2,36}$=22.242. (B) pCREB – fc – $*F_{1,36}$=4170.897; $\#F_{2,36}$=830.689; crb – $*F_{1,36}$=2863.435; $\#F_{2,36}$=445.299; hp – $*F_{1,36}$=2165.132; $\#F_{2,36}$=425.605; st – $*F_{1,36}$=1199.87; $\#F_{2,36}$=260.21. VEH – vehicle control group; DHS –developmental hyperserotonemia group; V10/20 – Vinpocetine 10/20 mg/kg; fc – frontal cortex; crb – cerebellum; hp – hippocampus; st – striatum.

different areas of the brain in the DHS group when compared to control group (P<0.05; n=8). Administration of vinpocetine (10 mg/kg and 20 mg/kg) resulted in a significant increase in the levels of BDNF and pCREB/CREB in different brain areas, when compared with DHS (P<0.05; n=8). BDNF and pCREB/CREB showed dose dependent improvements in different brain regions. This indicated beneficial effects of vinpocetine by correcting the levels of neuronal function markers.

Effect on brain inflammation markers

Rats in the DHS group had a significant increase in the levels of IL-6 (Fig. 7B) and TNF-α (Fig. 6A) in the frontal cortex, cerebellum, hippocampus, and striatum, respectively, when compared with the rats in the control group (P<0.05; n=8). Treatment with vinpocetine (10 mg/kg and 20 mg/kg) showed a significant dose dependent reduction in the levels of IL-6 and TNF-α in all brain areas when compared with the rats in the DHS group (P<0.05; n=8). In relation to the above findings, the levels of IL-10 (Fig. 7C) were found to be significantly reduced (P<0.05; n=8) in different brain areas of the animals in DHS group in comparison with the control group. Treatment with vinpocetine (10 mg/kg and 20 mg/kg) significantly increased the levels of IL-10 in different brain areas in a dose dependent manner when compared with the DHS group (P<0.05; n=8), indicating a reduction in inflammation of several brain regions.

Effect on brain oxidative stress

In comparison with control rats, rats in the DHS group exhibited a significant reduction in GSH (Fig. 8B) levels and a significant increase in TBARS (Fig. 8A) levels in all brain areas (P<0.05; n=8). Animals in the DHS group and treated postnatally with vinpocetine (10 mg/kg and 20 mg/kg) displayed a marked increase in GSH levels and a significant reduction in TBARS levels in all brain areas when compared with animals in the DHS group (P<0.05; n=8). This indicated a probable amelioration of increased brain oxidative stress due to vinpocetine treatment.

Correlation analysis of behavior with biochemistry

Pearson's correlations was performed between key behavioral parameters and all biochemical parameters (each brain area) (Table 1). In all cases the Pearson's coefficient (r) (Table 1) was significantly associated (P<0.0001) with all biochemical parameters in all brain regions. This suggests that correction of behavioral parameters may depend on the correction of biochemical markers *via* PDE1 inhibition with vinpocetine.

DISCUSSION

In the present study, we explored the effects of PDE1 inhibition in a DHS rat model exhibiting ASD like

