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Behavioral fragmentation in the D1CT-7 mouse model of Tourette's syndrome

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Summary

Aim: The transgenic D1CT-7 mouse is one of the best-characterized animal models of Tourette's syndrome (TS), exhibiting spontaneous tic-like Head-Body Twitches (HBT) and deficits in sensorimotor gating. This study is aimed at evaluating the behavioral dynamics of these mutants and their potential relevance to TS.

Methods: The behavior of D1CT-7 and Wild Type littermates was firstly assessed by considering frequencies and durations. To detect recurrent real-time behavioral sequences, the multivariate T-pattern analysis was employed. Analyses of transition probabilities among behaviors further provided an overall picture of the behavioral dynamics.

Results: T-patterns and transition matrices revealed in D1CT-7 mice a clear-cut hyperactivity compared to controls, with a lower behavioral organization and a marked shift from cautious sniffing toward locomotion. Moreover, the behavioral patterns of the transgenic mice were pervasively disturbed by intrusive tic-like HBT leading to a marked fragmentation of the behavior. Novel exposure to open field provoked a transient inhibitory control over the disrupting phenotype.

Conclusion: The results of this study show that the D1CT-7 mouse model is subjected to a behavioral fragmentation, with repercussions going beyond the simple tic-like phenomenon. These phenotypes are strikingly akin to behavioral problems observed in patients with TS and further validate the power of this model in summarizing pivotal behavioral aspects of TS.

KEYWORDS
D1CT-7, mice, tic disorders, Tourette's syndrome, T-pattern analysis, transition matrices

1 | INTRODUCTION

Tics are rapid, recurrent, nonrhythmic movements or vocalizations, which are often executed in a partially involuntary, contextually inappropriate fashion.[1,2] The most severe tic disorder, Tourette's syndrome (TS), is characterized by multiple motor and phonic tics, emerging before 18 years of age.[3] Tourette's syndrome (TS) is often comorbid with several psychiatric conditions, including obsessive-compulsive disorder, attention deficit/hyperactivity disorder, and anxiety disorders,[4] which further reduce the quality of life of affected individuals.[5] Even in the absence of comorbidities, the behavioral disturbances and the impulsivity found in patients with TS may be in part explained on the basis of deficits in inhibitory control.[6]

Increasing efforts have been recently devoted to the analysis of the neurobiological basis of tics and tic-related disorders; in particular, a valuable instrument to study these mechanisms is

afforded by animal models.[7] One of the best-characterized models of TS, the D1CT-7 transgenic mouse, was originally developed by Campbell and colleagues[8] by coupling the promoter of the D1 dopamine receptor gene with a sequence encoding the noncytotoxic A1-subunit of the cholera toxin. As a result, D1CT-7 mice harbor a neuropotentiating construct in a D1+ neuronal subpopulation in the somatosensory/insular and piriform cortices and intercalated nucleus of the amygdala which is thought to increase chronic cortical-limbic excitation of the striatum as in TS.[8] D1CT-7 mice exhibit brief (~0.1 seconds), spontaneous stereotypic head or body twitches, which are typically exacerbated by acute stress.[9] These recurring tic-like movements are accompanied by locomotor hyperactivity, perseverative allogrooming, digging, gnawing and leaping/rearing behaviors,[8,10-13] as well as sensorimotor gating deficits and gait impairments.[9,14,15] These deficits are consistent with clinical features in patients with TS,[16-18] supporting the face validity of the dyskinetic manifestations of D1CT-7 mice as reliable models of tics.[19]

Despite these initial characterizations, further research into the overall behavioral dynamics of the D1CT-7 mouse model and its relevance to tic disorders is needed.

Therefore, this study was aimed at evaluating the impact of the D1CT-7 genotype on the behavioral organization displayed by these transgenic mice at their first exposure to the open-field test, which is a widely used and simple trial to assess novelty and exploratory behavior.[20,21] The behaviors of D1CT-7 and Wild Type (WT) mice were analyzed employing quantitative analyses and two different multivariate techniques. First, the T-pattern analysis[22,23] was used to detect real-time behavioral patterns of the two genotypes. In addition, the analysis of transition probabilities[24] was applied to statistically depict the overall likelihood of the components of the behavioral repertoire to be sequentially related.

2 | MATERIALS AND METHODS

2.1 | Subjects and housing

Sixteen (8 D1CT-7 and 8 WT littermates) adult (3- to 4-month-old, weighing 20-30 g), experimentally naïve male Balb/c mice were used. Animals were purchased by Jackson Labs (Bar Harbor, ME, USA) and bred and genotyped as reported by Campbell et al.[8]

As the pattern of inheritance of D1CT-7 mice is autosomal dominant, WT females were bred with heterozygous D1CT-7 sires; this breeding scheme was selected to standardize maternal behavior. To control for litter effects, only 2 mice (1 D1CT-7 and 1 WT, respectively) per litter were used. Animals were housed in group cages with ad libitum access to food and water. The room was maintained at 22°C, on a 12:12 hours light/dark cycle from 08:00 to 20:00 hours. Animals were tested during their light cycle between 12:00 and 16:00 hours to minimize any potential circadian effects. All experimental procedures were in compliance with the National Institute of Health Guidelines and approved by the Institutional Animal Use Committees of the University of Utah.

