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Cerebellar BDNF Promotes Exploration and Seeking for Novelty

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Abstract

Background: Approach system considered a motivational system that activates reward-seeking behavior is associated with exploration/impulsivity, whereas avoidance system considered an attentional system that promotes inhibition of appetitive responses is associated with active overt withdrawal. Approach and avoidance dispositions are modulated by distinct neurochemical profiles and synaptic patterns. However, the precise working of neurons and trafficking of molecules in the brain activity predisposing to approach and avoidance are yet unclear.
Methods: In 3 phenotypes of inbred mice, avoiding, balancing, and approaching mice, selected by using the Approach/Avoidance Y-maze, we analyzed endogenous brain levels of brain derived neurotrophic factor, one of the main secretory proteins with pleiotropic action. To verify the effects of the acute increase of brain derived neurotrophic factor, balancing and avoiding mice were bilaterally brain derived neurotrophic factor-infused in the cortical cerebellar regions.
Results: Approaching animals showed high levels of explorative behavior and response to novelty and exhibited higher brain derived neurotrophic factor levels in the cerebellar structures in comparison to the other 2 phenotypes of mice. Interestingly, brain derived neurotrophic factor-infused balancing and avoiding mice significantly increased their explorative behavior and response to novelty.
Conclusions: Cerebellar brain derived neurotrophic factor may play a role in explorative and novelty-seeking responses that sustain the approach predisposition.

Keywords: approach-avoidance behavior, brain derived neurotrophic factor, cerebellar infusion, individual differences, open field task

Introduction

Novelty- and reward-seeking behavior associated with exploration is typical of the motivational approach system, whereas appetitive response inhibition and active withdrawal are representative of the attentional avoidance system (McNaughton and Gray, 2000; Pickering and Gray, 2001; Carver and Miller, 2006). Within the same species, some individuals have an overt tendency toward positive (e.g., rewarding) or away from negative (e.g., dangerous) stimuli, neophilic or neophobic responses,

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Significance Statement

The present research deals with the neurobiological basis of individual differences, in particular the predispositions in approach and avoidance. The findings indicated that the individual differences in the inherent predisposition to approach were associated with the basal cerebellar brain derived neurotrophic factor (BDNF) levels, and that the cerebellar BDNF levels were causally related with the acute response of approach in its double component of exploration and search for novelty. Here, we are proposing that the basal endogenous or acutely increased cerebellar BDNF levels promote explorative response and searching for novelty, components that sustain the approach pattern.

exploratory or withdrawal behaviors (Greenberg, 2003; Laricchiuta and Petrosini, 2014; Laricchiuta, 2015). Thus, individual differences in approach and avoidance may be considered constitutionally ingrained stable traits (Elliot, 2005, 2008; Sullivan et al., 2008; Helfinstein et al., 2011).

Approach and avoidance predispositions emerge from mechanisms operative in the whole brain, from spinal cord (Schutter et al., 2011) to brainstem (Nelson and Panksepp, 1998; Challis et al., 2013), to cortical regions (Nasser and McNally, 2012). Even if it is known that the intensity of the appetitive or defensive behavior is modulated by the levels of specific neurotransmitters and neuromodulators (Berridge, 2000; Linfoot et al., 2009; Groppe et al., 2013; Mogi et al., 2014), the precise working of neurons and trafficking of molecules in determining approach and avoidance patterns are yet unclear.

In an attempt to clarify this issue, we selected 3 phenotypes of inbred mice that spontaneously react to conflicting (simultaneously rewarding and threating) stimuli with withdrawing (avoiding [AV] mice), balanced (balancing [BA] mice), or advancing (approaching [AP] mice) responses (Laricchiuta et al., 2012a, 2012b, 2014a, 2016). The presynaptic control of cannabinoid type-1 (CB_1) receptors on GABAergic transmission in the dorsostriatal medium spiny neurons is nearly absent in the AV mice and conversely markedly increased in the AP mice in comparison with BA mice (Laricchiuta et al., 2012b). Further, when compared with BA animals, both AP and AV animals have greater CB_1 receptor density in the amygdaloid nuclei and ventromedial hypothalamic nucleus, and only AP animals have also higher CB_1 receptor functionality in the amygdaloid nuclei and dorsomedial hypothalamic nucleus (Laricchiuta et al., 2012a). Evidence suggests that density and functionality of CB_1 receptors in the corticolimbic, striatal, and cerebellar areas correlate with the levels of brain derived neurotrophic factor (BDNF), an activity-regulated secretory protein with pleiotropic action widely expressed within the previously quoted areas. In fact, BDNF brain levels are lower in mice lacking CB_1 receptors (Aso et al., 2008), whereas CB_1 activation increases BDNF levels in rodents (Butovsky et al., 2005) and humans (D'Souza et al., 2009). The increased BDNF release triggered by CB_1 stimulation mediates in turn the neuroprotective effects of endocannabinoids (Khaspekov et al., 2004). The mutant DISC1 mice, model of schizophrenia-like endophenotype, display impaired preference for social novelty, reduced BDNF receptor levels in prefrontal cortex, and diminished CB_1 expression in hippocampus (Kaminitz et al., 2014). BDNF inhibits CB_1 response in the visual cortex (Huang et al., 2008) and increases the expression of CB_1 receptor transcripts in cultured cerebellar granule neurons (Maison et al., 2009). However, striatal BDNF inhibits CB_1 functionality, and this interplay crucially controls the emotional consequences of stressful or rewarding experiences (Berton et al., 2006; De Chiara et al., 2010). Furthermore, prolonged exposure to palatable food suppresses CB_1 receptor gene expression and reduces BDNF levels (Martire et al., 2014). Interestingly, adult offspring of dams treated with corticosterone and fed a tryptophan-deficient diet show increased avoidance behaviors and anhedonia toward highly palatable reward, reduced striatal and increased hypothalamic BDNF levels, and reduced dopamine and serotonin levels in prefrontal cortex (Zoratto et al., 2013).

Starting from BDNF's role as CB_1 mediator and from observation that endocannabinoid activity is linked to the individual differences in approach and avoidance stable predispositions (Laricchiuta et al., 2012a, 2012b), it is possible to hypothesize that the difference in basal BDNF levels can represent a neurobiological marker of individual differences in approach and avoidance enduring tendencies. Thus, the present research firstly analyzed the endogenous BDNF levels in the 3 AV, BA, and AP phenotypes of mice in frontal cortex, hippocampus, striatum, and cerebellum, regions involved in approach and avoidance stable traits (Laricchiuta et al., 2012a, 2012b; Picerni et al., 2013; Laricchiuta et al., 2014a, 2014b, 2014c, 2016) and expressing high BDNF levels (Angelucci et al., 2009; De Chiara et al., 2010; Caporali et al., 2014; Cutuli et al., 2015). The present results indicate that in comparison with AV and BA mice, AP mice showed higher BDNF levels only in the cerebellum. Subsequently, we thus performed bilateral BDNF infusions in the cerebellar cortical regions to investigate whether even acutely increased cerebellar BDNF levels promoted explorative and novelty-seeking responses, typical components of the approach predisposition.

Methods

Subjects and Experimental Procedure

Male C57BL/6JOlaHsd mice (n = 198; 40 d old at study onset) (Envigo) were housed 4 per cage, with food (Mucedola) and water ad libitum. The mice were kept under a 12-h-light/-dark cycle, controlled temperature (22°C–23°C), and constant humidity (60% ± 5%). All efforts were made to minimize animal suffering and reduce the number of animals used, per the European Directive (2010/63/EU).

The timeline of the experimental procedure is reported in Figure 1. Based on the distribution of responses in the Approach/Avoidance (A/A) Y-maze, we selected AV (n = 5), BA (n = 5), and AP (n = 5) animals. After 2 weeks, the animals were tested in the open field with novel object (OFo) task. To analyze the BDNF role in approach and avoidance stable dispositions, 2 weeks later the animals were killed to determine endogenous BDNF brain levels.

