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Age-related alteration of emotional regulation in the BACHD rat model of Huntington disease

Charlotte Lamirault¹, Huu Phuc Nguyen², Valérie Doyère and Nicole El Massioui

¹Institut des Neurosciences Paris-Saclay (Neuro-PSI), UMR 9197, Université Paris Sud, CNRS, Université Paris Saclay, Orsay, 91405, France

²Department of Human Genetics, Faculty of Medicine, Ruhr University Bochum, Bochum, 44801, Germany

³Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Tuebingen, 72076, Germany

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Corresponding author: N. El Massioui, Paris-Saclay Institute of Neuroscience (NeuroPSI), UMR9197, Bt 446, Paris Sud University, 91405 Orsay Cedex, France.

Phone: 01 69 15 49 78; Fax: 01 69 15 77 26; email: nicole.elmassioui@u-psud.fr

ABSTRACT

Huntington's disease (HD) is a genetic neurodegenerative disorder, caused by an expanded CAG repeat in the gene encoding the huntingtin protein. At the premanifest phase, before motor symptoms occur, psychiatric and emotional disorders are observed with high prevalence in HD patients. Agitation, anxiety and irritability are often described but also depression and/or apathy, associated with a lack of emotional control. The aim of the present study was to better circumscribe and understand the emotional symptoms and assess their evolution according to the progression of the disease using a transgenic HD model, BACHD rats, at the age of 4, 12 and 18 months. To achieve this goal, we confronted animals to two types of tests: first, tests assessing anxiety like the light/dark box and the conflict test, which are situations that did not involve an obvious threat and tests assessing the reactivity to a present threat using confrontation with an unknown conspecific (social behavior test) or with an aversive stimulus (fear conditioning test). In all animals, results show an age-dependent anxiety-like behavior, particularly marked in situation requiring passive responses (light/dark box and fear conditioning tests). BACHD rats exhibited a more profound alteration than WT animals in these tests from an early stage of the disease whereas, in tasks requiring some kind of motivation (for food or for social contacts), only old BACHD rats showed high anxiety-like behavior compared to WT, may be partly due to the other symptoms' occurrence at this stage: locomotor difficulties and/or apathy.

INTRODUCTION

Huntington disease (HD) is an autosomal dominantly inherited, progressive neurodegenerative disorder, caused by an expanded CAG repeat in the gene encoding the protein huntingtin (htt). Clinical features of HD include motor, cognitive and psychiatric symptoms. Motor symptoms, which define the symptomatic stage of HD, are well documented. In contrast, psychiatric disorders are less studied, although they often occur before motor symptoms and are significant burdens to patients and family (1-3). At a premanifest stage, exaggerated reactions to changing situations commonly characterize personality changes in HD, with agitation and hyper-anxiety, but also irritability, possibly leading to aggressiveness (4). These frequently reported pathological emotional behaviors also often include depression and/or apathy (5). Moreover, deficits in recognition of facial emotions have also been described with emotion such as anger, disgust and fear being the most poorly recognized in premanifest as well as manifest HD (5-10). Recognition of positive emotions could also be impaired (12,13). However, emotional dysregulation is poorly studied in HD, even though these symptoms are often associated with the number of CAG repeats and the proximity of onset of motor symptoms, and could thus serve as markers of disease progression (13,14).

In animal models of HD, studies of emotional symptoms have described quite contradictory results depending on the age of the animals, the model and the behavioral tests used. Five-six weeks old CAG140 KI and R6/2 mice showed hyper-anxiety in the light/dark box test (15,16), whereas decreased levels of anxiety in 6-week-old R6/2 mice were reported in the elevated plus-maze (17) and in the center of an activity monitor (18). Nine months old BACHD mice showed increased anxiety in the zero-maze test (19,20) and in the light/dark box test, in the open field test and in the elevated plus-maze test when 4 weeks old (21-23). Similarly, 2 months old YAC128 mice displayed anxiety-like behavior in the elevated plus

maze, associated with depressive-like features as assessed by the Porsolt forced swim test and the sucrose preference test (24,25), but did not display anxiety-like behaviour in the hyperthermia test of anxiety (25). Moreover, in the TgHD rat model, 1 or 2 months old animals showed a decrease of anxiety level in social interaction and elevated plus maze tests (26-28), persisting when 6 months old in the social interaction test (29), in the open field test and the elevated zero maze (30). Seven months old TgHD animals showed also a reduced disruption of temporal processing by an emotional cue (31). In symptomatic animals at 12 months of age, emotional blunting associated with an hypersensitivity to negative emotional situations also appeared (32), evidencing a dysfunctional emotional regulation. In another rat model, at 4 months old, BACHD rats showed a reduced anxiety in the elevated plus maze (33) and a reduced impact of emotion on executive functions from 2 months of age (34). In conclusion, studies of emotional regulation remains sparse and have contradictory results, with some of them showing hyper-anxiety, as in patients, whereas most of the others suggesting a phenotype of hypo-anxiety, opposite results that may reflect age-related modulations the time-course of which may depend on the animal model used.

The aim of the present study was thus to further characterize the age-related emotional symptoms in the BACHD rat model (with 97 mixed CAG-CAA repeats), expressing full length mutant human huntingtin (mHTT) in the same developmental and tissue- and cell-specific manner seen as in patients with the disease (33). Anxious behaviors can occur without the presence of a threatening stimulus and can emerge in situations perceived as unavoidable, thus resulting in deficits in concentration, irritability or agitation (35). On the other hand, fear behaviors are expressed in the presence of a potential threat in order to react with the best adapted response (escape, fight or freezing). Accordingly, we assessed the evolution of different facets of emotional behavior, from a premanifest or early stage to later stages of the disease, in independent groups of 4, 12 or 18 months old BACHD rats, using a battery of behavioral tests

assessing (1) anxiety with the light/dark box and the conflict test, and (2) reactivity to a potential threat using confrontation with an unknown conspecific (social behavior) and during fear conditioning.

