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Changes in dopamine signalling does not underlie aberrant hippocampal plasticity in a mouse model of Huntington's disease

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Abstract:	Altered dopamine (DA) receptor labelling has been demonstrated in presymptomatic and symptomatic Huntington's disease (HD) gene carriers, indicating that alterations in dopaminergic signalling is an early event in HD. We have previously described early alterations in synaptic transmission and plasticity in both the cortex and hippocampus of the R6/1 mouse model of Huntington's disease. Deficits in cortical synaptic plasticity were associated with altered dopaminergic signalling and could be reversed by D1- or D2-like dopamine receptor activation. In light of these findings we here investigated whether defects in dopamine signalling could also contribute to the marked alteration in hippocampal synaptic function. To this end we performed dopamine receptor labelling and pharmacology in the R6/1 hippocampus and report a marked, age-dependent elevation of hippocampal D1 and D2 receptor labelling in R6/1 hippocampal subfields. Yet, pharmacological inhibition or activation of D1- or D2-like receptors did not modify the aberrant synaptic plasticity observed in R6/1 mice. These findings demonstrate that global perturbations to dopamine receptor expression do occur in HD transgenic mice, similarly in HD gene carriers and patients. However, the direction of change and the lack of effect of dopaminergic pharmacological agents on synaptic function demonstrates that the perturbations are heterogeneous and region-specific, a finding that may explain the mixed results of dopamine therapy in HD.
Response to Reviewers:	Reviewer #1: In this manuscript, the authors detected expression of dopamine receptors by immunostaining in HD mice and they found the levels of D1 and D2-like receptors were increased along with age in R6/1 HD hippocampus. Further they tested the effect of D1 or D2-like receptor agonists or antagonists on LTD of R6/1 mice hippocampal slices. There is no alteration on LTD properties presented by

manipulation of dopamine receptors. The results along with their previous finding provide systemic understanding of dopamine signaling and synaptic dysfunction in HD. Several concerns and suggestions are listed below:
1. What's the CAG repeat number in R6/1 mice? Did the authors check the repeat size occasionally since sometimes repeat size is quite not stable through generations in HD mice?
All the mice used in the study were genotyped as described in Vatsavayai et al. 2007 as part of a pedigree study (Vatsavayai et al. 2007). As shown in Figure R1, the primer set used in our genotyping enabled us to verify the repeat length of the transgene in tail samples collected from each animal prior to weaning. The PCR products representing approximately 116 repeats are in the region of 394bp (as shown in Figure R1 lane 1-4 and 6-9). For comparison, lane 5 (Figure R1) shows the PCR product of a different R6 line containing only 89 CAG repeats, here the size of the PCR product is 300bp. Stability of the repeat length across generations was maintained by breeding from male mice that had repeat length of 116.

2. Please replace the representative image of 1m, 3m HD mice in Fig 1 D1 receptor. The coronal level or presented region is not consistent with other pictures.
We understand that it may seem as if the sections used in Fig1 are from different coronal planes. This is however likely due to the orientation of the images acquired, notably with regard to the dendate gyrus. We were indeed careful to pick sections from -1.8 to -2.0 mm relative to bregma. In order to avoid such ambiguity, we re-centered images to only show the CA1 region in the correct orientation both in Fig 1 and Fig 2 and updated the captions accordingly.
3. Page 9, "Dopamine receptor expression increases in R6/1 transgenic mice" need specific to hippocampus since previously the authors found decreased levels of dopamine receptors in perirhinal cortex and other regions.
We agree and thank the reviewer for this suggestion. We have now replaced the title "Dopamine receptor expression increases in R6/1 transgenic mice" by "CA1 dopamine receptor expression increases in R6/1 transgenic mice" p9, l199.

4. Did the authors run western blot for dopamine D1 or D2- like receptors to confirm their finding? Especially in 7m HD hippocampus they found significant increase of D2-like receptor by immunostaining.
Western blots are indeed used to quantify protein expression but can lack sufficient spatial and cellular resolution, the latter is better addressed using fluorescence immunohistochemistry. In our investigation we aimed at assessing dopamine receptor expression of different regions of the CA1 area of the hippocampus. Western blots performed on hippocampal extracts is not the method of choice in our study as the changes we report would most likely be masked by heterogeneity from different hippocampal regions.
5. Evidences showed increased DA in early stage and reduced DA in late-stage HD patients and animal models. Manipulation DA receptor depends on the level of DA tone. Thus except DA receptors, determine DA level in hippocampus of R6/1 mice may provide more comprehensive information.
DA levels are indeed altered in HD patients and mice, and we have actually previously found that striatal release of this important neuromodulator is increased at early disease stages whilst it is markedly decreased in a late HD mouse model (Dallérac et al. 2015). We agree that studying DA release and tone in the hippocampus is relevant in light of the results we report here and of a recent study showing that dopamine content is reduced by ~30% in symptomatic R6/2 mice (Mochel et al. 2011). We thank the reviewer for the suggestion, this will however be addressed in a future investigation as it is beyond the scope of the current negative findings manuscript. In light of this sensible comment, we have nevertheless improved the discussion of our manuscript p13 l288:
" The significance of a large increase in dopamine receptor labelling is unclear, but it might reflect an up-regulation in dopamine receptor number in response to decreased dopaminergic innervation or signalling. Such a view is supported by a recent study reporting more than 30% decrease in hippocampal dopamine content in 12 weeks old symptomatic R6/2 mice (Mochel et al. 2011)."
References reviewer 1

Dallérac, G. M., Levasseur, G., Vatsavayai, S. C., Milnerwood, A. J., Cummings, D. M., Kraev, I., et al. (2015). Dysfunctional Dopaminergic Neurones in Mouse Models of

Huntington’s Disease: A Role for SK3 Channels. Neuro-degenerative diseases, 15(2), 93–108.

Mochel, F., Durant, B., Durr, A., & Schiffmann, R. (2011). Altered dopamine and serotonin metabolism in motorically asymptomatic R6/2 mice. PloS one, 6(3), e18336.

Vatsavayai, S. C., Dallérac, G. M., Milnerwood, A. J., Cummings, D. M., Rezaie, P., Murphy, K. P. S. J., & Hirst, M. C. (2007). Progressive CAG expansion in the brain of a novel R6 / 1-89Q mouse model of Huntington ’ s disease with delayed phenotypic onset. Brain Research Bulletin, 72, 98–102.

Reviewer #2: The manuscript by Dallerac et al. nicely demonstrates that in the R6/1 mouse model of Huntington's disease, aberrant LTD in the aged hippocampus is not due to alterations in dopamine detection. Specifically, the authors show that aberrant LTD is dissociated from pathologically elevated hippocampal expression of both D1 and D2 type receptors. This is important, as abnormal plasticity in the disease state is clearly linked to abnormal dopaminergic signaling in other brain regions, including the cortex. This dissociation helps shed light on one of the many potential limitations of dopamine-related therapies posited to be useful for HD. The study is from a well-established HD group, and should be of interest to researchers in the HD field. I only have a few minor comments.

1. For quantification of fluorescence: how was fluorescence intensity compared between different slices? Were wt and mutant slices processed and analyzed in parallel? Can you please clarify what is meant by "internally normalized" in the methods section?
Both transgenic and non-transgenic slices were indeed processed and analysed in parallel. Following the reviewer's advice we have now replaced the mention "internally normalized" by a more detailed description of the procedure p6 l140:
"Transgenic and non-transgenic slices were processed and analysed in parallel. Image stacks (6 m) of 12 sequential scans (0.5 m) were performed and collected for each section using Leica Confocal Software (Version 2.5, Leica, Heidelberg, Germany). Fluorescence was calculated by manually selecting the 3 brightest scans from each stack and generating a composite average. Fluorescence was quantified by generating a mean fluorescence value (in arbitrary units) from three manually placed non-overlapping sampling boxes (2000 µm2) in each region of interest (ROI) through the CA1 field of the hippocampus (capillaries were avoided). Fluorescence intensity was standardized between slices by imaging sections on the same day using the same laser and parameters; i.e. gain, offset and PMT intensity."

2. It may be useful to show where the recording electrode was for LTD experiments (fig 3), in relation to the immune data shown in figures 1-2. Perhaps a label in figure(s) 1 or 2.

This is a sensible suggestion and we have now inserted a schematic diagram showing placement of the electrodes in Figure 3. Figure caption has been amended accordingly (p21 l543).

3. Recently, evidence has been published that points to non-dopaminergic pathologies in HD that lead to impaired synaptic plasticity. For example, Surmeier's group recently showed that diminished TrkBR signaling in the striatum impairs LTP. It would be benSeficial to add references supporting the findings that non-dopaminergic impairments alter plasticity in HD models.

We agree with the reviewer and have now improved our manuscript by discussing non-dopaminergic alterations in synaptic plasticity, p12 l269:

" This indicates that although dopaminergic changes play an important role in HD, the etiology of the disease is more complex and involves multiple mechanisms. Focusing on synaptic plasticity, alteration in brain derived neurotrophic factor (BDNF) availability has for example been reported as an important modifier of synaptic efficacy (Lynch et al. 2007; Simmons et al. 2009; Zuccato et al. 2003). In this regard, two recent reports further indicate that in HD mice striatum (Plotkin et al. 2014) and hippocampus (Brito

et al. 2014), signalling downstream the BDNF tyrosine-related kinase B (TrkB) receptors and and p75 neurotrophin receptors (p75NTR) would also be deficient. Other identified molecular abnormalities underlying synaptic dysfunction in HD include NMDA receptor composition with an increased NR2B function (Li et al. 2004; Milnerwood et al. 2006; Zeron et al. 2002) and cell adhesion molecules such as PSA-NCAM (van der Borght and Brundin 2007). Finally, a recent report indicates that astroglial Kir4.1 channels are deficient in HD (Tong et al. 2014); these astroglial channels are involved in the regulation of synaptic function (Dallerac et al. 2013) and are therefore also likely to contribute to abnormal neurotransmission in HD. ".

