Deficit in motor skill learning-dependent synaptic plasticity at motor cortex to Dorso Lateral Striatum synapses in a mouse model of Huntington’s disease

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Abstract
Huntington’s disease (HD) is a neurodegenerative disease notably characterized by progressive motor symptoms. Although the loss of Medium Spiny Neurons (MSNs) in the striatum has been associated with motor deficits, premanifest patients already present cognitive deficiencies and show early signs of motor disabilities. Here in a YAC128 HD mouse model, we identified impairment in motor skill learning at the age of 11 - 14 weeks. Using optogenetic stimulation, we found that excitatory synaptic transmission from motor cortex to MSNs located in the Dorso Lateral part of the Striatum (DLS) is altered. Using single pellet reaching task, we observed that while motor skill learning is accompanied by a dynamic change in AMPA/NMDA ratio in wild type mice, this form of synaptic plasticity does not occur in YAC128 mice. This study not only proposes new meaningful insight the synaptopathic mechanisms of HD, but also highlights that deficit in motor skill learning-dependent synaptic plasticity at motor cortex to DLS synapses represents an early biomarker for Huntington’s disease.
Introduction

Huntington’s disease (HD) is an autosomal dominant neurodegenerative disease caused by CAG repetition in the gene encoding huntingtin protein (HTT) and is characterized by progressive motor, cognitive and psychiatric symptoms. Neurodegeneration of Medium Spiny Neurons (MSNs) in the striatum is the principal pathological hallmark of HD (la Monte et al., 1988). Although MSNs degeneration has been associated with motor deficits, premanifest patients not only already present cognitive deficiencies (Giralt et al., 2012) but also show subtle signs of motor disabilities (Tabrizi et al., 2011). Indeed, slight impairment in motor coordination, in fine motor control of upper extremities and in motor sequence learning have been described in premanifest HD patients (de Boo et al., 1997; Kirkwood et al., 1999; Kirkwood et al., 2000; Tabrizi et al., 2009; Schneider et al., 2010). Thus, it has been hypothesized that these fine motor evaluations could represent early biomarkers of the disease, a key step to propose potential neuroprotective treatments in clinical trials (Duff et al., 2008).

Several mechanisms attempted to explain how mutated HTT (mHTT) protein eventually lead to neuronal dysfunction without cell death in premanifest HD. Reduced striatal activity and changes in synaptic properties in the striatum have been described both in human and in mouse models (Wolf et al., 2012; Milnerwood and Raymond, 2010). In particular, increased sensitivity to NMDA (Levine et al., 1999) as well as an increase in extrasynaptic NMDAR signalling (Milnerwood et al., 2010) have been observed at early stage of the disease suggesting that dysfunctions in NMDA transmission may occur early in the disease progression. NMDARs are heteromeric receptors typically containing two GluN1 subunits together with a combination of two GluN2 (A-D) or one GluN2 and one GluN3 (A, B) subunit. Not only subunit composition determines the receptor’s biophysical and pharmacological properties, but changes in NMDAR subunit composition contribute to the pathophysiology of several neurological diseases (Paoletti et al., 2013). The expression of both GluN2B and GluN3A subunits have been previously linked to HD. Extrasynaptic GluN2B-containing NMDARs are enriched in the striatum of transgenic mice expressing mutated full-length human HD gene (YAC128) at an age preceding motor dysfunctions and neuronal loss (Milnerwood et al., 2010). Elevated GluN3A expression has been observed in both HD mouse models and human patients and linked to abnormal excitation of MSNs in the striatum (Marco et al., 2013; Mahfooz et al., 2016). Although early postsynaptic changes in NMDAR-mediated transmission have been described in premanifest HD mouse models, it is still an open question whether these changes
only impact survival/death signalling balance and consequent neuronal degeneration or whether they could also be causally link to early behavioural phenotypes.

New motor skill learning is often characterized by a fast-initial phase of improvement of the performance followed by a gradual improvement of motor skills. After consolidation, memory becomes long-lasting and can persist for the entire life. It had been previously shown that consolidation of motor skills is accompanied by long-lasting changes in glutamatergic transmission onto MSN of the DLS and requires striatal NMDAR (Dang et al., 2006; Yin et al., 2009; Lambot et al., 2016). Interestingly subjects with premanifest HD exhibit learning impairment that is not explained by differences in initial performance (Shabbott et al., 2013). It has been therefore hypothesized that deficits in motor skill learning happen prior to the deficits in motor execution in HD.

