Loss of SNAP-25 and Rabphilin 3a in sensory-motor cortex in Huntington's disease

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ABSTRACT

Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a CAG-expansion in the gene encoding the protein huntingtin. The disease is characterized by progressive motor disturbances, cognitive defects, dementia and weight loss. Using Western blotting and immunohistochemistry we have assessed the expression levels and patterns of a number of proteins involved in neurotransmitter release in post-mortem frontal cortex samples from ten HD cases with different disease grades. We report a loss of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) protein, synaptosomal associated protein 25 (SNAP 25) in HD brains of grades I to IV. Moreover, in brains of grade III and IV we found a reduction in rabphilin 3a, a protein involved in vesicle docking and recycling. These losses appear to be specific and not due to a general loss of synapses in the HD cortex. Thus, levels of synaptobrevin II, syntaxin 1, rab3a or synaptophysin are unaltered in the same patient samples. SNAP 25 and rabphilin 3a are crucial for neurotransmitter release. Therefore, we suggest that a deficient presynaptic transmitter release may underlie some of the symptoms of HD.

KEYWORDS:

Huntington's disease, synapse, transmitter release, SNARE, Rabphilin 3a

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant disease affecting mainly the nervous system. Patients with HD typically present both with psychiatric problems such as personality changes and depression and with hyperkinetic involuntary movements (Harper 1996). Subtle cognitive defects are sometimes detected several years before the onset of motor symptoms (Robins Wahlin et al. 2007). As the disease progresses it develops into a severe dementia combined with a hypokinesia and a relentless weight loss and wasting (Harper 1996). Neuropathologically the disease is characterized by a pronounced atrophy of the striatum and a thinning of the neocortex (Vonsattel et al. 1985; Vonsattel and DiFiglia 1998). Cell loss can also be detected in the hypothalamus (Kremer et al. 1991). At the subcellular level, the pathology is characterized by intranuclear and cytoplasmic protein aggregates (Davies et al. 1997; DiFiglia et al. 1997).

Following the discovery of the causative, mutated gene (*IT15*), encoding the protein huntingtin in 1993 (MacDonald et al. 1993), HD pathogenesis has been extensively studied. The mutation causes widespread intracellular downstream effects, such as alterations of gene transcription (Luthi-Carter et al. 2000; Hodges et al. 2006); protein-protein interactions (Li et al. 1995; Sun et al. 2001; Modregger et al. 2002; Singaraja et al. 2002; Li and Li 2004); intracellular transport (Gunawardena et al. 2003; Szebenyi et al. 2003; Gauthier et al. 2004; Trushina et al. 2004; del Toro et al. 2006); neurotransmitter synthesis, release (Petersen et al. 2002; Vetter et al. 2003; Smith et al. 2006) and neurotransmitter receptors (Arzberger et al. 1997; Cha et al. 1998; Cha et al. 1999; Bibb et al. 2000; Jarabek et al. 2004).

Interestingly, in contrast to the massive cell death seen at late stages of the disease in humans, animal models exhibit clear symptoms and dysfunctional neuronal connectivity without a widespread loss of neurons (for reviews see (Li et al. 2003; Smith et al. 2005a)). Therefore we have hypothesized that neuronal dysfunction may be responsible for some of the symptoms that appear in early stages of the disease (Smith et al. 2005a). In the R6/1 mouse model of HD (Smith et al. 2005b) we found a selective down-regulation of the exocytotic protein rabphilin 3a, both in the striatum and in the cerebral cortex, while the other proteins we examined were unaffected. Rabphilin 3a is believed to be an effector protein that binds to the protein rab3a (Shirataki et al. 1993; Yamaguchi et al. 1993; Shirataki et al. 1994). Rabphilin 3a and rab3a jointly assist in the process of docking vesicles to the plasma membrane (Chung et al. 1995; Komuro et al. 1996; Arribas et al. 1997; Burns et al. 1998), thereby promoting synaptic vesicle release. Recent evidence also suggests a role for rabphilin 3a in recycling of synaptic vesicles (Deak et al. 2006). For a proper release of neurotransmitters via the fusion of synaptic vesicles to the plasma membrane multiple proteins are needed. Among the most thoroughly studied ones are the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins, syntaxin 1, SNAP 25 (synaptosomal associated protein 25) and synaptobrevin II (for review see (Jahn et al. 2003)). The SNARE proteins are essential for an efficient Ca^{2+}-stimulated vesicle release (Washbourne et al. 2002; Jahn et al. 2003).

