Pharmacology, Biochemistry and Behavior 97 (2011) 647–655

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

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

Behavioural and pharmacological examinations in a transgenic mouse model of early-onset torsion dystonia

Nikola Lange [a,*], Melanie Hamann [a], Pullani Shashidharan [b], Angelika Richter [a]

[a] Institute of Pharmacology and Toxicology, Department of Veterinary Medicine, Freie Universität Berlin, Koserstr. 20, 14195 Berlin, Germany
[b] Department of Neurology, Mount Sinai School of Medicine, One Gustave L. Levy Place, NY 10029, USA

ARTICLE INFO

Article history:
Received 30 May 2010
Received in revised form 31 October 2010
Accepted 4 November 2010
Available online 13 November 2010

Keywords:
Dopamine
Movement disorder
DYT1
Basal ganglia
Animal model

ABSTRACT

Early-onset torsion dystonia is an autosomal dominant movement disorder associated with the DYT1 gene (TOR1A) defect which results in a deletion of a glutamic acid residue in the protein torsinA. The pathophysiology of dystonia is poorly understood. Well characterized animal models can help to give insights into the underlying mechanisms and thereby to develop new therapeutics. In the present study, we further characterized transgenic DYT1 mice, which were initially described to exhibit "dystonia-like" postures. In the present study, several behavioural tests in untreated animals did not show strong differences between transgenic and control mice, but nearly all transgenic mice showed "dystonia-like" postures. However, these movements, also observed in control mice, have to be regarded as a clasping reflex. Since dystonia is thought to be related to dopaminergic dysfunctions, pharmacological investigations have been performed to clarify if dopaminergic substances alter motor behaviour in transgenic mice. Chronic treatment with L-DOPA (combined with carbidopa) enhanced the hindlimb claspings only in transgenic mice, while acute applications of drugs, which exert more selective effects on the dopaminergic system, caused similar reactions in transgenic mice and control mice. Therefore, these data do not provide clear evidence for dysfunctions of the dopaminergic system in this mouse model.

1. Introduction

Dystonia is a movement disorder characterized by involuntary, sustained, patterned, and often repetitive muscle contractions of opposing muscles, frequently causing twisting movements or abnormal postures (Fahn et al., 1998; Jankovic, 2006). The pathogenesis of idiopathic types is poorly understood and probably heterogeneous in the different phenotypic and genotypic subtypes (Breakefield et al., 2008). Thus, there is a need for animal models which are clearly defined for various types of dystonia, which can be helpful for giving insights into the underlying mechanisms and for the development of more effective therapeutics (Richter and Löscher, 2000).

Early-onset torsion dystonia is the most common form of primary generalized dystonia. This type of dystonia usually occurs between the 5th and 28th year of age and has a penetrance of only 30–40% in gene carriers (Bandmann and Müler, 2002; Bressman, 2006). Most cases are caused by a 3 bp (GAG) deletion in the DYT1 gene (TOR1A) on chromosome 9q34, resulting in the loss of a glutamic acid residue in the torsinA protein (ΔE-torsinA) (Ozelius et al., 1997). Protein torsinA is a member of AAA+ proteins which are involved in many cellular functions, e.g., vesicle fusion and protein folding (Ogura and Wilkinson, 2001). However, the mechanisms by which the gene defect and the ΔE-torsinA cause dystonia are still unknown, but dopaminergic dysfunctions have been suggested to be involved. TorsinA is expressed in dopaminergic neurons of the substantia nigra pars compacta but also in other non-dopaminergic brain regions (Augood et al., 2003; Rostasy et al., 2003). Furthermore, significant increases in the turnover of dopamine and a reduction in dopamine D_1 and D_2 receptor binding have been reported in the striatum of DYT1 patients (Asanuma et al., 2005; Augood et al., 2002).

Different mouse lines which carry the human gene defect are recommended as models for the early-onset torsion dystonia (Grundmann et al., 2007; Sharma et al., 2005; Shashidharan et al., 2005). The initial descriptions by Shashidharan et al. (2005) led to the conclusion that these transgenic mice show "dystonic-like" postures, whereas other DYT1 mouse models have failed to exhibit such symptoms (Grundmann et al., 2007; Sharma et al., 2005). Since a dystonic phenotype is important for preclinical drug testing, the mouse model generated by Shashidharan et al. (2005) was chosen for further characterization by behavioural and pharmacological experiments in the present study. Initially, several tests were performed in untreated transgenic mice for the evaluation of motor behaviour. Heiman et al. (2004) described anxiety-related behaviour in DYT1 patients. Therefore, the elevated plus maze test was included in the present study. These investigations were carried out in mice at different ages (3, 6, 9 and 12 months) to recognize a possible progression of the disease, as known in DYT1 patients. In order to

* Corresponding author. Institute of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Freie Universität Berlin, Koserstr. 20, 14195 Berlin, Germany. Tel.: +49 3083853532; fax: +49 3083853112.
E-mail address: lange.nikola@vetmed.fu-berlin.de (N. Lange).

0091-3057/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2010.11.005

determine the possible functional role of dopaminergic dysfunctions in transgenic mice, different dopaminergic drugs were acutely administered in the present study. Thereby, attention was paid to the effects on the initially described "dystonic-like" postures (Shashidharan et al., 2005). Since these postures were also observed in control mice, we considered these postures as clasping reflexes. We also determined if possible changes of the clasping reflex could be provoked by long-lasting manipulations of the dopaminergic system.

2. Materials and methods

2.1. Animals

Breeding pairs of transgenic mice carrying the human mutant torsinA were housed in our laboratory. DNA from the offspring was isolated from tail biopsies using the Invisorb® Spin Tissue Mini Kit (Invitek, Berlin, Germany). Genotyping via PCR was performed as reported previously (Shashidharan et al., 2005). Non-littermate mice from the background strain C57Bl/6J were used as control groups. Transgenic and wildtype control mice were kept under the same environmental conditions (room temperature 23 °C ± 1 °C, humidity 50–70%, 12-hour light cycle) and had free access to food (ssnif® Lignocell 3–4 S) and water. All experiments were performed in compliance with international ethical standards and the German Animal Welfare Act (Reg. G 0355/05, G 0005/08 and O 0119/07).

2.2. Behavioural examinations in untreated animals

Untreated transgenic mice (n = 10) and control animals (n = 10) were tested at 3, 6, 9 and 12 months of age. The experiments were carried out from 8.30 am–1 pm at controlled temperature (23–25 °C) and at a light intensity of 320–480 lux. The behavioural tests were performed in a blinded manner in 3-month old transgenic mice, i.e., the experimentator was unaware of the genotype of the mice.

Neurological reflexes were tested as previously described (Crawley, 2000; Hamann et al., 2003), including the eye blink, ear twitch and whisker-orienting reflex. The righting reflex was the latency from turning the mice onto their back to the righting onto all four feet. All experiments were performed by the same experimentator in order to minimize experimental variations.

Locomotor activity was investigated in an activity cage (41 × 41 × 33 cm; Ugo Basile, Comerio, Italy) for 5 min. The number of transitions was registered by the interruptions of photocell beams. The mice were initially placed into the centre of the activity cage, and then the number of transitions (horizontal activity) was measured. Furthermore, the number of rearings (vertical activity) was monitored.

The *rotarod test* was used for evaluation of motor abnormalities. The session started at 4 rpm for 60 s and was then accelerated to 40 rpm within 300 s. The latency to fall off the rotating rod (in s) was noted.