Here, using YAC128 HD mouse model, we found that between the age of 11 - 14 weeks, mice show impairment in motor skill learning. Using optogenetic stimulation, we have observed a decrease in AMPA/NMDA ratio at motor cortex to DLS MSNs synapses. This change was accompanied by a change in NMDA receptor subunit composition while no modifications in presynaptic properties or in AMPAR mediated synaptic transmission were detected. Interestingly, YAC128 showed a stronger NMDA-dependent form of long-term depression at motor cortex to DLS when compared to control mice. Altogether, these data suggest that changes in AMPA/NMDA ratio in YAC128 MSN mice are driven by an increase in NMDAR mediated transmission. Finally, using single pellet reaching task, we found that motor skill learning was accompanied by a reduction in AMPA/NMDA ratio in wild type mice. This form of synaptic plasticity is absent in YAC128 mice suggesting that the decreased AMPA/NMDA ratio in YAC128 mice is a factor that limits learning of motor skills in premanifest HD.
Materials and Methods

Animals
One mouse model of HD was used in this study, YAC128 homozygote (line 55, as previously described in (Marco et al., 2013), obtained from Perez Otano laboratory). Age and genetic background matched C57Bl/6j mice were used as controls. YAC128 homozygous were intercrossed with Tg(Drd1-dTomato) transgenic mice (generous gift from Pr. N. Deglon) to generate YAC128 homozygous-Drd1-dTomato mice (YAC128-D1) after real-time quantitative PCR analysis. Both males and females were respectively housed in groups with food and water ad libitum under controlled conditions (22-23°C, humidity 50 ± 5 %, 12 h light-dark cycle with light on at 7.00 a.m). All the procedures performed at the UNIL and UNIGE compiled with the Swiss National Institutional Guidelines on Animal experimentation and were approved by the Swiss Cantonal Veterinary Office Committee for Animal Experimentation. VD 3016.d license authorization.

Electrophysiology
250 μm thick coronal slices containing dorsolateral striatum were prepared following the experimental injection protocols described in the text. Slices were kept in artificial cerebrospinal fluid containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgCl2, 2.5 mM CaCl2, 1.0 mM NaH2PO4, 26.2 mM NaHCO3 and 11 mM glucose, bubbled with 95% O2 and 5% CO2. Slices were maintained 30 min in bath at 30°C and then at room temperature. Whole-cell voltage-clamp recording techniques were used (37°C, 2–3 ml min−1, submerged slices) to measure the holding currents and synaptic responses of dorsolateral striatum MSN. The internal solution contained 130 mM CsCl, 4 mM NaCl, 2 mM MgCl2, 1.1 mM EGTA, 5 mM HEPES, 2 mM Na2ATP, 5 mM sodium creatine phosphate, 0.6 mM Na3GTP and 0.1 mM spermine. Currents were amplified, filtered at 5 kHz and digitized at 20 kHz.
Access resistance was monitored by a hyperpolarizing step of -4 mV at each sweep, every 10 s. The cells were recorded at the access resistance from 10–25 MΩ for MSN. Data were excluded when the resistance changed > 25%. Synaptic currents were evoked by intrastriatal electrical stimulation at 0.1 Hz and 0.05–0.1 msec of duration. For optogenetic experiments, we stimulated the glutamatergic fibers from motor cortex or from the thalamus in the dorsolateral striatum. The stimuli was delivered at 0.1 Hz and the duration was 1-3 msec. The experiments were carried out in the presence of GABAA receptor antagonist picrotoxin (100
µM); the AMPAR-EPSCs were pharmacologically isolated by application of the NMDAR antagonist D-APV (50 µM) and NMDAR EPSCs were recorded at +40 mV in presence of the AMPAR blocker NBQX (10 µM). Representative example traces are shown as the average of 15-20 consecutive EPSCs typically obtained at each potential. The rectification index of AMPARs is the ratio of the chord conductance calculated at negative potential (-60 mV) divided by the chord conductance at positive potential (+40 mV). The analysis of the decay time of NMDAR-mediated EPSC was conducted as described previously and the Ifenprodil sensitivity was calculated as the percentage of NMDAR-EPSC amplitude reduction (at + 40 mV) after 20-25 minutes of continuous Ifenprodil (3 µM, GluN2B-containing NMDAR antagonist) bath-application compared to baseline. The time interval between the two stimulations for the Paired Pulse Ratio (PPR) measurement was 50, 100 and 300 msec (Inter Stimulation Interval, ISI) and the ratio was obtained by dividing the EPSC2 by EPSC1 amplitude at -60 mV.

For the in vitro validation of the optogenetic experiment and the strontium chloride experiment, the internal solution contained 140 mM K-Gluconate, 2 mM MgCl2, 5 mM KCl, 0.2 mM EGTA, 10 mM HEPES, 4 mM Na2ATP, 0.3 mM Na3GTP and 10 mM Creatine-Phosphate. Blue-light was delivered through the 40X objective focused on the cell soma. The Synaptic responses were collected with a Multiclamp 700B-amplifier (Axon Instruments, Foster City, CA), filtered at 2.2 kHz, digitized at 10 Hz, and analyzed online using Igor Pro 6 software (Wavemetrics, Lake Oswego, OR).

I-V curves of pharmacologically isolated NMDARs were generated holding the cells at different membrane potential for 5 min each and normalizing EPSCs at 40 mV.

Asynchronous evoked EPSC (aEPSC). For this experiment, 3 mM of SrCl2 was added in the aCSF solution instead of the CaCl2. The internal solution contained: 140 mM K-Gluconate, 2 mM MgCl2, 5 mM KCl, 0.2 mM EGTA, 10 mM HEPES, 4 mM Na2ATP, 0.3 mM Na3GTP and 10 mM Creatine-Phosphate. Cells were hold at –70mV, and picrotoxin was added in this external bath. Asynchronous events were measured during 180 ms period, between 20 ms to 200 ms after stimulation (Choi and Lovinger 1997). Quantal events were detected and analysed using Mini analysis program version 6.0.

