

# Immunization with DISC1 protein in an animal model of ADHD influences behavior and excitatory amino acids in prefrontal cortex and striatum

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**Abstract** The *Disrupted-in-schizophrenia 1 (DISC1)* gene is involved in vulnerability to neuropsychiatric disorders. Naples high-excitability (NHE) rat model neuropsychiatric problems characterized by an unbalanced mesocortical dopamine system. Here, we assessed behavioral and neurochemical effects of immunization against multimeric rat DISC1 protein in adult NHE rats, an animal model of attention-deficit hyperactivity disorder and their Random-Bred (NRB) controls. Males of both lines received subcutaneous injections of vehicle (PB), adjuvant only (AD) or recombinant rat DISC1 protein purified from *E. coli*, suspended in AD

(anti-DISC1) at age of 30, 45 and 60 postnatal days (pnd). At 75 pnd, the rats were exposed to a Låt maze and 2 days later to an Olton eight-arm radial maze, and horizontal (HA) and vertical activities (VA) were monitored. Non-selective (NSA) and selective spatial attention (SSA) were monitored in the Låt and in the Olton maze by duration of rearings and working memory, respectively. Post mortem neurochemistry in the prefrontal cortex (PFC), dorsal (DS) and ventral (VS) striatum of L-Glutamate, L-Aspartate and L-Leucine was performed. All immunized rats showed a clear humoral IgM (but not IgG) immune response against the immunogen, indicating that immunological self-tolerance to DISC1 can be overcome by immunization. NHE rats exhibited a higher

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unspecific IgM response to adjuvant, indicating an immunological abnormality. The sole anti-DISC1 immunization-specific behavioral in the NHE rats was an increased horizontal activity in the Lât maze. Adjuvant treatment increased vertical activity in both lines, but in the NRB controls it increased rearing and decreased horizontal activity. Liquid chromatography/tandem mass spectrometry analysis of soluble or membrane-trapped neurotransmitters aspartate, glutamate and leucine revealed increased soluble aspartate levels in the ventral striatum of NRB controls after anti-DISC1 immunization. Immune activation by adjuvant independent of simultaneous DISC1 immunization led to other specific changes in NHE and control NRB rats. In DISC1-immunized NHE rats, horizontal activity in Lat maze correlated with membrane-trapped glutamate in PFC and in the NRB rats, duration of rearing in Olton maze correlated with membrane-trapped glutamate in PFC and aspartate in dorsal striatum. In addition to non-specific immune activation (by AD), the postnatal anti-DISC1 immune treatment led to behavioral changes related to mechanisms of activity and attention and had influenced amino acids and synaptic markers in striatum and neocortex in the adult NHE as well as control animals.

**Keywords** DISC1 protein · Schizophrenia · Animal models · Prefrontal cortex · L-Glutamate · L-Aspartate · Working memory · Attention · Attention-deficit hyperactivity disorder · Immunization

#### Abbreviations

AD	Adjuvant
ADHD	Attention-deficit hyperactivity disorder
anti-DISC1	Anti-DISC1 protein immunization
CE	Collision energy
DA	Dopamine
DAT	Dopamine transporter
DISC1	Disrupted-in-schizophrenia 1
DS	Dorsal striatum
EAA	Excitatory amino acids
FE	Position of first repetitive arm visit
HA	Horizontal activity
MTF	Membrane-trapped form
NHE	Naples high-excitability rat
NRB	Naples random bred
NSA	Non-selective attention
NVTC	Number of arm visits before all arms were visited

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PB	Phosphate buffer vehicle
PFC	Prefrontal cortex
Pnd	Post natal days
RT	Retention time
Sc	Subcutaneous
SF	Soluble form
SRM	Selected reaction monitoring
SSA	Selective spatial attention
VA	Vertical activity
VS	Ventral striatum

#### Introduction

The *Disrupted-in-schizophrenia 1 (DISC1)* gene was originally discovered through genetic linkage in a Scottish pedigree and has been linked to several psychiatric diseases (Millar et al. 2000). Genetic association studies in various ethnicities (reviewed by Chubb et al. 2008), as well as transgenic animal models (reviewed by Brandon and Sawa 2011), have corroborated a key role of DISC1 in brain functions regulating cognitive and mood-related behaviors. *DISC1* has also been shown to be involved in dopamine neurotransmission (Niwa et al. 2010; Pogorelov et al. 2012; Vomund et al. 2013) and glutamate (Hayashi-Takagi et al. 2010; Wei et al. 2013), although the molecular links and mechanisms are still obscure.

In post mortem brains of patients with chronic mental illness, we identified insoluble DISC1 in a subset of 15 % of patients with clinical diagnoses like schizophrenia or recurrent affective disorders in a limited cohort (Leliveld et al. 2008). The discovery of protein pathology in chronic mental illness concurs with evidence that aberrant, chronic brain conditions seem to be invariably concomitant with disturbed proteostasis (Bader et al. 2012b; Korth 2012) and that, for example in neurodegenerative diseases, the same proteins that are mutant in familial forms of the disease also become insoluble in sporadic forms of the specific diseases (Prusiner 2001). Subsequently, we demonstrated that DISC1 was cell-invasive in vitro and in vivo (Ottis et al. 2011; Bader et al. 2012a) and therefore, that DISC1 protein pathology in so-called DISC1opathies (Korth 2012) fulfills an important criterion of a protein conformational disorder.

In several protein-aggregation diseases, immunization with the respective aggregated protein has been shown to provide beneficial effects on elicited disease (Schenk et al. 1999; Masliah et al. 2005; Urushitani et al. 2007). We, therefore, hypothesized that immunization with recombinant DISC1 could have beneficial effects in suitable animal models of psychiatric disease, similar to what has been shown for neurodegenerative diseases (Schenk et al. 1999; Masliah et al. 2005; Urushitani et al. 2007).

Based on evidence for interactions between DISC1 and the dopamine systems, we set out to examine the effects of immunization on behavioral and neurochemical parameters in an animal model of attention-deficit hyperactivity (ADHD) which responds to dopaminergic agonists and displays other dopaminergic phenotypes. For this purpose we employed the Naples high-excitability rat, which models behavioral problems characterized by an unbalanced mesocortical dopamine system. This animal displays hyperactivity, impulsiveness and inattentiveness: for reviews see Vigliano et al. 2002; Gironi Carnevale et al. 2007 and Ruocco et al. 2009b. Our working hypothesis was that DISC1 could be involved in hyperactivity, altered selective attention, working memory and executive functions in NHE rats. Of note, the *DISC1* gene has been associated with ADHD in adults (Jacobsen et al. 2013). Therefore, the aim of these studies was to investigate behavioral and neurochemical effects in adult rats after immunization against DISC1 protein, carried out during postnatal development.

At the age of 75 pnd, animals were tested in two different spatial novelty paradigms, i.e., the Låt maze and eight-arm radial maze, and we assessed indices of activity, selective- and non-selective attention. After the experiments, blood samples were taken and serum was stored for determination of immune activation and antibody levels. Prefrontal cortex, dorsal and ventral striatum were microdissected for neurochemical determination of functional markers, including excitatory amino acids (L-Glu, L-Asp) and CAMKII and DAT proteins as synaptic markers.

## Materials and methods

### Animals

Thirty-day-old male rat of the Sprague Dawley-derived Naples high-excitability line (NHE;  $n = 30$ ) and the random bred line (NRB;  $n = 24$ ) was used. They were housed in groups of two in makrolon cages a reversed 12:12 light-dark cycle with lights on from 7 PM to 7 AM. All experiments fulfilled the requirements of the Guide for the Care and Use of Laboratory Animals, implemented by the Italian Legislative Decree 116/1992 authorized by the Ministero della Salute, for which ethical advice was issued from Istituto Superiore di Sanità. The 3Rs principles were taken into account to seek for alternatives to animal use, to reduce the number of animals used and to minimize animal suffering.

### Generation of DISC1 immunogen

ratDISC1(598-785) with an N-terminal His-tag was cloned into the pET15b vector (Novagen, USA), expressed in BL21 bacteria, and purified on NiNTA agarose as described

(Leliveld et al. 2009). The particular construct was chosen because we had demonstrated that this domain harbors a multimerization domain leading to an exclusively multimeric form of protein which is, therefore, particularly suited to represent epitopes of aggregated DISC1 (Leliveld et al. 2009).

### Measurement of the elicited unspecific immune response

For measuring the elicited unspecific immune response, rat IgG total ELISA Ready-SET-Go!<sup>®</sup> eBioscience 88-50490-22: (<http://www.ebioscience.com/rat-igg-total-ready-set-go-elisa-kit.htm>) and rat IgM ELISA Ready-SET-Go!<sup>®</sup> eBioscience 88-50540-22: (<http://www.ebioscience.com/rat-igm-ready-set-go-elisa-kit.htm>) were used according to the manufacturer.