MATERIALS AND METHODS

Animals

The study was performed on cohorts of male rats that were 4-months-old (20 WT and 20 BACHD, Group 1), 12-months-old (10 WT and 8 BACHD, Group 2) and 18-months-old (14 WT and 10 BACHD, Group 3). All groups run the different tests, except in the conflict test in which only 20 (10 WT and 10 BACHD) of the 4-months-old animals were used.

These were generated from in-house breeding, using hemizygous BACHD males from the TG 5 line (33) paired with WT females (Harlan, France). All animals were on the Sprague-Dawley background. Animals were genotyped according to previously published protocols (33). They performed social behavior, light/dark test, conflict test and fear conditioning in this order. The battery of tests lasted about 2 months. All animals were naïve for all these tests even though groups 1 and 3 previously performed a rat gambling task in another study (34).

Rats were housed by pairs in a temperature- and humidity-controlled colony room and maintained under a 12:12h light/dark cycle with *ad libitum* access to water and food. Experiments were conducted in accordance with the guidelines established by the European Communities Council Directive (2010/63/EU Council Directive Decree) for compliance and use of laboratory animals. The protocol was approved by the ethical committee Paris-Sud (CEEA N°59).

Anxiety tasks

Tests presented below are classically used to study anxiety of "trait", i.e. innate or basal, rather than anxiety of "state", i.e. in reaction to an anxiogenic stimulus (36). In these tests, animals can explore freely their environment.

Light/dark box test: it assesses the conflict between avoidance of a lit place (rats are nocturnal animals) and exploratory behavior. A squared open field (OF; 50cm × 50cm × 40cm) was divided into two compartments: a dark closed compartment (2 lux) and an illuminated open compartment (40 lux). An opening in the center permitted animals to move from one compartment to the other. Rats were first placed in the dark compartment. Latencies of the first exit and time spent in the lit compartment were measured for 10 minutes. An exit was counted when rats' four paws were out of the dark compartment.

Conflict test: This test analyzes the conflict between an appetitive motivation (sweetened concentrated milk) and an anxiogenic context (central lit area). Rats were under food restriction for 2 days before the test (10 g / day / rat). Sweetened condensed milk was previously presented to the rats in their home cages, in order to avoid neophobic bias and ensure a high motivational value. On the first day (habituation phase), rats were individually placed in a circular black open field (OF; 1m in diameter) with a peripheral area dimly enlightened (25 lux) and a center zone (31cm diameter) highly enlightened (90 lux). Rats could freely explore this environment for 10 minutes. On the second day (test phase), a white paper covered the central zone to increase its aversiveness, and a cup of sweetened concentrated milk was placed in its middle. Rats were then placed in the OF (in the peripheral zone) for 10 minutes. The Any-maze software (Ugobasile, Italy) was used to measure the distance traveled, the latency and the duration of consumption of the sweetened concentrated milk.

Reactivity tasks

The following tests measured reactivity to a threatening stimulus. Thus, the observed behaviors intended to measure the level of anxiety of "state", i.e. how the animal reacts, adapts or not, to

an imposed situation. Our tests permitted analyzing responses to different stimuli: reactivity to an unknown conspecific (social behavior) and reactivity to an aversive stimulus (Pavlovian conditioning).

Social behavior: The Social Behavior test was composed of two phases: first, during a 5-min habituation phase, rats could freely explore an open squared field (1m x 1m x 50cm) in which two small empty wire-cages (20cm x 15cm x 14cm) were placed on each side (70cm between the two cages); second, rats were removed from the OF and objects (legos) were placed in one cage and an unfamiliar conspecific in the other cage. Rats could then immediately explore this new configuration for 10 minutes. For analyses, the arena was divided in different areas: the cages (with object and with rat), larger areas (1800cm²) around the cages, and spaces between cages and on the sides. Time spent sniffing the objects and the conspecific rat (nose touching the cage), distance from the object or from the rat, as well as the total distance traveled in the arena were recorded.

Fear conditioning: This test was run in chambers (Coulbourn Instruments, USA; 34.3 x 34.3 x 50.8 cm) placed in a dark sound attenuating cubicle, ventilated by an exhaust fan mounted on the back wall (background noise intensity: 60 dB). The floor of the chambers was composed of 18 rods (0.47 cm in diameter made out of stainless steel and spaced 0.95 cm apart) through which scrambled shocks could be delivered. Pavlovian fear conditioning consisted in pairing a conditioned stimulus (CS, a tone), initially neutral, with an aversive unconditioned stimulus (US) (electrical footshock), so that presentation of the CS alone becomes sufficient to induce a conditioned response of fear (CR, freezing). It was then possible to measure and compare the freezing rate of animals to the conditioning context and to the CS. Four consecutive days were necessary for this test:

- Conditioning in context A (Day 1): rats were individually placed in the operant chambers with the grid, the loudspeaker and a red ambient light (context A). After three minutes of habituation

in this context, 5 trials composed of the CS presentation (Tone: 4 kHz, 80 dB, 30 sec duration) immediately followed by the US (electric footshock: 0.5 sec, 0.8mA) were delivered with an ITI of 4 minutes on average (range 2-6 min). After the last CS-US presentation, the rat remained in the box for 3 additional minutes.