The plasticity experiments were recorded at -40 mV holding potential, we measured 10 minutes of baseline response at 0.1 Hz, followed by 5 minutes of stimulation at 1 Hz. Then we recorded the EPSC’s for 40 minutes.
**Stereotaxic injections**

AAV5-CamKII-hChR2(H134R)-EGFP virus has been injected in the Motor Cortex in 4 to 7 weeks old mice. Anesthesia was induced and maintained with a mixture of Oxygen and Isoflurane. The animals were then placed on the stereotaxic frame (Stoelting Co., USA) and a single or bilateral craniotomy was made over Motor Cortex at following stereotaxic coordinates: M1: AP +1.18 mm, ML 1.21 mm, DV 0.65 mm from Bregma; M2: AP +1.18 mm, ML 0.60 mm, DV 0.50 mm from Bregma and for the thalamus at following coordinates: AP -2.30 mm, ML 0.6 mm, DV 3 mm from Bregma. The virus was injected with graduated pipettes (Drummond Scientific Company, Broomall, PA) at the rate of 100 nL/min for a total volume of 200 nL per injection side. For all the experiments the virus was incubated for at least 4 weeks, when expression was clearly identifiable by the reporter protein expression, before proceeding with further manipulations.

**Circular corridor test**

Mice were placed in a circular corridor (30 cm diameter) and were allowed to freely explore the circular corridor for a 30 min period in 11 lux illumination condition. Total distance travelled and velocity during the session were automatically recorded (Ethovision, Noldus, Wageningen, the Netherlands). Arena was cleaned with 1% acetic acid and dried between each test.

**Open field test**

Mice were placed in a square open field (42×42 cm) and were allowed to freely explore the open field for a 10 min period in 11 lux illumination condition. Total distance travelled and velocity during the session were automatically reported (Ethovision, Noldus, Wageningen, the Netherlands). The arena was cleaned with 1% acetic acid and dried between each test.

**Single pellet reaching task test**

To evaluate motor skill learning, a single pellet reaching task was performed. This paradigm requires a precise and coordinated sequence of movements of the forelimb in a serial order. Mice are trained to extend their forelimbs through a narrow slit to grasp and retrieve millet pellets (food) positioned at a fixed location as described in Chen et al 2014. First, mice are placed on a food restriction schedule, 90% of their free feeding body weight (Chen et al 2014, Lambot et al 2016). In detail, food restriction starts two days prior experiment to initiate
bodyweight loss. In a second step, a group and individual habituation has been done in the training chamber. In details, two cagemate mice are placed in the training chamber (custom made transparent Plexiglas training chamber 20 cm tall, 15 cm deep, 8.5 cm wide that contains three vertical slits) at the same time with 20 millet pellets inside the chamber for 20 min. The next day, a single habituation as previously described has been accessed. Then, to determine the forelimb dominance, a food platform with millet pellets was placed in front of the training chamber to allow the accessibility of the pellets to the mouse through vertical slit of the training chamber. This shaping phase is achieved when two criteria were encountered 1) the mouse conducts 20 reaching attempts within 20 min and 2) more than 70% reaching attempts are performed with one forelimb. If the mouse does no attempt these criteria within one week, the mouse was then excluded from the experiment. After this shaping phase (5 days), a single-pellet training started (8 days). During the training phase, mice are trained to reach single pellet for 20 min per day. After training, mice returned to their home cage and were kept under food restriction. Three responses were manually scored: success (grasp the pellet with the preferred paw and put it in the mouth), drop (grasp the pellet with the preferred paw and release it before to put in the mouth) and fail (can’t grasp the pellet with the preferred paw). Speed of success is defined as the number of successful reaches per minute. Success rate is the number of successful reaches divided by total reaching attempts (success/(success+ drop+ fail attempts)) expressed in percentage. Fail rate is the number of fail reaches divided by total reaching attempts (fail /(success+ drop+ fail attempts)) expressed in percentage. During the entire experiment, mice are group-housed (age and sex matches). All the procedure was recorded with a camera JVC model No. GZ-R430BE.

**Rotarod test**

Mice were brought 30 min prior the experiment in the room to allow acclimatization. The rotarod apparatus (Ugo Basile, Biological Research Apparatus, Varese, Italy) consisted of a plastic roller with small grooves running along its turning axis. Mice received 2 trials per day for 4 consecutive days, then the training were interrupted for 2 days, after this period the training restarted for 2 additional days. The protocol consists in a classical accelerated rotarod (Southwell et al., 2009) from 5 rotations per minute (RPM) to 40 RPM within 240 sec ramping over a maximum duration of 300s with 10 min interval session break. We scored the mouse fall latency in seconds of each last trial session per day. Mice that did not fall during experiment were scored as 300s.
**Swimming tank test**

