Neuroscience Research 65 (2009) 228–235

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Increased c-fos expression in the central nucleus of the amygdala and enhancement of cued fear memory in *Dyt1* ΔGAG knock-in mice

Fumiaki Yokoi [a], Mai T. Dang [b], Courtney A. Miller [d,e], Andrea G. Marshall [c], Susan L. Campbell [d], J. David Sweatt [d,e], Yuqing Li [a,c,d,*]

[a] Center for Neurodegeneration and Experimental Therapeutics, Department of Neurology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[b] Medical Scholars Program, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
[c] Neuroscience Program, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[d] Department of Neurobiology, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA
[e] Evelyn F. McKnight Brain Institute, School of Medicine, University of Alabama at Birmingham, Birmingham, AL 35294, USA

ARTICLE INFO

Article history:
Received 13 April 2009
Received in revised form 3 July 2009
Accepted 10 July 2009
Available online 18 July 2009

Keywords:
Amygdala
Anxiety
c-fos
Dystonia
Fear memory
Knock-in mouse

ABSTRACT

DYT1 dystonia is caused by a trinucleotide deletion of GAG (ΔGAG) in *DYT1*, which codes for torsinA. A previous epidemiologic study suggested an association of *DYT1* ΔGAG mutation with early-onset recurrent major depression. However, another study reported no significant association with depression, but instead showed an association with anxiety and dystonia. In this study, we analyzed these related behaviors in *Dyt1* ΔGAG heterozygous knock-in mice. The knock-in mice showed a subtle anxiety-like behavior but did not show depression-like behaviors. The mutant mice also displayed normal sensorimotor gating function in a prepulse inhibition test. While normal hippocampus-dependent contextual fear memory and hippocampal CA1 long-term potentiation (LTP) were observed, the knock-in mice exhibited an enhancement in the formation of cued fear memories. Anatomical analysis indicated that the number of c-fos positive cells was significantly increased while the size of the central nucleus of the amygdala (CE) was significantly reduced in the knock-in mice. These results suggest that the *Dyt1* ΔGAG mutation increased the activity of the CE and enhanced the acquisition of the cued fear memory.

1. Introduction

Dystonia is a movement disorder characterized by involuntary, repetitive, sustained muscle contractions or postures. Dystonia is defined by its symptoms and caused by a variety of factors. Acute dystonic symptoms are often observed as side effects of dopamine-receptor blockers used as antipsychotics (neuroleptics) in clinical practice (Dressler and Benecke, 2005). Writer's cramp and musician's cramp, examples of focal dystonia, are caused by intensive training of the fingers (Hallett, 2006; Watson, 2006). Dystonia can also arise from other disorders or injuries, such as brain trauma (O'Suilleabhain and Dewey, 2004). These are classified as secondary dystonia. Dystonia that arises spontaneously without any obvious cause or associated disease is primary in nature, and often has a hereditary component. Although there are 16 classifications of genetic dystonia, only about half of them have identifiable gene mutations associated with them (Breakefield et al., 2008; Camargos et al., 2008; Fuchs et al., 2009).

DYT1 primary generalized early-onset torsion dystonia is caused by mutations in *DYT1* (*TOR1A*) that codes for torsinA. Most patients have a 3 base pair (bp) deletion, ΔGAG, in *DYT1* corresponding to a loss of a glutamic acid residue in the C-terminal region of torsinA (Ozelius et al., 1997). In a rare case, an 18-bp deletion in *DYT1* was also reported in a family (Leung et al., 2001; Doheny et al., 2002). Recently, another transition mutation which causes an Arg288Gln exchange was found in a dystonia patient (Zirn et al., 2008). Some biochemical and cellular studies suggest that the mutation in torsinA causes functional defects that contribute to the pathology of this disease (Hewett et al., 2000; Liu et al., 2003; Cao et al., 2005; Pham et al., 2006). Recent genetic studies suggest that it is the partial loss of torsinA function that contributes to the pathology (Goodchild et al., 2005; Dang et al., 2006a; Yokoi et al., 2008).

Depression and anxiety have been observed in a variety of movement disorder patients (Lauterbach et al., 2003, 2004; Miller et al., 2007). In Parkinson's disease, it was suggested that anxiety and depression may manifest as the first symptoms of Parkinson's disease many years before motor symptoms appear (Lemke et al., 2004). Primary generalized dystonia has been believed to be a purely motor disease, without any associated psychiatric symptoms. However, an epidemiologic study suggested that

* Corresponding author at: CIRC 560, 1530 3rd Avenue South, Birmingham, AL 35294-0017, USA. Tel.: +1 205 996 6299; fax: +1 205 996 7200.
E-mail address: yli@uab.edu (Y. Li).

