Lévy statistics define anxiety and depression in mice subjected to chronic stress

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Abstract

Anxiety and depression are considered coping mechanisms organisms adopt in response to external stressors. We employed Lévy statistics to investigate the movement patterns of rodent models subjected to stress and compared them to non-stressed controls. Our analysis revealed that stressed mice exhibited heavy-tailed distributions in open fields, consistent with the Lévy flights utilized by animal predators in search of scarce food sources. In contrast, non-stressed mice demonstrated a normal distribution of speed. We used the two-dimensional Kolmogorov-Smirnov test to identify significant differences in the γ and μ parameters derived from Lévy flight between anxiety, depression, and control mice. Additionally, we employed the support vector machine algorithm to determine the optimal separation of each group. Furthermore, our mathematical model demonstrated the therapeutic effects of fluoxetine. These findings have the potential to contribute to the development of more precise definitions of anxiety and depression, as well as facilitate the evaluation of pharmaceutical developments.

Introduction

In 2019, the World Health Organization reported that one out of every eight individuals worldwide lives with one or more mental disorders[1]. The COVID-19 pandemic resulted in a significant increase in depression and anxiety cases within a year[2]. Chronic stress is the principal environmental factor that triggers a decline in mood leading to depression and anxiety[3,4]. To mimic these disorders, rodent models of stress, particularly the chronic unpredictable mild stress (CUMS) model, have been extensively employed since the early 1980s[5]. In CUMS and its derived models, mice or rats are subjected to constant but unpredictable mild stressors, resulting in the development of depression-like or anxiety-like behaviors, mimicking the core symptoms of clinical depression and anxiety, such as anhedonia[6,7]. CUMS has demonstrated excellent reliability and validity in studying underlying mechanisms and serves as a tool for drug discovery[5].

Different species or strains of experimental animals exhibit varying responses to CUMS due to their diverse genetic backgrounds. For instance, DBA/2 stress is more resilient than C57BL/6 and even more so than BALB/c[8]. Furthermore, the variability in response to CUMS results in various manifestations of anxiety-like and depression-like behaviors. Although anxiety and depression have distinct underlying neural circuits[4], they often coexist in many human patients[9]. CUMS mice are widely recognized as a classical model for depression since a significant induced symptom is anhedonia[6]. Given the desire of the pharmaceutical industry to evaluate drug effects, the ability to reliably and quantitatively assess mood disorders is crucial[10,11].

Rodents' response to stress is typically observed through sequential behaviors, including anhedonia, abnormal weight, and poor coat condition. To quantify the severity of mental disorders and differentiate anxiety and depression-like behaviors, traditional neurobehavioral studies such as the elevated plus maze (EPM) for anxiety and forced swim test (FST) and tail suspension test (TST) for depression are applied. However, each of these classic methods has limitations. For instance, Cheryl D. Conrad explained that assessing anxiety with open field test (OFT) does not necessarily predict anxiety with EPM[12]. Additionally, TST and FST only reflect one aspect of depression[13,14]. whereas EPM may induce anxiety during testing, complicating the evaluation process[9,15]. Furthermore, the experimental procedures themselves exert additional stress on animals[16], interfering with data interpretation, and limiting the study's translational ability.

Animals exhibiting varying mental states demonstrate different movement patterns in nature. For example, marine predators' foraging routes often exhibit a unique Lévy flight (LF) pattern when experiencing stress induced by hunger or lack of resources[17]. In recent years, LF has garnered

considerable inter-disciplinary attention as a theoretical framework encompassing migratory birds, fish, insects, and large terrestrial animals' moving patterns[18-20].

This study aimed to test the hypothesis that CUMS induces characteristic statistical behavioral patterns in animals. Using a minimally intrusive behavioral test incorporating LF analysis, we sought to differentiate anxiety and depression in CUMS mice. Furthermore, this novel approach enabled us to distinguish between animal behaviors before and after administration of anti-depression and anti-anxiety drugs. Our data indicated that mice with anxiety-like, depression-like, and non-stressed dispositions exhibited unique mathematical movement speed distributions. Both anxiety-like and depression-like behaviors demonstrated clear LF distribution patterns and differentiated from each other by two parameters. These findings have practical applications in developing an efficient one-step experimental method for quantifying stress-induced anxiety and depression severity. Additionally, this approach may reduce the unnecessary stress experienced by animals during repetitive experimental procedures and increase the efficiency of drug discovery.

Materials and Methods

Animals

Experiments were conducted using 641 male and female C57Bl/6 mice (6-8 weeks old, weighing 18-24 g) obtained from Charles River Laboratories Animal Technology Co., Ltd. (Beijing, China). The animals were housed under specific pathogen-free conditions, provided standard laboratory chow and distilled water, and maintained on a 12-hour light-dark cycle in standard cages with corn cobs in a room maintained at an ambient temperature of 23 ± 1 ℃ at Kangcheng Biotech, Ltd, Co. (Sichuan, China) facilities [animal production license number: SYXK (Chuan) 2019-215]. All procedures followed the guidelines of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and were approved by the Institutional Animal Care and Use Committee (IACUC) of the West China Hospital, Sichuan University (Approval No. 2019194A).

Chronic unpredictable mild stress

The CUMS modeling process was adjusted slightly and consisted of sequential application of mild stressors, including cage tilting, heat stress, noise stimulation, wet bedding, loneliness, fasting, inversion of day-night cycle for 24 hours, water deprivation, reciprocating sway, random foot shock for 120 seconds, crowding, cold stress, flash stimulation for 12 hours, constraint for 4 hours, and cage replacement for 24 hours. Each stimulus was randomly arranged, and two different stressors were performed consecutively every day throughout the experiment[6,21]. Prior to the experiment (day -2), we evaluated the sucrose preference of mice. Those with a preference rate lower than 80%

were excluded, resulting in the removal of 11 mice (Supplementary Fig. 1a). Prior to CUMS induction, all mice underwent a SP test to exclude subjects with low cognitive activity (Supplementary Fig. 1b). On day 14, both stressed and non-stressed mice underwent another SP test. Subjects with a SP greater than 80% were selected for further experiments in the non-stressed group whereas subjects with a sucrose preference of less than 80% were chosen for the stress group in mice subjected to CUMS (Supplementary Fig. 1c). On Day 14, the CUMS mice with fewer than five entries in the center in the OFT were excluded, leading to the elimination of seven mice (Supplementary Fig. 1d).

Chronic restraint Stress (CRS)

The CRS protocol was slightly modified from previous descriptions. One week prior to the start of stress exposure, animals were divided into control and experimental groups to acclimate to new cages. Mice were then placed in a cylinder for 4 hours per day (8:00-12:00) for 14 consecutive days, almost immobilizing them. The size of the cylinder matched the size of the animal. Non-stressed controls were moved to a test room and handled gently for 5 minutes before returning to their holding room 4 hours later[22].

Electric shocks (ES)

The modeling session was performed as described previously with minor modifications1. After a 30-minute adaptation period, mice underwent 10 intermittent inescapable electric foot shocks delivered by an isolated shock generator (Shanghai Jilang Information Technology Co., Ltd, China) through the grid floor. The shocks had an intensity of 0.8 mA, an interval of 15 seconds, and a duration of 15 seconds. Control mice were placed in the same chamber for 5 minutes without undergoing electric foot shocks[23].

Physical health assessment

Body weight, food intake, and coat state score were assessed every Wednesday from 10-12 am until day 49. Food consumption over 24 hours was recorded. The coat state score evaluated the condition of seven areas: head, neck, dorsal, ventral coat, tail, anterior claw, and hind claw.

Sucrose preference (SP) test

In preparation for the SP test, 1% sucrose solution and distilled water were both placed simultaneously in each mouse's cage two days prior to the test for taste adaptation. On the third day, following a six-hour fast and water deprivation, mice were raised individually in cages with one pre-weighed bottle of 1% sucrose solution and another of distilled water. The positions of the two bottles

were swapped after six hours. After an additional twelve hours, both bottles were removed, and the remaining liquid was weighed to calculate mouse sucrose preference using the following formula[24]:

$$Sucrose\ preference\ percentage = \frac{Sucrose\ consumption}{Total\ liquid\ consumption} \times\ 100\ \%$$

Behavioral assessment

The mice were acclimated to the testing room for 12 hours prior to assessment. Maintaining a clean, odor-free, and quiet environment was crucial for the experiment, and the operator remained concealed during testing. The Topscan Package (Clever Sys Inc., USA) was used to record and analyze mouse movements.

Open field test

Each mouse was gently placed in a 50 * 50 * 50 cm open field box for 5 minutes to explore freely under weak (50 lx) illumination. Total distance traveled (cm) and number of entries into the central area (25 * 25 cm) were recorded and analyzed using software. IT% was calculated as the time spent in the inner area/300 s * 100, while ID% was calculated as the inner area distance/total distance * 100[25].

Elevated plus-maze test

The EPM had two open and two closed arms. Each arm measured 30 cm in length and 5 cm in width, with walls that were 20 cm high in the closed arms. The EPM was elevated to a height of 60 cm above the ground, with a 90°angle between the arms. Prior to the test, mice were placed in the central area facing the open arm and allowed to explore for 5 minutes. Open Arm Entries Percent (OE%) was calculated as Entries into the Open Arm divided by (Entries into the Open Arm + Entries into the Closed Arm) multiplied by 100. Open Arm Time Percent (OT%) was calculated as Time in the Open Arm divided by 300 seconds multiplied by 100[24].

Light/dark box test

Anxiety was tested in the LDB as previously described[26]. Each mouse was placed in an apparatus consisting of two identical opaque Plexiglas compartments (18 * 12 * 12 cm) joined by an opening (5 cm * 5 cm). The mice were positioned in the light box with their heads facing the dark box, and their exploratory behavior was monitored for 10 minutes following their initial crossing.

Tail suspension test

The mice were suspended by their tails using adhesive tape, which was hooked onto a horizontal rod. The distance between the tip of the mouse's nose and the floor was approximately 25 cm. After being suspended for 6 minutes, the time spent immobile during the last 4 minutes was recorded[27].

Forced swimming test

Prior to testing, the mice were placed in plastic cylinders filled with water (25 ± 1 ℃; depth: 15 cm) for 15 minutes. The next day, the mice were placed in a 10-cm-wide cylinder (height: 30 cm) and the time spent immobile during the final 4 minutes of the 6-minute forced swimming test was recorded[28].

Criteria importance through intercriteria correlation (CRITIC) method

Diakoulaki employed the Analytic Hierarchy Process (AHP), a method for objectively weighting evaluation indicators. AHP breaks down complex decisions into a hierarchy of criteria, sub-criteria, and alternatives. Pairwise comparisons are made between criteria to determine their relative importance on a scale from 1 to 9. The consistency of these comparisons is checked mathematically, and the weights of each criterion are calculated by normalizing the pairwise comparison matrix using the following formula[29]:

$$w_i = (1/n) * \sum(j=1 \text{ to } n)\ a_ij$$

Standard depression and anxiety disorder scales

Depression and anxiety disorder scales were slightly adjusted according to the previous descriptions7. We classified mice in the CUMS group with anxiety scores below 30 as "anxiety". Similarly, we used the CRITIC method to obtain the percentage of each depression indicator. Then normalizing depression indicators for each group, we scored the results based on the corresponding rate. We classified mice in the CUMS group with depression scores below 50 as "depression". We established a new standard scale using a score of 1 as the highest point and 0 as the lowest. The normalized scores were determined based on the behavioral indicators in Tables 1 and 2 in the Supplementary Appendix. After normalizing anxiety indicators for each group, we scored the results using the corresponding percentage.

Drug

Fluoxetine hydrochloride (FXT, Sigma Chemical Company, St. Louis, MO, USA) was dissolved in saline and administered once daily via the intraperitoneal route for 37 days at a dosage of 20 mg/kg. The dosage of fluoxetine hydrochloride was selected based on previous studies[30].

LF fitting

The mice were placed in a standard open field apparatus for behavioral studies (Clever Sys., Inc. US), and their behavior was recorded through a series of videos. The recorded videos were analyzed using a custom MATLAB script (Math Works Inc. US, version R2019a). The mice were segmented from the videos frame-by-frame, and the mean coordinates of the segmented pixels were found to determine the center of each mouse. Walking speed was calculated by measuring the distance a mouse traveled between a specific number of frames, divided by the time interval. To maintain balance between measurement accuracy and sensitivity to speed changes, speed was calculated every five frames, which correspond to approximately 0.2 seconds apart. We fitted two PDF' s for the walking speed statistics of each mouse from all the categories in Fig. 2d-f. The first PDF is the LF distribution[31]:

$$L(s,\gamma,\mu) = \begin{cases} \sqrt{\frac{\gamma}{2\pi}} e^{\left[-\frac{\gamma}{2(s-\mu)}\right]} \frac{1}{(s-\mu)^{3/2}}, 0 < \mu < s < \infty \\ 0, \quad Otherwise \end{cases} \qquad \text{Equation 1}$$

The other PDF is the normal distribution[32]:

$$f(s,\sigma,\mu) = \frac{1}{\sqrt{2\pi}} e^{-\frac{(s-\mu)^2}{2\sigma^2}} \qquad \text{Equation 2}$$

LF and the normal distribution described random walks with different probability distributions. One of the unique properties of the LF model is its slightly larger distribution values in the far right region than the normal distribution has.