In this study, we studied the expression of exocytotic proteins in brain samples from ten patients with HD. To do this, we generated three antibodies against human rabphilin 3a. We also investigated the expression levels of several proteins in the synapse essential for neurotransmitter release. We observed a decrease in the levels of the SNARE protein

SNAP-25 that is accompanied by a loss of rabphilin 3a in cases with a higher HD pathological grade. The protein loss does not involve the other SNARE proteins syntaxin 1 and synaptobrevin II or the vesicular proteins rab3a and synaptophysin. Our findings suggest a defect of the transmitter release machinery in HD patients occurring from a time-point when there are only limited neuropathological findings.

METHODS

Human brain samples

The tissues used in these experiments were obtained from the Neurological Foundation of New Zealand Human Brain Bank, Department of Anatomy with Radiology, University of Auckland, New Zealand. The collection procedures were approved by the University of Auckland Human Subjects Ethics Committee. Control cases were age matched and all had no known history of neurological disease. Immediately following autopsy the brains were taken to the Department of Anatomy with Radiology, University of Auckland. One hemisphere was perfused through the cerebral arteries with 15% (vol/vol) formalin, blocked, postfixed in the same fixative and stored at -80^{o}C until sections were cut for the immunohistochemical studies (see also (Waldvogel et al. 2006)). The other hemisphere was cut into blocks, frozen on dry ice and stored at -80°C until further processing. The post-mortem delay in each case is described as the time interval between death and the perfusing or freezing of the tissue blocks (see Table 1 and 2). The patients had been diagnosed clinically and genetically with HD, and graded according to the grading scale criteria described by Vonsattel and co-workers (Vonsattel et al. 1985; Vonsattel and DiFiglia 1998).

Rabphilin 3a antibody generation

Generating protein expression vectors

cDNA for human rabphilin 3a (Ultimate ORF clone IOH14025) was ordered from (Invitrogen). Primers: (Forward, pos 210) 5'-ataggatcctctactATGACTGACACCGTG-3' containg a BamHI site; (Forward, pos 810) 5'-CGAGGTGACAGTGAAGATAG-3';

(Reverse, pos 1761) 5'-TCACTCGCTCCAGGCAGATG-3'; (Reverse, pos 2288) 5'-atagtcgacctaaTCACTTGACACGTGG-3' containing a SalI site were ordered from (Sigma/Genosys). The full length (Rph-FL; aa 1- 690) coding sequence was PCR-amplified using the Forward 210 - Reverse 2288 primers and cloned into a dephosphorylated pGEX-6P1 vector (Amersham Biosciences) after BamHI/SalI digestion. Two more amplicons containing parts of the coding sequence were generated using the Forward 810 – Reverse 1761 (Rph-R2; aa 199 - 515) and Forward 810 – Reverse 2288 (Rph-R4; aa 199 - 690) primers. The Rph-R2 and Rph-R4 amplicons were inserted blunt ended into pBlueScript (Stratagene) vectors after an EcoRV digestion of the vector. White colonies were picked and verified to be correct. The plasmids were digested using BamHI/XhoI (Rph-R2) and BamHI/SalI (Rph-R4) and inserted into BamHI/XhoI-digested, dephosphorylated pGEX-6P1 vectors. All constructs were sequenced and found to be correct and in frame with the GST-tag of the pGEX-vectors.

Protein production

BL21 bacteria were transformed with the pGEX-constructs and were let to grow until the optical density at 600nm was > 0.5 at which point isopropyl B-D-Thiogalactoside (IPTG; 250 µM end conc.) was added. The cultures were let shaking under aerobic conditions at room temperature over night. The bacterial suspensions were spun at 4500 x rpm for 15 min and the pellets were resuspended in 10 ml ice cold PBS containing protease inhibitor cocktail (Sigma; P8340). Lysozyme (Sigma, 20 mg/ml)) was added and the bacteria were incubated for 20 min on ice. The lysate was sonicated 3 x 5 seconds. 250 µl 20% (vol/vol) Triton X-100 was added and the lysate was incubated rotating at 4°C for 25

min. The lysate was spun for 15 min at 4500 rpm and the supernatant transferred to a new vial. 100 µl Glutathione-sepharose (Amersham Biosciences) beads were added and incubated rotating with the supernatant at 4 °C for 4 hours. The Glutathione-beads were spun down briefly (500 x g, 5 min) and washed four times in PBS with 0.1% (vol/vol) Triton X-100. The bound proteins were eluted in 200 µl elution buffer (10 mM reduced glutathion in 50 mM Tris-HCl, pH 8.0). 10 µl of the eluate was dissolved in 10 µl 2x Laemmli buffer and run on a 10% SDS-PAGE gel.

