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ORIGINAL PAPER

Effects of Anti-NMDA Antibodies on Functional Recovery and Synaptic Rearrangement Following Hemicerebellectomy

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Abstract The compensation that follows cerebellar lesions is based on synaptic modifications in many cortical and subcortical regions, although its cellular mechanisms are still unclear. Changes in glutamatergic receptor expression may represent the synaptic basis of the compensated state. We analyzed in rats the involvement of glutamatergic system of the cerebello-frontal network in the compensation following a right hemicerebellectomy. We evaluated motor performances, spatial competencies and molecular correlates in compensated hemicerebellectomized rats which in the frontal cortex contralateral to the hemicerebellectomy side received injections of anti-NMDA antibodies from patients affected by anti-NMDA encephalitis. In the compensated hemicerebellectomized rats, the frontal injections of anti-NMDA antibodies elicited a marked decompensation state characterized by slight worsening of the motor symptoms as well as severe impairment of spatial mnesic and procedural performances. Conversely, in the sham-operated group the frontal injections of anti-NMDA antibodies elicited slight motor and spatial impairment. The molecular analyses indicated that cerebellar compensatory processes were related to a relevant rearrangement of glutamatergic synapses (NMDA and AMPA receptors and other glutamatergic components) along the entire cortico-cerebellar network. The long-term maintenance of the rearranged glutamatergic activity plays a crucial role in the maintenance of recovered function.

Keywords Anti-NMDA encephalitis · Cerebellar compensation · Glutamate receptors · Morris water maze · Postural and motor behaviors · Rat

Introduction

Since cerebellar circuits are endowed with great plastic potentialities, the compensation that follows cerebellar lesions represents an excellent model of lesion-induced plasticity (Burello et al. 2012). As classically observed in patients and animals following surgical ablation or stroke injury of the cerebellar structures (Holmes 1917; Luciani 1891), unilateral cerebellar lesions (as the hemicerebellectomy, HCb) cause a dramatic syndrome that subsides with time in a process of functional recovery known as "cerebellar compensation." During the compensated state, the severity of static symptoms (including ocular nystagmus, head and body tilt) gradually decreases, whereas dynamic symptoms (including complex and coordinated motor behaviors) compensate much less completely and

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more variably (Cutuli et al. 2011; Manni and Dow 1963; Molinari et al. 1990). Finally, a residual motor symptomatology with ataxic, dysmetric and asthenic symptoms remains present together with a multifaceted cognitive impairment, which affects visuo-spatial, mnesic, attentional, and executive functions (Buckner 2013; Timmann et al. 2010). It is assumed that cerebellar compensation is based on profound synaptic modifications in many cortical and subcortical regions, but the detailed cellular mechanisms are still a matter of debate (Federico et al. 2006b).

Glutamatergic synapses mediate most of brain excitatory neurotransmission, and changes in their strength or efficiency have emerged as a cellular basis for learning and memory processes. According to the mechanism by which their activation gives rise to a postsynaptic current, ionotropic (NMDA, AMPA and kainate receptors) and metabotropic receptors may be identified (Conn et al. 2005; Lisman et al. 2002). In the neocortex, the most abundant subtypes of NMDA receptors (NMDAR) are composed of GluN1 subunits associated with GluN2A and/or GluN2B subunits (Cull-Candy and Leszkiewicz 2004). The ratio between GluN2A and GluN2B varies during synaptic refinements following sensory experience (Lau and Zukin 2007). Thus, depending on the context, synapses adapt their GluN2 signaling in order to appropriately modulate their integrative capacity. Animal models and human pharmacological trials (Gunduz-Bruce 2009; Honnorat et al. 2001) have established that synaptic localization and trafficking of the NMDAR and AMPA receptors (AMPAR) are the basis of many motor and cognitive functions (Lau and Zukin 2007; Shepherd and Huganir 2007).

Getting back to the cerebellar compensation, changes in NMDAR expression, affinity or efficacy may represent the synaptic basis of compensated state. Indeed, we previously showed that systemic administration of an NMDAR antagonist (CGS 19,755) to compensated hemicerebellectomized rats provokes a dramatic loss of the compensated state (decompensation) (Federico et al. 2006a). However, because of the systemic administration of the drug, the action sites of the NMDAR antagonist could not be defined. Recently, an autoimmune "anti-NMDA encephalitis" has been described (Dalmau et al. 2008; Sansing et al. 2007). It has been shown that the extracellular domain of the N1 subunit of the NMDAR is directly targeted by autoantibodies present in patients' serum and cerebrospinal fluid (CSF). The impairment of glutamate signaling via the NMDAR leads to the prominent psychiatric and behavioral symptoms, rapid memory loss, dyskinesias, seizures, and autonomic instability characteristic of the disease (Dalmau et al. 2008, 2011; Sansing et al. 2007). The NMDAR reduction decreases the ability of neurons to respond to glutamate and, by doing so, harms multiple essential neuronal functions that depend on, and are mediated by, glutamate signaling (Hughes et al. 2010). More recently, it has been demonstrated that blocking NMDAR activity with autoimmune anti-NMDA antibodies (Ab) prevents the mobility of the same receptors at synaptic sites causing long-term potentiation (LTP) impairment (Dupuis et al. 2014).

On such a basis, in the present research we studied in rats the involvement of NMDAR of the frontal cortex (FC) in the cerebellar compensation that follows a hemicerebellar ablation. In fact, given the extensive bidirectional cortico-cerebellar connections (Middleton and Strick 2001), the FC and the spared hemicerebellum may be preferential sites of the synaptic remodeling characterizing the compensated state. We analyzed motor (postural and locomotor) performances, spatial (mnesic and procedural) competencies and molecular (composition of postsynaptic density, PSD) correlates in compensated HCb rats which in the FC contralateral to HCb side received injections of anti-NMDA Ab from anti-NMDA encephalitic patients or injections of serum without Ab. We expected that the dramatic hypofunction of NMDAR caused by encephalitic patients' Ab injections could disrupt the cortico-cerebellar glutamatergic synaptic rearrangement featuring the compensated state.

Materials and Methods

CSF and IgG Purification

The methodology used for the control CSF has been explained earlier (Manto et al. 2010, 2011). We used patients' CSF positive for NMDAR Ab and subsequently confirmed the results using purified IgG. Patients' CSF positive for NMDAR Ab was obtained from two patients with anti-NMDA encephalitis (University of Berlin, Germany). The composition of the CSF is given in the Supplementary Table S1. NMDAR Ab concentrations were 1:32 and 1:100 in CSF1 and CSF2, respectively. The method used to demonstrate the presence of Ab has been reported previously (Wandinger et al. 2011). Identification of Ab was performed by Euroimmun (Germany). IgGs were purified from the serum of a patient (SNP1037; University of Lyon, France) presenting the anti-NMDA encephalitis. Serum was collected at symptom presentation, before any treatment. Briefly, IgG were adsorbed to protein A-Sepharose beads (protein A Sepharose 4 fast flow, Sigma, L'Isle-d'Abeau, France) and eluted with glycine buffer (0.1 M, pH 2.8). After neutralization with Tris 1.5 M pH 8.8, IgGs were dialyzed overnight at 4 °C against PBS buffer, pH 7.4 and sterilized by filtration with 0.22-μm filters. Protein concentration was estimated with the nanodrop spectrophotometer (Thermoscientific, Villebon, France). IgG concentration was 2.2 mg/ml (Manto et al. 2007, 2010, 2011).

Experimental Procedures

The present data were collected from 48 adult male Wistar rats (300–450 g; Harlan, Italy) housed in standard conditions. They were housed two animals to a cage with temperature (22–23 °C) and humidity controlled (60 ± 5 %), under a 12:12 h light/dark cycle (light on between 07.00 and 19.00 h), with free access to food and water. All efforts were made to minimize animal suffering and to reduce their number in accordance with the European Union Directive of September 22, 2010 (2010/63/EU). All procedures were approved by the Italian Ministry of Health.