### ELISA against recombinant rat DISC1(598-785)

MaxiSorp 96-well plates (Nunc, Denmark) were coated with 1 µg/well of purified immunogen ratDISC1(598-854) in 100 mM sodium carbonate buffer, pH 8.3 and then blocked overnight with 5 % BSA. A serial dilution of rat sera was then exposed to the ELISA plates for 30 min at 37 °C, washed three times with PBS-T, incubated with POD-labeled secondary antibody (dianova/Jackson 112-035-003), washed again and then substrate (Opteia, Beckton Dickinson, USA) was added.

### Rabbit anti-DISC1 antiserum

The antiserum was generated in NZW rabbits by immunizing 100 µg rat DISC1 (598-785) three times with ABM-ZK (Linaris, Germany) as adjuvant. Final antiserum was affinity purified on a column with immobilized immunogen. The antiserum is called anti-C-term ratDISC1 and recognition of rat DISC1 was validated by comparing wild-type rat to a DISC1-knockout rat (Wei et al. 2013).

### Immunization procedure

Rats were immunized with either ratDISC1 (598-785) in 100 µL adjuvant (ABM-ZK vial suspended in 2 ml PBS, Linaris, Germany), adjuvant only (100 µL), or just PBS (100 µL) was injected subcutaneously at 30, 45 and 60 postnatal days. At 75 pnd the behavioral studies were initiated and at 90 pnd the rats were killed, and serum was taken for immunoassays (see above).

### Behavioral testing

Two groups of each rat line were used. Animals were randomly assigned to treatment and control groups ( $n = 10$ )

group). Group A received phosphate buffer (PB) vehicle as control, group B adjuvant (AD) and group C DISC1 immunogen (anti-DISC1). Rats were given a single subcutaneous (sc) injection of vehicle or either treatment at 30, 45 and 60 PND. At age of 70–75 days, they were tested in the Låt maze for 10 min. Two days later they were tested in the Olton maze for 10 min. In both cases they were tested at the beginning of the dark phase of the LD cycle. All animals were handled by picking them up and allowing them to adapt to the experimenters' hand three times per week over their lifespan.

**Låt maze:** As described elsewhere (Viggiano et al. 2002), the Låt maze consisted of 60 × 60 × 40 cm box made of special poly-vinyl chloride (PVC) material (KÖMACEL<sup>®</sup>), with a smaller plastic transparent box (30 × 30 × 40 cm) within the center. Rats were allowed to explore the resulting corridor (60-cm long, 15-cm wide and 40-cm high). Two such boxes were located in a sound attenuating room. The box was illuminated by a white, cold, 4 W lamp placed 60 cm above the floor in the center of the wooden cover, providing 0.1–0.2  $\mu\text{W}/\text{cm}^2$  diffuse light. For the Låt maze, the behavioral variables measured were corner crossing (HA) and frequency (VA) and duration (RD) of rearing on the hind limbs.

**Eight-arm radial maze (Olton maze):** The apparatus consisted of eight arms (8 × 60 cm) extending from an octagonal center platform (diameter 18.5 cm). The distance from the platform center to the end of each arm was 69.25 cm. The apparatus was constructed of grey poly-vinyl chloride with a smooth surface and 14-cm-high side walls of transparent Plexiglas. The maze was placed on the floor of a dimly lit room, surrounded by a circular higher wall without visual cues.

A non-reinforced exploration procedure was used; i.e., none of the end arms of the maze was ever baited with food. The animals had no prior experience in the maze. This test assesses the degree of efficiency of exploring a novel environment. A perfectly efficient animal would enter each of the eight arms only once to fully explore this maze. Procedure: each rat was placed onto the center platform surrounded by a cardboard cylinder to avoid their immediately escaping into an arm. The trial began with the removal of the cylinder and the rat being allowed to explore it for 10 min. Behaviors assessed were horizontal (HA) and vertical activity (VA) and duration of rearing as scanning time (RD), position of first repetitive arm entry (FE) and number of alleys visited before completion of all arm visits (NVTC). The duration of rearing has been used as a measure of non-selective attention (NSA) (Aspide et al. 1998, 2000), whereas the position of first repetitive arm entry and number of alleys visited were used as measures of selective spatial attention (SSA) and working memory (Ruocco et al. 2009a, 2010, 2014c). Behavioral variables were monitored

by CCD video camera, stored on DVD and analyzed offline. After each test, the number of fecal boluses was counted to provide a defecation score a measure of emotionality (Broadhurst 1960) and the floor carefully cleaned with a wet sponge.

#### Dissection of brain areas

After behavioral testing, the rats were killed by guillotine; blood samples were collected, centrifuged at low speed (3,200 rpm for 8 min) and supernatants were stored at  $-80\text{ }^{\circ}\text{C}$  for determining immune activation and anti-DISC1 antibody titers. As previously described (Ruocco et al. 2014a), the forebrains were dissected out and the following areas were stored at  $-80\text{ }^{\circ}\text{C}$  for further analysis: prefrontal cortex (PFC), dorsal striatum (DS) and ventral striatum (VS). The samples from the right and the left hemisphere were used for determination of L-glutamate and L-aspartate. After removal of the olfactory tubercles, the first coronal cut was made at 4.20 AP from bregma (Paxinos and Watson 2007). Thus, a tiny tissue included the PFC and cingulate cortex area1. The upper portion of the striatum (i.e., DS) was removed by a sagittal pinch extending between 2.20 and  $-3.8$  AP, up to 7-mm D-V in depth, which included globus pallidus. Then, the ventral striatum was removed by sagittal pinch between 2.70 and 0.48 AP, at 1 mm from the midline and at about 1 mm in depth.

#### Excitatory amino acids

**Extraction procedure** Following a compartmentalization procedure previously described, brain samples were homogenized in 1 ml ice-cold saline in an Eppendorf and centrifuged at 7,500g at  $+4\text{ }^{\circ}\text{C}$  for 20 min. The supernatant was filtered and used for the analysis of this soluble fraction (SF). The precipitate was then shocked in bi-distilled water and centrifuged at 7,500g at  $4\text{ }^{\circ}\text{C}$  for 20 min to produce the membrane-trapped form (MTF). For both SF and MTF forms, the free amino acids L-Glu, L-Asp and L-Leu were measured by LC/MS/MS technique (Ruocco et al. 2014c).

**Liquid chromatography/tandem mass spectrometry** The detection was performed on Varian 310-MS triple quadrupole mass spectrometer (Varian, Palo Alto, CA, USA). All the analytes were detected in positive ionization mode and with selected reaction monitoring (SRM) mode (Table 1). The settings of the ESI source were as follows: spray voltage, 5,000 V; capillary temperature  $300\text{ }^{\circ}\text{C}$ ; sheath gas pressure (spraying), 20 arbitrary units; auxiliary gas pressure (desolvating), 10 arbitrary units; ion sweep gas pressure (curtain), 5 arbitrary units. The collision cell (Q2) pressure was 2.2 mTorr of argon. The collision energies were optimized for a maximum detection of each product ion

**Table 1** Liquid chromatography–tandem mass spectrometry parameters

Analyte	RT (min)	SRM transitions ( <i>m/z</i> )	CE (eV)
Aspartate	1.36	134.0 → 134.0	4.0
		134.0 → 116.0	4.0
Glutamate	1.38	148.0 → 148.0	4.0
		148.0 → 130.0	7.5
Leucine	1.42	132.2 → 132.2	4.0
		132.2 → 86.0	10.0

RT retention time, SRM selected reaction monitoring, CE collision energy

(Table 1). Chromatographic separation was achieved with a ProStar™ 300 HPLC system (Varian, Palo Alto, CA, USA) on an Varian Polaris® C<sub>18</sub> column (5 μm, 2.1 × 100 mm) at a flow rate of 0.3 mL/min. The mobile phase consisted of aqueous 0.1 % formic acid (A) and acetonitrile (B). Samples were eluted with a linear gradient from 10 to 90 % B in 5 min. At 5:01 min, solvent B was decreased from 90 to 10 % and remained constant for 5 min. The total run time was 10 min.

#### Preparation of protein extracts

Membrane and cytosolic protein fractions were prepared as described before with minor modification. Tissues were homogenized in a glass–glass potter in cold 0.32 M sucrose buffer pH 7.4 containing 1 mM HEPES, 0.1 mM EGTA and 0.1 mM phenylmethylsulfonyl fluoride, in the presence of commercial cocktails of protease (Roche, Monza, Italy) and phosphatase (Sigma–Aldrich) inhibitors. The homogenate was clarified at 1,000g for 10 min, obtaining a pellet (P1) corresponding to the nuclear fraction. The supernatant (S1) was centrifuged at 13,000g for 15 min to obtain a clarified fraction of cytosolic proteins (S2) and a pellet (P2) corresponding to the crude membrane fraction, which was homogenized in a glass–glass potter in 1 % Triton X-100 buffer containing: 50 mM Tris–HCl pH 7, 4–300 mM NaCl, 5 mM EDTA, 0.02 % sodium azide. Total protein content was measured according to the Bradford Protein Assay (Bio-Rad, Milan, Italy), using bovine serum albumin as the calibration standard.