Two weeks after the A/A Y-Maze, other AV (n = 8) and BA (n = 36) mice were bilaterally implanted with guide cannulas into the cerebellar cortical regions. Twenty-four hours later, the AV and BA (n = 21 of 36) animals were injected with phosphate-buffered saline (PBS) (group names: BA-PBS, n = 7; AV-PBS, n = 4) or 0.25 μg/μL PBS/side BDNF (group name: BA-BDNF 0.25, n = 7) or 0.75 μg/μL PBS/side BDNF (group names: BA-BDNF 0.75, n = 7; AV-BDNF 0.75, n = 4). Two hours later, all animals performed the OFo task.

Seventy-two hours after cannula implantations, the remaining BA (n = 15) mice were injected with PBS (n = 5) or 0.25 (n = 5) or 0.75 (n = 5) μg/μL PBS/side BDNF. In parallel, the AV mice were re-injected with PBS or 0.75 μg/μL PBS/side BDNF. Two hours later, all animals were re-tested in the A/A Y-Maze.

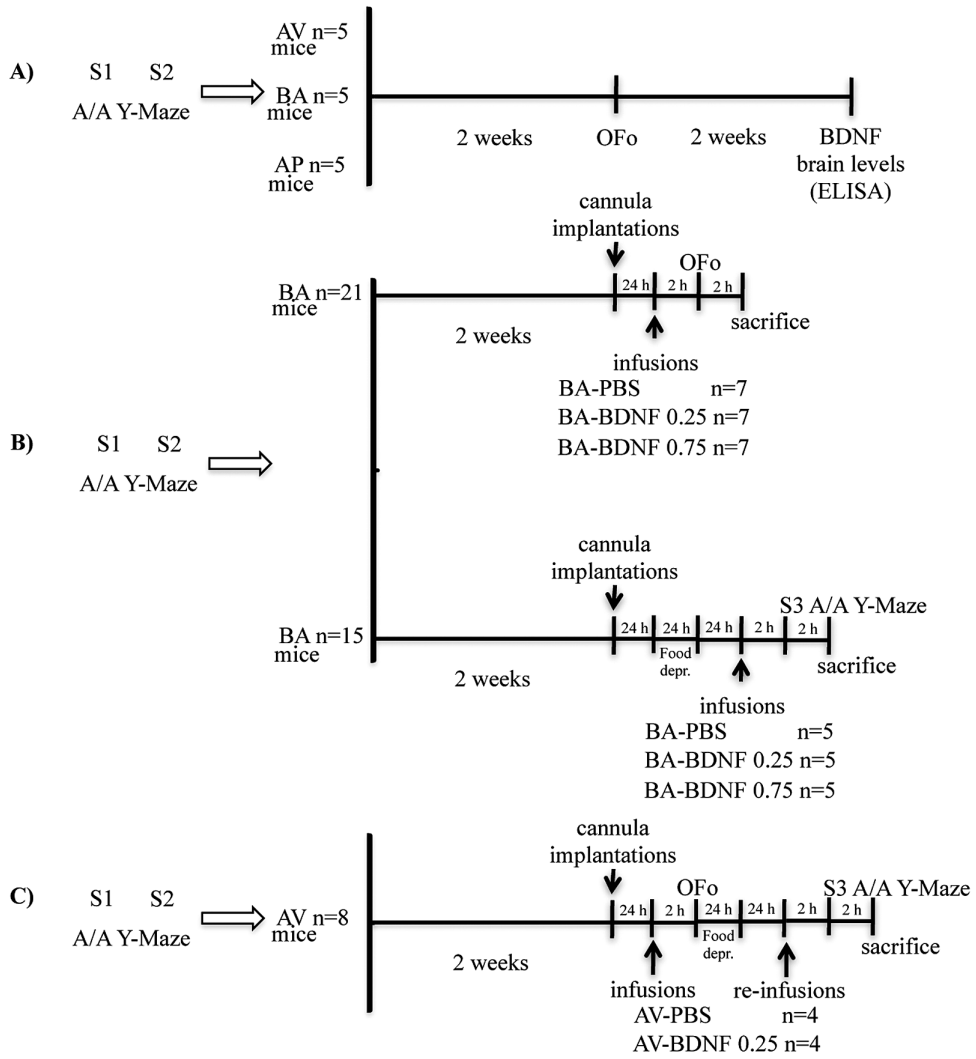

Figure 1. Timeline of the experimental procedure. (A) Avoiding (AV, n = 5), balancing (BA, n = 5), and approaching (AP, n = 5) mice were selected based on the distribution of responses in the session 1 (S1) and session 2 (S2) of the Approach/Avoidance (A/A) Y-maze. Two weeks later, the animals were tested in the Open Field with novel object (OFo) task. Two weeks later the animals were killed to determine endogenous BDNF brain levels through enzyme-linked immunosorbent assay procedure. (B) Other BA (n = 36) mice were bilaterally implanted with guide cannulas into the cerebellar cortical regions. Twenty-four hours later, 21 of 36 BA animals were injected with PBS (BA-PBS, n = 7), 0.25 μg/μL PBS/side BDNF (BA-BDNF 0.25, n = 7), or 0.75 μg/μL PBS/side BDNF (BA-BDNF 0.75, n = 7). Two hours later, the animals performed the OFo task. Seventy-two hours after cannula implantations, the remaining 15 BA mice were injected with PBS (n = 5) or 0.25 (n = 5) or 0.75 (n = 5) μg/μL PBS/side BDNF. Two hours later, the animals were retested in session 3 (S3) of A/A Y-Maze. All BA (n = 36) infused animals were injected with 1 μL/side of PBS containing methylene blue to perform histological control of BDNF injection sites and diffusion. Two hours later, they were killed by decapitation. (C) Other AV (n = 8) mice were bilaterally implanted with guide cannulas into the cerebellar cortical regions. Twenty-four hours later, they were injected with PBS (AV-PBS, n = 4), or 0.75 μg/μL PBS/side BDNF (AV-BDNF 0.75, n = 4). Two hours later, the animals performed the OFo task. Forty-eight hours later, the AV mice were re-injected with PBS or BDNF. Two hours later, the animals were retested in A/A Y-Maze S3. The AV infused-animals were injected with 1 μL/side of PBS containing methylene blue and 2 h later they were killed by decapitation.

To perform histological control of BDNF injection sites and diffusion, the mice infused with PBS or BDNF were injected with 1 μL/side of PBS containing methylene blue, and 2 h later they were killed by decapitation (supplementary Methods; Figure 2).

Behavioral Testing

A/A Y-Maze

The test implemented to select approach/avoidance phenotypes has been previously described (Laricchiuta et al., 2012a, 2014a, 2014b, 2016) and is accurately detailed in the supplementary Methods. The apparatus consisted of a Y-maze with a starting gray arm and 2 choice arms: 1 black and dark, the other one white and lit.

In Session 1 (S1), the slightly food-deprived animal could choose to enter one of the 2 arms, both containing the same standard food reward. During Session 2 (S2), which started 24 h after S1, the white arm was rewarded with a new palatable food (Fonzies, KP Snack Foods) (Bassareo et al., 2002), while the black arm remained rewarded with the standard food. Notably, the S2 of A/A test required to choose between 2 conflicting drives: reaching the new palatable reward placed in an aversive (white and lighted) environment or reaching the familiar standard food placed in a reassuring (black and dark) environment. The slightly food-deprived animals to be re-tested were submitted to a new session (S3) applying the S2 protocol (Laricchiuta et al., 2012b, 2014a).