2.2 | Experimental procedure and apparatus

The open-field apparatus used in the present experiment consists of a square 40 × 40 cm arena surrounded by three opaque Plexiglas walls and a front transparent one. At the beginning of the test, each mouse was placed in the center of the arena and allowed to freely explore for 10 minutes. After each session, the open field was carefully cleaned with ethyl alcohol (70%) to remove possible scent cues left by the preceding animal. The experiments were recorded with a digital video camera, and video files were stored in a PC for the following analyses.

2.3 | Data analyses

A formal description of each component of the behavioral repertoire, namely an ethogram, is represented in Table 1. Particular effort was made to select discrete and easily detectable behavioral components, to confer reproducibility and reliability to each observation. Behavioral responses in the open field included: *Walking* (Wa), *Immobile Sniffing* (IS), *Stretched Sniffing* (SS), *Climbing* (Cl), *Head-Body Twitch* (HBT; describing the tic-like, sudden axial jerks observed in D1CT-7 mice, Figure 1), *Rearing* (Re), and the "*Grooming & Immobility*" cluster. Results from "Grooming & Immobility" cluster were computed altogether due to low occurrences. Jumping was excluded from statistics due to its extremely low occurrence (found only in one D1CT-7 mouse). Recorded behaviors

TABLE 1 Description of studied behavioral components

Horizontal activity cluster
Walking (Wa): the mouse walks around.
Immobile Sniffing (IS): the mouse sniffs the surrounding environment without walking activity. Head and vibrissae movements are produced.
Stretched Sniffing (SS): the mouse stretches its head and shoulders forward and then returns to the original position. Anterior limbs stand still.
Vertical activity cluster
Climbing (Cl): the mouse maintains an erect posture leaning against the walls.
Jumping (Ju): the mouse jumps from the surface of the open field. The body is erected against the walls of the arena.
Head-Body Twitch (HBT): the mouse performs a rapid bottom-up vertical movement. Fast forelimb swing may be present.
Rearing (Re): the mouse retains an erect posture without leaning against the walls.
Grooming and immobility cluster
Face Grooming (FG): the mouse rubs its face with rapid moves of its fore limbs.
Body Grooming (BG): the mouse licks or rubs its body fur.
Front Paw Licking (FPL): the mouse licks or grooms its anterior limbs.
Hind Paw Licking (HPL): the mouse licks or grooms its posterior limbs.
Immobility (Im): the mouse maintains a fixed posture.

were annotated using The Observer (Noldus Information Technology bv, Wageningen, The Netherlands), a PC software similar, in some aspects, to the earlier EthoMac freeware, first designed to quantify and graphically display behavioral patterns of D1CT-7 vs normal mice.[8,10,25] The Observer's generated time-stamped lists of the aforementioned behavioral components were used to run subsequent analyses.

2.3.1 | Quantitative analyses

For each genotype (D1CT-7 or WT), overall behavioral components, irrespective to their specific nature, were reported as mean number ± standard error (SE). In addition, the frequency and the total duration of each component of the behavioral repertoire were calculated, respectively, as mean number and mean time ± SE spent during the testing session.

2.3.2 | T-pattern analysis

To test the hypothesis that the D1CT-7 neuropotentiating transgene affects recurring behavioral sequences, we employed a T-pattern analysis. Such a multivariate analysis was designed to detect the relationship among events through time. To this purpose, we used Theme, a specifically developed software (PatternVision, Ltd, Reykjavik, Iceland). As previously described,[26] the Theme algorithm processes behavioral lists obtained for each mouse with the aforementioned coding process detecting recurring sequences of events characterized by statistically significant constraints among the interval length separating them. Simply stated, the Theme algorithm executes a statistical comparison of the distribution of each possible pair of behavioral components following a bottom-up procedure. For instance, assuming "A" and "B" are two behavioral components with a given distribution along the time window, the "A B" pair is defined as a "T-pattern" only if a statistically significant time interval between the two events is found. In this case, such a T-pattern is indicated as "(A B)" and considered, by the algorithm, as a potential "A" or "B" term in higher order patterns, for example, "((A B) C)." This recursive procedure continues up to any level, and it is completed when no more patterns are found.