- Extinction in a new context B (Days 2 and 3): rats were placed in a modified operant chamber: solid floor, one of the corners filled with a board to change the shape of the box, a green ambient light and peppermint odor. In this new context, 16 CS alone (without footshock) were delivered to the rats with an ITI of 2 minutes on average (range 2-6 min).

- Extinction in context A (Day 4): rats were placed back in context A for 10 minutes without any CS or US. At the end of these 10 minutes, 3 CS were presented (without footshock) with an ITI of 4 minutes in average (range 2-6 min).

On days 2, 3 and 4, a camera recorded the rats' behavior in order to measure off-line their freezing rates to the CSs and to the context. Some data were lost due to video recording dysfunction, so the concerned rats were excluded from the results

Statistics

ANOVAs were used for statistical analysis. Data are expressed as means \pm SEM. Differences were considered statistically significant when $p < 0.05$. Statistics were based on *a priori* hypothesis of an effect of neurodegeneration with age in BACHD rats.

RESULTS

Light/dark box

Latency to enter the lit compartment (Figure 1A): ANOVAs with genotype (WT vs. BACHD) and age (4, 12 or 18 months) as group factors demonstrated that BACHD rats needed more time than WT animals to enter the lit compartment ($F(1,76)=21.39$; $p < .001$). This difference was significant at 4 months ($F(1,38)=21.39$, $p < .001$) and 18 months ($F(1,22)=5.77$,

$p < .05$) of age. Moreover, latencies increased with age for both groups (WT: $F(2,41)=11.20$, $p < .001$; BACHD: $F(2,35)=9.37$, $p < .001$). There was no age x genotype interaction ($F(2,76)=0.006$).

Time spent in the lit compartment (Figure 1B): BACHD rats spent less time in the lit compartment than WT rats ($F(1,76)=27.88$, $p < .001$), particularly at 4 months ($F(1,38)=41.18$, $p < .001$) and 12 months ($F(1,16)=4.87$, $p < .05$) of age. For both genotypes, the time decreased with age (WT: $F(2,41)=35.78$, $p < .001$; BACHD: $F(2,35)=9.19$, $p < .001$) and the genotype x age interaction was significant ($F(2,76)=11.12$, $p < .001$), likely due to a floor effect reached in the oldest animals.

These results indicate an increasing level of anxiety with age for all animals in this test, and a higher level of anxiety-like behavior in BACHD rats compared to WT rats.

Conflict test

Locomotor activity (Figure 2A): ANOVAs evidenced a significant genotype effect ($F(1,56)=10.24$, $p < .001$) and an effect of age ($F(2,56)=24.86$, $p < .001$). There was no age x genotype interaction ($F < 1$). We also tested whether the effect of age was significant for each genotype as well as whether the effect of genotype was significant at all ages, or only at a specific age. While both genotypes showed a significant decrease in distance travelled with age (WT: $F(2,31)=14.54$; $p < .001$; BACHD: $F(2,25)=11.14$; $p < .001$), a significant effect of genotype was found only in 18 months rats with a distance traveled lower in BACHD than WT animals ($F(1,22)=7.73$, $p < .01$) and not in 4 and 12 months old animals (4 months: $F < 1$; 12 months: $F(1,16)=3.42$, $p = .08$) (Figure 2A).

Latency and duration of consumption: In this task, the balance between anxiety and motivation was analyzed through the latency to reach the center of the OF (where the sweetened concentrated milk was placed) and the duration of consumption of the milk. Statistical analyses

of latencies (Figure 2B) showed a significant genotype effect ($F(1,56)=5.31$, $p<.05$), a significant effect of age ($F(2,56)=7.48$, $p<.001$) and a significant age x genotype interaction ($F(2,56)=6.03$, $p<.001$). Indeed, the effect of age was not significant in WT animals ($F<1$), whereas it was significant in BACHD rats ($F(2,25)=15.17$, $p<.001$). Similarly, there was an effect of age ($F(2,56)=9.15$; $p<.001$) for the duration of consumption (Figure 2C) and a significant age x genotype interaction ($F(2,56)=3.17$, $p<.05$) with no significant genotype effect ($F(1,56)=1.4$). The effect of age was significant in BACHD rats ($F(2,25)=11.04$, $p<.001$), but was not in WT animals ($F(2,31)=0.86$). Parsing these effects demonstrate that 18 months old BACHD rats were slower in consuming the sweetened concentrated milk compared to WT rats (latency: $F(1,22)=18.10$, $p<.001$; duration: $F(1,22)=6.63$, $p<.01$), indicating an increased level of anxiety and/or a decreased motivation in the older BACHD rats. No other comparison involving age or genotype was found significant or close to significance (all $ps>.10$).

Social behavior

Locomotor activity (Figure 3A): As during the conflict test, statistical analyses demonstrated a reduced distance travelled in BACHD rats (genotype effect, $F(1,76)=7.36$, $p<.001$) and in older animals (effect of age, $F(2,76)=5.20$, $p<.001$), with no age x genotype interaction ($F<1$). Parsing the genotype effect showed that only 12- and 18-months old BACHD rats travelled significantly less distance than WT animals (4 months: $F<1$; 12 months: $F(1,16)=13.33$, $p<.001$); 18 months: $F(1,22)=30.61$, $p<.001$).