References reviewer 2

Brito, V., Giralt, A., Enriquez-Barreto, L., Puigdellívol, M., Suelves, N., Zamora-Moratalla, A., et al. (2014). Neurotrophin receptor p75(NTR) mediates Huntington's disease-associated synaptic and memory dysfunction. The Journal of clinical investigation, 124(10), 4411–28.

Dallerac, G., Chever, O., & Rouach, N. (2013). How do astrocytes shape synaptic transmission? Insights from electrophysiology. Front Cell Neurosci, 7, 159. doi:10.3389/fncel.2013.00159

Li, L., Murphy, T. H., Hayden, M. R., & Raymond, L. A. (2004). Enhanced striatal NR2B-containing N-methyl-D-aspartate receptor-mediated synaptic currents in a mouse model of Huntington disease. J Neurophysiol, 92(5), 2738–2746.

Lynch, G., Kramar, E. A., Rex, C. S., Jia, Y., Chappas, D., Gall, C. M., & Simmons, D. A. (2007). Brain-derived neurotrophic factor restores synaptic plasticity in a knock-in mouse model of Huntington's disease. The Journal of neuroscience: the official journal of the Society for Neuroscience, 27(16), 4424–34.

Milnerwood, A. J., Cummings, D. M., Dallerac, G. M., Brown, J. Y., Vatsavayai, S. C., Hirst, M. C., et al. (2006). Early development of aberrant synaptic plasticity in a mouse model of Huntington's disease. Hum Mol Genet, 15(10), 1690–1703.

Plotkin, J. L., Day, M., Peterson, J. D., Xie, Z., Kress, G. J., Rafalovich, I., et al. (2014). Impaired TrkB receptor signaling underlies corticostriatal dysfunction in Huntington's disease. Neuron, 83(1), 178–88.
Simmons, D. A., Rex, C. S., Palmer, L., Pandyarajan, V., Fedulov, V., Gall, C. M., & Lynch, G. (2009). Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. Proceedings of the National Academy of Sciences of the United States of America, 106(12), 4906–11.

Tong, X., Ao, Y., Faas, G. C., Nwaobi, S. E., Xu, J., Haustein, M. D., et al. (2014). Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. Nature neuroscience, 17(5), 694–703.

van der Borght, K., & Brundin, P. (2007). Reduced expression of PSA-NCAM in the hippocampus and piriform cortex of the R6/1 and R6/2 mouse models of Huntington's disease. Experimental neurology, 204(1), 473–8.

Zeron, M. M., Hansson, O., Chen, N., Wellington, C. L., Leavitt, B. R., Brundin, P., et al. (2002). Increased Sensitivity to N-Methyl-D-Aspartate in a Mouse Model of Huntington ' s Disease, 33, 849–860.

Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., Conti, L., et al. (2003). Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. Nature Genetics, 35(1), 76–83.

Changes in dopamine signalling do not underlie aberrant hippocampal plasticity in a mouse model of Huntington's disease

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Abstract

Altered dopamine (DA) receptor labelling has been demonstrated in presymptomatic and symptomatic Huntington's disease (HD) gene carriers, indicating that alterations in dopaminergic signalling is an early event in HD. We have previously described early alterations in synaptic transmission and plasticity in both the cortex and hippocampus of the R6/1 mouse model of Huntington's disease. Deficits in cortical synaptic plasticity were associated with altered dopaminergic signalling and could be reversed by D1- or D2-like dopamine receptor activation. In light of these findings we here investigated whether defects in dopamine signalling could also contribute to the marked alteration in hippocampal synaptic function. To this end we performed dopamine receptor labelling and pharmacology in the R6/1 hippocampus and report a marked, age-dependent elevation of hippocampal D1 and D2 receptor labelling in R6/1 hippocampal subfields. Yet, pharmacological inhibition or activation of D1- or D2-like receptors did not modify the aberrant synaptic plasticity observed in R6/1 mice. These findings demonstrate that global perturbations to dopamine receptor expression do occur in HD transgenic mice, similarly in HD gene carriers and patients. However, the direction of change and the lack of effect of dopaminergic pharmacological agents on synaptic function demonstrates that the perturbations are heterogeneous and region-specific, a finding that may explain the mixed results of dopamine therapy in HD.

Introduction

Huntington's disease (HD) is a late-onset and fatal neurological disorder caused by the repetition of a CAG repeat codon in the first exon of the gene that codes for the protein huntingtin. This translates into a protein with an expanded polyglutamine repeat that confers a toxic gain of function, which induces neurodegenerative changes and neuronal cell death. A number of studies, including ours (Cummings et al. 2006; Dallérac et al. 2011; Dallérac et al. 2015; Milnerwood et al. 2006; Murphy et al. 2000), have demonstrated that neuronal dysfunction occurs prior to neurodegeneration. In particular, the loss of neuromodulatory receptors for dopamine, adenosine, and cannabinoids has been described in post-mortem human tissues (Glass et al. 2000), in prodomal and overt HD patients (Andrews et al. 1999; Antonini et al. 1998; Ginovart et al. 1997; Weeks 1997), as well as in several HD mouse models (André et al. 2010).

Dopaminergic signalling is involved in both cognition and the control of movement (Korchounov et al. 2010; Shohamy and Adcock 2010; Smith and Villalba 2008), processes that are affected in HD, though the etiology is poorly understood. Many studies have demonstrated progressive loss of D1 and D2 dopamine receptor in striatal medium spiny neurones and cortical areas of symptomatic patients as well as asymptomatic HD gene carriers (André et al. 2010) demonstrating that striatal and cortical changes in the dopaminergic system are detected before clinical diagnosis and prior to gross neuropathological changes. Such findings support the notion that the early

cognitive and emotional disturbances seen in HD gene carriers occur as a consequence of cellular dysfunction, rather than neuronal loss.

We have previously found that altered cortical plasticity in prodomal and symptomatic HD mouse models is attributable to dopaminergic dysfunction in the perirhinal as well as prefrontal areas, brain regions that are highly sensitive to dopaminergic neuromodulation (Cummings et al. 2006; Dallérac et al. 2011). Others have shown that long-term potentiation (LTP) is affected in the striatum of HD mice, a form of plasticity that is also modulated by dopamine (Kung et al. 2007). Strikingly, the impairment of perirhinal long-term depression (LTD) in R6/1 mice could be reversed by the administration of a D2R agonist (Cummings et al. 2006) whilst prefrontal long-term potentiation (LTP) was fully rescued by administration of a D1R agonist; suggesting that dopaminergic tone is altered in HD (Dallérac et al. 2011). Recent findings support further the view that dopaminergic modulation is abnormal in HD (Dallerac et al 2015). Dopaminergic neuronal excitability was shown to be abnormally high in HD mice; importantly, evoked dopamine release from dopaminergic neurones was increased in the prodomal state and markedly decreased in symptomatic HD mouse models (Dallérac et al. 2015).

Cognition is altered in HD patients (Harper 1996) and the hippocampus plays a central role in memory formation (Colgin et al. 2008). A number of investigations have reported that hippocampal-dependent cognitive functions are modulated by midbrain dopaminergic inputs (González-Burgos and Feria-Velasco 2008; Hansen and Manahan-Vaughan 2014; Jay 2003). We and others

have previously described markedly altered hippocampal synaptic plasticity in several HD mouse models (Hodgson et al. 1999; Milnerwood et al. 2006; Murphy et al. 2000; Usdin et al. 1999). In R6/1 and R6/2 mice this is manifest as impaired LTP and aberrant LTD (Milnerwood et al. 2006; Murphy et al. 2000). In light of the finding that alterations in cortical synaptic plasticity are highly sensitive to dopaminergic modulation in HD mice (André et al. 2010; Cepeda et al. 2014; Cummings et al. 2006; Dallérac et al. 2011; Dallérac et al. 2015), we hypothesized that abnormal dopaminergic signalling might also underlie the changes in synaptic plasticity seen in the hippocampus of HD mice. Therefore, using immunohistochemistry and electrophysiology, we have assessed the expression and regulatory functions of D1 and D2 receptors in the hippocampus of R6/1 mice.

Materials & Methods

Mice

Hemizygotic R6/1 males (Mangiarini et al. 1996), were mated with CBAxC57BL/6 females, resulting in ~50% of the offspring being hemizygotic for the R6/1 transgene. At weaning (3 weeks), all mice were given identity marks and tail-tip samples were taken for genotyping by PCR (Mangiarini et al. 1996). R6/1 and aged-matched non-transgenic littermates (WT) mice were killed by cervical dislocation and immediate decapitation in accordance with UK legislation (Animal (Scientific Procedures) Act 1986).