#### Gel electrophoresis and immunoblotting

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transfer of proteins to nitrocellulose membranes were performed according to the conventional methods with minor modifications. The proteins were solubilized in loading buffer at 54 °C for 45 min and then separated

on 10 % polyacrylamide gels and transferred to nitrocellulose membranes (Bio-Rad) in transfer buffer (25 mM Tris, 192 mM glycine, pH 8.3, 20 % methanol). The membranes were incubated with blocking buffer [5 % ECL blocking agent (GE Healthcare) in PBST (0.1 M phosphate buffered saline, pH 7.4, 0.1 % Tween-20)] for 1 h at room temperature. The conditions of the primary antibodies were as follows: CaMKII alfa [1:2000 Anti-CaM Kinase II alfa, Clone 6G9 Upstate (Millipore) #05-532], DAT (1:500 anti-Dopamine Transporter Millipore AB2231); Actin (1:500 anti-Actin Sigma A2066) or Actin (1:3,000-anti-Actin clone C4-Chemicon-Millipore MAB1501). After three 10-min washes in PBST, the blots were incubated for 1 h at room temperature with horseradish peroxidase-conjugated secondary antibody (anti-rabbit or anti-mouse ECL Anti-rabbit IgG, HRP-Linked Whole Ab GE Healthcare NA934, NA931) and immunocomplexes were visualized by chemiluminescence using the ECL Western blotting kit (GE Healthcare) according to the manufacturer's instructions.

All protein bands used were within linear range of standard curves and were normalized for actin level in the same membrane. Quantity One software (BioRad Laboratories, Hercules, CA, USA) was used for standardization and quantization of protein bands obtained with Western blot analysis. The molecular weight of the bands obtained by Western blot incubated with the relevant antibody sera was estimated using as a molecular weight standard product BioRad:prestained PRECISION PLUS PROTEIN STANDARD DUAL COLOR code 1610374. The standard is composed of a series of bands of different weights: 10–20, 25, 37, 50, 75, 100, 150, 250 kD, respectively (<http://www.bio-rad.com/en-us/sku/161-0374-precision-plus-protein-dual-color-standards>).

The anti-DAT antibody was used with 1:500 dilution as for the other experiments.

#### Statistics

Data are expressed as means and standard error of the mean. Behavioral variables were frequency of corner crossings or alley visits and frequency and duration of rearing in both mazes. In the Olton maze, the number of arm visits before all arms are visited (NVTC) and position of first repetitive arm visit (first error: FE) were considered as indices of selective spatial attention and orientation (SSA), respectively. The values of the EAAs Glu and Asp (corrected for Leu, which does not participate in neurotransmission) were obtained for the soluble and membrane-trapped forms and blood levels of IgG and IgM were determined. All data were checked for the fulfillment of requirements for parametric analysis (Levene test for univariate analysis, and Box test of equality of covariance matrices for repeated measures analysis) by use of SPSS software (Version 11.0). For comparison of the two



rat lines, two-way ANOVAs line per treatment were used. For body weight and each behavioral parameter, separate two-way repeated measures ANOVA was carried out, with treatment as between-groups factor and time as repeated measures factor. For spatial orientation in the Olton maze, emotionality index and biochemical measures, one-way ANOVAs were conducted with treatment as between-groups factor. Post-hoc analysis was performed by Tukey HSD method. Correlation analyses between behavioral parameters and biochemical measures were performed by Spearman non-parametric method. The rejection level was set at  $p < 0.05$ , after correction by the Hölm procedure (Hölm 1979).

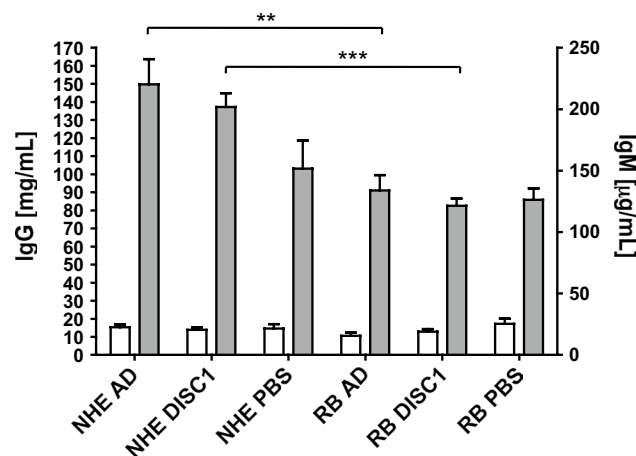
## Results

### Body weight

At 30, 45 and 60 postnatal days, body weight of animals receiving PB, AD or anti-DISC1 protein antibody did not differ between groups. Two-way repeated measures ANOVA (time  $\times$  treatment) showed a significant main effect only for time ( $F = 416.08$  and  $509.08$ ,  $df = 2/47$  and  $2/43$ ,  $p < 0.0001$ ) with no interaction. The two rat lines exhibited a similar increase in body weight.

### Immune response

A specific immune response was elicited in both the NHE and NRB rats receiving adjuvant either with or without DISC1 (Fig. 1). There were no significant changes in IgG titers in any of the experimental groups. As to IgM levels, the NHE rats had an elevated IgM response when adjuvant (AD) was administered either alone ( $149.8 \pm \text{SEM } 14.0$ ;



**Fig. 1** Immune response. IgG (open bars) and IgM (black bars) levels of NHE or NRB rats receiving ratDISC1 (598-785) suspended in adjuvant, adjuvant-only control, or PBS control

Mean  $\pm$  SEM) or with anti-DISC1 ( $137.3 \pm \text{SEM } 7.6$ ) (Fig. 1). These values were significantly higher in comparison with the vehicle group ( $103.1 \pm \text{SEM } 15.6$ ). Thus, no increase in unspecific immune activation by adding recombinant ratSIC1 (598-785) effect was observed. Conversely, the values for the NRB rats were  $91.1 \pm \text{SEM } 8.5$  for AD,  $82.5 \pm \text{SEM } 4.0$  for anti-DISC1 and  $85.9 \pm \text{SEM } 6.4$  for vehicle, respectively, with no significant differences.

As to the ratDISC1 (598-785)-specific immune response, an ELISA against ratDISC1 (598-785) indicated that all rats that received ratDISC (598-785), but not those that either received AD alone or PBS, had antibody titers against ratDISC (598-785)  $\geq 1:800$ , thus indicating an immunogen-specific high anti-DISC1 antibody response.

### Behavior

#### Activity and non-selective attention in the Låt maze

A two-way ANOVA line  $\times$  treatment confirmed the well-known hyperactivity of the NHE vs. the NRB rats. In particular, the NHE exhibited a higher frequency of corner crossings and rearing ( $F = 7.053$  and  $35.966$ ,  $df 1/54$ ,  $p < 0.010$ ,  $0.0001$  respectively).

**Horizontal activity** In the NHE rats, two-way ANOVA time  $\times$  treatment revealed significant main effects for time ( $F = 18.055$ ,  $df = 6/155$ ,  $p < 0.0001$ ) and for treatment ( $F = 6.168$ ,  $df 2/27$ ,  $p < 0.006$ ), with no interaction effects (Fig. 2). Post-hoc analysis by Tukey HSD test showed that the increase in corner crossings was due to the anti-DISC1 protein treatment ( $p < 0.020$ ) and that it pertained mainly to the second part of the test as confirmed by two-way ANOVA time  $\times$  treatment ( $F = 7.139$ ,  $df = 2/27$ ,  $p < 0.003$ ) on data from min 6 to 10 of the testing period. Post-hoc analysis showed a significant difference between PB and anti-DISC1 treatment ( $p < 0.022$ ). In contrast, in the NRB rats there was no main effect for treatment, but only for the time block ( $F = 39.37$ ,  $6/155$ ,  $p < 0.0001$ ) with no interaction effects.