Distance from and sniffing an unfamiliar conspecific: It is classically accepted that a shorter time interacting with an unfamiliar conspecific indicates a high level of anxiety in the tested rat. For the mean distance from the rat during the test (Figure 3B), there was a global genotype effect ($F(1,76)=3.82$, $p=0.054$), an effect of age ($F(2,76)=12.13$, $p<.001$) and no age x genotype interaction ($F(2,76)=2.27$, $p=.11$). Further analyses showed that only 18-months old

rats stayed further from the conspecific than WT rats ($F(1,22)= 12.49, p<.001$; for 4- and 12-months old rats: $F_s<1$).

Classically, normal rats favor social contacts with conspecific over exploration of inanimate objects. However, the more anxious an animal is, the less it will interact with a congener. A sniffing preference index was calculated by subtracting the time spent sniffing the object from the time spent sniffing the conspecific. This measure (Figure 3C) revealed no difference between genotypes ($F(1,76)=2.05, p=.156$), a tendency for an effect of age ($F(2,76)= 2.68, p=.075$) and no age x genotype interaction ($F(2,76)= 2.01, p=.14$). Specific analyses of 18-months old rats showed a lower sniffing index in BACHD rats compared to WT animals ($F(1,22)=7.54, p<.01$). These tests demonstrated no particular fear of the unfamiliar conspecific in 4- and 12-months old animals independent of their genotype and highlighted an increased level of anxiety in 18-months old BACHD rats.

Fear conditioning

Extinction in context B: the percentage of freezing during the 30 sec preceding the first CS presentation in context B (preCS1) reflects the basal anxiety level of animals and/or the generalization of the anxiety to another context. ANOVAs showed no genotype difference ($F(1,55)= 1.58$), no age x genotype interaction ($F(2,55)= 1.27$) but a progressive increase of the rate of freezing with age ($F(2,55)= 3.72 ; p<.05$). When the CS was presented in extinction, ANOVAs with genotype (WT vs. BACHD), age (4, 12 or 18 months) and CS repetition (first and last blocks of 4 trials of day 1 and day 2) demonstrated a global genotype effect ($F(1,55)= 6.21, p<.05$), an age effect ($F(2,55)= 14.51, p<.001$), a CS repetition effect ($F(3,165)= 57.60, p<.001$) and a significant CS x age interaction ($F(6,165)= 6.23, p<.001$). The CS x genotype ($F(3,165)= 1.45$), age x genotype ($F(2,55)= 0.84$) and CS x age x genotype ($F(6,165)=0.725254$) interactions were not significant (Figure 4A). The observed increased of freezing with age was significant for both WT ($F(2,30)= 6.10, p<.001$) and

BACHD ($F(2,25)= 7.94, p<.001$) rats. Further analyses showed an extinction in both groups at 4 months of age (WT: ($F(1,18)= 5.11, p<.05$; BACHD: $F(3,27)=22.24, p<.001$) with less freezing to the CSs in BACHD rats than in WT rats ($F(1,18)=5.11, p<.05$), but no CS x genotype interaction ($F<1$). At 12 months of age, there was no difference between genotypes ($F(1,15)= 1.54, ns$). WT rats showed significant extinction of freezing ($F(3,24)= 4.36, p<.05$) while BACHD did not ($F<1$). At 18 months of age, both groups had similar high levels of freezing during the two days of extinction (no genotype effect: $F<1$), with an extinction of freezing with CS repetition (WT: $F(3,39)= 25.19, p<.001$; BACHD: $F(3,27)= 7.82, p<.001$), and no CS x genotype interaction ($F<1$).

Bouts of freezing (Figure 4B): The mean duration of freezing episodes (bouts) during CS presentations indicates the ability of animals to maintain immobility. The duration of freezing bouts increased with age ($F(2,55)=21.07, p<.001$) and decreased with CS repetition ($F(3,165)=37.68, p<.001$). There was no difference between genotypes ($F(1,55)=2.18, p=.14$). However, there were a CS x genotype interaction ($F(3,165)= 2.63, p<.05$) and a CS x age interaction ($F(6,165)= 5.62, p<.001$). Parsing these interactions showed that only 4 months old BACHD rats did shorter bouts of freezing than WT animals of the same age ($F(1,18)= 12.98, p<.01$; 12 and 18 months: $F_s<1$). The decrease of freezing bouts' duration with CS repetition was significant for WT animals at 4 ($F(3,27)=9.61, p<.001$), 12 ($F(3,24)= 4.64, p<.05$) and 18 months ($F(3,39)= 24.17, p<.001$), but only at 4 ($F(3,27)=7.89, p<.001$) and 18 months ($F(3,27)= 6.63, p<.01$) for BACHD rats (12 months: $F<1$).

Freezing in the conditioning context (context A): Returning the animals back to the conditioning context induced a lower rate of freezing to the context in BACHD than in WT rats ($F(1,55)= 12.99, p<.001$), a difference significant in 4- ($F(1,18)= 7.48, p<.01$) and 12- months old animals ($F(1,15)= 7.59, p<.01$), but not in 18-months old rats ($F(1,22)= 1.78, p>.05$) (Figure 4C). There was no effect of age ($F(2,55)= 1.32, p=.17$) and no age x genotype interaction

($F(2,55)= 1.99, p=.15$). Presenting the 3 CSs in this context induced a lower rate of freezing in BACHD than in WT rats ($F(1,55)= 7.31, p<.001$), an increasing level of freezing with age ($F(2,55)= 10.05, p<.001$) and a significant extinction of freezing with CS repetition ($F(2,110)= 18.57, p<.001$). There was also a significant CS x age interaction ($F(4,110)= 2.60, p<.05$) and a tendency for an age x genotype x CS interaction ($F(4,110)= 2.17, p=.077$). The age x genotype ($F(2,55)= 2.01$) and CS x genotype ($F(2,110)= 1.21$) interactions were not significant. Further analyses showed that the genotype effect was close to significance in the 4-months old rats ($F(1,18)= 3.68, p=.071$), significant in 12-months old rats ($F(1,15)= 9.05, p<.001$) and non-significant in 18-months old animals ($F<1$). These results demonstrate a more rapid extinction of freezing to the CS when repeated without footshock in BACHD rats, especially in the youngest animals, in both contexts, as well as a virtually absence of freezing to the conditioning context. There was no more difference in 18-months old animals with a high level of freezing to the CS for both groups.