To measure swimming behavior, we used a swimming tank apparatus build of Plexiglas, the dimensions were 100 cm long, 30 cm high and 6 cm wide with an escape platform in one extremity (6x6 cm and 20 cm high) (Carter et al., 1999). The tank was filled with water (26-27°C) until the escape platform protrudes 1-2 cm above the water level. In the opposite side of the platform, a vertical red line indicates the starting point located at 60 cm from the platform. The first day of training, the animals were deposited in the tank and when necessary were conducted to reach the platform. From day 2 of training, animals were deposited and slightly conducted until the red line. The task consists in three consecutive trials (approximately 10 seconds between trails), performed daily from Day 1 to 3, then, a rest time window of 3 days was left until Day 7 that correspond to the last day of training. We measured the time to swim the 60 cm of distance from the red line to the platform. Trials were finished when mice reach and climb on the platform. Given that YAC128 mice expressed a floating behavior during this task we set a threshold time of 30 seconds, if mice completed the task in more than 30 s, the trials were counted as failed.

**Elevated plus maze**

The elevated plus maze consisted in a platform of four opposite arms (40 cm) two of them are open and two are closed arms (enclosed by 15 cm high walls). The apparatus was elevated at 55 cm from the floor. The task was recorded and analyzed with the software Ethovision (Noldus, Wageningen, the Netherlands) and we measured the time spent in each arm in trials of 5 min. The luminosity of the room was 11-12 Lux in the open arms.

**Real-Time PCR**

Real time PCR was performed by microsynth company to determine the genotype of the transgenic YAC128 mice. Genomic DNA was isolated from ear punch or post mortem tail biopsies and analyzed by real–time PCR specific for huntingtin gene and β-actin. The resulting Ct values are used for relative quantification of the copy-number of human specific huntingtin (HD) in the provided samples according to the following equation: ΔCt=Ct (β actin) – Ct (HD). The gene expression fold change, normalized to the β-actin and relative to the control sample, was calculated as $2^{ΔCt}$. Values close to 1 corresponds to HD homozygous while values lower
than 0.5 corresponds to HD heterozygous mice. All samples were run in triplicate. The following primers used for the real-time PCR reaction were:

**HD primer:**

F 5' GAAAGTCAGTCCGGGTAGAACTTC 3'

R 5' CAGATACCCGCTCCATAAGCAA 3'

**mouse b-actin primers:**

F 5' ACGGCCAGGTCATCACTATTG 3'

R 5' CAAGAAGGAAGGCTGGAAAGA 3'

Briefly, real time PCR was assayed in a total volume of 20 µL reaction mixture containing 2.5 µL of diluted cDNA, 10 µL SYBR Green PCR master mix (Applied Biosystem), 1µL primer F (5pmol/µL), 1 µL primer R (5pmol/µL), 5.5 µL H2O. PCR thermal conditions were done with a step at 50 °C for 2 min, a 10 min at 95 °C, followed by 40 cycles of denaturation for 15 s at 95 °C and annealing/primer elongation for 1 min at 60 °C.

**Drug and viruses**

AAV5-CamKII-hChR2(H134R)-EYFP virus (2.8x10e12 viral molecules/mL, UNC GTC Vector core), D-AP5 (0106, Tocris), Picrotoxin (1128, Tocris), NBQX (0373,tocris), Ifenprodil hemitartrate (0545, Tocris).

**Statistical Analysis**

Normality was checked with the Shapiro-Wilk criterion and when violated, non-parametric statistics were applied (Mann-Whitney and Kruskal-Wallis). When samples were normally distributed, data were analyzed with independent or paired two-tailed samples t-tests, one-way, two-way or repeated measures analysis of variance (ANOVA) followed if significant by post hoc tests. All error bars represent the mean ± the standard error of the mean (SEM) and the significance was set at p < 0.05. Data were analyzed using the Graphpad Prism 5 and 7 and graphs were created using the Graphpad Prism 5 and 7 (San Diego, CA, USA). Outliers were defined as higher than mean ± 2 SD (standard deviation).
RESULTS

YAC128 mice show strong motor dysfunctions when they reached the age 10 months (Marco et al., 2013) while earlier detection of motor disturbance in this model is more controversial (Slow et al., 2003; Southwell et al., 2009). To verify whether subtle deficits in motor behavior could be observed at earlier time points, we tested mice between 11 and 14 weeks. We did not detect any gross impairment in locomotor activity as indicated by the absence of differences in travelled distance and velocity between Wild Type (WT) and YAC128 mice in the circular corridor (Fig. 1A-C) and in the open field tests (Fig. 1D-F). However, in the swimming tank test (Fig. 1G) while WT and YAC128 mice present equivalent initial performance at day 1, WT mice improved the swimming latency over the subsequent days, whereas YAC128 became slower in their swim performance (Fig. 1H). In YAC128 mice we also observed an increased number of failed trials (Supplementary Fig. 1A) compared to controls and floating behavior (Supplementary Fig. 1B-C). Remarkably, we noted that YAC128 spent less time in open arms in the elevated plus maze (Supplementary Fig. 1F-G). Together these results indicate that although no major motor deficits were observed, anxiety traits together with deficits in late phase of motor skill learning are already present at this early stage of the disease.

