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Chronic Administration of the Neurotrophic Agent Cerebrolysin Ameliorates the Behavioral and Morphological Changes Induced by Neonatal Ventral Hippocampus Lesion in a Rat Model of Schizophrenia

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Neonatal ventral hippocampal lesion (nVHL) in rats has been widely used as a neurodevelopmental model to mimic schizophrenia-like behaviors. Recently, we reported that nVHLs result in dendritic retraction and spine loss in prefrontal cortex (PFC) pyramidal neurons and medium spiny neurons of the nucleus accumbens (NAcc). Cerebrolysin (Cbl), a neurotrophic peptide mixture, has been reported to ameliorate the synaptic and dendritic pathology in models of aging and neurodevelopmental disorder such as Rett syndrome. This study sought to determine whether Cbl was capable of reducing behavioral and neuronal alterations in nVHL rats. The behavioral analysis included locomotor activity induced by novel environment and amphetamine, social interaction, and sensoriomotor gating. The morphological evaluation included dendritic analysis by using the Golgi-Cox procedure and stereology to quantify the total cell number in PFC and NAcc. Behavioral data show a reduction in the hyperresponsiveness to novel environment- and amphetamine-induced locomotion, with an increase in the total time spent in social interactions and in prepulse inhibition in Cbl-treated nVHL rats. In addition, neuropathological analysis of the limbic regions also showed amelioration of dendritic retraction and spine loss in Cbl-treated nVHL rats. Cbl treatment also ameliorated dendritic pathology and neuronal loss in the PFC and NAcc in nVHL rats. This study demonstrates that Cbl promotes behavioral improvements and recovery of dendritic neuronal damage in postpubertal nVHL rats and suggests that Cbl may have neurotrophic effects in this neurodevelopmental model of schizophrenia. These findings support the possibility that Cbl has beneficial effects in the management of schizophrenia symptoms.

Key words: cerebrolysin; neonatal ventral hippocampal lesion; schizophrenia; prefrontal cortex; nucleus accumbens; Golgi-Cox stain; stereology

Rats with a bilateral neonatal ventral hippocampal (nVH) lesion are a widely used heuristic neurodevelopmental animal model for studying schizophrenia and have been reported to mimic many schizophrenia-like behaviors (for review see Tseng et al., 2009). These rats exhibit behavioral changes that manifest themselves fully only after puberty (Lipska and Weinberger, 2000; Marcotte et al., 2001), with normal behaviors at a prepubertal age. Behavioral changes include locomotor hyperresponsiveness to stress (Lipska et al., 1993; Flores et al., 1996a; Silva-Gomez et al., 2003a; Alquicer et al., 2008), deficits in social interaction (Sams-Dodd et al., 1997; Flores et al., 2005b), sensorimotor gating (Le Pen and Moreau, 2002; Le Pen et al., 2003b), spatial learning and working memory problems (Chambers et al., 1996; Silva-Gómez et al., 2003a), and decreased attention (Le Pen et al., 2003a). In addition to these behavioral alterations, multiple neurochemical, molecular, and morphological changes have been reported in these

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rats, including low levels of brain-derived neurotrophic factor (BDNF; Lipska et al., 2001; Molteni et al., 2001) and nerve growth factor-inducible B (NGFB; Bhardwaj et al., 2003) and atrophy of prefrontal cortex (PFC) pyramidal neurons and medium spiny neurons of the nucleus accumbens (NAcc; Flores et al., 2005a; Alquicer et al., 2008). All of these changes suggest that a deficit in neurotrophic factors may participate in the development of functional alterations between hippocampus and PFC, interconnecting neural circuits implicated in several aspects of memory and cognition (for review see Thierry et al., 2000). Together with these changes, high levels of histone deacetylases (HDAC) have been reported in the PFC of nVH-lesioned rats (Sandner et al., 2011).

A recent report suggests that excitotoxic lesions of the nVH with ibotenic acid led to significant and persistent astrogliosis and microglial activation associated with the production of inflammatory mediators (Drouen-Ouellet et al., 2011). In opposition, cerebrolysin (Cbl), a peptide preparation produced by the biotechnological process using enzymatic breakdown of purified porcine brain proteins displaying a neuroimmunetrophic activity, reduces the microglial activation, restraining the inflammatory process (Alvarez et al., 2000). In addition, many of beneficial effects of Cbl administration are thought to be related to its ability to mimic the action of neurotrophic factors (Veinbergs et al., 2000; Tatebayashi et al., 2003; Zhang et al., 2010). This neurotrophic action mediated by Cbl has been shown to interfere with excitotoxicity, free radical formation, and inflammatory responses (González et al., 1998; Hutter-Paier et al., 1998; Veinbergs et al., 2000). In vitro studies have shown that Cbl can counteract the destructive effects of glutamate, resulting in increased neuronal viability (Hutter-Paier et al., 1996, 1998; Riley et al., 2006). In a murine model of neurodegeneration induced by injection of the glutamate analogue kainic acid, pretreatment with Cbl supported integrity of the dendritic morphology in the hippocampus and neocortex (Veinbergs et al., 2000). Our recent data (Juarez et al., 2011) suggest that Cbl may improve the dendritic length and dendritic spine density of the PFC pyramidal neurons in age mice.

The present study assessed the effect of Cbl administration from postnatal day (PD) 30 to PD60 on behavioral, morphological, and immunohistochemical changes reported in the nVH lesion animal model. The behavioral analysis included locomotor activity induced by novel environment and amphetamine, social interaction, and sensorimotor gating. The morphological evaluation included analysis of the dendrites using the Golgi-Cox procedure and stereology to quantify the total cell number in three regions: PFC, NAcc, and caudate-putamen (CPu). Finally, immunohistochemical analysis using antibodies against tyrosine hydroxylase (TH) was performed for the NAcc to examine the effects of Cbl treatment on axonal projections of DA neurons to these regions.

MATERIALS AND METHODS

Animals

The protocol used for neonatal lesions has been previously described in detail (Flores et al., 1996a, 2005a; Alquicer et al., 2008; Sierra et al., 2009). Pregnant Sprague-Dawley rats were obtained at gestational days 14–17 from our facilities (University of Puebla). Animals were individually housed in a temperature- and humidity-controlled environment on a 12:12-hr light:dark cycle with free access to food and water. On the day following birth, litters of eight male pups were regrouped, and on PD7 each pup (weighing 15–17 g) was assigned to either a sham or a lesion group. All surgical procedures were in accordance with the *Guide for care and use of laboratory animals* of the Mexican Council for Animal Care (Norma Oficial Mexicana NOM-062-ZOO-1999) and with the National Institutes of Health *Guide for the care and use of laboratory animals*. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Surgical Procedures

For this study, the pups were anesthetized by placing them on wet ice for 18–20 min. The pups were then positioned on a modified platform (Sierra et al., 2009) fixed to a stereotaxic Kopf instrument, and subsequently 0.3 μl ibotenic acid (10 μg/μl; Sigma, St. Louis, MO) or an equal volume of vehicle (0.1 M phosphate-buffered saline [PBS], pH 7.4) was bilaterally injected into the ventral hippocampus over a 2-min period through a 30-gauge stainless-steel cannula positioned at the following coordinates from Paxinos and Watson: AP –3.0 mm, ML ±3.5 mm to bregma, and DV –4.9 mm from dura. After the procedure, the pups were placed on a heating pad for recovery and then returned to their dams. On PD21, the animals were weaned, and similar numbers of sham and lesioned rats were placed per cage (four animals per cage).

Cerebrolysin Administration

To assess possible beneficial effects of Cbl on behavioral and neuronal alterations in nVHL rats, five sets of experiments were performed, which were used in 1) locomotor activity, 2) social interaction, 3) prepulse inhibition of the acoustic startle response, 4) Golgi-Cox stain, and 5) stereology. Three weeks (PD30) following the neonatal lesioning, sham- and ibotenic acid-lesioned rats were injected (i.p.) every day for 30 days either with 5 ml/kg of Cbl (215.2 mg/1 ml; Ever Neuro Pharma GmbH, Unterach, Austria) or vehicle (saline solution). Four groups of animals were formed: 1) vehicle-treated sham, 2) Cbl-treated sham, 3) vehicle-treated nVHL, and 4) Cbl-treated nVHL (Fig. 1A). All of the behavioral and morphological studies were performed 1 day after finishing the Cbl administration.

Behavioral Testing

Locomotor activity. Tests were conducted as previously described in detail (Flores et al., 1996a,b; Juarez et al., 2003; Flores-Tochihuitl et al., 2008; Morales-Medina et al., 2008). Locomotor activity was tested between 8:00 AM and noon and was monitored in 16 individual cages (20 × 40 × 30 cm), each of which was equipped with an eight-photo-

beam detector connected to a computer counter (Tecnologia Digital Mexico). After Cbl administration, at a postpuberal age (PD60, n = 10 animals per group), each male rat was assessed with the next protocol: 1) after exposure to a novel environment, unacclimatized rats were placed in an activity box for 120 min, during which the locomotor activity score was recorded, or 2) 2 days after the first test, rats were again placed in the activity boxes, and basal locomotor activity was recorded for 60 min. Animals were first injected with 1 ml/kg 0.9% NaCl (sc) and 120 min later with a 1 mg/ml solution of d-amphetamine sulfate (Sigma) dissolved in 0.9% NaCl (1 mg/kg free base, sc), and the locomotor activity was recorded for the next 120 min. All movements were quantified and analyzed statistically with Graph Pad 4.0. The mean values from each animal were treated as a single measurement for the data analysis. Data on locomotor activity were analyzed by two-way ANOVA, followed by the Newman-Keuls test for post hoc comparisons, with lesion and Cbl as independent factors ($P < 0.05$ was considered significant). Immediately after measuring the locomotor activity, all sham and lesioned rats were anesthetized with sodium pentobarbital (75 mg/kg ip). Brains were rapidly removed, frozen in isopentane, maintained at −40°C for a short period, and stored long term at −80°C until use.