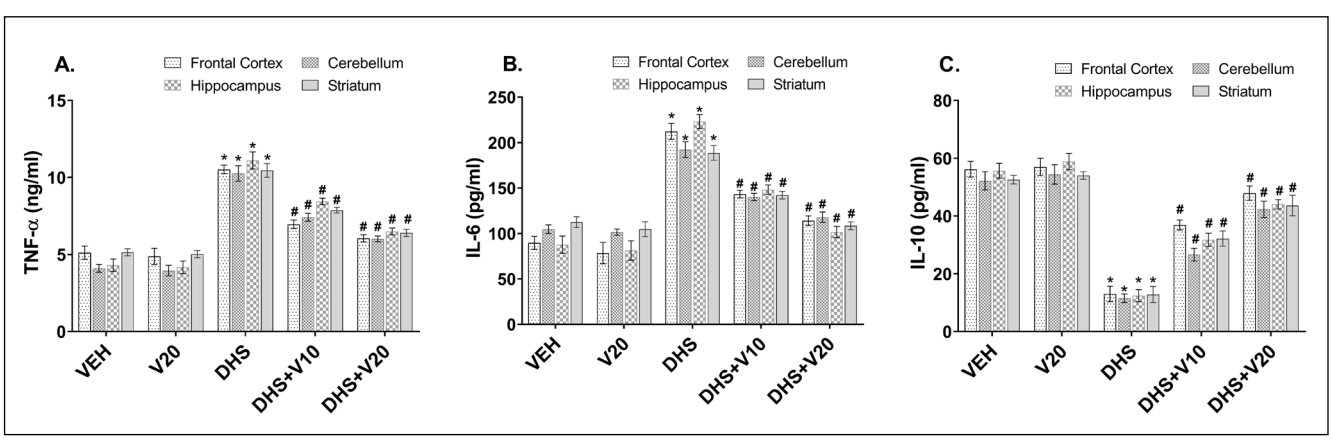

Fig. 7. Effect of PDE1 inhibition on brain inflammation. The graphs represent gross protein values of the indicated protein in four different brain areas. Data are presented as the mean ± SD (n=8, males), two-way ANOVA followed by Tukey's test. *P<0.05 *versus* control group; #P<0.05 *versus* DHS group. (A) TNF-α levels – fc – $*F_{1,36}$=125.288; $\#F_{2,36}$=40.216; crb – $*F_{1,36}$=130.282; $\#F_{2,36}$=19.998; hp – $*F_{1,36}$=229.979; $\#F_{2,36}$=36.516; st – $*F_{1,36}$=121.805; $\#F_{2,36}$=26.219. (B) IL-6 levels – fc – $*F_{1,36}$=181.934; $\#F_{2,36}$=60.657; crb – $*F_{1,36}$=110.515; $\#F_{2,36}$=91.614; hp – $*F_{1,36}$=69.894; $\#F_{2,36}$=24.638; st – $*F_{1,36}$=77.735; $\#F_{2,36}$=51.934. (C) IL-10 levels – fc – $*F_{1,36}$=106.656; $\#F_{2,36}$=28.235; crb – $*F_{1,36}$=106.213; $\#F_{2,36}$=20.783; hp – $*F_{1,36}$=141.370; $\#F_{2,36}$=26.501; st – $*F_{1,36}$=148.909; $\#F_{2,36}$=33.98. VEH – vehicle control group; DHS – developmental hyperserotonemia group; V10/20 – vinpocetine 10/20 mg/kg; fc – frontal cortex; crb – cerebellum; hp – hippocampus; st – striatum.