To assess motor learning abilities of YAC128 mice, we used the accelerated rotarod task (Fig. 1I). While no differences in the time to fall were observed at day 1 (Fig. 1J), YAC128 mice did not improved their performance over the days and differences between mice were observed at day 7 (Fig. 1R and Supplementary Fig. 1D-E).

To study fine motor skill learning involving forelimb dexterity, we then adopted the single-pellet reaching task. In this task, food restricted mice are trained to extend their forelimbs through a narrow slit to grasp and retrieve food pellets positioned in a fixed location. After 2 days of habituation, mice undergo 5 days of shaping followed by a period of training (Fig. 2A-C; See Material and method session). YAC128 and control mice show a similar performance at the first training session. Strikingly, while control mice improved the successful attempts per minute over training days, YAC128 mice did not enhance their performance at day 8 (Fig. 2D). In addition, successful rate increased between day 1 versus day 8 in WT while it remained unchanged in YAC128 mice (Fig 2E, F). Alteration in single pellet reaching task in YAC128 mice does not account for difference in bodyweight compared to WT (Supplementary Fig. 2A and B), neither to default motivation to perform this task. Indeed, no difference in total
attempts across days between groups (Supplementary Fig. 2C) was observed between control and YAC128 mice.

These data suggest that at early stages of the disease, difficulties in motor skills learning can be observed in HD mice.

Changes in excitatory synaptic transmission in the striatum have been previously described in symptomatic HD mouse models. Here we first investigated whether early behavioral traits were accompanied by specific changes in glutamatergic synaptic transmission onto MSN in the DLS. Using intra-striatal electrical stimulation (Fig. 3A), we did not detect changes in presynaptic release properties (Fig. 3B), neither in the amplitude and frequency of strontium-evoked asynchronous AMPAR events (Fig 3 C) neither in the strength of synaptic transmission measured as AMPA/NMDA ratio (Fig. 3E). Furthermore, by pharmacological isolation of AMPARs or NMDARs, we could not detect any change in rectification index (Fig. 3D), Ifenprodil sensitivity (Fig.3F, G), a GluN2B-containing NMDAR antagonist or NMDA-EPSC decay time kinetics (Fig. 3H, I) suggesting that AMPAR and NMDA subunit composition was not changed in YAC128 mice compare to control.

Because previous work showed changes in excitatory transmission onto DLS during late motor skill learning (Yin et al., 2009), we decided to focus our attention on glutamatergic projections from motor cortex and thalamus, two of the major inputs onto MSN in the DLS, (Smith:2004ia) (Wall et al., 2013; Guo et al., 2015). Using optogenetic stimulation, we wanted to investigate whether the observed motor skill learning deficits were accompanied by deficits in transmission within defined circuits. First, we injected Channelrhodopsin (ChR2) expressing virus in the motor cortex and in the thalamus in control and YAC128 mice (Fig. 4A). After 6 weeks, acute brain slices were obtained and recording were performed from MSN of the DLS. We did not detect differences in the strength of transmission at thalamo-DLS synapses (Fig. 4B). By contrast, when recording from DLS MSNs were obtained with light-evoked synaptic transmission from motor cortex inputs, we found a decreased AMPA/NMDA ratio in YAC128 compared to WT (Fig. 4C). Paired pulse ratio measured at different time intervals (Supplementary Fig. 3A), amplitude of the optically-induced AMPAR mediated currents (Fig. 4E) as well as the amplitude of strontium-evoked asynchronous AMPAR events (Fig. 4F) and the rectification index of AMPAR-mediated transmission (Fig. 4D) did not differ between control and YAC128 mice.
These data indicate that the decrease in AMPA/NMDA ratio specifically observed at motor cortex to DLS synapses was not accompanied by major changes in AMPAR-mediated transmission and suggest therefore that the reduction of the ratio may be the consequence of an increase in NMDA-mediated current.

To better describe possible changes in NMDAR-mediated current at motor cortex to DLS synapses, we pharmacologically isolated optogenetically-induced NMDAR currents and characterized the NMDAR subunit composition. NMDA-EPSC recorded from YAC128 mice presented a slower decay time (Fig. 5A) and an increased ifenprodil sensitivity (Fig. 5B, C). Furthermore, we could not find changes in current/voltage relationship compared to control mice (Fig. 5D).

These data indicated an enriched GluN2B subunit composition at motor cortex to DLS synapses at early stages of HD diseases but no changes in GluN3A contents. NMDAR subunit composition is crucial for the induction of Long Term Depression (LTD) in the DLS (Brigman et al., 2010). We predicted that changes in NMDAR current would therefore impact the induction of this form of synaptic plasticity. We observed that 1Hz stimulation for 5 min induced a significantly stronger NMDA-dependent Long-Term Depression (LTD) at motor cortex to DLS synapses in YAC128 compared to control mice (Fig. 6 A-D). In addition, this stimulation protocol didn’t elicit changes in paired pulse ratio in both groups (Fig. 6 E, F). Altogether these data indicate that at early stage of HD disease there are specific changes in NMDA-mediated currents at motor cortex to DLS synapses and that these changes prime the synapses for changes in NMDA-dependent form of synaptic plasticity.