Protein purification and antibody production

The theoretical isoelectrical point of Rph-FL was calculated to 8.20. To purify the Rph-FL sample the pH was set to 5.5, the sample was loaded onto a column and washed with 20 mM Na-Acetate, pH 5.0. The protein was eluted with increasing NaCl concentration (0 M – 5 M over 60 ml; 1 ml samples collected). 10 µl samples from every third sample were analyzed on a SDS-PAGE gel. The samples in the elution peak were collected and pooled. The Rph-R2 and Rph-R4 samples did not need further purification. 500 µg of the purified proteins were lyophilized and sent off for immunization of rabbits (Pineda Antikörper Service, Berlin). After 90 days (3 immunizations) the total serum was collected, aliquoted and frozen at –80°C.

Western blotting

Gray matter from brain cortex samples was dissected and homogenized on ice in homogenization buffer containing 4 mM HEPES-NaOH (pH 7.3), 2 mM EDTA, 1 x protease inhibitors (Sigma, Stockholm, Sweden), using a Vibra-cell sonicator (Sonics &

Materials Inc., Danbury, CT, USA). The homogenate was spun at 1,000 x g for 10 min, the supernatant saved and the protein concentration was determined using BioRad D_C protein assay (BioRad, Hercules, CA). 1% SDS (final concentration) was added. Samples were diluted to 1mg/ml in 3x Laemmli buffer containing ß-mercaptoethanol. Samples were heated to 95°C for 5 minutes, and were then kept at –20°C. 20 µg of protein was loaded per lane and analyzed by SDS-PAGE and immunoblotting onto PVDF membranes (GE Healthcare, Uppsala, Sweden). The membranes were incubated with the primary antibodies in 0.05% Tween-20 in PBS. Primary antibodies used were; Rph-R2 (rabbit, 1:20000); Rph-R4 (rabbit, 1:10000) Rph-FL (rabbit, 1:20000); rab3a (Synaptic Systems, Göttingen, Germany, mouse, 1:100000); SNAP-25 (Synaptic Systems, clone 71.2, mouse, 1:25000); syntaxin 1 (Synaptic Systems, clone 78.2, mouse, 1:100000); synaptophysin (Synaptic Systems, clone 7.2, mouse 1:10000); synaptobrevin II (SySy, clone 69.1; 1:10000) and GAPDH (Chemicon (mAB374), mouse, 1:50000). After rinsing, secondary antibodies (goat anti-mouse or donkey anti-rabbit IgG) conjugated with HRP (GE Healthcare) were used; the signals were detected using ECL (1.25 mM Luminol (Sigma), 225µM p-Coumaric acid (Sigma) in 100mM Tris-HCl, pH 8.5, activated by addition of 0.03% (vol/vol) H_2O_2). ECL exposed films were scanned and the intensities of the bands were measured in all films using the ImageJ freeware.

Immunohistochemistry

C57/Bl6 mice were anaesthetized and transcardially perfused with 4% (wt/vol) paraformaldehyde, the brain dissected and serially sectioned into 30µm coronal sections. The sections were preincubated free floating in blocking solution (5% (vol/vol) normal

horse or goat serum, 0.3% (vol/vol) triton-X-100 in 0.1M PBS, pH 7.4) for 1 hour. Primary antibody incubations (in 2% (vol/vol) serum, 0.3 % (vol/vol) triton-X-100) were carried out over night at room temperature. Primary antibodies used were Rph-R4 (rabbit, 1:1000) and murine rabphilin (BD, #610509, 1:1000). Human brain blocks from the superior frontal gyrus were serially sectioned in a cryostat (40 µm). The sections were immunostained free floating as described above. For double labeling with fluorescent secondary antibodies rabbit Rph-R4 (1:1000) and SNAP25 (Synaptic Systems, #111 002, 1:1000) were combined with mouse EM48 (Chemicon, Mab5374 1:1000). Mouse syntaxin 1 antibody (Synaptic Systems, clone 78.2, 1:1000) or synaptobrevin II (Synaptic Systems, Clone 69.1, 1:800) were combined with rabbit ubiquitin (Dako, Z0458, 1:1000). Cy-2 or Cy-3 labeled secondary antibodies (Jackson) were used. To minimize the auto-fluorescence in the human sections, they were incubated in 5 mM $CuSO_4$ in 50 mM ammonium acetate pH 5.0, before mounting.

Statistics

To compare protein levels in different disease stages Jonckheere-Terpstra Trend tests were used. For comparison of age and post-mortem interval effects on protein expression non-parametric Spearman correlation analyses were performed. For two group comparisons Mann-Whitney U-tests were used. Statistical significance was considered being $p < 0.05$.

RESULTS

Antibody generation.