The *strength* of the forelimbs was measured by the grip strength test, while the strength of fore- and hindlimbs was determined by the wire hang test. The grip strength meter (Ugo Basile, Comerio, Italy) was horizontally positioned. The mice were held at their tail and lowered toward the apparatus so that they could grasp a triangular pull bar with their forepaws. Then, the animals were pulled backward in the horizontal plane. The peak force (in g) was measured at the moment the grasp was released. The test was repeated 5 consecutive times within the same session and the average value was calculated. For the wire hang test, the mice were placed on a wire cage lid, which was slightly shaken, causing the mouse to grip the wire. After 60 s, the wire was turned to about 90° approximately 20 cm above the surface of a soft bedding material. Latency to fall onto the bedding was measured for the maximum of 60 s. Finally, the lid was turned upside down (180°) and the latency to fall was again recorded for the maximum of 60 s.

The *footprint test* was used for gait analyses. The mice were placed in a 5 cm wide, 85 cm long corridor. The floor of this corridor was covered with white absorbing paper. The paws were stained with different water based non-toxic inks (red for the forepaws and black for the hindpaws). Subsequently, mice were placed into the corridor in order to walk through it. Three footprint steps from the middle part of the run were measured for forelimb and hindlimb stride length (distance of forward movement between each stride), hind-base width (the distance between the right and left hindpaw), front-base width (the distance between the right and left forepaw) and the overlap of the left respectively right side (distance between forepaw and hindpaw placement). Mean values were used for statistical analysis.

The *staircase test* was used for testing fine motor skills. Therefore, we modified the staircase and the test method described by Baird et al. (2001), previously. We constructed a sliding lid and closed the open front of the staircase. Mice were food deprived for 16 h prior to the test. Immediately after the test session the mice had access to standard lab chow, sunflower seeds and wet food for 8 h. There were 3 training days followed by 5 testing days. Duration of the sessions was 15 min per mouse either for training or for testing. For the test, one 20 mg pellet (Dustless Precision Pellet, TSE Systems, Bad Homburg, Germany) was put into each well of the 8 stairs on the left and right side. Thus, a maximum of 16 pellets was available. The sessions were recorded with a digital camera (DCR-PC100E, Sony, Berlin, Germany). The parameters "eaten pellets", "scattered pellets" and "not eaten pellets" were monitored and counted afterwards. Mean values from the test days were used for statistical analysis.

Anxiety-related behaviour was tested in the *elevated plus maze*. It consisted of two open arms (30 cm × 5 cm) and two enclosed arms (30 cm × 5 cm × 10 cm) at a height of 45 cm and was connected to a video system (TSE Systems, Bad Homburg, Germany). Animals were placed into the centre (5 cm × 5 cm) of the maze facing to an open arm. The time each mouse spent on the open and on the closed arms and the entries into the open and closed arms were recorded during the observation period of 10 min. An entry was counted when the mouse placed all four paws into the arm. In addition, the number of head dips on the open arms and the number of rearings were recorded. The classical parameters related to anxiety behaviour were the time spent on the open arms and entries into the open arms given as percentage of total arm entries. The number of head dips were considered as parameter of risk assessment, while rearings are related to the locomotor activity (Rodgers and Cole, 1994).

2.3. Pharmacological examinations

The effects of different drugs on motor behaviour were tested in the activity cage, the rotarod, the wire hang test, the grip strength test and the footprint test (see above). Vehicle and drug injections were encoded, i.e., the rater of these experiments was blinded to the treatment condition. Some mice received more than one substance. However, there was a wash-out phase of at least two weeks between the administration of different substances. The tests started 20 min after application of the test substance or vehicle, respectively. After measurements in the activity cage, the rotarod test was carried out 25 min, the wire hang test 30–35 min, the grip strength test 40–45 min and the footprint test 50 min after injection.

Additionally, a score-system was established for a better quantification of the previously described "dystonic-like" postures (Shashidharan et al., 2005). Since these postures were also observed in control mice, we considered these postures as clasping reflexes. Therefore, the mice were placed into an empty plastic cage and then observed for 3 h by one rater who was blinded to the treatment condition.

Rating of the clasping reflex was done at the time points 0 (prior to injection) and 10, 20, 30, 45, 60, 90, 120, 150 and 180 min following injections. A severity scale from 0 to 4 was used (Table 1). Rating was carried out in mice hanging at the tail (Fig. 1) as well as in mice walking in the cage. For this purpose the mice were put by the tail approximately 20 cm above the surface of the cage for about 10 s, comparable to the procedure previously described by Yamamoto et al. (2000). Afterwards they were placed back into the cage in order to observe walking for about 10 s. Subsequently, both scores were added, resulting in an achievable total maximum score of 8. Likewise, a scoring-system was used for locomotor activity and stereotype behaviour (sniffing, grooming and Straub tail) at the same time-points as used for the scoring of the clasping reflex. Locomotor activity was rated on a severity scale from −4 to 4 (Table 1). Stereotype behaviour was scored on a severity scale from 0 to 3 (Table 1). The catalepsy response was determined by quantifying the descent latency (in s) in the block test. Mice were placed with the forelimbs on a block with a height of 4 cm and the descent latency, i.e., the duration animals persisted in this position, was noted for a maximum of 30 s.

For pharmacological investigations, the mice received an i.p. injection of vehicle (control trials) or freshly dissolved test substances (injection volume: 10 ml/kg). Each mouse was their own control, i.e., the mouse received the vehicle and one week later the substance. L-dihydroxyphenylalanine (L-DOPA) and carbidopa (Sigma-Aldrich, Munich, Germany), amphetamine (Merck, Darmstadt, Germany), A 68930, quinpirole and raclopride (Tocris, Bristol, UK) were dissolved in isotonic saline (DeltaSelect, Dreieich, Germany), while GBR 12935 (Tocris, Bristol, UK) was dissolved in 0.3% Tween® 80 (Carl Roth, Karlsruhe, Germany) at the lower dose or 2% Tween® 80 at the higher dose. SCH 39166 (Tocris, Bristol, UK) was dissolved in 1% DMSO (Sigma-Aldrich, Munich, Germany). The doses of these drugs were chosen on the basis of several previously performed behavioural experiments in rats and mice (e.g., Adriani and Laviola, 2002; Daly and Waddington, 1992; Darmani, 1998; Deveney and Waddington, 1997; Horvitz et al., 2001; Parra et al., 1999; Quinn et al., 2006).

The dopamine precursor L-DOPA (100 mg/kg) in combination with the decarboxylase inhibitor carbidopa (10 mg/kg) was tested in 3-, 6-, 9-, and 12-months old mice, (n = 10) respectively. The dopamine uptake inhibitor GBR 12935 (dose: 15 mg/kg) was tested in groups of 6, 9 and 12 months old mice (n = 10) by carrying out behavioural tests and score-systems as described above.

Table 1
Rating scale for clasping reflex, motor activity and stereotype behaviour.

Clasping reflex	
0	Normal posture
1	One hindlimb not permanently affected
2	One hindlimb permanently affected
3	Both hindlimbs not permanently affected
4	Both hindlimbs permanently affected
Activity	
−4	Hypolocomotion present all the time and not suppressible by tactile stimuli
−3	Hypolocomotion present all the time but suppressible by tactile stimuli
−2	Hypolocomotion present during more than half of the observation time
−1	Hypolocomotion present during less than half of the observation time
0	Normal activity
1	Hyperlocomotion present during less than half of the observation time
2	Hyperlocomotion present during more than half of the observation time
3	Hyperlocomotion present all the time with short interruptions
4	Hyperlocomotion present all the time without interruptions
Stereotype behaviour	
0	No stereotype behaviour
1	Present during less than half of the observation time
2	Present during more than half of the observation time
3	Present all the time.