Immunohistochemistry

Brains were rapidly removed and 400µm coronal slices were prepared on a vibrating microtome (Campden Instruments Inc. USA). Slices were fixed in 4% paraformaldehyde (PFA, Sigma-Aldrich, UK) then 2% PFA overnight and transferred to 0.1M phosphate buffered saline (PBS pH 7.4) and stored at 4ºC. Slices were temporarily mounted in 5% agar and re-sectioned to 50µm on a vibrating microtome (VT1000S; Leica, Milton Keynes, UK) washed in PBS, blocked/permeabilized (2% Fish gelatine; 0.01% sodium azide; 0.1% TritonX-100 in PBS) for 2 h, and peroxidase quenched (3% H_2O_2 30 min). Subsequently, sections were incubated with the relevant primary antibody (AB1765P, rabbit polyclonal anti-dopamine D1A receptor or AB5840P rabbit polyclonal anti-dopamine D2 receptor; 1:1600 dilution of 1 mg/ml stock, Chemicon International Inc., UK) made up in 2% blocking solution for 48 h. Next, sections were rinsed (PBS) prior to O/N incubation with peroxidase-conjugated anti-rabbit antibody (tyramide signal amplification kit, Molecular Probes Inc., USA). Sections were incubated in a 1:50 dilution of the amplification reagent and 0.0015% H_2O_2 for 5 h, rinsed in PBS (3x15 min), coverslipped with fluorescence mounting medium, and left to dry for 48–62h. Consecutive slices were visualized on an inverted confocal microscope (Leica DM IBRE scanning confocal microscope, Leica Microsystems, Heidelberg, Germany) under 568 nm excitation (PMT 907) with the TRIT-C channel optimized for emission at 576 nm. Image stacks (6 mm) of 12 sequential scans (0.5 mm) were collated for each section using Leica Confocal Software (Version 2.5, Leica). **Transgenic and non-transgenic slices were processed and analysed in parallel. Image stacks (6 µm) of 12**

sequential scans (0.5 µm) were performed and collected for each section using Leica Confocal Software (Version 2.5, Leica, Heidelberg, Germany). Fluorescence was calculated by manually selecting the 3 brightest scans from each stack and generating a composite average. Fluorescence was quantified by generating a mean fluorescence value (in arbitrary units) from three manually placed non-overlapping sampling boxes (2000 µm^2) in each region of interest (ROI) through the CA1 field of the hippocampus (capillaries were avoided). Fluorescence intensity was standardized between slices by imaging sections on the same day using the same laser and parameters; i.e. gain, offset and PMT intensity. A minimum of three consecutive sections (3 measurements were collected per slice, and slice values collapsed to an animal mean) was used per animal (WT, R6/1 *n* = 3 animals) and age (1, 3 and 7 months; three animals per genotype per time point). Negative control sections were included where the primary antibody was omitted. Antibody specificity was further confirmed on sections of the brain from mice deficient in D2 dopamine receptors (Kelly et al. 1997) that were a gift from Professor Michael Levine (Intellectual and Developmental Disabilities Research Center, UCLA, USA). Sections prepared from D2 knock-out brains were processed for D2 immunoreactivity together with control and R6/1 tissue. No immunoreactivity was observed in the D2 knock-out material or negative controls

Electrophysiology

Transverse hippocampal slices (400 µm) were prepared as previously reported (Milnerwood et al. 2006), area CA3 was excised and slices were

transferred to an interface recording chamber (Scientific Systems Design Inc., USA) maintained at 28°C and constantly perfused with oxygenated (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF; containing in mM: 120 NaCl, 3 KCl, 2 $MgSO_4$, 2 $CaCl_2$, 1.2 NaH_2PO_4, 23 $NaHCO_3$, 11 glucose) and left to incubate for a minimum of 1.5 h prior to experimentation. Hippocampal CA1 field potentials were evoked by constant current stimuli (40 μs) applied via monopolar stimulating electrodes (impedance 5 MΩ; AM Systems, USA) to CA3 Schaffer-collateral commissural projections. Field potentials were recorded via extracellular glass microelectrodes (impedance 5-8 MΩ, filled with 1 M NaCl and 2% pontamine blue) placed in the stratum radiatum of CA1 using either a Neurolog AC-preamp or Axoclamp 2B amplifier (Digitimer, UK; Axon Instruments Inc., USA, respectively). Low frequency stimulation (LFS) consisted of 900 shocks at 1 Hz. For the purposes of assessing the probability of the induction of LTD it was defined as a stable reduction (>10%) of the fEPSP slope 1 h post-conditioning. The fEPSP initial linear slope set at a fixed latency (software: A/Dvance 3.6) was used as an index of synaptic efficacy. Data are presented as mean±SEM (*n* = slice/experiment) and statistical analysis performed by one-way ANOVA. Stimulus intensity was set to produce a response just below the threshold for population spike activity detected in the fEPSP, and evoked at 0.033 Hz for at least 20 min, to ensure a stable baseline prior to conditioning. All drugs (purchased from Tocris Bioscience, UK and Sigma-Aldrich Company Ltd.) were diluted in ACSF and perfused into the recording chamber for a minimum of 20 minutes prior to experimentation. The D2 dopamine receptor agonist

quinpirole (10µM, Cummings et al., 2006; Dallérac et al., 2015), the D2 dopamine receptor antagonist remoxipride (10µM, Cummings et al. 2006), the D1 dopamine receptor antagonist SCH 23390 (10µM, Huang et al. 2004) and the D1 dopamine receptor partial agonist SKF 38393 (10µM, Dallérac et al. 2011) were used to investigate dopamine receptor activity.

Statistical analyses

Data for each condition were pooled and are expressed as mean±SEM. One - or Two-way ANOVA were performed using Statistica 6.1 (StatSoft Inc.). Fisher LSD test was used for post-hoc analysis.

Results

CA1 dopamine receptor expression increases in R6/1 transgenic mice

In order to investigate the potential role of altered dopaminergic signalling in the R6/1 hippocampus, immunohistochemical investigation of the distribution of both D1 and D2 dopamine receptors was conducted. Representative confocal micrographs are shown in figures 1 & 2 for D1 and D2 receptor labelling respectively. Regions of interest (ROIs: white matter, WM; stratum oriens, SO; stratum pyramidale, SP; stratum radiatum proximal to SP, SRp; stratum radiatum distal from SP, SRd; molecular layer, ML) were sampled for fluorescence quantification.

Two-way ANOVA demonstrated significant effect of age and genotype upon D1 receptor labelling ($p<0.00001$, $F_{2,226}=18.4$), relative to WT. At 1 month of age there was a trend towards less D1 receptor labelling in all ROIs in R6/1 sections

(figure 1). D1 labelling was significantly lower in the SP (42.2%, $p<0.03$) and SRp (36.9%, $p<0.04$). By 3 months D1 labelling had increased relative to WT sections and significantly greater fluorescence was observed in the stratum radiatum (SRp, 62.9%, $p<0.03$ & SRd, 75.9%, $p<0.03$), suggesting that D1 receptor numbers are altered specifically in the R6/1 stratum radiatum. In the 7-month age group, D1 labelling also appeared to be increased, although this did not reach significance.

Significant effects of age and genotype were also observed in D2 receptor labelling by ANOVA ($p<0.00001$, $F_{2,220}=22.9$). As detailed in figure 2, no significant differences between R6/1 and WT sections were observed at 1 month of age. At 3 months D2 labelling was significantly increased in the WM (32.9%, $p<0.02$), SR (SRp, 49.6%, $p<0.01$ & SRd, 63.9%, $p<0.001$) and SLM (47.4%, $p<0.01$). There was no significant difference between the degree of labelling in WT and R6/1 SP ($p=0.4$) or SO, although the latter approached significance ($p=0.06$). At seven months of age there was a highly significant increase in D2 labelling in the WM (99.7%, $p<0.001$), SO (93.7%, $p<0.001$), SR (SRp, 83.1%, $p<0.001$ & SRd, 141.4%, $p<0.001$) and SLM (86.3%, $p<0.001$) relative to WT sections. The data suggest that D2 receptor numbers are greatly altered in the R6/1 CA1 field at three months and older. Taken together, these observations suggest that large alterations in D1 and D2 receptor expression occur in the R6/1 mouse hippocampus (albeit later for D2) compared to WT littermates, and furthermore that these differences occur months prior to the onset of the overt motor phenotype.

Dopamine signalling does not underlie aberrant synaptic function

Pharmacological manipulation of D1 and D2 receptors was employed to investigate whether altered dopaminergic transmission could account for the aberrant LTD observed in adult R6/1 mice (Milnerwood et al. 2006), which is normally down-regulated by 1 month in wild type control mice (Milner et al. 2004). As shown in figure 3, neither D1 nor D2 receptor agonists nor antagonists (all delivered at 10μM) altered the likelihood or magnitude of LTD induced by LFS in slices prepared from R6/1 mice aged 7-8 months. Indeed, as we reported previously (Milnerwood et al. 2006), in aged-matched untreated R6/1 slices, LFS induced significant LTD (–12.1 ± 1.4%, n=41, $p<0.000001$). In the presence of the D1 receptor antagonist SCH 23390 (23), LTD was also induced (–9.3 ± 3.8%, n=8, $p<0.04$) in 63% of experiments. Similarly, LTD was induced (–14.5 ± 2.2%, n=7, $p<0.001$) in the presence of the D2 receptor agonist quinpirole (Cummings et al. 2006) in 86% of experiments. The presence of the D1 receptor partial agonist SKF 38393 (Dallérac et al. 2011) did not alter LTD either as it was found to be induced (–14.0 ± 1.4%, n=11, $p<0.00005$) in 82% of experiments. Finally, the proportion of LTD induction (–12.4 ± 1.9%, n=5, $p<0.02$) in the presence of the D2 receptor antagonist remoxipride (Cummings et al. 2006) reached an equally comparable 80%. There were no significant differences in the mean LTD produced between activation and inhibition of either D1 ($p>0.2$) or D2 receptors ($p>0.3$), and none of the four drug conditions produced LTD that was significantly different from that seen in age-matched untreated R6/1 slices. Therefore the data

suggest that, despite alterations to dopamine receptor expression, the mechanisms responsible for the induction of LTD in adult R6/1 mice is unperturbed by modulation of dopaminergic neurotransmission.