We have previously demonstrated that rabphilin 3a is reduced in different brain regions of HD transgenic mice (Smith et al. 2005b). However, commercially available rabphilin 3a antibodies work poorly in human brain tissue. We therefore generated antibodies specifically against epitopes of the human rabphilin 3A. cDNA of full-length human rabphilin 3a was used to generate GST fusions with either full length protein (Rph-FL, aa 1-690), with a central fragment (Rph-R2, aa 198-515) and with a C-terminal fragment (Rph-R4, aa 198-690) (Fig 1a). The GST-proteins were produced in *E. coli*, purified by affinity precipitation and ion exchange chromatography before being used to immunize rabbits. In Western blots of brain tissue from patients all antibodies detect a single band of ~80 kDa, representing rabphilin 3a (Fig 1b). Full-length human rabphilin 3a has 87% sequence homology to mouse and 86% to rat rabphilins. The antibodies generated against human rabphilin 3a show patterns very similar to the signal obtained using antibodies generated against mouse rabphilin 3a in immunohistochemistry on mouse brain sections (Fig 1c (Rph-R4) and data not shown). Western blots on mouse brain homogenates gives a single band of ~80 kDa (data not shown).

Expression levels of exocytotic proteins in human sensory motor cortex.

We studied the expression levels of a number of proteins involved in exocytotic processes in samples from the grey matter of sensory / motor cortex from 10 patients with HD (HC, Table 1) and 10 neurologically normal and age-matched controls (H; Table 1). There were no significant differences in post-mortem interval between HD patients and

controls. The levels of rabphilin 3a decrease in brains from HD patients with higher neuropathological grade and higher CAG repeats (Fig 2 d; Jonckheere Terpstra Trend Test, $p < 0.05$). In a similar manner, the levels of SNAP 25, one of the three essential SNARE complex proteins, was significantly decreased in HD cases (Fig 2 a; Jonckheere Terpstra Trend Test, $p < 0.01$). The earliest changes were seen in SNAP 25, the protein levels are lowered already in HD brains with limited neuropathology (Grade I). Importantly, the levels of synaptobrevin II, syntaxin 1, rab3a and synaptophysin (Fig 2 b-c. e-f and g) remain unchanged in all pathological grades. This indicates that the reductions of rabphilin 3a and SNAP 25 do not merely reflect a loss of synapses in the grey matter of the sensory/motor cortex.

To elucidate whether there were any effects of age, post mortem interval or gender on levels of protein, we sorted the control sample results for rabphilin 3a according to these parameters (Fig 2 h). There was neither any detectable effect of post mortem interval ($p = 0.73$, Spearman correlation), nor of age ($p = 0.24$, Spearman correlation), nor gender ($p = 0.58$, Mann-Whitney) on the expression of rabphilin 3a.

No association of SNARE proteins with huntingtin inclusions

In order to assess morphological changes, we first stained sections from patients and controls from the superior frontal gyrus or Broca's area 9 (Table 2) for rabphilin 3a (Rph R4) using a diaminobenzidine (DAB) based detection (data not shown). We did not observe any aggregation of the protein or other changes in the subcellular expression pattern of the protein in the samples studied.

A recent study reported that syntaxin 1 is recruited into mutant huntingtin inclusions in cell models of HD (Swayne and Braun 2007) we therefore studied the association between rabphilin 3a, SNAP 25, syntaxin 1 and synaptobrevin II with huntingtin aggregates (Fig 3 a-d). Intranuclear and cytoplasmic aggregates were clearly demonstrated by immunohistochemistry with antibodies either against mutant huntingtin (EM48) or ubiquitin. However, we never detect any localization of the SNARE proteins or rabphilin 3a in huntingtin aggregates/inclusions. The absence of rabphilin 3a from inclusions is in line with the observation from the R6/1 mouse model (Smith et al. 2005b). The immunostaining of rabphilin 3a and SNAP 25 indicate a general decrease in protein levels in HD cases, however, we did not find any detectable alterations in the subcellular localization of any of the four proteins in patient samples as compared to control sections (Fig. 3 a-d).

DISCUSSION

In the neocortex from HD cases, we observed a loss of one of the SNARE proteins, SNAP 25 and a loss of rabphilin 3a with higher disease severity. These changes are specific as there is no concomitant decrease in the other synaptic or SNARE proteins we analyzed, namely syntaxin 1, synaptobrevin II, rab3a or synaptophysin. Moreover, we found no association between rabphilin 3a or the SNARE proteins and mutant huntingtin aggregates. Probably, losses of cells and synapses to some extent are responsible for these decreases.. Interestingly, loss of cells cannot fully explain the decreased protein levels since the presynaptic markers rab3a and synaptophysin remain virtually unchanged. Taken together, these data suggest that there is a selective loss of SNAP 25 and rabphilin 3a in the HD neocortex.