Twenty-four rats received a right HCb and 24 rats a sham surgery. Once recovered (the recovery had occurred 2 months after HCb or sham surgery), the animals were injected with anti-NMDA Ab or serum 24 h before the behavioral testing. The injections of serum or anti-NMDA Ab were performed in the left FC (contralaterally to the HCb side). Thus, 4 groups of animals (12 rats/group) were used: HCb + serum (H–S), HCb + anti-NMDA Ab (H–A), Sham + serum (S–S), Sham + anti-NMDA Ab (S–A).

Since we found that action of the anti-NMDA Ab on motor and spatial performances was temporary (see "Results" section), we sacrificed a subsample of animals 24 h after frontal injections to perform the biochemical analyses when Ab effects were still well marked. Thus, out of 12 rats of each group, 7 animals were behaviorally tested twice, at 24 and 48 h after the frontal injections, while the remaining 5 rats were behaviorally tested only at 24 h after the injections and then killed. Behavioral data of these four 5-animal groups are described in detail as Supplementary Material. To verify the homogeneity of the differently sized experimental groups, we compared the performances of the four 7-animal groups with those of the four 5-animal groups on all behavioral parameters by means of three-way MANOVA (sample size × group × parameter). This analysis revealed a not significant difference between the differently sized groups ($F_{1,40} = 0.19$; $p = 0.66$), while the differences among four experimental groups ($F_{3,40} = 9.6$; $p < 0.0001$) and parameters ($F_{33,1320} = 122.29$; $p < 0.000001$) were significant. The second-order interaction (sample size × group × parameter) was not significant ($F_{99,1320} = 0.24$; $p = 1$). The only significant first-order interaction was group × parameter ($F_{99,1320} = 4.14$; $p < 0.000001$). Thus, we considered the differently sized groups as closely matching.

Surgery

All rats were anesthetized with Zoletil 100 (tiletamine and zolazepam: 50 mg/kg i.p.—Virbac s.r.l., Milan, Italy) and Rompun (xylazine: 10 mg/Kg i.p.—Bayer s.p.a., Milan, Italy), and a craniotomy was performed over the right hemicerebellum. In the animals undergoing the surgical ablation of the cerebellar regions, the dura was excised, and the right cerebellar hemisphere and hemivermis as well as the fastigial, interpositus, and dentate cerebellar nuclei of the right side were ablated by suction. Care was taken not to lesion the extra-cerebellar structures. The cavity was filled with sterile gel foam, the wound edges were sutured and the animals were allowed to recover from anesthesia and surgical stress. In the animals belonging to the sham surgery group, no cerebellar ablation was performed. The wound edges were then sutured, and the animals were allowed to recover from anesthesia and surgical stress.

Lesioned animals were included in the present study only if they had received a total ablation of the right side of the cerebellum. The extent of the cerebellar lesion was determined by visual inspection.

Neurological Evaluation

The presence of postural and locomotor symptoms as well as of deficient complex motor behaviors elicited by the right HCb was assessed by means of a behavioral rating scale from the very first post-lesional days until a stable compensation stage. A score from 0 to 2 was assigned to each symptom according to its degree of severity (0 = absent; 1 = slight; 2 = marked) (Foti et al. 2011). As 23 behaviors were taken into account, the total score ranged from 0 (complete absence of any deficits) to 46 (presence of all symptoms to the highest degree). The behavioral scores were attributed by an expert investigator unaware of the individual specimen's group. The neurological evaluation started 24 h after HCb or sham lesion, and was performed at variable time intervals for 2 months. After 2 months, insignificant changes in the lesioned animals' test scores were observed, indicating a stable motor and behavioral symptomatology (Supplementary Fig. S1, Supplementary Table S2). The presence of symptoms was again assessed before (pre-injection day) and after (1st and 2nd post-injection day) the frontal injections of anti-NMDA Ab or serum.

FC Injections

After 2 months from HCb or sham surgery, under anesthesia all rats received three injections of 7.5 µl each one of anti-NMDA Ab (H–A and S–A groups) or serum without Ab (H–S and S–S groups) performed at three different sites of the left FC through 25-µL Hamilton syringe (stereotaxic coordinates: Cg/M2: AP +3.2, ML +1, DV -2; M1: AP +3.2, ML +2.2, DV -2; M1: AP +3.2, ML +3.4, DV -3) (Paxinos and Watson 1998). Solutions were injected in each site at flow rates of 1.5 µl/min. The needle was left in situ 5 min after each injection. The animals were

allowed to recover from anesthesia and surgical stress for 24 h.

Morris Water Maze (MWM)

Following (1st and 2nd post-injection day) the frontal injections of anti-NMDA Ab or serum, the animals were submitted to the MWM to evaluate their spatial mnesic (localization abilities) and procedural (navigational strategies) competencies (Federico et al. 2006a; Foti et al. 2011; Gandhi et al. 2000; Leggio et al. 1999). Notably, postural and locomotor symptoms do not severely affect swimming performances, and do not alter forelimb movement inhibition, hindlimb thrusting or turning abilities, because swimming functions are under multisystem control, so that extra-cerebellar circuits can efficiently operate to provide compensatory support (Molinari et al. 1990; Petrosini et al. 1990). Consequently, testing cerebellar animals on cognitive tasks that require swimming performances is advantageous for analyzing spatial procedural abilities not influenced by motor impairment or motivational failures.

Short-lasting anti-NMDA Ab effects were evaluated through an MWM protocol detailing 24–48 h effects (Federico et al. 2006a, b; Hampe et al. 2013).

The rats were placed in a circular white pool (diameter 140 cm) filled with 24 °C water made opaque by the addition of non-toxic acrylic black color (Giotto, Italy). An escape platform (diameter 10 cm) was placed in the middle of one quadrant, 30 cm from the side walls. It was either submerged 2 cm or raised 2 cm above the water level. On day 1 (24 post-injection h), each rat was submitted to the 10-trial Place 1 phase (hidden platform put in the SE quadrant), followed by 1 trial with no platform in the pool (Probe) and then to the 3-trial Cue phase (visible platform put in the NW quadrant). On day 2 (48 post-injection h), the animals performed the 10-trial Place 2 phase (hidden platform put in the NW quadrant).

The rat was released into the water from randomly varied starting points and allowed to search for the hidden or visible platform for a maximum of 120 s. When the rat reached the platform, it was allowed to remain there for 30 s. The inter-trial interval was 1 min. In the Probe phase, the platform was removed and rats were allowed 60 s to search for it. Navigational trajectories were recorded by a video camera whose signal was relayed to a monitor and to an image analyzer (Ethovision, Noldus, Wageningen, The Netherlands).

The following MWM parameters were considered: escape latency (in seconds) to find the platform; total distance (in cm) swum in the pool; peripheral distance, considered as percentage of total distance swum in a 20-cm peripheral annulus; and swimming velocity (in cm/s). All parameters have been considered in the 10 trials (scores averaged pair-wise) of Place 1 and 2 phases as well as in the 3 trials of Cue phase. Furthermore, during Probe phase the percentage of total distance travelled in the previously rewarded (platform) quadrant was calculated. The following navigational strategies put into action in reaching the platform regardless of whether the platform was reached or not were considered: Circling (C), circular swimming with counterclockwise and clockwise turnings and loop swimming with compulsive restricted turnings; Searching (S), swimming around the pool in all or some quadrants; Finding (F), reaching the platform by swimming through a brief semicircular or direct trajectory without any searching around the pool. Animals were not trained in MWM prior to frontal injections to avoid prior acquisition of skills. Two researchers unaware of the individual specimen's group categorized the swimming trajectories drawn by the image analyzer. They attributed the dominant behavior in each trial to a specific category. Categorization was considered reliable only when their judgments were consistent.