The Goodness of Fit (R-good) is a quantitative representation of the curve fitting quality, which is defined as:

$$\text{R-good} = 1 - \frac{SSE}{SST} \qquad \text{Equation 3}$$

Where SSE (error sum of squares) is defined as SSE = $\sum_{i=1}^{n} (x_i - \hat{x}_i)^2$ and SST is defined as SST $= \sum_{i=1}^{n} (x_i - \overline{x}_i)^2$. x_i is defined as the average value of this set of data. The value of R-good is between 0 and 1. The closer it is to one, the better the fit is.

The R-good value for each mouse was noted as a point in Fig. 2a, b.

Support vector machine (SVM)

SVM is a supervised pattern recognition scheme method. It is suitable for the feature reduction of high-dimensional data with small sample size. A general mathematical model of support vector machines is shown in Equation 4 and Equation 5[33].

$$\min_{\omega,\varepsilon} \frac{1}{2}\omega_2^2 + C\sum_{i=1}^{n} \varepsilon_i l_i \qquad \text{Equation 4}$$

$$s.t.\left(\boldsymbol{\omega}^{\boldsymbol{T}}\varphi(\boldsymbol{x}_i) + b\right) \geq 1 - \varepsilon_i, \varepsilon_i \geq 0, n = 1, \ldots, n \qquad \text{Equation 5}$$

The detailed solution process of the SVM problem is found in Refs[34,35].

Statistical information

Data obeying normal distributions were performed using SPSS Statistics v.20 (IBM Ltd, UK) and were represented as means ± standard errors of the mean (s.e.m). The cartograms were performed using GraphPad Prism 8.00 (GraphPad Software, San Diego, California, USA). Repeated measures analysis of variance (ANOVA) was performed to determine differences in body weight, food intake, coat state scores, and SPT among groups, followed by Bonferroni's multiple comparisons test. Differences among each group were analyzed by one-way ANOVA, followed by the post hoc Bonferroni. A p-value < 0.05 was regarded as significantly different.

Data transformation, calculation of unified scores. Outcome measures for each test were normalized to obtain a 'measure score' between 0 (low anxiety/depression) and 1 (highly anxiety/depression) for each individual, using the following formula:

$$\mathrm{X(i)} = \frac{M(i)}{M(m)}$$

The 2-Dimensional Kolmogorov−Smirnov (KS) test[36] is a nonparametric, bivariate statistical test designed to verify whether two two-dimensional samples follow the same distribution. The point defined four quadrants for any given point in a two-dimensional space. The integration probability is defined as the probability of sample data distribution in one of the quadrants that accounted for the total number of samples. By iterating over all data points and quadrants, the test statistic DFF,1 is defined by the maximal difference of the integrated probabilities between samples in any quadrant for any origin from the first sample. Similarly, after going through all points of another sample, the test statistic DFF,2 is obtained. DFF,1 and DFF,2 are then averaged to compute the overall DFF for hypothesis testing, DFF=(DFF,1+DFF,2)/2.

In the large sample limit (n ≥ 80), it was shown that DFF converged in distribution[29].

$$D_{FF} \xrightarrow{d} \Phi(\lambda) = 2\sum_{k=1}^{\infty}(-1)^{k-1}e^{-2k^2\lambda^2}$$

For a single sample of size n

$$P(d_{FF} > D_{FF}) = \Phi\left(\frac{D_{FF}\sqrt{n}}{1+\sqrt{1-r^2}\,(0.25-0.75/\sqrt{n})}\right)$$

The two-sample case used the same formula as above, where n is defined as

$$n = \frac{n_1 n_2}{n_1 + n_2}$$

and r is defined as

$$r = \frac{\sum_i (X_i - \overline{X})(Y_i - \overline{Y})}{\sqrt{\sum_i (X_i - \overline{X})^2}\sqrt{\sum_i (Y_i - \overline{Y})^2}}$$

Results

Mice exposed to CUMS displayed characteristic anxiety-like or depression-like behaviors

Using a modified CUMS method (Fig. 1. a), we successfully induced anxiety or depression in mice models, which were validated using industry standards (Fig. 1.). To exclude subjects with naturally low activity levels, either physically or mentally, as described in the method section, 145 mice underwent a SP test (Supplementary Fig. 1. a) and an OFT (Supplementary Fig. 1. b). After two weeks of modeling, we utilized SP and OFT to assess the efficacy of CUMS induction. The control group excluded a preference rate of less than 80% for SP, whereas the CUMS group eliminated a preference rate higher than 80% (Supplementary Fig. 1. c). Seven mice were eliminated from the study on Day 14, as they did not enter the center of the OFT fewer than five times, following the exclusion criteria (Supplementary Fig. 1. d). The SP test was conducted thrice throughout the entire experiment, and the CUMS + Saline group displayed a significantly lower SP rate compared to the control group after CUMS (Fig. 1. b). The mice subjected to CUMS for 42 days showed lower body weight (Fig. 1. c), lower food intake (Fig. 1. d), and worse coat state (Fig. 1. e), compared with their non-stressed counterparts (Control). During 37 days of treatment, the success of the CUMS model was validated by administering fluoxetine (FXT) to CUMS mice, with the observation that the CUMS + Saline mice showed fewer center entries, and distance during OFT (Fig. 1. f-h). Neurobehavioral tests, including

EMP and LDB, were conducted on all mice during day 44 to day 50 to evaluate anxiety-like behavior. Additionally, TST and FST were employed to assess depressive-like behavior. The CUMS + Saline group exhibited less exploration time in the open arm of EPM and light box in LDB (Fig. 1. i-n), as well as reduced struggling time in TST and FST, as shown in Figure 1 o-r. Conversely, FXT treatment increased the number of struggle times in the CUMS + FXT group.

The velocity of mice walking in an OF showed a pattern of LF

Due to spatial confinement in limited arenas, rodent trajectories are often truncated by the boundaries, making statistical distributions a better model for walking speed than rodents' walking trajectories to reveal mental states under stress. In this study, the walking rates of each mouse group were plotted against time in Figure 2a, and the frequency of walking speed was computed and represented as a bar graph in Figure 2b-c. Additionally, two probability density functions (PDFs), the LF distribution and normal distribution (Equation. 1, 2), were fitted to the walking speed statistics for each mouse category. The goodness of Fit (R-good) quantitatively represented curve fitting quality. Results showed that control mice's walking behavior followed normal and LF distributions, while CUMS mice had an LF distribution as depicted in Figures 1d-e.

LF pattern in the OFT distinguished control and stress in mice

The significant difference between control and CUMS groups led us to fit the LF distribution model to walking speed of animals from each group at every experimental stage. A mouse's walking speed distribution was represented by a curve with two parameters, γ and μ (Equation 1, Method Section), and each mouse was denoted as a dot on the γ-μ plane. Control mice were represented by black dots, while CUMS mice on day 14 were depicted with red and blue dots in Figure 3a. The 2D Kolmogorov-Smirnov (2D K-S) test was utilized to determine whether the two-dimensional sets of variables followed the same distribution. Results indicated a substantial difference between the control and CUMS groups (Fig. 3. a, $p < 0.001$). The CUMS-stressed mice were divided into CUMS + Saline and CUMS + FXT groups after day 14. On day 42, there was a significant difference between the CUMS + Saline and CUMS + FXT groups (Fig. 3. b, $p < 0.001$). Furthermore, compared to control, the CUMS + Saline group exhibited larger differences during OFT (Fig. 3. b, $p < 0.001$), whereas no significant difference was observed between the control and CUMS + FXT group. Similar methods were used to compare OF data of the CUMS + Saline group on day 0, 14, and 42, revealing significant differences between day 0 and day 14 (Fig. 3. c, $p < 0.001$), day 14 and day 42 (Fig. 3. c, $p = 0.040$), and day 0 and day 42 (Fig. 3. c, $p < 0.001$). By comparing OF data differences at the three-time points in the CUMS + FXT group, significant differences were observed in pairwise comparisons at each time point (Fig. 3. d, $p < 0.001$). These results provide a quantitative approach to determine the mood state of CUMS mice.

Conventional and novel methods to differentiate between depression and anxiety in mice

The CUMS model can exhibit both anxiety-like and depression-like behaviors. In this study, remaining mice underwent various classical neurobehavioral tests, including OFT, EPM, LDB, TST, and FST (Fig. 1. a). A new standard scale was set up based on previous studies' anxiety/depression indicators, which were obtained according to the CRITIC method and normalized using a percentage (Supplementary Table. 1, 2). The normalized scores were then added to obtain a total anxiety or depression score for each mouse (Fig. 4. a, c). The CRITIC-derived percentages were multiplied by the normalized value of each indicator and summed to acquire the total anxiety or depression score for each mouse, with the control group used as the reference point. Based on the scores, CUMS mice were categorized into four groups, with nine mice exhibiting anxiety-like behavior (Anxiety) (Fig. 4. b), seven mice showing depression-like behaviors (Depression) (Fig. 4. d), and eight mice with comorbid depression and anxiety (A & D). Upon re-examination of the behavioral indicators of the four groups following differentiation, it was observed that not all indicators could effectively differentiate between depression and/or anxiety (Supplementary Fig. 2).

The LF fitting analysis was conducted twice on OFT video recordings, on the 14th and 42nd day of CUMS modeling, for the control, depression, anxiety, and A&D mice groups. On day 14, there were significant differences observed between the control group and the remaining three groups ($p < 0.001$, respectively). Notably, no significant difference was detected in the 2D K-S test between the Anxiety, Depression, and A & D groups (Fig. 5. a). On day 42, significant differences were observed between the control group and the remaining three groups (Fig. 5. b, $p < 0.001$, respectively). Furthermore, significant differences were detected between Anxiety and Depression (Fig. 5. b, $p = 0.001$). The data pertaining to Depression and A & D also exhibited significant differences.

Preliminary LF sorting for anxiety and depression

Given the above results, we next focused on establishing a preliminary LF-based standard to sort mice into three groups, namely depression, anxiety, and healthy controls. We realized that multiple classical stress means existed to produce animal models. To incorporate these potential models, we enlisted 99 anxiety-like mice produced with the CUMS procedure, 46 depression-like with CUMS, 89 anxiety-like with the electric shock method (ES), 69 depression-like with chronic restraint stress (CRS), and 237 controls. Each diseased mouse was verified with the traditional methods, and each mouse underwent the LF procedure. Their coordinates in the γ -μ plane was marked in figure 5a. Given the distribution of these data, we hence used the support vector machine (SVM) algorithm to find the optimal separation of the three mice group in the γ - μ planes. The optimal linear separation

between the control and the anxiety was found as L_1: $\mu = -0.59\gamma + 0.79$. The optimal linear separation between the control and the anxiety was L_2: $\mu = 6.54\gamma - 3.91$ (Fig. 6. a).

We used the L_1 as distinguish between the control and the anxiety, and the L_2 as distinguish between the depression and the anxiety mice in this test. We found that 6 mice in the depression group were in the depression-like behavior region, and all the anxiety mice were in the anxiety-like behavior region (Fig. 6. b).

Discussion/Conclusion

The above results reveal a previously unknown statistical walking pattern of mice in anxiety and depression mental stages. This finding offers a novel illustrative method to differentiate anxiety, depression and healthy mice. This method is advantageous because it provides a one-step gentle approach (free walk in an OF) as opposed to the traditional multi-step stressful tests. This innovative approach has the potential to alleviate the industry's long-standing concerns regarding the strong stress that traditional tests impose on animals. While some previous studies have also reported the behavioral features of stressed mice, few statistical models have been developed to help differentiate and quantize stressed rodent models[7,21]. For example, Jin Jin et al. interpreted in the journal Cell that the anxiety-like mice tend to spend more time in the outer portion of the box and less time in the centre of the box[24]. Belzung et al. mentioned that mice unable to escape in a confined space with increased immobility time have depressive-like behavior[6]. These observations shine light on the correlation between rodents' mental stages and their behaviors. With our new approach, it is possible to process the OFT videos of rodents in an automated batch fashion, enabling the acquisition of quantitative results with minimal stress being imposed on the animals.