The parameters considered were: white choices, frequency of entry into the white arm in S1, S2, and S3; A/A conflict index,

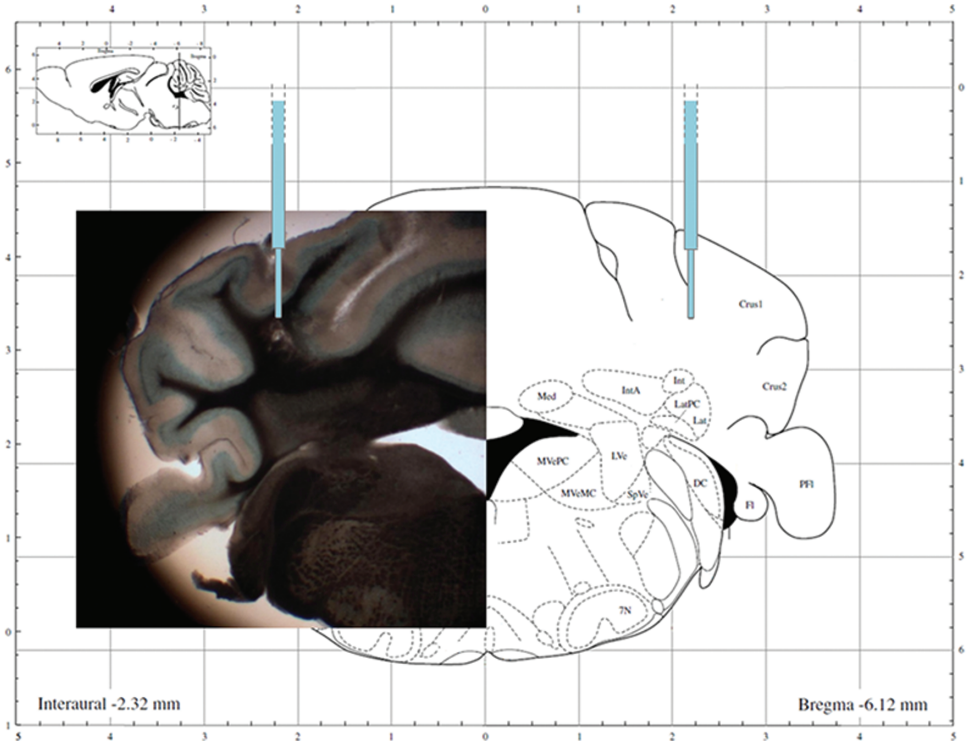

Figure 2. Histological control of bilateral brain derived neurotrophic factor (BDNF) injection sites and diffusion in the cerebellar cortex. A representative photomicrograph (2x magnification) showing the left methylene blue injection site and the cerebellar cortical diffusion is superimposed to a drawing adapted from Franklin and Paxinos (1997). Abbreviations: Med, medial (fastigial) cerebellar nucleus; Int, interpositus cerebellar nucleus; IntA, interpositus cerebellar nucleus, anterior; Lat, lateral (dentate) cerebellar nucleus; LatPC, lateral cerebellar nucleus, parvicellular; PFl, paraflocculus; Fl, flocculus; MVePC, medial vestibular nucleus, parvicellular; MVeMC, medial vestibular nucleus, magnocellular; LVe, lateral vestibular nucleus; SpVe, spinal vestibular nucleus; DC, dorsal cochlear nucleus; 7N, facial nucleus.

the difference in the number of white choices between S2 and S1. Given that this index was normally distributed, it allowed us to identify the 3 AV, BA, and AP phenotypes. In particular, BA animals (22% of mice) showed values in the A/A conflict index corresponding to the mean of the distribution. The 2 tails of the distribution curve represented the few subjects that exhibited responses unbalanced toward one of the conflicting inputs: AV animals (7% of mice) had A/A conflict index values corresponding to -2 SDs of the mean, while AP animals (6% of mice) had values corresponding to + 2 SDs of the mean.

OFo Task

To eliminate the "food" and "palatability" dimensions and maintain the conflicting drives given by an appealing new object placed in an anxiogenic central location of a wide arena, the OFo task (detailed in the supplementary Methods) was used (Laricchiuta et al., 2012b, 2014a). In S1 the animal was allowed to explore an empty 60-cm circular arena, while in S2 an object (a gray plastic cone: 10×6 cm; base diameter: 9.5 cm) was put in the arena center. Notably, the approach to the object requires the subject to overcome its innate fear toward open spaces and indicates thus that the animal is reacting to the mismatch between the initial (empty arena) and new (presence of the object) situations.

The parameters considered were: total distance (in cm) traveled in the arena in each session; peripheral distance, the percentage of total distance traveled in a 6-cm peripheral annulus in each session; central distance, the percentage of total distance traveled in a central circular area (diameter 21.5 cm) in each session; mean velocity in each session; contact time with the object.

BDNF Determination

Tissue Dissection

The AV, BA, and AP mice (n=5/group) were decapitated, and the brains were removed and dissected on ice using a binocular dissection microscope. Frontal cortex, hippocampus, striatum, and cerebellum were bilaterally collected according to Glowinski and Iversen's method (1966). All regions were extracted in 1 mL extraction buffer/100 mg tissue. Brain tissue samples were homogenized in an ice-cold lysis buffer containing 137 mM NaCl, 20 mM Tris-HCl (pH 8.0), 1% NP40, 10% glycerol, 1 mM phenylmethanesulfonylfluoride, 10 mg/mL aprotinin, 1 mg/mL leupetin, and 0.5 mM sodium vanadate. The tissue homogenate solutions were centrifuged at 14000g for 25 minutes at 4°C. The supernatants were collected and stored at -80°C until analysis for quantification of BDNF.

Enzyme-Linked Immunosorbent Assay

BDNF concentrations were assessed using a 2-site enzyme immunoassay kit (G7610 Promega). In brief, 96-well immunoplates (NUNC) were coated with 50 µL/well with the corresponding capture antibody and stored overnight at 4°C. The next day, serial dilutions of known amounts of BDNF ranging from 0 to 500 pg/mL were performed in duplicate to generate a standard curve. The plates were washed 3 times with wash buffer, and the standard curves and supernatants of brain tissue homogenates were incubated in the coated wells (100 µL each) for 2 h at room temperature (RT) with shaking. After additional washes, the antigen was incubated with second specific antibody for 2 h

at RT. The plates were washed again with wash buffer and then incubated with an anti-IgY HRP for 1 h at RT. After another wash, the plates were incubated with a TMB/peroxidase substrate solution for 15 min and phosphoric acid 1 M (100 μL/well) was added to the wells. The colorimetric reaction product was measured at 450 nm using a microplate reader (Dynatech MR 5000). BDNF concentrations were determined from the regression line for the BDNF standard (ranging from 7.8 to 500 pg/mL-purified mice BDNF) incubated under similar conditions in each assay. As declared by the company (Promega), the cross-reactivity with other related neurotrophic factors (NGF, NT-3, and NT-4) was <3%. BDNF concentration was expressed as pg/g wet weight. All assays were performed in triplicate.

Cerebellar BDNF Infusion

Mice were anesthetized by using Zoletil 100 (tiletamine HCl 50 mg/mL + zolazepam HCl 50 mg/mL; Virbac) and Rompun 20 (xylazine 20 mg/mL; Bayer S.p.A) dissolved in a volume of 4.1 mg/mL and 1.6 mg/mL, respectively, in saline and i.p. injected in a volume of 7.3 mL/kg. Mice were mounted onto a stereotaxic frame (David Kopf Instruments) equipped with a mouse adapter and bilaterally implanted with guide cannulas (stainless steel, shaft outer diameter 0.38 mm, Metalant AB) lowered 0.3 mm from the scalp in cerebellar cortical regions. The coordinates from bregma, measured according to the atlas of Franklin and Paxinos (1997) and Mouse Brain Atlases (The Mouse Brain Library, www.nervenet.org), were: AP -6.1; L ±2.2. The guide cannulas were fixed with epoxy glue and dental cement. The length of the guide cannulas was 4.5 mm. Twenty-four hours after the guide cannula implantations, the infusion cannulas (diameter 0.25 mm; Unimed) were bilaterally inserted into the guide cannulas, so that 0.6 mm of the infusion cannula extended past the end of the guide cannula. According to Saylor and McGinty (2010), human recombinant BDNF (Tocris Bioscience, R & D Systems) at 2 concentrations (0.25 or 0.75 μg/μL PBS/side) or sterile PBS was infused into cerebellar regions by using 10-μL Hamilton syringes and an infusion pump (Harvard Apparatus). Volumes of 1 μL/side of BDNF or PBS were infused over 5 min, and the infusion cannulas remained in the guide cannulas for 5 min before and after the infusion. Two hours later, mice were submitted to behavioral testing.