A previous publication reported normal levels of SNAP 25 and syntaxin 1, but decreased levels of synaptobrevin II in a small number of human HD striata and normal or increased levels in hippocampal samples (Morton et al. 2001). However, in yet another study mRNA-levels of striatal SNAP 25 and rabphilin 3a in HD patient striatum were decreased to 35% and 61% of control patient levels respectively (Supplementary data in (Hodges et al. 2006)). A decrease in mRNA levels of the two proteins is in line with our previous study in R6-mice (Smith et al. 2005b) and indicates a decrease in protein levels due to a transcriptional down-regulation of the SNAP 25 and rabphilin 3a genes.

The SNARE proteins are generally considered being essential for Ca^{2+}-triggered neurotransmitter release (Jahn et al. 2003). Knockout of SNAP 25 (Washbourne et al. 2002) and synaptobrevin II (Schoch et al. 2001) results in embryonic or early post-natal

lethality and a total absence of vesicle fusion and transmitter release in response to action potentials and increased intracellular levels of Ca^{2+}. Interestingly, cultures from embryonic neurons in these two knockout models show a slight residual SNARE-independent fusion activity and a low frequency of spontaneous excitatory post-synaptic activity. There is clear gene dose-dependence in the studied effects since heterozygous are less affected and viable (Schoch et al. 2001; Washbourne et al. 2002). Recently Deak and co-workers showed that rabphilin 3a interacts with the SNARE complex via SNAP 25, and that this interaction is involved in the recycling and reuse of synaptic vesicles after transmitter release (Deak et al. 2006). Another recent study (Anggono et al. 2006) has provided further leads for a possible vesicular disturbance in the presynapse of HD patients, by showing that the protein PACSIN I/syndapin I is involved in presynaptic vesicular recycling (Anggono et al. 2006). Previously PACSIN I was shown to have an altered subcellular location in low grade HD cases (Modregger et al. 2002).

Our results suggest that there may be a defect in the machinery fusing the synaptic vesicles to the plasma membrane and in the recycling and docking of vesicles after transmitter release. Cepeda and coworkers demonstrated changes in the cortico-striatal pathway in a mouse model of HD (R6/2), the changes are suggested to be of presynaptic origin (Cepeda et al. 2003). We therefore speculate that some of these defects could be caused by changes in the SNARE-protein levels and an altered SNARE efficacy in fusing vesicles to the plasma membrane.

A defect in the presynaptic release machinery could also potentially affect other processes of relevance to the pathogenesis of HD, such as BDNF release from cortical neurons, supporting the survival of the striatal medium sized spiny neurons (Altar et al.

1997). A decreased synthesis (Zuccato et al. 2001) and transport (Gauthier et al. 2004) of BDNF in tissues and cells expressing mutant huntingtin has previously been shown. Our data suggest a possible additional defect in route of delivery of BDNF to the target cells. Moreover, it is likely that some of the symptoms of HD could be caused or aggravated by a cortical signaling defect. Cognitive defects are seen early in the disease, with slight disturbances appearing up to 15 years prior to the onset of motor symptoms (Robins Wahlin et al. 2007). At such a stage, cell loss in the cortex is likely to be minimal.

In conclusion, we have shown that there is a down-regulation of the synaptic proteins SNAP 25 and rabphilin 3a in the neocortex of HD patients. The data indicate a possible neurotransmitter release defect in human HD cortical neurons. Moreover, cortical release of BDNF to the striatum is a key factor for striatal neuronal survival and a loss of this neurotrophic support may be detrimental for striatal neurons (Zuccato et al. 2001).

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TABLES

Table 1: Samples for western blotting

Case	Gender /age	CAG-number	Vonsattel grade	Post mortem interval (h)
H 114	M/42	N/A	-	14
H 121	F/64	18/23	-	6.5
H 109	M/81	15/18	-	7
H 164	M/73	N/A	-	13
H 142	M/41	N/A	-	16
H 155	M/61	17/19	-	7
H 128	F/34	17/19	-	18.5
H 129	M/48	20/21	-	12
H 132	F/63	15/19	-	12
H 111	M/46	17/20	-	10
HC 103	M/41	19/39	1	11
HC 105	F/67	15/42	1	9
HC 83	M/80	20/40	1	9
HC 107	M/75	19/43	3	3
HC 104	M/40	18/51	3	15
HC 102	M/64	17/42	3	10
HC 96	F/39	22/53	3	20
HC 77	F/53	16/55	4	9
HC 87	M/45	21/50	4	18
HC 109	F/59	23/47	4	7

Table 2: Samples used for immunohistochemistry

Case	Gender/age	CAG-number	Vonsattel Grade	Post-mortem interval (h)	Region
H109	M/81	15/18	-	7	BA9
H114	M/42	N/A	-	14	SFG
H152L	M/79	N/A	-	18	SFG
H164	M/73	N/A	-	13	SFG
HC92	M/72	N/A	1	5	SFG
HC82	M/74	N/A	2	16	SFG
HC96	F/39	22/53	3	20	SFG
HC102	M/64	17/42	3	10	SFG
HC104	M/40	18/51	3	15	SFG
HC77	F/53	16/55	4	9	SFG
HC87	M/45	21/50	4	18	SFG
HC109	F/59	23/47	4	7	SFG

BA9 – Brodmann area 9; SFG – superior frontal gyrus; N/A – not available.