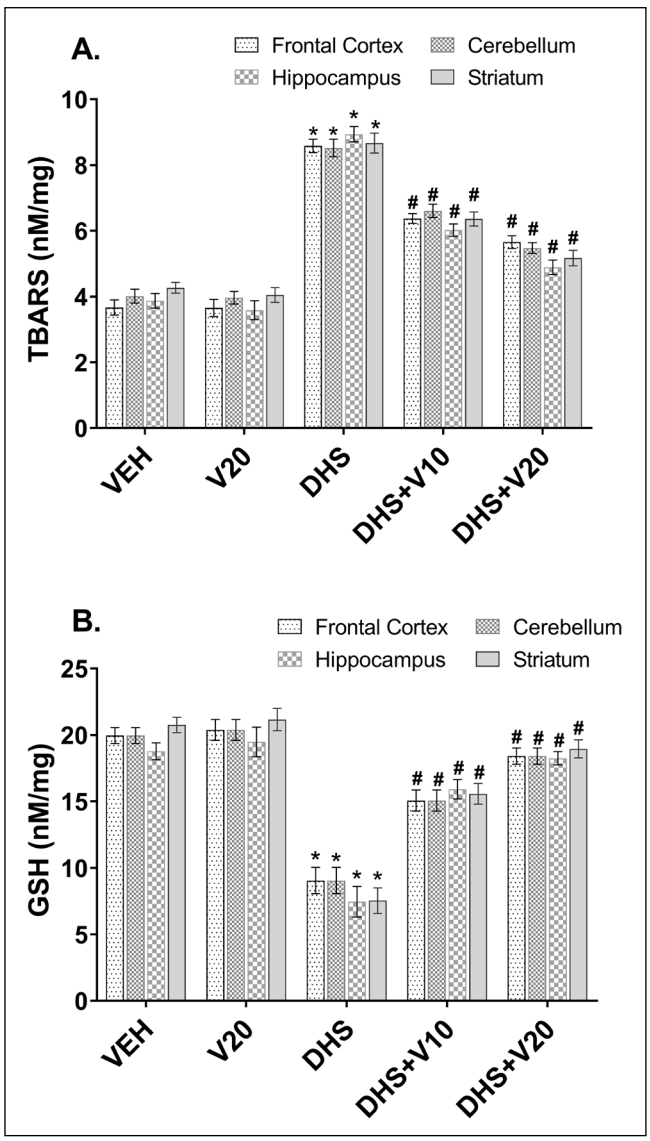

Fig. 8. Effect of PDE1 inhibition on brain oxidative stress. The graphs represent gross protein values of the indicated protein in four different brain areas. Data is presented as the mean ± SD (n=8, males). A two-way ANOVA was used followed by Tukey's test. *P<0.05 *versus* control group; #P<0.05 *versus* DHS group. (A) TBARS levels – fc – *$F_{1,36}$=280.153; #$F_{2,36}$=33.186; crb – *$F_{1,36}$=159.628; #$F_{2,36}$=23.112; hp – *$F_{1,36}$=96.997; #$F_{2,36}$=24.99; st – *$F_{1,36}$=123.622; #$F_{2,36}$=30.891. (B) GSH levels – fc – *$F_{1,36}$=87.915; #$F_{2,36}$=27.407; crb – *$F_{1,36}$=114.168; #$F_{2,36}$=37.761; hp – *$F_{1,36}$=196.324; #$F_{2,36}$=95.117; st – *$F_{1,36}$=121.278; #$F_{2,36}$=41.070. VEH – vehicle control group; DHS – developmental hyperserotonemia group; V10/20 – vinpocetine 10/20 mg/kg; fc – frontal cortex; crb – cerebellum; hp – hippocampus; st – striatum.

symptomatology. Induction of DHS in animals produced a deleterious effect on social behavior as well as on the levels of neuronal function markers (BDNF and pCREB/CREB), brain inflammation (TNF–α, IL–6, IL–10) and brain oxidative stress (TBARS and GSH), in selected brain areas (frontal cortex, cerebellum, hippocampus, striatum). Administration of PDE1 inhibitor, vinpocetine (10/20 mg/kg p.o.), to rats with induced hyperserotonemia resulted in amelioration of spontaneous hyperlocomotion, social deficits, repetitive behavior, and anxiety. Similarly, behavioral improvements were accompanied by significant improvements in biochemical markers of selected brain areas. Further, the effect on the aforementioned biochemical markers in various brain areas of DHS and vinpocetine-administered animals was measured and is being reported for the first time.