Sub-Synaptic Fractionation and Western Blot

At the end of the 1st behavioral evaluation day, the animals devoted to biochemical analyses were killed by decapitation under deep anesthesia. Brains were removed and frontal cortices and left hemicerebellum were dissected to isolate the PSD (D'Amelio et al. 2011). Briefly, tissues were homogenized in cold homogenization buffer (320 mM sucrose, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 1 mM PMSF, 1 mM Na_3VO_4, 5 mMNaF, 20 mM 2-glycerophosphate, protease inhibitors cocktail-Sigma) with ten strokes of a tight-fitting glass Dounce tissue grinder. The homogenate was centrifuged at $1000\times g$ for 10 min, and the resulting supernatant was centrifuged at $10{,}000\times g$ for 15 min. The pellet was re-suspended in homogenization buffer containing 0.5 % Triton X-100 with ten strokes of a tight-fitting glass Dounce tissue grinder, incubated 40 min on ice and centrifuged at $32{,}000\times g$ for 20 min. The resulting pellet (TxP) containing PSD fraction was re-suspended in RIPA buffer (50 mM Tris-HCl (pH 7.4), 1 % Triton X-100, 0.25 % sodium deoxycholate, 150 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.1 % SDS, protease inhibitors cocktail), sonicated and incubated on ice for 20 min. The samples were centrifuged at $11{,}500\times g$ for 10 min and the protein concentration of resulting supernatant was determined by Bradford method.

Equal amounts of proteins were applied to SDS-PAGE and electroblotted on a PVDF membrane. Immunoblotting analysis was performed using a chemiluminescence detection kit. The relative levels of immunoreactivity were determined by densitometry by using the software Image J.

Primary antibodies were the following: GluA1 (1:1000; Millipore-Upstate); PSD-95 (1:1000; Millipore-Chemicon); GluN1, GluN2A (1:500; Santa Cruz Biotechnology).

Secondary antibodies were the following: goat anti-rabbit IgG HRP conjugate (1:3000; Bio-Rad), goat anti-mouse IgG HRP conjugate (1:3000; Bio-Rad); rabbit anti-goat IgG HRP conjugate (1:1000; Bio-Rad).

Statistical Analyses

Data presented as mean ± SEM were first tested for normality (Wilk-Shapiro's test) and homoscedasticity (Levene's test). Then, data were analyzed by using one-way ANOVAs (group) or two-way ANOVAs for independent (group) and repeated measures (trial/day/navigational strategy) followed by Newman-Keuls' test, when appropriate. Significance level was set at $p \leq 0.05$. Statistical analyses were performed by using STATISTICA 8.0 (StatSoft, Italy).

Results

Neurological Evaluation

The analysis of the motor behaviors revealed that before the injections (pre-injection day) some postural and locomotor symptoms (head bobbing, head tilt, tremor, wide base, collapsing on the belly) and some deficient complex behaviors (as those displayed in testing abilities of descending a ladder, rearing, vestibular drop) were still slightly present in both HCb groups (H–S and H–A) (Fig. 1). No motor deficit was present in the sham-operated (S–S and S–A) animals. In H–A rats, the anti-NMDA Ab injections induced a worsening of some HCb symptoms (head and body tilt, hyperflexion of right hindlimb, collapse on the belly, side falls), without affecting nystagmus, hyperactivity and turning. In sham-operated rats, the frontal anti-NMDA Ab injections slightly impaired postural and locomotor abilities and more severely the motor abilities, as rearing and vestibular drop, once again leaving unaffected eye nystagmus, hyperactivity and turning. All effects of anti-NMDA Ab faded away within 48 h from the injection (2nd post-injection evaluation).

Statistical Comparisons

A two-way ANOVA (group × day) on the scores of the behavioral rating scale revealed significant group ($F_{(3,24)} = 27.21$; $p < 0.00001$) and day ($F_{(2,48)} = 10.51$; $p = 0.0002$) effects. The interaction was also significant ($F_{(6,48)} = 2.51$; $p = 0.03$). Post hoc comparisons on interaction revealed that both H–S and H–A groups were significantly different (at least $p = 0.03$) from S–S and S–A groups in the three evaluations (pre-injection, 1st and 2nd post-injection day). Interestingly, in both groups

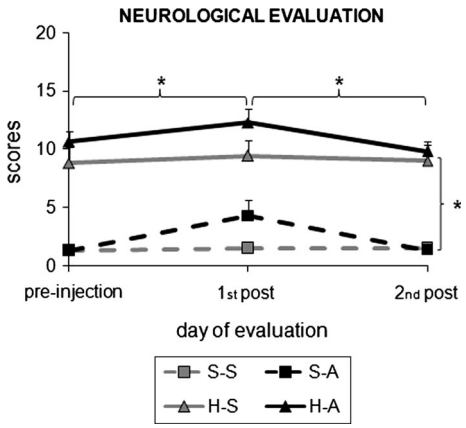

Fig. 1 Effects of frontal anti-NMDA antibodies or serum injections on postural and locomotor symptoms in hemicerebellectomized or sham-lesioned rats. Abscissa: pre-injection and 1st and 2nd post-injection days. Ordinate: degree of severity of symptoms evaluated according to the rating scale described in the text. *Data* are presented as mean ± SEM. Intra- and inter-group post hoc comparisons: at least * $p < 0.03$. In this and the following figures: S–S: Sham + Serum; S–A: Sham + anti-NMDAR Ab; H–S: HCb + serum; H–A: HCb + anti-NMDAR Ab, groups

treated with anti-NMDA Ab (H–A, S–A) there were significant within-group differences among the three evaluations. Namely, in H–A and S–A groups a significant (at least $p = 0.02$) neurological impairment was observed when pre-injection and 1st post-injection evaluations were compared and a complete recovery from anti-NMDA Ab effect was observed when 1st and 2nd post-injection evaluations were compared.

Spatial Performances in MWM

As depicted in Figs. 2 and 3, S–S group exhibited the best spatial (mnesic and procedural) competencies and searched for (and found) the platform with the most direct strategies during the entire MWM task including Probe phase. In S–A group, the anti-NMDA Ab injections resulted in a spatial impairment (mnesic and procedural) as severe as the impairment displayed by H–S animals in Place 1 and Probe phase when both groups persisted in displaying a compulsive Circling in the peripheral pool sectors. In Place 2 (2nd post-injection day), the S–A group reached the platform through the strategy of direct Finding and showed tuned spatial abilities similar to those of S–S animals, demonstrating thus that in the presence of intact cerebello-frontal circuit the anti-NMDA Ab effect completely ended within 48 h. The H–S group displayed mnesic and procedural performances compromised in all MWM phases. In H–A group, the injections of anti-NMDA Ab induced the most severely impaired spatial performances and elicited a compulsive Circling during Place 1 and Probe phase. In Place 2, the H–A group still exhibited spatial deficits, at