Social interaction. Another separate cohort of animals was used (n = 8–10 animals per group) to evaluate the effects of Cbl administration on social interaction. The animals were tested under low lighting (30 lx) and unfamiliar conditions. A modified version of the original method (File, 1980) was used to evaluate the social abilities of the animals with lesions. Briefly, a pair of rats was randomly selected within the same test group (vehicle-treated sham/vehicle-treated sham, Cbl-treated sham/Cbl-treated sham, vehicle-treated lesion/vehicle-treated lesion, and Cbl-treated lesion/Cbl-treated lesion) and placed into an acrylic cage (80 × 90 × 30 cm) with bedding of Beta Chips (Harwood Laboratory Bedding, Northeastern, NY). Their activity was recorded for 10 min. Only the following behaviors were considered as active social interaction: sniffing, following, grooming, mounting, wrestling, and jumping on or crawling under or over the partner. The session was videotaped and scored by an investigator blind to the experimental status of the rat, using PC software developed at the Universidad Autónoma de Puebla (Flores et al., 2005b). The data are expressed as the total number of interactions and the time spent in the active social behaviors. For each pair of animals, individual data were calculated as the average of four trials. Data on time and number of encounters were analyzed by two-way ANOVA, followed by the Newman-Keuls test for post hoc comparisons, with lesion and Cbl as independent

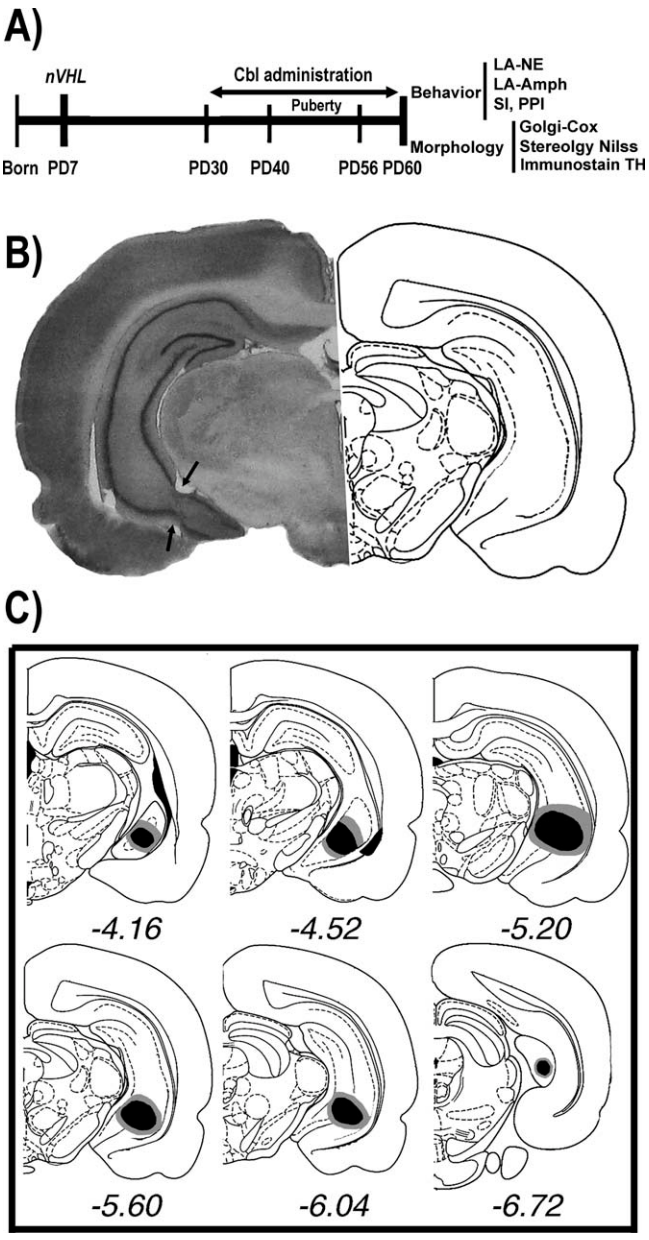

Fig. 1. **A**: Experimental design. At postnatal day (PD) 7, a bilateral ventral hippocampus lesion (nVHL) was made in male Sprage-Dawley rats by ibotenic acid. Sham-operated animals received only PBS. Cerebrolysin (Cbl) was administered from PD30 to PD60. At PD30, four groups of animal were formed: vehicle-treated sham-operated, Cbl-treated sham-operated, vehicle-treated nVHL and Cbl-treated nVHL rats. At PD60, behavioral tests (locomotor activity in response to novel environment [LA-NE], locomotor activity in response to amphetamine [LA-AMPH], social interaction [SI], and percentage prepulse inhibition [PPI]) and morphological analysis (dendritic analysis by Golgi-Cox stain, stereological analysis by Nissl stain and tyrosine hydroxylase [TH] immunostain) were performed. **B**: Photograph of a coronal section of the ventral hippocampus stained with cresyl violet showing retraction of the tissue, gliosis, and neural loss from rats with a neonatal-ventral hippocampus lesion after puberty. The arrow indicates the zone of the lesion. **C**: Schematic drawing of coronal sections illustrating areas of least and greatest VH lesion as determined by Nissl-stained sections of the hippocampus of animals with nVHL at a postpubertal age. Gray, reconstruction of the neuronal loss and gliosis in the hippocampus of the rat with the most widespread lesion. Black, extent of the lesion in the rat with the minimal lesion considered significant. Numbers indicate distance (mm) posterior from bregma according to Paxinos and Watson (1986).

factors ($P < 0.05$ was considered significant). After the social interaction analysis, all the animals were anesthetized with sodium pentobarbital (75 mg/kg ip). Brains were rapidly removed, frozen in isopentane, maintained at −40°C for a short period, and stored long term at −80°C until use.

Acoustic Startle Response and Prepulse Inhibition

Apparatus. Acoustic startle response (ASR) and prepulse inhibition (PPI) experiments were performed using a single automated Responder-X system (Columbus Instruments, Columbus, OH). The startle chamber consisted of a polycarbonate plastic cage (15.4 × 28.1 × 17.5 cm; w, l, h), with a load cell aluminum platform on the floor. This system works with unrestrained subjects, and the load cell platform records the force (in grams) of the startle reflex. Background noise (65 dB) and acoustic stimuli were provided via a loudspeaker placed 10 cm aside the test chamber. The system was kept within a sound-attenuating, ventilated cabinet. Stimuli were delivered and responses were measured using the Responder-X software (Columbus Instruments) running on a PC. Startle response was taken as the first positive peak in force from calibrated zero; this measure is the sum of the subject's mass and the startle response. For all the measures of the startle response, we subtracted the subject's mass (in grams) from the peak amplitude.

Procedure. Another cohort of animals was used to assess the effects of Cbl on PPI (n = 8–10 animals per group). Rats were brought to the PPI experiment room 1 hr before testing for familiarization. Afterward, rats were placed in the startle chamber for a 5-min acclimation period with a 65-dB background noise. ASR and PPI were measured at the same session, as reported previously (Ralph-Williams et al., 2003; Vinkers et al., 2007). Each experimental session consisted of baseline (BL) pulses, nonstimulus trials (nonstim), startle trials (pulse-alone), and prepulse trials (prepulse + pulse); the background noise was present throughout the session. After the acclimation period, six BL pulse trials of broadband noise (120 dB, 40 msec) were presented in order to achieve a relatively stable level of startle response amplitude. The data of these BL trials were not considered in the ASR and PPI analysis. After that, 80 trials of eight different types were presented in pseudorandom order with an interval of 30 sec. Ten nonstim trials consisted of background noise only; pulse-alone included 10 each of three different trials consisting of 40-msec acoustic broadband noise pulses with intensities of 85, 105, and 120 dB; and four different prepulse + pulse trials consisted of a 40-msec noise prepulse (75 or 85 dB), a 100-msec delay, and then a 40-msec startle pulse (105 or 120 dB broadband noise). Thus, the four prepulse + pulse trials were 10 each of 75–105 dB, 85–105 dB, 75–120 dB, and 85–120 dB. The startle apparatus was wiped with detergent solution between tests of each animal. After finishing the test, all the animals were anesthetized with sodium pentobarbital (75 mg/kg ip). Brains were rapidly removed, frozen in isopentane, maintained at −40°C for a short period, and stored long-term at −80°C until use.

Data analysis. For each rat, individual startle responses were calculated as the average of 10 same-type pulse or prepulse + pulse trials. The acoustic startle responses for sham-operated and nVH-lesioned groups with Cbl or vehicle are expressed as the mean ± SEM. Percentage of PPI was calculated as the difference between pulse alone trial and the respective prepulse + pulse (same pulse intensity) trial divided by the pulse alone trial × 100. The results of the ASR experiment were not normally distributed, so a square root transformation was applied to the data before of the two-way analysis of variance (ANOVA), with lesion (lesion/sham) and treatment (Cbl/Veh) as independent factors. For each prepulse + pulse intensity (75–105, 85–105, 75–120, and 85–120 dB) trial, a two-way ANOVA with lesion and treatment as independent factors was used. The Student-Newman-Keuls test was used to make multiple, pairwise group comparisons. The level of significance was set at $P < 0.05$. For analyses, SigmaStat version 3.5 was used.

Morphological Assessment

Golgi-Cox stain method. A separate cohort of animals was used to study the effects of Cbl on dendritic morphology (n = 9–10 animals per group). One day after the final Cbl treatment, the rats were deeply anesthetized with sodium pentobarbital (75 mg/kg body weight, ip) and perfused intracardially with 0.9% saline solution. The brains were removed and stained by modified Golgi-Cox method described previously (Flores et al., 2005a). Coronal sections of 200-μm thickness from the PFC, CPu, and Nacc were obtained using a vibrotome (MA752; Campden Instrument, Leicester, United Kingdom). These sections were collected on clean, gelatin-coated microscope slides and treated with ammonium hydroxide for 30 min, followed by 30 min in Kodak Film Fixer, and finally were rinsed with distilled water and mounted with resinous medium (Robinson and Kolb, 1997; Gibb and Kolb, 1998).

Microscopic observation and Sholl analysis. Pyramidal cells from layers 3 and 5 of the PFC (area Cg1 and prelimbic cortex; plate 7– 9 of Paxinos and Watson, 1986) and medium spiny neurons from the CPu and NAcc (plate 10–13 of Paxinos and Watson, 1986) were selected for study. For each animal, neurons from the left and right PFC, CPu, and NAcc were drawn using a camera lucida at a magnification of ×250 (DMLS Leica Microscope) by a trained observer who was blind to the experimental conditions (Kolb et al., 1998). PFC pyramidal neurons were readily identified by their characteristic triangular soma, apical dendrites extending toward the pial surface, and numerous dendritic spines, whereas the medium spiny neurons from the NAcc and CPu were identified by soma size and dendritic extension, as described by Robinson and Kolb (1997). The criteria used to select neurons for reconstruction have been fully described previously (Silva-Gomez et al., 2003b; Vega et al., 2004; Flores et al., 2005a; Martinez-Tellez et al., 2005; Juarez et al., 2008). Briefly, only complete, fully impregnated pyramidal neurons with no apparent truncation of the basal dendritic arbor were included in our analyses; the ends of the dendrites were positively identified by their characteristic conical shape. In the case of PFC pyramidal neurons, the present analyses were performed on the basal dendrites, because these run parallel to the coronal plane. Sequential two-dimensional reconstructions

of the entire dendritic tree were generated for each neuron, and the dendritic tracings were quantified by Sholl analysis (Sholl, 1953) as follows. A transparent grid with equidistant (10 μm) concentric rings was centered over the dendritic tree tracings, and the number of ring intersections was used to estimate the total dendritic length and dendritic arborization (Kolb et al, 1998; Silva-Gomez et al., 2003b; Vega et al., 2004; Flores et al., 2005a; Martinez-Tellez et al., 2005). Another estimate of dendritic arborization, the total number of dendritic branches (branching indicated by Y bifurcation), was counted at each order away from the cell body or dendritic shaft. To calculate the spine density, a length of dendrite (at least 10 μm long) was traced (at ×1,000), the exact length of the dendritic segment was calculated, and the number of spines along the length was counted (to yield spines/10 μm).