A consistent finding in clinical as well as experimental ASD is the increased levels of peripheral circulating serotonin and decreased function of serotonin in the brain (Madden and Zup, 2014; Tanaka et al., 2018). In conditions such as ASD, the lack of serotonergic function in the brain is evident from many studies, whereby substances increasing the levels of serotonin directly or by indirect means help ameliorate symptoms associated with the lack of it (Cai et al., 2019; Chakraborti et al., 2019; Kumar et al., 2015). The use of non-selective serotonin receptor agonist such as 5–MT during the peak serotonergic development stage in rats (GD 12 to PND 20) is known to cause pervasive harm to the serotonergic system (Kahne et al., 2002; McNamara et al., 2008; Musumeci et al., 2014), including symptoms associated with ASD such as deficits in social interactions, presence of repetitive behavior, spontaneous hyperlocomotion (McNamara et al., 2008; Tanaka et al., 2018), anxiety, and lack of exploratory activity (Yang et al., 2014; Zangrossi and Graeff, 2014). Phenotypical features such as sexual dimorphic characteristic of ASD, alterations to oxytocin system (McNamara et al., 2008), serotonin transporters (SERT), 5- hydroxytryptamine (5-HT1 and 5-HT2) receptor system (Lawson et al., 2016) and increased peripheral serotonin (Muller et al., 2016) along with reduced central levels of 5- HT expression, suggests similarity of DHS model with other well established models mimicking ASD symptomatology (Kahne et al., 2002; Kumar and Sharma, 2016a; McNamara et al., 2008). Alterations in serotonergic development due to changes in prenatal and early postnatal serotonin levels are well established clinically and experimentally. The increased levels of circulating serotonin may affect the behavioral and biochemical outcome in an organism (Castrogiovanni et al., 2014; McNamara et al., 2008; Montgomery et al., 2018; Tanaka et al., 2018). Similarly, in the present study, animals that exhibited high peripheral serotonin levels on PND 21 later exhibited ASD related behavioral changes and biochemical changes. Also, the results from the pup retrieval task and return to dam behavior show that 5-MT (at the dose administered in the present study) preferentially affected developing brain function in contrast

Table 1. Pearson's correlation of key behavioral parameters with biochemical assessment.

Parameters		Locomotor Activity		Social Behavior		Repetitive Behavior	Elevated Plus Maze	
	Brain area	Number of line crossings (0–5 min)	Number of line crossings (5–10 min)	Sociability index	Social preference index	Percentage of spontaneous alternation	Percentage of time spent in open arm	Percentage of open arm entries
BDNF	Fc	-0.8599	-0.9122	0.9323	0.9501	0.8629	0.8656	0.9138
	Crb	-0.7760	-0.8316	0.8854	0.9055	0.7995	0.7929	0.8831
	Hp	-0.8933	-0.9207	0.9492	0.9648	0.9058	0.8915	0.9043
	St	-0.828	-0.8699	0.9217	0.9322	0.8471	0.8373	0.8711
pCREB/CREB	Fc	-0.8767	-0.8993	0.9385	0.9643	0.87	0.8716	0.9087
	Crb	-0.8789	-0.9159	0.9365	0.9576	0.8894	0.8856	0.9151
	Hp	-0.8903	-0.9063	0.9579	0.9595	0.8816	0.8956	0.9186
	St	-0.9226	-0.9354	0.9583	0.9587	0.9167	0.9062	0.9063
TNF-α	Fc	0.9474	0.9529	-0.9612	-0.9615	-0.9150	-0.9464	-0.9435
	Crb	0.8898	0.936	-0.9533	-0.9721	-0.9034	-0.9047	-0.9283
	Hp	0.8827	0.9178	-0.9438	-0.9614	-0.8788	-0.8701	-0.9157
	St	0.9274	0.9355	-0.9548	-0.9709	-0.9075	-0.9187	-0.9383
IL-6	Fc	0.9350	0.9374	-0.9638	-0.9696	-0.9124	-0.9087	-0.9311
	Crb	0.9468	0.9498	-0.9548	-0.9687	-0.9177	-0.9274	-0.9323
	Hp	0.9497	0.9418	-0.9465	-0.961	-0.9478	-0.9406	-0.9181
	St	0.9492	0.9315	-0.9233	-0.9415	-0.9173	-0.9283	-0.891
IL-10	Fc	-0.9506	-0.9508	0.959	0.9721	0.916	0.9449	0.9489
	Crb	-0.8916	-0.9141	0.9588	0.9598	0.8812	0.8919	0.8966
	Hp	-0.9054	-0.9282	0.9578	0.9712	0.916	0.8928	0.9182
	St	-0.9186	-0.9298	0.9658	0.9727	0.9075	0.912	0.9247
TBARS	Fc	0.8710	0.9193	-0.933	-0.9497	-0.8777	-0.8676	-0.9173
	Crb	0.9016	0.9162	-0.9566	-0.9715	-0.8962	-0.8899	-0.9316
	Hp	0.9434	0.9551	-0.9649	-0.971	-0.9219	-0.9282	-0.9356
	St	0.9462	0.9455	-0.9721	-0.9766	-0.9174	-0.9311	-0.9399
GSH	Fc	-0.9469	-0.9509	0.9534	0.9665	0.9152	0.9286	0.9277
	Crb	-0.9573	-0.9417	0.9551	0.9529	0.9236	0.9298	0.9316
	Hp	-0.965	-0.9425	0.9407	0.9455	0.9181	0.9473	0.9178
	St	-0.9548	-0.9568	0.9491	0.9665	0.9323	0.9343	0.9249