FIGURE LEGENDS

Figure 1

Generation of rabphilin 3a antibodies. (a) Three constructs were generated, full-length (Rph-FL); central (Rph-R2) and C-terminal (Rph-R4) constructs were cloned from human rabphilin 3a cDNA. (b) Representative blots using the three antibodies on human cortical lysates. All antibodies detect a single band of approximately 80 kDa. (c) Colocalization studies of mouse hippocampal sections using a mouse rabphilin 3a antibody and the Rph-R4 antibody. Images were acquired in a Leica confocal microscope. Scale bars = 250µm (upper panel); 50 µm (middle panel); and 10µm (lower panel) in c).

Figure 2

Western blots of human sensory/motor cortex. (a) SNAP 25, $p < 0.01$. (b) synaptobrevin II, p = n.s. (c) syntaxin 1, p = n.s. (d) rabphilin 3a, $p < 0.05$ (e) rab 3a, p = n.s. (f) synaptophysin, p = n.s. (g) GAPDH, p = n.s. All results were tested for statistical significance using Jonckheere-Terpstra trend tests. (h) Representative blots of the analyzed proteins. (i) rabphilin 3a control samples plotted against Post-mortem interval (upper panel), Age (middle panel) and Gender (lower panel). a.u. – arbitrary units.

Figure 3

Double immunofluorescence labeling of SNARE proteins and rabphilin 3a with EM48 or ubiquitin. (a) SNAP 25 (b) synaptobrevin II (c) syntaxin 1 (d) rabphilin 3a. None of the

proteins show any colocalization with mutant huntingtin aggregates in the human cortex. Scale bars = 40 μm.