**Vertical activity** Frequency and duration of rearing were not significantly influenced by treatments.

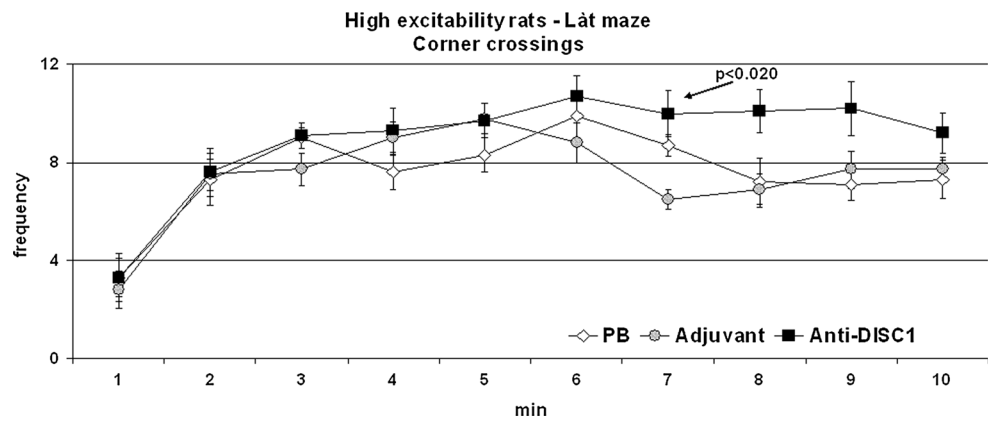
**Rearing frequency** In the NHE and the NRB rats, two-way ANOVAs revealed significant main effects only for time ( $F = 6.67$  and  $14.64$ ,  $df 9/243$ ,  $p < 0.0001$  respectively), with no interaction effects.

**Rearing duration** In the NHE and the NRB rats, two-way ANOVAs revealed no significant main effects for time and treatment and/or interaction effect.

#### Activity, attention and working memory in the Olton maze

A two-way ANOVA time  $\times$  treatment confirmed hyperactivity of the NHE vs. the NRB rats with the NHE rats

**Fig. 2** Låt maze. Behavioral effects of AD and anti-DISC1 immunization on horizontal activity in the Låt maze in NHE rats. Frequency of corner crossings plotted over the 10-min exposure in 1-min blocks. Data are given as mean  $\pm$  SEM



exhibiting a higher frequency of corner crossings and rearings ( $F = 15.062$  and  $10.589$ ,  $df$  1/51,  $p < 0.0001$  and  $P < 0.002$ , respectively).

**Horizontal activity** In the NHE rats, two-way ANOVA time  $\times$  treatment revealed significant main effects only for time ( $F = 9.408$ ,  $df$  9/243,  $p < 0.0001$ ), with no interaction effects. By contrast, in the NRB rats there was a main effect for time ( $F = 4.679$ ,  $5/130$ ,  $p < 0.0001$ ) and for treatment ( $F = 5.065$ ,  $df$  2/24,  $p < 0.015$ ), with no interaction effects, as shown in Fig. 3a. Post-hoc analysis by Tukey HSD test showed that the decrease in frequency of alley visits was due to the AD ( $p < 0.012$ ).

**Vertical activity** For rearing frequency in both NHE (Fig. 3b) and NRB (Fig. 3c) rats two-way ANOVAs revealed significant main effects for time ( $F = 13.021$  and  $9.770$ ,  $df$  9/243 and 9/216, respectively,  $p < 0.0001$  for both) and for treatment ( $F = 14.919$  and  $6.142$ ,  $df$  2/27, 2/24,  $p < 0.0001$  and  $0.007$ , respectively), with no interaction effects. Post-hoc analysis showed that the increase in the measure of non-selective attention was due both to the AD ( $p < 0.001$  and  $0.018$ ) and to anti-DISC1 ( $p < 0.0001$  and  $0.016$ ).

**Rearing duration** In the NHE rats, two-way ANOVA revealed significant main effects only for time ( $F = 3.971$ ,  $df$  5/128,  $p < 0.003$ ), whereas in the NRB rats there were significant main effects for time ( $F = 3.034$ ,  $df$  5/131,  $p < 0.010$ ) and for treatment ( $F = 6.906$ ,  $df$  2/24,  $p < 0.004$ ), without an interaction effect (Fig. 3d). Post-hoc analysis by Tukey HSD test showed that the increase in non-selective attention index was due to the AD ( $p < 0.004$ ).

**Spatial orientation (selective spatial attention)** Effects of DISC1 immunization upon selective spatial attention (SSA) in the eight-arm radial maze were analyzed in terms of the number of arm visits before all arms were visited (NVTC) and position of first repetitive arm visit (first error: FE) in both rat lines. An efficient rat will explore the eight arms only one time; thus, the best possible score is 8 for NVTC and 9 for FE. One-way ANOVA showed no significant treatment effects for these indices of attention and exploration in either the NHE or NRB rats.

#### Emotionality index

No significant differences across treatment groups were found in terms of fecal bolus counts. Separate one-way ANOVAs showed no treatment effect in the two mazes, whereas in the Olton Maze a line effect between the NHE and the NRB, with the latter showing the higher score, was found ( $F = 6.950$ ,  $df = 1/59$ ,  $p < 0.011$ ).

#### L-Glutamate and L-aspartate in the anterior forebrain

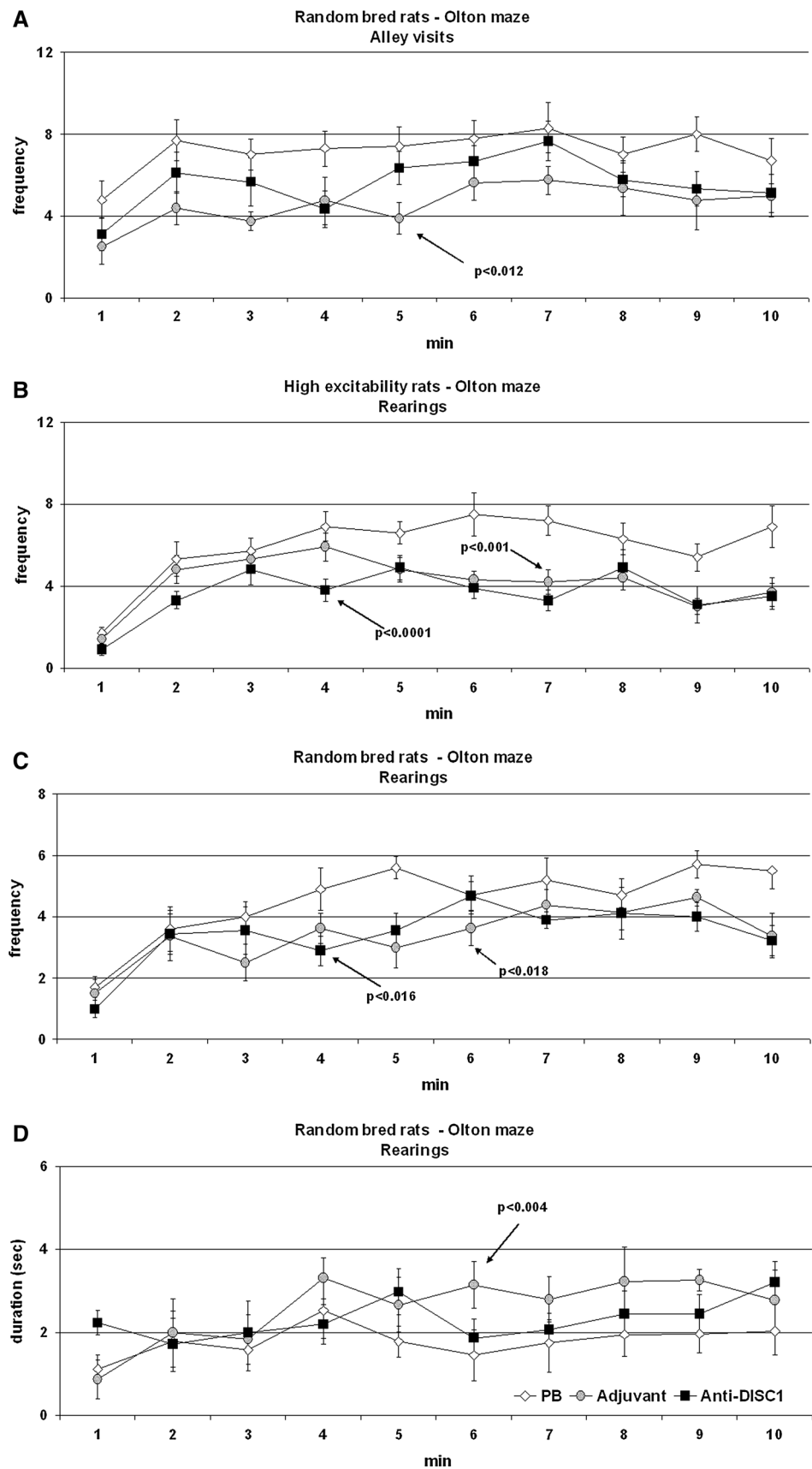
The effect of treatments on the values of the EEAs Glu and Asp corrected for Leu (which does not participate in neurotransmission) for the soluble and membrane-trapped form were analyzed in the NHE and the NRB rats.