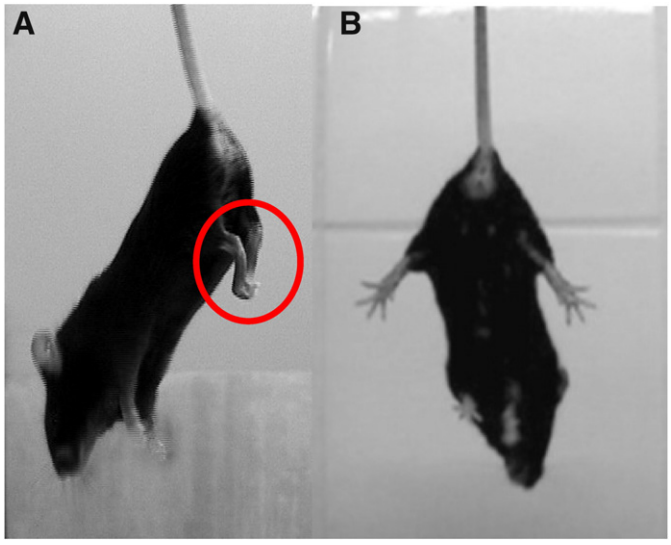

Fig. 1. Hindlimb claspings in a transgenic mouse (A) and a control mouse (B).

Other drugs and doses were only tested in groups of 5 to 10 mice at the age of 6 months. Behaviour and the clasping reflex were scored as described above.

Chronic treatments with L-DOPA were performed to examine if the clasping reflex can be enhanced by long-lasting manipulations of the dopaminergic system. For this purpose, 25 mg/kg L-DOPA + 10 mg/kg carbidopa were administered daily over a period of 20 days in 9 transgenic and 10 wildtype control mice. In addition, 9 transgenic and control mice received the vehicle. Animals were investigated at injection days 1, 4, 8, 11, 15, 18, and 20 with the scoring-systems as described above.

3. Statistical analyses

All calculations were performed with the statistical program SigmaStat (version 3.0). Data are expressed as mean ± SE. The significance of differences in behavioural parameters between different ages within one genotype was calculated with the Friedman variance analyses. If differences were statistically significant (at least $P<0.05$), the Wilcoxon signed rank test for paired replicates was used post hoc to determine which pairs differed (two-sided; $P<0.05$ was considered significant). Differences between age-matched transgenic mice and control mice were calculated with the Mann–Whitney U test. For pharmacological examinations the significance of differences between control trials (vehicle) and test trials (test substances) in the same group of animals was calculated by the Wilcoxon signed rank test. For other comparisons (see above) the difference between substance and vehicle trial was used. To analyse differences in the scoring-system, the maximum score from each animal achieved during 3 hours following vehicle or drug trial was applied. Varieties between different ages within one genotype or differences between the genotypes were calculated with the Mann–Whitney U test.

Significant differences during the 20-day test session within one group (L-DOPA treated transgenic mice, vehicle treated transgenic mice, L-DOPA treated control mice, vehicle treated control mice) were calculated with the Friedman repeated measures analysis of variance. If the difference was statistically significant (at least $P<0.05$), the Wilcoxon signed rank test for paired replicates (two-sided; $P<0.05$ was considered significant) was used post hoc to determine which day differed in comparison with day 1. Significant differences between the groups at the test days were calculate with the Mann–Whitney U test.

4. Results

4.1. Phenotype of transgenic mice

All reflexes of the transgenic and control mice were unaltered (not illustrated). In the *activity cage*, 3-months old mice of both groups showed significant higher horizontal locomotor activity than older mice (Fig. 2A). Only the 3-months old control mice showed more rearings than older control mice. The transgenic mice showed significant less rearings than the control mice at the age of 3 months, while older transgenic mice exhibited comparable rearing rates as control mice (Fig. 2B). Control mice had a better *rotarod* performance at the age of 6, 9 and 12 months than at the age of 3 months (Fig. 2 C). However, no differences were observed between transgenic and control animals at any age. In both groups, a reduction of the *grip strength* occurred in 9- and 12-months old animals in comparison to mice at the age of 3 and 6 months (Fig. 2D) which probably based on habituation. No differences were observed between transgenic and control animals at any age. The *wire hang test* (Fig. 2E) revealed a difference in muscle strength only between month 3 and 6 vs. month 12 in control mice, while transgenic mice did not show age-dependent changes or differences when compared to the control group. In the *footprint test,* the transgenic animals showed a reduced forelimb and hindlimb stride length (Fig. 3) in comparison with the control group at the age of 9 months. Age-dependent increases were observed in both transgenic and control groups and were statistically significant in transgenic mice between the age of 3 vs. 6 and 12, 6 vs. 9 and 12 months as well as in control mice between 3 vs. 6, 9, and 12 months and between 9 and 12 months.

In the *staircase test*, a comparable number of "eaten pellets", "scattered pellets" and "not eaten pellets" (not illustrated) indicates that transgenic mice show no impairments in clasping the pellets, i.e., the skill was unaltered and there was no lateralisation.

In the *elevated plus maze test*, a reduction of all parameters (time spend on open arms, open arm entries, head dips and rearings) between the age of 3 months and the age of 6 months was observed in control mice (Fig. 4) but not in transgenic animals. Between the age of 3 and 9 months the parameters time spend on open arms, open arm entries and head dips were decreased in control mice but not in transgenic animals. All parameters (time spend on open arms, open arm entries, head dips and rearings) were reduced between the age of 3 months and the age of 12 months in control mice, while in transgenic animals only the time spend on open arms was reduced. At the age of 9 months transgenic mice entered the open arms more often than control mice and showed more head dips than control mice at the age of 3 and 12 months.