Discussion

Neither agonism nor antagonism of D1 or D2 dopamine receptors significantly altered LTD in R6/1 hippocampal slices (figure 3). This result is in stark contrast with the full rescue of LTP in the R6/1 prefrontal cortex by D1 receptor activation as well as restoration of LTD in the R6/1 perirhinal cortex by D2 agonist applied at similar concentrations (Cummings et al. 2006; Dallérac et al. 2011). The lack of effect upon hippocampal LTD is not attributable to a loss of dopamine receptors as we find an increase rather than a decrease in immunostaining for these receptors in R6/1 CA1 fields, with respect to wild type controls. **This indicates that although dopaminergic changes play an important role in HD, the etiology of the disease is more complex and involves multiple mechanisms. Focusing on synaptic plasticity, alteration in brain derived neurotrophic factor (BDNF) availability has for example been reported as an important modifier of synaptic efficacy (Lynch et al. 2007; Simmons et al. 2009; Zuccato et al. 2003). In this regard, two recent reports further indicate that in HD mice striatum (Plotkin et al. 2014) and hippocampus (Brito et al. 2014), signalling downstream the BDNF tyrosine-related kinase B (TrkB) receptors and and p75 neurotrophin receptors (p75NTR) would also be deficient. Other identified molecular abnormalities underlying**

synaptic dysfunction in HD include NMDA receptor composition with an increased NR2B function (Li et al. 2004; Milnerwood et al. 2006; Zeron et al. 2002) and cell adhesion molecules such as PSA-NCAM (van der Borght and Brundin 2007). Finally, a recent report indicates that astroglial Kir4.1 channels are deficient in HD (Tong et al. 2014); these astroglial channels are involved in the regulation of synaptic function (Dallerac et al. 2013) and are therefore also likely to contribute to abnormal neurotransmission in HD.

The significance of a large increase in dopamine receptor labelling is unclear, but it might reflect an up-regulation in dopamine receptor number in response to decreased dopaminergic innervation. **Such a view is supported by a recent study reporting more than 30% decrease in hippocampal dopamine content in 12 weeks old symptomatic R6/2 mice (Mochel et al. 2011).** Another possibility is that the dopamine receptors are dysfunctional, thus leading to a compensatory increase in their expression levels. DA release has been found to be severely reduced in both R6/1 and R6/2 HD mice (Dallérac et al. 2015; Johnson et al. 2006; Ortiz et al. 2011). Chemical enervation and depletion of the dopaminergic system in rats, by chronic treatment with 6-hydroxydopamaine, results in behavioural hyperactivity in the case of limited destruction and hypoactivity with larger lesions (Koob et al. 1981), reminiscent of the behaviour of R6/1 mice as they age (Bolivar et al. 2004). This treatment causes a priming effect in intact rats; subsequent application of D1 and D2 agonists results in greatly exaggerated behavioural responses (e.g., explosive jumping) in

303 comparison to the same agonism of non-treated animals (LaHoste and Marshall
304 1989). This priming effect is correlated with large increases in D2 receptor
305 labelling (LaHoste and Marshall 1989; Savasta et al. 1992) and mRNA levels
306 (Chritin et al. 1992). The lack of any observed effect of D1 and D2 agonism and
307 antagonism suggests that although there is an increase in number, the
308 localisation, activity or downstream cascades resulting from DA receptor
309 activation are either non-functional or severely impaired.

310 Interestingly, changes were not uniform for D1 and D2 labelling throughout
311 hippocampal subfields, results reminiscent of the changes in dopamine receptors
312 expression during ageing (Amenta et al. 2001). There is also an important
313 heterogeneity between brain regions as reduction were seen in the cortex and
314 striatum of various mouse models of HD including R6/1 and R6/2 mice (Ariano et
315 al. 2002; Cummings et al. 2006; Heng et al. 2007) whereas we observe an
316 augmentation in the hippocampus. We thus propose that dynamic modulations of
317 dopamine receptors occur as a function of the changes in dopamine
318 bioavailability (Dallérac et al. 2015) that results from transgene expression.

319 Dopamine therapy has long been used in the palliative treatment of HD with
320 limited success (van Vugt and Roos 1999); likely because of the diverse actions
321 of dopaminergic signalling in the brain. Our previous reports (Cummings et al.
322 2006; Dallérac et al. 2011; Dallérac et al. 2015) together with the data presented
323 here demonstrate that pharmacological manipulations may have very different
324 effects depending on the brain region in which they are active. The results of this

study add weight to the suggestion that targeted dopamine therapy might better alleviate symptoms in HD.

Disclosure/Conflict of interest

None

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References

Amenta, F., Mignini, F., Ricci, A., Sabbatini, M., Tomassoni, D., & Tayebati, S. K. (2001). Age-related changes of dopamine receptors in the rat hippocampus: a light microscope autoradiography study. *Mechanisms of ageing and development*, *122*(16), 2071–83.

André, V. M., Cepeda, C., & Levine, M. S. (2010). Dopamine and glutamate in Huntington's disease: A balancing act. *CNS neuroscience & therapeutics*, *16*(3), 163–78.

Andrews, T. C., Weeks, R. A., Turjanski, N., Gunn, R. N., Watkins, L. H., Sahakian, B., et al. (1999). Huntington's disease progression: PET and clinical observations. *Brain*, *122*(12), 2353–2363.

352 Antonini, A., Leenders, K. L., & Eidelberg, D. (1998). [11C]raclopride-PET studies
353 of the Huntington's disease rate of progression: relevance of the
354 trinucleotide repeat length. *Ann Neurol*, *43*(2), 253–255.

355 Ariano, M. A., Aronin, N., Difiglia, M., Tagle, D. A., Sibley, D. R., Leavitt, B. R., et
356 al. (2002). Striatal neurochemical changes in transgenic models of
357 Huntington's disease. *Journal of neuroscience research*, *68*(6), 716–29.

358 Bolivar, V. J., Manley, K., & Messer, A. (2004). Early exploratory behavior
359 abnormalities in R6/1 Huntington's disease transgenic mice. *Brain Res*,
360 *1005*(1-2), 29–35.

361 Brito, V., Giralt, A., Enriquez-Barreto, L., Puigdellívol, M., Suelves, N., Zamora-
362 Moratalla, A., et al. (2014). Neurotrophin receptor p75(NTR) mediates
363 Huntington's disease-associated synaptic and memory dysfunction. *The*
364 *Journal of clinical investigation*, *124*(10), 4411–28.

365 Cepeda, C., Murphy, K. P. S., Parent, M., & Levine, M. S. (2014). The role of
366 dopamine in Huntington's disease. *Progress in brain research*, *211*, 235–54.

367 Chritin, M., Savasta, M., Mennicken, F., Bal, A., Abrous, D. N., Le Moal, M., et al.
368 (1992). Intrastriatal Dopamine-rich Implants Reverse the Increase of
369 Dopamine D2 Receptor mRNA Levels Caused by Lesion of the Nigrostriatal
370 Pathway: A Quantitative In Situ Hybridization Study. *The European journal of*
371 *neuroscience*, *4*(7), 663–672.

372 Colgin, L. L., Moser, E. I., & Moser, M.-B. (2008). Understanding memory
373 through hippocampal remapping. *Trends in neurosciences*, *31*(9), 469–77.

374 Cummings, D. M., Milnerwood, A. J., Dallérac, G. M., Waights, V., Brown, J. Y.,
375 Vatsavayai, S. C., et al. (2006). Aberrant cortical synaptic plasticity and
376 dopaminergic dysfunction in a mouse model of Huntington's disease. *Human*
377 *molecular genetics*, 15(19), 2856–68.
378
379 Dallerac, G., Chever, O., & Rouach, N. (2013). How do astrocytes shape
380 synaptic transmission? Insights from electrophysiology. *Front Cell Neurosci*,
381 *7*, 159.

382 Dallérac, G. M., Levasseur, G., Vatsavayai, S. C., Milnerwood, A. J., Cummings,
383 D. M., Kraev, I., et al. (2015). Dysfunctional Dopaminergic Neurones in
384 Mouse Models of Huntington's Disease: A Role for SK3 Channels. *Neuro-*
385 *degenerative diseases*, *15*(2), 93–108.

386 Dallérac, G. M., Vatsavayai, S. C., Cummings, D. M., Milnerwood, A. J., Peddie,
387 C. J., Evans, K. A., et al. (2011). Impaired Long-Term Potentiation in the

388 Prefrontal Cortex of Huntington's Disease Mouse Models: Rescue by D(1)
389 Dopamine Receptor Activation. *Neurodegenerative Diseases*, *8*(4), 230–9.

390 Ginovart, N., Lundin, A., Farde, L., Halldin, C., Bäckman, L., Swahn, C. G., et al.
391 (1997). PET study of the pre- and post-synaptic dopaminergic markers for
392 the neurodegenerative process in Huntington's disease. *Brain*, *120*(3), 503–
393 514.

394 Glass, M., Dragunow, M., & Faull, R. L. M. L. (2000). The pattern of
395 neurodegeneration in Huntington's disease: a comparative study of
396 cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the
397 human basal ganglia in Huntington's disease. *Neuroscience*, *97*(3), 505–
398 519.

399 González-Burgos, I., & Feria-Velasco, A. (2008). Serotonin/dopamine interaction
400 in memory formation. *Progress in brain research*, *172*, 603–23.

401 Hansen, N., & Manahan-Vaughan, D. (2014). Dopamine D1/D5 receptors
402 mediate informational saliency that promotes persistent hippocampal long-
403 term plasticity. *Cerebral cortex (New York, N.Y. : 1991)*, *24*(4), 845–58.

