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The role of isolated diffuse axonal brain injury on post-traumatic depressive- and anxiety-like behavior in rats

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Traumatic brain injury (TBI) is a significant global health concern and is associated with short-term and long-term comorbidities such as mood disorders and reduced quality of life. Diffuse axonal brain injury (DABI) is a common but severe type of TBI. The role of DABI in the development of psychiatric sequelae after TBI is not well understood due to the challenge of isolating DABI from general TBI in the human population. Here we investigate the role of DABI in the occurrence of post-TBI depressive- and anxiety-like behaviors in a rat model. Forty rats were randomly assigned to two groups, with 20 receiving DABI and 20 receiving sham treatment. We used a magnetic resonance imaging (MRI) protocol developed for DABI using a 3-T clinical scanner to confirm DABI. We then compared neuroimaging, neurological and behavioral assessments across experimental groups. There was a significant difference between DABI and sham groups on sucrose preference, a measurement of depressive-like behavior ($p < 0.012$), and time spent on open arms on a plus maze test, a measurement of anxiety-like behavior ($p < 0.032$). For MRI-detected injury, there was a difference in diffusion-weighted imaging with relative anisotropy ($p < 0.001$) and fractional anisotropy ($p < 0.001$) mapping. We found that isolated DABI in our model led to post-traumatic depressive-like behavior in 30% of cases and anxiety-like behavior in 35%. Additionally, we established diagnostic cut-offs for depressive-like and anxiety-like behaviors in injured rats. We also documented comorbidity between the development of depression and anxiety in DABI-exposed rats. We anticipate that this study will greatly enhance the understanding of the relationship between DABI, TBI, and mood disorders like depression and anxiety, and aid in developing treatment options for these interconnected conditions.

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INTRODUCTION

Traumatic brain injury (TBI) has a significant global impact [1] as a leading cause of death and disability [2, 3]. TBI accounts for up to one-third of all accidental deaths and is the primary cause of trauma-related deaths in hospitals [4–7].

Diffuse axonal brain injury (DABI) is a common type of TBI that results when the brain's long connective nerve fibers are torn due to rapid movement inside the skull as an injury is occurring [8–10]. DABI is considered one of the most severe forms of TBI due to its widespread impact on brain function and the potential for long-term disability [11]. Impairments can range from confusion and memory issues to a shortened attention span and amnesia, affecting social reintegration and overall quality of life. Though its exact prevalence can vary, it is estimated to occur in approximately 40–50% of all TBI patients with a mortality rate around 42–62% [1, 2]. Despite being a critical aspect of TBI, DABI has not been as extensively studied, often limited to research using unspecialized animal models [12–16].

Survivors of TBI are at increased risk for severe, long-term psychiatric disorders, including major depression [17, 18], anxiety [19], post-traumatic stress disorder [20, 21], social withdrawal [22], apathy [22, 23], and aggression [24–28]. These conditions can persist for decades [22, 29] and significantly hinder rehabilitation and restoration of daily functioning [30, 31]. Depression and anxiety, in particular, severely impact health, productivity, and quality of life, correlating with poorer global outcomes, reduced social functioning, and lower health-related quality of life, even when other factors are considered [32–37]. The role of DABI in the development of psychiatric sequelae following TBI remains poorly understood, largely because it is impossible to isolate DABI from general TBI in the human population.

In order to better understand the mechanisms responsible for the development of behavioral disorders after TBI, it is essential to isolate and examine the specific impact of DABI on post-TBI depression and anxiety. To the best of our knowledge, these

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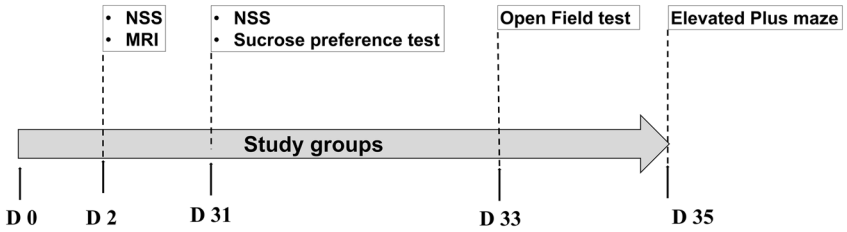

0	**Day (D)**	Start of study - surgical intervention.
2	**D**	NSS, MRI.
31	**D**	Sucrose preference test and NSS (2 days duration).
33	**D**	Open field test (1 day duration).
35	**D**	Elevated plus maze (1 day duration).