DISCUSSION

The main aim of the present study was to better circumscribe and understand the different emotional symptoms in BACHD rats at three ages (4, 12 and 18 months), likely targeting different levels of neurodegeneration. To achieve this goal, we analyzed animals' behavior when confronted with two types of tests: first, tests assessing reactivity to situations that did not involve an obvious threat, such as light-dark and conflict tests, and tests in which rats were really exposed to a potential threat, such as facing an unknown conspecific in the social test and a stimulus predicting an aversive event in the fear conditioning task.

Beside the effect of genotype, a progressive effect of age was clearly apparent in all rats. In the light/dark test, all rats expressed a progressive increase of latency to enter in the illuminated compartment with age and a progressive decrease in time spent in it. Similarly, all

rats were found to display an increasing level of freezing to the CS with age and longer bouts of freezing in the fear conditioning task. These results evidenced a progressive increase of emotional sensitivity with aging in rats, with intact capabilities to identify the CS as the best predictor of the shock (low contextual freezing registered in the conditioning context, i.e. before CS presentation). This confirms previous results showing an age-dependent increase in anxiety-like behaviors in rats (37-44). Moreover, the progressive reduction of the distance travelled (especially in the conflict test) in all animals indicates also an obvious reduction of locomotor capability with increasing age. The constant progressive alterations of behaviour in 4-, 12- and 18-month old rats (BACHD and WT) exclude an impact of the history of each group on their behaviour in the present tests.

Concerning the genotype effect, different types of disruption were observed in the BACHD rat model for Huntington disease, some of which potentially related to altered emotional processing abilities, while others to motor alterations. These alterations increased progressively with age or appeared solely in the older animals, thus likely reflecting the neurodegenerative process. Interestingly, two experimental situations (light/dark box and fear conditioning) permit to highlight early pathological anxiety-like behavior despite mobilizing two different types of anxiogenic situations (without or with obvious threatening stimulus). These two situations require a passive inhibitory response (stay in the dark compartment or freezing) in contrast to the two other tests in which animals had to move to approach or avoid a situation. In the Light/dark test, BACHD rats needed more time than WT animals to enter the lit compartment and stayed less time in it, from 4 to 18 months of age. These results evidenced a higher level of anxiety from the earlier age and strengthen previous studies using mouse models of HD (15,45,22). Similarly, in the fear conditioning task, BACHD rats showed an initial similar level of conditioning to the CS (freezing to the first CSs in context B) than WT animals thus evidencing correct associative learning abilities as described in other studies

(34,46-48). However, with repetition of CSs presentation, 4 months old BACHD rats showed less freezing to the CS whatever the context, with significantly shorter bouts of freezing. The reduced level of freezing in the younger (4 and 12 months) BACHD rats was mainly due to a more rapid extinction of freezing responses compared to WT, a difference which was not observed in 18 months old rats, with both groups exhibiting a high level of freezing. Shorter durations of each bout of immobility suggest that the younger animals were less able to stand still during the CS, maybe due to the high level of impulsivity already described in 4 months old BACHD rats (48) or in TgHD rats, another rat model of HD (49). This effect disappeared in older groups of age. The more rapid extinction of freezing in the younger BACHD rats could thus be explained by a default of inhibitory control, interfering with behavioral expression of fear/anxiety. Alternatively, an enhanced anxious state could result in an increased US expectancy (50,51). A violation of the US expectancy during the tests could have accelerated the extinction to the CS (50,52,53). Thus, presentations of the CSs alone could lead to a surprising absence of the US and result in a rapid decrease in the associative strength of the CS, inducing a more rapid extinction of the behavioral response (freezing) of BACHD rats. Interestingly, a marked reduced freezing to the conditioning context (context A) was observed in BACHD rats at all ages. This difference, already noticed in previous studies in 4, 6 and 12 months old BACHD rats (46) and in mouse models of HD (18,45), could express a different memory of fear to the CS or to the context in BACHD rats, suggesting a differential alteration of pathways involved in fear conditioning (amygdala and hippocampus).