The data is represented as Pearson's coefficient value (r) with a $P<0.0001$ for all comparisons. A negative r-value represents an inverse correlation and positive r-value represents a positive correlation between the parameters. BDNF – brain derived neurotrophic factor; CREB-cAMP response element binding protein; IL – interleukin; Fc – frontal cortex; Crb – cerebellum; Hp – hippocampus; St – striatum.

to a developed/adult brain during gestational delivery (Shah et al., 2018).

The levels of BDNF and 5-HT are inter-dependent and alterations in this system might result in altered cerebral development (Popova and Naumenko, 2019). The loss of BDNF is associated with expression of hyperlocomotive traits in animals (Simchon and Tenenbaum et al., 2015). Yoo and colleagues (2014) have noted that factors such as BDNF and pCREB, play a pivotal role in neuronal formation, synapse function, neuroplasticity, neurogenesis, and neuronal survival, thus affecting repetitive and stereotypyical behavior. The levels of pCREB in animals positively correlate with cell survival, proliferation and migration, as a consequence of increased cAMP levels (Jagasia et al., 2009). According to Zhang and colleagues (2016), anxiety-like behavior is exhibited by mice with CREB gene knocked out and reduced pCREB in the limbic region and cerebellum. In rodents, an increase in the central levels of BDNF has been shown to ameliorate anxiety, maybe *via* the activation of the pCREB/BDNF pathway (Bahi, 2017; Zhang et al., 2016).

The levels of inflammatory mediators and oxidative stress markers are dependent on the levels of pCREB (Guo et al., 2017; Motaghinejad et al., 2017; Wu et al., 2017). The activation of microglia can result in an increased level of pro-inflammatory mediators such as TNF-α, IL-1β, IL-6 and oxidative stress markers such as TBARS, GSH and SOD.

Also, 5-HT levels in the brain regulate immune function and release of cytokines and inflammation (Goeden et al., 2016; Ruiz-Miyazawa et al., 2015; Wu et al., 2018; Yang et al., 2015). The developmental changes induced by 5-MT are known to alter several key areas of the brain such as frontal cortex, hippocampus, cerebellum, striatum, and amygdala. It is important to note that these are also the critical areas which are innervated by serotonergic pathways directly or indirectly (Courchesne et al., 2011; Kahne et al., 2002; Pierce and Courchesne, 2001; Whitaker-Azmitia, 2005). Thus, we have performed various biochemical assessments in these critical areas of the brain. In the current study, the biochemical alterations were observed in these critical areas of the brain. This could indicate that developmental hyper-serotonergic state was successfully created in the animals resulting in biochemical and behavioral changes. Further, our correlation analysis indicated that behavioral deficits were associated with the alterations in biochemical parameters.

PDE1 inhibitors prevent the hydrolysis of cAMP/cGMP and thereby increase the activity of dependent protein kinases (protein kinase A and protein kinase C). This results in phosphorylation and activation of CREB (Medina, 2011; Wu et al., 2018). Radiolabeled assay of vinpocetine has revealed CNS entry and binding with PDE1 enzymes with a heterogeneous distribution upon oral administration, suggesting the drug easily crosses the blood brain barrier and is accumulated in the brain upon oral administration (Gulyás et al., 2002). The effect of vinpocetine (5 mg/kg p.o. and 20 mg/kg p.o.) has been studied on brain metabolism by analyzing glucose metabolism, suggesting therapeutic effects in the brain at the selected doses (Shibota et al., 1982).