**PFC** In the PFC of the NHE rats, one-way ANOVA revealed significant treatment effect only for the membrane-trapped L-Glu/ L-Leu ( $F = 11.384$ ,  $df = 2/12$ ,  $p < 0.002$ ). Post-hoc analysis by Tukey HSD test showed that the increase was due to AD ( $p < 0.013$ ) and to anti-DISC1 ( $p < 0.001$ ). In the PFC of the NRB rats, a treatment effect was present for soluble L-Glu/ L-Leu ( $F = 16.512$ ,  $df = 2/12$ ,  $p < 0.0001$ ) and membrane-trapped L-Glu/ L-Leu ( $F = 12.924$ ,  $df = 2/12$ ,  $p < 0.001$ ). Post-hoc analysis showed that the decrease was due to AD ( $p < 0.001$  and  $p < 0.044$  respectively) and to anti-DISC1 ( $p < 0.0001$  and  $p < 0.001$  respectively). No significant effects were observed in either rat line on the soluble or membrane-trapped forms of aspartate/leucine (Fig. 4a, b).

**Dorsal striatum** In the dorsal striatum (Fig. 5a, b) of the NHE rats, treatment significantly modified soluble L-Glu/ L-Leu (one-way ANOVA:  $F = 4.075$ ,  $df = 2/12$ ,  $p < 0.045$ ) and membrane-trapped L-Glu/ L-Leu ( $F = 9.240$ ,  $df = 2/12$ ,  $p < 0.004$ ) levels. The anti-DISC1 increased both values ( $p < 0.039$  and  $0.003$  respectively). As to aspartate/leucine in both forms, no significant effects were observed in NHE rats.

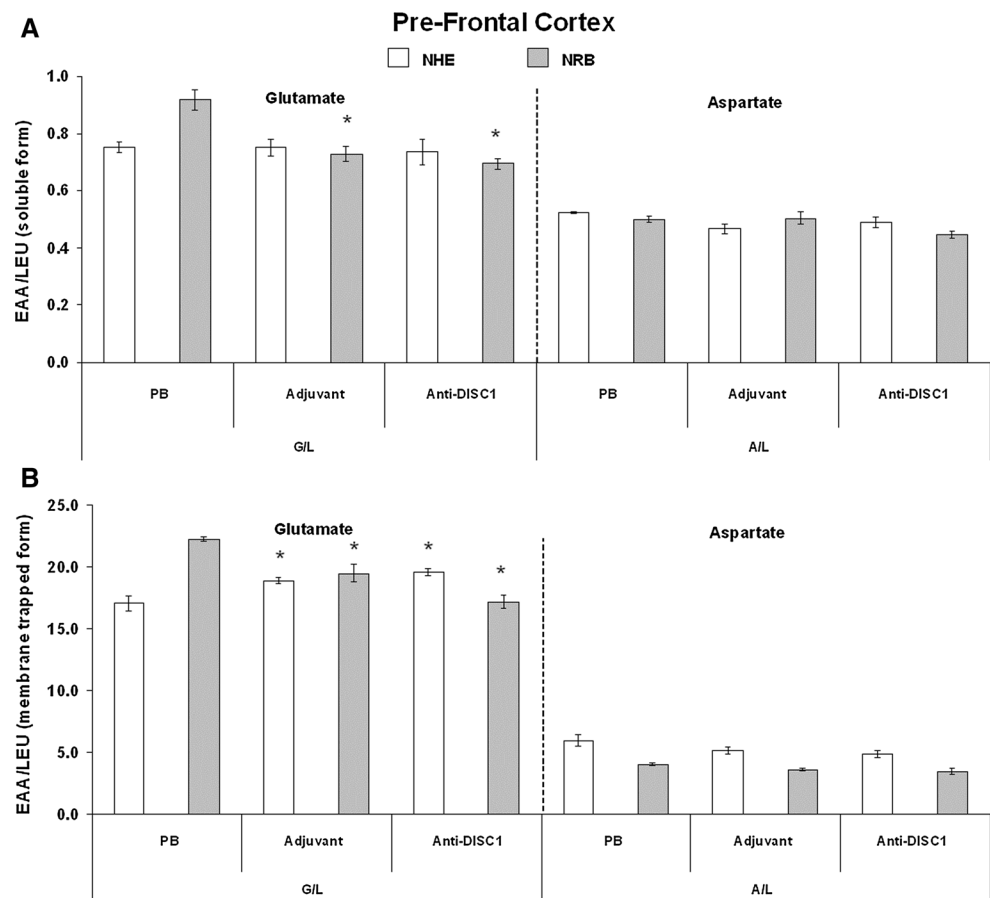
In the NRB rats, one-way ANOVA revealed significant treatment effects for soluble L-Asp/ L-Leu ( $F = 13.588$ ,

**Fig. 3** Olton maze. Behavioral effects of AD and anti-DISC1 immunization on: horizontal activity in the Olton maze in NRB rats (a); vertical activity in the Olton maze in NHE (b) and NRB (c) rats; duration of rearing in the Olton maze in NRB (d) rats. Frequency and duration are plotted over the 10-min exposure in 1-min blocks. Data are given as mean  $\pm$  SEM





**Fig. 4** EAA in the PFC. L-Glu/L-Leu (G/L) and L-Asp/L-Leu (A/L) ratios in the soluble form (a) and in the membrane-trapped form (b) in the prefrontal cortex of NHE and NRB rats for PB, AD and anti-DISC1 immunization. Data given as mean  $\pm$  SEM (\* $p < 0.05$  for comparisons vs. PB)



$df = 2/12, p < 0.001$ ), soluble L-Glu/L-Leu ( $F = 7.007, df = 2/12, p < 0.010$ ) and membrane-trapped L-Asp/L-Leu ( $F = 5.901, df = 2/12, p < 0.016$ ). Post-hoc tests demonstrated that for soluble L-Asp/L-Leu, the increase was induced both by AD ( $p < 0.006$ ) and by anti-DISC1 ( $p < 0.001$ ), whereas only anti-DISC1 significantly increased soluble L-Glu/L-Leu ( $p < 0.008$ ) and membrane-trapped L-Asp/L-Leu (0.013).

**Ventral striatum** In the ventral striatum (Fig. 6a, b) of the NHE rats, treatment effect was significant only for soluble L-Glu/L-Leu ( $F = 27.113, df = 2/12, p < 0.0001$ ) and this increase was due both to AD ( $p < 0.0001$ ) and to anti-DISC1 ( $p < 0.0001$ ). As to aspartate/leucine in both forms, no significant effects were observed in the NHE rats.

In the NRB rats, treatment affected only soluble L-Asp/L-Leu ( $F = 6.250, df = 2/12, p < 0.014$ ) and this increase was due only to anti-DISC1 ( $p < 0.011$ ).

**Neurobehavioral co-variations** Several co-variations emerged between behavioral measures and amino acid levels in relation to treatment. The anti-DISC1-treated NHE rats showed a positive correlation between membrane-trapped L-Glu/L-Leu in the PFC and horizontal activity in the Låt maze ( $R_s = 0.927; p < 0.008$ ). Similarly, duration of rearing in the Olton maze was positively correlated with membrane-trapped