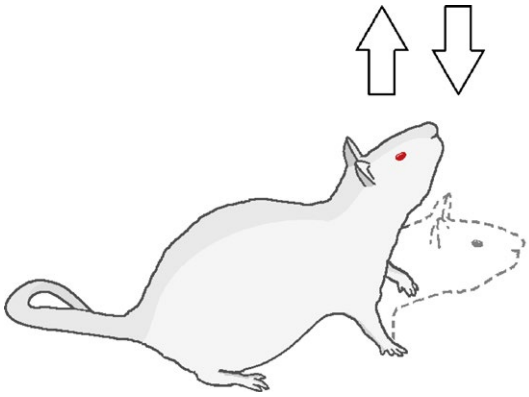

FIGURE 1 Graphical representation of the tic-like component "Head-Body Twitch." See Table 1 for description

Further details, theories, and concepts behind T-pattern analysis can be found in our recent works.[22,23]

2.3.3 | Analysis of transition probabilities

To evaluate the influence of the D1CT-7 transgene on behavioral dynamics, we analyzed the transitions from a given behavioral component to each other reported in the ethogram. The resulting transition matrices (TMs) of all the subjects were summed to obtain a total TM for each group. For each total TM, the following two conditions were met: (i) the number of empty cells was less than 20%; (ii) minimum number of transitions was at least 5 times the number of components.[27] The second step was to transform total TMs into probability matrices, where (i) transition probability from a component to all others is 1 (100%), (ii) each row must sum to 1, and (iii) each transition must be between 0 and 1. Probability matrices, for each group, were graphically expressed through path diagrams, where different transition probabilities were represented by connecting arrows of different thickness. Transition matrices (TMs) were obtained and processed by means of a specific software for matrix manipulation and analysis[28] (Matman version 1.1, Noldus Information Technology).

2.4 | Statistics

To test the influence of genotype on the mean number of total components encoded per group, we performed a *t* test for independent samples. To measure statistically significant differences among mean frequencies and mean durations of each component listed in the ethogram, we employed a multivariate analysis of variance (MANOVA) as described in "Results" section. A two-way mixed analysis of variance (mixed-ANOVA) was employed to test the effect of genotype and time on T-pattern onset-time distributions. The Theme algorithm was employed to detect recurrent behavioral sequences. To this aim, Theme requires specific parameters to be set before a search run for T-patterns is performed.[22,23,29] The detection of any T-pattern relies on a statistical procedure determining a critical interval which significantly underlies timing and recurrence of its behavioral components. The definition of a proper "significance level" determines the highest accepted probability of any critical interval relationship to occur by chance. Additional relevant parameters are: "Minimum occurrences" = minimum number of times a T-pattern must occur to be detected; "Lumping factor" = forward and backward transition probability in which "A" and "B" of a T-pattern (A B) are lumped.

In this study, a significance level of 0.0001 and a lumping factor of 0.90 were selected.

Albeit the critical interval implies a statistical significance, the number of detection could reach the thousands, which raises the question whether T-patterns could be detected only by chance. To exclude this possibility, we performed five shuffling randomizations of the behavioral components encoded for each animal, and then we reanalyzed these data, applying the same search parameters as above. The mean number of patterns from the analysis of randomized data was then compared with patterns from the original dataset.

3 | RESULTS

3.1 | Quantitative analyses

The coding process resulted in a mean number of 831.88 ± 33.03 behavioral components in the D1CT-7 mice and 320.00 ± 48.89 in the WT group. A *t* test for independent samples confirmed a statistical difference between groups ($t_{(14)} = -8.68$; $P < 0.001$). Mean number ± SE of the specific components of the ethogram is shown in Figure 2. The MANOVA was used to compare Wa, IS, SS, CI, HBT, Re, and "Grooming & Immobility" across genotypes. The results of these analyses identified a statistically significant difference in the number of behavioral occurrences comparing the D1CT-7 group with the WT group, $F_{(7,8)} = 14.14$, $P < 0.001$; Wilk's $\Lambda = 0.075$, partial $\eta^2 = 0.93$. D1CT-7 mice were significantly different from WT with respect to the following behavioral components: Wa ($F_{(1,14)} = 70.32$; $P < 0.001$; partial $\eta^2 = 0.83$); IS ($F_{(1,14)} = 12.03$; $P < 0.005$; partial $\eta^2 = 0.46$); SS ($F_{(1,14)} = 17.39$; $P < 0.001$; partial $\eta^2 = 0.55$); CI ($F_{(1,14)} = 24.97$; $P < 0.001$; partial $\eta^2 = 0.64$); HBT ($F_{(1,14)} = 50.66$; $P < 0.001$; partial $\eta^2 = 0.78$); Re ($F_{(1,14)} = 34.26$; $P < 0.001$; partial $\eta^2 = 0.71$). No difference was found comparing occurrences of "Grooming & Immobility" cluster ($F_{(1,14)} = 3.60$; $P = 0.079$; partial $\eta^2 = 0.21$). See Table 1 for abbreviations.