404 Harper, P. (1996). Huntington's Disease. In W. B. Saunders (Ed.), *Major
405 Problems of Neurology. 2nd ed. Philadelphia, Pa: WB Saunders; 1996.* PA,
406 USA.

407 Heng, M. Y., Tallaksen-Greene, S. J., Detloff, P. J., & Albin, R. L. (2007).
408 Longitudinal evaluation of the Hdh(CAG)150 knock-in murine model of
409 Huntington's disease. *The Journal of neuroscience : the official journal of the
410 Society for Neuroscience*, *27*(34), 8989–98.

411 Hodgson, J. G. G., Agopyan, N., Gutekunst, C.-A. A., Leavitt, B. R., LePiane, F.,
412 Singaraja, R., et al. (1999). A YAC mouse model for Huntington's disease
413 with full-length mutant huntingtin, cytoplasmic toxicity, and selective striatal
414 neurodegeneration. *Neuron*, *23*(1), 181–192.

415 Huang, Y.-Y. Y., Simpson, E., Kellendonk, C., & Kandel, E. R. (2004). Genetic
416 evidence for the bidirectional modulation of synaptic plasticity in the
417 prefrontal cortex by D1 receptors. *Proc Natl Acad Sci U S A*, *101*(9), 3236–
418 3241.

419 Jay, T. M. (2003). Dopamine: a potential substrate for synaptic plasticity and
420 memory mechanisms. *Progress in neurobiology*, *69*(6), 375–90.

421 Johnson, M. A., Rajan, V., Miller, C. E., & Wightman, R. M. (2006). Dopamine
422 release is severely compromised in the R6/2 mouse model of Huntington's
423 disease. *Journal of neurochemistry*, *97*(3), 737–46.

Kelly, M. A., Rubinstein, M., Asa, S. L., Zhang, G., Saez, C., Bunzow, J. R., et al. (1997). Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. *Neuron*, *19*(1), 103–13.

Koob, G. F., Stinus, L., & Le Moal, M. (1981). Hyperactivity and hypoactivity produced by lesions to the mesolimbic dopamine system. *Behavioural brain research*, *3*(3), 341–59.

Korchounov, A., Meyer, M. F., & Krasnianski, M. (2010). Postsynaptic nigrostriatal dopamine receptors and their role in movement regulation. *Journal of neural transmission (Vienna, Austria : 1996)*, *117*(12), 1359–69.

Kung, V. W. S., Hassam, R., Morton, A. J., & Jones, S. (2007). Dopamine-dependent long term potentiation in the dorsal striatum is reduced in the R6/2 mouse model of Huntington's disease. *Neuroscience*, *146*(4), 1571–80.

LaHoste, G. J., & Marshall, J. F. (1989). Non-additivity of D2 receptor proliferation induced by dopamine denervation and chronic selective antagonist administration: evidence from quantitative autoradiography indicates a single mechanism of action. *Brain research*, *502*(2), 223–32.

Li, L., Murphy, T. H., Hayden, M. R., & Raymond, L. A. (2004). Enhanced striatal NR2B-containing N-methyl-D-aspartate receptor-mediated synaptic currents in a mouse model of Huntington disease. *J Neurophysiol*, *92*(5), 2738–2746.

Lynch, G., Kramar, E. A., Rex, C. S., Jia, Y., Chappas, D., Gall, C. M., & Simmons, D. A. (2007). Brain-derived neurotrophic factor restores synaptic plasticity in a knock-in mouse model of Huntington's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *27*(16), 4424–34.

Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., et al. (1996). Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. *Cell*, *87*(3), 493–506.

Milner, A. J., Cummings, D. M., Spencer, J. P., & Murphy, K. P. S. J. (2004). Bi-directional plasticity and age-dependent long-term depression at mouse CA3-CA1 hippocampal synapses. *Neurosci Lett*, *367*(1), 1–5.

Milnerwood, A. J., Cummings, D. M., Dallerac, G. M., Brown, J. Y., Vatsavayai, S. C., Hirst, M. C., et al. (2006). Early development of aberrant synaptic plasticity in a mouse model of Huntington's disease. *Hum Mol Genet*, *15*(10), 1690–1703.

Mochel, F., Durant, B., Durr, A., & Schiffmann, R. (2011). Altered dopamine and serotonin metabolism in motorically asymptomatic R6/2 mice. *PloS one*, *6*(3), e18336.

Murphy, K. P. S. J., Carter, R. J., Lione, L. A., Mangiarini, L., Mahal, A., Bates, G. P., et al. (2000). Abnormal synaptic plasticity and impaired spatial cognition in mice transgenic for exon 1 of the human Huntington's disease mutation. *J Neurosci*, *20*(13), 5115–5123.

Ortiz, A. N., Kurth, B. J., Osterhaus, G. L., & Johnson, M. a. (2011). Impaired dopamine release and uptake in R6/1 Huntington's disease model mice. *Neuroscience letters*, *492*(1), 11–4.

Plotkin, J. L., Day, M., Peterson, J. D., Xie, Z., Kress, G. J., Rafalovich, I., et al. (2014). Impaired TrkB receptor signaling underlies corticostriatal dysfunction in Huntington's disease. *Neuron*, *83*(1), 178–88.

Savasta, M., Mennicken, F., Chritin, M., Abrous, D. N., Feuerstein, C., Le Moal, M., & Herman, J. P. (1992). Intrastriatal dopamine-rich implants reverse the changes in dopamine D2 receptor densities caused by 6-hydroxydopamine lesion of the nigrostriatal pathway in rats: an autoradiographic study. *Neuroscience*, *46*(3), 729–38.

Shohamy, D., & Adcock, R. A. (2010). Dopamine and adaptive memory. *Trends in cognitive sciences*, *14*(10), 464–72.

Simmons, D. A., Rex, C. S., Palmer, L., Pandyarajan, V., Fedulov, V., Gall, C. M., & Lynch, G. (2009). Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(12), 4906–11.

Smith, Y., & Villalba, R. (2008). Striatal and extrastriatal dopamine in the basal ganglia: an overview of its anatomical organization in normal and Parkinsonian brains. *Movement disorders : official journal of the Movement Disorder Society*, *23 Suppl 3*, S534–47.

Tong, X., Ao, Y., Faas, G. C., Nwaobi, S. E., Xu, J., Haustein, M. D., et al. (2014). Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. *Nature neuroscience*, *17*(5), 694–703.

Usdin, M. T., Shelbourne, P. F., Myers, R. M., & Madison, D. V. (1999). Impaired synaptic plasticity in mice carrying the Huntington's disease mutation. *Hum Mol Genet*, *8*(5), 839–846.

van der Borght, K., & Brundin, P. (2007). Reduced expression of PSA-NCAM in the hippocampus and piriform cortex of the R6/1 and R6/2 mouse models of Huntington's disease. *Experimental neurology*, *204*(1), 473–8.

Van Vugt, J., & Roos, R. (1999). Huntington's Disease: Options for Controlling Symptoms. *CNS Drugs*, *11*(2), 105–123.

Weeks, R. (1997). Cortical control of movement in Huntington's disease. A PET activation study. *Brain*, *120*(9), 1569–1578.

Zeron, M. M., Hansson, O., Chen, N., Wellington, C. L., Leavitt, B. R., Brundin, P., et al. (2002). Increased Sensitivity to N-Methyl-D-Aspartate in a Mouse Model of Huntington ' s Disease, *33*, 849–860.

Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., Conti, L., et al. (2003). Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nature Genetics*, *35*(1), 76–83.

Figure captions

Figure 1. Hippocampal CA1 D1 receptor labelling.

Representative confocal micrographs (x40 objective) of D1 immunofluorescence in the **CA1 area of the** hippocampus of WT (left) and R6/1 (right) mice age as indicated (months). Regions of interest are marked for reference (top left): WM, white matter; SO stratum oriens; SP, stratum pyramidale; SRp/d, stratum radiatum proximal/distal to SP; SLM, stratum lanculosum-moleculare; hf, hippocampal fissure; dg, dentate gyrus. Bar = 100 µm. Quantification of D1 receptor immunofluorescence is also shown. R6/1 (n=8(3)) sections had significantly less D1 receptor labelling than WT sections (n=9(3)) in the SRp (∗p<0.03) and SP (∗p<0.04) at 1 month. At 3 months D1 receptor labelling was significantly increased in the R6/1 stratum radiatum (∗p<0.03. R6/1, n=9(3). WT,

n=9(3)). R6/1 labelling was not significantly different from WT at 7 months (∗p>0.1. R6/1, n=5(2). WT, n=5(3)).

Figure 2. Hippocampal CA1 D2 receptor labelling.

Representative confocal micrographs (x40) of D2 immunofluorescence in the **CA1 area of the** hippocampus of WT (left) and R6/1 (right) mice from the ages indicated. Regions of interest are marked for reference in the top left panel: WM, white matter; SO stratum oriens; SP, stratum pyramidale; SRp/d, stratum radiatum proximal/distal to SP; SLM, stratum lanculosum-moleculare; hf, hippocampal fissure; dg, dentate gyrus. Bar = 100 µm. Quantification of D2 immunofluorescence is also shown. R6/1 (n=8(3)) and WT (n=6(2)) D2 receptor labelling is similar at one month. At 3 months D2 receptor labelling is significantly increased (with respect to WT) in the R6/1 stratum radiatum and WM. At seven months a highly significant increase in R6/1 D2 labelling was observed in all ROIs except the SP (R6/1, n=8(3). WT, n=6(2), ∗p<0.05, ∗∗p<0.01, ∗∗∗p<0.001).

Figure 3. LTD in R6/1 adults is not blocked by pharmacological manipulation of dopamine receptors.