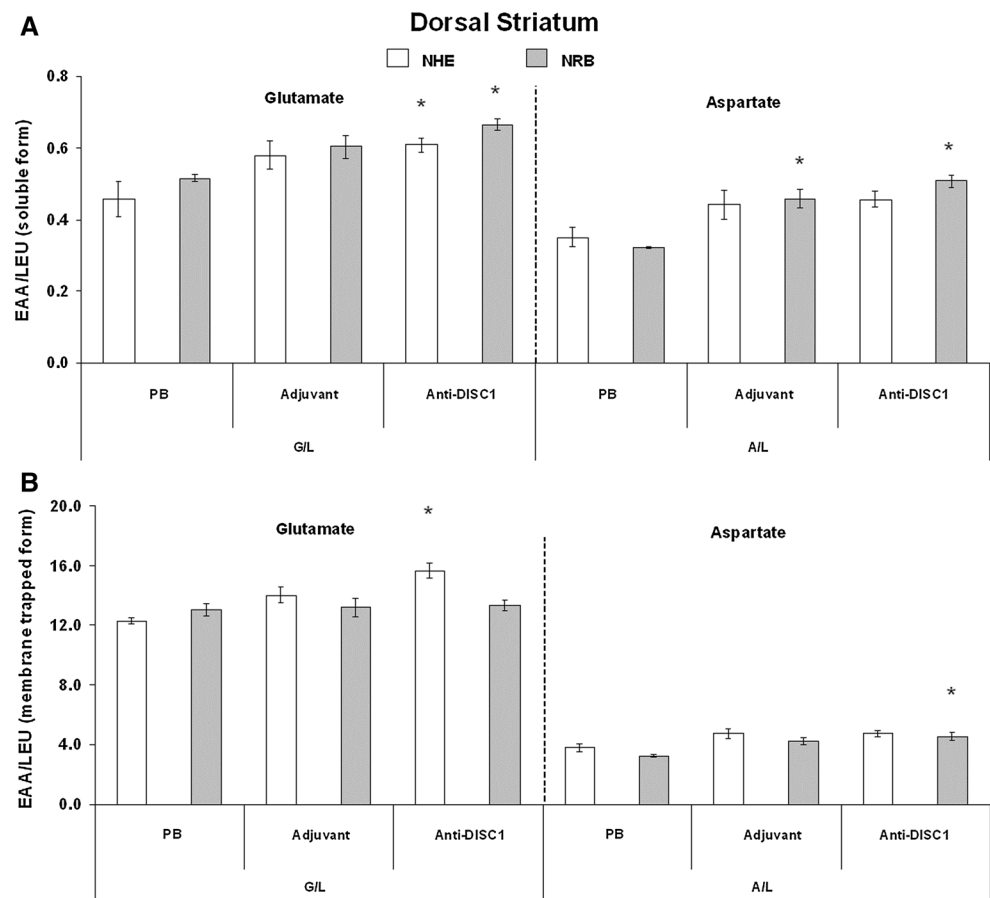
L-Glu/L-Leu in PFC ( $R_s = 0.829; p < 0.042$ ) and membrane-trapped L-Asp/L-Leu in DS ( $R_s = 0.829; p < 0.042$ ) of NRB anti-DISC1-treated rats. Finally, AD-treated NRB rats showed an inverse correlation between horizontal activity in the Olton maze and membrane-trapped L-Glu/L-Leu in the PFC ( $R_s = -0.812; p < 0.05$ ).

**Synaptic markers: preliminary measurements of DAT and CAMKII**

**CAMKII protein** Following immunization with DISC1 immunogen, the level of CAMKII protein was reduced, though not significantly, in the medial PFC in both NHE and NRB rat lines, as measured by densitometry in Western blots. In the NHE rats, the mean protein levels ( $\pm$ SEM), expressed as ratio of marker over Actin level, were  $0.49 \pm 0.170$  for vehicle-treated,  $0.36 \pm 0.079$  for AD-treated and  $0.26 \pm 0.056$  for anti-DISC1-treated rats. Similarly, in the NRB rats the values were  $0.30 \pm 0.175$  for vehicle-treated,  $0.22 \pm 0.070$  for AD-treated and  $0.13 \pm 0.021$  for the anti-DISC1-immunized rats.

**DAT protein** Of the various DAT proteins with different KD, we focused upon the 53 types, given its stability in a highly “reduced” medium. Immunization led to a significant

**Fig. 5** EAA in the DS. L-Glu/L-Leu (G/L) and L-Asp/L-Leu (A/L) ratios in the soluble form (a) and in the membrane-trapped form (b) in the dorsal striatum of NHE and NRB rats for PB, AD and anti-DISC1 immunization. Data given as mean  $\pm$  SEM (\* $p < 0.05$  for comparisons vs. PB)



increase in DAT only in the NHE rat line. The DAT protein level, expressed as ratio of marker over Actin level, was  $6.08 \pm 1.463$  for AD-treated and  $11.19 \pm 1.975$  in the anti-DISC1 immunized NHE rats ( $t$  test for unpaired data:  $p < 0.037$ ), whereas it was  $10.86 \pm 2.163$  for AD-treated and  $11.74 \pm 2.148$  in the anti-DISC1-immunized NRB rats.

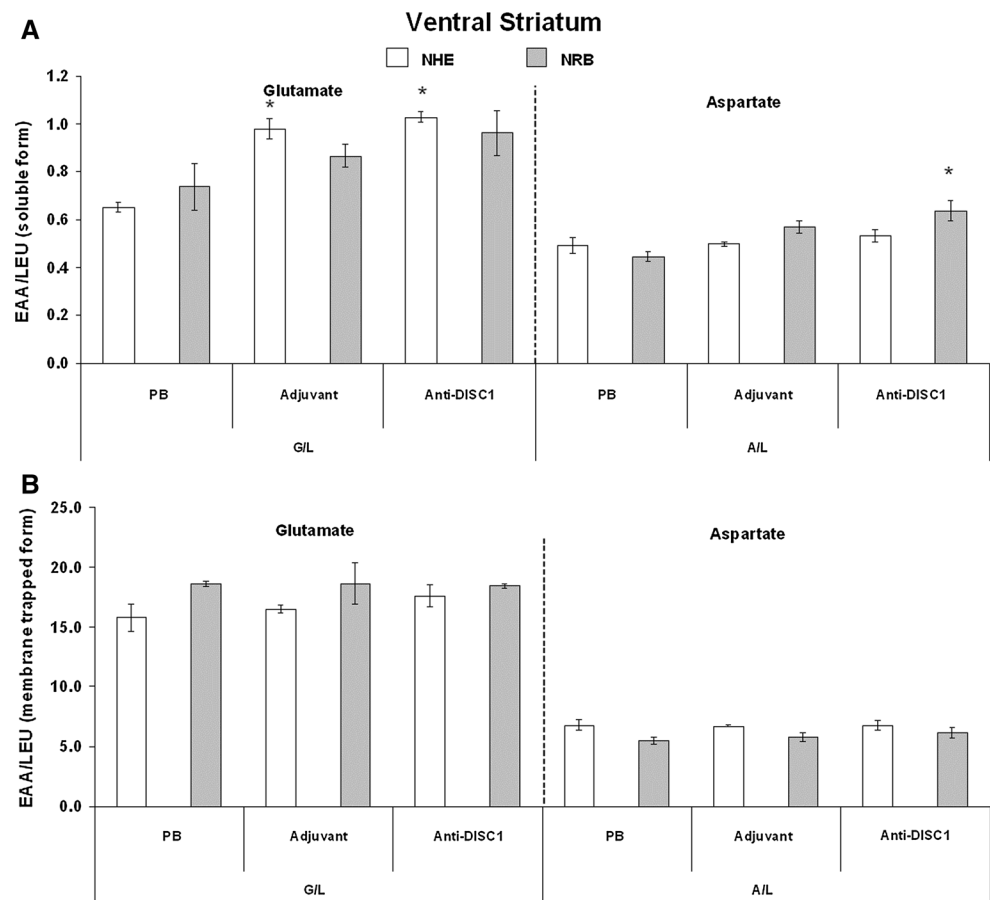
## Discussion

Given the evidence for a relationship between DISC1 and the dopamine system, we examined the effects of DISC1 immunization in the Naples high-excitability rat, an animal model for ADHD. This model has been characterized by prefrontal and striatal DAT hyperfunction, suggestive of DA hyperactivity and prefrontal and striatal hyperfunction of NMDA receptors, indicative of net EAA hyperactivity (Ruocco et al. 2014c). The rationale of these experiments was that the DISC1 protein could be a basis of hyperactivity, altered selective attention, working memory and executive functions in NHE rats. We aimed to determine whether postnatal immunization of NHE rats and NRB controls with DISC1 immunogen would modify their behavioral and neurochemical profiles. The NHE rats exhibited an

increased unspecific immune response after adjuvant treatment (Fig. 1). Moreover, ratDISC1 was immunogenic in all those rats receiving ratDISC1 (598-785), indicating that immunological self-tolerance to the DISC1 protein can be overcome.

For the behavioral analysis, two spatial novelty paradigms were utilized, namely the Låt maze and eight-arm radial maze. Though they differ in complexity, both require low motivational level and invoke relatively little stress. However, as previously reported, even a simple exposure to an open-field can trigger a neuro-vegetative response with high defecation score and corticosterone plasma level (Sadile 1996). A new finding from the present experiments is that the plasma level of IgM was elevated in both DISC1-immunized and AD-only receiving NHE rats. Thus, following behavioral testing, an immunological response persisted differentially only in the NHE rats and for IgM type, revealing a non-specific nature due to adjuvant but a particular immunological susceptibility that has not been described far. NHE rats, immunized with anti-DISC1 protein, differed from those receiving either or vehicle only in the Låt maze. More specifically, the sole behavioral effect in the Låt maze consisted of an increase in frequency of horizontal activity.