Cumulative mean time ± SE of each specific component of the ethogram is shown in Figure 2. MANOVA showed a statistically significant difference when comparing the D1CT-7 group and the WT group

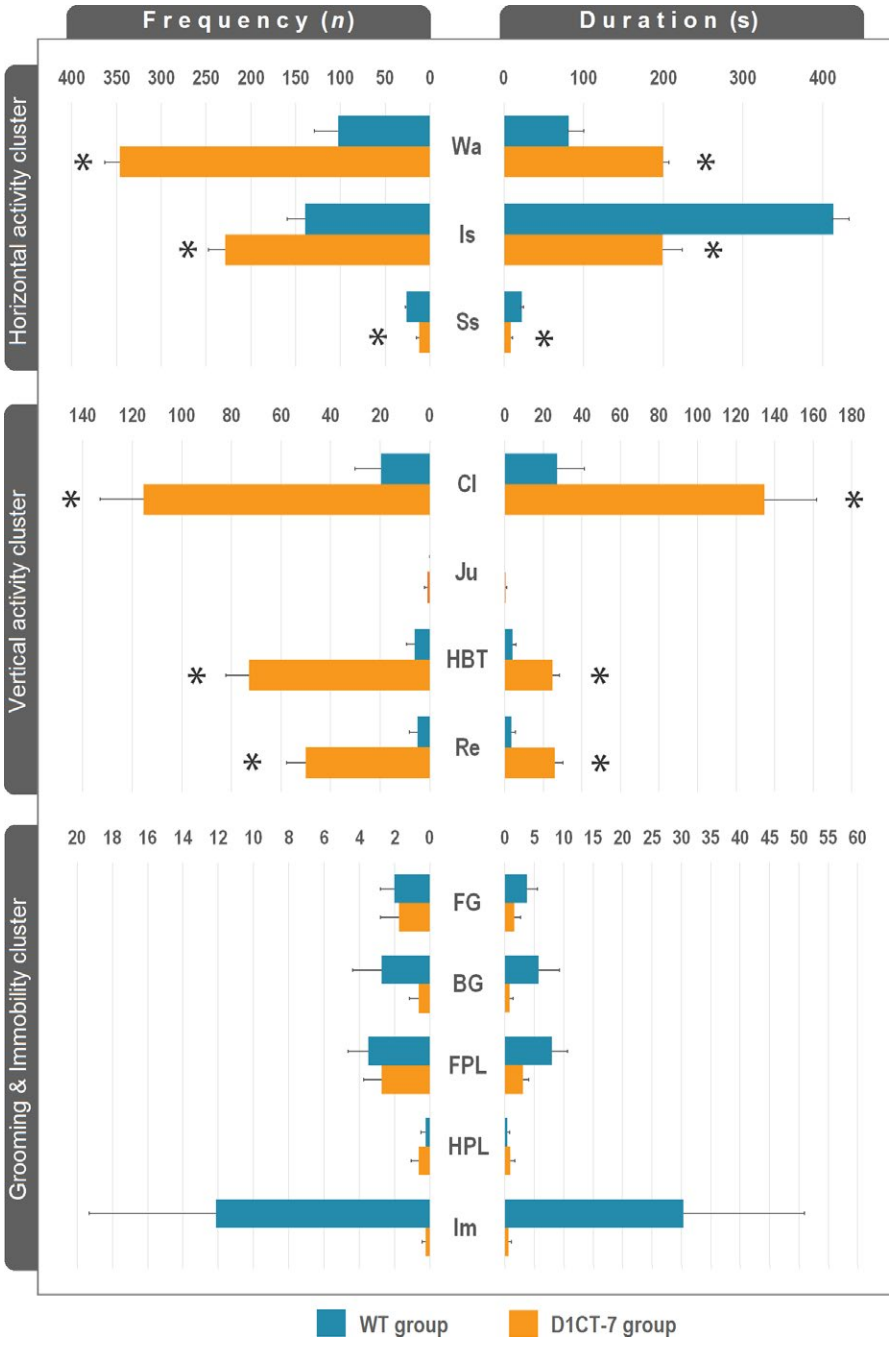

FIGURE 2 Quantitative results. Left: frequencies of all the behavioral components of the ethogram in open field (10 min). *X*-axis: mean number ± SE. Right: durations of the behavioral components in open field. *X*-axis: mean time (seconds) ± SE. *Y*-axis: components of the ethogram. See Table 1 for abbreviations. *$P < 0.05$, MANOVA

for the mean durations of the following dependent variables: Wa, IS, SS, CI, HBT, Re, and the "Grooming & Immobility" cluster ($F_{(7,8)}$ = 11.84, P < 0.001; Wilk's Λ = 0.088, partial η^2 = 0.91). The influence of genotype was statistically significant for Wa ($F_{(1,14)}$ = 37.18; P < 0.001; partial η^2 = 0.73), IS ($F_{(1,14)}$ = 53.07; P < 0.001; partial η^2 = 0.79), SS ($F_{(1,14)}$ = 21.84; P < 0.001; partial η^2 = 0.61), CI ($F_{(1,14)}$ = 14.21; P < 0.005; partial η^2 = 0.50), HBT ($F_{(1,14)}$ = 28.88; P < 0.001; partial η^2 = 0.67), Re ($F_{(1,14)}$ = 27.83; P < 0.001; partial η^2 = 0.67). There were no statistical differences for the "Grooming & Immobility" cluster ($F_{(1,14)}$ = 4.51; P = 0.052; partial η^2 = 0.24).