When completely ignorant to the orientation and distance of the pray, an animal may unintentionally choose a searching strategy among a few choice of styles[11]. Ralf Metzler et al. calculated that searching trajectories that follow the LF statistics have higher efficiency than those following the normal statistics when the pray is relatively far from the initial starting location[37]. On the contrary, if the pray is initially close to the starting position, a normal statistics trajectory is of higher efficiency. Therefore, it is intuitive to assume that evolution has taught many animals a surviving strategy such that a decision is made between the normal distribution and the LF distribution based on the expected pray distance. Animals in various stressed mental stages may have various pray distance expectations thus adopt various searching strategies. Our findings further imply that the severity of a mental stage may be quantized by the governing parameters of the LF statistics.

Lack of food is the most common stress animal faces in nature[19]. When food and other resources are abundant, mobile organisms tend to forage in a pattern of Brownian motion[38]. In contrast, when faced with food and other resources shortage pressure, they often present a LF searching strategy, which means the distribution of traveling distances decays as a power law[39]. The stress response of food deficiency in nature is similar to the fear of the unknown for the anxiety and depression mice. The anxiety and depression mice display heavy-tailed distributions in an open field and are consistent with the LF employed by animal predators searching for scarce food sources. In contrast, the speed of non-stress mice performed a normal distribution. A random walk is a nature-inspired algorithm consisting of consecutive unexpected steps or velocities. The LF distribution model usually exists in animal trajectories under survival pressure[11]. This movement trajectory can effectively improve the survival rate when food is scarce[18,19,38,40]. LF has also been applied to other research fields related to bionics[17,41,42]. Although little is known about how LF searching systems developed, evidence indicates that this strategy may have evolved to allow animals to cover the most expansive area with the least energy, which may explain the widespread occurrence of such patterns among modern animals. We suspect that this pattern of behavior, imprinted in animal brains by survival advantage in evolution, should also exist in laboratory animals.

FXT has been well reported to alleviate the symptoms of CUMS[43]. As anticipated, it was observed that the administration of the drug caused a shift in the mice's behaviors on the γ - μ plane towards the healthy region from Figure 3a towards Figure 3b (blue dots). On the other hand, the symptoms of the CUMS + saline group appear to be more severe (as indicated by the pink dots). Next, we conducted a time-based analysis of the behavioral tests of each mouse in the group identified as the CUMS + saline group. Figure 3c illustrates that their positions on the γ – μ plane shifted gradually from the upper right region (black) to the middle region (purple) and then to the lower left region (red) through time, indicative of an increase in their symptoms. Similarly, the behaviors of the CUMS + FXT group were analyzed over time, and it was observed that they initially resided in the upper right region (black) of the γ – μ plane, which then transitioned to the middle region (red), and eventually recovered to the upper right region (blue). These observations suggest that our quantification method is capable of demonstrating the effects caused by drug administration. The ability to demonstrate the effects of drug administration is a critical requirement for the pharmaceutical industry, as it enables researchers to accurately evaluate the efficacy of new drug candidates. With further rigorous validations in the future, there is reason to be optimistic that the length of the dot-shifting-vector in the γ-μ plane could serve as a quantitative measure of a drug's therapeutic efficacy.

In this work, we proposed for the first time that depression and anxiety mice can be distinguished by LF in an OF test. Furthermore, we used speed as a surrogate indicator since stressed animals should subconsciously increase their speed after activating LF search patterns. Finally, we found that the velocity distribution of the stressed mice in the OF matched that of LF. The r-good value was significantly different from that of the normal mice. At the same time, depression and anxiety groups of mice also showed significant differences in γ and μ parameters, which we could easily distinguish. Based on the velocity LF fitting pattern of the stressed mice in OF, we could qualitatively evaluate the anxious or depressive state of the tested mice and the efficacy of novel therapeutic agents in a rapid, straightforward, and low-cost way.

In the upcoming discussion, we aim to analyse the significance of the parameters associated with the LF distribution. Given the inherent abstractness of these parameters, they may be difficult to visualize, thereby warranting a need for appropriate visualization methods. To this end, we propose the utilization of a visualization technique known as "typical mice" to represent each category and to demonstrate the statistical behavior of the respective groups. To construct the typical healthy mouse, we computed the average γ and μ parameters of the control mice group, yielding $\gamma = 1.182$ and $\mu = 0.205$, respectively. This typical mouse was named Healthy Doe. In a similar manner, we derived the Depress Doe ($\gamma = 0.380$ and $\mu = 0.005$) and the Anxiety Doe ($\gamma = 0.917$ and $\mu = 0.095$) by computing the average values of the corresponding groups. These parameters are plugged into equation 1 in the Method section and the typical velocity distributions of each group is plotted. In order to plot the typical velocity distributions for each group, the γ and μ parameters obtained for the respective group were inputted into Equation 1, as described in the Method section. The resulting output from this equation provides a graphical representation of the distribution of velocities exhibited by each group. These distributions were then plotted and can be used to compare and contrast the statistical behavior of each group (Supplementary Fig. 3).

Statement of Ethics

This study protocol was reviewed and approved by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and were approved by the Institutional Animal Care and Use Committee (IACUC) of the West China Hospital, Sichuan University (Approval No. 2019194A).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Z.H.Z. designed the thought and conceived the whole article. Z.W. directed and coordinated the study. Q.X.L. wrote the article’ s first draft, analyzed the results, and cooperated to construct the results shown in the figures. Y.R.N. wrote computer codes for data analysis and analyzed the results. Y.Q.Z. designed experiments and analyzed the behavior data. M.X. and Y.P.L. collected data and proofread the manuscript. B.C.Z, C.T, Q.L and Y.H. performed the relevant experiments. C.H.M, X.J.L, and T.Z. performed part animal behavior studies. D.J.C, N.Z. and W.K. helped with the theoretical analysis. All authors read and corrected the article.

Data Availability Statement

The data could be available at the request of the editor, and experimental data has been uploaded in a separate manner.

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Figure Legends

Fig. 1. Timeline and validation of the experimental design for the CUMS Model.

a The experimental design for CUMS demonstrated classic anxiety-like and depression behaviors. After a seven-day adaptive feeding period, mice were randomly assigned to three groups based on OFT results. The CUMS model was established from day 0 to day 51, with drug administration initiated on day 14 after successful model establishment continuing until day 51. Behavioral tests, including OFT, EPM, LDB, TST, and FST, were performed once on days 0, 14, and 42, and again on days 44, 46, 48, and 50, respectively. Additionally, SPT evaluations were conducted on days -2, 14, and 42. Weekly measurements of body weight, food intake, and coat state scores were taken (red dots in Figure 1a). The experiment concluded on day 51 with the sacrifice of the mice for further analysis. **b** Exposure to the CUMS paradigm resulted in significant anhedonia, as demonstrated by the SP test ($p < 0.001$ for Control vs. CUMS + Saline). **c-e** Throughout the duration of the CUMS model, changes in coat state score (c), body weight (d), and food intake (e) were monitored weekly ($p < 0.001$ for Control vs. CUMS + Saline, respectively). **f** On the 42nd day, OFT trajectories were recorded and analyzed. **g** On the 42nd day, during the OFT, mice in the CUMS + FXT group exhibited a higher number of center entries ($F_{2,76} = 59.74$, $p < 0.001$ for CUMS + Saline vs. CUMS + FXT). **h** CUMS + FXT mice exhibited reduced anxiety- and depression-like behaviors, as indicated by significantly greater distances traveled during the OFT ($F_{2,76} = 25.86$, $p < 0.001$ for CUMS + Saline vs. CUMS + FXT). **i** During the EPM test, mice in the CUMS + Saline group spent significantly less time exploring the open arm, with a notable difference observed between the CUMS + Saline and CUMS + FXT groups ($F_{2,76} =40.08$, $p = 0.002$) (**j**). No significant difference in behavior was observed between the CUMS + Saline and CUMS + FXT groups (**k**). **l** During the LDB test, mice in the CUMS + Saline group exhibited significantly reduced exploration time in the light box ($F_{2,76} =13.13$, $p = 0.027$ for CUMS + Saline vs. CUMS + FXT) (**m**). **n** On the 46th day, during the LDB test, mice in the control group exhibited a significantly greater number of entries ($F_{2,76} =12.17$, $p < 0.001$ for Control vs. CUMS + Saline). **o-r** During the TST and FST, mice in the CUMS + Saline group exhibited significantly longer periods of immobility (TST: $F_{2,76} =42.62$, FST: $F_{2,76} =29.44$, $p < 0.001$ for Control vs CUMS + Saline and CUMS + FXT, respectively). The presented data represent mean ± s.e.m. Statistical significance was established when p values were below 0.05. The number of animals is indicated on graphs. Differences among groups were analyzed using one-way ANOVA, followed by post hoc Bonferroni analysis. Repeated measures analysis of variance (ANOVA) was performed to determine differences

in SP test, coat state scores, body weight, and food intake among groups, followed by Bonferroni's multiple comparisons test.

Fig. 2. The walking velocity of mice exhibited a pattern of LF.

a A mouse was selected randomly, and the walking speed of the two groups was recorded as a function of time. **b-c** Random selection was performed for a control mouse and a CUMS mouse to apply the normal distribution and LF distribution models. **d-e** The comparison between the r-good of the normal distribution (**d**) and LF distribution model (**e**) was performed in each group. The data were presented as means ± s.e.m., and the number of animals is indicated on the graphs.

Fig. 3. Each mouse was represented as a dot on the γ - μ plane.

a The black dots on the γ-μ plane represented control mice (n = 27), while red and blue dots represented CUMS mice (n = 52). Our analysis indicated that LF patterns in the OFT distinguished Control and CUMS mice. The OF data for the two groups were analyzed at the 14th-day time point after CUMS modeling. A substantial difference between control and CUMS mice was observed on the γ-μ plane ($p < 0.001$ for Control vs CUMS). **b** After day 14, CUMS-stressed mice were divided into CUMS + Saline and CUMS + FXT groups. On day 42, a significant difference between the CUMS + Saline and CUMS + FXT groups was observed ($p < 0.001$). The comparison between Control and CUMS + Saline during OFT indicated a substantial difference ($p < 0.001$), while no significant difference was found between Control and CUMS + FXT group. **c** The OF data for the CUMS + Saline group were compared using the same method at 0, 14, and 42 days. A significant difference was observed between day 0 and day 14 ($p < 0.001$). Significant differences were also noted between day 14 and day 42 of the CUMS + Saline group ($p = 0.040$). The difference between day 0 and day 42 was highly evident ($p < 0.001$). **d** Significant differences were observed in pairwise comparisons of OF data at the three-time points in the CUMS + FXT group ($p < 0.001$, respectively). The number of animals is indicated on the graphs. The null hypothesis that the 2D sets of variables followed the same distribution was tested using the two-dimensional Kolmogorov-Smirnov (2D K-S) test.

Fig. 4. Mice exposed to CUMS exhibited typical symptoms of anxiety, depression, and comorbid anxiety and depression (A&D).

a The percentage of each anxiety index was determined by utilizing the Criteria Importance Through Intercriteria Correlation (CRITIC) analysis method. **b** Following the normalization of anxiety indicators

within each group, the CRITIC-derived percentage was multiplied by the normalized value of each indicator and subsequently summed to obtain the total anxiety score for each mouse. Using the control group's score as a reference point, mice with an anxiety score below 30 were deemed anxious in the CUMS group. **c** The percentage of each depression index was determined by utilizing the CRITIC analysis method. **d** Upon normalization of depression indicators within each group, the CRITIC-calculated percentage was added to the normalized value of each indicator to obtain the total depression score for each mouse. Using the control group's score as a reference, mice with a depression score below 30 were classified as depressed in the CUMS group. The data is presented as mean ± standard error of the mean (s.e.m); the number of animals is indicated on the graphs.

Fig. 5. Assessment of the reliability of LF analysis in characterizing anxiety and depression in mice.

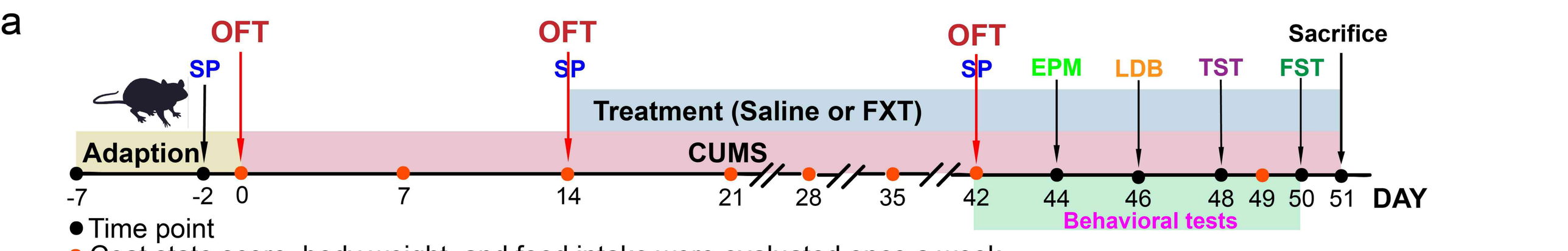
a The black region indicates the data distribution range for the control mice, whereas the red, blue, and green regions represent the data distribution range for the anxiety, depression, and A&D mice respectively on day 14. Significant differences were observed between the control group and the other three groups ($p < 0.001$ for all comparisons) on day 14; however, no significant difference was observed in the two-dimensional Kolmogorov-Smirnov (2D K-S) test between the Anxiety, Depression, and A&D groups. **b** Significant differences were observed between the control group and the other three groups on day 42 ($p < 0.001$ for all comparisons). Furthermore, significant differences were noted between the Anxiety and Depression groups ($p = 0.001$), and between the Depression and A&D groups ($p = 0.009$). The number of animals is indicated on the graphs. The two-dimensional Kolmogorov-Smirnov (2D K-S) test was employed to evaluate the null hypothesis that the 2D sets of variables conform to the same distribution.