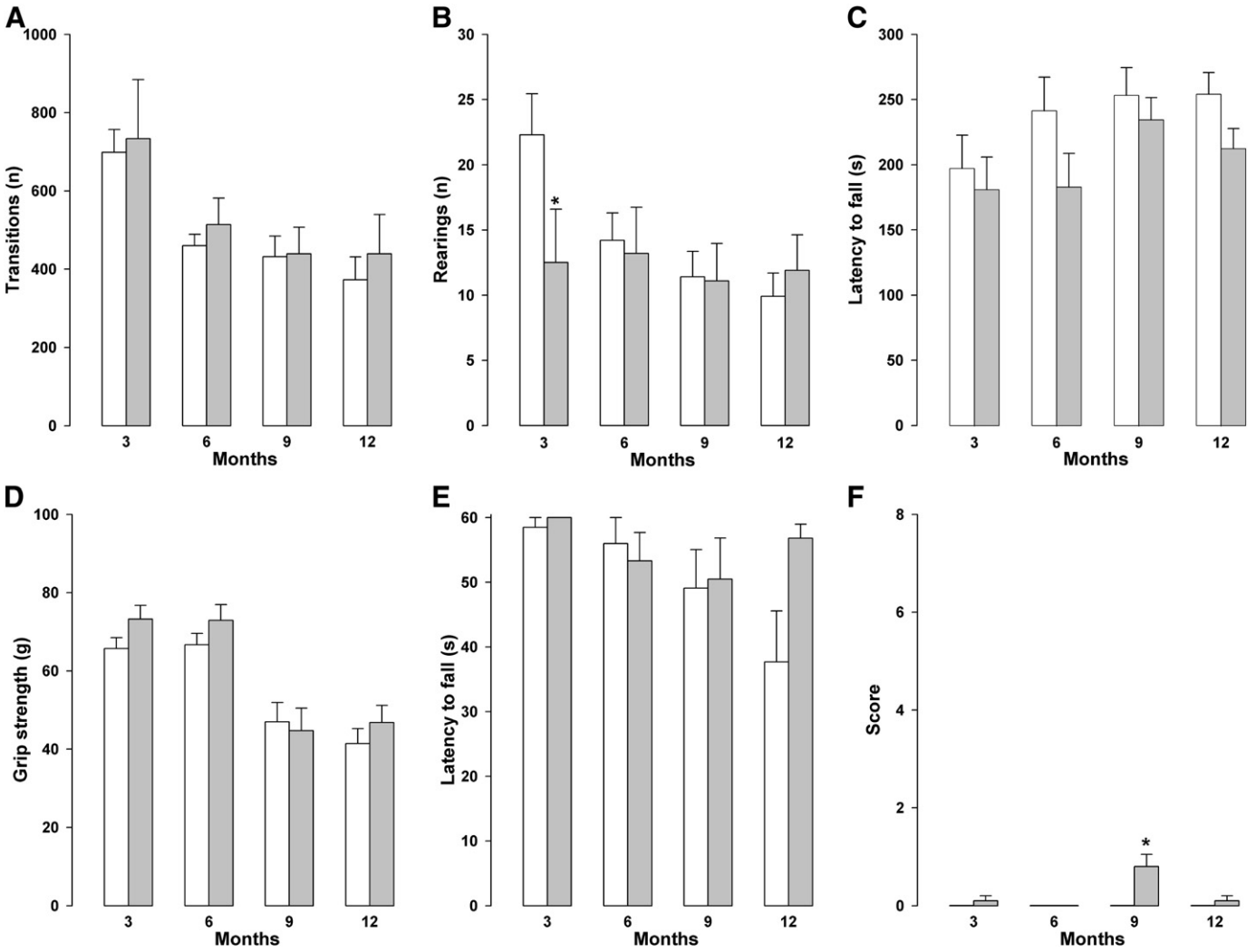

Fig. 2. Spontaneous horizontal (A, number of transitions) and vertical (B, number of rearings) locomotor activity measured in the activity cage, coordination, measured by rotarod (C, latency to fall in s) and muscular strength determined by the grip strength test (D, in g), the wire hang test at 180° (E, latency to fall in s) and the "clasping reflex"-score of untreated animals (F). Data are shown as means + SE of groups of 10 transgenic mice (grey bars) and 10 control mice (white bars) at different ages (3, 6, 9 and 12 months). Asterisks indicate significant differences between transgenic mice in comparison to age matched control mice (*$p<0.05$). Significant differences between different ages are described in the results.

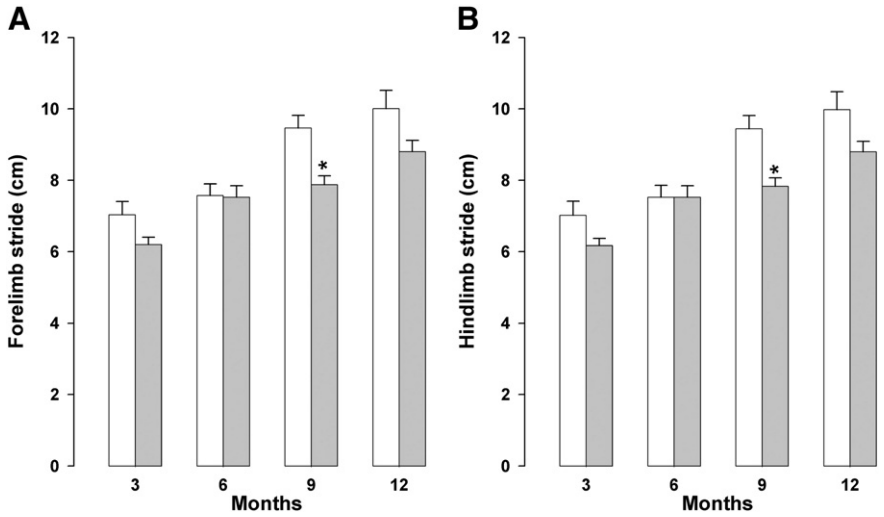

Fig. 3. Stride lengths (forelimb stride A and hindlimb stride B) determined in the footprint test. Data are shown as means + SE of groups of 10 transgenic mice (grey bars) and 10 control mice (white bars) at different ages (3, 6, 9 and 12 months). Asterisks indicate significant differences between transgenic mice in comparison to age matched control mice (*$p<0.01$). Significant differences between different ages are described in the results.

At the age of 9 months transgenic mice showed the clasping reflex significantly more frequently than control mice (Fig. 2F). Occasionally, hindlimb claspings also occurred in untreated control mice (see also vehicle treated mice Fig. 5).

4.2. Pharmacological examinations

In order to determine age-dependent varieties in drug response, the dopamine precursor L-DOPA at a dose of 100 mg/kg in combination with the decarboxylase inhibitor carbidopa at a dose of 10 mg/kg and the dopamine uptake inhibitor GBR 12935 at a dose of 15 mg/kg were tested at different ages. Since there were no age related differences in the drug effects between transgenic and control animals, other compounds and doses were only tested in 6-month old mice.

As shown in Fig. 5A, the "clasping reflex"-score was increased after injection of L-DOPA (100 mg/kg + 10 mg/kg carbidopa) in both animal groups at different ages. No differences were observed between transgenic and control mice. There was a reduction of locomotor activity (Table 2) measured in the activity cage after treatment with L-DOPA in comparison with the vehicle control in both, transgenic and control mice, except of the horizontal activity in 3-month old transgenic mice ($p=0.074$). At the age of 9 months, reduction of locomotor activity was more pronounced after substance application in transgenic animals than in control mice. Comparable results were achieved when using the scoring-system (not illustrated) except of the 3-month old control mice in which only a tendency towards reduction of activity ($p=0.055$) could be observed. The rotarod performance was reduced in L-DOPA treated transgenic and control mice in comparison with control trials which was independent from the age and the genotype (not illustrated). The muscle strength was not affected by L-DOPA in transgenic and control mice (not illustrated). In the footprint test, the stride length of the forelimbs and hindlimbs was reduced after treatment in 6- (hindlimbs: $p=0.004$; forelimbs: $p=0.006$), 9- (forelimbs and hindlimbs: $p=0.020$), and 12-month (forelimbs and hindlimbs: $p=0.002$) old control mice, while in transgenic mice only the forelimb stride was reduced at the age of 9 months ($p=0.049$). At the age of 12 months the stride length of the forelimbs of transgenic mice differed significantly from control mice ($p=0.045$). The hind-