3.2 | T-pattern analysis

The first exposure to an open field elicits complex motor sequences (henceforth defined as T-patterns) in mice.[30] We identified, at a significance level of 0.0001, a number of 16 T-patterns of different composition in the WT group including: 1 encompassing eight events, 2 encompassing six events, 2 encompassing four events, 2 with three events, and 9 with two behavioral events. The D1CT-7 group performed 17 T-patterns of different composition arranged as follows: 5 encompassing four events, 3 patterns encompassing three events, and 9 of two events (Figure 3). Each T-pattern of different composition occurred several times in each group as shown in the occurrences (occs) columns of Figure 3. Comprehensively, we found a mean number of 286.00 ± 80.51 T-patterns per mouse in the WT group and 719.63 ± 60.27 T-patterns in the D1CT-7 group. A t test analysis for independent samples found a statistical difference between the two groups, $t_{(14)}$ = −4.31; P < 0.001.

Behavioral components of vertical and horizontal activity clustered together in the composition of patterns of exploratory activity in both groups of this study. We did not find T-patterns encompassing grooming behaviors; immobility and jumping did not enter in the composition of any T-pattern. No statistical difference was found comparing the T-pattern mean length: 2.14 ± 0.03 for WT and 2.17 ± 0.02 for D1CT-7 mice ($t_{(14)}$ = 0.98; P = 0.34).

From a qualitative point of view, the T-patterns characterizing D1CT-7 behavior were markedly different in their specific composition in comparison with WT ones (Figure 3). For instance, the behavioral components IS and SS, which represent highly relevant assessment-related rodent behaviors,[31] took part in composing 12 of the 16 T-patterns detected in WT mice, whereas the same behavioral components were found only in 5 of the 17 T-patterns displayed by D1CT-7 mice. On the contrary, behavioral components of vertical activities were largely more common among T-patterns of transgenic mice: HBT took part in composing 4 of the 16 T-patterns of WT mice, whereas it took part in composing 13 of the 17 D1CT-7 mice' sequences. Re was not present in any of the T-patterns of WT mice, whereas it was present in 6 of the T-patterns of the D1CT-7 group (Figure 3).

Wild type group

#	T-pattern string	Occs	Length	Tree structure
1	(wa wa)	762	2	wa wa
2	(is wa)	657	2	is wa
3	(ss ss)	154	2	ss ss
4	(cl is)	139	2	cl is
5	(cl cl)	133	2	cl cl
6	(cl wa)	91	2	cl wa
7	(ss wa)	47	2	ss wa
8	(hbt is)	45	2	hbt is
9	(wa hbt)	33	2	wa hbt
10	((cl is) cl)	110	3	cl is cl
11	((is wa) hbt)	28	3	is wa hbt
12	((is wa)(hbt is))	30	4	is wa hbt is
13	((is wa)(ss wa))	19	4	is wa ss wa
14	((wa wa)((is wa)(ss wa)))	16	6	wa wa is wa ss wa
15	((is wa)((is wa)(ss wa)))	13	6	is wa is wa ss wa
16	(((is wa)((is wa)(ss wa)))(is wa))	11	8	is wa is wa ss wa is wa
		2288		

D1CT-7 group

#	T-pattern string	Occs	Length	Tree structure
1	(is is)	1720	2	is is
2	(cl cl)	864	2	cl cl
3	(hbt wa)	548	2	hbt wa
4	(wa hbt)	546	2	wa hbt
5	(hbt hbt)	350	2	hbt hbt
6	(re re)	349	2	re re
7	(hbt is)	260	2	hbt is
8	(hbt re)	133	2	hbt re
9	(ss ss)	77	2	ss ss
10	((hbt wa) hbt)	381	3	hbt wa hbt
11	(hbt (wa hbt))	328	3	hbt wa hbt
12	(re (wa hbt))	51	3	re wa hbt
13	((hbt re)(wa hbt))	57	4	hbt re wa hbt
14	((hbt is)(wa hbt))	42	4	hbt is wa hbt
15	((hbt is)(hbt wa))	22	4	hbt is hbt wa
16	((re (wa hbt)) re)	17	4	re wa hbt re
17	(hbt (re (wa hbt)))	12	4	hbt re wa hbt
		5757		