Statistical analysis. The mean values from each brain region of each animal were treated as a single measurement for the data analysis. Data on dendritic length and the spine densities were analyzed by two-way ANOVA, followed by the Newman-Keuls test for post hoc comparisons, with lesion and Cbl as independent factors ($P < 0.05$ was considered significant). Data on the length per branch order also were analyzed by two-way ANOVA, followed by the Newman-Keuls test for post hoc comparisons, with lesion and branch order as independent factors ($P < 0.05$ being significant).

Stereological analysis. A separate subset of rats (four animals per group) was deeply anesthetized with sodium pentobarbital (75 mg/kg ip) and perfused through the heart with 0.9% saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer. One day before sectioning, each brain was refrigerated and stored in distilled water. Forty-micrometer-thick coronal sections from the PFC and NAcc were obtained using a vibratome (model 2000; Leica). For stereological analysis of neuronal populations, briefly, sections were mounted on glass slides and stained with cresyl violet and were analyzed with the optical dissector as previously described (Everall et al., 1997; Chana et al., 2003; Kuczenski et al., 2007). Cells were sampled within a volume in the PFC and NAcc by optical sectioning, at a 10-μm distance, within the vibratome section, using an Olympus BH2 microscope with a digital color camera attached to a DataCell computer-assisted image analysis system (Stereo Investigator System; MicroBrightField, Williston, VT) for stereology. From each case, at least four random sections within a given area of about 400 μm were analyzed, and results were averaged and expressed as total number per cubic millimeter.

Data were analyzed in Graph Pad 4.0 conducted in triplicate on blind-coded samples. After the results had been obtained, the code was broken, and the data were analyzed by two-way ANOVA, followed by the Newman-Keuls test for post hoc comparisons, with lesion and Cbl as independent factors ($P < 0.05$ was considered significant).

TH immunoreactivity. Inmunohistochemistry was conducted on 40-μm vibratome sections, which were washed in PBS (pH 7.4) and incubated with 0.3% hydrogen peroxide for 15 min to block endogenous peroxidases. After washing in PBS, sections were incubated for 1 hr in 10% normal horse serum/0.3% Triton X-100 in PBS, then incubated overnight at 4°C in the primary antibody against the dopaminergic marker tyrosine hydroxylase (MAB318; 1:250; Millipore, Bedford, MA). After washing in PBS, sections were incubated with the corresponding biotynilated secondary antibody anti-mouse IgG (1:100; Vector Laboratories, Burlingame, CA) at room temperature for 2 hr. The avidin-biotin complex method was used to detect the secondary antibody (ABC Elite kit; Vector Laboratories), and the reaction product was visualized by 3,3′-diaminobenzidine tetrachloride (DAB; Sigma) containing 0.001% H_2O_2 and nickel chloride for visualization.

To evaluate the levels of TH immunoreactivity in the CPu and NAcc, sections (three per animal) imunostained with antibodies against TH were digitally imaged (groups of 10 digital images per section). Images were acquired after calibration of the system to ensure adequate exposure and avoid saturation of gray levels. Images were analyzed in Image J to determine optical density levels per field using a 160 threshold in each case. Individuals values were averaged and expressed as mean value.

All analyses were conducted in triplicate on blind-coded samples. After the results had been obtained, the code was broken, and data were analyzed in Graph Pad 4.0. Comparisons among the groups were performed by two-way ANOVA followed by the Newman-Keuls test for post hoc comparisons, with lesion and Cbl as independent factors ($P < 0.05$ was considered significant). All results were expressed as mean ± SEM.

nVH lesion assessment. For assessment of lesion size, the frozen brains were sectioned into 15-μm-thick slices on the coronal plane using a Leica CM-1100 cryostat. Sections at the level of the ventral hippocampus were collected on cleaned, gelatin-coated microscope slides (four sections/slide) and then stored at –80°C until the day of staining. Sections were stained with 0.5% cresyl violet and examined under a microscope where the lesions and probe placement could be seen.

RESULTS

Verification of the Lesion

Cresyl violet-stained sections obtained from the animals with nVH lesions at a postpubertal age (PD60) revealed significant bilateral damage of the ventral hippocampus, with neuronal loss, atrophy, and apparent retraction of the ventral hippocampus (Fig. 1B). Cavities resulting from the lesions were also frequently seen. Only animals with a bilateral lesion of the nVH were included in the present study. The brains of the sham animals did not show any morphological alterations. Representative schematic drawings of the histological analyses from the brains of the animals with lesions are shown in Figure 1C.

Behavioral Results

Locomotor activity. Previous reports have shown that neonatal bilateral lesion of the VH results in an enhanced locomotion in response to novel environment (Lipska et al., 1993; Flores et al., 1996a, 2005a,b; Becker et al., 1999; Brake et al., 1999). Both sham- and nVH-lesioned animals with or without Cbl initially

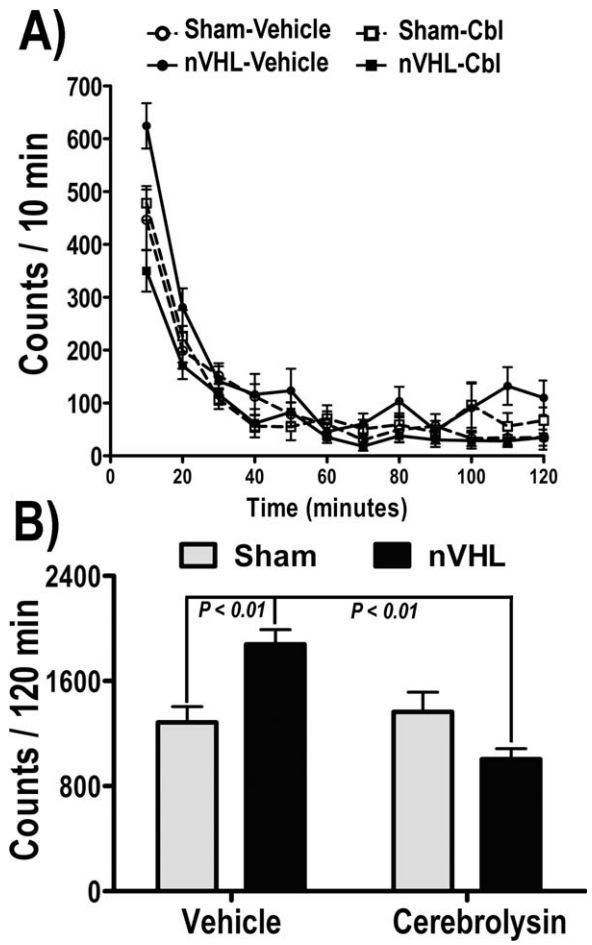

Fig. 2. Locomotor activity (mean number of beam interruptions per 10 min ± SEM, n = 10 per group) in a novel environment for vehicle-treated and cerebrolysin-treated sham-operated or nVHL animals tested at PD60. **A**: Temporal profile of the locomotor activity at PD60. **B**: Analysis of total activity scores reveals that vehicle-treated nVHL animals are more active compared with their corresponding vehicle-treated sham-operated rats. Cerebrolysin (Cbl) treatment reduced the hyperresponse to novel environment observed in the vehicle-treated nVHL rats.

showed increased locomotor activity reflecting active exploratory behavior in a novel environment. The locomotor activity gradually declined to a stable level within 60 min (Fig. 2A). An analysis of the data for the entire 120-min period (two-way ANOVA, Cbl; $F_{1,38} = 11.6$, $P < 0.001$; interaction of lesion with Cbl; $F_{1,38} = 16.8$, $P < 0.001$) showed a significant increase in the locomotor activity in the vehicle-treated nVHL animals ($P < 0.01$) compared with the vehicle-treated sham (Fig. 2B). Cbl treatment significantly reduces the hyperlocomotion observed in the vehicle-treated nVHL animals (Fig 2B). No significant differences in locomotor activity were observed between vehicle- or Cbl-treated sham-control rats (Fig. 2B).

Several reports have demonstrated that nVHL rats display hyperresponsiveness to d-amphetamine (AMPH) after puberty (Lipska et al., 1993; Lipska and Weinberger, 1995; Flores et al., 1996a; Wan et al., 1996). Consistent with these findings, our results also show that locomotor activity (two-way ANOVA, interaction of lesion with Cbl; $F_{1,28} = 7.8$, $P < 0.01$) was higher in vehicle-treated lesioned rats than in vehicle-treated sham-controls following AMPH at a dose of 1 mg/kg ($P < 0.01$, Fig. 3B). Cbl treatment significantly ameliorated this amphetamine-induced hyperresponsiveness in Cbl-treated nVHL rats compared with vehicle-treated nVHL animals (Fig. 3B). No significant differences in locomotor activity induced by amphetamine were observed between vehicle- or Cbl-treated sham-control rats (Fig. 3B). In addition, no significant effect of vehicle injection was observed in any of these groups (Fig. 3C).

Social interaction. Consistent with previous studies (Sams-Dodd et al., 1997; Becker et al., 1999; Silva-Gomez et al., 2003a; Flores et al., 2005b), social behavior analysis of the vehicle-treated nVHL rats showed a decreased in total time spent in social interactions (two-way ANOVA, lesion; $F_{1,18} = 4.9$, $P < 0.05$) compared with vehicle-treated sham-operated animals (Fig. 4A), whereas the number of episodes of social encounters was not affected by the lesion (Fig. 4B). Interestingly, Cbl treatment significantly ameliorated this alteration of social behavior in Cbl-treated nVHL rats compared with vehicle-treated nVHL animals (Fig. 4A). Total time spent in social interactions in Cbl-treated sham-operated rats was comparable to that seen in vehicle-treated sham-operated animals (Fig. 4A).