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Fig1

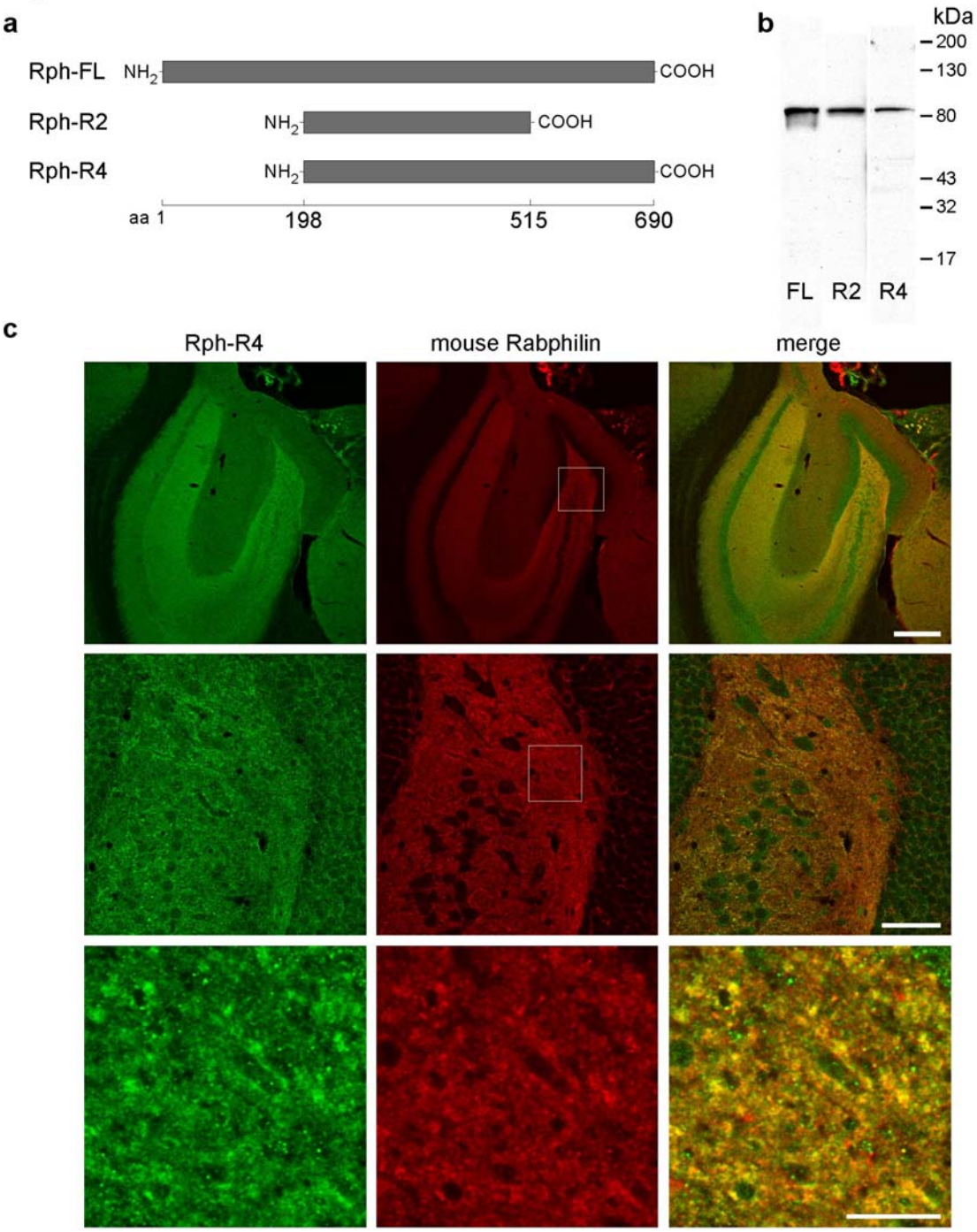
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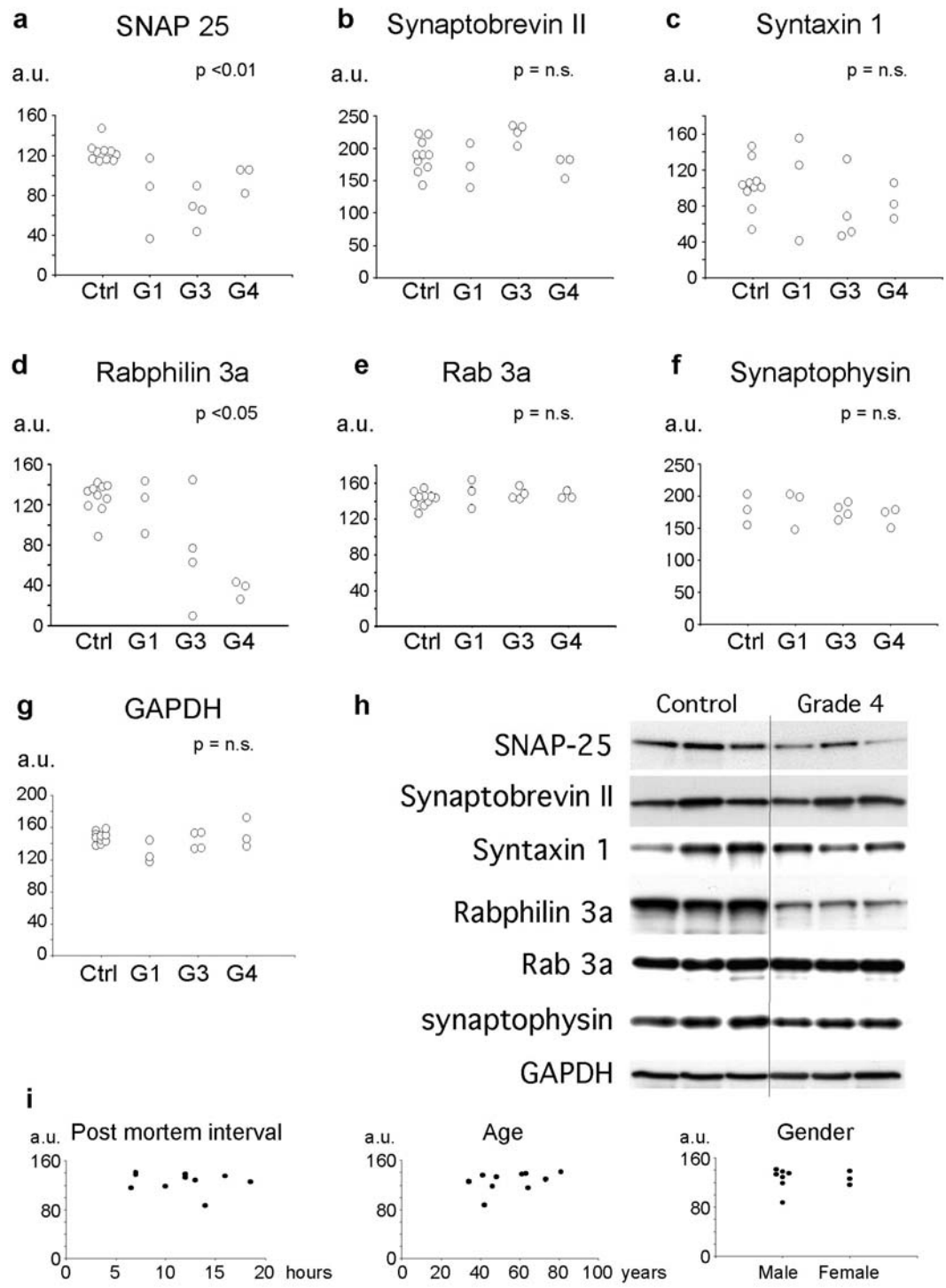