FIGURE 3 Terminal strings and tree structures of the T-patterns detected in WT (top) and D1CT-7 (bottom) groups. The number on the left of each string indicates the corresponding tree structure illustrated on the right. Numbers on the right of each string indicate their overall occurrences (Occs) and length. The tic-like component "Head-Body Twitch" (HBT) is highlighted in red and bold font. See Table 1 for abbreviations. Data obtained from the analysis of 8 WT and 8 D1CT-7 mice

An additional question of interest concerned the distribution of these sequences along the 10-minute (min) time window of observation. To this aim, we calculated a min-by-min distribution of the onset-time of all the patterns found in the two study groups (Figure 4A). A mixed analysis of variance (mixed-ANOVA) was performed to evaluate the effect of the (between subjects) genotype factor and the (within subjects) min-by-min time factor on T-pattern mean number (dependent variable). There was a statistically significant interaction between the genotype and time on T-pattern mean number, $F_{(2.960,41.443)} = 2.908$, $P < 0.05$, partial $\eta^2 = 0.172$, Greenhouse-Geisser correction, $\varepsilon = 0.329$. The T-pattern mean number was statistically higher in D1CT-7 mice, compared to WT mice, from minute 2 to 10 (Figure 4A).

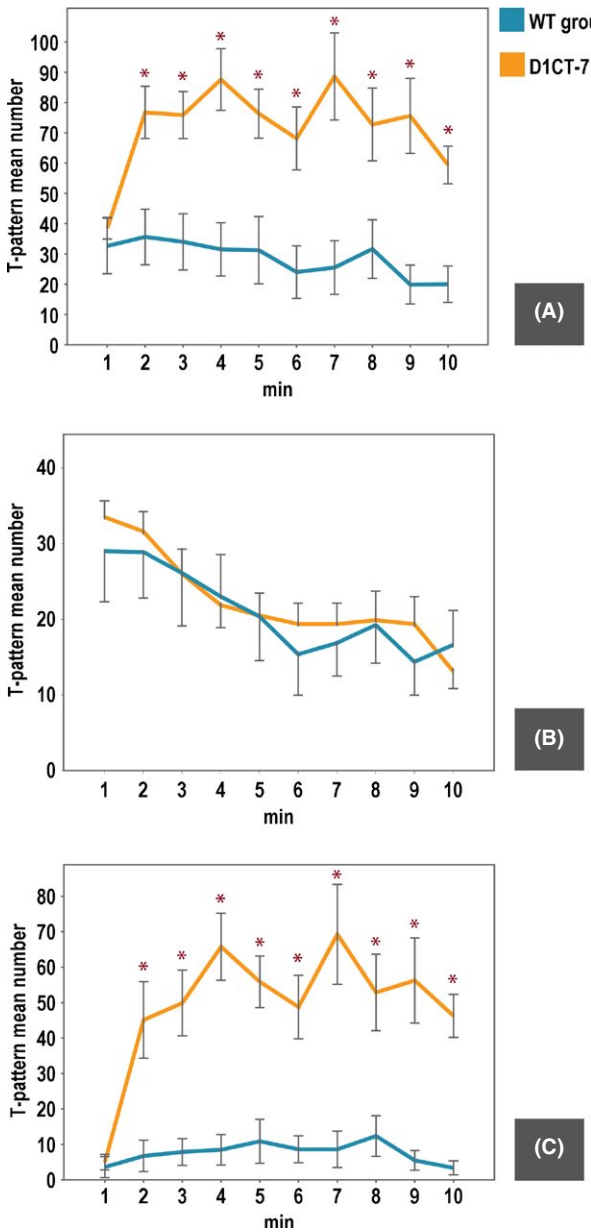

FIGURE 4 Min-by-min distribution of T-pattern's mean number ± SE detected in WT and D1CT-7 groups. (A) Mean number of overall T-patterns. Extended textual and graphical representations are shown in Figure 3. (B) Mean number of T-patterns which do not encompass components of vertical activity cluster (Table 1). (C) Mean number of T-patterns which encompass components of vertical activity cluster. *$P < 0.05$, *t* test for independent samples

Vertical activities such as HBT, Re, and CI took part in composing most of the T-patterns of the D1CT-7 group and only a few of the T-patterns of the WT group. Thus, we tested the hypothesis that there was a differential (min-by-min) onset-time distribution of T-patterns either containing or not containing these vertical activity cluster components. Figure 4B describes the genotype effect on min-by-min distribution of T-patterns that do not comprise vertical activity cluster components. The mixed-ANOVA showed no statistically significant interaction between genotype and time on T-pattern mean number, $F_{(3.815,53.403)} = 0.600$, $P = 0.657$, partial $\eta^2 = 0.041$, Greenhouse-Geisser correction, $\varepsilon = 0.424$. The main effect of time showed a statistically significant difference in T-pattern mean number along time, $F_{(3.815,53.403)} = 10.399$, $P < 0.001$, partial $\eta^2 = 0.426$. The main effect of group showed no statistically significant differences between groups $F_{(1,14)} = 0.076$, $P = 0.786$, partial $\eta^2 = 0.005$. On the contrary, Figure 4C illustrates the genotype effect on the min-by-min distribution of T-patterns comprising vertical activity cluster components. The mixed-ANOVA showed a statistically significant interaction between genotype and time on T-pattern mean number $F_{(2.929,41.005)} = 3.704$, $P < 0.05$, partial $\eta^2 = 0.209$, Greenhouse-Geisser correction, $\varepsilon = 0.325$. The T-pattern mean number was statistically higher in the D1CT-7 group, compared to the WT group, from minute 2 to 10 (Figure 4C), but not in minute 1.