PPI of the ASR

The analysis of the ASR magnitude (Fig. 5A) revealed that Cbl and lesion did not result in differences at any intensity range examined. The effects of Cbl on the mean of percentage PPI are shown in Figure 5B. The two-way ANOVA revealed a significant main effects of the Cbl treatment ($F_{1,28} = 5.82$; $P < 0.05$) and lesion ($F_{1,28} = 10.53$; $P < 0.01$) at 75–105 dB prepulse + pulse intensity trial. There were no significant differences in the effects of Cbl or lesion in the others prepulse + pulse intensities (85–105, 75–120, and 85–120 dB). The Student-Newman-Keuls test demonstrated that postpubertal rats with vehicle-treated nVHL exhibited significant reduction in PPI at the 75–105 dB prepulse + pulse intensity trial ($P < 0.05$; Fig. 5B); these results are in accordance with previous studies (Le Pen et al., 2003b; Lipska et al., 1995). There were no significant differences in the effects of Cbl on PPI between Cbl-treated nVHL rats compared with their corresponding control (Cbl-treated sham-operated; Fig. 5B).

Morphological Data

Golgi-Cox procedure. The morphological analysis presented here is based on a total of 1,600 neurons from 40 animals. Estimates of dendritic length and spine density were obtained from 800 PFC pyramidal

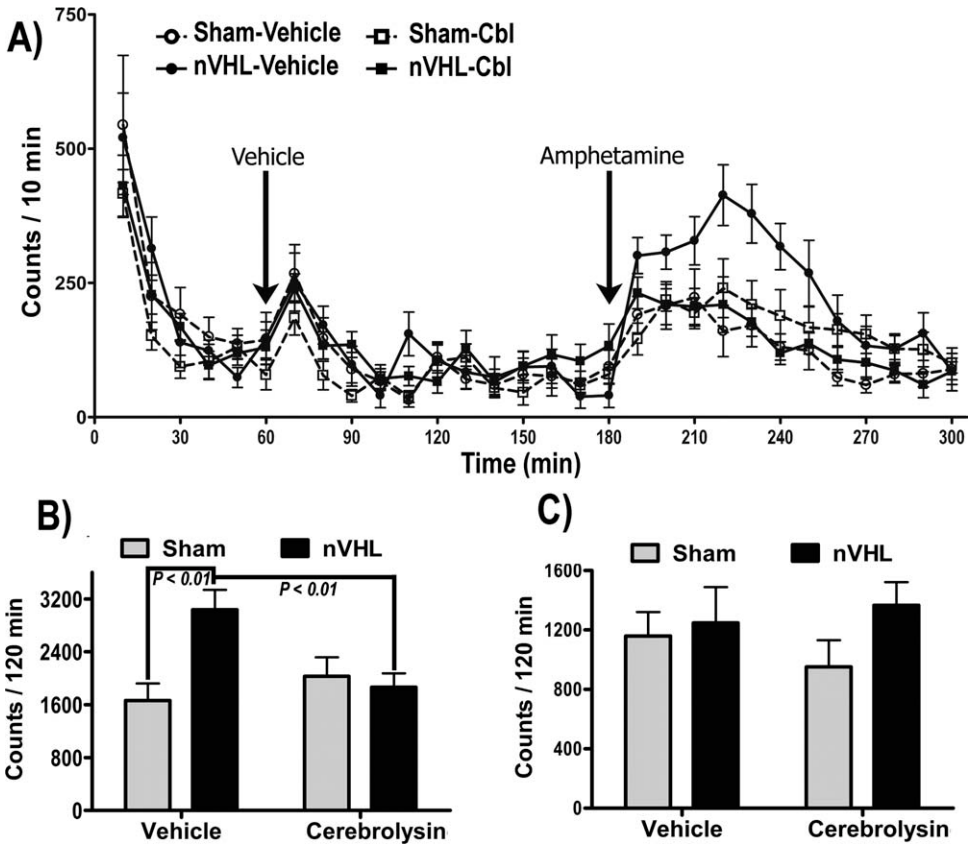

Fig. 3. Locomotor activity after vehicle (saline) and d-amphetamine administration (1 mg/kg, s.c.) of vehicle-treated and cerebrolysin-treated sham-operated or nVHL animals tested at PD60 (mean number of beam interruptions per 10 min ± SEM; n = 9–10 per group). **A**: Temporal profile of locomotor activity at a postpubertal age. **B**: Analysis of total activity scores after d-amphetamine injection showed that the vehicle-treated nVHL animals were more active after d-amphetamine administration than their corresponding vehicle-treated sham animals. Interestingly, cerebrolysin (Cbl) treatment ameliorated this hyperresponsiveness to amphetamine in Cbl-treated nVHL rats compared with vehicle-treated nVHL animals. **C**: Analysis of total activity scores after vehicle injection showed no significant effect of vehicle injection in any of the groups.

neurons, from 400 NAcc and 400 CPu medium spiny neurons.

As observed in our previous studies, the Golgi-Cox impregnation procedure clearly filled the dendritic shafts and spines of layer III and V pyramidal neurons of the PFC and medium spiny neurons of the NAcc and CPu (Silva-Gomez et al., 2003b; Vega et al., 2004; Flores et al., 2005a; Martinez-Tellez et al., 2005; Solis et al., 2007). Examples of impregnated PFC, NAcc, and CPu neurons from control animals are shown in Figures 6–9.

Our previous studies have shown that nVHL induced dendritic retraction and spine loss of the pyramidal neurons from layer III of the PFC (Flores et al., 2005a; Alquicer et al., 2008). Compared with vehicle-treated sham animals, rats with nVHL displayed decreased in the total dendritic length of the pyramidal neurons from layer III (two-way ANOVA, interaction of lesion with Cbl; $F_{1,36} = 8.6$, $P < 0.001$) and layer V (two-way ANOVA, lesion; $F_{1,36} = 20$, $P < 0.001$; Cbl; $F_{1,36} = 17$, $P < 0.001$; interaction of lesion with Cbl; $F_{1,36} = 30$, $P < 0.001$) of the PFC (Figs. 6D, 7D) and medium spiny neurons of the NAcc (two-way ANOVA, lesion; $F_{1,36} = 8.3$, $P < 0.01$; Fig. 8D). Interestingly, Cbl treatment significantly ameliorated the dendritic length hypotrophy observed in the pyramidal neurons of the PFC and medium spiny neurons of the NAcc of the vehicle-treated nVHL rats. In the same analysis for the CPu, no significant effect of nVHL and Cbl was observed in any of these groups (Fig. 9C). No significant differences in total dendritic length were observed between vehicle- and Cbl-treated sham-operated rats (Figs. 6–9).

Analyses of the dendritic spine density of neurons are shown in Figures 6–9. Consistently with our previous studies (Flores et al., 2005a; Alquicer et al., 2008), nVHL rats treated with vehicle displayed a decrease in the dendritic spine number of pyramidal neurons from layer III (two-way ANOVA, interaction of lesion with Cbl; $F_{1,36} = 7.6$, $P < 0.01$; Fig. 6C) without changes in layer V of the prefrontal cortex compared with sham-treated vehicle animals (Fig. 7C). No significant differences in dendritic spine number of the medium

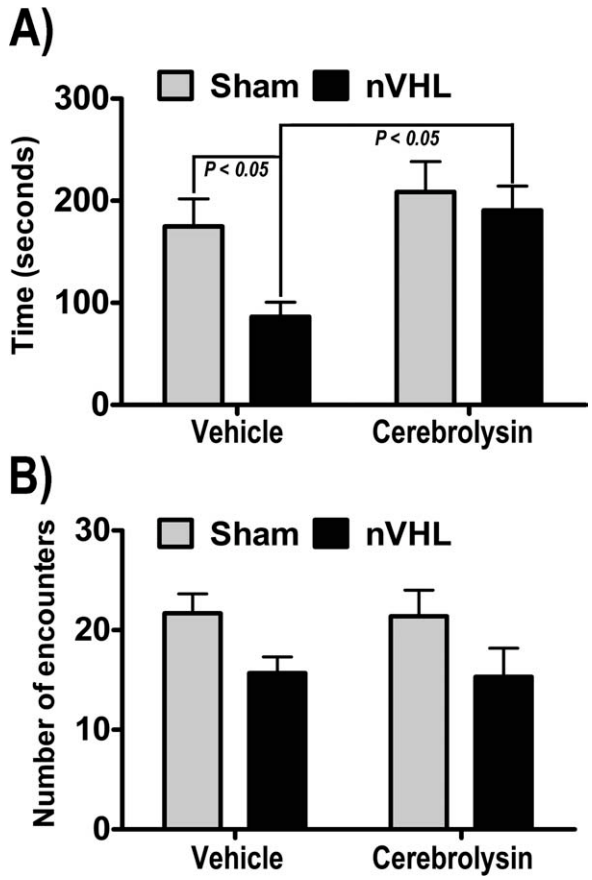

Fig. 4. Social behavior in vehicle-treated and cerebrolysin-treated sham-operated or nVHL animals. Age at testing was 60 days. **A**: Time (seconds) spent in social interaction. **B**: Number of encounters. The vehicle-treated nVHL spent less time in social encounters (A). The cerebrolysin (Cbl) treatment reduced this altered social behavior in Cbl-treated nVHL rats compared with vehicle-terated nVHL animals (A). No significant difference in the number of encounters was observed among groups.