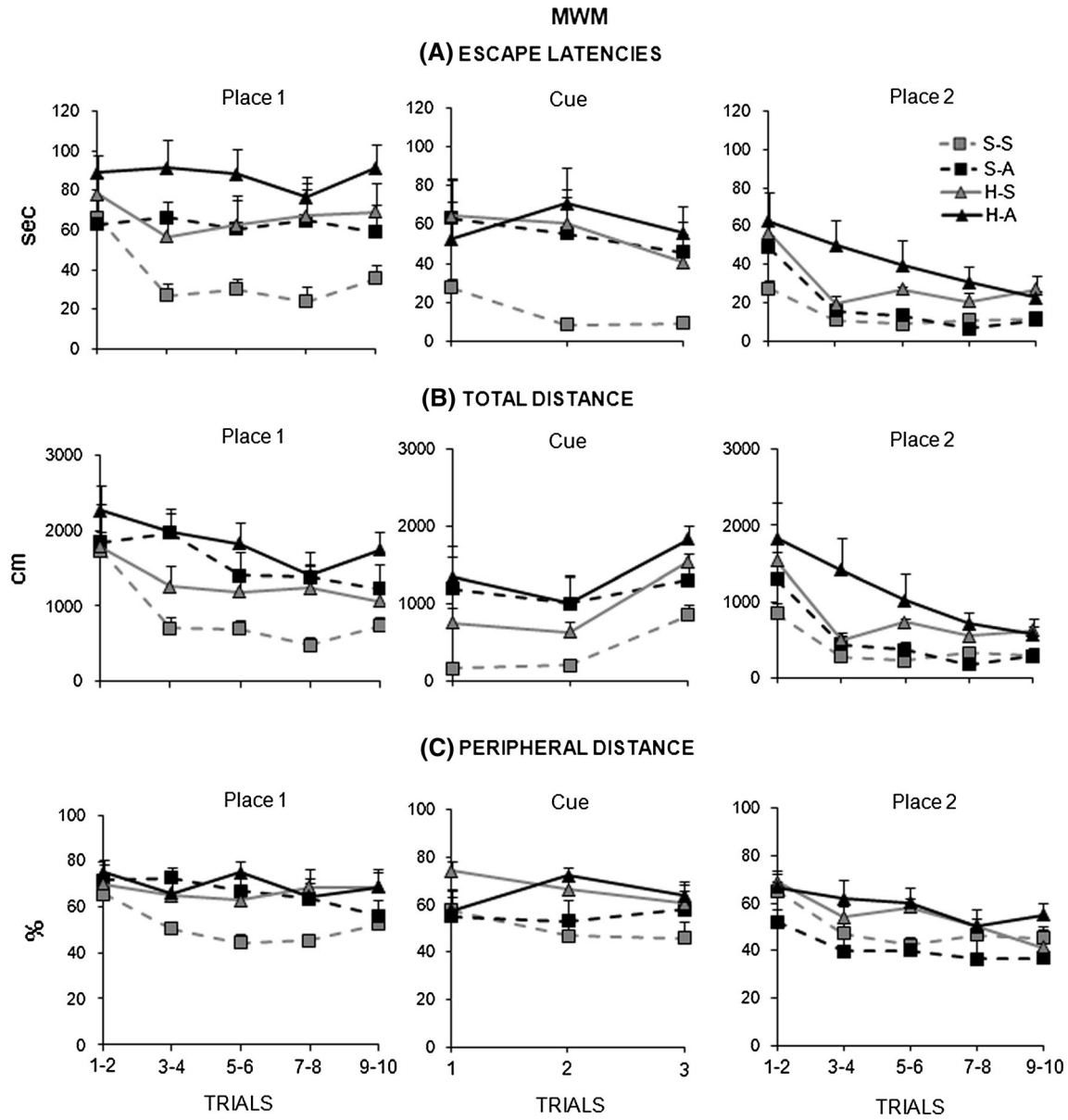

Fig. 2 Effects of frontal anti-NMDA antibodies or serum injections on the Morris Water Maze (MWM) performance in hemicerebellectomized or sham-lesioned rats analyzed in Place 1, Cue and Place 2 phases. Escape latencies (**a**), total distance (**b**) and peripheral distance (**c**) are depicted. Data are presented as mean ± SEM

odds of the disappearance of anti-NMDA Ab effects in S-A group. Thus, in the HCb animals when tested in MWM, the anti-NMDA Ab injections caused the cerebellar impairment to get worse eliciting thus a marked and long-lasting decompensation.

Statistical Comparisons

Escape Latencies (Fig. 2a) A two-way ANOVA (group x trial) on escape latency of Place 1 phase revealed a significant group effect ($F_{(3,24)} = 7.78$; $p = 0.0008$) and no significant trial effect ($F_{(4,96)} = 1.51$; $p = 0.20$). The interaction was not significant ($F_{(12,96)} = 0.78$; $p = 0.67$). Post hoc comparisons on group effect revealed that S–S animals showed the lowest latencies in comparison with the remaining groups (at least $p = 0.02$) and that H–S and S–A animals exhibited latencies lower than H–A animals (at least $p = 0.04$). A two-way ANOVA (group x trial) on escape latency of Cue phase revealed a significant group effect ($F_{(3,24)} = 3.32$; $p = 0.04$) and no significant trial effect ($F_{(2,48)} = 2.02$; $p = 0.14$). The interaction was not significant ($F_{(6,48)} = 0.71$; $p = 0.64$). Post hoc comparisons on group effect revealed that the S–S animals showed the lowest latencies in comparison with the remaining

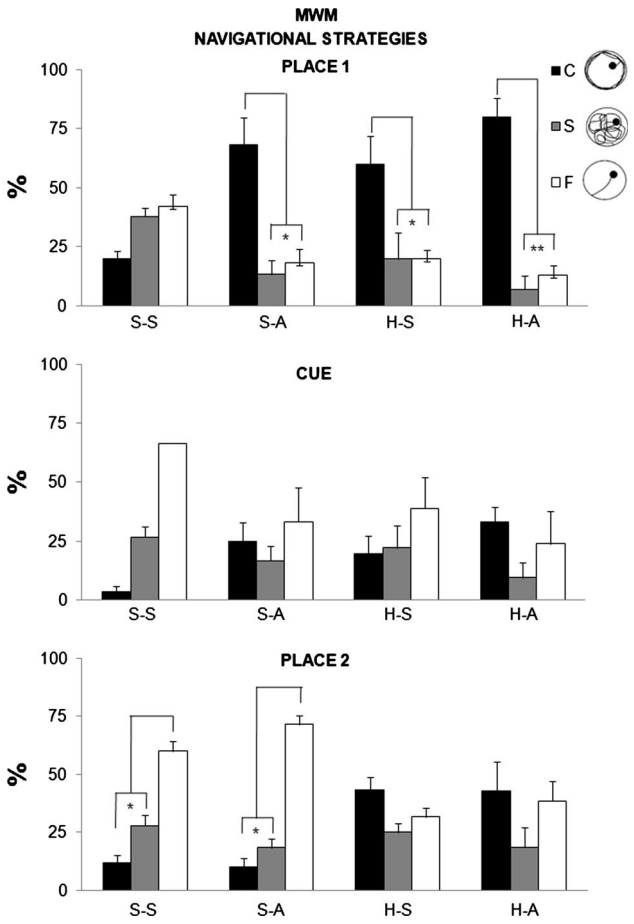

Fig. 3 Effects of frontal anti-NMDA antibodies or serum injections on the Morris Water Maze (MWM) navigational strategies exhibited in the three phases by hemicerebellectomized or sham-lesioned rats. The circular figurines at the top of the figure illustrate the typical explorative patterns of the three main navigational strategies. The *black filled circles* indicate platform position. *C* circling, *S* searching, *F* finding strategies. Data are presented as mean ± SEM. Intra-group post hoc comparisons: at least * $p < 0.03$; ** $p < 0.01$

groups (at least $p = 0.04$). A two-way ANOVA (group x trial) on escape latency of Place 2 phase revealed significant group ($F_{(3,24)} = 12.35$; $p = 0.00004$) and trial ($F_{(4,96)} = 16.61$; $p < 0.00001$) effects. The interaction was not significant ($F_{(12,96)} = 1.18$; $p = 0.31$). Post hoc comparisons on group effect revealed that S–S and S–A groups showed latencies lower than H–S (at least $p = 0.03$) and H–A (at least $p = 0.0005$) animals. Interestingly, H–S animals showed latencies lower than H–A animals ($p = 0.03$).

Total distance (Fig. 2b) A two-way ANOVA (group x trial) on total distance of Place 1 phase revealed significant group ($F_{(3,24)} = 6.30$; $p = 0.003$) and trial ($F_{(4,96)} = 7.16$; $p = 0.00004$) effects. The interaction was not significant ($F_{(12,96)} = 0.77$; $p = 0.68$). Post hoc comparisons on group effect revealed that S–S animals showed the lowest total distances in comparison with the remaining groups (at least $p = 0.05$) and that H–S animals showed total distances lower than H–A animals ($p = 0.04$). A two-way ANOVA (group x trial) on total distance of Cue phase revealed a significant group effect ($F_{(3,24)} = 3.22$; $p = 0.04$) and no significant trial effect ($F_{(2,48)} = 1.14$; $p = 0.33$). The interaction was not significant ($F_{(6,48)} = 1.10$; $p = 0.38$). Post hoc comparisons on group effect revealed that S–S animals showed total distance lower than S–A ($p = 0.04$) and H–A ($p = 0.03$) animals. A two-way ANOVA (group x trial) on total distance of Place 2 phase revealed significant group ($F_{(3,24)} = 10.59$; $p = 0.0001$) and trial ($F_{(4,96)} = 18.70$; $p < 0.00001$) effects. The interaction was not significant ($F_{(12,96)} = 1.13$; $p = 0.34$). Post hoc comparisons on group effect revealed that S–S and S–A animals showed total distances lower than H–S (at least $p = 0.04$) and H–A (at least $p = 0.0008$) animals, and that H–S animals showed total distance lower than H–A animals ($p = 0.03$).