Statistical Analysis

Data presented as mean ± SEM were tested for normality (Will-Shapiro's test) and homoscedasticity (Levene's test). Behavioral and neurochemical data were compared by using ANOVAs, followed by Tukey's HSD test when appropriate. When data did not fully meet parametric assumptions, nonparametric analyses (Friedman ANOVA, Wilcoxon Signed Rank Test, and Mann–Whitney U) were used. Linear regression analyses were run to determine the associations between cerebellar BDNF levels and A/A conflict index and white choices in the S2 of A/A Y-Maze, or distances, velocity, and contact time with the novel object in the OFo task sessions. The differences were considered significant at the $P < .05$ level.

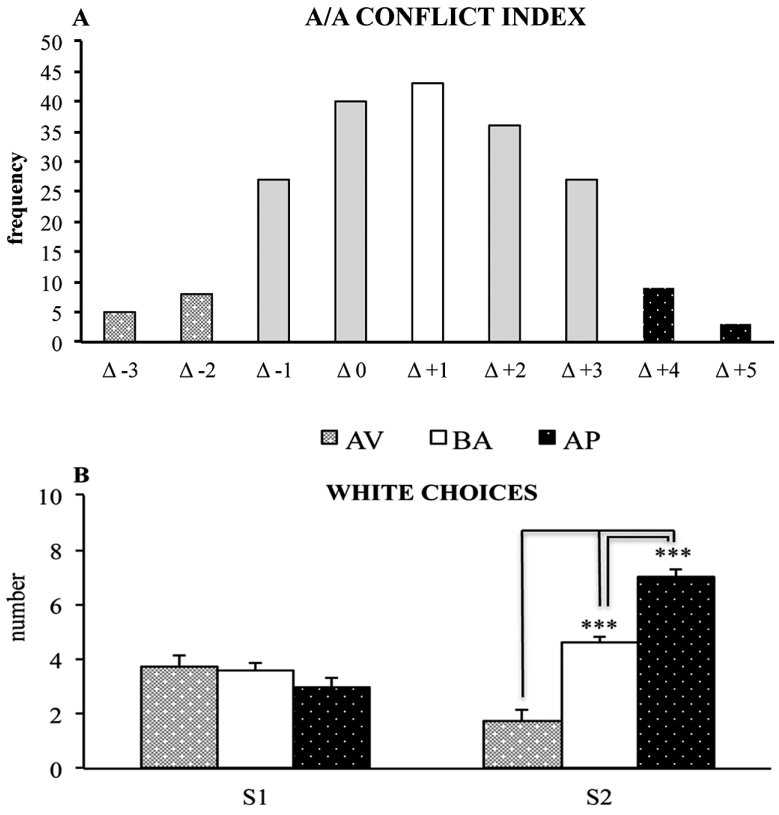

Figure 3. Approach/Avoidance (A/A) Y-Maze performances of avoiding (AV), balancing (BA), and approaching (AP) mice. (A) The curve of distribution of the A/A conflict index (the difference [Δ] in the number of white choices between session 2 [S2] and 1 [S1]) indicates that the white choice frequency increased (mean = Δ + 1). (B) The white choices were similar among phenotypes in S1, while AV mice showed the lowest number of white choices (***P = .0002), and BA mice showed a number of white choices lower than AP mice (***P = .0002) in S2. Between S1 and S2, the number of white choices within groups was different (***P = .0002), given that it decreased in the AV mice and increased in the BA and AP mice. In B, data are presented as means ± SEM.

Results

A/A Y-Maze Performances of AV, BA, and AP Mice

The A/A conflict index was normally distributed (Figure 3A), and its bell-shaped curve indicated that in S2 the new palatable food, even if placed in the aversive white environment, was salient enough to increase white choices number.

When white choices in S1 and S2 were analyzed in relation to the phenotype of the animals (Figure 3B), a 2-way ANOVA (phenotype x session) revealed significant phenotype ($F_{2,12}$=12.93, P=.001) and session ($F_{1,12}$=14.3, P=.001) effects. The interaction was significant ($F_{2,12}$=42.0, P=.0001). Posthoc comparisons on interaction revealed no significant differences in S1 among AV, BA, and AP mice. In S2, while AV mice showed the lowest number of white choices (always P=.0002), BA mice showed a number of white choices lower than AP mice (P=.0002). Between S1 and S2, the number of white choices was significantly different (always P=.0002) in the 3 phenotypes, given that it decreased in the AV mice and increased in the BA and AP mice.

OFo Performances of AV, BA, and AP Mice

AP mice were significantly more active and explorative than AV and BA animals. Two-way ANOVA (phenotype × session) on total distances (Figure 4A) revealed a significant phenotype effect ($F_{2,12}$=8.82, P=.004), while session effect ($F_{1,12}$=2.67, P=.13) and interaction ($F_{2,12}$=3.19, P=.08) were not significant. Posthoc comparisons on phenotype effect revealed that AP animals explored the environment more actively than BA (P=.003) and AV (P=.05) animals. Two-way ANOVA (phenotype × session) on peripheral distances (Figure 4B) revealed no significant phenotype effect ($F_{2,12}$=1.30, P=.31), while session effect ($F_{1,12}$=187.66, P<.00001) was significant. Interaction ($F_{2,12}$=1.43, P=.28) was not significant. Two-way ANOVA (phenotype × session) on central distances (mean ± SE: AV: S1 12.5 ± 1.9, S2 41.0 ± 5.0; BA: S1 10.5 ± 2.5, S2 49.8 ± 2.9; AP: S1 13.7 ± 3.7, S2 47.6 ± 2.3) showed no significant phenotype effect ($F_{2,12}$=1.39, P=.29), while session effect ($F_{1,12}$=194.62, P<.00001) was significant. Interaction ($F_{2,12}$=1.66, P=.23) was not significant. Two-way ANOVA (phenotype × session) on velocity (Figure 4C) revealed a significant phenotype effect ($F_{2,12}$=8.88, P=.004), while session effect ($F_{1,12}$=2.70, P=.13) and interaction ($F_{2,12}$=3.31, P=.07) were not significant. Posthoc comparisons on phenotype effect revealed that AP animals were more rapid than BA (P=.003) and AV (P=.05) animals. One-way ANOVA on contact time with the novel object (Figure 4D) revealed a significant phenotype effect ($F_{2,12}$=15.46, P=.0005). In fact, the AP animals contacted the novel object longer than BA (P=.003) and AV (P=.007) animals.

Brain BDNF Determination in AV, BA, and AP Mice

One-way ANOVA on BDNF levels in the cerebellum revealed a significant phenotype effect ($F_{2,12}$=6.64, P=.01). Posthoc comparisons revealed that AP animals exhibited the highest BDNF