Neither D1 nor D2 receptor agonists nor antagonists (10µM) significantly altered the magnitude (**A, B, C, D, E**) or probability (**F**) of LTD induction in slices prepared from R6/1 mice at 8 months of age. **Insert in (A) shows the**

stimulating and recording electrode placement. Double arrows represents cutting of the CA3 area for which the excised part is depicted in grey.

Dear Dr Mattson,

We thank you and the reviewers for examining our work and for the positive comments it received. Please find below our point-by-point answer to reviewers' comments.

Best regards

Kerry Murphy & Glenn Dallérac.

Reviewer #1: In this manuscript, the authors detected expression of dopamine receptors by immunostaining in HD mice and they found the levels of D1 and D2-like receptors were increased along with age in R6/1 HD hippocampus. Further they tested the effect of D1 or D2-like receptor agonists or antagonists on LTD of R6/1 mice hippocampal slices. There is no alteration on LTD properties presented by manipulation of dopamine receptors. The results along with their previous finding provide systemic understanding of dopamine signaling and synaptic dysfunction in HD.

Several concerns and suggestions are listed below:

1. What's the CAG repeat number in R6/1 mice? Did the authors check the repeat size occasionally since sometimes repeat size is quite not stable through generations in HD mice?

All the mice used in the study were genotyped as described in *Vatsavayai et al. 2007* as part of a pedigree study (Vatsavayai et al. 2007). As shown in Figure R1, the primer set used in our genotyping enabled us to verify the repeat length of the transgene in tail samples collected from each animal prior to weaning. The PCR products representing approximately 116 repeats are in the region of 394bp (as shown in Figure R1 lane 1-4 and 6-9). For comparison, lane 5 (Figure R1) shows the PCR product of a different R6 line containing only 89 CAG repeats, here the size of the PCR product is 300bp. Stability of the repeat length across generations was maintained by breeding from male mice that had repeat length of 116.

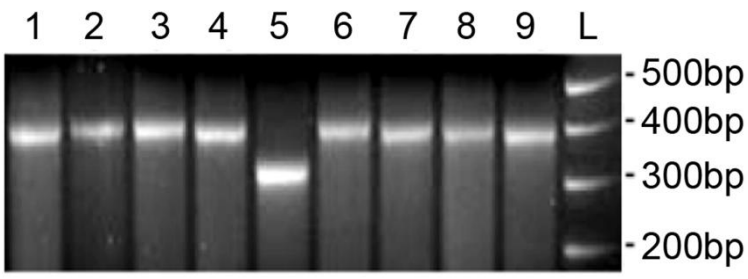

Figure R1. Example of PCR products enabling verification of the transgene CAG repeat length.

2. Please replace the representative image of 1m, 3m HD mice in Fig 1 D1 receptor. The coronal level or presented region is not consistent with other pictures.

We understand that it may seem as if the sections used in Fig1 are from different coronal planes. This is however likely due to the orientation of the images acquired, notably with regard to the dendate gyrus. We were indeed careful to pick sections from -1.8 to -2.0 mm relative to bregma. In order to avoid such ambiguity, we re-centered images to only show the CA1 region in the correct orientation both in Fig 1 and Fig 2 and updated the captions accordingly.

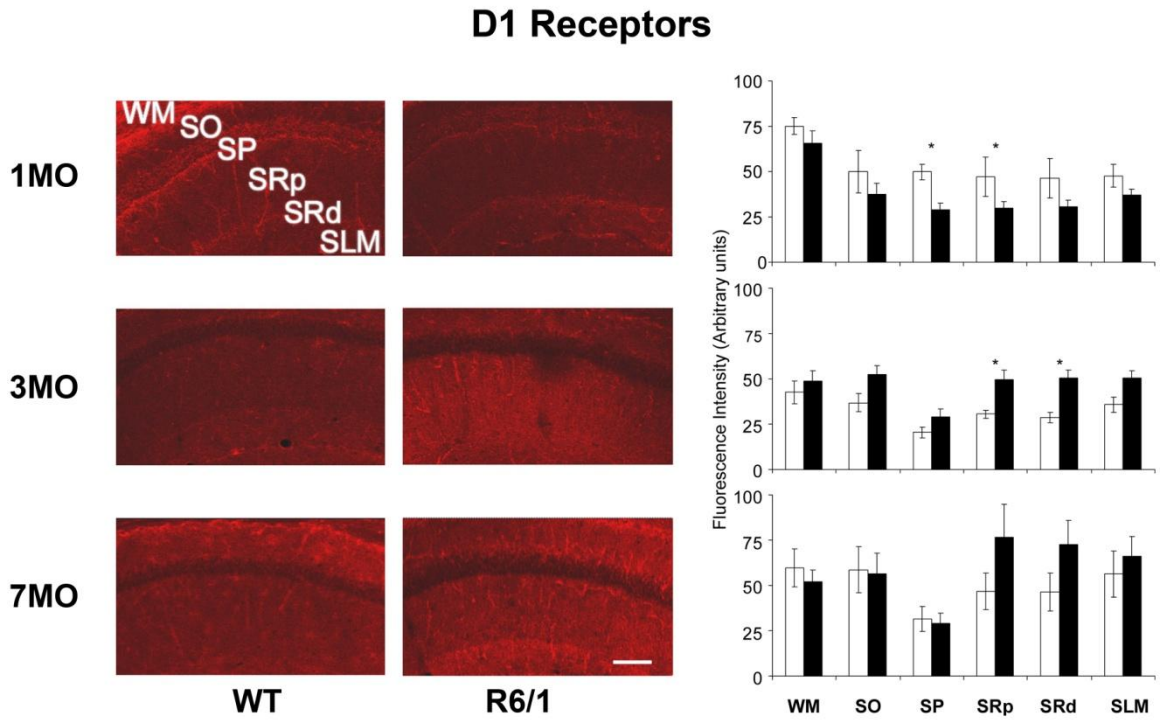

Figure 1. Hippocampal CA1 D1 receptor labelling. (p20 l512)

Representative confocal micrographs (x40 objective) of D1 immunofluorescence **in the CA1 area of the hippocampus** of WT (left) and R6/1 (right) mice age as indicated (months).

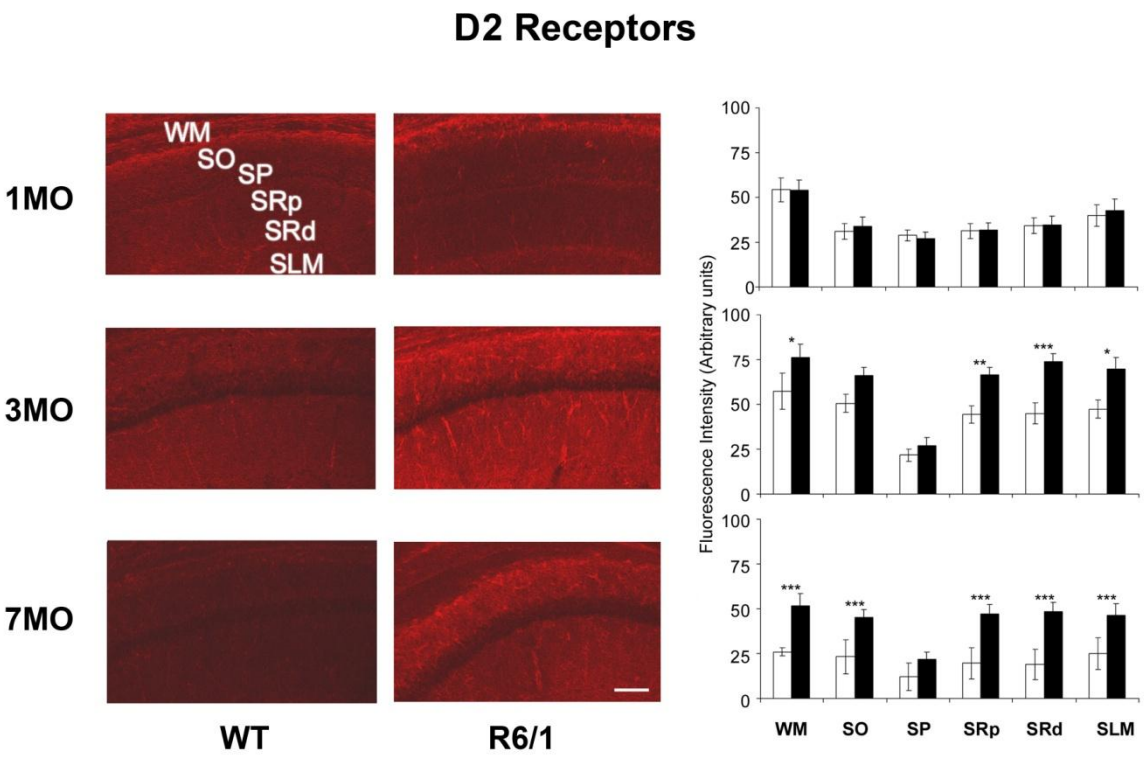

Figure 2. Hippocampal CA1 D2 receptor labelling. (p21 l526)

Representative confocal micrographs (x40) of D2 immunofluorescence **in the CA1 area of the hippocampus** of WT (left) and R6/1 (right) mice from the ages indicated.

3. Page 9, "Dopamine receptor expression increases in R6/1 transgenic mice" need specific to hippocampus since previously the authors found decreased levels of dopamine receptors in perirhinal cortex and other regions.

We agree and thank the reviewer for this suggestion. We have now replaced the title *"Dopamine receptor expression increases in R6/1 transgenic mice"* by *"CA1 dopamine receptor expression increases in R6/1 transgenic mice" p9, l199.*

4. Did the authors run western blot for dopamine D1 or D2- like receptors to confirm their finding? Especially in 7m HD hippocampus they found significant increase of D2- like receptor by immunostaining.

