Research report

Anxiety and risk assessment-related traits in a rat model of Spinocerebellar ataxia type 17

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A R T I C L E   I N F O
Article history:
Received 6 July 2016
Received in revised form 13 December 2016
Accepted 16 December 2016
Available online 23 December 2016

Keywords:
Polyglutamine disease
Model characterization
Disease development
C-Fos neuronal activation
Elevated plus maze

A B S T R A C T
Anxiety as a common feature of several neurodegenerative/polyglutamine diseases is an important aspect for the face validity of an animal model for Spinocerebellar Ataxia type 17 (SCA17). Risk assessment and anxiety-like traits were characterised in 3–6–9 months old rats of a transgenic model for SCA17 using the standard behavioural test elevated plus maze. In addition, c-Fos immunostainings in the basolateral amygdala evaluated neuronal activation in correlation to the behavioural responses. The most prominent behavioural effect was a higher level of risk assessment in the transgenic rats. In addition, an increase in anxiety-related behaviour in these rats was found. Although the EPM caused no overall effect on c-Fos expression, a negative correlation with the anxiety-like behavioural response was observed. Our results suggest that the SCA17 rat model displays an anxious phenotype already at 3 months of age resembling the generalized anxiety in early symptomatic SCA17 patients, thus confirming the validity of this rat model.

1. Introduction

The unraveling and treatment of neurodegenerative diseases is a constant challenge for the scientific community. One substantial group of the neurodegenerative diseases group involves the polyglutamine diseases which consist of Huntington’s, seven types of spinocerebellar ataxia, dentatorubropallidoluysian atrophy (DRPLA), and Spinobulbar muscular atrophy. Spinocerebellar ataxia type 17 is one of the rarest forms of SCA caused by the abnormal expansion of the polyglutamine tract in the TATA box binding protein. The normal PolyQ stretch ranges between 29 and 42 glutamines while the abnormal expansion of 49–63 repeats leads to the onset of SCA17 in humans. Clinical features related to this disease, like many other neurodegenerative diseases, manifest a wide spectrum. In 2004 a human clinical study covered a 5 generation family study which recorded that from its 230 members, 16 members were affected by the disease. Thirteen of the 16 patients had undergone behavioural testing and it was observed that 54% of the affected patients suffered from anxiety as one of the disease symptoms [4]. In addition, in 2003, Rollfs and colleagues studied 4 families with autosomal dominant SCA17, and onset of disease appeared as early as 8 years of age and as late as 43 years of age. All patients suffered from at least one form of ataxia which included gait or limb ataxia, dysarthria, dysmetria and dysphagia, while psychiatric symptoms included schizophrenia, dementia, paranoia, depression and/or anxiety [23].

Due to the deleterious effects on patients’ lives, the importance of studying the psychiatric disorders along with the motor symptoms at both clinical and preclinical level is clearly important. Therefore, including psychiatric symptoms in the modeling of the disease with animals is crucial for the validity of preclinical studies. However, one has to keep in mind that the complexity of such symptoms makes it sometimes challenging to study such symptoms in a fully translational way in animal models. In this study we focused on anxiety as one of the non-motoric symptoms seen in SCA17 patients, and using the standard behavioural test elevated plus maze we concentrated on replicating the anxiety phenotype in an animal model for SCA17. As discussed in literature,
rums are a more suitable model than mice for certain behavioural aspects such as cognition, anxiety, impulsivity and other neuropsychiatric behaviours (Kuijpers, 1999). Therefore, for our study we use a recently generated SCA17 transgenic rat model that carries the full-length human TBP-cDNA with an abnormal CAG/CAA repeat of 64 codons. Primary characterization of the SCA17 rats showed a severe neurological phenotype, loss of body weight, early death [17]. However, the levels of anxiety-like behaviours across different developmental stages of the disease and their maintenance at the symptomatic stage are still unknown, which is important in order to identify whether anxiety can be related to the actual disease development. As in the Kelp and colleagues study there were indications of an anxiety phenotype only at the age of 4 months and taking into account the human situation, we decided to go further with the validation of this rat model and investigate the development of anxiety in one earlier and two later age points. The ages are chosen in relation to the progression and manifestation of the motor symptoms as these have been described in literature: 3 months (pre-symptomatic), 6 months (early symptomatic) and 9 months (late stage of the disease). Thus, we aim at assessing the anxiety-like phenotype as a whole by using the well-established elevated plus maze (EPM) and open field (OF) tests. One other aspect of our design is that the use of automated measurements allows us to expand our analysis and check whether the anxiety-like phenotype can be influenced by the impaired motor capacities of these animals. Our analysis includes the traditional anxiety-related behaviour measured in the EPM (time spent in the open arms) but also the risk assessment behaviour (head dips in the open arms). The reasoning behind including the risk assessment is in order to enrich our knowledge about the anxiety phenotype in this model and detect potentially subtle differences already in the pre-symptomatic stage. Notably, risk assessment is an important element when testing anxiety on the EPM, as it is part of the defensive behaviours in rodents which may mimic the symptoms of generalized anxiety in humans, and its analysis is believed to enhance the sensitivity of the task to detect subtle differences in anxiety [28]. Since anxiety states and anxiety-related behaviours appear to be regulated by a distributed and highly interconnected system of forebrain structures including the basolateral amygdaloid complex [13], we correlate the behavioural response to the neuronal response in the basolateral amygdala, which has been described as the brain center for anxiety [9]. As very little is known about the functionality of the brain circuitry of anxiety in this SCA17 rat model, we chose the basolateral amygdala as our first target expecting the biggest changes (if any) to be presented there among all brain areas. To confirm the anxiety-related phenotype in the SCA17 rats we followed a separate cohort of animals longitudinally over the 3, 6 and 9 months and analysed their responses in the open field. In the open field our analysis included quantification of the locomotor activity over the three age points, but also the velocity with which the animals move in the arena and time spent in the central area as an index of anxiety in the SCA17 rats [25].