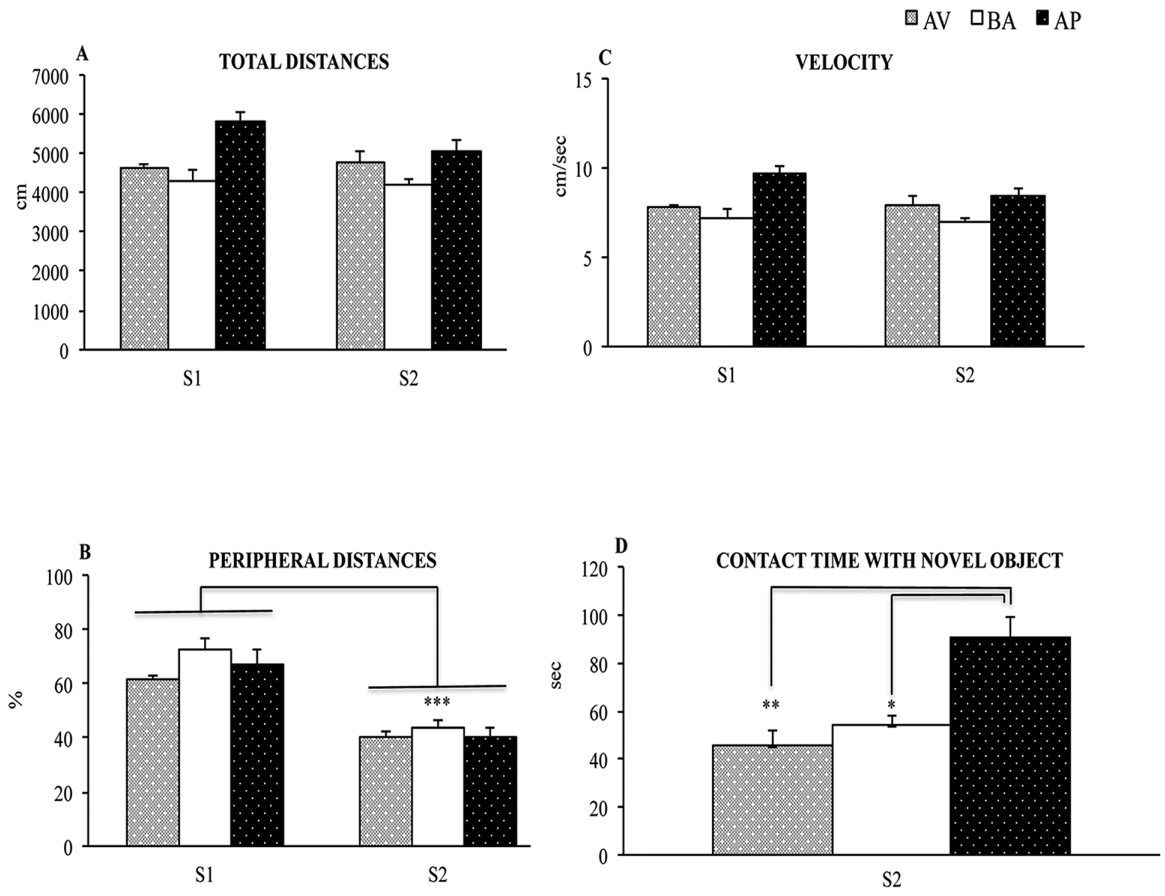

Figure 4. Open Field with object (OFo) performances of avoiding (AV), balancing (BA), and approaching (AP) mice. Total distances (A), peripheral distances (B), and velocity (C) in session 1 (S1) and 2 (S2) are reported. Peripheral distances (B) decreased between S1 and S2 (***P=.001). AP animals were more active and rapid in exploring the environment (A, C), and contacted the novel object (D) longer than BA (**P=.003) and AV (*P=.007) animals. Data are presented as means ± SEM.

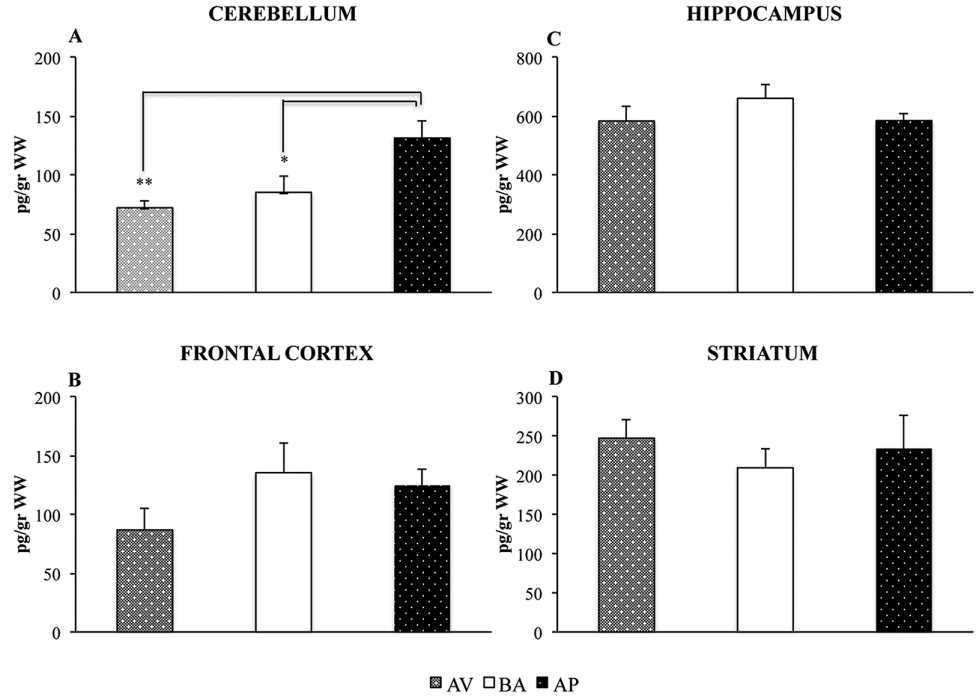

Figure 5. Brain derived neurotrophic factor (BDNF) determination in avoiding (AV), balancing (BA), and approaching (AP) mice in the cerebellum (A), frontal cortex (B), hippocampus (C), and striatum (D). AP animals showed the highest cerebellar BDNF levels (*P=.05; **P=.01). Data are presented as means ± SEM and values are expressed in pg/g of wet weight (WW).

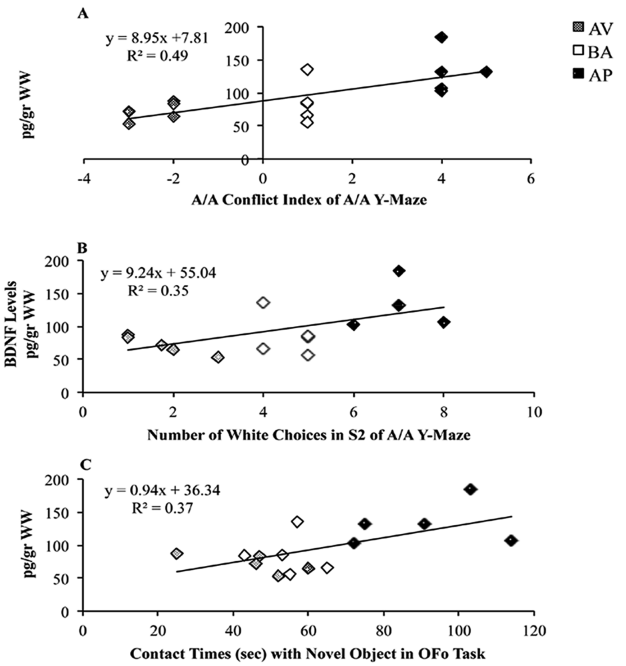

Figure 6. Linear regressions between cerebellar brain derived neurotrophic factor (BDNF) levels and behavioral data in avoiding (AV), balancing (BA), and approaching (AP) mice. A positive significant association was found between cerebellar BDNF levels and conflict index of the Approach/Avoidance (A/A) Y-Maze (A). A positive association was found between cerebellar BDNF levels and number of white choices in the session 2 (S2) of the A/A Y-Maze (B). Positive association was also found between cerebellar BDNF levels and contact times with the novel object in the Open Field (OFo) task (C). In the scatterplots are reported the linear fits (solid black lines), equations and R^2.

cerebellar levels (AP vs BA: P=.05; AP vs AV: P=.01; BA vs AV: P=.73) (Figure 5A). Conversely, 1-way ANOVAs on BDNF levels in the frontal cortex ($F_{2,12}$=1.63, P=.24), hippocampus ($F_{2,12}$=1.13, P=.35), and striatum ($F_{2,12}$=0.38, P=.69) failed to reveal any significant difference among the 3 phenotypes (Figure 5B-D).

Linear Regressions between Cerebellar BDNF Levels and Behavioral Data in AV, BA, and AP Mice

Positive significant associations were found between cerebellar BDNF levels and A/A conflict index (ß=0.69, $F_{1,14}$=12.03, P=.0041), number of white choices in the S2 of the A/A Y-Maze (ß=0.59, $F_{1,14}$=6.89, P=.02), and contact times with the novel object (ß=0.61, $F_{1,14}$=7.83, P=.01) in the OFo task (Figure 6A-C). No significant associations were found between cerebellar BDNF levels and total (S1: ß=0.47, $F_{1,14}$=3.65, P=.08; S2: ß=0.21, $F_{1,14}$=0.63, P=.44), peripheral (S1: ß=0.33, $F_{1,14}$=1.55, P=.23; S2: ß=0.09, $F_{1,14}$=0.11, P=.75), and central (S1: ß=0.38, $F_{1,14}$=1.75, P=.28; S2: ß=0.17, $F_{1,14}$=0.18, P=.74) distances, as well as mean velocity (S1: ß=0.47, $F_{1,14}$=3.72, P=.07; S2: ß=0.21, $F_{1,14}$=0.62, P=.45).