Peripheral Distance (Fig. 2C) A two-way ANOVA (group × trial) on peripheral distance of Place 1 phase revealed significant group ($F_{(3,24)} = 3.42$; $p = 0.03$) and trial ($F_{(4,96)} = 3.34$; $p = 0.01$) effects. The interaction was not significant ($F_{(12,96)} = 1.46$; $p = 0.15$). Post hoc comparisons on group effect revealed that S–S animals showed the lowest percentage of peripheral distance in comparison with the remaining groups (at least $p = 0.03$).Two-way ANOVA on peripheral distance of Cue phase revealed no significant group ($F_{(3,24)} = 2.52$; $p = 0.08$) and trial ($F_{(2,48)} = 0.42$; $p = 0.66$) effects. Also the interaction was not significant ($F_{(6,48)} = 1.10$; $p = 0.37$). Two-way ANOVA on peripheral distance of Place 2 phase revealed significant group ($F_{(3,24)} = 3.95$; $p = 0.03$) and trial ($F_{(4,96)} = 12.83$; $p < 0.00001$) effects. The interaction was not significant ($F_{(12,96)} = 0.96$; $p = 0.49$). Post hoc comparisons on group effect revealed that S–A animals showed percentages of peripheral distance lower than H–A animals ($p = 0.04$).

Swimming Velocity A one-way ANOVA on mean swimming velocities revealed no significant differences ($F_{(3,24)} = 2.19$; $p = 0.11$) among S–S ($\bar{x} = 24.3$ cm/s), S-A ($\bar{x} = 24.4$), H–S ($\bar{x} = 21.6$) and H–A ($\bar{x} = 22.1$) groups.

Total Distance During Probe Phase A one-way ANOVA on percentage of total distance travelled in the previously rewarded quadrant during the Probe phase revealed significant differences among groups ($F_{(3,24)} = 6.32$; $p = 0.002$). Post hoc comparisons revealed that S–S animals ($\bar{x} = 31.2$ %) showed percentage of total distance higher than S–A ($\bar{x} = 25.4$ %), H–S ($\bar{x} = 22.9$ %) and H–A ($\bar{x} = 20.1$ %) (at least $p = 0.03$) animals.

Navigational Strategies In the Place 1, while S–S animals already put into action the efficient navigational strategy of Finding, S–A, H–S and H–A animals exhibited high percentages of the inefficient Circling (Fig. 3). A two-way ANOVA (group × strategy) on navigational strategies of Place 1 revealed a not significant group effect ($F_{(3,24)} = 1.94$; $p = 0.15$) and a significant strategy effect ($F_{(2,48)} = 21.27$; $p < 0.00001$). The interaction was significant ($F_{(6,48)} = 5.60$; $p = 0.0002$). Post hoc comparisons on interaction revealed that S–A, H–S and H–A groups, but not S–S animals, put into action mainly the Circling strategy in comparison with other strategies (at least $p = 0.02$).

A two-way ANOVA on navigational strategies of Cue phase revealed a not significant group effect ($F_{(3,24)} = 0.92$; $p = 0.44$) and a significant strategy effect ($F_{(2,48)} = 5.78$; $p = 0.005$). The interaction was not significant ($F_{(6,48)} = 1.25$; $p = 0.29$).

Two-way ANOVA on navigational strategies of Place 2 revealed significant group ($F_{(3,24)} = 3.11$; $p = 0.04$) and strategy ($F_{(2,48)} = 16.11$; $p < 0.00001$) effects. The interaction was significant ($F_{(6,48)} = 6.48$; $p = 0.00005$). Post hoc comparisons on interaction revealed that the Finding was the most used strategy (at least $p = 0.04$) in comparison with other strategies by S–S and S–A animals. H–S and H–A animals used similar percentages of all strategies.

Significant differences were found by comparing each navigational strategy during Place 1 and Place 2 phases. While no difference was found in the Searching strategy, post hoc comparisons of significant interactions of two-way ANOVAs (group × phase) on Circling ($F_{(3,24)} = 6.19$; $p = 0.002$) and Finding ($F_{(3,24)} = 11.84$; $p = 0.00006$) strategies indicated that in Place 2 the Circling was significantly (at least $p = 0.0005$) reduced in S–A and H–A animals and the Finding was significantly (at least $p = 0.002$) increased in S–S, S–A and H–A animals.

PSD Analysis

To evaluate the effects of cerebellar lesion and of frontal anti-NMDA Ab injections on PSD composition, we isolated PSD from left (injected and contralateral to the HCb) and right (non-injected and ipsilateral to the HCb) frontal cortices (Fig. 4), and left (spared) hemicerebellum (Fig. 5) of all groups at 24 h after the frontal injections.

In comparison with S–S group, in S–A animals the anti-NMDA Ab significantly reduced (p at least 0.04) the NMDAR subunits GluN1 and GluN2A at postsynaptic sites in the injected FC, but not in the non-injected one and in the spared hemicerebellum. The reduction was found only on NMDAR, given the AMPAR subunit GLUA1 and PDS-95 levels were not affected in both frontal cortices as well as in the spared hemicerebellum.

In comparison with S–S group, in H–S rats the NMDAR subunits GluN1 and GluN2A as well as GluA1 and PSD-95 levels were significantly increased (p ranging from 0.04 to 0.005) in both frontal cortices. Also in the spared hemicerebellum, the GluA1 and GluN1 (but not GluN2A and PSD-95) levels were significantly increased ($p < 0.01$). Thus, the synaptic changes occurring during the cerebellar compensation involve a heavy synaptic

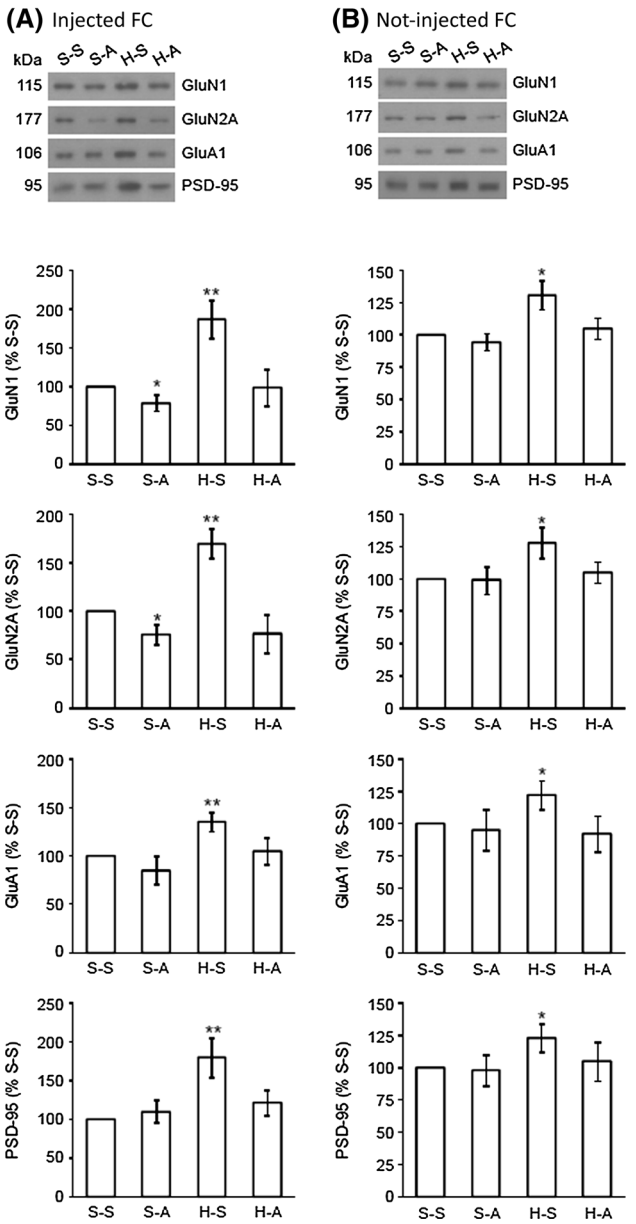

Fig. 4 Effects of frontal anti-NMDA antibodies or serum injections on postsynaptic density (PSD) composition in the frontal cortices. Representative immunoblots of PSD proteins isolated from the injected (**a**) and not-injected (**b**) frontal cortex (FC) of S–S, S–A, H–S and H–A groups. The histograms show the relative levels of GluN1, GluN2B, GluA1 and PSD-95 (S–S percentage). Data are expressed as mean ± SEM ($n = 5$ per group). * $p < 0.05$; ** $p < 0.01$