Fig 2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SNAP-25
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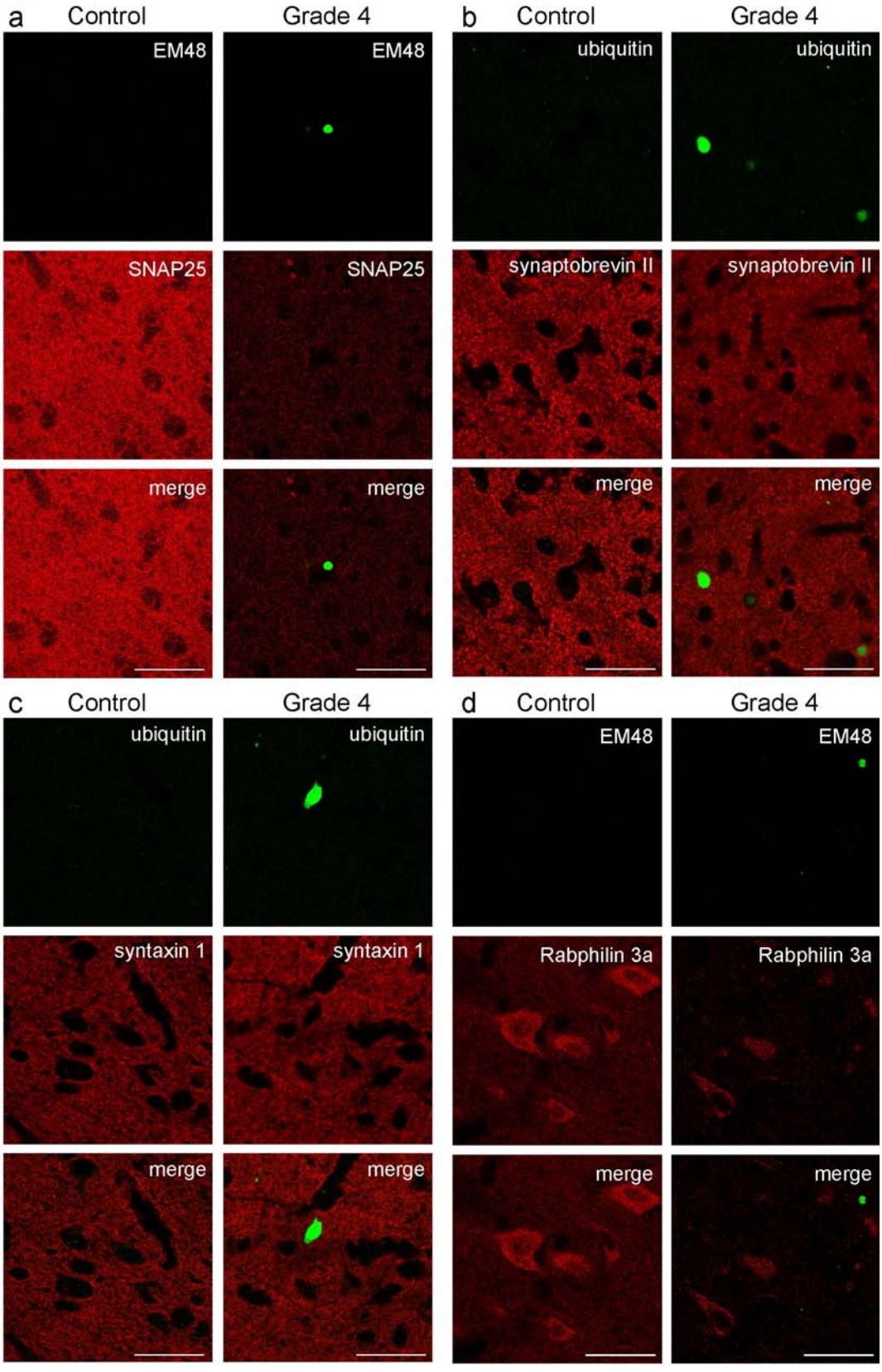
Fig 3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