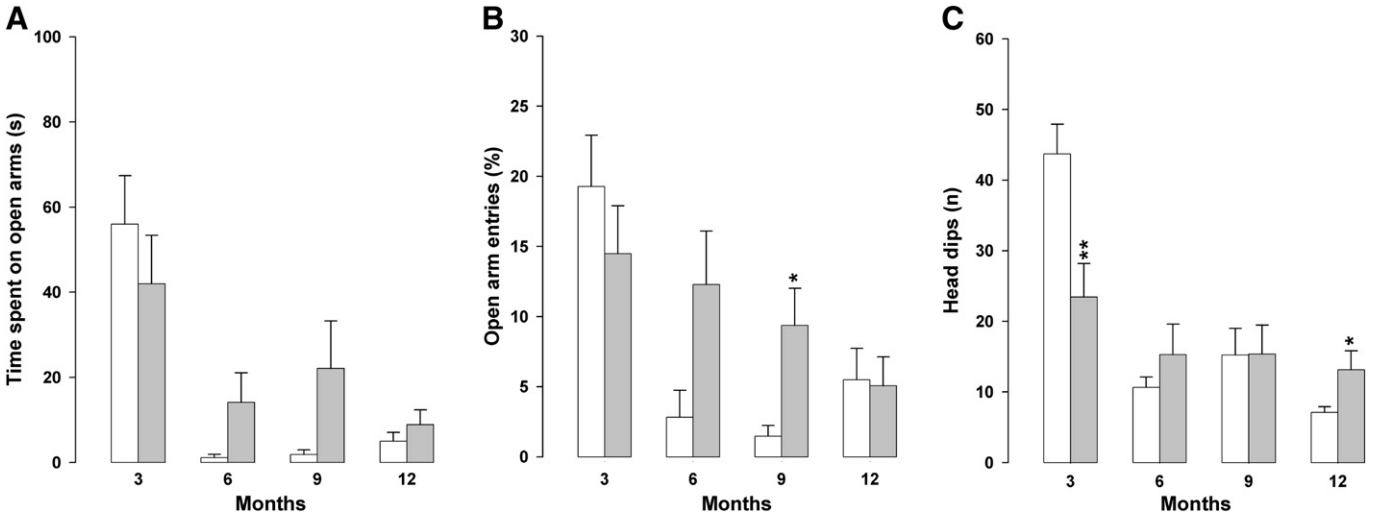

Fig. 4. Elevated plus maze: time spent on the open arms (A), open arm entries (B) and head dips (C). Data are shown as means + SE of groups of 10 transgenic mice (grey bars) and 10 control mice (white bars) at different ages (3, 6, 9 and 12 months). Asterisks indicate significant differences between transgenic mice in comparison to age matched control mice (*$p<0.05$, **$p<0.01$). Significant differences between different ages are described in the results.

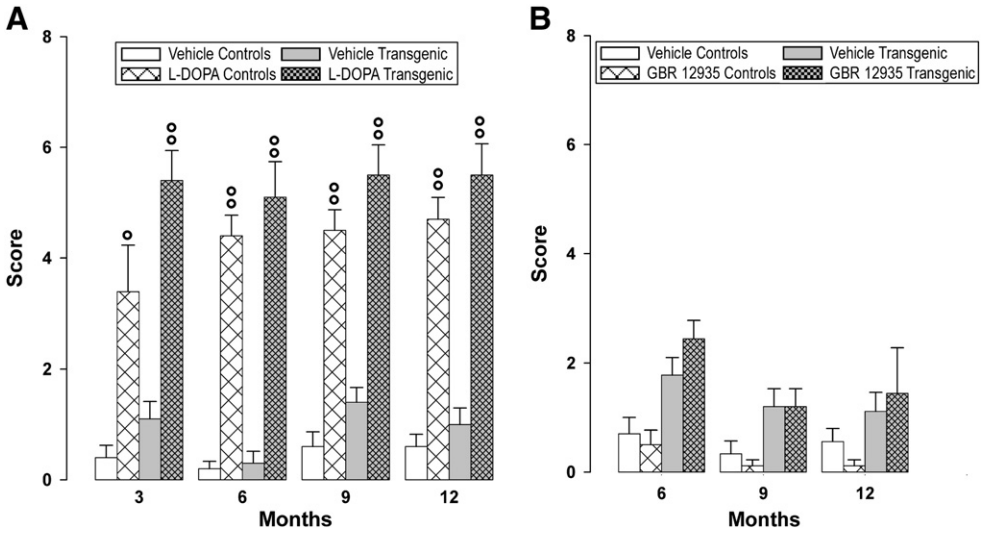

Fig. 5. Maximal "clasping reflex"-score reached during 3 hours after injection of 100 mg/kg L-DOPA + 10 mg/kg carbidopa (A) and 15 mg/kg GBR 12935 (B) or of vehicle in groups of 10 transgenic and 10 control mice (vehicle treated control mice white bars, L-DOPA treated control mice white crossed bars, vehicle treated transgenic mice gray bars, L-DOPA treated transgenic mice gray crossed bars). Data are shown as means + SE of the score individually reached as a maximum within 3 hours after injection. Circles indicate significant increases after treatment with L-DOPA in comparison to vehicle injections in control mice and transgenic mice (°$p<0.05$, °°$p<0.01$). No changes became evident between transgenic and control mice.

base was also decreased in the control mice at the age of 6 ($p=0.002$), 9 ($p=0.008$), and 12 ($p=0.002$) months and was significantly more decreased than in transgenic mice at the age of 6 ($p=0.002$) and 9 ($p=0.025$) months. At the age of 3 months, the overlap of the left side was smaller after L-DOPA treatment in control mice than in transgenic mice ($p=0.014$) (not illustrated). Other behavioural effects of L-DOPA, such as sniffing, grooming and Straub tail, did not significantly differ between transgenic and control mice (not illustrated).

As shown in Fig. 5B, there was no effect of GBR 12935 on the "clasping reflex"-score at a dose of 15 mg/kg. GBR 12935 enhanced the horizontal activity in both groups at all ages (Table 2). The vertical activity was also significantly increased in transgenic mice (6 months) and in control mice (at all ages). The sole difference between transgenic and control mice was a greater enhancement of rearings after drug administration in 6 months old control mice. The rotarod performance and muscle strength were comparable in transgenic and control mice and no drug effects were observed (not illustrated). Sniffing was induced at all ages in both groups except in the group of 6 months old transgenic mice (not illustrated).

The acute effects of drugs on the severity of the "clasping reflex"-score in 6 months old mice are summarized in Table 3. L-DOPA significantly increased the score at the lower dose (25 mg/kg L-DOPA + 10 mg/kg carbidopa) in transgenic and control groups, but the effect was less pronounced than at the higher dose and was therefore chosen for the chronic experiments with L-DOPA (Fig. 6). There was only a tendency towards a higher increase of the "clasping reflex"-score in transgenic mice in comparison to controls ($p=0.050$). The other tested compounds did not exert any significant effects on clasping reflex and there were no differences between transgenic and control mice. However, after administration of quinpirole (1 and 5 mg/kg), the transgenic as well as the control mice tended to show a higher score ($p=0.063$).

The behavioural effects of the tested drugs did not differ between transgenic and control mice (not illustrated). In contrast to the higher dose, L-DOPA (25 mg/kg) did not reduce the locomotor activity and had no effect on sniffing, grooming and Straub tail. Adverse effects of GBR 12935 (45 mg/kg) were stereotypic biting, observed in a few transgenic and control animals. Amphetamine caused licking in 3 transgenic and 3 control animals at a dose of 10 mg/kg amphetamine. After injection of the D_1 agonist A 68930 at a dose of 2 mg/kg, convulsions were observed in both groups, while the D_1 antagonist SCH 39166 did not induce behavioural effects at the administered doses. The D_2/D_3 agonist quinpirole and the D_2 antagonist raclopride had no behavioural effects in both animal groups at the lower doses. At the higher doses of the agonist and the antagonist, transgenic animals showed a higher reduction of locomotor activity after substance-application in comparison with vehicle application than the control mice (not illustrated). There were no behavioural effects, including sniffing, Straub tail and grooming, in control and transgenic animals (not illustrated).