Fig. 1 Experimental timeline. MRI magnetic resonance imaging, NSS neurological severity score.

conditions has never been studied in an isolated DABI model, neither in humans nor animals. In this study, we applied behavioral tests that have been successfully used in other TBI models—such as weight-drop, fluid percussion injury, and controlled cortical impact [38–40]—to assess depressive- and anxiety-like behaviors in rodent models specifically tailored for the DABI protocol.

We have already established and validated an isolated DABI model using histologic markers [41], and we designed a complementary protocol utilizing magnetic resonance imaging (MRI) technology, which is critical for non-invasive assessment of brain damage in studying animal behavior [16]. Our prior work explored the development and progression of depression and anxiety in various contexts, including TBI [38–40], stroke [42–44], subarachnoid hemorrhage [45], and in a rat model of chronic unpredictable stress [46], early life stress [47], and other conditions causing behavioral changes [48].

The goals of this study are twofold: first, to investigate the role of DABI in the onset of depressive and anxiety-related behaviors; and second, to establish diagnostic criteria for identifying these behavioral disorders in rats. This study is the first to investigate the role of DABI in the development of depression and anxiety, and we hope that our findings will help provide new insights into mechanisms driving this relationship.

MATERIALS AND METHODS

The experiments were carried out following the guidelines of the Declarations of Helsinki and Tokyo, as well as the European Community's Guidelines for the Use of Experimental Animals. Approval for the experiments was granted by the Animal Care Committee at Ben-Gurion University of the Negev, Beer-Sheva, Israel.

Animals

The experiments utilized 20 male and 20 female Sprague-Dawley rats from Harlan Laboratories in Israel, each weighing between 280 and 320 grams. The rats were provided with Purina Chow and water ad libitum. They were kept under a 12-h light/12-h dark cycle at a constant temperature of 22 ± 1 °C. All experimental procedures were conducted during the dark phase, between 08:00 and 16:00.

Experimental design

The study was conducted using a 2 × 2 design. The dependent variables were DABI (20 rats) sham (20 rats) and sex (male/female). Rats with motor deficits after four weeks were excluded from the study so that the behavioral results would not be confounded by motor deficits. Magnetic resonance imaging and neurological status were assessed 48 h after the intervention. At one month after the start of treatment, all rats from each experimental group underwent a series of behavioral tests (Fig. 1).

Neurological severity score (NSS)

NSS was recorded by two individual blinded observers, as previously described [41, 49]. Scores were assigned based on observed changes in motor functions and behavior. The highest possible score of 15 indicated the most severe neurological dysfunction, while a score of 0 represented an intact neurological condition. The evaluated parameters included: gait on a wide surface (3-point scale), gait on a narrow surface (4-point scale), effort to remain on a narrow surface (2-point scale), beam walking (3-point scale), and beam balance (3-point scale).

Induction of moderate DABI

The apparatus was previously described by our lab [41]. The device has four main components: (1) a transparent plastic cylinder; (2) an iron weight; (3) a rotation mechanism with a cylindrical tube, two bearings, and head fixation for ear pins; and (4) a horizontal platform with two bearings. In this study, a force of 2.3 kg was applied to the animal's head to induce DABI, as described previously [41]. Rats were anesthetized with isoflurane (5% for induction, 1.5–2.5% for maintenance) using equal parts medical air and oxygen. After induction, the rat's head was fixed in the device, and an ear pin was inserted into the ipsilateral external auditory canal. A free-falling weight dropped from 100 cm hit the bolt, activating the rotation mechanism, causing the rodent's head to turn rapidly from 0–90°. The rat was then awakened and moved to a recovery room.

Diffusion-weighted imaging (DWI)

A 3 T MRI (Ingenia, Philips Medical Systems, Best, The Netherlands) with an eight-channel receive-only coil was used to perform DWI 48 h after the intervention, as previously described [16, 49]. The animals were kept under general anesthesia (1.5% isoflurane in oxygen). Axial diffusion tensor imaging in six directions was performed using a multi-shot, spin-echo, echo-planar sequence (TR/TE = 1419/138 msec, epi factor of 19, SENSE factor of 1.5, b-factor of 1000 s/mm^2, and fat suppression). Seven slices with zero gaps were acquired at a resolution of 0.55 × 0.55 × 2.0 mm. Five signal averages were collected over 11:19 min. The Intellispace Portal workstation (V5.0.0.20030, Philips Medical Systems) was used for post-processing.