In contrast, the younger BACHD rats exhibited similar behavior as WT animals in the conflict task and in the social behavior test, and differences appeared with age. In effect, in the conflict test, 18 months old BACHD animals needed more time to go to the rewarded place in the arena and spent less time consuming the sweetened concentrated milk than WT rats. Similarly, they exhibited fewer interactions with the unknown conspecific in the social behavior

task. One possible cause of the BACHD rats' behavior in the conflict test is a reduction of motivation for food especially in old animals. BACHD rats were shown to be less motivated to perform a progressive ratio test and to consume less food compared to their wild type littermates, even though their body weight were similar (34; 54). It is possible however that the reduced consumption rate found in the BACHD rats may be due to motor impairments rather than differences in hunger or motivation (55). Another cause could be the development of apathy with age in HD, related to cognitive deterioration and functional decline intrinsic to the evolution and progression of the disease (56-58). Apathy and/or increased anxiety with age in 18-month old BACHD rats could also explain the reduced interaction with the unknown conspecific in the social behavior test, with no preference for exploring the objects. However, it is worth noting that the oldest BACHD rats exhibited reduced locomotor activity (reduced distance traveled in the arena) in both Conflict and Social behavior tasks, suggesting motor difficulties linked to the progression of the disease. Our results were recorded in male BACHD and WT rats. There is currently no data with female animals in the BACHD model. Unpublished data from our laboratory however show no difference between BACHD female and male rats in cognitive tasks or in reactivity to mild electric footshocks. Nevertheless, it should be interesting to further study female animals in degenerative models.

Emotional disorders, anxiety, and personality changes have been commonly described as appearing before motor symptoms in HD patients (5-12). Even though the primary neuropathological hallmark of the disease is atrophy and neuronal loss in the striatum and the cortex, neurodegeneration rapidly appears in other brain structures (i.e. limbic structures). Atrophies and/or changes in grey matter volume in emotion-relevant and memory related structures (insula, orbito-frontal cortex, amygdala and hippocampus) were observed as correlating with emotion recognition deficits in symptomatic patients (59-68). In rat models of HD, a volume reduction of the amygdala (central nucleus) associated with an increased level of

cellular activity was observed in tgHD transgenic rats, associated with emotional blunting of hedonic perception and high level of impulsive behaviors (49,32). In BACHD rats, a basal pathological hyper-reactivity of amygdala to threat compared to WT rats was observed (47). As the amygdala is known to be a critical structure in the regulation and expression of emotional behavior, its dysfunction may suggest that it could be specifically implicated in the affective symptoms appearing at the early stages of HD.

In HD, a progressive neurodegenerative disease, marked by an age-dependent functional decline, it is quite difficult to disentangle the contributions from different factors (anxiety, motor decline or apathy) which can all impact behavior in old subjects. However, our results showed clear alteration of emotional regulation from an early stage of the disease in the light/dark box and fear conditioning tests, i.e. in tasks where the expected response results from passive inhibition. In contrast, in tests where the expected response is an active behavior (going to the area center to drink or interact with an unknown conspecific), the disorders were only visible in advanced stage of the disease in parallel to the occurrence of motor and apathy symptoms.

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LEGENDS

Figure 1: Light/dark box test: **A:** Mean latency (sec) to enter in the lit compartment for WT (white bars) and BACHD rats (black bars) for the different groups of age (4, 12 and 18 months-old). **B:** Mean time (sec) spent in the lit compartment for WT (white bars) and BACHD rats (black bars) for the three groups of age (4, 12 and 18 months-old). Data are presented as mean + SEM; *: $p < 0.05$.

Figure 2: Conflict test: **A:** Mean distance (m) travelled in the open field during the test for WT (white bars) and BACHD rats (black bars) as a function of age (4, 12 and 18 months-old). **B:** Mean latency (sec) to reach the center of the open field to drink the sweetened concentrated milk for WT (white bars) and BACHD rats (black bars) as a function of age (4, 12 and 18 months-old). **C:** Mean time (sec) spent consuming the sweetened concentrated milk placed in the center of the open field for WT (white bars) and BACHD rats (black bars) for the three groups of age (4, 12 and 18 months-old). Data are presented as mean + SEM; *: $p < 0.05$.

Figure 3: Social behaviour test: **A:** Mean distance (m) travelled in the open field during the test for WT (white bars) and BACHD rats (black bars) as a function of age (4, 12 and 18 months-old). **B:** Mean distance (m) from the conspecific rat, located in a small box in the open field, for WT (white bars) and BACHD rats (black bars) as a function of age (4, 12 and 18 months-old). **C:** Mean sniffing (sec) preference index (time spent sniffing the conspecific - time spent sniffing the objects) for WT (white bars) and BACHD rats (black bars) as a function of age (4, 12 and 18 months-old).

Figure 4: Fear conditioning. **A:** Percentage of freezing during the two extinction days in context B (preCS: mean percent freezing during the 30 sec before the first CS presentation in day 1; CS1-4 and CS13-16: mean percent freezing during the first four and last four 30 sec CS

presentations of each day) for WT (open squares) and BACHD rats (black triangles) for the 4 (left graph), 12 (middle graph) and 18 (right graph) months-old animals. **B:** Mean duration (sec) of bouts of freezing during the first four (CS1-4) and last four (CS13-16) CS presentations of each day for WT (white bars) and BACHD rats (black bars) for the 4 (left graph), 12 (middle graph) and 18 (right graph) months-old animals. **C:** Percentage of freezing during the extinction day when animals are returned back to the conditioning context A (Context: percent freezing during the first 5 minutes in context A without CS presentation; CS1, CS2, CS3: percent freezing during the three CS presentations in this context A) for WT (open squares) and BACHD rats (black triangles) for the 4 (left graph), 12 (middle graph) and 18 (right graph) months-old animals. Data are presented as mean \pm SEM; *: $p < 0.05$.