0168-0102/$ – see front matter © 2009 Elsevier Ireland Ltd and the Japan Neuroscience Society. All rights reserved.
doi:10.1016/j.neures.2009.07.004

early-onset, recurrent major depression occurs more frequently in *DYT1* ΔGAG mutation carriers, regardless of the manifestation of dystonic symptoms (Heiman et al., 2004). The report raised the possibility that *DYT1* ΔGAG mutation functions as a "blue" gene (Richard and McDonald, 2004). However, when the data were analyzed after combining both single and recurrent major depression as a group, there was no significant difference found in the frequency between carriers and non-carriers. Another report suggested that there was no difference in the occurrence of depression between the *DYT1* ΔGAG mutation carriers and non-carriers, although symptomatic DYT1 dystonia patients exhibited increased anxiety, verbal memory retroactive interference, and semantic fluency performance (Balas et al., 2006). These conflicting findings led us to investigate whether the *DYT1* ΔGAG mutation by itself truly causes anxiety and depression.

We previously reported the production of *Dyt1* ΔGAG knock-in heterozygous (KI) mice as a genetic animal model of DYT1 dystonia (Dang et al., 2005). KI mice showed motor and dopaminergic deficits, and brainstem protein aggregation similar to DYT1 dystonia patients. In this study, we performed standard tests for anxiety- and depression-like behaviors, sensorimotor gating, and fear memory formation on these KI mice. KI mice showed significantly enhanced cued fear memory while their contextual fear memory was normal. To determine the mechanism of this alteration, we also performed electrophysiological recordings in hippocampal slices and quantified the number of c-fos positive cells within and the size of the CE and the lateral nucleus of the amygdala (LA).

2. Materials and methods

2.1. Animals

Two groups of KI mice and their littermates were prepared for behavior tests as previously described (Dang et al., 2005). Mice were genotyped using Tcko1 and Tcko2 primer sets (Dang et al., 2005) before the behavioral experiments. A group of 21 KI (11 males and 10 females) and 20 wild-type (WT; 10 males and 10 females) littermates from 6 litters was used in anxiety and depression-like behavioral tests. Mice were housed under a 12-h-light and 12-h-dark cycle. Behavior tests were performed within the last 6 h of the light period after acclimation to a sound-attenuated testing room for 1 h. Behavior tests were performed in the following order: elevated-plus maze, open field, light-dark box, tail suspension, and forced swimming tests. Mice were allowed to rest for one week in between the tests. The second group for prepulse inhibition and fear conditioning tests consisted of 15 KI and 13 control WT males from 6 litters. All of the behavior tests and immunohistochemistry were performed by investigators blind to the genotypes.

2.2. Elevated-plus maze test

Anxiety-related behavior was tested by an elevated-plus maze (PlusMaze version 1.41; AccuScan Instruments, Inc., OH) that consisted of a black plexiglass apparatus with four arms (30 cm long × 5 cm wide) positioned in a cross from a neutral central square (5 cm × 5 cm) as described previously (Cao and Li, 2002). Two opposite arms were surrounded from the three sides by vertical transparent plastic walls 20 cm in height (closed), while the other arms were without walls (open). The plus maze was located 50 cm above the floor under dim indirect lighting (approximately 100 lx on the maze platform). Each mouse (120–137 days old) was placed in the center of the maze facing an open arm. The activity of the mouse was videotaped for 5 min. The number of entries and the time spent in each arm were measured.

2.3. Open-field test

Locomotor activities were measured by infrared sensors of an open-field apparatus connected to a computerized Digiscan System (Accuscan Instruments, Inc., OH) as described previously (Cao and Li, 2002). Each mouse (128–146 days old) was placed in the center of the chamber under bright illumination (approximately 1 klx at the center by a 60 W white bulb) focused on the center of the field. Locomotor activities were automatically recorded by counting the number of breaks in the infrared beams for 15 min.

2.4. Light-dark box test

Anxiety-related locomotion was also measured in a light-dark box as described previously (File et al., 2004). A light box made of transparent plastic (27 cm × 21 cm × 21 cm; illuminated by a 60 W white bulb) and a dark box made of black plastic (27 cm × 21 cm × 21 cm) were connected by a small opening (5 cm × 12 cm) (Cao and Li, 2002). Each mouse (137–154 days old) was placed in the center of the light box facing away from the dark box. Transition events between the two boxes as well as the activity and time spent in the dark box were automatically recorded for 10 min by the infrared sensors of the apparatus (Accuscan Instruments, Inc., OH).