We also performed statistical analysis of shuffling randomizations of behavioral components, as described in Section 2.4., and confirmed such randomization diminished detected T-patterns, from a mean number of 9 T-patterns of two components in either WT or D1CT-7 groups, to 1.25 T-patterns of two components in the WT group and 0.8 patterns in the D1CT-7 group. Taken together, results from shuffling randomization confirm the validity of the results obtained from T-pattern analysis.

3.3 | Analysis of transition probabilities

Transition probabilities are illustrated by means of path diagrams (Figure 5). Arrows of four different thickness levels were employed to describe different transition probability ranges. The path diagram of the WT group shows a high likelihood (50% or higher) of transition from all the behavioral components toward IS. Alternatively, the D1CT-7 group presented a statistically significant shift of the weight of transition probabilities toward Wa (*t* test for independent samples; Figure 5). This result was more evident for the transitions deriving from CI ($t = 6.063$, $P < 0.001$), Re ($t = 6.546$, $P < 0.001$), and HBT ($t = 6.925$, $P < 0.001$), the components of vertical activity cluster.

4 | DISCUSSION

The current study was aimed at evaluating the behavioral organization displayed by D1CT-7 transgenic mice upon their first exposure

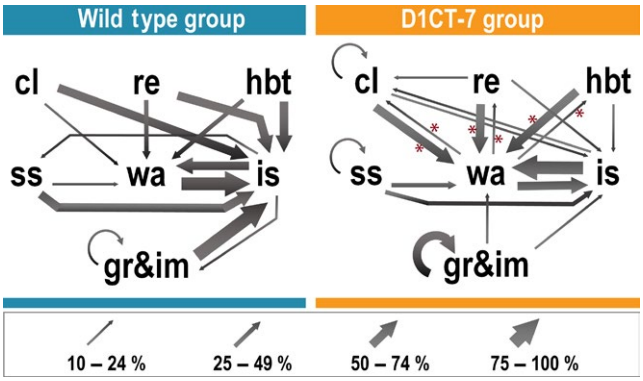

FIGURE 5 Path diagrams illustrating transition probabilities among the components of the ethogram by means of arrows of different thickness. Left: Wild type group, n = 8. Right: D1CT-7 group, n = 8. See Table 1 for abbreviations. The components of grooming and immobility cluster are represented together due to their low frequencies. *$P < 0.001$, *t* test for independent samples

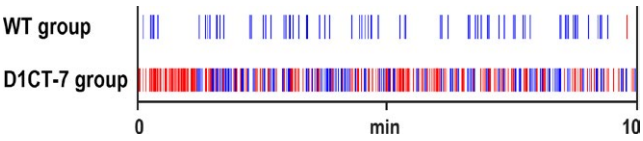

FIGURE 6 Inside/outside T-pattern distribution of the behavioral component Walking (Wa) during the 10-min test. Example from one WT subject and one D1CT-7 subject. Vertical bars indicate the onset of each detected Walking occurrence. Blue: Walking included in the composition of T-patterns. Red: Walking not included in the composition of T-patterns. Adapted from Theme software output, Section 2.3.2

to the open-field test, which is a widely used and simple test to assess novelty-induced responses and exploratory behavior.[20,21] The results were obtained following two steps: firstly, a quantitative measure was carried out to report frequencies and durations of each component of the behavioral repertoire; secondly, by means of T-pattern and transition probability analyses, the behavior of D1CT-7 transgenic mice was described analyzing the statistically relevant relationships occurring among the above-mentioned individual components of the behavior. Our results show that such T-pattern analysis of the relationships among single acts, which can unveil emergent behavioral dynamics,[22,29,32] similarly provides new insights on the functional impact of the transgenic mutation characterizing the D1CT-7 mice.

Quantitative results showed that the D1CT-7 mice exhibited a strikingly higher number of behavioral components, which exceeded twice the behaviors observed in the WT littermates along the same time windows (831.88 ± 33.03 for D1CT-7 and 320.00 ± 48.89 for WT). As illustrated in Figure 2, these differences in frequencies were particularly evident for Wa and for the components of vertical activities: Cl, Re and, as expected, HBT. This hyperactivity confirms what has been previously reported in the literature.[8-10,13,14] Our quantitative data suggest that the D1+ neuropotentiating transgene reduces assessment-related sniffing behaviors. D1CT-7 mice displayed a statistically lower number and duration of SS behaviors than their WT littermates. Furthermore, although the mean number of IS occurrences was higher than in WT mice, the mean duration of this behavior was, in comparison, much decreased (Figure 2).