Western blots are indeed used to quantify protein expression but can lack sufficient spatial and cellular resolution, the latter is better addressed using fluorescence immunohistochemistry. In our investigation we aimed at assessing dopamine receptor expression of different regions of the CA1 area of the hippocampus. Western blots performed on hippocampal extracts is not the method of choice in our study as the changes we report would most likely be masked by heterogeneity from different hippocampal regions.

5. Evidences showed increased DA in early stage and reduced DA in late-stage HD patients and animal models. Manipulation DA receptor depends on the level of DA tone. Thus except DA receptors, determine DA level in hippocampus of R6/1 mice may provide more comprehensive information.

DA levels are indeed altered in HD patients and mice, and we have actually previously found that striatal release of this important neuromodulator is increased at early disease stages whilst it is markedly decreased in a late HD mouse model (Dallérac et al. 2015). We agree that studying DA release and tone in the hippocampus is relevant in light of the results we report here and of a recent study showing that dopamine content is reduced by ~30% in symptomatic R6/2 mice (Mochel et al. 2011). We thank the reviewer for the suggestion, this will however be addressed in a future investigation as it is beyond the scope of the current negative findings manuscript. In light of this sensible comment, we have nevertheless improved the discussion of our manuscript p13 l288:

" The significance of a large increase in dopamine receptor labelling is unclear, but it might reflect an up-regulation in dopamine receptor number in response to decreased dopaminergic innervation or signalling**. Such a view is supported by a recent study reporting more than 30% decrease in hippocampal dopamine content in 12 weeks old symptomatic R6/2 mice (Mochel et al. 2011)."**

References reviewer 1

Dallérac, G. M., Levasseur, G., Vatsavayai, S. C., Milnerwood, A. J., Cummings, D. M., Kraev, I., et al. (2015). Dysfunctional Dopaminergic Neurones in Mouse Models of Huntington's Disease: A Role for SK3 Channels. *Neuro-degenerative diseases*, *15*(2), 93–108.

Mochel, F., Durant, B., Durr, A., & Schiffmann, R. (2011). Altered dopamine and serotonin metabolism in motorically asymptomatic R6/2 mice. *PloS one*, *6*(3), e18336.

Vatsavayai, S. C., Dallérac, G. M., Milnerwood, A. J., Cummings, D. M., Rezaie, P., Murphy, K. P. S. J., & Hirst, M. C. (2007). Progressive CAG expansion in the brain of a novel R6 / 1-89Q mouse model of Huntington ' s disease with delayed phenotypic onset. *Brain Research Bulletin*, *72*, 98–102.

Reviewer #2: The manuscript by Dallerac et al. nicely demonstrates that in the R6/1 mouse model of Huntington's disease, aberrant LTD in the aged hippocampus is not due to alterations in dopamine detection. Specifically, the authors show that aberrant LTD is dissociated from pathologically elevated hippocampal expression of both D1 and D2 type receptors. This is important, as abnormal plasticity in the disease state is clearly linked to abnormal dopaminergic signaling in other brain regions, including the cortex. This dissociation helps shed light on one of the many potential limitations of dopamine-related therapies posited to be useful for HD. The study is from a well-established HD group, and should be of interest to researchers in the HD field. I only have a few minor comments.

1. For quantification of fluorescence: how was fluorescence intensity compared between different slices? Were wt and mutant slices processed and analyzed in parallel? Can you please clarify what is meant by "internally normalized" in the methods section?

Both transgenic and non-transgenic slices were indeed processed and analysed in parallel. Following the reviewer's advice we have now replaced the mention "internally normalized" by a more detailed description of the procedure p6 l140:

"Transgenic and non-transgenic slices were processed and analysed in parallel. Image stacks (6 µm) of 12 sequential scans (0.5 µm) were performed and collected for each section using Leica Confocal Software (Version 2.5, Leica, Heidelberg, Germany). Fluorescence was calculated by manually

selecting the 3 brightest scans from each stack and generating a composite average. Fluorescence was quantified by generating a mean fluorescence value (in arbitrary units) from three manually placed non-overlapping sampling boxes (2000 µm^2) in each region of interest (ROI) through the CA1 field of the hippocampus (capillaries were avoided). Fluorescence intensity was standardized between slices by imaging sections on the same day using the same laser and parameters; i.e. gain, offset and PMT intensity."

2. It may be useful to show where the recording electrode was for LTD experiments (fig 3), in relation to the immune data shown in figures 1-2. Perhaps a label in figure(s) 1 or 2.

This is a sensible suggestion and we have now inserted a schematic diagram showing placement of the electrodes in Figure 3. Figure caption has been amended accordingly (p21 l543).

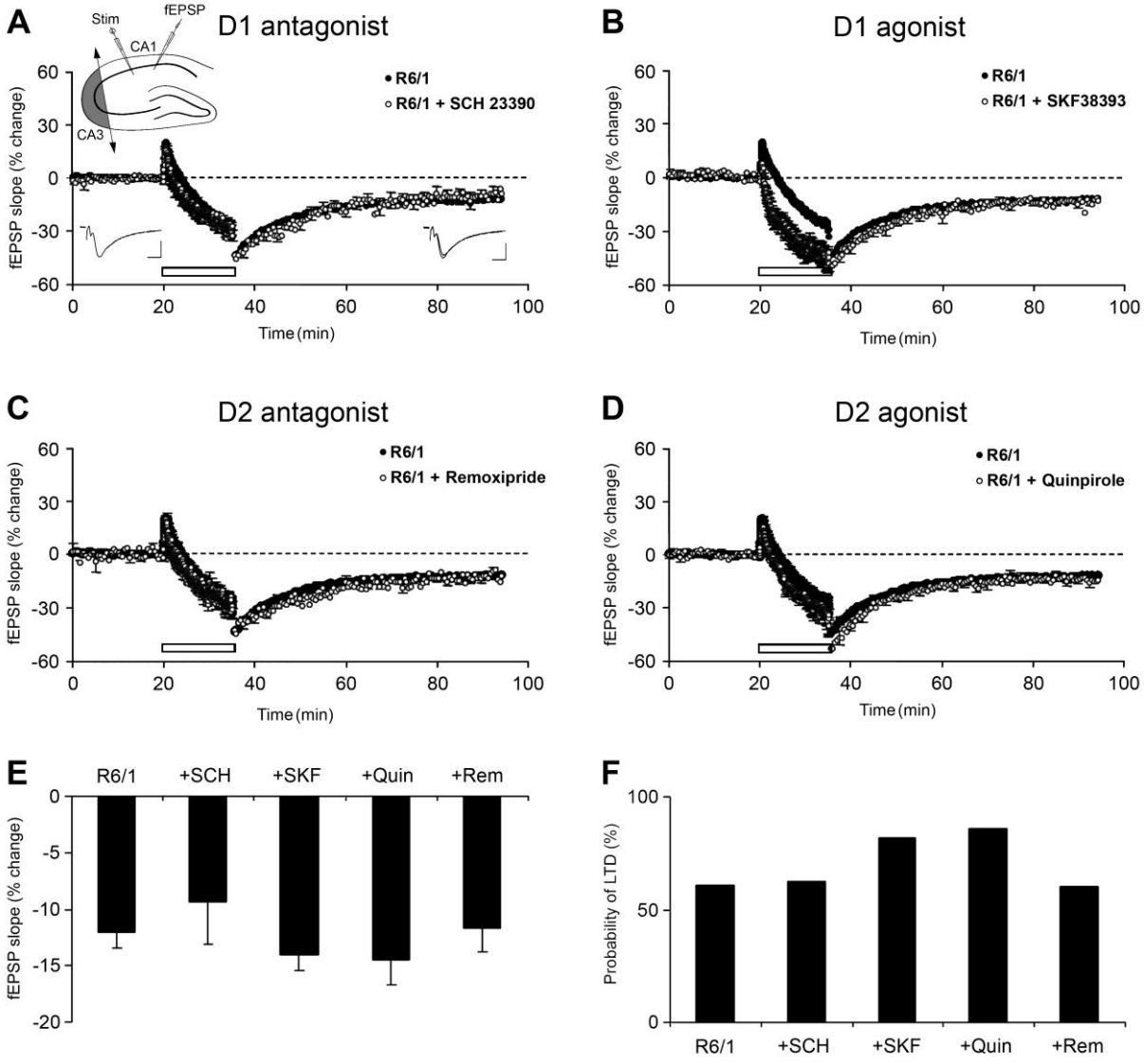

Figure 3. LTD in R6/1 adults is not blocked by pharmacological manipulation of dopamine receptors
Neither D1 nor D2 receptor agonists nor antagonists (10µM) significantly altered the magnitude (A, B, C, D, E) or probability (F) of LTD induction in slices prepared from R6/1 mice at 8 months of age. **Insert in (A) shows the stimulating and recording electrode placement. Double arrows represents cutting of the CA3 area for which the excised part is depicted in grey. (p21 l543)**

3. Recently, evidence has been published that points to non-dopaminergic pathologies in HD that lead to impaired synaptic plasticity. For example, Surmeier's group recently showed that diminished TrkBR signaling in the striatum impairs LTP. It would be benSeficial to add references supporting the findings that non-dopaminergic impairments alter plasticity in HD models.