As shown in Fig. 6, chronic treatment with daily injections of 25 mg/kg L-DOPA + 10 mg/kg carbidopa over a time period of 20 days led to an increase of the "clasping reflex"-score in transgenic animals

Table 2
Age-dependence of acute effects of L-DOPA (100 mg/kg i.p. combined with 10 mg/kg i.p. carbidopa) and GBR 12935 (15 mg/kg) on locomotor behaviour in 3-, 6-, 9- and 12-month old transgenic and control mice measured 20 min after drug administration in the activity cage in groups of 10 animals. Data are shown as means ± SE. Circles indicate significant differences between L-DOPA in comparison to vehicle in control and transgenic mice (°$p<0.05$, °°$p<0.01$). Significant differences between different ages are described in the results.

			3 months		6 months		9 months		12 months	
			Controls	DYT1	Controls	DYT1	Controls	DYT1	Controls	DYT1
L-DOPA	Transitions	Substance	92.6 ± 37.3°	279 ± 133	62.9 ± 10.0°°	83.1 ± 39.3°°	51.3 ± 9.35°°	109 ± 27.8°°	97.2 ± 35.3°°	42.3 ± 9.20°°
		Vehicle	358 ± 67.2	630 ± 122	350 ± 51.2	438 ± 95.5	292 ± 35.0	542 ± 75.8	282 ± 23.3	317 ± 93.2
	Rearings	Substance	0.70 ± 0.70°	0.00 ± 0.00°°	0.00 ± 0.00°°	0.78 ± 0.66°	0.00 ± 0.00°°	0.40 ± 0.40°°	1.60 ± 1.30°	0.22 ± 0.22°
		Vehicle	10.4 ± 2.64	17.3 ± 4.56	10.2 ± 2.03	15.1 ± 4.92	6.33 ± 1.35	14.3 ± 3.12	6.90 ± 1.31	11.4 ± 4.38
GBR 12935	Transitions	Substance	n.d.	n.d.	811 ± 98.7°°	1267 ± 208°	723 ± 82.7°°	1227 ± 177°°	708 ± 131°°	908 ± 267°
		Vehicle	n.d.	n.d.	444 ± 57.8	845 ± 78.4	418 ± 63.5	711 ± 145	430 ± 80.5	650 ± 253
	Rearings	Substance	n.d.	n.d.	38.4 ± 3.96°°	38.3 ± 7.22°°	27.6 ± 3.48°	32.4 ± 4.45	22.8 ± 4.12°°	21.5 ± 4.53
		Vehicle	n.d.	n.d.	10.4 ± 1.89	23.2 ± 3.73	11.0 ± 2.04	21.8 ± 6.39	11.8 ± 2.46	19.9 ± 7.53

Table 3
Maximal "clasping reflex"-score reached during 3 hours after injection of L-DOPA (10 mice), GBR 12935, amphetamine, A 68930, SCH 39166, quinpirole and raclopride (each group 5 mice) at an age of 6 months. Data are shown as means ± SE of the score individually reached as a maximum within 3 h after injection. Circles indicate significant changes between L-DOPA and vehicle treated mice in control mice respectively in transgenic mice (°$p<0.01$).

Substance	Dose	Genotype	Max score	
			Substance	Vehicle
L-DOPA	25 mg/kg	controls	1.20 ± 0.20°	0.10 ± 0.10
		DYT1	2.60 ± 0.43°	0.50 ± 0.22
GBR 12935	45 mg/kg	controls	0.80 ± 0.49	0.60 ± 0.40
		DYT1	0.60 ± 0.40	0.60 ± 0.40
Amphetamine	5 mg/kg	controls	0.20 ± 0.20	0.00 ± 0.00
		DYT1	1.40 ± 0.51	1.00 ± 0.32
	10 mg/kg	controls	1.20 ± 0.80	0.60 ± 0.40
		DYT1	1.60 ± 0.60	0.80 ± 0.37
A 68930	1 mg/kg	controls	0.00 ± 0.00	0.60 ± 0.40
		DYT1	0.60 ± 0.00	0.80 ± 0.37
	2 mg/kg	controls	0.40 ± 0.40	0.60 ± 0.40
		DYT1	1.20 ± 0.37	0.80 ± 0.37
SCH 39166	0.3 mg/kg	controls	0.20 ± 0.20	0.20 ± 0.20
		DYT1	1.80 ± 0.73	1.40 ± 0.40
	0.6 mg/kg	controls	0.20 ± 0.20	0.20 ± 0.20
		DYT1	2.20 ± 0.58	1.40 ± 0.40
Quinpirole	1 mg/kg	controls	3.20 ± 0.58	1.00 ± 0.32
		DYT1	5.60 ± 0.98	1.80 ± 0.49
	5 mg/kg	controls	2.80 ± 0.58	0.40 ± 0.40
		DYT1	6.40 ± 0.98	2.00 ± 0.00
Raclopride	0.5 mg/kg	controls	0.00 ± 0.00	0.40 ± 0.24
		DYT1	1.40 ± 0.40	2.40 ± 0.40
	3 mg/kg	controls	0.80 ± 0.37	0.60 ± 0.24
		DYT1	0.80 ± 0.37	1.40 ± 0.40

but not in L-DOPA treated control mice nor in both vehicle treated groups. The enhancement was statistically significant in comparison with the vehicle treated transgenic mice at day 4, 8, 15, 18, and 20 and compared to L-DOPA treated control mice at day 8, 15, 18, and 20. L-DOPA did not exert effects on locomotor activity or other parameters (sniffing, Straub tail and grooming) in transgenic and control mice (not illustrated).

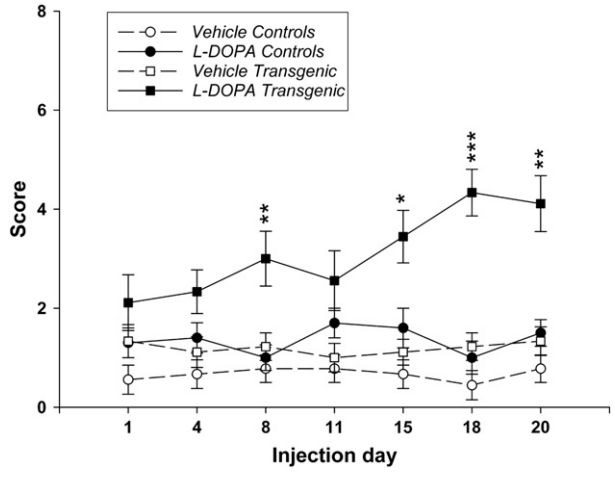

Fig. 6. Effects of chronic treatment with 25 mg/kg L-DOPA + 10 mg/kg carbidopa over 20 days on "clasping reflex"-score in groups of 9 transgenic and 10 control mice and 9 vehicle treated transgenic and control mice. Data are shown as means of the individual maximal scores over 3 hours after injection ± SE. Asterisks indicate significant differences between L-DOPA treated transgenic mice and the L-DOPA treated control mice ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$).