Finally, we tested whether input specific changes in NMDAR-mediated transmission relate to deficits in motor skills learning. We injected mice with ChR2 expressing virus in the motor cortex in control and YAC128 mice and 6 weeks after the injection we performed single pellet reaching task (Fig 7A). Five minutes after the last training session (day 8 and 9), we sacrificed the animals and cut coronal slices. Interestingly we found that motor training promoted a decrease in AMPA/NMDA ratio at motor cortex to DLS synapses in WT mice (Fig. 7B). Remarkably we found that motor skills learning-induced synaptic plasticity was absent in YAC128 mice (Fig. 7C, D).
These data not only indicated a circuit-identified form of synaptic plasticity associated with motor skills learning, but also suggest that this form of plasticity is needed during the consolidation of motor skill learning.

**DISCUSSION**

We found motor learning alterations in 3-month-old YAC128 mice compared to WT without major motor dysfunctions. While during initial task exposition, motor abilities were equivalent between groups, control mice improved their motor performance across training both in rotarod, swimming tank test and single pellet reaching tasks whereas YAC128 mice didn’t. Associated to these late motor skill learning disruptions, we showed a specific decrease in AMPA/NMDA ratio at motor cortex to DLS synapses in YAC128 mice. Together with a modified NMDAR transmission and enhanced ifenprodil sensitivity in motor cortex to DLS synapses in these mice, we also observed an aberrant NMDAR-dependent LTD induced by optogenetic low frequency stimulation protocol. Moreover, we highlighted that motor cortex to DLS synaptic plasticity occurring in WT mice after single pellet reaching task training was occluded in YAC128 mice.

As previously reported (Chiu et al., 2011) and contrary to another report (Slow et al., 2003) we didn’t observe any change in locomotor activity in YAC128 mice compared to control between 11 and 14 weeks in the circular corridor and in the open field task. Despite the absence of any gross motor impairment, here for the first time we observed a deficit in late motor skill learning in YAC128 compared to wild type. Intriguingly YAC128 mice presented an early phase of motor skill learning during single pellet reaching task and swimming tank that is comparable with the one observed in WT mice. After a pause in the training, while WT mice maintained their acquired learning, YAC128 mice showed a worsening of the motor skills. Our data strongly suggest that YAC128 mice present deficits in motor learning consolidation. Future studies will be needed to investigate whether these deficits are specific within the context of motor learning or if similar deficits in memory consolidation could be observed in different skills. Furthermore, we revealed a lack of improvement in success speed response in single pellet training in YAC128 mice while their error responses remained unaffected compared to WT (Supplementary Fig. 2D). These data suggest that YAC128 mice can perform fine motor movement but in a slower and less reliable manner compared to WT.
Importantly, skill reaching task can be considered as a useful motor learning task that can be performed in premanifest HD mouse model where new alternative protective treatments may be tested with a strong potential translational perspective for HD patients (Klein et al., 2011; 2012). Consequently, a detailed analysis of distinct motor tasks seems essential to better dissect motor alterations in initial stage of the disease both in rodent HD model and in humans.

Although we decreased potent stressful environment effects and considered anxiogenic trait of YAC128 mice by performing handling, habituation and using low light setting conditions, motor tasks cannot be exclusively restricted to motor function and deficits in the performance of motor task could be also highly influenced by anxiety phenotype, attention and motivation deficits, or by depressive like behaviours. Elevated plus maze and the increase floating behaviour during the swimming tank test suggest that premanifest HD mice present anxiety-like and depressive-like phenotypes. Interestingly previous studies have reported similar results in YAC128 mice and in the R6/2 mice model of Huntington disease (Carter et al., 1999; Chiu et al., 2011). Future studies will need to further address these behaviour traits in YAC128 mice and investigate the relevant circuits. Importantly, we reported the same number of total attempts in single pellet reaching task across days in WT and YAC128 mice suggesting no difference in the motivation to perform this task. Even no major anhedonia has been suggested here, the integrity of reward circuits and of dopamine neuromodulation should be further investigated.

Previous studies pinpointed abnormalities in glutamatergic transmission onto MSN in different HD mouse model across age (André et al., 2011; Marco et al., 2013). It has been shown that striatal MSN express higher level of extrasynaptic NMDARs at pre-symptomatic stages (Okamoto et al., 2009; Milnerwood et al., 2010) and that changes in NMDAR localization in MSN recorded from YAC128 mice are independent of the source of glutamatergic input (Kolodziejczyk and Raymond, 2016). Here, we unraveled specific deficiencies of glutamatergic transmission at motor cortex to DLS synapses whereas no AMPA/NMDA ratio changes were observed at thalamostratial synapses at that stage.