Regions of interest (ROI)

Regions of interest (ROIs) were defined using anatomical landmarks according to the Paxinos and Watson rat brain atlas [50] (see Supplementary Material 1). Diffusion tensor imaging (DTI) values from the bregma −3.14 mm image slice (right and left hemispheres) were averaged for each anatomical region.

DWI parameter map analysis

An expert who was blind to the group assignments analyzed the images. Relative anisotropy (RA) and fractional anisotropy (FA) maps were produced using the Philips software package, Version 5.7.1.2 (Ingenia, Philips Medical Systems, Best, The Netherlands). The values of DTI metrics were averaged once for each ROI [51] and subsequently analyzed [16, 51, 52].

Sucrose preference test

The sucrose preference test, used to evaluate anhedonia and depressive-like symptoms in rodents, was conducted as previously described [48]. Each rat was given two bottles of 1% (w/v) sucrose solution in their cage. After 24 h, one bottle was replaced with water to acclimate the rats to having both water and sucrose. Following this habituation, the rats were deprived of food and water for 12 h. They were then placed in individual cages with access to two bottles, one containing 100 ml of 1% sucrose solution and the other 100 ml of water, for 4 h. The volumes of consumed sucrose solution and water were recorded. Sucrose preference was calculated as the volume of sucrose consumed divided by the total volume of both sucrose and water consumed, multiplied by 100 to get a percentage [48].

Open field test

The standard open field test assesses locomotor, exploratory, and depressive-like behaviors in animals [48]. The test involves placing animals individually in a round, black plastic arena (2 m diameter, 60 cm high) in a darkened room. The arena was cleaned with 10% ethanol after each session to prevent residual smells from previous rats. A video camera mounted 200 cm above recorded the rat's activity for 5 min using a Logitech HD Pro Webcam C920. The recordings were analyzed with Ethovision XT software (Noldus, Wageningen, Netherlands) based on the total distance traveled [38].

Elevated plus maze task

The plus maze was placed in a dark room and consisted of two open and two closed arms (each 16 × 46 cm), constructed from black plastic and positioned 100 cm above the floor. The closed arms had 40 cm high surrounding walls. The maze was cleaned with 10% ethanol before each trial. Rats were tested individually in random order, starting in the center of the maze facing an open arm. Their behavior was recorded for 5 min with a Logitech HD Pro Webcam C920 and analyzed using Ethovision XT software (Noldus, Wageningen, Netherlands) [40].

Statistical analysis

Statistical evaluation was carried out using the SPSS-24 package (SPSS Inc., Chicago, USA). A Kolmogorov–Smirnov test was used to determine whether to employ parametric or non-parametric analysis for the comparisons between the different parameters. The data were analyzed with a 2 way-ANOVA for the effects of brain trauma and sex. Prior to conducting the t-tests, the assumption of homogeneity of variances between the two groups was evaluated using Levene's test for equality of variances. When Levene's test indicated significant differences in variance between the groups ($p < 0.05$), indicating unequal variances, we adjusted the degree of freedom for the subsequent t-tests according to the Welch–Satterthwaite equation. The significance of comparisons between groups was determined using the Mann–Whitney, 2-sided (for non-parametric data and NSS) and t test, 2-sided (for parametric data, MRI parameters and behavioral results). To establish the diagnostic criterion cut-off value for post-traumatic depressive, emotionality and locomotor hyperactivity, and anxiety in DABI rats, we used the mean ± 2 SD method [53–55]. Statistical significance of the diagnostic criterion cut-off values for sucrose preference, total distance traveled, and time spent in open arms in DABI rats was analyzed with a chi square, Fisher's exact test, 1-sided. The phi coefficient (φ) for dichotomous variables and Pearson coefficient for quantitative variables were used in the chi-squared test to assess the correlation between two variables. Normally distributed data and continuous variables were presented as mean ± SD. Nonparametric data were presented as a median ± inner quartile range. Results were considered statistically significant when $p < 0.05$.

Table 1. Neurological severity score 48 h after DABI.