Figure 1: Light/dark box test

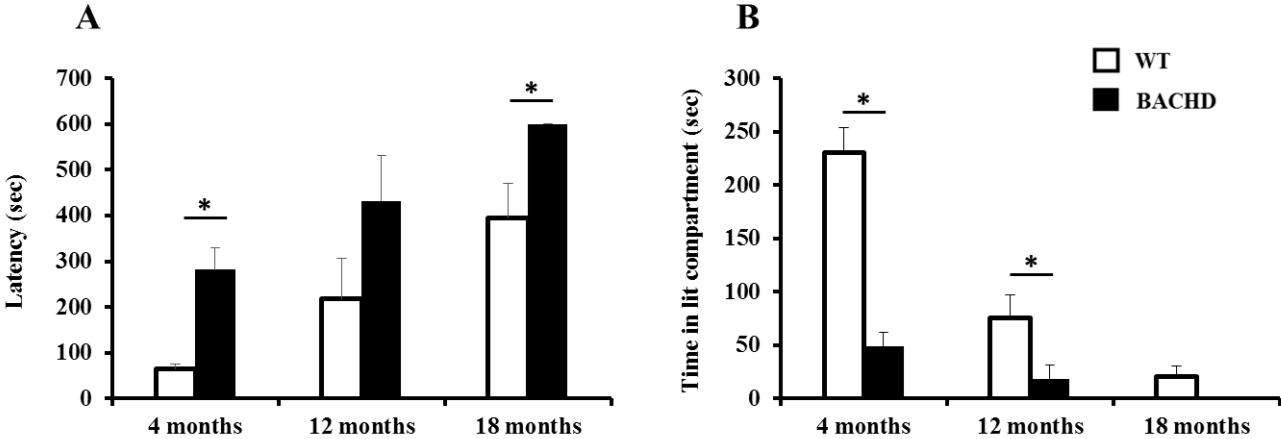


Figure 2: Conflict test

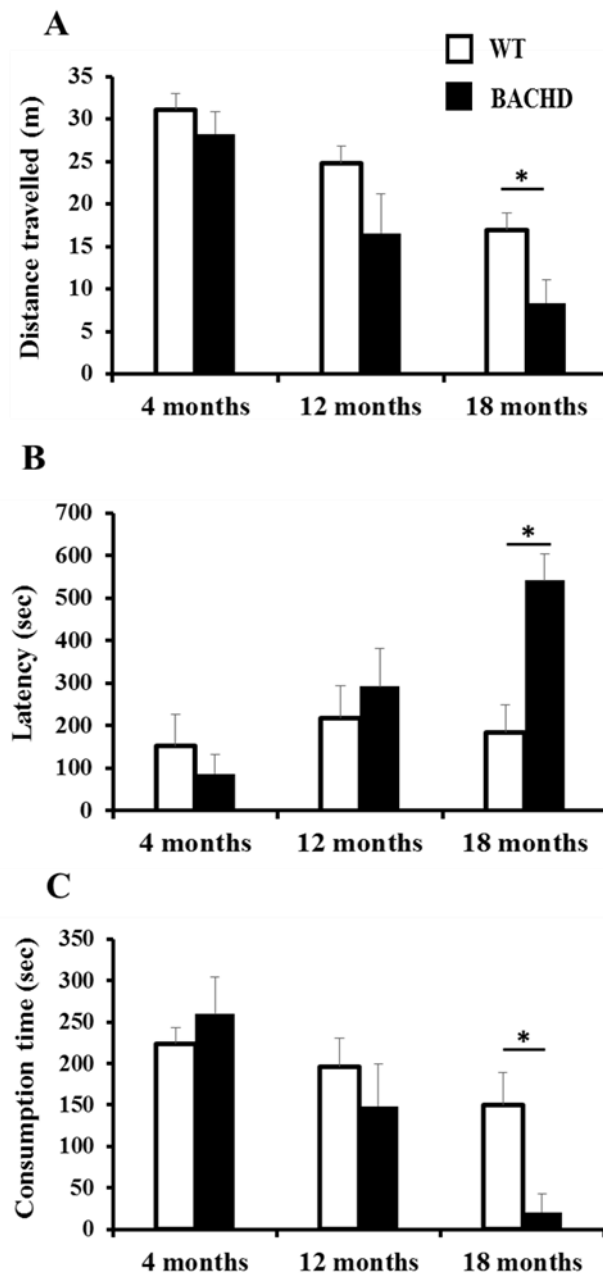


Figure 3: Social behaviour test