Motor learning deficit in HD mouse models have been linked to deficits in striatal plasticity and to aberrant function of NMDARs. Indeed, deletion of striatal NMDAR abolished striatal LTP and impaired learning (Dang et al., 2006). Furthermore, R6/2 mice show less NMDA-dependent LTP in the striatum compared to WT control (Kung et al., 2007) while deficit in
endocannabinoid-dependent LTD was observed in the YAC128 mice (Sepers et al., 2018). Here we show that at motor cortex to DLS synapses, the increased contribution of NMDAR is accompanied by an increase in GluN2B-containing NMDARs. Interestingly, an increase in NMDAR-mediated over an AMPAR-mediated current has been shown in DLS synapses after extended motor training (Yin et al., 2009). Here we show that motor learning-induced synaptic plasticity is occluded in YAC128 mice suggesting that the increased number of NMDARs at motor cortex occludes the motor learning dependent insertion of NMDARs. Remarkably we also found that NMDA-dependent LTD increases at motor cortex to DLS synapses suggesting that depotentiation of NMDA synaptic transmission in this pathway may restore synaptic transmission and may therefore represent a possible therapeutic intervention.

In general, this study proposed new meaningful insight in the synaptopathic mechanisms of HD. We highlight that deficit in motor skill learning-dependent synaptic plasticity at motor cortex to DLS synapses represents an early biomarker for Huntington’s disease. Lastly, we encourage detailed motor investigations at premanifest stage to further screen new potential therapeutically preventive strategies for HD.

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Authors contributions
Ex vivo electrophysiology experiments and behavioural tasks were performed by C.G and P.E.. P.E. and C.G. analysed the in vitro electrophysiology data and the behavioural experiments. P.E. and C.G. performed the statistical analyses for the in vitro electrophysiology and the behavioral experiments. The study was designed and the manuscript written by C.B., C.G., with assistance from P.E.
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FIGURE LEGENDS

Fig. 1. Motor learning alterations in swimming tank test and rotarod task in premanifest stage in YAC128 mice.

A, D. Left: experimental schematic; right: activity trail plots. B, E. Scatter plot and group mean of the distance travelled in circular corridor (b) or in open field (e) in WT and YAC128 mice. C, F. Scatter plot and group mean of the velocity in circular corridor (c) or in open field (f) in WT and YAC128 mice. G. experimental schematic. H. Time course of cross latency in swimming tank test in WT and YAC128 mice. I. Experimental schematic. J. Time course of the fall latency in rotarod in WT and YAC128 mice. Error bars show SEM.

Fig. 2. Forelimb motor skill learning is impaired in premanifest stage in YAC128 mice.

A, B. Timeline (a) and experimental schematic (b) of single pellet reaching task. C. Heat map of successful attempts in WT and YAC128 mice at day 1 and day 8. Each horizontal line in the heat map represents the performance of an individual mouse. D. Average speed of success over the training phase of single pellet reaching task in WT and YAC128 mice. E, F. Scatter plot and group mean of the success rate at day 1 versus day 8 of the single pellet reaching task within WT (e) and YAC128 mice (f). Error bars show SEM.

Fig. 3. No major differences on synaptic strength with electrical stimulation in YAC128 MSN.

A. Experimental schematic. B. Group mean Paired Pulses Ratio for WT and YAC128 MSN. Right: example traces of AMPAR-EPSC at -60 mV in WT and YAC128 MSN. Scale bar 20 ms, 50 pA. C. Group mean amplitude and frequency of asynchronous evoked events in WT and YAC128 MSN. Right: example traces of evoked AMPAR-aEPSCs recorded at -70 mV. Scale bar 50 ms, 25 pA. D. Group mean RI calculated in WT and YAC128 MSN. Right: example traces of evoked AMPAR-EPSCs recorded at -60 mV, 0 mV and + 40 mV. Scale bar 20 ms, 50 pA. E. Group mean AMPA/NMDAR ratio calculated in WT and YAC128 MSN. Right: example traces of evoked AMPAR- and NMDAR-EPSCs at + 40 mV. Scale bar 20 ms, 50 pA. F. Time course of NMDAR-EPSC amplitude during ifenprodil application for WT and YAC128 MSN. G. Group mean ifenprodil inhibition calculated in WT and YAC128 MSN. Scale bar 50 ms, 50 pA. H, I Group mean decay time of NMDAR-EPSCs at + 40 mV before and after ifenprodil in WT (h) and YAC128 (i) MSN. Error bars show SEM.
Fig. 4. AMPA/NMDA ratio is decreased at motor cortex to DLS MSN synapses in YAC128 mice.

A. Left: Experimental schematic; left down: *in vitro* validation of 20 Hz blue light stimulation protocol. Scale: 0.1s, 10 mV; right: epifluorescent image of AAV5-CamKII-hChR2(H134R)-EGFP injection in the thalamus (top) or in the Motor Cortex (down). B, C. Group mean AMPA/NMDAR ratio calculated in WT and YAC128 MSN at thalamo-dorsolateral synapses (b) or at motor cortex to dorsolateral synapses (c). Right: example traces of evoked AMPAR- and NMDAR-EPSCs at + 40 mV. Scale bar 25 ms, 50 pA. D. Group mean RI calculated at motor cortex to DLS synapses in WT and YAC128 MSN. Right: example traces of evoked AMPAR-EPSCs recorded at -60 mV, 0 mV and + 40 mV. Scale bar 20 ms, 50 pA for YAC128 and scale bar 20 ms, 25 pA for WT. E. I-O relationship of motor cortex glutamatergic transmission established by the stimulation duration (synaptic input) and the amplitude of the EPSC (output) in slices from WT and YAC128 MSN. Right: Representative EPSCs evoked by motor cortex terminal stimulation in dorsolateral striatum recorded at -60 mV in WT and YAC128 MSN. F. Group mean amplitude of asynchronous evoked events in WT and YAC128 MSN. Right: example traces of evoked AMPAR-aEPSCs recorded at - 70 mV. Scale bar 50 ms, 25 pA.