Fig. 6. The LF method was employed to differentiate open field videos of mice exhibiting depression or anxiety, which were validated in prior experiments.

a Three distinct models, namely chronic unpredictable mild stress (CUMS), electric shocks (ES), and chronic restraint stress (CRS) were utilized. CUMS was categorized into two groups based on behavior, anxiety-like (CUMS1; n = 99) and depression-like (CUMS2; n = 46). Additionally, ES (n = 89) represented an anxiety-like model, while CRS (n = 69) depicted a depression-like model. The control group (n = 237) was also included. Following successful modeling, LF fitting was performed on OFT videos to determine the precise values of γ and μ. A support vector machine (SVM) calculated two lines that distinguished the three data groups based on training data. The optimal linear separation

between the control and anxiety-like groups was determined as L_1: $\mu = -0.59\gamma + 0.79$, while L_2: $\mu = 6.54\gamma - 3.91$ represented the optimal linear separation between the control and anxiety-like groups. **b** The two-dimensional map distribution of the four groups of mice, validated in this experiment, was shown using two curves fitted by 540 mice. Rephrased sentence: The control group selected the data of OFT tested for the first time (Day 0), and the other groups selected the anxiety group, depression group and A&D group distinguished by CUMS modeling (Day 42). The number of animals is indicated on the graphs.

a

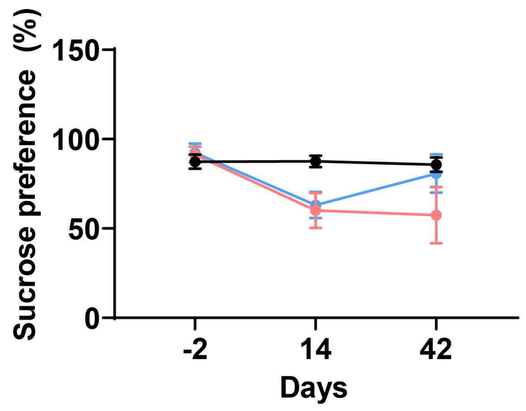


● Time point

● Coat state score, body weight, and food intake were evaluated once a week

Abbreviations: Chronic unpredictable mild stress (CUMS), fluoxetine (FXT), sucrose preference (SP), open field test (OFT), elevated plus maze (EPM), light/dark box (LDB), tail suspension test (TST), forced swim test (FST).

b



Control vs. CUMS + Saline, $P < 0.001$
Control vs. CUMS + FXT, $P < 0.001$

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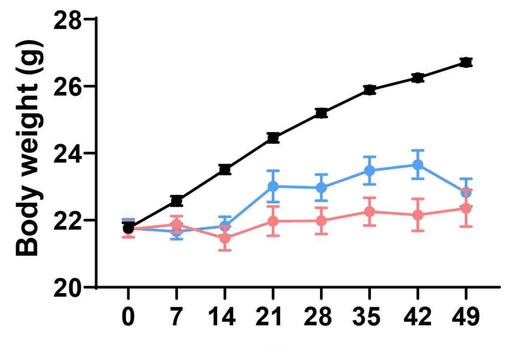

Control vs. CUMS + Saline, $P < 0.001$
Control vs. CUMS + FXT, $P < 0.001$

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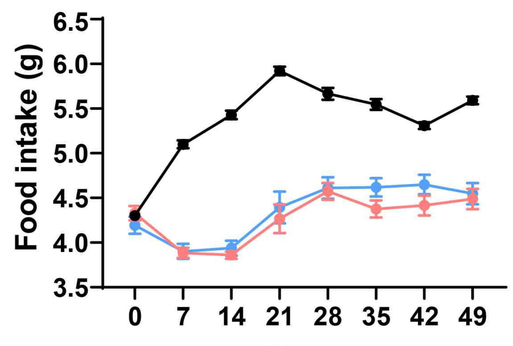

Control vs. CUMS + Saline, $P < 0.001$
Control vs. CUMS + FXT, $P < 0.001$

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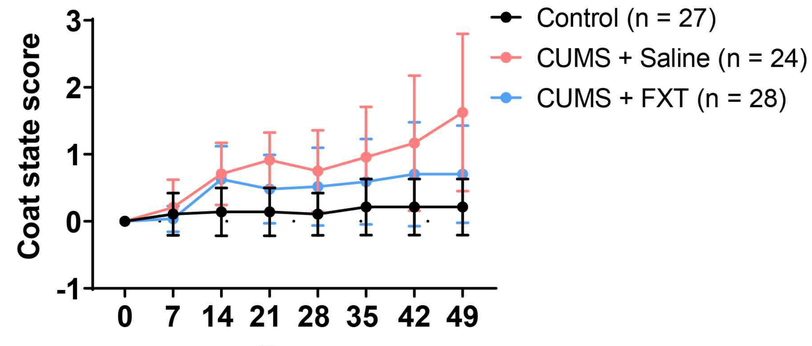

Control vs. CUMS + Saline, $P < 0.001$
Control vs. CUMS + FXT, $P = 0.001$

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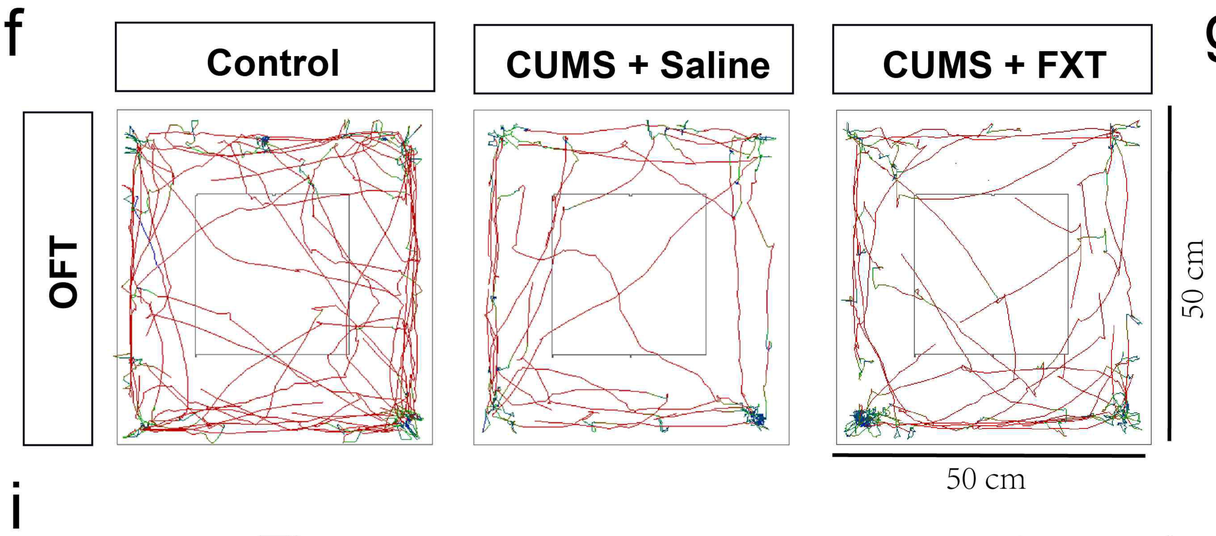

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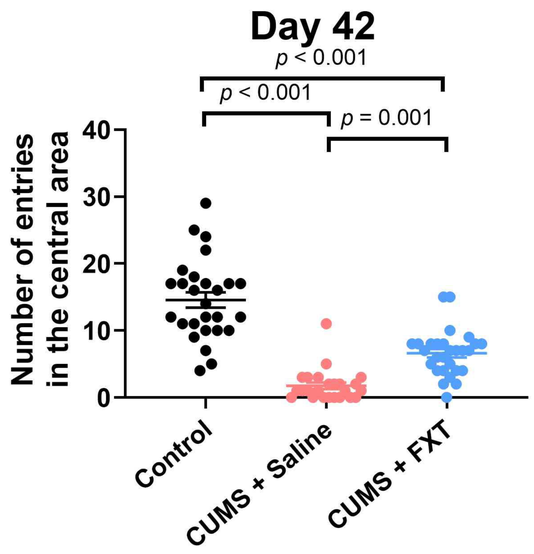

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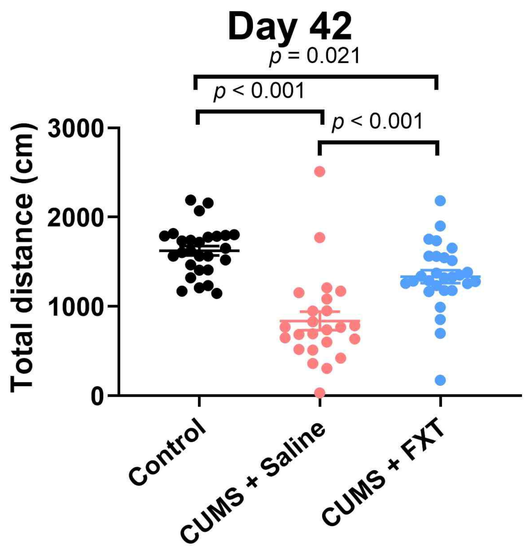

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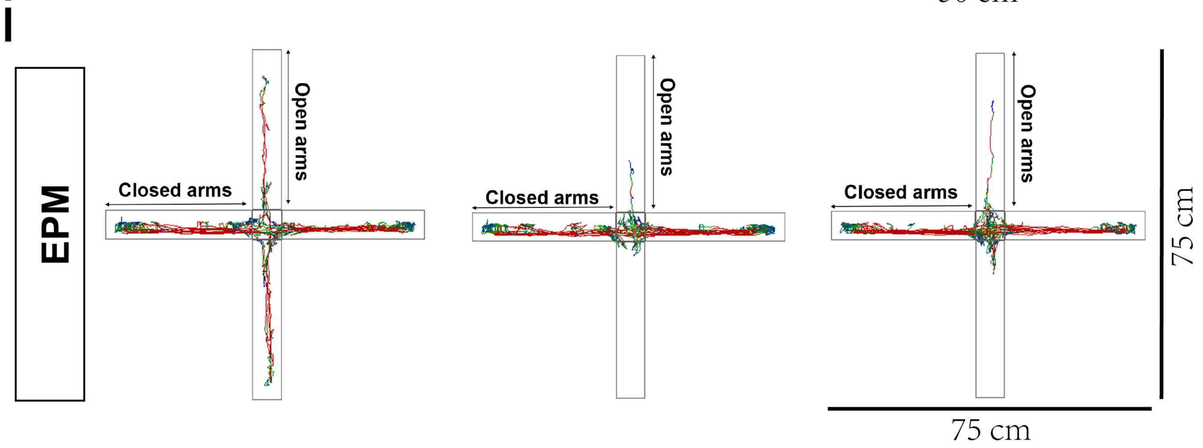

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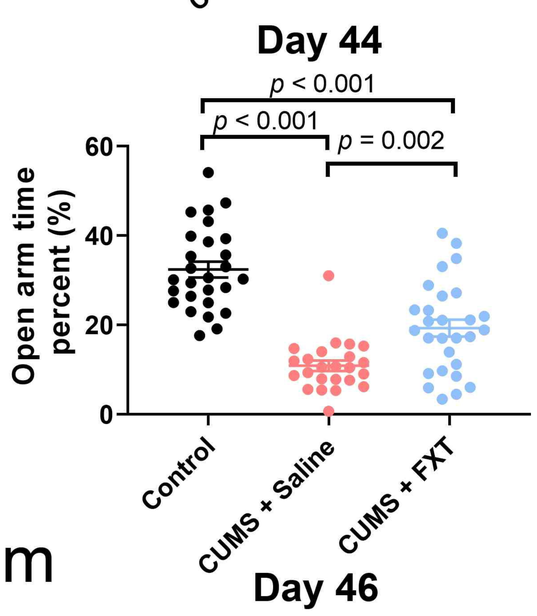