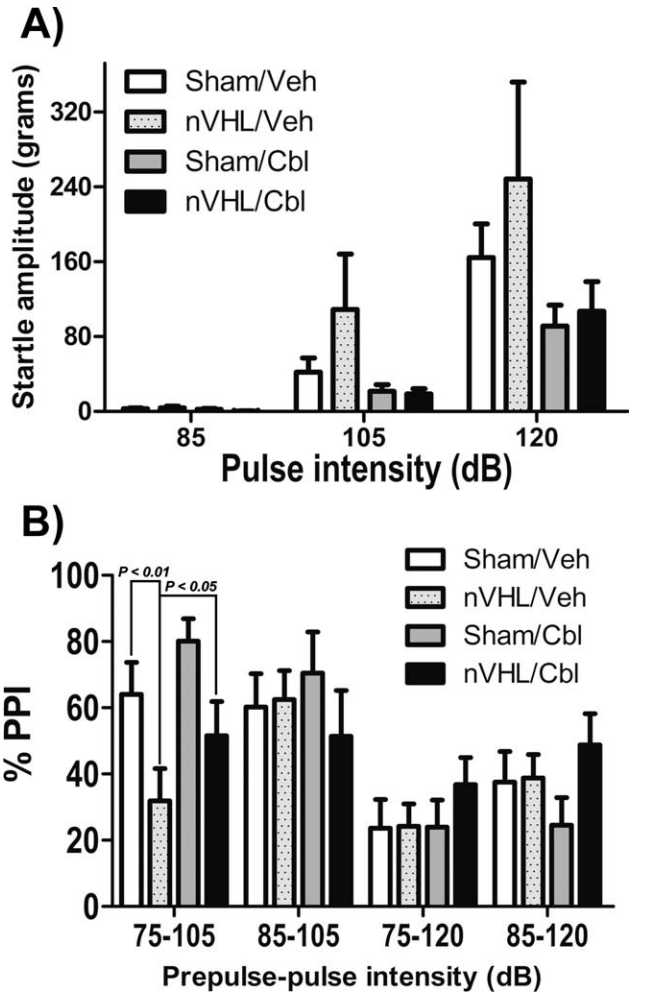

Fig. 5. Effects of cerebrolysin on acoustic startle response in neonatal ventral hippocampal lesion rats. Age at testing was 60 days. **A**: Analysis of the starle amplitude revealed no significant vehicle-treated or cerebrolysin effects on the mean acoustic startle response magnitude (grams). **B**: Analysis of the percentage of prepulse inhibition (PPI) on acoustic startle reflex revealed that vehicle-treated nVHL rats exhibited a reduction in PPI at 75–105 prepulse + pulse intensity compared with vehicle-treated sham animals. The Cbl treatment attenuated the deficits in prepulse inhibition of the startle reflex induced by neonatal lesions (B).

spiny neurons of the NAcc and CPu were observed between vehicle-treated nVHL and vehicle-treated sham-operated rats (Figs. 8C, 9B). Interestingly, Cbl treatment significantly ameliorated the decreased number of dendritic spines observed in the pyramidal neurons from layer III of the PFC in the vehicle-treated nVHL rats.

Another measure obtained from the Sholl analysis was length per branch order. The branch-order analysis also revealed that dendritic length of layer III (two-way ANOVA, Cbl: $F_{3,288} = 2.86$, $P < 0.05$; branch order: $F_{1,288} = 349$, $P < 0.001$) and layer V (two-way ANOVA, Cbl: $F_{3,245} = 22.7$, $P < 0.001$; branch order: $F_{1,245} = 376$, $P < 0.001$; interaction of branch order with Cbl: $F_{18,245} = 4.66$, $P < 0.001$) of the PFC was less in the vehicle-treated nVHL animals at the level of the fourth and fifth ($P < 0.05$) orders to layer III and at the level of the third to fifth ($P < 0.01$) orders to layer V compared with vehicle-treated sham rats (Figs. 6E, 7E). Cbl treatment ameliorated these changes in the branch order observed in the pyramidal neurons from layers III and V of the PFC in the vehicle-treated nVHL rats. The same analysis of the medium spiny neurons of the NAcc (two-way ANOVA, Cbl: $F_{3,210} = 4.4$, $P < 0.01$; branch order: $F_{5,210} = 357$, $P < 0.001$) revealed that vehicle-treated nVHL animals showed a decrease in dendritic length only at the level of the third ($P < 0.01$) order compared with vehicle-treated sham rats (Fig. 8E). Cbl treatment significantly ameliorated this dendritic hypotrophy at the level of the third order observed in the medium spiny neurons of the NAcc in the vehicle-treated nVHL rats. In the branch-order analysis for the

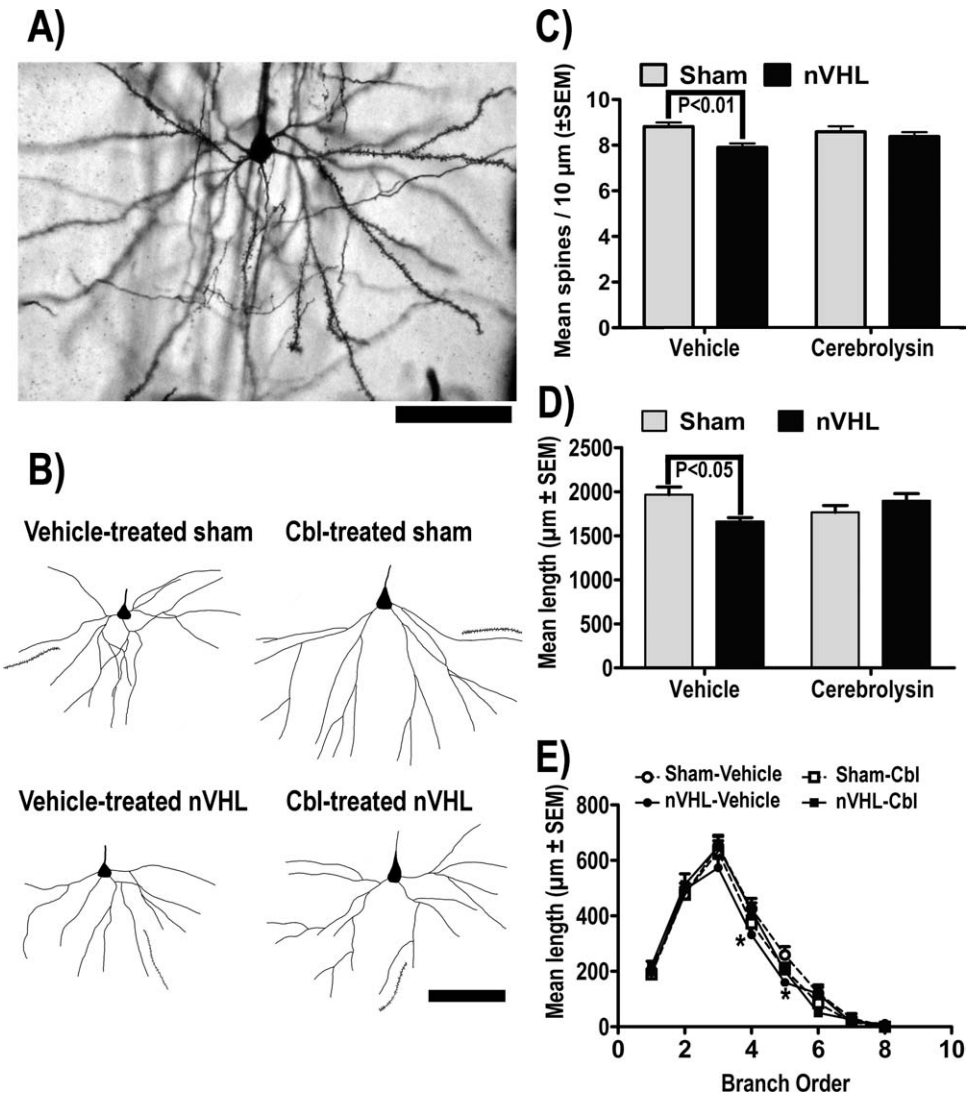

Fig. 6. Analysis of the cerebrolysin effect on the pyramidal neurons from layer III of the prefrontal cortex (PFC) in postpubertal (PD60) animals (n = 8–10 animals per group). **A**: Photomicrograph showing a representative Golgi-Cox-impregnated pyramidal neuron of the PFC from vehicle-treated sham animal. **B**: Representative schematic drawings of the dendritic basilar arbor of the PFC neurons. **C**: Dendritic spiny neuron density. The density of the dendritic spines decreased in vehicle-treated nVHL animals compared with the corresponding sham rats. Cbl treatment significantly reduced the spines loss observed in the pyramidal neurons of the PFC of the vehicle-treated nVHL rats. **D**: Total dendritic length analysis also revealed that vehicle-treated nVHL rats shown a reduction in the dendritic length compared with vehicle-treated sham animals. Interestingly, Cbl treatment also significantly ameliorated the dendritic length hypotrophy observed in the pyramidal neurons of the PFC of the vehicle-treated nVHL rats. **E**: Length of branch-order analysis revealed that dendritic length of the layer III of the PFC was less in the vehicle-treated nVHL animals at the level of the fourth and fifth ($*P < 0.05$) orders compared with vehicle-treated sham rats. Cbl treatment ameliorated these reduction of the length in fourth and fifth order. Scale bar = 100 µm.

CPu, no significant effect of nVHL and Cbl was observed in any of these groups (Fig. 9D).

Stereological analysis. To examine whether there is a relationship between the nVHL and anatomical measures of the limbic subregions, we estimated total number of cells in the PFC and shell and core parts of the NAcc. Stereological analyses of neurons with cresyl violet are shown in Figure 10. The PFC assessment suggests that vehicle-treated nVHL rats have a reduction in cells in the PFC (two-way ANOVA, interaction of lesion with Cbl; $F_{1,12} = 21$, $P < 0.001$) in comparison with vehicle-sham controls ($P < 0.01$, Fig. 10A). Cbl treatment significantly ameliorated this loss in Cbl-treated nVHL rats compared with vehicle-treated nVHL animals (Fig. 10A). Interestingly, Cbl treatment significantly increases the number of NAcc core cells (two-way ANOVA, interaction of lesion with Cbl; $F_{1,12} = 5.8$, $P < 0.05$) in the Cbl-treated nVHL rats compared with vehicle-treated nVHL animals ($P < 0.05$; Fig. 10C). In addition, nVHL per se did not cause a significant difference compared with the