OFo Performances of BA Mice Infused with BDNF into the Cerebellar Cortices

BA animals infused with 0.25 or 0.75 µg/µL PBS/side BDNF were significantly more active than BA animals bilaterally infused with PBS, and BA animals infused with 0.75 µg/µL PBS/side BDNF contacted the object significantly longest. Two-way ANOVA (group × session) on total distances (Figure 7A) revealed significant group ($F_{2,18}$=5.89, P=.01) and session ($F_{1,18}$=5.47, P=.03) effects. The interaction ($F_{2,18}$=1.61, P=.22) was not significant. Posthoc comparisons on group effect revealed that the BA-PBS group was less active in moving into the arena than the BA-BDNF

0.25 ($P=.02$) and BA-BDNF 0.75 ($P=.01$) groups. Two-way ANOVA (group × session) on peripheral distances (Figure 7B) showed no significant group effect ($F_{2,18}=1.41$, $P=.27$), while session effect ($F_{1,18}=104.85$, $P<.00001$) was significant. Interaction ($F_{2,18}=2.15$, $P=.15$) was not significant. Two-way ANOVA (group × session) on central distances (mean ± SE: BA-PBS: - S1 9.4 ± 1.4, - S2 41.9 ± 0.7; BA-BDNF 0.25: - S1 11.5 ± 0.8, - S2 30.7 ± 2.6; BA-BDNF 0.75: - S1 12.0 ± 1.5, - S2 40.9 ± 6.0) showed no significant phenotype effect ($F_{2,12}=2.07$, $P=.16$), while session effect ($F_{1,12}=6522.00$, $P<.00001$) was significant. Interaction ($F_{2,12}=3.02$, $P=.08$) was not significant.

Two-way ANOVA (group × session) on velocity (Figure 7C) showed significant group ($F_{2,18}=6.03$, $P=.01$) and session ($F_{1,18}=5.78$, $P=.03$) effects. The interaction ($F_{2,18}=1.39$, $P=.27$) was not significant. Posthoc comparisons on group effect revealed that the BA-PBS group was less rapid in moving into the environment than the BA-BDNF 0.25 ($P=.02$) and BA-BDNF 0.75 ($P=.01$) groups. One-way ANOVA on contact time with the novel object (Figure 7D) was significant ($F_{2,18}=6.16$, $P=.01$), with the BA-BDNF 0.75 group contacting the novel object more than the BA-BDNF 0.25 and BA-PBS (always $P=.02$) groups.

A/A Y-Maze Performances of BA Mice Infused with BDNF into the Cerebellar Cortices

BA animals were infused with PBS or 0.25 or 0.75 μg/μL PBS/side BDNF before S3. When white choices were analyzed (Figure 8), a 2-way ANOVA (group x session) revealed no significant group effect ($F_{2,12}=2.90$, $P=.09$), while the session effect ($F_{1,12}=12.26$, $P=.0002$) was significant. The interaction ($F_{4,24}=2.81$, $P=.05$) was significant. Posthoc comparisons on significant interaction revealed no significant difference among groups in S1 and S2, while in S3, BA-BDNF 0.75 mice showed a number of white choices similar to the BA-BDNF 0.25 group ($P=.72$) and increased compared with the BA-PBS group ($P=.05$). The number of white choices was similar between BA-BDNF 0.25 and BA-PBS groups ($P=.72$).

OFo Performances of AV Mice Infused with BDNF into the Cerebellar Cortices

AV animals infused with 0.75 μg/μL PBS/side BDNF were significantly more active and contacted the object significantly longer than AV animals bilaterally infused with PBS. Nonparametric analyses (Mann–Whitney U) on total distances (Figure 9A) revealed a significant difference between groups in S1 ($U=0$, $P=.02$) but not in S2 ($U=8$, $P=1$). Mann–Whitney U test on peripheral distances (Figure 9B) revealed no significant difference between groups in S1 ($U=4$, $P=.25$) and a significant difference in S2 ($U=0$, $P=.02$). Mann–Whitney U test on central distances (mean ± SE: AV-PBS: - S1 4.0 ± 0.7, - S2 37.9 ± 5.2; AV-BDNF 0.75: - S1 6.9 ± 2.5, - S2 38.6 ± 1.8) revealed no significant differences between groups in S1 ($U=6$, $P=.56$) and S2 ($U=8$, $P=1$). Mann–Whitney U test on mean velocity (Figure 9C) revealed a significant difference between groups in S1 ($U=0$, $P=.02$) but not in S2 ($U=8$, $P=1$). Mann–Whitney U test on contact time with the novel object (Figure 9D) revealed a significant difference between groups ($U=1$, $P=.05$).

As regards the AV-PBS group, Wilcoxon Signed Rank Test on total, peripheral, and central distances as well as mean velocity revealed significant differences between S1 and S2 (always $P=.05$) (Figure 9A-C). As regards the AV-BDNF 0.75 groups, Wilcoxon Signed Rank Test showed that peripheral and central distances were significantly different between S1 and S2 (always $P=.05$) (Figure 9B).

A/A Y-Maze Performances of AV Mice Infused with BDNF into the Cerebellar Cortices

AV animals were re-infused with PBS or 0.75 μg/μL PBS/side BDNF before S3. When white choices were analyzed (Figure 10), Mann–Whitney U test revealed no significant differences in S1 ($U=2$, $P=.08$) and S2 ($U=2$, $P=.08$), while a significant difference between groups was found in S3 ($U=1$, $P=.04$).

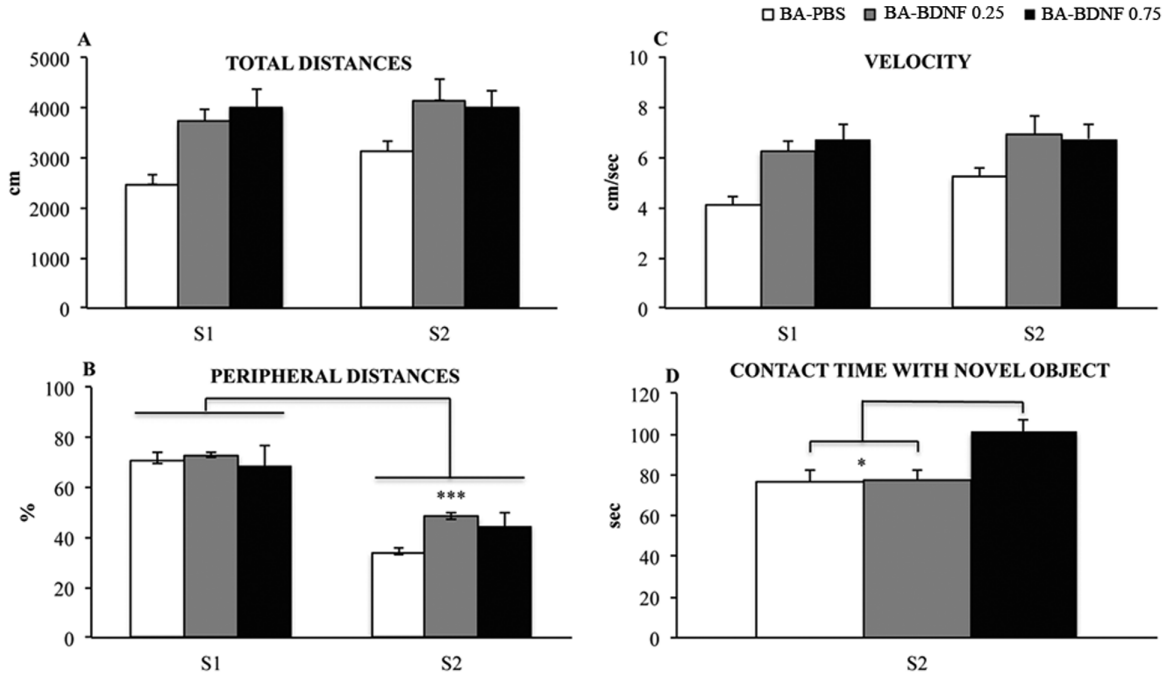

Figure 7. Open Field with novel object (OFo) performances of balancing (BA) brain derived neurotrophic factor (BDNF)-infused mice into the cerebellar cortices. Total distances (A), peripheral distances (B), and velocity (C) are reported. Animals infused with 0.25 (BA-BDNF 0.25) or 0.75 (BA-BDNF 0.75) μg/μL PBS/side BDNF were more active and rapid in exploring the environment in comparison to BA-PBS animals. The BA-BDNF 0.75 group contacted the novel object longer (D) than the BA-BDNF 0.25 and BA-PBS (*$P=.02$) groups. Data are presented as means ± SEM.