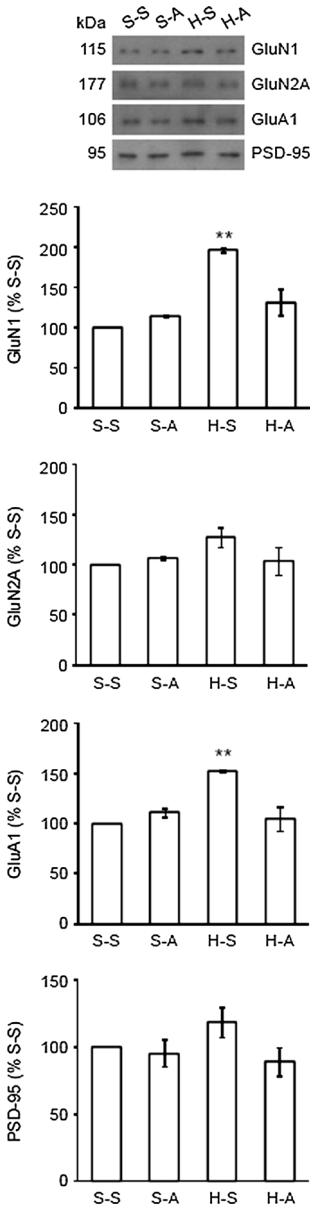

Fig. 5 Effects of frontal anti-NMDA antibodies or serum injections on postsynaptic density (PSD) composition in the spared hemicerebellum. Representative immunoblots of PSD proteins isolated from the spared hemicerebellum of S–S, S–A, H–S and H–A groups. The *histograms* show the relative levels of GluN1, GluN2B, GluA1 and PSD-95 (S–S percentage). Data are expressed as mean ± SEM ($n = 5$ per group). ** $p < 0.01$

glutamatergic reorganization of the PSD at neocortical and cerebellar level.

In comparison with H–S group, in the H–A group the anti-NMDA Ab injections caused a significant reduction (p at least 0.04) of the NMDAR subunits GluN1 and GluN2A, as well as of GluA1 levels in both frontal cortices. Conversely, PSD-95 levels significantly diminished ($p = 0.04$) only in the injected FC. In the spared hemicerebellum, the GluA1 and GluN1 levels were significantly reduced ($p < 0.01$).

These findings indicate that in HCb animals the decompensating effect of anti-NMDA Ab was linked to the marked reduction not only of NMDAR but also of the other glutamatergic components either in frontal or cerebellar sites.

Discussion

Although unilateral cerebellar ablations result in massive destruction of neural networks, alterations to neurotransmitter systems, and disruption to vasculature, dramatic recovery mainly of motor functions characterizes the compensated state. While the typical pattern and time course of the functional recovery following cerebellar lesions are well described (Holmes 1917; Luciani 1891; Manni and Dow 1963), the underlying neurophysiological mechanisms remain still unclear. The present research addressed the synaptic remodeling that follows HCb by altering the glutamatergic transmission in the cortico-cerebello-cortical circuitry.

The compensated HCb animals when treated with frontal injections of serum (H–S group) maintained the slight ataxic and dysmetric symptoms they exhibited as residual cerebellar symptomatology in the pre-injection phase. Further, H–S group displayed severely impaired procedural and mnesic spatial performances. Namely, in MWM Place 1 the H–S rats exhibited disrupted explorative procedures (compulsive Circling strategy). During the Probe phase, their lack of efficient explorative behaviors resulted in a swimming not biased toward the previously rewarded (platform) quadrant (as in Federico et al. 2006b; Petrosini et al. 1996). In the H–S group, the molecular analysis of PSD composition of the injected and non-injected frontal cortices evidenced marked increases of NMDAR GluN1 and GluN2A, AMPAR GluA1, and PSD-95 expression consistently with the wide cerebello-frontal connections. Synaptic rearrangement of GluN1 and GluA1 subunits was evident also in the spared hemicerebellum. Such a pattern of synaptic rearrangement is consistent with recent evidence in the peri-lesional areas of increased expression of PSD-95, whose activity is considered important for structural and functional changes (Cooper-rider et al. 2014; Pagnussat et al. 2012). It is also accepted that GluA1 recruitment is an activity-dependent process and that GluA1 plays a critical role in synaptic plasticity paradigms, as LTP and long-term depression (LTD) (Lee et al. 2010). Finally, also NMDAR activity is heavily involved in LTP- and LTD-related synaptic plasticity, as well as in synapse elimination or stabilization, and inhibition or promotion of axonal sprouting (Colonnese et al.

2005; Dupuis et al. 2014). Therefore, we observed for the first time that the concomitant changes in ionotropic glutamatergic receptors in the frontal cortices and spared hemicerebellum of the HCb animals reflect the deep synaptic reorganization that follows cerebellar lesions. This synaptic reorganization appears to be a compensatory mechanism for recovery from cerebellar symptoms.

The frontal anti-NMDA Ab injections in the absence of cerebellar lesion (S-A group) resulted in only slightly impaired postural and locomotor abilities, severely affected complex motor behaviors, and dramatically impaired mnesic and procedural spatial performances as severe as those of H–S animals. However, such a symptomatology fully disappeared within 48 h. In S-A animals, the analysis of synaptic markers indicated that the anti-NMDA Ab reduced only the NMDAR subunits and only in the injected frontal regions, leaving unaffected the not-injected homologue cortex and the remote cerebellar regions. Such a selective and reversible action of the anti-NMDA Ab on NMDAR subunits is fully consistent with the reduction in surface and synaptic NMDAR density and function described by Hughes et al. (2010) in in vitro and in vivo studies using Ab of patients with anti-NMDA encephalitis.

Further deepening on the synaptic rearrangement that follows HCb resulted from the behavioral and molecular findings of the compensated HCb rats injected with anti-NMDA Ab (H–A group). In H–A rats, the frontal anti-NMDA Ab injections provoked a multifaceted motor and cognitive impairment. Namely, the H–A rats exhibited a worsening of several postural and locomotor symptoms that lasted 24 h and fully recovered within 48 h. Conversely, in MWM the H–A animals exhibited a long-lasting impairment of spatial abilities and they still displayed the highest latencies and the longest distances to reach the platform at the 2nd post-injection day. As a collateral note, it is important to underline that the overlapping values of latency and distance during the last trials of MWM Place 2 as well as the similar navigational strategies put into action by H–S and H-A groups during the whole Place 2 indicate the reversibility of symptoms induced by the frontal anti-NMDA Ab injections. Anyway, the different time course of the anti-NMDA Ab effects on motor and spatial symptoms was probably linked to the different afferent and efferent connectivity of vermian and hemispherical cerebellar regions. While the vermis is bidirectionally connected mainly with the spinal cord and brain stem, and it plays a primary role in controlling body and limb posture and movement, the cerebellar hemispheres are bidirectionally connected mainly with neocortical areas and are involved in the cognitive functions and in planning complex motor behaviors (Ito 2006; Manto and Oulad BenTaib 2010). Consequently, the frontal anti-NMDA Ab injections impacted more directly (and severely) on the neocortico-hemispherical cerebellar network, affecting thus more lasting cognitive (spatial) performances. The prolonged impairment of the spatial performances of H–A animals can be interpreted as a decompensating action of the anti-NMDA Ab on the entire cortico-cerebellar circuit. In fact, in the H–A group when compared to H–S group the anti-NMDA Ab injections affected not only the NMDAR subunits but also the AMPAR and PSD-95 glutamatergic components, and not only in the injected FC, but also in the not-injected one and in the spared cerebellar regions. However, an alternative explanation may be considered. The spatial behavior could have been more affected than motor behavior by the disruption of NMDAR activity in the FC because of a residual (however slight) pharmacological block, or even by altered number of receptors. Future studies will allow clarifying the various alternatives.