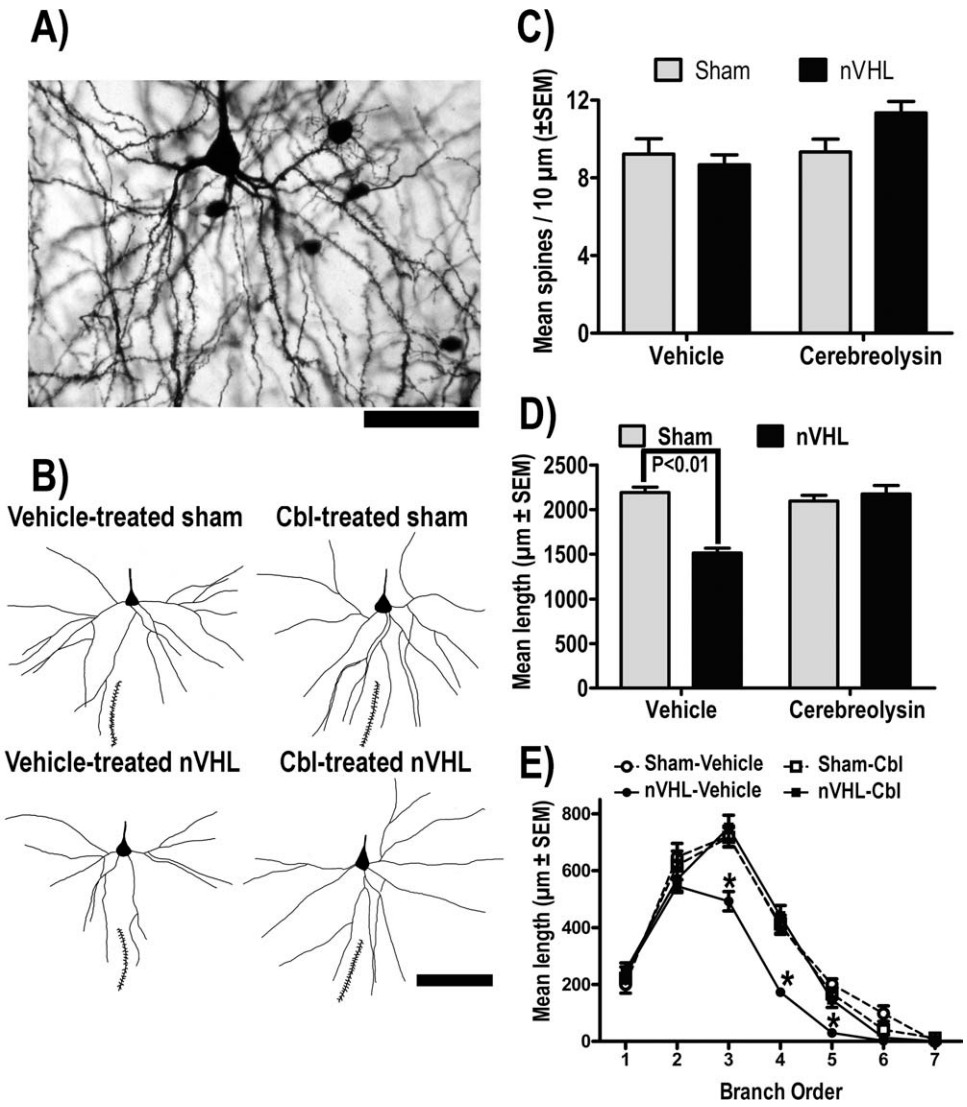

Fig. 7. The cerebrolysin (Cbl) effect on the pyramidal neurons from layer V of the prefrontal cortex (PFC) in postpubertal (PD60) animals (n = 8–10 animals per group). **A**: Photomicrograph showing a representative Golgi-Cox-impregnated pyramidal neuron from layer V of the PFC from vehicle-treated sham animal. **B**: Representative schematic drawings of the dendritic basilar arbor of the PFC neurons. **C**: Dendritic spine number analysis revealed no differences among groups. **D**: Total dendritic length analysis revealed that vehicle-treated nVHL rats showed a reduction in the dendritic length compared with vehicle-treated sham animals. Cbl treatment significantly ameliorated the dendritic length hypotrophy observed in the pyramidal neurons of the PFC of the vehicle-treated nVHL rats. **E**: Length of branch-order analysis revealed that vehicle-treated nVHL rats shown a reduction in the dendritic length between three and five branch orders ($^*P < 0.01$) compared with vehicle-treated sham animals; however, Cbl treatment reduced this effect. Scale bar = 100 µm.

vehicle-treated sham group in the NAcc shell (Fig. 10B). Finally, no significant difference in the number of cells stained with cresyl violet was evident between vehicle- or Cbl-treated nVHL in the shell part of the NAcc (Fig. 10B).

Image analysis of TH immunoreactivity. Consistent with behavioral and neurochemical data, which suggest that nVH lesioning results in an enhancement of dopaminergic function in the NAcc (Lipska et al., 1993; Flores et al., 1996a; Wan et al., 1996; Brake et al., 1999), neuropathological analysis of the TH immunoreactivity in the CPu and NAcc showed an increase in the shell part of the NAcc (two-way ANOVA, interaction of lesion with Cbl; $F_{1,12} = 30$, $P < 0.001$) in the vehicle-treated nVHL rats compared with vehicle-treated sham animals ($P < 0.01$; Fig. 11A). No significant difference in the TH immunoreactivity was evident between vehicle-treated nVHL and vehicle-treated sham-operated rats in the core part of the NAcc and CPu (Fig. 11B,C). Cbl treatment significantly ameliorated this high level of NAcc shell TH immunoreactivity in Cbl-treated nVHL rats compared with vehicle-treated nVHL animals ($P < 0.05$, Fig. 11A).

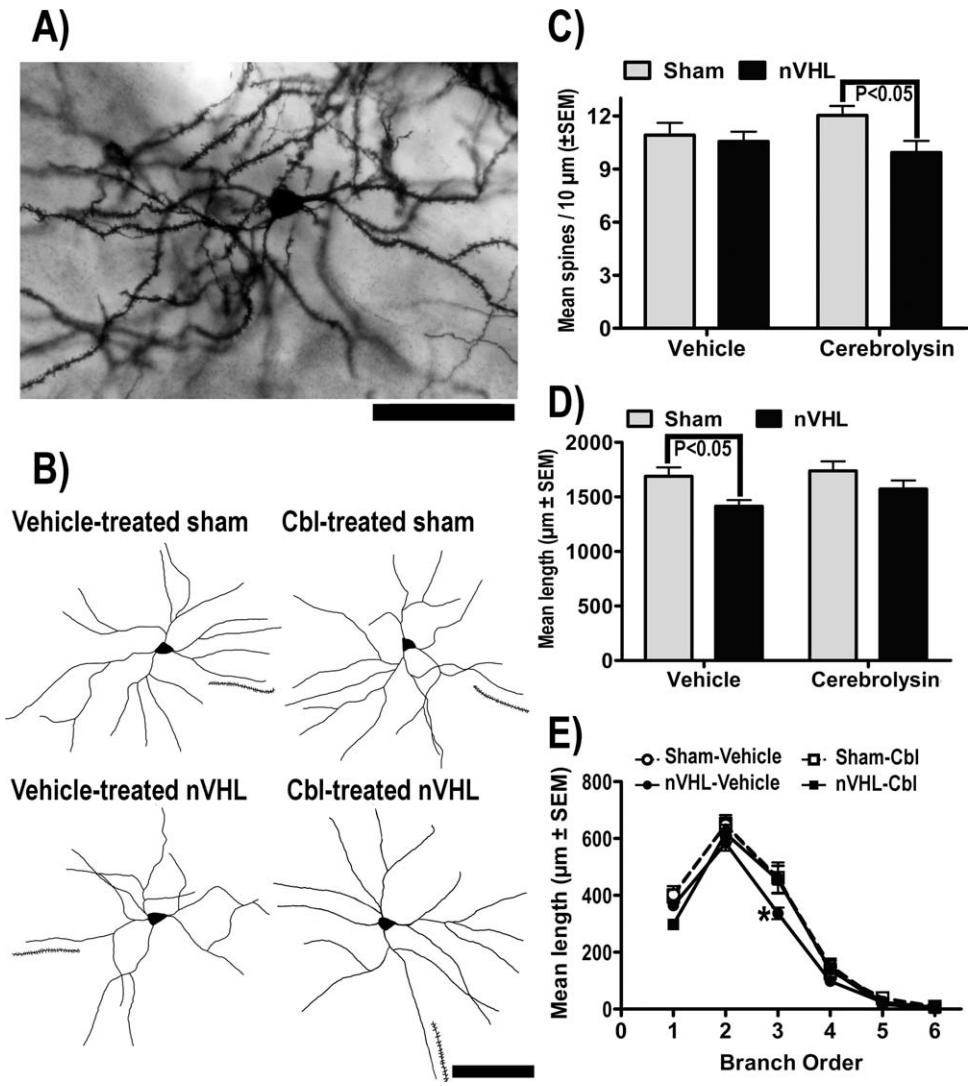

Fig. 8. The cerebrolysin (Cbl) effect on medium spiny neurons of the nucleus acumbens (NAcc) in postpubertal (PD60) animals (n = 8–10 animals per group). **A**: Photomicrograph showing a representative Golgi-Cox-impregnated medium spiny neuron from the NAcc from a vehicle-treated sham animal. **B**: Representative schematic drawings of the dendritic arbor of the medium spiny neurons of the NAcc. **C**: Dendritic spine number analysis revealed that Cbl-treated nVHL rats showed a reduction in dendritic length compared with Cbl-treated sham animals. **D**: Total dendritic length analysis revealed that vehicle-treated nVHL rats showed a reduction in dendritic length compared with vehicle-treated sham animals. Cbl treatment significantly ameliorated the dendritic length hypotrophy observed in the medium spiny neurons of the NAcc of the vehicle-treated nVHL rats. **E**: Length of branch-order analysis revealed that vehicle-treated nVHL rats showed a reduction in the dendritic length at the third branch order ($*P < 0.01$) compared with Cbl-treated nVHL and Cbl-treated sham animals. Scale bar = 100 µm.