2.5. Tail suspension test

Depression-like behavior was assessed by the standard tail suspension test (TST) using mice ranging from 144 to 161 days old (Porsolt et al., 2001; Yokoi et al., 2006). Each mouse was suspended by its tail with adhesive tape attached to a rope and videotaped. The total immobility time (s) during a 6 min interval was evaluated. Mice that climbed up the rope were excluded from the statistical analysis (3 KI males, 5 KI female, 3 WT males and 2 WT females were excluded).

2.6. Forced swimming test

Depression-like behavior was also assessed by the forced swimming test (FST), one week after TST (Porsolt et al., 2001). Each mouse was placed in a glass jar (diameter = 22 cm, height = 26 cm) filled with water at about 20 °C to the height of 11 cm for 6 min. The test was videotaped and the total immobility time (s) during the last 4 min was evaluated.

2.7. Prepulse inhibition

Prepulse inhibition of acoustic startle responses was measured using the Med-Associates System (Med Associates, St. Albans, VT). Mice ranging from 166 to 181 days old were first habituated to the startle chamber and plexiglass cylinder for 5 min a day for 3 days to reduce stress and unnecessary movements during the test session. On the fourth day, prepulse inhibition testing was performed. The test began with a 5 min acclimation period where the mice were left in the chamber's cylinder undisturbed. The remainder of the test session consisted of three blocks of trials. The first block consisted of six 40 ms, 120 dB sound bursts used as a startle stimulus. The second and third blocks consisted of 26 trials each, with five different trial types: startle only stimulus, no stimulus, or a 20 ms prepulse sound at 4, 8 or 16 dB above the background noise level (65 dB) presented 100 ms before the startle stimulus. The trial types were presented in pseudorandom order throughout each block with a variable inter-trial interval (ranged from 8 to 23 s). The maximum startle amplitude recorded during the 50 ms sampling window was used as the dependent variable. The percent prepulse inhibition of the startle response was calculated as: (startle response to the startle stimuli alone − startle with the prepulse/startle to startle stimuli alone) × 100.

2.8. Hippocampal slice preparation and electrophysiology

Preparation of hippocampal slice and electrophysiological analysis were performed as described previously (Levenson et al., 2004). The mouse brain was immersed in ice-cold cutting saline [CS (in mM): 110 sucrose, 60 NaCl, 3 KCl, 1.25 NaH_2PO_4, 28 $NaHCO_3$, 0.5 $CaCl_2$, 7 $MgCl_2$, 5 glucose, 0.6 ascorbate] prior to isolation of the caudal portion containing the hippocampus and entorhinal cortex. Transverse slices (400 μm) were prepared with a Vibratome (The Vibratome Company, St. Louis, MO). After isolation, cortical tissue was removed and hippocampal slices were equilibrated in a mixture of 50% CS and 50% artificial cerebrospinal fluid [ACSF (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2 $CaCl_2$, 1 $MgCl_2$, 25 glucose] at room temperature. Slices were further equilibrated in 100% ACSF for 45 min at room temperature, followed by a final incubation in 100% ACSF at 32 °C for 1 h. All solutions were saturated with 95% O_2/5% CO_2. Electrophysiological analysis was performed in an interface chamber (Fine Science Tools, Foster City, CA). Oxygenated ACSF (95% O_2/5% CO_2) was perfused into the recording chamber at a rate of 1 ml/min. Electrophysiological traces were digitized and stored using Digidata (models 1200 and 1320A) and Clampex software (Axon Instruments, Union City, CA). Extracellular stimuli were administered on the border of areas CA3 and CA1 along the Schaffer-collaterals using Teflon-coated, bipolar platinum electrodes. Field excitatory postsynaptic potentials (fEPSPs) were recorded in *stratum radiatum* with an ACSF-filled glass recording electrode (1–3 MΩ). All subsequent experimental stimuli were set to an intensity that evoked a fEPSP that had a slope of 50% of the maximum fEPSP slope. LTP was induced by administering three trains of burst stimulation. Each train consisted of 10 sets of bursts (4 stimuli, 100 Hz) with an inter-burst interval of 200 ms. There was a 20 s interval between each stimulus train. Synaptic efficacy was monitored 30 min prior to and 120 min following induction of LTP by recording fEPSPs every 20 s (traces were average for every 2-min interval).

2.9. Contextual and cued fear conditioning

Emotional memory formation and recall was assessed by contextual and cued fear conditioning test as described (Shalin et al., 2006). Fifteen KI and 13 WT male mice from 188 to 203 days old were tested in an automated fear conditioning system (Video Freeze Ver. 1.20.0.0, Med Associates Inc.). Mice were trained by a