Further qualitative analyses also unveiled a profound influence of the D1CT-7 genotype on the behavioral dynamics compared to WT controls. T-pattern analysis, indeed, showed that the D1CT-7 phenotype, noticeably hyperactive, was associated with an increased number of patterns (Figure 4A). At the same time, we reported relevant differences in pattern composition. For example, the tic-like component HBT (Figure 1) showed a pervasive recurrence within the behavioral sequences displayed by D1CT-7 mice, as it was a component of 13 of the 17 sequences detected (Figure 3). This count reaches 15 if we include the other highly frequent components of vertical activity such as Re and Cl. T-patterns encompassing such components are responsible for the greater number of behavioral sequences displayed by the transgenic group (Figure 4C), whereas the number of T-patterns consisting only of horizontal activity components was not significantly different from WT (Figure 4B). As reported elsewhere,[20,21,30,33] the first 5-10 minutes in open field are largely devoted to horizontal activity such locomotion and sniffing. In our hands, quantitative results and T-patterns characterizing WT group behavior were in accordance with such findings. On the contrary, the diffuse recurrence of disrupting tic-like behaviors and other vertical activities in the composition of D1CT-7 real-time motor sequences could therefore suggest a functional impairment and/or interference on the exploratory drive. T-pattern tree structures, represented in Figure 3, offer a clear view of such sequences. For example, the pattern (Wa HBT) occurred only 33 times in the WT group but occurred 546 times in the D1CT-7 group.

In addition to these repercussions on the detected T-patterns, we found, as a direct marker of behavioral disorganization, a sensitive reduction in the relative number of Wa incidences involved in the composition of T-patterns, which dropped from 99.4% (817 of the total 822 Wa) in the WT group to 34.1% (944 of the total 2770 Wa) in the D1CT-7 group (Figure 6). These data further suggest a fragmented and less organized behavior in the D1CT-7 mice in comparison with the WT mice. Such an aspect might contribute to the prominent habituation impairments observed in the transgenic mice.[14]

Lastly, D1CT-7 tic-related patterns showed a peculiar distribution during the 10 minutes of observation. Figure 4C illustrates a clear rise in pattern mean number only from the second minute of the test. We suggest that this difference is not observed in the first minute due to initial novel stimuli which provoke an increased inhibitory control over the expression of T-patterns containing disrupting and context-unrelated tic-like behaviors. This is consistent with reported suppression of twitches in D1CT-7 mice while engaged in more concentration-dependent and/or goal-directed behaviors (ie, during eating, drinking, bar-hanging, digging, and grooming).[13] Notably, this is in line with clinical data on tic fluctuations, which are affected by contextual factors such as goal-directed activity and concentration, both of which are acknowledged to facilitate tic attenuation.[18,34]

Beside the analysis of real-time patterns, we assessed D1CT-7 behavior through an analysis of transition probabilities. The results are illustrated in Figure 5 by means of path diagrams which provide

a general overview summarizing the overall dynamics of D1CT-7 and WT behavior. Even at very first glance, the path diagram shows that IS plays a pivotal role in the economy of WT behavior. Each single behavioral component in WT mice shows at least a 50% chance of being followed by IS, which itself is subsequently followed at high probability by Wa and again by IS. Such a simple behavioral patterning, together with the results of the T-pattern analysis, indicates that the WT mice were engaged in cautious sniffing and exploration of the novel environment, as expected. However, the path diagram illustrates a significant difference in the D1CT-7 mice (Figure 5, right). Although IS still represents an important node, the highest chances of transition converge in the direction of Wa, with less emphasis placed on sniffing activities (IS and SS). Since D1CT-7 mice showed an increase in anxiety-like behavior[35] and aggravation of their hyperactivity by anxiogenic stimuli,[36] the reduced relevance of IS in their behavioral transitions in this anxiogenic open-field assay is likely due to their baseline or anxiety-aggravated hyperactivity.

5 | CONCLUSION

Using both quantitative and multivariate techniques of investigation, our results show how the behavioral dynamics observed in WT mice are modified in the transgenic D1CT-7 mouse model of TS. Moreover, a measure of the extent of the latter functional deficit is provided. The comparison between WT and D1CT-7 mice revealed a clear-cut hyperactivity in the transgenic group, with a marked shift from cautious sniffing to proneness toward walking. Importantly, transgenic mice showed a lower behavioral organization, a markedly fragmented behavior, and patterns pervasively disturbed by intrusive tic-like twitches of the head-body segments. These aspects strengthen the face validity of D1CT-7 mice as a model of TS and may represent a reliable support to complement the analysis of tic-like behaviors in these mutants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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