Friedman ANOVAs on white choices revealed significant differences among S1, S2, and S3 in AV-PBS ($P = .02$) and AV-BDNF 0.75 groups ($P = .02$).

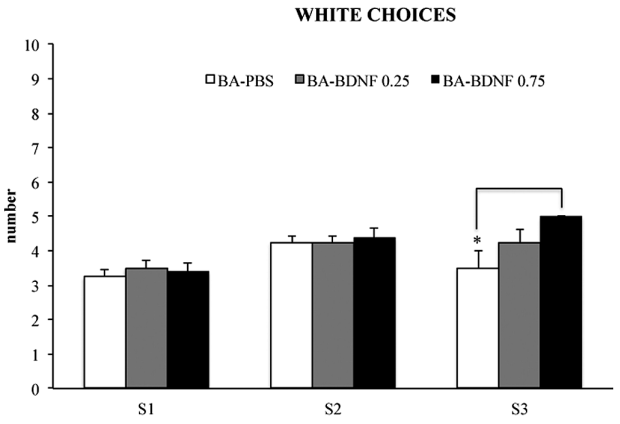

Figure 8. Approach/Avoidance (A/A) Y-Maze performances of balancing (BA) brain derived neurotrophic factor (BDNF)-infused mice into the cerebellar cortices. The white choices were similar among groups in session 1 (S1) and session 2 (S2), while the session 3 (S3) animals infused with 0.75 μg/μL PBS/side BDNF (BA-BDNF 0.75) showed a number of white choices similar to animals infused with 0.25 μg/μL PBS/side BDNF (BA-BDNF 0.25), but higher (*$P = .05$) than BA-PBS animals. Data are presented as means ± SEM.

Discussion

According to Elliot (2008), a subject characterized by a stable predisposition of approach is explorative, curious, and seeks novelty. In fact, the AP mice were more active, rapid, and prone to explore the OFo arena by travelling longer distances in comparison with the BA and AV mice. Notably, the longest distances moved by the AP animals were not related to anxious behaviors, given that in all animals the percentages of peripheral and central distances were similar and decreased between S1 and S2. Even more importantly, the AP animals made contact with the novel object longer than BA and AV animals. To verify whether the basal BDNF levels were associated with a specific and stable predisposition to approach or avoidance, the BDNF levels in frontal cortex, hippocampus, striatum, and cerebellum were evaluated in the 3 phenotypes. These analyses were made at a time-point distant from any behavioral testing to rule out any acute effect of the behavioral performance on BDNF levels. Interestingly, the AP mice showed the highest cerebellar BDNF levels. Regression analyses indicated that the basal cerebellar BDNF levels were positively associated with the conflict index and white choices in the S2 of the A/A Y-Maze (when the aversive white environment was rewarded with the new palatable food, thus making it worth risking to enter the "threatening and anxiogenic" white and lighted arm to obtain the reward). Furthermore, the cerebellar BDNF levels were positively associated with the contact times with the novel object placed in the "threatening and anxiogenic" central location of OFo arena. Our

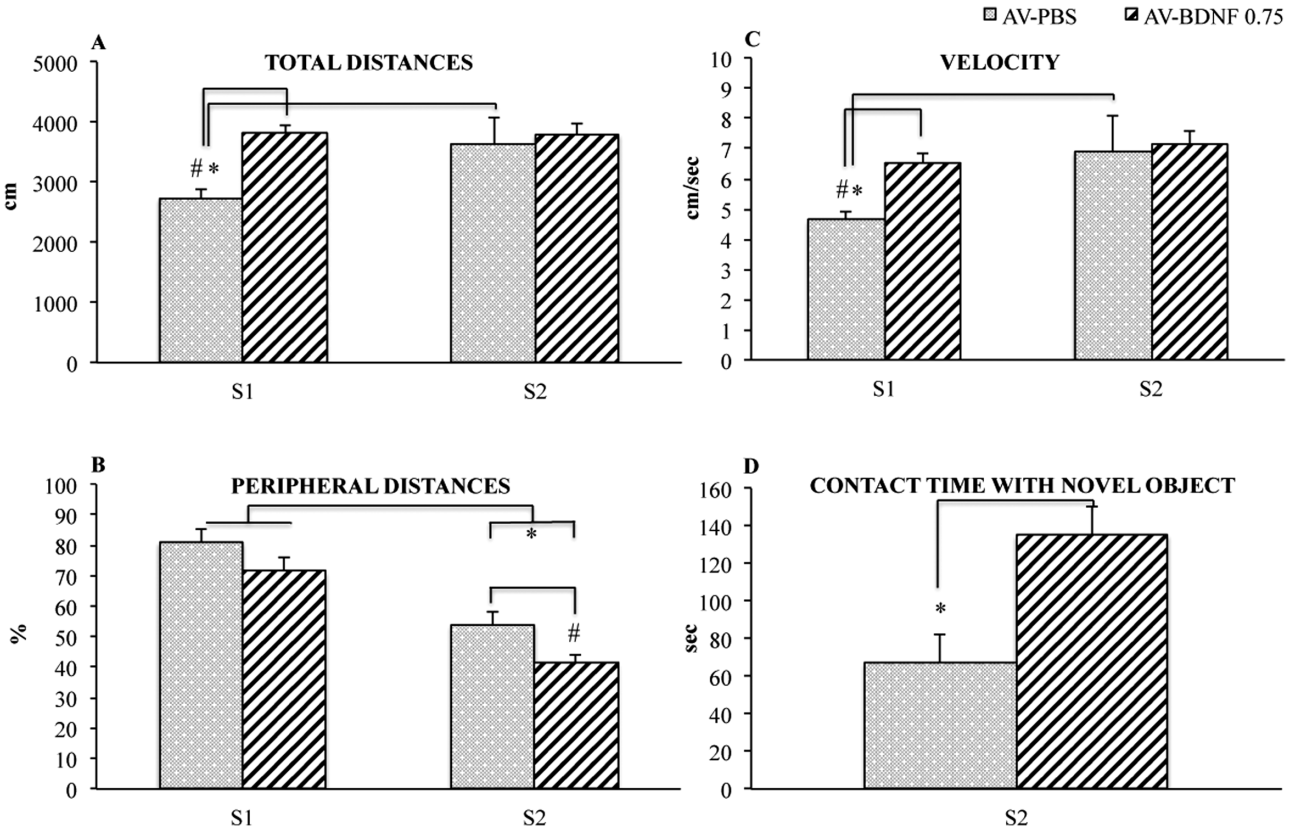

Figure 9. Open Field with novel object (OFo) performances of avoiding (AV) brain derived neurotrophic factor (BDNF)-infused mice into the cerebellar cortices. Total distances (A), peripheral distances (B), velocity (C), and contact time with the novel object (D) in session 1 (S1) and session 2 (S2) are reported. AV animals infused with 0.75 μg/μL PBS/side BDNF (AV-BDNF 0.75) were more active (#$P = .02$) and rapid (#$P = .02$) in exploring the environment and contacted the object longer (*$P = .05$) than AV-PBS animals. In the AV-PBS group, total and peripheral distances and mean velocity were significantly different between S1 and S2 (*$P = .05$). In the AV-BDNF 0.75 group, peripheral distances were significantly different between S1 and S2 (*$P = .05$). Data are presented as means ± SEM.