NSS values of the study groups		
Animal Groups	**N**	**Median (range)**
Sham-operated	20	0(0–0)
DABI	20	1(0–3)*

A significant difference in NSS was observed at 48 h between the DABI group and the sham-operated group, indicated with an asterisk ($p < 0.01$). The data are measured as counts and presented as median and range

RESULTS

Neurological severity score (NSS)

The control sham did not indicate any neurological deficit. The NSS at 48 h was significantly greater in DABI rats compared to the sham group (1(0–3) vs. 0(0–0), U = 78, $p < 0.001$, r = 0.585, Mann–Whitney U test). The data are measured as a count and expressed as median and 25–75 percentile range, see Table 1. No significant difference was observed between males and females.

Analysis of neuroimaging outcomes from MRI

The data were analyzed with a 2-way ANOVA to determine the effect of the brain trauma (DABI or sham) and sex (male or female).

Fractional anisotropy (FA) for the thalamus

A 2-way ANOVA showed a significant effect of trauma ($F1,36 = 73.6$, $p < 0.001$, $pη2 = 0.672$). No effects of sex or interaction between sex and trauma on FA were found. The analysis using Student's t-test showed a significantly lower effect between the 20 DABI rats and the 20 sham-operated rats in the thalamus at 48 h after injury (0.3 ± 0.08, $\bar{x} \pm SD$, vs. 0.51 ± 0.07, $\bar{x} \pm SD$; $t(38) = 8.8$; $p < 0.001$; $d = 2.8$) (see Fig. 2a).

Fractional anisotropy for cerebral cortex

A 2-way ANOVA showed a significant effect of trauma ($F1,36 = 79.4$, $p < 0.001$, $pη2 = 0.688$). No effects of sex or interaction between sex and trauma on FA were found. The

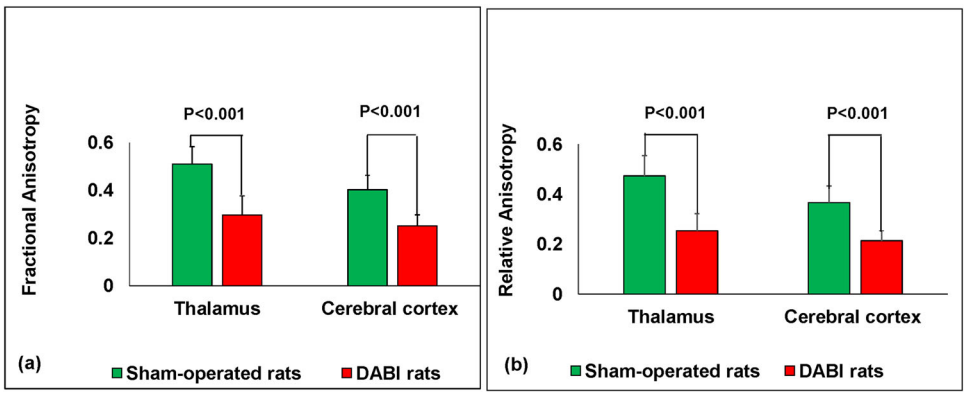

Fig. 2 MRI-determined outcome parameters 48 h after DABI. a Fractional anisotropy. In the thalamus and cerebral cortex, fractional anisotropy is significantly lower in DABI rats (red bars) compared to sham-operated rats (green bars). **b** Relative anisotropy. In the thalamus and cerebral cortex, the relative anisotropy values are significantly lower in DABI rats (red bars) compared to sham-operated rats (green bars).