Vinpocetine is a specific inhibitor of PDE1 enzyme that has been shown to ameliorate hyperlocomotion, social deficits, repetitive behavior, anxiety, and nociception in experimental model of fetal alcohol syndrome (Nunes et al., 2011), schizophrenia (Ahmed et al., 2018), Huntington's disease (Gupta and Sharma, 2014) and lipopolysaccharide induced inflammatory pain (Ruiz-Miyazawa et al., 2015). In line with these observations, in our study vinpocetine administration resulted in amelioration of behavioral deficits. PDE1 inhibition by vinpocetine is known to improve cAMP levels and pCREB levels *via* the cAMP/CREB pathway in both the cerebellum and hippocampus, areas of the brain which are implicated in locomotor activity (Abreu-Villaça et al., 2018). The molecule pCREB is known to play an important role in long-term plasticity, memory formation and circuitry development, potentially *via* its modulation of BDNF (Paldino et al., 2019) through cAMP/CREB dependent pathways, as impairments in social behavior and stereotypical behavior (Yoo et al., 2014) are linked with reduced expression of pCREB and BDNF. Xu and colleagues (2019), has noted that social deficits and repetitive/stereotypical behavior in animal models of schizophrenia might be due to decreased levels of BDNF and pCREB in the frontal cortex, and this was attenuated using vinpocetine.

PDE1 inhibition by vinpocetine has been shown to reduce neuro-inflammation by reducing the levels of TNF-α (Ishola et al., 2018; Ruiz-Miyazawa et al., 2015), IL-6 and increasing the levels of IL-10 (Yoshikawa et al., 1999) in animal models of inflammation. Zhang and colleagues (2018) noted that vinpocetine administration to post-stroke patients significantly improved their neurological function and clinical outcomes in comparison to control patients, and this effect was attributed to modulation of NF-κB and its effect on markers such as TNF-α and IL-6 (F. Zhang et al., 2018). The cAMP/pCREB pathway signaling has been reported to be involved in the reduction of inflammation following activation by PDE1 inhibition (O'Brien et al., 2020). Also, the pCREB/BDNF pathway may have been responsible for the reduction of inflammation (Motaghinejad et al., 2017). Further, vinpocetine is reported to reduce brain oxidative stress

by reducing TBARS level and increasing GSH levels in the brain (Gupta and Sharma, 2014; Ruiz-Miyazawa et al., 2015). Reduction in levels of GSH and TBARS might be linked to the activity of cAMP/pCREB pathway (Abreu-Villaça et al., 2018). These findings were corroborated in the present study. PDE1 inhibition resulted in reduction of inflammation and oxidative stress while improving neuronal function marker levels in brain. Also, amelioration of behavioral deficits associated with the DHS model was observed in vinpocetine treated animals.

Although direct effects of PDE inhibition on 5-HT levels and its possible outcome is beyond the scope of this study, PDE4 inhibition has been indicated to support the function of serotonergic receptors, hinting at a relationship between 5-HT cell signaling and PDE enzyme cell signaling (Fujita et al., 2017; Levallet et al., 2009). The secondary messenger cAMP is regulated by both serotonergic cell signaling as well as by PDE enzyme activity and it is known that both are hampered in ASD, clinically as well as experimentally (Braun et al., 2007; Cai et al., 2019; Muller et al., 2016; Tanaka et al., 2018). The effects of PDE1 on serotonin are yet to be elucidated, and we are unable to find any previous work directly comparing the relationship specifically of PDE1 with 5- HT in the brain.

CONCLUSION

It is clear from the present study that early developmental exposure of rats to 5-MT resulted in generation of ASD related behavioral and biochemical phenotypes later in their life. Administration of vinpocetine resulted in amelioration of ASD related behavioral phenotypes such as spontaneous hyperlocomotion, social deficits, repetitive behavior, and anxiety. Also, vinpocetine administration increased the levels of pCREB, BDNF, IL-10, and GSH along with a reduction in the levels of TNF-α, IL-6 and TBARS in several important brain areas. Thus, the observed effects of PDE1 inhibition by vinpocetine might be a result of pCREB/BDNF pathway activation, reducing inflammation and oxidative stress in important brain areas.

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