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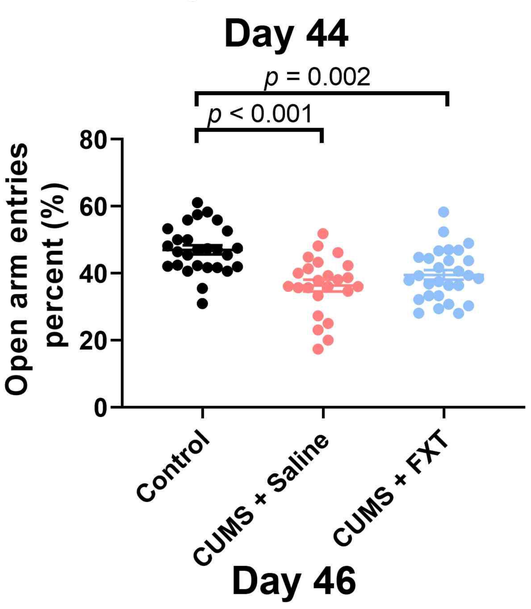

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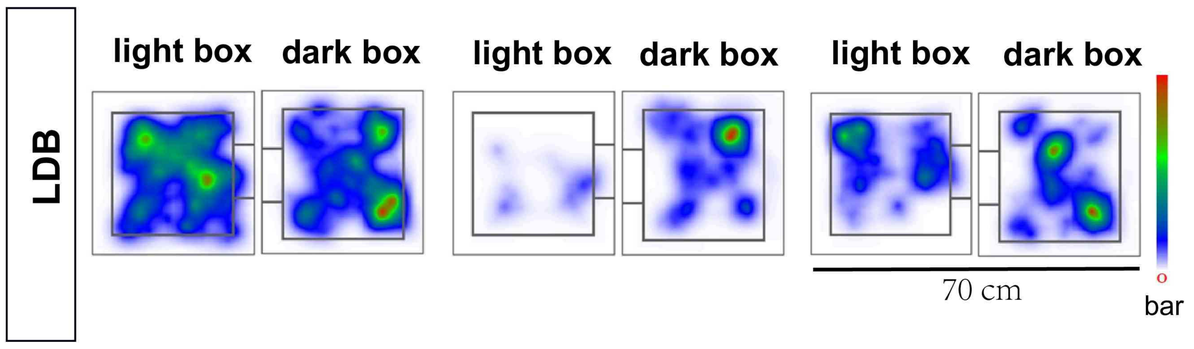

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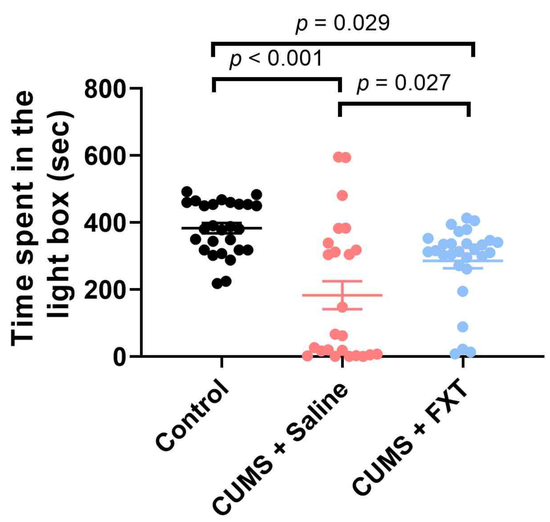
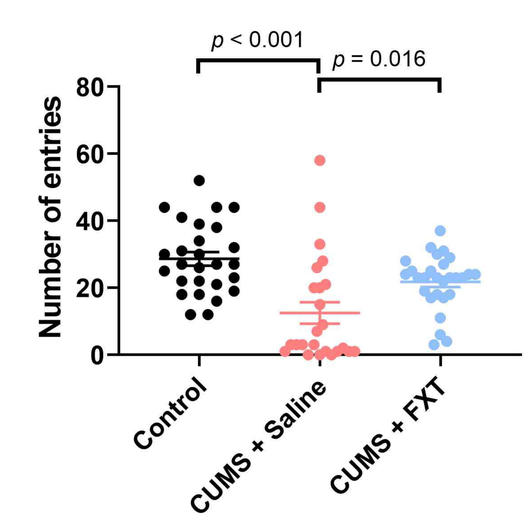
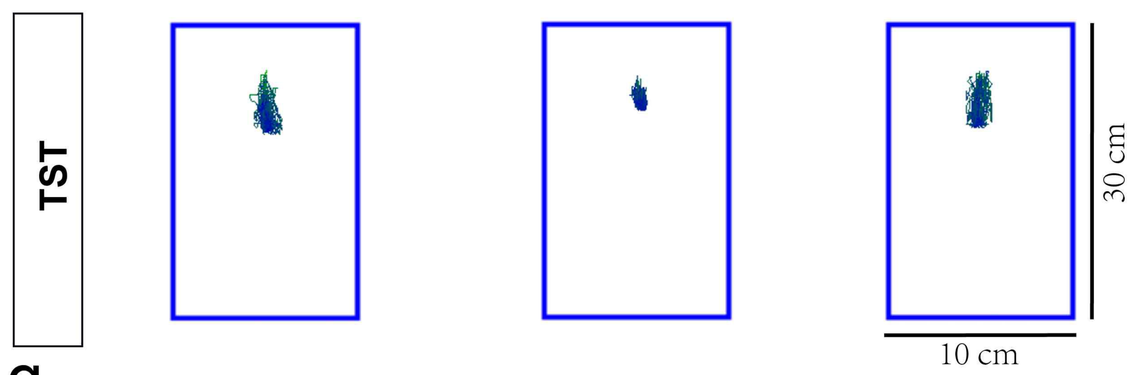