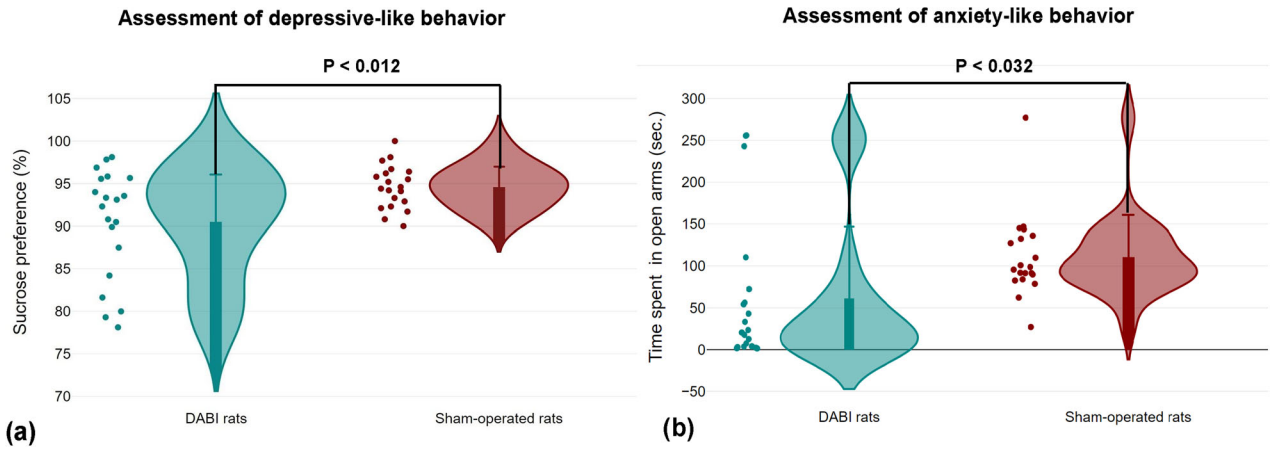

Fig. 3 Behavioral tests. a Assessment of depressive-like behavior. The shape of the violin plot shows the distribution of sucrose preference within each group. The width at different points indicates the density of the data, i.e., how many rats fell into a particular sucrose preference range. The DABI rats (teal) had a wider distribution but generally lower sucrose preference compared to the sham-operated rats (red). **b** Assessment of anxiety-like behavior. The DABI rats (teal) spent less time in the open arms compared to sham-operated rats (red), shown by the lower distribution and median. This suggests more anxiety-like behavior compared to the sham-operated rats, who spend more time in the open arms on the elevated plus maze.

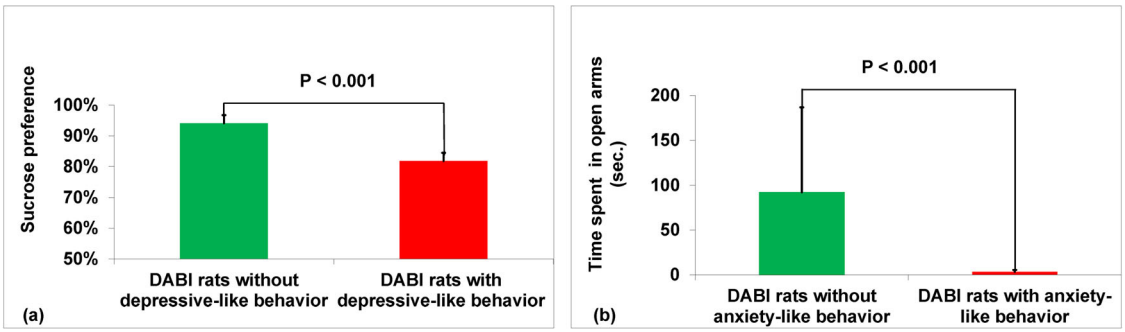

Fig. 4 Behavioral assessment of depressive-like and anxiety-like behaviors in DABI (Diffuse Axonal Brain Injury) rats. a Sucrose preference test shows a significant reduction in sucrose consumption in DABI rats with depressive-like behavior compared to those without depressive-like behavior ($P < 0.001$), indicating anhedonia. **b** Elevated plus maze test reveals a significant decrease in the time spent in the open arms in DABI rats with anxiety-like behavior compared to those without anxiety-like behavior ($P < 0.001$), reflecting heightened anxiety levels.

analysis using the Student's t-test showed a significantly lower effect between the 20 DABI rats and the 20 sham-operated rats in the cerebral cortex at 48 h after injury (0.25 ± 0.046, $\bar{x} \pm$ SD, vs. 0.402 ± 0.061, $\bar{x} \pm$ SD; $t(38) = 8.61$; $p < 0.001$; $d = 2.81$) (see Fig. 2a).

Relative anisotropy (RA) for the thalamus

A 2-way ANOVA showed a significant effect of trauma ($F1,36 = 81.53$, $p < 0.001$, $p\eta 2 = 0.694$). No effects of sex or interaction between sex and trauma on RA were found. The analysis using the Student's t-test showed a significantly lower effect between the 20 DABI rats and the 20 sham-operated rats in the cerebral cortex at 48 h after injury (0.254 ± 0.048, $\bar{x} \pm$ SD, vs. 0.474 ± 0.081, $\bar{x} \pm$ SD; $t(38) = 9.26$; $p < 0.001$; $d = 2.93$) (see Fig. 2b).