5. Discussion

In the present study, transgenic mice which carry the human DYT1 gene (TOR1A) (Shashidharan et al., 2005) were investigated in comparison to control mice in order to clarify if these mice exhibit behavioural abnormalities or abnormal responses to dopaminergic drugs. Transgenic mice did not show any abnormalities in the activity cage, the rotarod test, the wire-hang test or the grip-strength test, while Shashidharan et al. (2005) had described hyperactivity and circling in about 40% of transgenic mice. It is not clear if this discrepancy might be related to the background of mice in which it was bread subsequently in our laboratory or to a loss of over-expression of transgenic torsinA, as recently described in a succeeding generation of these mice. However, a recent study in a succeeding generation of these mice demonstrated electrophysiological alterations that are similar to human dystonia (Chiken et al., 2008). As shown in the present study, moderate behavioural abnormalities, such as a reduced hindlimb stride, could be also observed in this generation of mice. Since reduced hindlimb strides can be induced by the dopamine depleting drug reserpine and by the dopamine receptor antagonist haloperidol (Fernagut et al., 2002), this observation might point to alterations of the dopaminergic system in transgenic mice.

The hindlimb claspings, described by Shashidharan et al. (2005) as "dystonic-like" movements in the first generation, were also occasionally seen in untreated or vehicle treated control mice in the present study. In both groups, transgenic and control animals, a significantly higher score for the hindlimb claspings occurred after single treatment with L-DOPA. There was also a trend toward an increase after acute injection of the D_2/D_3 receptor agonist quinpirole, although it was not statistically significant in both groups. With regard to the observation of occasional hindlimb claspings in untreated control mice, hindlimb claspings should not be interpreted as a dystonic symptom but as a normal clasping reflex. It should be noted that more pronounced occurrence of this reflex has been also described in weaver mutant mice (Lalonde, 1987b), in staggerer mutant mice (Lalonde, 1987a), in spasmodic mice (Buckwalter et al., 1993), in a mouse model of Huntington's disease (Auerbach et al., 2001) and in a mouse model of Tay-Sachs disease (Miklyaeva et al., 2004), i.e., in mouse models with a different genetic background and different changes in brain functions. In the mouse model of Huntington's disease, the mutant protein huntingtin is also expressed in non nervous tissues (Heng et al., 2008). Thus, various mutations and probably different neurochemical alterations appear to result in enhanced expression of hindlimb claspings. As indicated by the lack of significant acute effects of compounds with more specific effects on the dopaminergic system than L-DOPA, such as the dopamine re-uptake inhibitor GBR 12935, the clasping reflex is not specifically related to the dopaminergic system.

In order to examine if a repeated activation of the dopaminergic system over a long time might provoke dystonic symptoms in transgenic mice, as known for L-DOPA-induced dyskinesias in parkinsonian animals (Lundblad et al., 2002), L-DOPA was administered over a period of 20 days. Although the "clasping reflex"-score increased in transgenic mice, but not in control mice, chronic treatment with L-DOPA did not provoke a dystonic phenotype. While the effects of chronic levodopa administration on neurotransmitters and proteins are well investigated in parkinsonian striata, the knowledge about the effects in normal animals is limited. As shown by Gross et al. (2003), treatment of unlesioned mice with 10 to 100 mg/kg L-DOPA over a period of 20 days did not lead to changes in dopamine receptor binding (D_1, D_2, D_3), whereas in dopamine-depleted mice D_3 receptor binding was increased. Previous studies have shown increased dopamine levels in homogenates of the striatum in transgenic mice which had no obvious behavioural phenotype, while the levels were decreased in mice which exhibited an abnormal behaviour (Shashidharan et al., 2005). Therefore, the more pronounced L-DOPA-induced hindlimb claspings in transgenic

mice (which showed no behavioural alterations) might be related to a higher increase in D_3 receptors.

In another line of DYT1 transgenic mice which do not exhibit a dystonic phenotype, more comprehensive neurochemical studies (including determinations of extracellular dopamine levels by microdialysis) did not reveal any basal changes in dopaminergic transmission (Balcioglu et al., 2007). This is in accordance with other mouse models of DYT1 dystonia (Dang et al., 2005, 2006). However, Balcioglu et al. (2007) described that the amphetamine-induced increase of extracellular dopamine levels was lower in transgenic mice, indicating that the mutation interferes with the release or transport of dopamine. In these mice, D_1 and D_2 receptor binding was unaltered but impaired striatal D_2 receptor function obviously led to enhanced acetylcholine release and GABAergic transmission (Pisani et al., 2006; Sciamanna et al., 2009). Indeed, several lines of evidence support the role of altered striatal dopaminergic neurotransmission in primary dystonia in humans and phenotypic genetic models (Augood et al., 2004; Hamann and Richter, 2004). The response to compounds which act on the dopaminergic system is heterogeneous in human dystonia. For example, in DOPA-responsive dystonia low doses of L-DOPA improve this type of dystonia, while it often aggravates other types of dystonia (Jankovic, 2006). In non-manifesting carriers of the DYT1 dystonia mutation, the striatal D_2 receptor binding was reduced (Asanuma et al., 2005; Breakefield et al., 2008; Rinne et al., 2004). Thus, the gene defect can be associated with neurochemical changes without a manifestation of dystonia in patients and in transgenic mice.

6. Conclusion

The present pharmacological manipulations of the dopaminergic system failed to show a clear evidence for dopaminergic dysfunctions, but chronic treatment with L-DOPA enhanced hindlimb claspings only in transgenic mice. Genotyping was performed to identify transgenic mice, but it remains unclear if the abnormal drug response is related to an overexpression of mutant torsinA. Neurochemical studies have to clarify the role of monoamines in this animal model. Although minor motor abnormalities were found in transgenic mice, it is important to note that the hindlimb claspings, which were also observed in control mice, should not be interpreted as a symptom of dystonia. Thus, a dystonic phenotype is absent and is not inducible by pharmacological manipulations of the dopaminergic system. The lack of a dystonic phenotype limits the use of these transgenic mice for preclinical drug testing.

Acknowledgements

We thank A. Russ for his excellent technical assistance. This study was supported by grants from the DFG (RI845/1-3) and the Forschungskommission of the Freie Universität Berlin and from NIH (NS 43038) to Pullani Shashidharan.