**Fig. 6** EAA in the VS. L-Glu/L-Leu (G/L) and L-Asp/L-Leu (A/L) ratios in the soluble form (a) and in the membrane-trapped form (b) in the ventral striatum of NHE and NRB rats for PB, AD and anti-DISC1 immunization. Data given as mean  $\pm$  SEM (\* $p < 0.05$  for comparisons vs. PB)



The data available do not allow us to tease out the relative contribution of *decreased anxiety* (see also (a) vs *reduced inhibition with poor habituation*) to the change in activity level. The increase in horizontal activity of NHE rats immunized with anti-DISC1 could be interpreted in terms of a decrease in anxiety level. This would imply that DISC1 protein is involved in the control of this non-cognitive emotional component of behavior. Alternatively, the increased horizontal activity in the Låt maze could be due to impaired inhibition, operationally defined as a decrease in short-term habituation. Rats patrolling the Låt maze corridor habituate (decrease horizontal activity) over time. If the increase in horizontal activity was a function of “cognitive” variables (e.g., reduced rate of habituation due to failure of mapping of the environment), then the NHE rats would have been expected to also show altered mapping in a different paradigm, such as the Olton maze. Yet, this was not the case, as they did not demonstrate impaired selective spatial attention assessed by position of first repetitive arm visit. However, spatial mapping in the two paradigms is likely to involve different neural mechanisms, namely, underlying egocentric orientation in the Låt maze and allocentric orientation in the radial maze (Berke et al. 2009). In fact, it has been demonstrated that conflicts between global

and local spatial frameworks dissociate neural representations of the lateral and medial entorhinal cortices (Neu-nuebel et al. 2013). Moreover, intra- and infra-pyramidal mossy fibers have been shown to be fewer in NHE in comparison to NRB rats in the hippocampal formation at the mid-septo temporal level (Lipp et al. 1987). In addition, a functional imaging study by cytochrome oxidase revealed a different architecture at the cortical level in the NHE rats versus NRB controls; e.g. the metabolic activity of entorhinal cortex of NHE rats was significantly higher at external, intermediate and internal levels (Gallo et al. 2002). Lastly, at the behavioral level, the altered intra-hippocampal circuitry has been associated to spatial mapping in a tunnel maze that relies on internal cues rather than on external ones (Sadile et al. 1988). Therefore, the appropriate interface to focus upon is the hippocampal cortex where several inputs converge and convey internal and external information (Buzsaki et al. 2013). In our opinion, the immunization procedure is likely to act upon a myriad of processes, such as control of inhibition and anxiety level, which are impossible to disentangle. We should keep in mind that both DISC1 protein and adjuvant treatments share a strong non-specific stimulation. The peripheral macrophages are rather heterogeneous (Auffray et al. 2009) and communicate with

macrophages in the brain (Schwartz and Shechter 2010a, b; Schwartz et al. 2013).

The second behavioral outcome of this experiment was that in the Olton eight-arm maze the NRB rats showed a reduced frequency of horizontal and vertical activity along with an increase in duration of rearing in relation to AD alone or associated with anti-DISC1. In fact, AD decreased horizontal activity in the NRB rats, whereas AD and anti-DISC1 decreased vertical activity in both rat lines and increased duration of rearing only in the NRB rats. It is worth noting that the robust effect of non-specific immunomodulation was exerted by AD on behavior only in the NRB rats. The present experiment should be envisioned as immunological stimulation on top of different basal responses.

High-resolution analysis of L-Glu and L-Asp by LC/MS/MS in different compartments revealed, first of all, an increased soluble form of L-Glu in DS and VS of NHE rats by anti-DISC1 or both treatments, respectively. A similar increase in the membrane-trapped form of L-Glu was found in PFC and DS by both treatments or anti-DISC1 treatment, respectively. Moreover, a decrease in soluble L-Glu was found in the PFC of NRB rats by both treatments, whereas an increase of soluble L-Asp and L-Glu was found in DS of NRB rats. Furthermore, an increase of soluble L-Asp was found in the VS of NRB rats. Lastly, a decreased membrane-trapped form of L-Glu in PFC and increased L-Asp were found in DS in NRB rats by both or anti-DISC1 treatments, respectively.

We found several co-variations between amino acid levels and indexes of activity and attention. Exposure to Lât Maze yielded a positive correlation between membrane-trapped L-Glu/L-Leu in the PFC and horizontal activity in the anti-DISC1-immunized NHE rats. Exposure to Olton radial maze yielded a positive correlation between membrane-trapped L-Glu/L-Leu in PFC and membrane-trapped L-Asp/L-Leu in the DS and duration of rearing in the anti-DISC1-immunized control rats. Conversely, in the NRB controls treated with the adjuvant, we found an inverse correlation between membrane-trapped L-Glu/L-Leu in the PFC and horizontal activity in the Olton maze.

Data obtained using CAMKII immunoblotting, though preliminary, showed a trend towards reduced levels of CAMKII protein in the medial PFC of both NHE and NRB rat lines, following immunization with DISC1 immunogen. CAMKII protein has also been studied in the forebrain of another model of ADHD, the spontaneously hypertensive rat (Moser et al. 1988) and reviewed (Marco et al. 2011). In this rat, CAMKII protein level was lower in the nucleus accumbens core and shell regions in comparison with controls (Papa et al. 2002) and was reversed by methamphetamine treatment (3 mg/Kg for 2 weeks) in a time-dependent manner within 24–72 h. Our results also show that immunization with DISC1 immunogen increased DAT

significantly in comparison with adjuvant in the NHE, but not NRB lines. These preliminary findings suggest interactions between the DISC1 protein and the mesocortical dopamine system as well as with CAMKII. Interactions between DISC1 and the dopamine systems have also been shown elsewhere (Niwa et al. 2010; Pogorelov et al. 2012; Vomund et al. 2013).

Altogether, our findings suggest that immunization with DISC1 protein leads to changes in behaviors as well in neurochemical markers in the forebrain of rats. The results also suggest that the significance of the DISC1 protein, originally proposed mainly in relation to schizophrenia and depression, may be broader and include other pathologies linked to the mesocorticolimbic dopamine system, such as ADHD.

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**Conflict of interest** None of the authors declare any conflict of interest.

## References

- Aspide R, Gironi Carnevale UA, Sergeant JA, Sadile AG (1998) Non-selective attention and nitric oxide in putative animal models of Attention-Deficit Hyperactivity Disorder. *Behav Brain Res* 95:123–133
- Aspide R, Fresiello A, De Filippis G, Gironi Carnevale UA, Sadile AG (2000) Non selective attention in a rat model of hyperactivity and attention deficit: subchronic methylphenidate and nitric oxide synthesis inhibitor treatment. *Neurosci Biobehav Rev* 24:59–71
- Auffray C, Sieweke MH, Geissmann F (2009) Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annu Rev Immunol* 27:669–692
- Bader V, Ottis P, Pum M, Huston JP, Korth C (2012a) Generation, purification, and characterization of cell-invasive DISC1 protein species. *J Vis Exp* 66:e4132. doi:10.3791/4132
- Bader V, Tomppo L, Trossbach SV, Bradshaw NJ, Prikulis I, Leliveld SR, Lin CY, Ishizuka K, Sawa A, Ramos A, Rosa I, García Á, Requena JR, Hipolito M, Rai N, Nwulia E, Henning U, Ferrea S, Luckhaus C, Ekelund J, Vejjola J, Järvelin MR, Hennah W, Korth C (2012b) Proteomic, genomic and translational approaches identify CRMP1 for a role in schizophrenia and its underlying traits. *Hum Mol Genet* 21:4406–4418
- Berke JD, Breck JT, Eichenbaum H (2009) Striatal versus hippocampal representations during win-stay maze performance. *J Neurophysiol* 101:1575–1587
- Brandon NJ, Sawa A (2011) Linking neurodevelopmental and synaptic theories of mental illness through DISC1. *Nat Rev Neurosci* 12:707–722
- Broadhurst PL (1960) Experiments in psychogenetics: application of biometrical genetics to the inheritance of behavior. In: Eysenck