As described in literature, the mechanisms of functional recovery can be grouped into two general classes, restitution and substitution of function (Kolb and Gibb 2014), both processes that alter function and efficacy of synapses and finely tune the functional state of neurons in response to varied synaptic inputs. It is known that postsynaptic cells, once deprived of their characteristic synaptic inputs, develop increased sensitivity to neurotransmitter via the emergence of new receptors and larger surface areas (Gonzalez-Forero et al. 2004). Thus, denervation hypersensitivity may facilitate post-lesional activation of pathways and restitution of function. The present findings indicate the up-regulation of cortical (and cerebellar) NMDAR and AMPAR to offset the loss of cerebellar glutamatergic input caused by HCb. In the presence of up-regulation of NMDAR, the entire cerebello-frontal system became vulnerable to the effects of the NMDA antagonist that therefore elicited the reinstatement of cerebellar deficits. Our results fit with previous studies demonstrating that changes in the strength of NMDAR activation contribute to the long-term modifications of pathway efficacy that mediate vestibular compensation (Kim et al. 1997; Kitahara et al. 1998, 2000).

However, the denervation hypersensitivity is probably only one of the neuronal re-adjustments responsible for the synaptic effects occurring during cerebellar compensation. As suggested by studies on injury-induced cortical plasticity, various neuronal readjustments tend to keep cell firing within a desirable range, and to produce compensatory reactions in the circuit excitability in order to re-establish the baseline firing rate set point (homeostatic readjustment) (Nahmani and Turrigiano 2014). In the case of cerebellar lesions, in compensated HCb animals we recently demonstrated an enhancement of density and size of dendritic spines of Purkinje cells in the spared hemicerebellum, an effect aimed at the maintenance of synaptic homeostasis conditions (Gelfo et al. 2015). Thus, it has to be taken into account that the increase in

glutamatergic synaptic markers in the spared hemicerebellum and frontal cortices here described may be the consequence of an enhanced spinogenesis associated with the wide functional rewiring of cortico-cerebellar loop already described (Burello et al. 2012). As further indication of the extensive synaptic readjustment that follows HCb, there is the altered activity of striatal spiny neurons in HCb rats that we previously demonstrated (Centonze et al. 2008; Cutuli et al. 2011).

A large amount of research indicates that NMDAR are crucially linked to learning and memory functions through LTP and LTD processes (Kim and Linden 2007). Most studies demonstrate that NMDAR antagonists disrupt recall and working memory, and prevent the acquisition of new behaviors (Dix et al. 2010; Driesen et al. 2013). Interestingly, to the degree that functional recovery may be considered a form of motor learning (Krakauer 2006), the cerebellar compensation comes to be a form of "re-learning" of motor skills. Thus, the frontal injections of anti-NMDA Ab may have affected the memory of the compensatory motor abilities re-learned in response to asymmetries and deficits elicited by HCb. Although there are aspects of synaptic reorganization probably unique to brain injury, there are aspects of brain plasticity linked to NMDAR (as for example generation of functional dendritic spines) that largely coincide with those occurring during motor learning. It is increasingly clear that learning-related plasticity, both at network and synaptic levels, contributes to promote recovery of function that follows brain damage.

The present findings indicate that the compensatory processes that follow cerebellar lesions are related to a relevant rearrangement of glutamatergic synapses along the cortico-cerebellar network. Following cerebellar damage, the long-term maintenance of the rearranged glutamatergic activity plays a key role in the maintenance of recovered function, assuming a significant translational value even for human post-injury rehabilitation.

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Compliance with Ethical Standards

Conflicts of interest All authors disclose any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within 3 years of beginning the submitted work that could inappropriately influence, or be perceived to influence, the work.

Human and Animals Rights Statement All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in the study involving animals were in accordance with the ethical standards of the institution or practice at which the study was conducted.

References

Buckner, R. L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. *Neuron, 80*(3), 807–815.

Burello, L., De Bartolo, P., Gelfo, F., Foti, F., Angelucci, F., & Petrosini, L. (2012). Functional recovery after cerebellar damage is related to GAP-43-mediated reactive responses of precerebellar and deep cerebellar nuclei. *Experimental Neurology, 233*(1), 273–282.

Centonze, D., Rossi, S., De Bartolo, P., De Chiara, V., Foti, F., Musella, A., et al. (2008). Adaptations of glutamatergic synapses in the striatum contribute to recovery from cerebellar damage. *European Journal of Neuroscience, 27*(8), 2188–2196.

Colonnese, M. T., Zhao, J. P., & Constantine-Paton, M. (2005). NMDA receptor currents suppress synapse formation on sprouting axons in vivo. *Journal of Neuroscience, 25*(5), 1291–1303.

Conn, P. J., Battaglia, G., Marino, M. J., & Nicoletti, F. (2005). Metabotropic glutamate receptors in the basal ganglia motor circuit. *Nature Reviews Neuroscience, 6*(10), 787–798.

Cooperrider, J., Furmaga, H., Plow, E., Park, H. J., Chen, Z., Kidd, G., et al. (2014). Chronic deep cerebellar stimulation promotes long-term potentiation, microstructural plasticity, and reorganization of perilesional cortical representation in a rodent model. *Journal of Neuroscience, 34*(27), 9040–9050.

Cull-Candy, S. G., & Leszkiewicz, D. N. (2004). Role of distinct NMDA receptor subtypes at central synapses. *Science's STKE, 255*, 16.

Cutuli, D., Rossi, S., Burello, L., Laricchiuta, D., De Chiara, V., Foti, F., et al. (2011). Before or after does it matter? Different protocols of environmental enrichment differently influence motor, synaptic and structural deficits of cerebellar origin. *Neurobiology of Disease, 42*(1), 9–20.

Dalmau, J., Gleichman, A. J., Hughes, E. G., Rossi, J. E., Peng, X., Lai, M., et al. (2008). Anti-NMDA-receptor encephalitis: Case series and analysis of the effects of antibodies. *The Lancet Neurology, 7*(12), 1091–1098.

Dalmau, J., Lancaster, E., Martinez-Hernandez, E., Rosenfeld, M. R., & Balice-Gordon, R. (2011). Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. *The Lancet Neurology, 10*(1), 63–74.

D'Amelio, M., Cavallucci, V., Middei, S., Marchetti, C., Pacioni, S., Ferri, A., et al. (2011). Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. *Nature Neuroscience, 14*(1), 69–76.

Dix, S., Gilmour, G., Potts, S., Smith, J. W., & Tricklebank, M. (2010). A within-subject cognitive battery in the rat: Differential effects of NMDA receptor antagonists. *Psychopharmacology (Berl), 212*(2), 227–242.

Driesen, N. R., McCarthy, G., Bhagwagar, Z., Bloch, M. H., Calhoun, V. D., D'Souza, D. C., et al. (2013). The impact of NMDA receptor blockade on human working memory-related prefrontal function and connectivity. *Neuropsychopharmacology, 38*(13), 2613–2622.

Dupuis, J. P., Ladépêche, L., Seth, H., Bard, L., Varela, J., Mikasova, L., et al. (2014). Surface dynamics of GluN2B-NMDA receptors controls plasticity of maturing glutamate synapses. *The EMBO Journal, 33*(8), 842–861.

Federico, F., Leggio, M. G., Mandolesi, L., & Petrosini, L. (2006a). The NMDA receptor antagonist CGS 19755 disrupts recovery following cerebellar lesions. *Restorative Neurology and Neuroscience, 24*(1), 1–7.

Federico, F., Leggio, M. G., Neri, P., Mandolesi, L., & Petrosini, L. (2006b). NMDA receptor activity in learning spatial procedural strategies II. The influence of cerebellar lesions. *Brain Research Bulletin, 70*(4–6), 356–367.