2. Methods

2.1. Animals

Three different age groups of maze-naive male rats of both genotypes were used (3 months: SCA17 n = 12 and WT n = 10; 6 months: SCA17 n = 11 and WT n = 10; 9 months: SCA17 n = 10 and WT n = 10). For the open field test, the two genotype groups were tested longitudinally at all 3 age points (3, 6, and 9 months, SCA17 n = 15; WT n = 13). Animals were housed socially1 under conditions in reversed day light cycle and water and food were available ad libitum. Testing was performed during the animals’ active (dark) phase. All experiments were performed after approval of the Ethical Committee for Animal Experiments of the Radboud University Nijmegen Medical Center for compliance to ethical standards and use of laboratory animals according to EU-guidelines.

2.2. Elevated plus maze (EPM)

This conventional test is used to evaluate anxiety-like behaviour in mice and rats based on the strong aversion of these animals to open areas and on the other hand their high motivation to explore, resulting in an approach-avoidance conflict. The test was performed under a general room light intensity of 101x. At the start of the test the rat was placed in the center of the EPM facing one of the open arms. Subsequently, behavioural parameters were recorded and calculated by the EthoVision XT9 tracking system for a total trial duration of 300 s.

The behavioural parameters recorded were: total distance moved, velocity, frequency of entries in open and closed arms, cumulative duration in open and closed arms, total frequency of arm visits, frequency of head dips in open arms (relatively short intensions to explore (“risk assessment”) as indicated by movement of the snout or head out of the closed area with the rest of the body and all four paws remaining in the closed arms), cumulative duration of head dips in open arms, ratio of open/closed arm entries and relative open and closed arms visits.

2.3. Immunohistochemistry

Male SCA17 and WT littermates at 3, 6 and 9 months of age were transcardially perfused 90 min after the exposure to the EPM, through the left ventricle with 4% paraformaldehyde (PFA) (3 months: SCA17 n = 8 and WT n = 8; 6 months: SCA17 n = 7 and WT n = 7; 9 months: SCA17 n = 8 and WT n = 8). The brain was fixed overnight with 4% PFA, and equilibrated for 2 days in 30% sucrose solution. Coronal sections of 40 μm were then cut using a sliding microtome. Brain sections of the basolateral amygdala were blocked to prevent nonspecific binding using a 1% BSA solution and incubated with primary antibody against rabbit c-fos (1:100) (Santa Cruz), and chicken NeuN (1:1000) (Millipore). Secondary antibodies used included anti-rabbit (1:200) (Jackson ImmunoResearch Laboratories, Inc.), anti-chicken (Jackson ImmunoResearch Laboratories, Inc.) Images (for examples see supplementary Fig. S6) were then analysed and compared using ImageJ software (NIH) version 1.50e as a tool for counting neurons number and c-fos positive (+) cells.

2.4. Open field

This assay has been generally used over the past decades to measure general locomotor activity levels and anxiety in rodents and is mainly based on rodents’ motivation to explore novel environments and hide from potential risks, thereby causing initial thigmotaxis, gradually (initial approach-avoidance conflict) evolving into more free exploration of the arena, including the center. The test was performed under red-light conditions and the appa-

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1 All animals were socially housed by 3 or 2 in Makrolon-IVS cages (Techniplast, Italy) until 6 days prior to the EPM testing, when the animals were housed individually in the PhenOType 4500 (Noldus IT, Wageningen, The Netherlands) for homecage monitoring (not included in this manuscript) with food and water provided ad libitum. The uneven number of animals per genotype and age group is due to the variation in the litter sizes.
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