References

Adriani W, Laviola G. Spontaneous novelty seeking and amphetamine-induced conditioning and sensitization in adult mice: evidence of dissociation as a function of age at weaning. Neuropsychopharmacology 2002;27:225–36.
Asanuma K, Ma Y, Okulski J, Dhawan V, Chaly T, Carbon M, et al. Decreased striatal D2 receptor binding in non-manifesting carriers of the DYT1 dystonia mutation. Neurology 2005;64:347–9.
Auerbach W, Hurlbert MS, Hilditch-Maguire P, Wadghiri YZ, Wheeler VC, Cohen SI, et al. The HD mutation causes progressive lethal neurological disease in mice expressing reduced levels of huntingtin. Hum Mol Genet 2001;10:2515–23.
Augood SJ, Hollingsworth Z, Albers DS, Yang L, Leung JC, Muller B, et al. Dopamine transmission in DYT1 dystonia: a biochemical and autoradiographical study. Neurology 2002;59:445–8.
Augood SJ, Keller-McGandy CE, Siriani A, Hewett J, Ramesh V, Sapp E, et al. Distribution and ultrastructural localization of torsinA immunoreactivity in the human brain. Brain Res 2003;986:12–21.
Augood SJ, Hollingsworth Z, Albers DS, Yang L, Leung J, Breakefield XO, et al. Dopamine transmission in DYT1 dystonia. Adv Neurol 2004;94:53–60.
Baird AL, Meldrum A, Dunnett SB. The staircase test of skilled reaching in mice. Brain Res Bull 2001;54:243–50.
Balcioglu A, Kim MO, Sharma N, Cha JH, Breakefield XO, Standaert DG. Dopamine release is impaired in a mouse model of DYT1 dystonia. J Neurochem 2007;102:783–8.
Bandmann O, Müler U. In: Rieß O, Schöls L, editors. Dystonie. NeurogenetikStuttgart; Berlin: Köln Kohlhammer; 2002. p. 338–45.
Breakefield XO, Blood AJ, Li Y, Hallett M, Hanson PI, Standaert DG. The pathophysiological basis of dystonias. Nat Rev Neurosci 2008;9:222–34.
Bressman S. Genetics of dystonia. J Neural Transm Suppl 2006:489–95.
Buckwalter MS, Testa CM, Noebels JL, Camper SA. Genetic mapping and evaluation of candidate genes for spasmodic, a neurological mouse mutation with abnormal startle response. Genomics 1993;17:279–86.
Chiken S, Shashidharan P, Nambu A. Cortically evoked long-lasting inhibition of pallidal neurons in a transgenic mouse model of dystonia. J Neurosci 2008;28:13967–77.
Crawley JN. What's Wrong with my Mouse. New York: Wiley-Liss; 2000.
Daly SA, Waddington JL. Two directions of dopamine D1/D2 receptor interaction in studies of behavioural regulation: a finding generic to four new, selective dopamine D1 receptor antagonists. Eur J Pharmacol 1992;213:251–8.
Dang MT, Yokoi F, McNaught KS, Jengelley TA, Jackson T, Li J, et al. Generation and characterization of Dyt1 DeltaGAG knock-in mouse as a model for early-onset dystonia. Exp Neurol 2005;196:452–63.
Dang MT, Yokoi F, Pence MA, Li Y. Motor deficits and hyperactivity in Dyt1 knockdown mice. Neurosci Res 2006;56:470–4.
Darmani NA. Cocaine and selective monoamine uptake blockers (sertraline, nisoxetine, and GBR 12935) prevent the d-fenfluramine-induced head-twitch response in mice. Pharmacol Biochem Behav 1998;60:83–90.
Deveney AM, Waddington JL. Psychopharmacological distinction between novel full-efficacy "D1-like" dopamine receptor agonists. Pharmacol Biochem Behav 1997;58:551–8.
Gross CE, Ravenscroft P, Dovero S, Jaber M, Bioulac B, Bezard E. Pattern of levodopa-induced striatal changes is different in normal and MPTP-lesioned mice. J. Neurochem. 2003;84:1246–55.
Fahn S, Bressman SB, Marsden CD. Classification of dystonia. Adv Neurol 1998;78:1-10.
Fernagut PO, Diguet E, Labattu B, Tison F. A simple method to measure stride length as an index of nigrostriatal dysfunction in mice. J Neurosci Meth 2002;113:123–30.
Grundmann K, Reischmann B, Vanhoutte G, Hubener J, Teismann P, Hauser TK, et al. Overexpression of human wildtype torsinA and human DeltaGAG torsinA in a transgenic mouse model causes phenotypic abnormalities. Neurobiol Dis 2007;27:190–206.
Hamann M, Richter A. Striatal increase of extracellular dopamine levels during dystonic episodes in a genetic model of paroxysmal dyskinesia. Neurobiol Dis 2004;16:78–84.
Hamann M, Meisler MH, Richter A. Motor disturbances in mice with deficiency of the sodium channel gene Scn8a show features of human dystonia. Exp Neurol 2003;184:830–8.
Heiman GA, Ottman R, Saunders-Pullman RJ, Ozelius LJ, Risch NJ, Bressman SB. Increased risk for recurrent major depression in DYT1 dystonia mutation carriers. Neurology 2004;63:631–7.
Heng MY, Detloff PJ, Albin RL. Rodent genetic models of Huntington disease. Neurobiol Dis 2008;32:1–9.
Horvitz JC, Williams G, Joy R. Time-dependent actions of D2 family agonist quinpirole on spontaneous behavior in the rat: dissociation between sniffing and locomotion. Psychopharmacology (Berl) 2001;154:350–5.
Jankovic J. Treatment of dystonia. Lancet Neurol 2006;5:864–72.
Lalonde R. Motor abnormalities in staggerer mutant mice. Exp Brain Res 1987a;68:417–20.
Lalonde R. Motor abnormalities in weaver mutant mice. Exp Brain Res 1987b;65:479–81.
Lundblad M, Andersson M, Winkler C, Kirik D, Wierup N, Cenci MA. Pharmacological validation of behavioural measures of akinesia and dyskinesia in a rat model of Parkinson's disease. Eur J Neurosci 2002;15:120–32.
Miklyaeva EI, Dong W, Bureau A, Fattahie R, Xu Y, Su M, et al. Late onset Tay-Sachs disease in mice with targeted disruption of the Hexa gene: behavioral changes and pathology of the central nervous system. Brain Res 2004;1001:37–50.
Ogura T, Wilkinson AJ. AAA+ superfamily ATPases: common structure–diverse function. Genes Cells 2001;6:575–97.
Ozelius LJ, Hewett JW, Page CE, Bressman SB, Kramer PL, Shalish C, et al. The early-onset torsion dystonia gene (DYT1) encodes an ATP-binding protein. Nat Genet 1997;17:40–8.
Parra A, Arenas MC, Monleon S, Vinader-Caerols C, Simon VM. Sex differences in the effects of neuroleptics on escape-avoidance behavior in mice: a review. Pharmacol Biochem Behav 1999;64:813–20.
Pisani A, Martella G, Tscherter A, Bonsi P, Sharma N, Bernardi G, et al. Altered responses to dopaminergic D2 receptor activation and N-type calcium currents in striatal cholinergic interneurons in a mouse model of DYT1 dystonia. Neurobiol Dis 2006;24:318–25.
Quinn LP, Stean TO, Chapman H, Brown M, Vidgeon-Hart M, Upton N, et al. Further validation of LABORAS using various dopaminergic manipulations in mice including MPTP-induced nigro-striatal degeneration. J Neurosci Meth 2006;156:218–27.
Richter A, Löscher W. Animal models of dystonia. Funct Neurol 2000;15:259–67.
Rinne JO, Iivanainen M, Metsahonkala L, Vainionpaa L, Paakkonen L, Nagren K, et al. Striatal dopaminergic system in dopa-responsive dystonia: a multi-tracer PET study shows increased D2 receptors. J Neural Transm 2004;111:59–67.

Rodgers RJ, Cole JC. Anxiolytic-like effect of (S)-WAY 100135, a 5-HT1A receptor antagonist, in the murine elevated plus-maze test. Eur J Pharmacol 1994;261:321–5.
Rostasy K, Augood SJ, Hewett JW, Leung JC, Sasaki H, Ozelius LJ, et al. TorsinA protein and neuropathology in early onset generalized dystonia with GAG deletion. Neurobiol Dis 2003;12:11–24.
Sciamanna G, Bonsi P, Tassone A, Cuomo D, Tscherter A, Viscomi MT, et al. Impaired striatal D2 receptor function leads to enhanced GABA transmission in a mouse model of DYT1 dystonia. Neurobiol Dis 2009;34:133–45.
Sharma N, Baxter MG, Petravicz J, Bragg DC, Schienda A, Standaert DG, et al. Impaired motor learning in mice expressing torsinA with the DYT1 dystonia mutation. J Neurosci 2005;25:5351–5.
Shashidharan P, Sandu D, Potla U, Armata IA, Walker RH, McNaught KS, et al. Transgenic mouse model of early-onset DYT1 dystonia. Hum Mol Genet 2005;14:125–33.
Yamamoto A, Lucas JJ, Hen R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 2000;101:57–66.