Fig. 5. NMDAR transmission dysfunction at motor cortex to DLS MSN in YAC128 mice.

A. Group mean decay time of NMDAR–EPSCs at + 40 mV in WT and YAC128 MSN. Right: example traces of NMDAR-EPSC at + 40 mV. B. Time course of NMDAR-EPSC amplitude during ifenprodil application for WT and YAC128 MSN. C. Group mean ifenprodil inhibition calculated in WT and YAC128 MSN. Right: example traces of NMDAR-EPSCs during ifenprodil (3 µM) bath application. D. I-V plots of normalized and averaged NMDAR-EPSCs of motor cortex to dorsolateral striatal MSN in WT and YAC128 mice and their associated example traces. Scale bar C, D E 10 ms, 50 pA and B, F, 50 ms, 50 pA.

Fig. 6. Aberrant NMDAR dependent LTD at motor cortex to DLS MSN in YAC128 mice

A. Experimental schematic. B, C. Kinetic of AMPA EPSC amplitude normalized to baseline at motor cortex to DLS MSN after low frequency stimulation (1Hz, 5min) in WT and YAC128 groups with picrotoxin (50 µM) (b) or with picrotoxin and APV (30 µM) (c). Top: example traces pre and post 1 Hz, 5min. Scale bar 10 ms, 50 pA. D. Quantification of AMPA EPSC amplitude normalized to baseline at motor cortex to DLS MSN after low frequency stimulation
in WT and YAC128 groups without and with APV application. E, F. Paired pulse ratio pre
and post low frequency stimulation protocol at motor cortex to DLS MSN in WT (e) and
YAC128 mice (f). Error bars show SEM.

Figure 7. Motor training induced motor cortex to DLS MSN plasticity is occluded in
YAC128 mice.
A. Experimental schematic. B, C. Group mean AMPA/NMDAR ratio calculated in WT and
YAC128 MSN in naïve group or after single pellet reaching task training. Right: example
traces of evoked AMPAR- and NMDAR-EPSCs at + 40 mV. Scale bar 10 ms, 50 pA. Error
bars show SEM

Supplementary Fig 1.
A. Quantification of failed trials at day 2 and day 7 of swimming tank test in WT and YAC128
mice. B. Quantification of floating behaviour across training in YAC128 mice. C. Floating
behaviour across swimming tank test training in YAC128 mice. Each horizontal line represents
the floating behaviour of an individual mouse during the 30 s trial for the trial 1 (t1), the trial
2 (t2) and the trial 3 (t3) at day 1, day 2, day 3 and day 7. Each episode of floating behaviour is
represented in dark blue. D, E. Time course of the time to fall in the rotarod for all training
days (d) and for all trials (e) in WT and YAC128 mice. F. Experimental schematic. G, H.
Histograms showing the time spent in the open arms in the elevated plus maze EPM in WT
and YAC128 expressed in seconds (g) or in percentage (h).

Supplementary Fig 2.
A. Histogram representing the body weight of WT and YAC128 mice before initiation of single
pellet reaching task. B. Kinetic of the bodyweight loss during single pellet reaching task in WT
and YAC128 mice. C. Kinetic of total attempts (including success, drop and failed attempts)
across single pellet reaching task training days in WT and YAC128 mice. D. Kinetic of fail
rate during single pellet training in WT and YAC128 mice. E, F, G, H. Scatter plots of
successful attempts per minute at day 1 versus day 5 in WT (e) and YAC128 mice (f) and at
day 5 versus day 8 in WT (g) and in YAC128 mice (h).

Supplementary Fig 3.
A. Group mean Paired Pulses Ratio recorded at interval of 50, 100, 300 ms for WT and YAC128 MSN evoked by motor cortex stimulation. Right: example traces of AMPAR-EPSC at -60 mV in WT and YAC128 MSN.
Circular corridor

30 min

Open field

10 min

Rotarod training

5 RPM to 40 RPM within 240s
2 trials per day with 10 min break

Swimming tank test

60 cm from the red line to the platform
3 trials per day without break

Figure 1. Glangetas, Espinosa and Bellone 2019
Figure 2. Glangetas, Espinosa and Bellone 2019
Figure 3. Glangetas, Espinosa and Bellone 2019
Figure 4. Glangetas, Espinosa and Bellone 2019
Figure 5. Glangetas, Espinosa and Bellone 2019
Figure 6. Glangetas, Espinosa and Bellone 2019
Figure 7. Glangetas, Espinosa and Bellone 2019
Supplementary Figure 1. Glangetas, Espinosa and Bellone 2019
Supplementary Figure 2. Glangetas, Espinosa and Bellone 2019
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