DISCUSSION

The present study demonstrates that the neurotrophic agent Cbl ameliorates the behavioral alterations in novel environmental- and amphetamine-induced locomotion, social interaction, and prepulse inhibition in a neurodevelopmental rat model of schizophrenia. The performance alterations in social interaction and the prepulse inhibition test observed in this study are consistent with previous studies using this model (Lipska et al., 1995; Sams-Dodd et al., 1997; Becker et al., 1999; Le Pen et al., 2003b; Silva-Gomez et al., 2003a; Flores et al., 2005b) and are reminiscent of the behavioral disturbances in patients with schizophrenia (for review see Tseng et al., 2009). Consistent with these improvements, morphological examination of the limbic subregions in this animal model found that Cbl administration also ameliorated the dendritic hypotrophy observed in the nVHL rats. Interestingly, stereological analysis showed for the first time that nVHL rats also displayed a reduction in cells in PFC and that Cbl treatment ameliorated this loss. In addition, we found that Cbl treatment reduced the hyperdopaminergic function of the NAcc suggested by behavioral reports (Flores et al., 1996a;

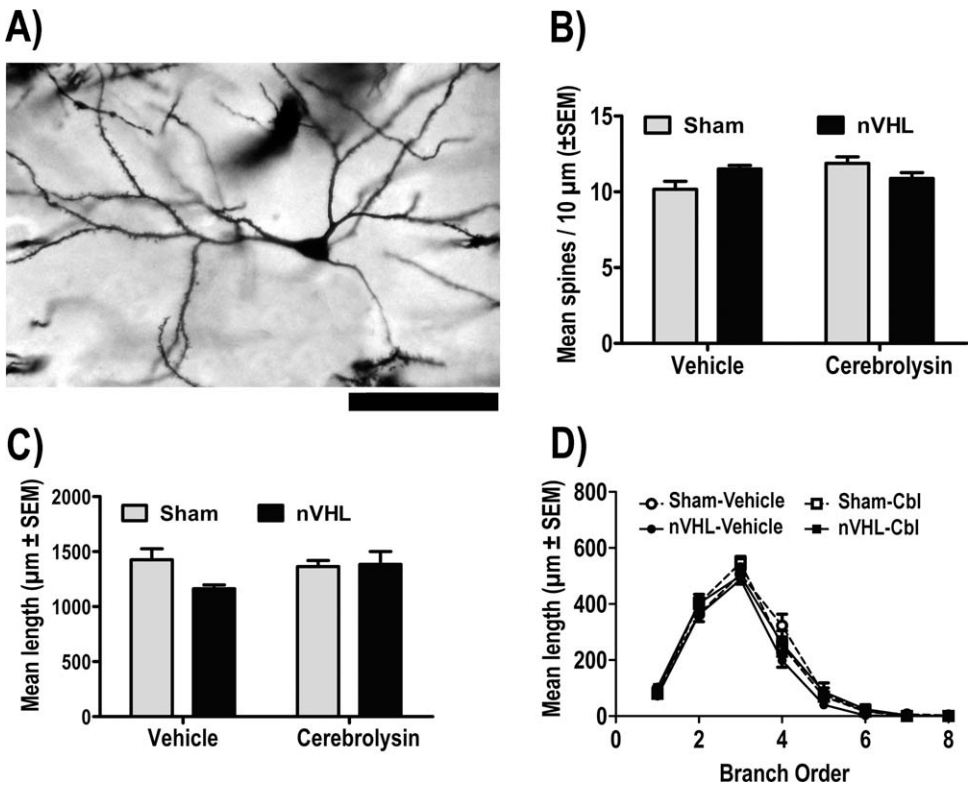

Fig. 9. The cerebrolysin (Cbl) effect on the medium spiny neurons of the caudate putamen (CPu) in postpubertal (PD60) animals (n = 8–10 animals per group). **A**: Photomicrograph showing a representative Golgi-Cox-impregnated medium spiny neuron from CPu from vehicle-treated sham animal. **B**: Dendritic spine number analysis revealed no differences among groups. **C**: Total dendritic length analysis also revealed no significant effect of nVHL or Cbl treatment. **D**: Length of branch-order analysis shown no differences among the groups. Scale bar = 100 µm.

Wan et al., 1996; Chrapusta et al., 2003). On the other hand, Cbl was used in the treatment of the extrapyramidal and somatovegetative side effects of neuroleptics in schizophrenic patients, with symptom relief in 59–62% of patients (Panteleeva et al., 1999).

Postpubertal, but not prepubertal, animals with nVH lesions exhibit increased locomotor activity in a slightly stressful novel environment, and, with stimuli such as amphetamine, these behavioral abnormalities are often related to increased mesolimbic-dopaminergic activity (Lispka et al., 1993; Flores et al., 1996a, 2005b; Wan et al., 1996; Brake et al., 1999; Chrapusta et al., 2003; Alquicer et al., 2004). The mechanism by which an nVHL produces changes in both hyperresponsiveness to stress and to amphetamine at a postpubertal age remains undetermined. However, several behavioral reports suggest that both alterations may be related to an augmented mesolimbic-dopaminergic activity, because treatment with haloperidol or clozapine-dopaminergic antagonism was effective at suppressing the hyperlocomotion in the nVH-lesioned animals (Lispka and Weinberger, 1994; Negrete-Diaz et al., 2010). Interestingly, in the present study, increased TH immunoreactivity levels in the NAcc shell were ameliorated by chronic Cbl treatment in the nVH lesioned rats. In addition, Cbl treatment also ameliorated the altered PPI in the vehicle-treated nVHL animals. PPI reflects a mechanism that allows an individual to filter incoming sensory information and is reportedly disrupted in schizophrenic patients. This disruption can be reversed by antipsychotics (Kumari and Sharma, 2002), PPI can also be disrupted either by several pharmacological treatments (i.e., dopamine receptor agonism, glutamate receptor antagonism, etc.; Geyer et al., 2001) or by nVHL (Lipska et al., 1995). Several brain regions have been shown to regulate PPI, including NAcc (core and shell; Swerdlow et al., 2000), and systemic and intra-accumbens administration of dopamine agonists disrupts PPI (Wan and Swerdlow, 1993; Weber and Swerdlow, 2008). Thus, the deficit in PPI observed in nVHL vehicle-treated rats could be caused by a dopamine hyperactivity, which was evidenced by the altered behavior related to an enhanced mesolimbic-dopaminergic activity. Moreover, clozapine or risperidone, both of which are dopamine antagonists, significantly attenuate nVHL-induced PPI deficits (Le Pen and Moreau, 2002; Rueter et al., 2004). Taken together, these data suggest that nVHL results in postpubertal development of a mesolimbic-dopaminergic hyperactivity, notably in the shell part of the NAcc, which in turn produces a PPI deficit; here, such an nVHL-induced PPI deficit was reversed by chronic Cbl treatment. Schizophrenic patients also

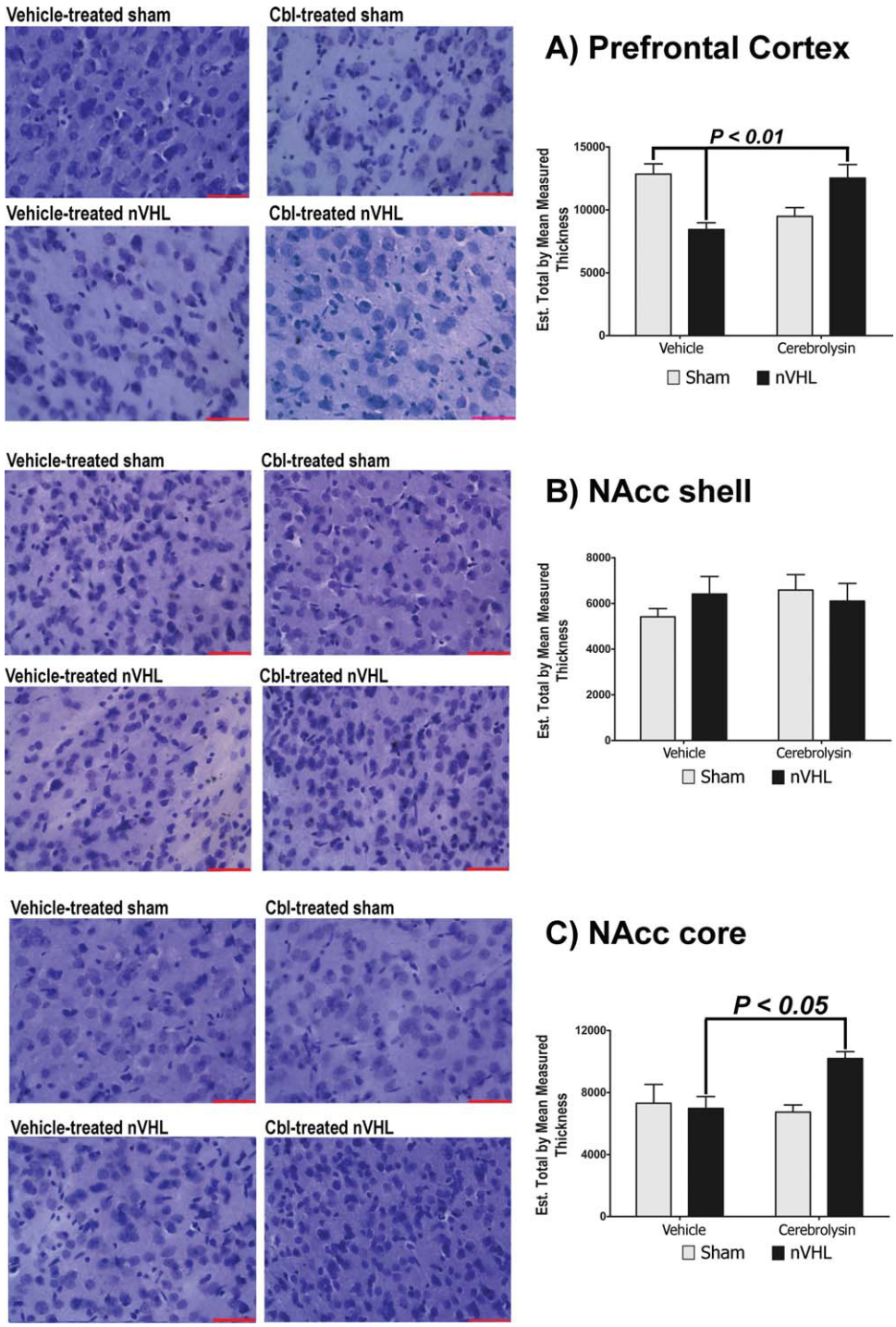

Fig. 10. Stereological analyses of neurons with cresyl violet from prefrontal cortex (PFC; **A**), nucleus accumbens (NAcc), NAcc shell (**B**), and NAcc core (**C**) The vehicle-treated nVHL rats showed a reduction in cells number in the PFC in comparison with vehicle-sham controls (A). Cbl treatment significantly ameliorated this loss in Cbl-treated nVHL rats compared with vehicle-treated nVHL animals. In addition, Cbl treatment increased the cell numbers in the NAcc core in the Cbl-treated nVHL rats compared with vehicle-treated nVHL animals (C), with no differences between vehicle- and Cbl-treated shams. Scale bar = 50 μm.

present so-called negative symptoms (i.e., avolition, alogia, apathy, and, diminished social interaction). In the nVHL model, after puberty, a reduction in active social interaction has been observed (Sams-Dodd et al., 1997; Becker et al., 1999). In the present study, nVH-lesioned animals shown a significant reduction in time spent in social inter-