- HJ (ed) Experiments in personality, vol I., Psychogenetics and Psychopharmacology/Routledge, Regan Paul, London, pp 1–102
- Buzsaki G, Logothetis N, Singer W (2013) Scaling brain size, keeping timing: evolutionary preservation of brain rhythms. *Neuron* 80:751–764
- Chubb JE, Bradshaw NJ, Soares DC, Porteous DJ, Millar JK (2008) The DISC locus in psychiatric illness. *Mol Psychiatry* 13:36–64
- Gallo A, Gonzalez-Lima F, Sadile AG (2002) Impaired metabolic capacity in the perirhinal and posterior parietal cortex lead to dissociation between attentional, motivational and spatial components of exploration in the Naples high excitability rat line. *Behav Brain Res* 130:133–140
- Gironi Carnevale UA, Vitullo E, Varriale B, Ruocco LA, Sadile AG (2007) A classical Mendelian cross-breeding study of the Naples high and low excitability rat lines. *Behav Brain Res* 183:130–140
- Hayashi-Takagi A, Takaki M, Graziane N, Seshadri S, Murdoch H, Dunlop AJ, Makino Y, Seshadri AJ, Ishizuka K, Srivastava DP, Xie Z, Baraban JM, Houslay MD, Tomoda T, Brandon NJ, Kamiya A, Yan Z, Penzes P, Sawa A (2010) Disrupted-in-Schizophrenia 1 (DISC1) regulates spines of the glutamate synapse via Rac1. *Nat Neurosci* 13:327–332
- Hölm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 6:65–70
- Jacobsen KK, Halmoy A, Sanchez-Mora C, Ramos-Quiroga JA, Cormand B, Haavik J, Johansson S (2013) DISC1 in adult ADHD patients: an association study in two European samples. *Am J Med Genet B Neuropsychiatr Genet* 162B:227–234
- Korth C (2012) Aggregated proteins in schizophrenia and other chronic mental diseases: DISC1opathies. *Prion* 6:134–141
- Leliveld SR, Bader V, Hendriks P, Prikulis I, Sajnani G, Requena JR, Korth C (2008) Insolubility of disrupted-in-schizophrenia 1 disrupts oligomer-dependent interactions with nuclear distribution element 1 and is associated with sporadic mental disease. *J Neurosci* 28:3839–3845
- Leliveld SR, Hendriks P, Michel M, Sajnani G, Bader V, Trossbach S, Prikulis I, Hartmann R, Jonas E, Willbold D, Requena JR, Korth C (2009) Oligomer assembly of the C-terminal DISC1 domain (640–854) is controlled by self-association motifs and disease-associated polymorphism S704C. *Biochemistry* 48:7746–7755
- Lipp HP, Schwegler H, Heimrich B, Cerbone A, Sadile AG (1987) Strain-specific correlations between hippocampal structural traits and habituation in a spatial novelty situation. *Behav Brain Res* 24:111–123
- Marco EM, Adriani W, Ruocco LA, Canese R, Sadile AG, Laviola G (2011) Neurobehavioral adaptations to methylphenidate: the issue of early adolescent exposure. *Neurosci Biobehav Rev* 35:1722–1739
- Masliah E, Rockenstein E, Adame A, Alford M, Crews L, Hashimoto M, Seubert P, Lee M, Goldstein J, Chilcote T, Games D, Schenk D (2005) Effects of alpha-synuclein immunization in a mouse model of Parkinson's disease. *Neuron* 46:857–868
- Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CA, Devon RS, Clair DM, Muir WJ, Blackwood DH, Porteous DJ (2000) Disruption of two novel genes by a translocation co-segregating with schizophrenia. *Hum Mol Genet* 9:1415–1423
- Moser MB, Moser EI, Wultz B, Sagvolden T (1988) Component analyses differentiate between exploratory behaviour of spontaneously hypertensive rats and Wistar Kyoto rats in a two-compartment free-exploration open field. *Scand J Psychol* 29:200–206
- Neunuebel JP, Yoganarasimha D, Rao G, Knierim JJ (2013) Conflicts between local and global spatial frameworks dissociate neural representations of the lateral and medial entorhinal cortex. *J Neurosci* 33:9246–9258
- Niwa M, Kamiya A, Murai R, Kubo K, Gruber AJ, Tomita K, Lu L, Tomasito S, Jaaro-Peled H, Seshadri S, Hiyama H, Huang B, Kohda K, Noda Y, O'Donnell P, Nakajima K, Sawa A, Nabeshima T (2010) Knockdown of DISC1 by in utero gene transfer disturbs postnatal dopaminergic maturation in the frontal cortex and leads to adult behavioral deficits. *Neuron* 65:480–489
- Ottis P, Bader V, Trossbach S, Kretschmar H, Michel M, Leliveld SR, Korth C (2011) Convergence of two independent mental disease genes on the protein level: recruitment of dysbindin to cell invasive DISC1 aggresomes. *Biol Psychiatry* 70:604–610
- Papa M, Diewald L, Carey MP, Esposito FJ, Gironi Carnevale UA, Sadile AG (2002) A rostro-caudal dissociation in the dorsal and ventral striatum of the juvenile SHR suggests an anterior hypo and a posterior hyperfunctioning mesocorticolimbic system. *Behav Brain Res* 130:171–179
- Paxinos G, Watson C (2007) *The Rat Brain in Stereotaxic Coordinates*. Academic Press, London
- Pogorelov VM, Nomura J, Kim J, Kannan G, Ayhan Y, Yang C, Taniguchi Y, Abazyan B, Valentine H, Krasnova IN, Kamiya A, Cadet JL, Wong DF, Pletnikov MV (2012) Mutant DISC1 affects methamphetamine-induced sensitization and conditioned place preference: a comorbidity model. *Neuropharmacology* 62:1242–1251
- Prusiner SB (2001) Shattuck Lecture—Neurodegenerative diseases and prions. *N Engl J Med* 344:1516–1526
- Ruocco LA, de Souza Silva MA, Topic B, Mattern C, Huston JP, Sadile AG (2009a) Intranasal application of dopamine reduces activity and improves attention in Naples High Excitability Rats that feature the mesocortical variant of ADHD. *Eur Neuropsychopharmacol* 19:693–701
- Ruocco LA, Sadile AG, Gironi Carnevale UA (2009b) Modeling the mesocortical variant of ADHD: the Naples high excitability rat. In: Gordon SM, Mitchell AM (eds) *Attention Deficit Hyperactivity Disorder*. Nova Science, New York, pp 85–106
- Ruocco LA, Gironi Carnevale UA, Treno C, Sadile AG, Melisi D, Arra C, Iba M, Schirru C, Carboni E (2010) Prepuberal subchronic methylphenidate and atomoxetine induce different long-term effects on adult behaviour and forebrain dopamine, norepinephrine and serotonin in Naples High-Excitability rats. *Behav Brain Res* 210:99–106
- Ruocco LA, Romano E, Treno C, Lacivita E, Claudio A, Gironi Carnevale UA, Travaglini D, Leopoldo M, Laviola G, Sadile AG, Adriani W (2014a) Emotional and risk seeking behavior after prepuberal subchronic or adult acute stimulation of 5-HT7-Rs in Naples high excitability rats. *Synapse* 68:159–167
- Ruocco LA, Treno C, Gironi Carnevale UA, Arra C, Mattern C, Huston JP, de Souza Silva MA, Nikolaus S, Scorziello A, Nieddu M, Boatto G, Illiano P, Pagano C, Tino A, Sadile AG (2014c) Prepuberal intranasal dopamine treatment in an animal model of ADHD ameliorates deficient spatial attention, working memory, amino acid transmitters and synaptic markers in prefrontal cortex, ventral and dorsal striatum. *Amino Acids* 46(9):2105–2122
- Sadile AG (1996) Long-term habituation of theta-related activity components of albino rats in the Låt-maze. In: Sanberg PR, Ossenkopp KP, Kavaliers M (eds) *Motor activity and movement disorders: measurement and analysis*. Humana Press, New York, pp 1–54
- Sadile AG, Gironi Carnevale UA, Vitullo E, Cioffi LA, Welzl H, Bätzig K (1988) Maze learning of the Naples High- and Low-Excitability rat lines. *Adv Biosci* 70:177–180
- Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandeventer C, Walker S, Wogulis M, Yednock T, Games D, Seubert P (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173–177
- Schwartz M, Shechter R (2010a) Systemic inflammatory cells fight off neurodegenerative disease. *Nat Rev Neurol* 6:405–410



- Schwartz M, Shechter R (2010b) Protective autoimmunity functions by intracranial immunosurveillance to support the mind: the missing link between health and disease. *Mol Psychiatry* 15:342–354
- Schwartz M, Kipnis J, Rivest S, Prat A (2013) How do immune cells support and shape the brain in health, disease, and aging? *J Neurosci* 33:17587–17596
- Urushitani M, Ezzi SA, Julien JP (2007) Therapeutic effects of immunization with mutant superoxide dismutase in mice models of amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 104:2495–2500
- Viggiano D, Vallone D, Welzl H, Sadile AG (2002) The Naples high- and low excitability rats: selective breeding, behavioral profile, morphometry and molecular biology of the mesocortical dopamine system. *Behav Genet* 32:315–333
- Vomund S, Tapir S, Reiner O, De Souza Silva AM, Korth C (2013) Generation of topically transgenic rats and in vivo screening by bioluminescence imaging. *J Vis Exp* 79:e50146. doi:[10.3791/50146](https://doi.org/10.3791/50146)
- Wei J, Graziane NM, Wang H, Zhong P, Wang Q, Liu W, Hayashi-Takagi A, Korth C, Sawa A, Brandon NJ, Yan Z (2013) Regulation of N-methyl-D-aspartate receptors by disrupted-in-schizophrenia-1. *Biol Psychiatry* 75:414–424