We agree with the reviewer and have now improved our manuscript by discussing non-dopaminergic alterations in synaptic plasticity, p12 l269:

" This indicates that although dopaminergic changes play an important role in HD, the etiology of the disease is more complex and involves multiple mechanisms. Focusing on synaptic plasticity, alteration in brain derived neurotrophic factor (BDNF) availability has for example been reported as an important modifier of synaptic efficacy (Lynch et al. 2007; Simmons et al. 2009; Zuccato et al. 2003). In this regard, two recent reports further indicate that in HD mice striatum (Plotkin et al. 2014) and hippocampus (Brito et al. 2014), signalling downstream the BDNF tyrosine-related kinase B (TrkB) receptors and and p75 neurotrophin receptors (p75NTR) would also be deficient. Other identified molecular abnormalities underlying synaptic dysfunction in HD include NMDA receptor composition with an increased NR2B function (Li et al. 2004; Milnerwood et al. 2006; Zeron et al. 2002) and cell adhesion molecules such as PSA-NCAM (van der Borght and Brundin 2007). Finally, a recent report indicates that astroglial Kir4.1 channels are deficient in HD (Tong et al. 2014); these astroglial channels are involved in the regulation of synaptic function (Dallerac et al. 2013) and are therefore also likely to contribute to abnormal neurotransmission in HD. ".

References reviewer 2

Brito, V., Giralt, A., Enriquez-Barreto, L., Puigdellívol, M., Suelves, N., Zamora-Moratalla, A., et al. (2014). Neurotrophin receptor p75(NTR) mediates Huntington's disease-associated synaptic and memory dysfunction. *The Journal of clinical investigation*, *124*(10), 4411–28.

Dallerac, G., Chever, O., & Rouach, N. (2013). How do astrocytes shape synaptic transmission? Insights from electrophysiology. *Front Cell Neurosci*, *7*, 159. doi:10.3389/fncel.2013.00159

Li, L., Murphy, T. H., Hayden, M. R., & Raymond, L. A. (2004). Enhanced striatal NR2B-containing N-methyl-D-aspartate receptor-mediated synaptic currents in a mouse model of Huntington disease. *J Neurophysiol*, *92*(5), 2738–2746.

Lynch, G., Kramar, E. A., Rex, C. S., Jia, Y., Chappas, D., Gall, C. M., & Simmons, D. A. (2007). Brain-derived neurotrophic factor restores synaptic plasticity in a knock-in mouse model of Huntington's disease. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *27*(16), 4424–34.

Milnerwood, A. J., Cummings, D. M., Dallerac, G. M., Brown, J. Y., Vatsavayai, S. C., Hirst, M. C., et al. (2006). Early development of aberrant synaptic plasticity in a mouse model of Huntington's disease. *Hum Mol Genet*, *15*(10), 1690–1703.

Plotkin, J. L., Day, M., Peterson, J. D., Xie, Z., Kress, G. J., Rafalovich, I., et al. (2014). Impaired TrkB receptor signaling underlies corticostriatal dysfunction in Huntington's disease. *Neuron*, *83*(1), 178–88.

Simmons, D. A., Rex, C. S., Palmer, L., Pandyarajan, V., Fedulov, V., Gall, C. M., & Lynch, G. (2009). Up-regulating BDNF with an ampakine rescues synaptic plasticity and memory in Huntington's disease knockin mice. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(12), 4906–11.

Tong, X., Ao, Y., Faas, G. C., Nwaobi, S. E., Xu, J., Haustein, M. D., et al. (2014). Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in Huntington's disease model mice. *Nature neuroscience*, *17*(5), 694–703.

van der Borght, K., & Brundin, P. (2007). Reduced expression of PSA-NCAM in the hippocampus and piriform cortex of the R6/1 and R6/2 mouse models of Huntington's disease. *Experimental neurology*, *204*(1), 473–8.

Zeron, M. M., Hansson, O., Chen, N., Wellington, C. L., Leavitt, B. R., Brundin, P., et al. (2002). Increased Sensitivity to N-Methyl-D-Aspartate in a Mouse Model of Huntington ' s Disease, *33*, 849–860.

Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., Conti, L., et al. (2003). Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nature Genetics*, *35*(1), 76–83.

Figure 1

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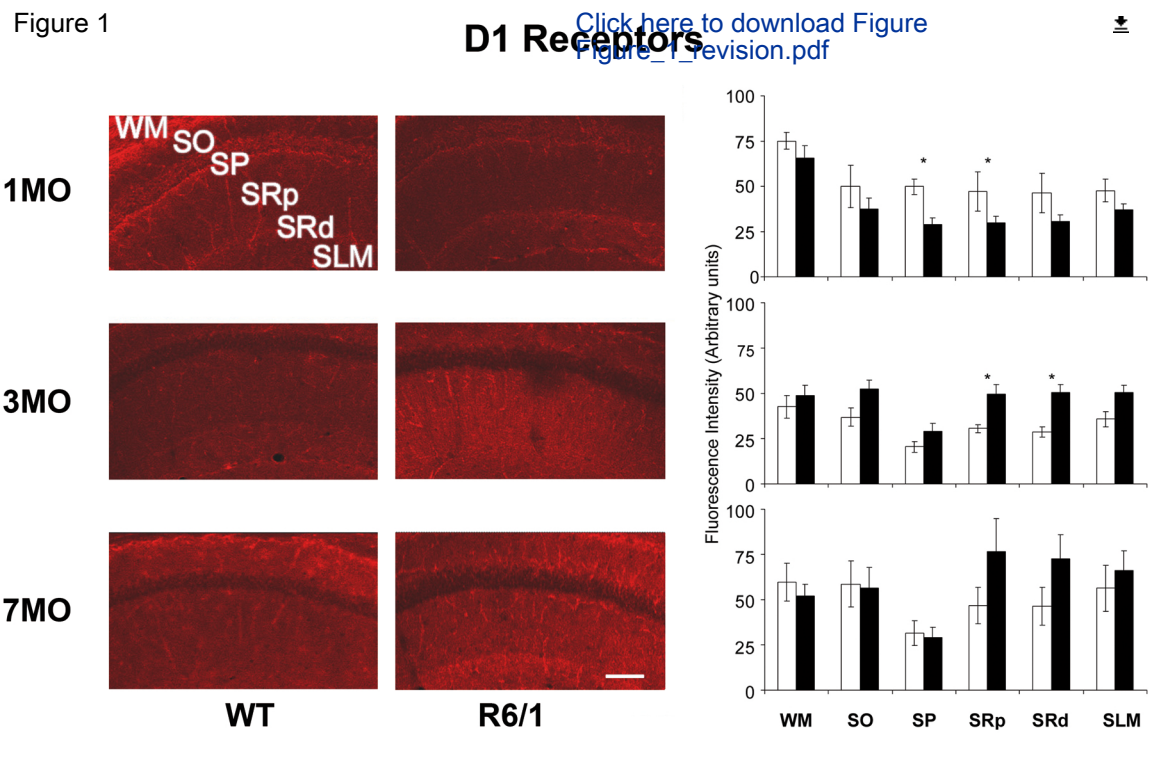

Figure 2

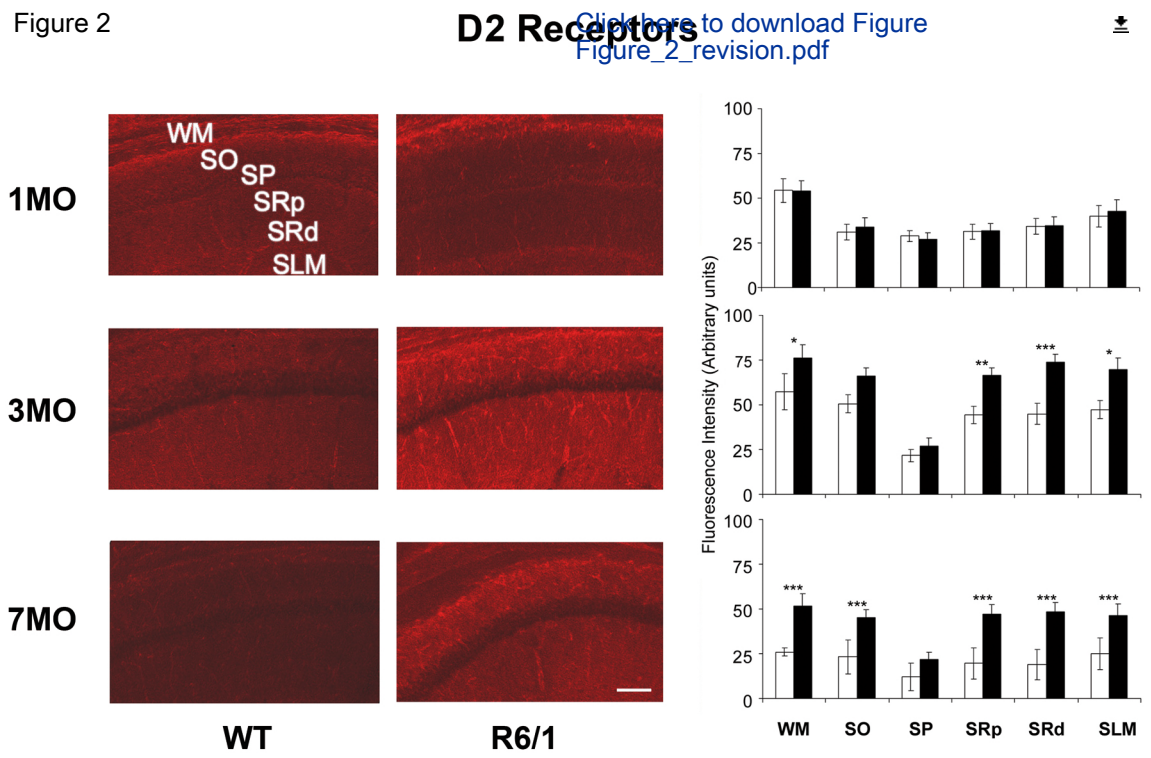

Figure 3