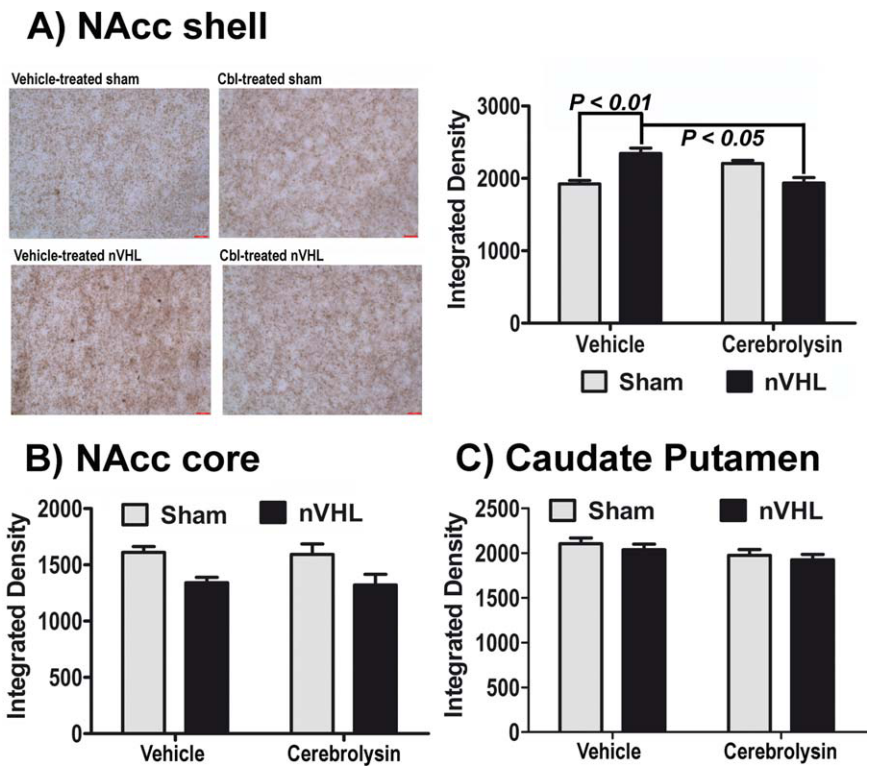

Fig. 11. Light microscopic images of immunostaining with an antibody against the tyrosine hydroxylase enzyme in the nucleus accumbens (NAcc) shell (**A**), NAcc core (**B**), and caudate-putamen (**C**). The analysis of the TH immunostaining at the level of the NAcc shell revealed an increase in TH immunoreactivity in vehicle-treated nVHL rats in comparison with vehicle-treated sham-operated controls. A significant decrease in TH immunoreactivity in the NAcc shell was observed following cerebrolysin treatment in nVHL rats in comparison with vehicle-treated nVHL rats (A). Finally, the analysis of the NAcc core (B) and CPu (C) revealed no differences among the groups. Scale bar = 20 μm.

action. Such reduction was restored in rats with nVHL by the Cbl treatment.

Findings on the DA efflux across shell and core of the NAcc are rather controversial. Corda et al. (2006), using microdialysis, found an increase in DA efflux induced by amphetamine in the core of the NAcc, with a concomitant attenuation of DA overflow in the shell of the NAcc in the nVH-lesion animals at postpuberal age. In contrast, Brake et al. (1999), using voltammetry, and Wan et al. (1996) and Lillrank et al. (1999), using microdialysis, found that amphetamine- and stress-induced activation of NAcc DA release is diminished in adult animals with neonatal damage to the VH, without differences in the baseline levels of DA. In addition, Chambers et al. (2010), using microdialysis, assessed the effect of cocaine on the DA efflux across shell and core of the NAcc in this animal model and found that cocaine-induced less DA efflux in shell and core of the NAcc, with no differences in baseline of levels DA in either part of the NAcc. All these results suggest that presynaptic release of DA had no major contribution to lesion-enhanced DA transmission in the mesolimbic DA system. However, an increase in TH was not implicated in increased DA release (for review see Daubner et al., 2011). TH is the rate-limiting enzyme of catecholamine biosynthesis, such as DA; this enzyme is needed to convert tyrosine to DOPA, a tetrahydrobiopterin, and molecular oxygen (for review see Daubner et al., 2011). TH is activated to make more DOPA, which after decarboxylation to dopamine is transferred into the synaptic vesicle by the vesicular monoamine transporter (VMAT). Interestingly, chronic stress reduces the VMAT2 density in the NAcc (Zucker et al., 2005) and increases the expression of TH in the ventral tegmental area (VTA; Ortiz et al., 1996). In addition, adult rats with nVH lesion also displayed an excessive reaction to stress (for review see Tseng et al., 2009), but there is no information on the VMAT density in the NAcc in this animal model. However, a recent report suggests that phenylcyclohexylpiperazine (PCP)-treated mice, another animal model related to schizophrenia, also showed a reduced expression of VMAT2 in the hippocampal formation (Iritani et al., 2010). Recently, several lines of evidence have suggested that altered functions of VMAT may be involved in the pathogenesis of certain neuropsychiatric diseases, such as schizophrenia (Westerink, 2006; Gutierrez et al., 2007).

A recent report (Sandner et al., 2011) suggests that chronic administration of phenylbutyrate, a histone deacetylases (HDAC) inhibitor (PD7–PD56) in rats, reduces long-term consequences of an nVH lesion, such as locomotion induced by apomorphine and alteration of reward learning. However, no effects are observed in

reducing anxiety and anatomical changes in the brain (Sandner et al., 2011). The HDAC activity has been reported to be enhanced in patients with schizophrenia and in nVH-lesioned rats, especially in the prefrontal cortex (Roth et al., 2009; Sander et al., 2011). A recent study (Doppler et al., 2008) evaluating the Cbl effects on H3 histone acelylation in $Mecp^{2308/Y}$ mutant mice, an animal model for Rett syndrome and with a hyperacetylation of histone (Shahbazian and Zoghbi, 2002), found that Cbl had no effect on histone acetylation. However, Cbl promotes recovery from dendritic and neuronal damage and behavioral improvements in young adult $Mecp^{2308/Y}$ mutant mice (Doppler et al., 2008). These results indicate that the beneficial effects of Cbl seen in this animal model of Rett syndrome are independent of the known histone deacetylase-containing complexes (Doppler et al., 2008).

The effects of Cbl on ameliorating the behavioral alterations in adult rats with nVHL may be related to its ability to promote dendritic regeneration. In nVHL rats, dendritic hypotrophy is observed in the PFC and NAcc (Flores et al., 2005a; Alquicer et al., 2008). These brain areas are in part responsible for some of the behavioral symptoms observed in the nVHL model and in schizophrenic patients (for review see Tseng et al., 2009). It is well known that excitatory inputs from the PFC are received by these dopaminergic neurons of the VTA, the source of the mesolimbic dopaminergic system (Sesack and Pickel, 1992). Moreover, the NAcc, the major component of the ventral striatum, occupies a key position to integrate a wide range of limbic and motor information (Mogenson et al., 1988; Meredith and Totterdell, 1999). Glutamatergic afferents from limbic-related brain areas influence the motor processes via NAcc connections with motor output structures such as the globus pallidus and substantia nigra pars reticulata (Nauta et al., 1978; Mogenson et al., 1983; Groenewegen et al., 1993). The main neurons in the NAcc, the medium spiny GABAergic cells, receive limbic afferents from the ventral subiculum of the hippocampus (Kelley and Domesick, 1982; Groenewegen et al., 1987), the basolateral amygdala (McDonald, 1991; Shinonaga et al., 1994; Petrovich et al., 1996), the PFC (Christie et al., 1985; Berendse et al., 1992), and the midline intralaminar thalamic nuclei (Berendse and Groenewegen, 1990). These distinct glutamatergic afferents innervate the central core and peripheral shell of the NAcc, forming intricate patterns of overlap and segregation (Wright and Groenewegen, 1995). The specific input–output characteristics of different NAcc subregions might represent circuits involving ensembles of neurons that could be modulated by specific sets of convergent afferent inputs (Pennartz et al., 1994; Groenewegen et al., 1999). Therefore, the NAcc is a site for integration of emotional salience and contextual constraints processed in the hippocampus and executive/motor plans from the PFC, with the output positioned toward controlling goal-directed behavior (for review see Goto and Grace, 2008).

Abnormal interactions between areas of the brain have been pointed out as possible causes for schizophrenia. Disturbances in PFC and striatum are evident in schizophrenia (Goldman-Rakic, 1994; Lewis, 1995; Kempf et al., 2008; Konrad and Winterer, 2008; Benes, 2010). Several reports have shown dendritic hypotrophy and spine loss in post-mortem brains of schizophrenics (Roberts et al., 1996; Garey et al., 1998; Glantz and Lewis, 2000; Rosoklija et al., 2000; Broadbelt et al., 2002; Blanpied and Ehlers, 2004). In addition, brain imaging studies in schizophrenia generally report lower volumes in limbic regions (for review see Puri, 2010). In addition, schizophrenia is widely believed to be a neurodevelopmental disorder of altered prefrontal connectivity and cognition (Marenco and Weinberger, 2000; Lewis and Levitt, 2002), and it is noteworthy that, similarly to our observations in nVHL animals, brains of human schizophrenic patients show reduced spine density in layer III prefrontal cortical pyramidal neurons (Glantz and Lewis, 2000) and lower cell density in limbic regions including the PFC (Thune and Pakkenbergh 2000; Witthaus et al., 2010; Yu et al., 2010; Schuster et al., 2011).

The effects of Cbl on dendritic arborization have been investigated in a chronic low-serum cell stress model (Hartbauer et al., 2001). After 4 days in cell culture, quantification of spontaneous outgrowth of embryonic chicken telencephalon neurons demonstrates an outgrowth promoting effect of Cbl. In contrast to the observed neuronal degeneration after 8 days in cells treated with a synthetic amino acid solution, Cbl-treated cells presented a well-differentiated neuronal network that was more pronounced than those seen in control cultures treated with brain-derived neurotrophic factor or serum supplementation. These results suggest a neurotrophic role of Cbl, which may in turn be due to the stabilizing effect of Cbl on the microtubule-associated protein MAP2. In addition, in animal models of excitotoxicity (Hutter-Paier et al., 1996, 1998), levels of MAP2 expression are increased by Cbl in a dose-dependent manner. The exact mechanism by which Cbl exerts its effect on MAP2 remains unclear. However, a recent study found that $Mecp^{2308/Y}$ mutant mice, an animal model for Rett syndrome, displayed a significant reduction in MAP2 expression in the basal ganglia, and Cbl was also able to ameliorate this reduction (Doppler et al., 2008).

The cumulative findings here demonstrate that the morphological effects of nVHL in the PFC and NAcc are complex; however, Cbl administration provides an opportunity for recovery from the dendritic hypotrophy observed in the PFC and NAcc, with an associated improvement in behavior. These findings support the possibility that Cbl has beneficial effects in the management of schizophrenic symptoms.

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