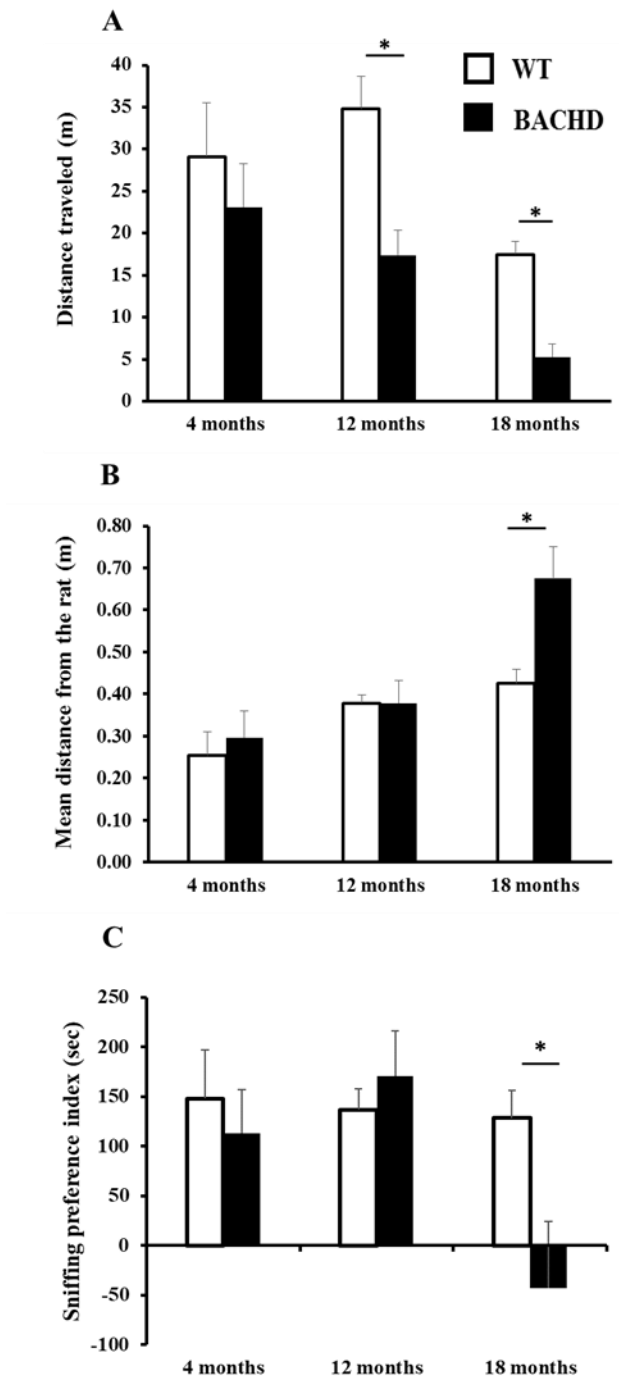
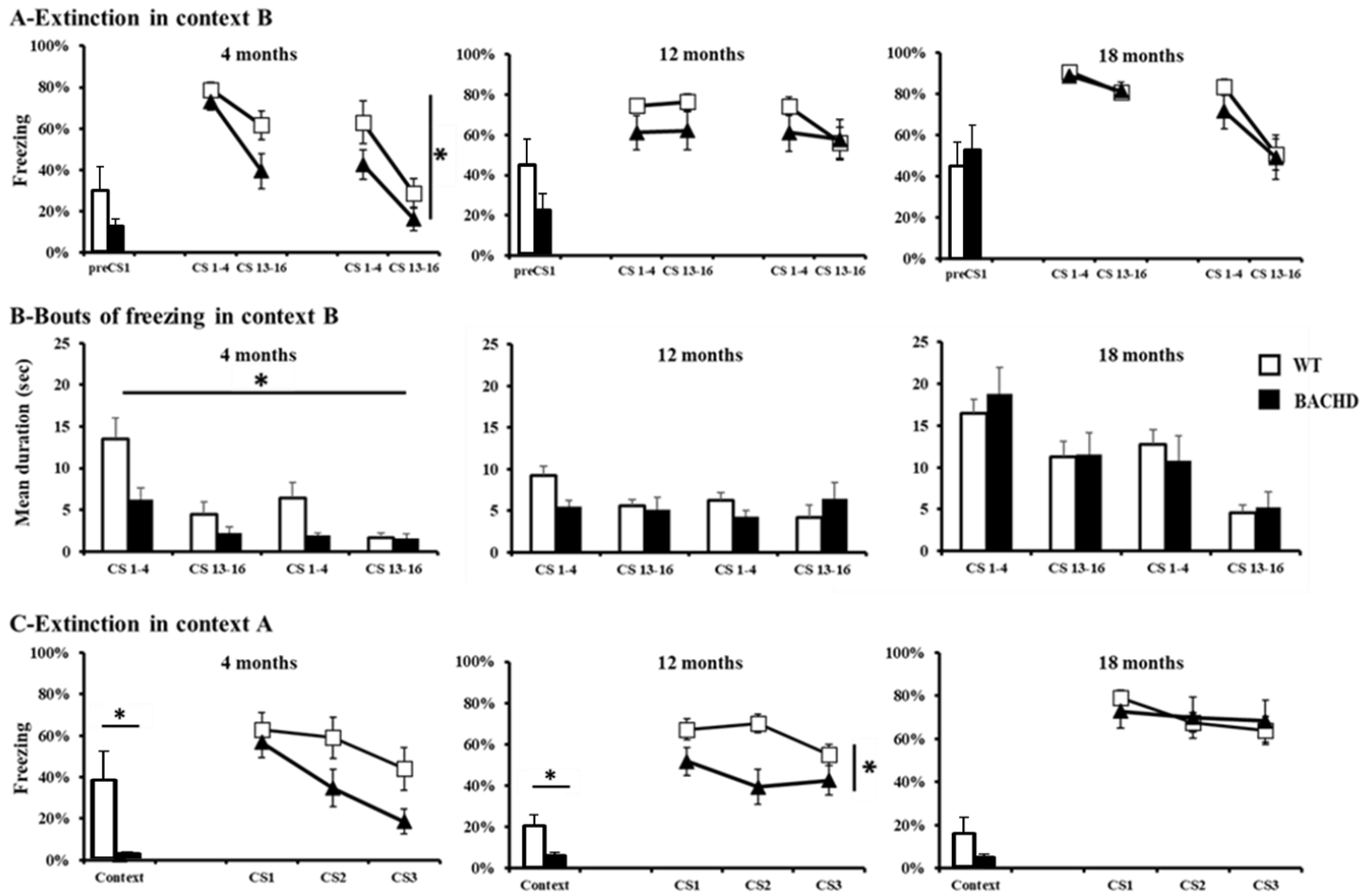


Figure 4: Fear conditioning



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