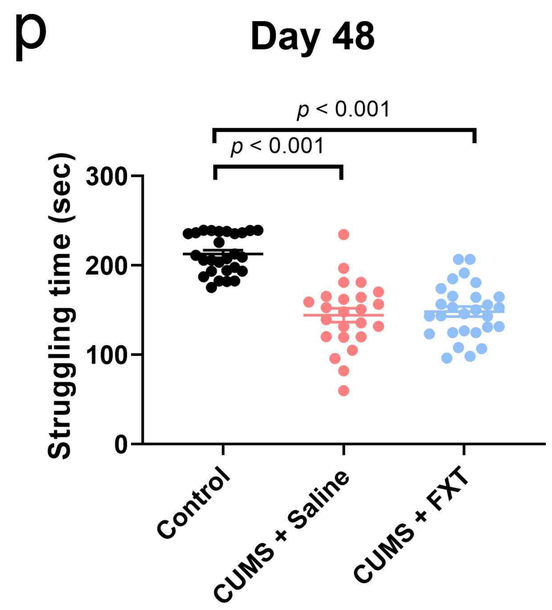
n



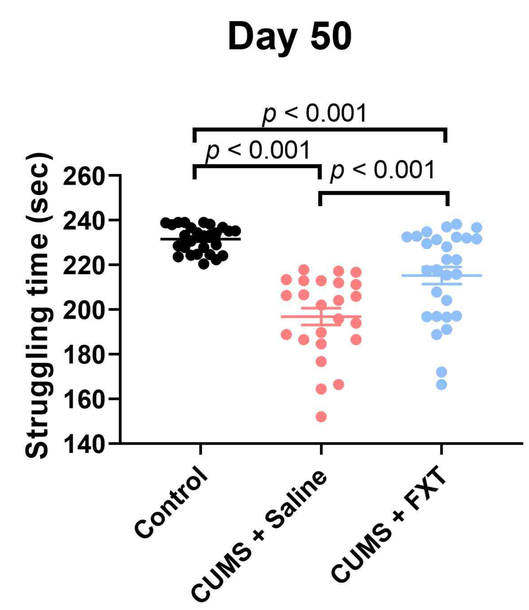
o



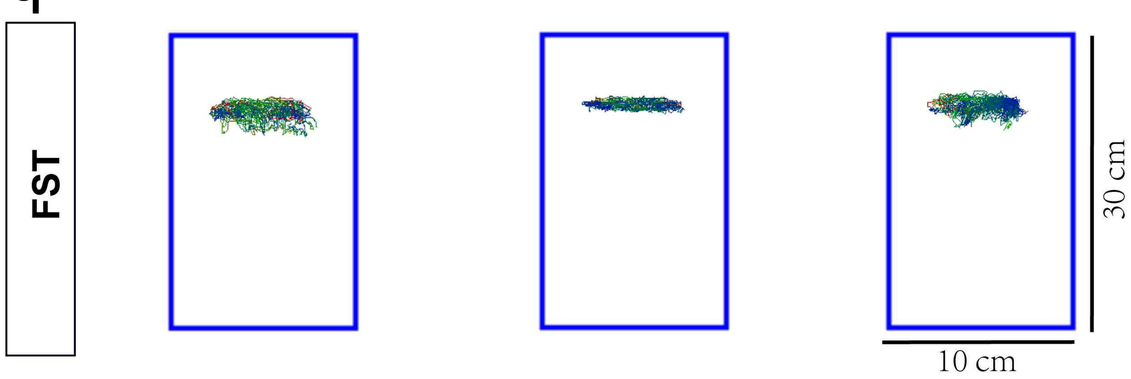
p

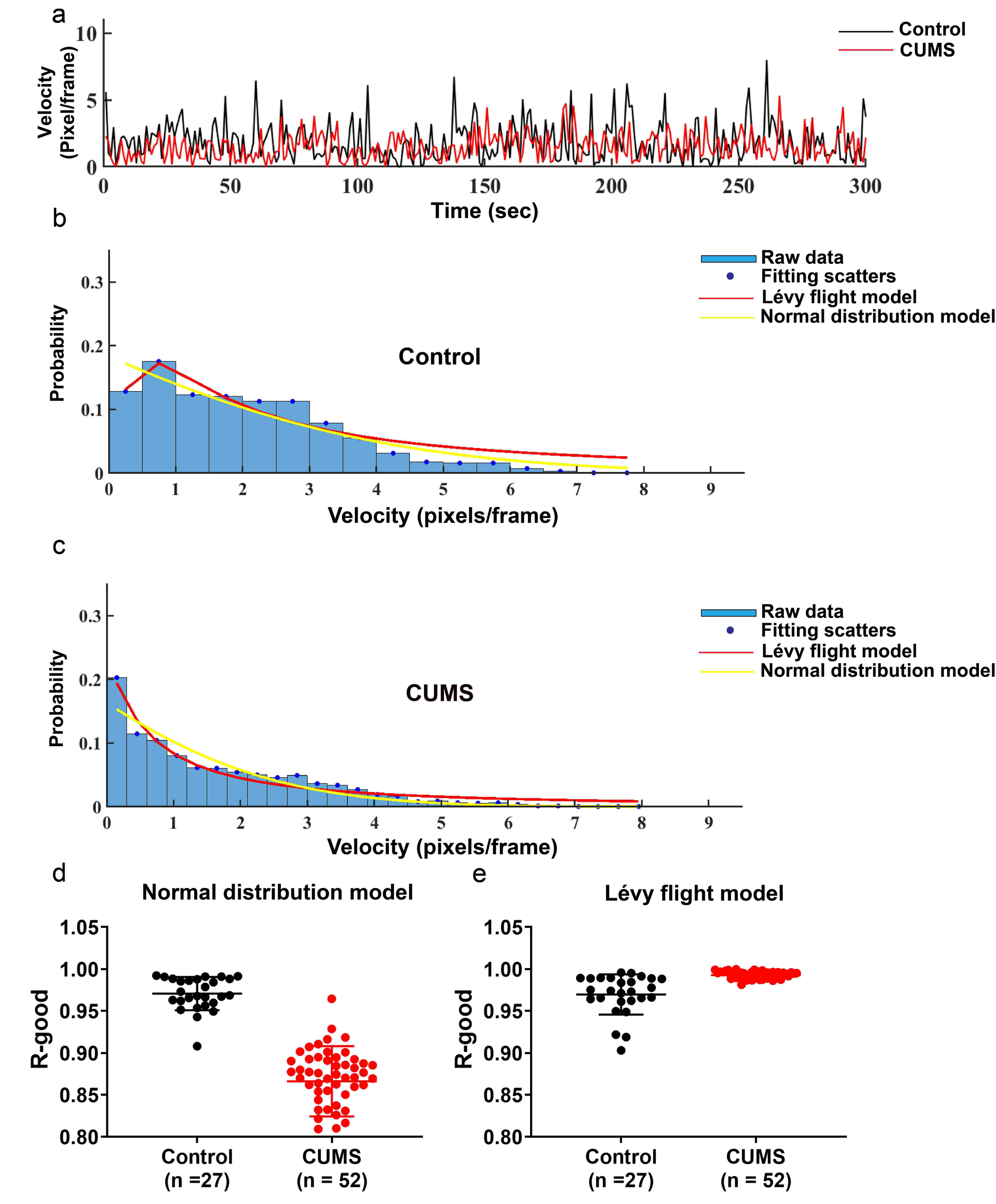


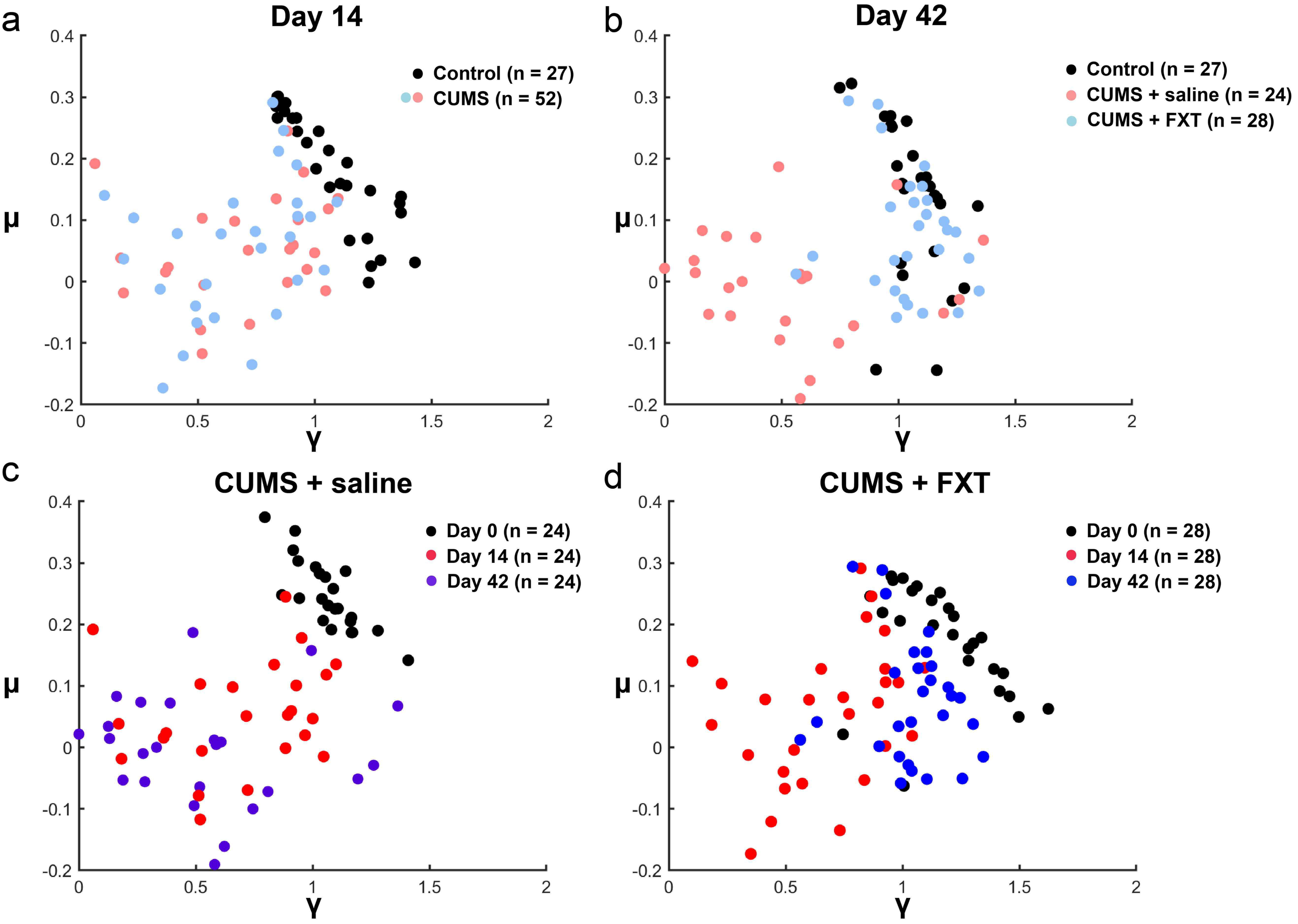
r

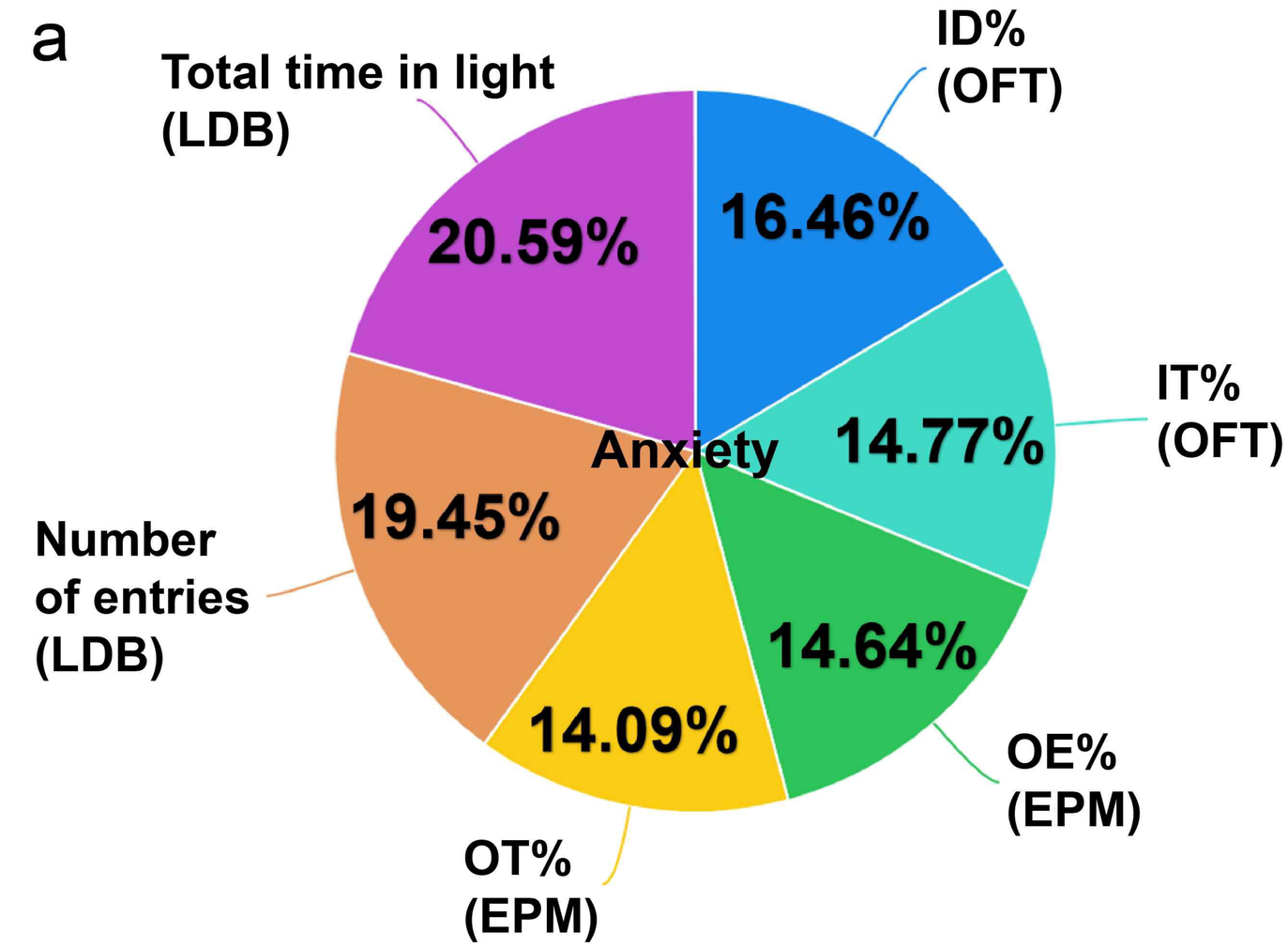


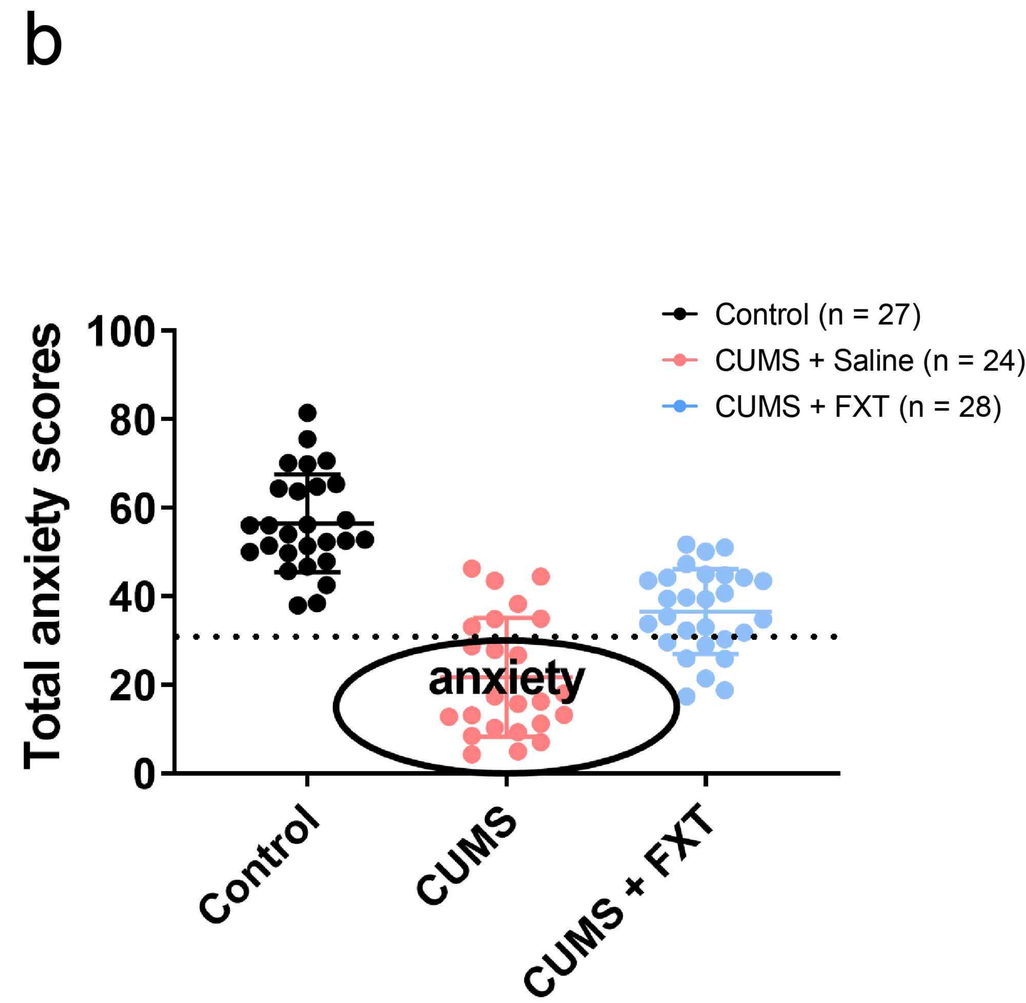
q

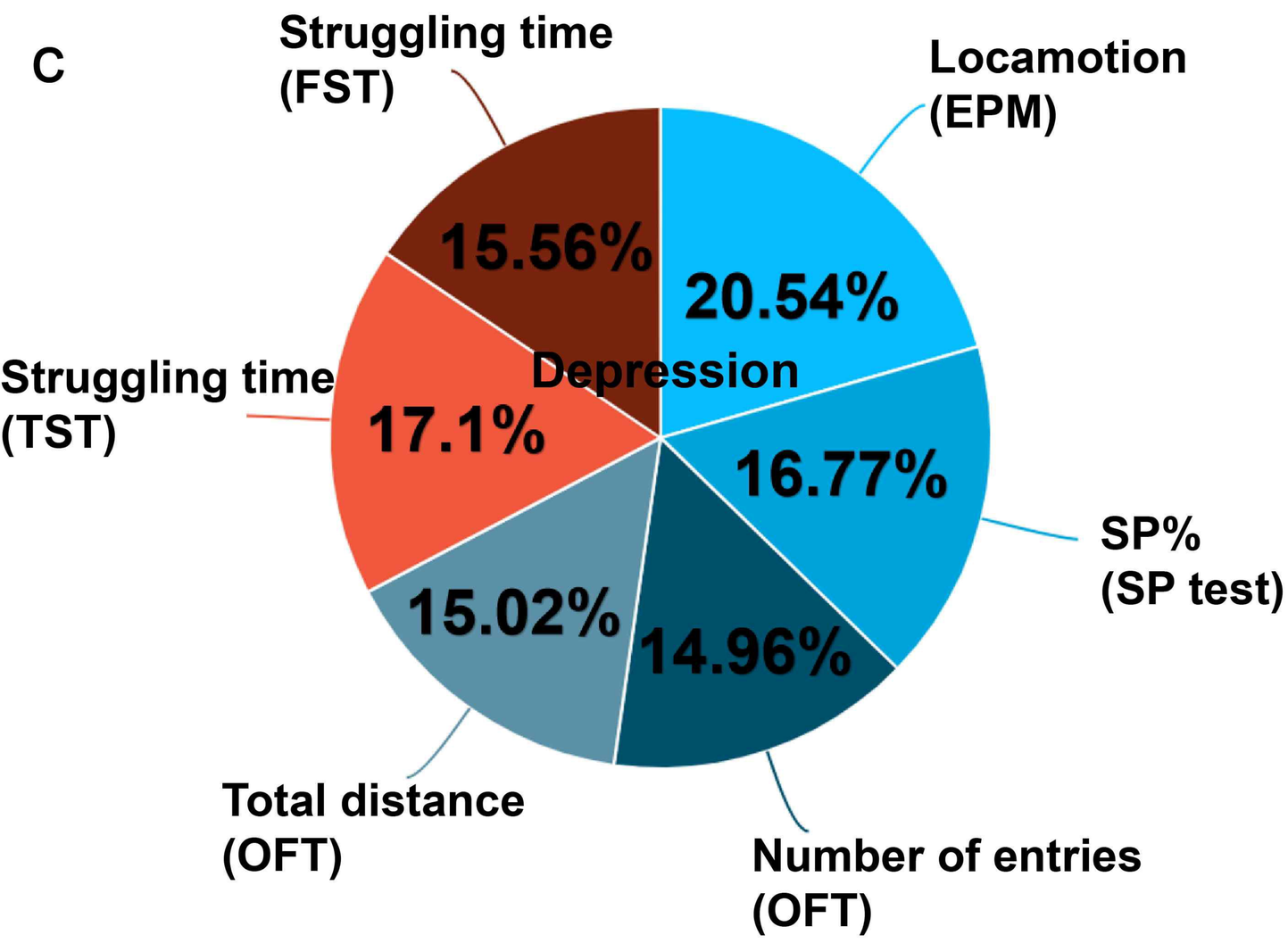


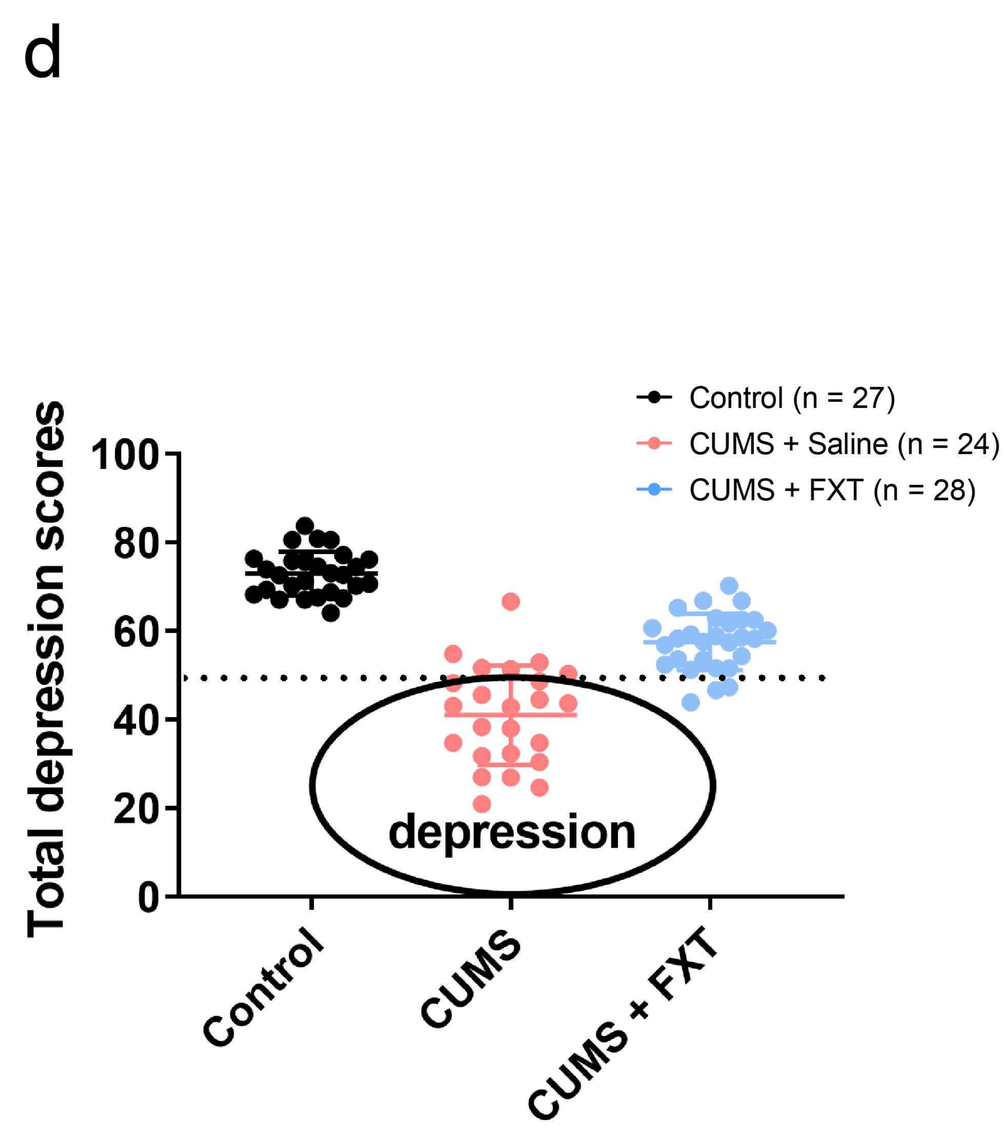

a
Velocity (Pixel/frame)
Time (sec)
Control
CUMS
b
Probability
Velocity (pixels/frame)
Control
Raw data
Fitting scatters
Lévy flight model
Normal distribution model
c
Probability
Velocity (pixels/frame)
CUMS
Raw data
Fitting scatters
Lévy flight model
Normal distribution model