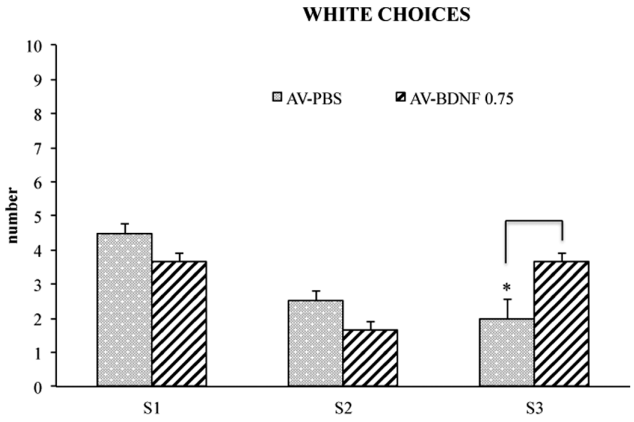

Figure 10. Approach/Avoidance (A/A) Y-Maze performances of avoiding (AV) mice brain derived neurotrophic factor (BDNF) re-infused into the cerebellar cortices before session 3 (S3). The AV-BDNF 0.75 group showed a higher number of white choices than the AV-PBS group (*P = .04). Data are presented as means ± SEM.

findings indicate that high cerebellar BDNF levels may represent a biomarker associated with stable predisposition to approach in its 2 components of exploration and search for novelty. These data are consistent with the observations that the endogenous concentrations and signaling of BDNF are associated with individual differences in specific temperamental traits and dispositions (Okuno et al., 2011; Duclot and Kabbaj, 2013; Yasui-Furukori et al., 2013). It was recently reported in healthy individuals a coupling between anxiety temperamental traits and basal resting blood flow in fronto-limbic circuitry, determined in part by genetically mediated BDNF signaling (Wei et al., 2017). Furthermore, the individuals with at least 1 copy of the methionine allele in the BDNF gene show increased predisposition to anxious and depressive behaviors (Gatt et al., 2009; Terracciano et al., 2010; Verhagen et al., 2010; Minelli et al., 2011). They also exhibit increased positive mood, lower perceived exertion, and increased motivation during exercise (Bryan et al., 2007, 2013; Caldwell Hooper et al., 2014). By using low and high exploratory mice differing in their OF exploratory behavior (Kazlauckas, 2005), Kazlauckas et al. (2011) demonstrated that low exploratory mice show less retention in the inhibitory avoidance and lower BDNF levels in the hippocampus (unfortunately the only brain area taken into account).

Nevertheless, it must be taken into account that BDNF is associated not only with stable temperamental predispositions but also with the ongoing behavioral performance. Namely, exercise acutely increases BDNF levels (Adlard et al., 2005; Ferris et al., 2007) and improves BDNF transcription (Oliff et al., 1998), thereby improving cognitive function (Berchtold et al., 2005, 2010). In parallel, single intraventricular injections of BDNF elicit anticataleptic effects (Naumenko et al., 2012; Tikhonova and Kulikov, 2012; Kulikov et al., 2014), and BDNF administration attenuates behavioral responses to stress (Schmidt and Duman, 2010; Ye et al., 2011).

Returning to the functions of BDNF in the cerebellar system, it has been demonstrated that BDNF transgene improves motor behavior in mutant mice characterized by severe cerebellar ataxia (Meng et al., 2007), and that BDNF is implicated in the cerebellar long-term plasticity induced by the environmental enrichment (Angelucci et al., 2009; Vazquez-Sanroman et al., 2013). It is noteworthy that the offspring of pre-reproductively enriched female rats show early maturation of complex motor abilities and increased cerebellar (and striatal) BDNF levels (Caporali et al., 2014).

Accordingly, after providing the indication that the individual differences in the inherent predisposition to approach were associated with the cerebellar BDNF levels, in the present study it was needed to verify whether the cerebellar BDNF levels were causally related with the acute response of approach, in its double component of exploration and novelty-seeking. BDNF-infused BA and AV animals were more explorative, rapid, and approaching toward novelty and reward than PBS-infused animals.

Overall, these data demonstrate that the approach behavior, in its 2 components of search for novelty and exploration, is a BDNF-mediated cerebellar process. The involvement of cerebellar system in the approach is an intriguing, but not unforeseeable, outcome. Besides motor coordination and learning, cerebellar system has been functionally implied in cognitive, emotional, and motivational processes (Ito, 2006; Zhu et al., 2006; De Smet et al., 2013; Laricchiuta et al., 2015), and more importantly (with regard to the present issue) in neural substrates of temperamental individual differences (Wei et al., 2011; Picerni et al., 2013; Laricchiuta et al., 2014b; Petrosini et al., 2015, 2016). It was asserted that the cerebellum is the site where new and familiar stimuli are compared to detect discordances, and where the novelty-related information is processed more and more efficiently and adaptively (Restuccia et al., 2007; O'Reilly et al., 2008). In accordance with the cerebellar functions of error/novelty detection and internal model formation, Ito (2008, 2013) proposed that the cerebellum may alert the prefrontal cortex about the absence of internal models matching the novel information, maintain the newly generated internal models, and incorporate them into routine schemes of thought. Also, its reciprocal connections with basal ganglia (Hoshi et al., 2005; Centonze et al., 2008; Rossi et al., 2008; Bostan et al., 2010) allow the cerebellum to influence reward-driven behavior and to process information related to motivational valence linked in turn to novelty detection and seeking. Not by chance, cerebellar BDNF infusions in AV and BA mice increased their approaching behavior toward the new palatable food. Crucially, in healthy individuals we found cerebellar volumes associated positively with Novelty Seeking scores and negatively with Harm Avoidance scores of the Temperament and Character Inventory by Cloninger (1986, 1987) (Picerni et al., 2013; Laricchiuta et al., 2014b).

Beyond seeking novelty, the cerebellar system has even been linked to the other component of approach, the explorative behavior that, by requiring close integration between environmental (sensory) information and searching (motor) acts, involves the sensory-motor role classically attributed to cerebellar networks. Several studies reported explorative deficits and spatial difficulties following cerebellar damage (Petrosini et al., 1996; 1998; Noblett and Swain, 2003; Molinari et al., 2004). In particular, hemicerebellectomized rats exhibit reduced exploration in the OF (Mandolesi et al., 2003), and cerebellar mutant mice (Rora(sg), Nna1(pcd-1J), nervous, Lurcher) exhibiting degeneration of cerebellar cortex or dentate nucleus or selective Purkinje cell loss display reduced exploration (Lalonde et al., 1988a, 1988b; Caston et al., 1998; Lalonde and Strazielle, 2003). Even in humans, the link between cerebellar function and exploration has been reported (Pierce and Courchesne, 2001; Kawa and Pisula, 2010). High scores in the exploratory excitability subscale of the novelty seeking scale were related with micro-structural variations in cerebellar lobules IV, V, and VI (Picerni et al., 2013). However, despite the interest of these human findings, it is still to be clarified whether specific functions associated with individual differences determine the cerebellar regional structure or conversely, the different structure determines the specific functions. Through the present experimental approach, we provide evidence for the existence of a link between BDNF-mediated

cerebellar processing and behaviors of exploration and novelty seeking. Even if an analysis of the possible BDNF action mechanisms on cerebellar processing is beyond the scope of the present paper, it is important to note that the BDNF has been recognized as a crucial modulator of synaptic plasticity in the adult brain (Alkadhi, 2017). By binding the extracellular domain of tyrosine kinase B receptor, BDNF may enhance the quantal release of glutamate and the functionality of NMDA receptors, acting pre- and postsynaptically, respectively (Lista and Sorrentino, 2010). These mechanisms influence various downstream signaling molecules involved in calcium entry and actin polymerization, improving structural plasticity and brain functionality (Vasuta et al., 2007).

In addition to the various events related to BDNF levels, including movement, physical activity, and exploration of a novel environment (Vaynman et al., 2004; Berchtold et al., 2010; Sleiman et al., 2016), here we propose that the endogenous basal or acutely increased cerebellar BDNF levels promote the responses of exploration and novelty seeking, components that sustain the approach pattern.

Supplementary Material

Supplementary data are available at *International Journal of Neuropsychopharmacology* online.

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Statement of Interest

None.

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