Foti, F., Laricchiuta, D., Cutuli, D., De Bartolo, P., Gelfo, F., Angelucci, F., et al. (2011). Exposure to an enriched environment accelerates recovery from cerebellar lesion. *Cerebellum, 10*(1), 104–119.

Gandhi, C. C., Kelly, R. M., Wiley, R. G., & Walsh, T. J. (2000). Impaired acquisition of a Morris water maze task following selective destruction of cerebellar Purkinje cells with OX7-saporin. *Behavioural Brain Research, 109*(1), 37–47.

Gelfo, F., Florenzano, F., Foti, F., Burello, L., Petrosini, L., & De Bartolo, P. (2015). Lesion-induced and activity-dependent structural plasticity of Purkinje cell dendritic spines in cerebellar vermis and hemisphere. *Brain Structure and Function,*. doi:10.1007/s00429-015-1109-5.

Gonzalez-Forero, D., Benitez-Temoino, B., de la Cruz, R., & Pastor, A. (2004). Functional recovery in the peripheral and central nervous system after injury. In T. Herdegen & J. Delgado-Garela (Eds.), *Brain damage and repair* (pp. 285–305). Dordrecht: Kluwer.

Gunduz-Bruce, H. (2009). The acute effects of NMDA antagonism: from the rodent to the human brain. *Brain Research Reviews, 60*(2), 279–286.

Hampe, C. S., Petrosini, L., De Bartolo, P., Caporali, P., Cutuli, D., Laricchiuta, D., et al. (2013). Monoclonal antibodies to 65 kDa glutamate decarboxylase induce epitope specific effects on motor and cognitive functions in rats. *Orphanet Journal of Rare Diseases, 8*, 82–92.

Holmes, G. (1917). The symptoms of acute cerebellar injuries due to gunshot injuries. *Brain, 40*, 461–535.

Honnorat, J., Saiz, A., Giometto, B., Vincent, A., Brieva, L., de Andres, C., et al. (2001). Cerebellar ataxia with anti-glutamic acid decarboxylase antibodies: study of 14 patients. *Archives of Neurology, 58*(2), 225–230.

Hughes, E. G., Peng, X., Gleichman, A. J., Lai, M., Zhou, L., Tsou, R., et al. (2010). Cellular and synaptic mechanisms of anti-NMDA receptor encephalitis. *Journal of Neuroscience, 30*(17), 5866–5875.

Ito, M. (2006). Cerebellar circuitry as a neuronal machine. *Progress in Neurobiology, 78*(3–5), 272–303.

Kim, M. S., Jin, B. K., Chun, S. W., Lee, M. Y., Lee, S. H., Kim, J. H., et al. (1997). Role of vestibulocerebellar N-methyl-D-aspartate receptors for behavioral recovery following unilateral labyrinthectomy in rats. *Neuroscience Letters, 222*(3), 171–174.

Kim, S. J., & Linden, D. J. (2007). Ubiquitous plasticity and memory storage. *Neuron, 56*(4), 582–592.

Kitahara, T., Fukushima, M., Takeda, N., Saika, T., & Kubo, T. (2000). Effects of pre-flocculectomy on Fos expression and NMDA receptor-mediated neural circuits in the central vestibular system after unilateral labyrinthectomy. *Acta Oto-Laryngologica, 120*(7), 866–871.

Kitahara, T., Takeda, N., Kiyama, H., & Kubo, T. (1998). Molecular mechanisms of vestibular compensation in the central vestibular system. *Acta Oto-Laryngologica, 539*, 19–27.

Kolb, B., & Gibb, R. (2014). Searching for the principles of brain plasticity and behavior. *Cortex, 58*, 251–260.

Krakauer, J. W. (2006). Motor learning: Its relevance to stroke recovery and neurorehabilitation. *Current Opinion in Neurology, 19*(1), 84–90.

Lau, C. G., & Zukin, R. S. (2007). NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nature Reviews Neuroscience, 8*(6), 413–426.

Lee, H. K., Takamiya, K., He, K., Song, L., & Huganir, R. L. (2010). Specific roles of AMPA receptor subunit GluR1 (GluA1) phosphorylation sites in regulating synaptic plasticity in the CA1 region of hippocampus. *Journal of Neurophysiology, 103*(1), 479–489.

Leggio, M. G., Neri, P., Graziano, A., Mandolesi, L., Molinari, M., & Petrosini, L. (1999). Cerebellar contribution to spatial event processing: Characterization of procedural learning. *Experimental Brain Research, 127*(1), 1–11.

Lisman, J., Schulman, H., & Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nature Reviews Neuroscience, 3*(3), 175–190.

Luciani, L. (1891). *Il cervelletto: Nuovi studi di fisiologia normale e patologica*. Firenze: Le Monnier.

Manni, E., & Dow, R. S. (1963). Some observations on the effects of cerebellectomy in the rat. *Journal of Comparative Neurology, 121*, 189–194.

Manto, M., Dalmau, J., Didelot, A., Rogemond, V., & Honnorat, J. (2010). In vivo effects of antibodies from patients with anti-NMDA receptor encephalitis: Further evidence of synaptic glutamatergic dysfunction. *Orphanet Journal of Rare Diseases, 5*, 31.

Manto, M., Dalmau, J., Didelot, A., Rogemond, V., & Honnorat, J. (2011). Afferent facilitation of corticomotor responses is increased by IgGs of patients with NMDA-receptor antibodies. *Journal of Neurology, 258*(1), 27–33.

Manto, M., Laute, M. A., Aguera, M., Rogemond, V., Pandolfo, M., & Honnorat, J. (2007). Effects of anti-glutamic acid decarboxylase antibodies associated with neurological diseases. *Annals of Neurology, 61*(6), 544–551.

Manto, M., & Oulad BenTaib, N. (2010). Cerebellar nuclei: Key roles for strategically located structures. *Cerebellum., 9*(1), 17–21.

Middleton, F. A., & Strick, P. L. (2001). Cerebellar projections to the prefrontal cortex of the primate. *Journal of Neuroscience, 21*(2), 700–712.

Molinari, M., Petrosini, L., & Gremoli, T. (1990). Hemicerebellectomy and motor behaviour in rats. II. Effects of cerebellar lesion performed at different developmental stages. *Experimental Brain Research, 82*(3), 483–492.

Nahmani, M., & Turrigiano, G. G. (2014). Adult cortical plasticity following injury: Recapitulation of critical period mechanisms? *Neuroscience, 283*, 4–16.

Pagnussat, A. S., Simao, F., Anastacio, J. R., Mestriner, R. G., Michaelsen, S. M., Castro, C. C., et al. (2012). Effects of skilled and unskilled training on functional recovery and brain plasticity after focal ischemia in adult rats. *Brain Research, 1486*, 53–61.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). San Diego: Academic.

Petrosini, L., Molinari, M., & Dell'Anna, M. E. (1996). Cerebellar contribution to spatial event processing: Morris water maze and T-maze. *European Journal of Neuroscience, 8*(9), 1882–1896.

Petrosini, L., Molinari, M., & Gremoli, T. (1990). Hemicerebellectomy and motor behaviour in rats. I. Effects of cerebellar lesion performed at different developmental stages. *Experimental Brain Research, 82*(3), 483–492.

Sansing, L. H., Tüzün, E., Ko, M. W., Baccon, J., Lynch, D. R., & Dalmau, J. (2007). A patient with encephalitis associated with NMDA receptor antibodies. *Nature Clinical Practice Neurology, 3*(5), 291–296.

Shepherd, J. D., & Huganir, R. L. (2007). The cell biology of synaptic plasticity: AMPA receptor trafficking. *Annual Review of Cell and Developmental Biology, 23*, 613–643.

Timmann, D., Drepper, J., Frings, M., Maschke, M., Richter, S., Gerwig, M., et al. (2010). The human cerebellum contributes to motor, emotional and cognitive associative learning. *A review. Cortex, 46*(7), 845–857.

Wandinger, K. P., Saschenbrecker, S., Stoecker, W., & Dalmau, J. (2011). Anti-NMDA-receptor encephalitis: A severe, multistage, treatable disorder presenting with psychosis. *Journal of Neuroimmunology, 231*(1–2), 86–91.