d
Normal distribution model
R-good
Control (n =27)
CUMS (n = 52)
e
Lévy flight model
R-good
Control (n =27)
CUMS (n = 52)

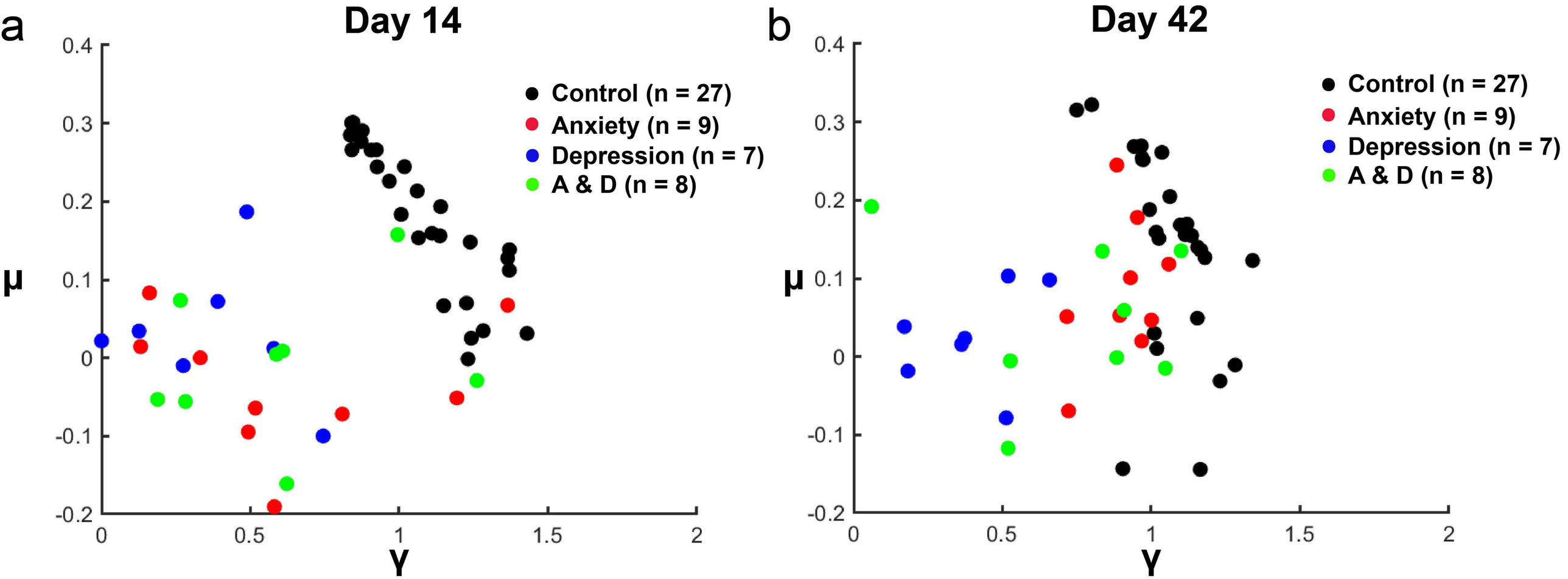

a
Day 14
Control (n = 27)
CUMS (n = 52)
b
Day 42
Control (n = 27)
CUMS + saline (n = 24)
CUMS + FXT (n = 28)
c
CUMS + saline
Day 0 (n = 24)
Day 14 (n = 24)
Day 42 (n = 24)
d
CUMS + FXT
Day 0 (n = 28)
Day 14 (n = 28)
Day 42 (n = 28)
μ
γ
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0
0.5
1
1.5
2

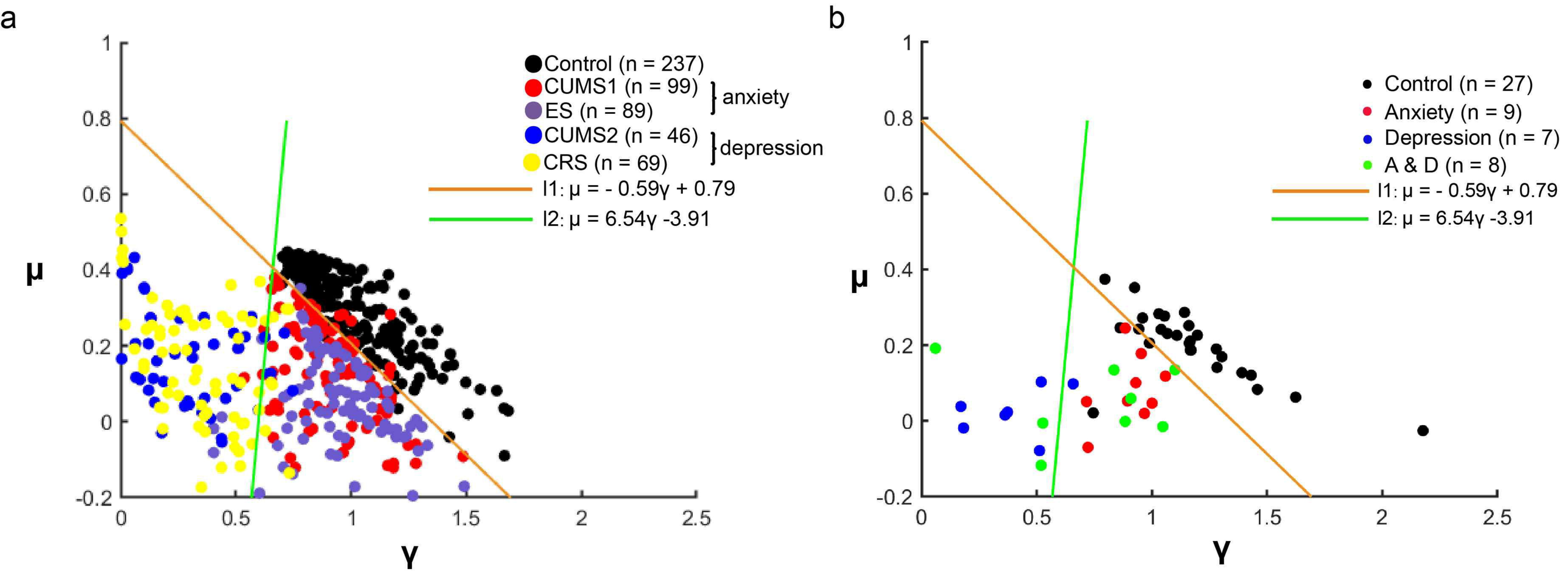

a
Total time in light (LDB)
ID% (OFT)
20.59%
16.46%
IT% (OFT)
Anxiety
14.77%
19.45%
Number of entries (LDB)
14.64%
14.09%
OE% (EPM)
OT% (EPM)

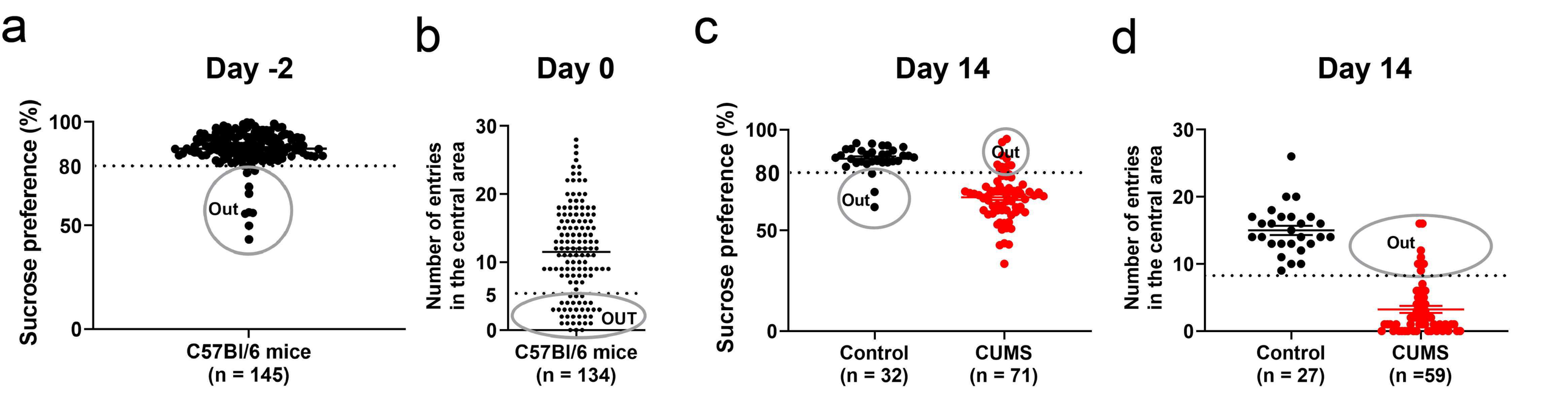

b
Control (n = 27)
CUMS + Saline (n = 24)
CUMS + FXT (n = 28)
Total anxiety scores
100
80
60
40
20
0
anxiety
Control
CUMS
CUMS + FXT

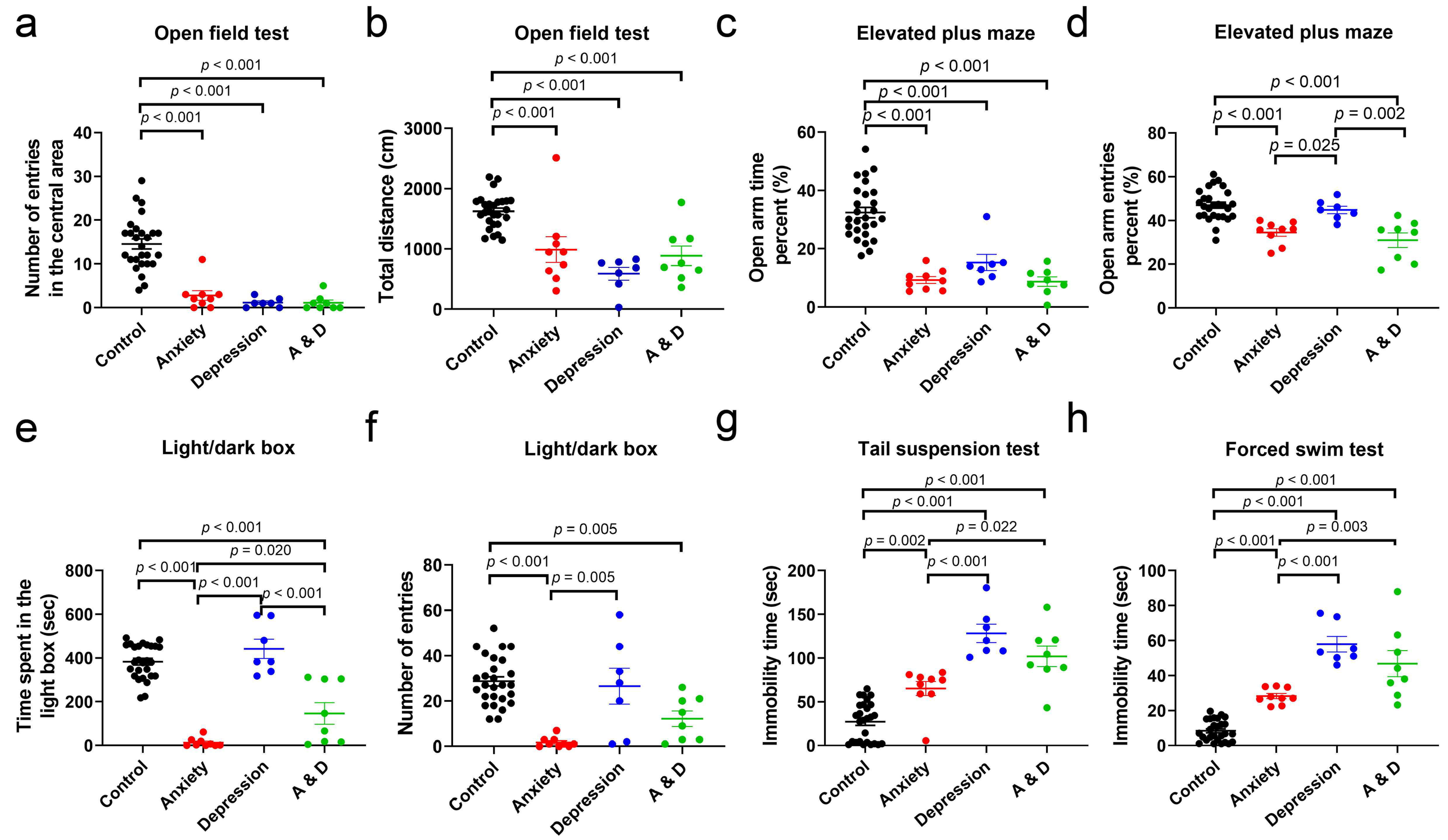

c
Struggling time (FST)
Locomotion (EPM)
15.56%
20.54%
Struggling time (TST)
Depression
17.1%
16.77%
SP% (SP test)
15.02%
14.96%
Total distance (OFT)
Number of entries (OFT)

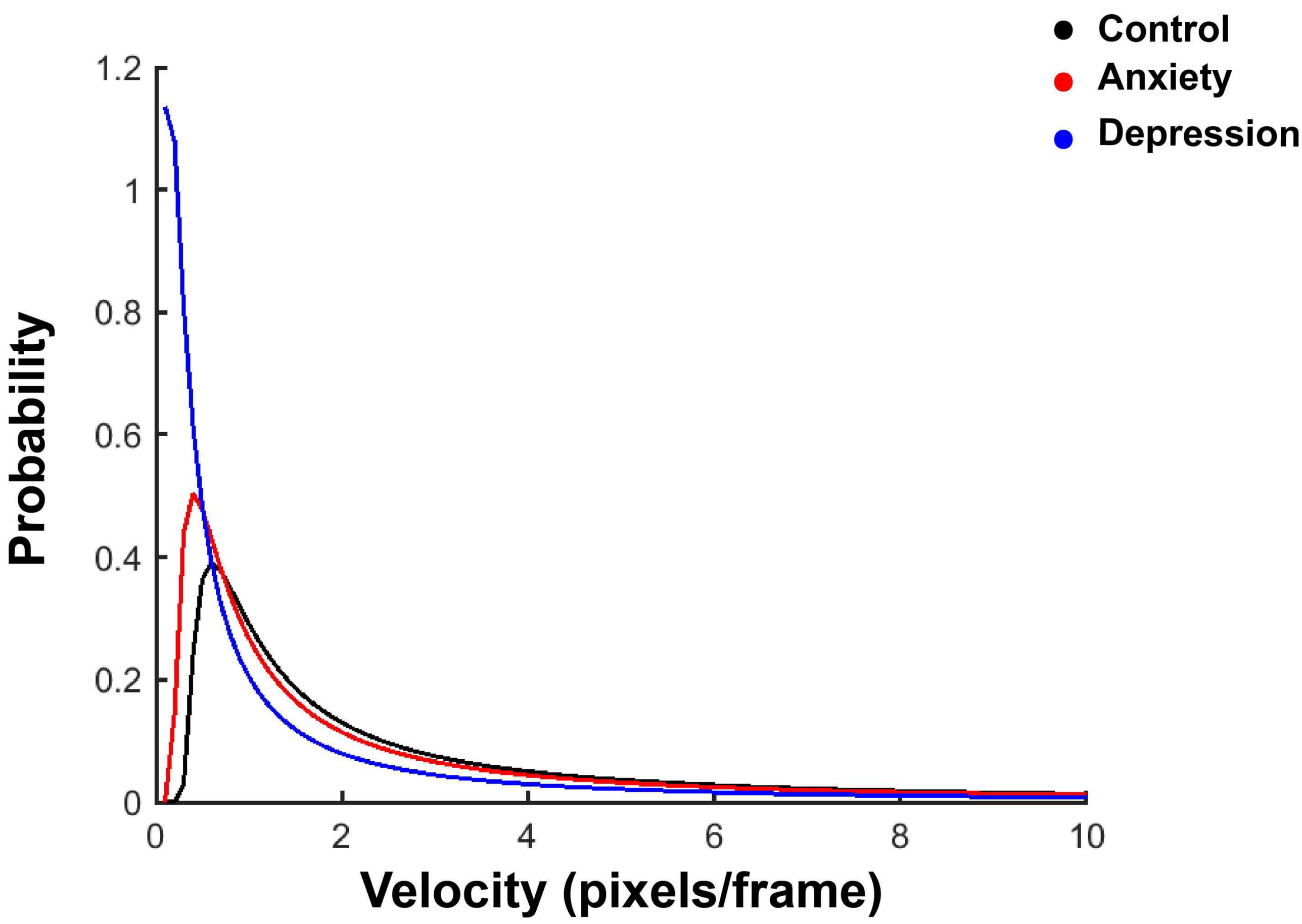

d
Control (n = 27)
CUMS + Saline (n = 24)
CUMS + FXT (n = 28)
Total depression scores
100
80
60
40
20
0
depression
Control
CUMS
CUMS + FXT


a
Day 14
b
Day 42
Control (n = 27)
Anxiety (n = 9)
Depression (n = 7)
A & D (n = 8)
μ
γ
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0
0.5
1
1.5
2


a
Control (n = 237)
CUMS1 (n = 99)
ES (n = 89)
anxiety
CUMS2 (n = 46)
CRS (n = 69)
depression
l1: μ = - 0.59γ + 0.79
l2: μ = 6.54γ -3.91
μ
γ
b
Control (n = 27)
Anxiety (n = 9)
Depression (n = 7)
A & D (n = 8)
l1: μ = - 0.59γ + 0.79
l2: μ = 6.54γ -3.91
μ
γ


a
Day -2
Sucrose preference (%)
100
80
50
0
Out
C57Bl/6 mice
(n = 145)
b
Day 0
Number of entries
in the central area
30
20
10
5
0
OUT
C57Bl/6 mice
(n = 134)
c
Day 14
Sucrose preference (%)
100
80
50
0
Out
Out
Control
(n = 32)
CUMS
(n = 71)
d
Day 14
Number of entries
in the central area
30
20
10
0
Out
Control
(n = 27)
CUMS
(n =59)


a
Open field test
Number of entries in the central area
p < 0.001
p < 0.001
p < 0.001
Control
Anxiety
Depression
A & D
b
Open field test
Total distance (cm)
p < 0.001
p < 0.001
p < 0.001
c
Elevated plus maze
Open arm time percent (%)
p < 0.001
p < 0.001
p < 0.001
d
Elevated plus maze
Open arm entries percent (%)
p < 0.001
p < 0.001
p = 0.002
p = 0.025
e
Light/dark box
Time spent in the light box (sec)
p < 0.001
p = 0.020
p < 0.001
p < 0.001
f
Light/dark box
Number of entries
p = 0.005
p < 0.001
p = 0.005
g
Tail suspension test
Immobility time (sec)
p < 0.001
p < 0.001
p = 0.022
p = 0.002
p < 0.001
h
Forced swim test
Immobility time (sec)
p < 0.001
p < 0.001
p = 0.003
p < 0.001
p < 0.001


Control
Anxiety
Depression
Probability
1.2
1
0.8
0.6
0.4
0.2
0
0
2
4
6
8
10
Velocity (pixels/frame)