Relative anisotropy for the cerebral cortex

A 2-way ANOVA showed a significant effect of trauma ($F1,36 = 72.64$, $p < 0.001$, $p\eta 2 = 0.669$). No effects of sex or interaction between sex and trauma on RA were found. The analysis using the Student's t-test showed a significantly lower effect between the 20 DABI rats and the 20 sham-operated rats in the cerebral cortex at 48 h after injury (0.214 ± 0.04, $\bar{x} \pm$ SD, vs. 0.366 ± 0.068, $\bar{x} \pm$ SD; $t(38) = 8.64$; $p < 0.001$; $d = 2.73$) Levene's test indicated unequal variances ($F = 7.42$, $p < 0.05$), so the degree of freedom was adjusted from 38–31 (see Fig. 2b).

Behavioral tests

Sucrose test. The data were analyzed with a 2-way ANOVA for the effect of trauma (DABI or sham) and sex (male or female). A 2-way ANOVA showed a significant effect of trauma ($F1,36 = 7.04$, $p < 0.011$, $p\eta 2 = 0.164$). No effects of sex or interaction between sex and trauma on sucrose preference were found. An independent-samples t-test indicated that sucrose preference was significantly lower in the DABI rats compared to the sham rats (DABI = $90.4 \pm 6.4\%$ vs. sham = $94.6 \pm 2.56\%$, $t(38) = 2.7$, $p < 0.012$, $d = 0.856$). A Mann–Whitney U test indicated that sucrose preference was significantly lower in the DABI rats with depressive-like behavior compared to the DABI rats without depressive-like behavior ($81.8 \pm 3.5\%$, $n = 6$ vs. $94.1 \pm 2.7\%$, $n = 14$, $U = 0$, $p < 0.001$, $r = 0.775$). The data are measured in ml and expressed as a percentage, presented as mean ± SD (see Figs. 3a and 4a). To establish the diagnostic criterion cut-off for depressive states in DABI rats, we used the mean ± 2 SD method [53–55], which revealed that 30% of the rats developed post-traumatic depressive-like states compared to sham rats within a month after injury (6 out of 20 DABI rats vs. 0 out of 20 sham rats; $p < 0.01$, according to a chi-square, Fisher's exact test).

Open field test. The data were analyzed with a 2-way ANOVA for the effect of trauma (DABI or sham) and sex (male or female) on locomotor activity. A 2-way ANOVA showed no significant effect of

sex, trauma, or interaction between sex and trauma on total distance traveled (DABI = 1543 ± 563 cm vs. sham = 1259 ± 316 cm). The data are measurements in cm and expressed as mean ± SD. To establish the diagnostic criterion cut-off for emotionality and locomotor hyperactivity in DABI rats, we used the mean + 2 SD method, which revealed that 20% of the rats (4 out of 20, not significant according to a chi-square, Fisher's exact test) developed post-traumatic emotionality and locomotor hyperactivity compared to sham rats (0 out of 20 sham rats) within a month after injury.

Elevated plus maze. The data were analyzed with a 2-way ANOVA for the effect of trauma (DABI or sham) and sex (male or female). A 2-way ANOVA showed a significant effect of trauma ($F1,36 = 5.06$, $p < 0.029$, $p\eta 2 = 0.123$). No effects of sex or interaction between sex and trauma on time spent in the open arms of the plus maze test were found. An independent-samples t-test indicated that time spent in open arms was significantly lower in the DABI rats compared to the sham rats (DABI = 61.06 ± 86.9 s. vs. sham = 110.7 ± 49.5 s, $t(38) = 2.2$, $p < 0.032$, $d = 0.702$). A Mann–Whitney U test indicated that time spent in the open arms was significantly lower in the DABI rats with anxiety-like behavior compared to the DABI rats without anxiety-like behavior (3.3 ± 2.1 s, $n = 7$ vs. 95.2 ± 94.6 s, $n = 13$, $U = 0$, $p < 0.001$, $r = 0.86$). The data were measured in sec. and expressed as mean ± SD (see Figs. 3b and 4b). To establish the diagnostic criterion cut-off for anxiety-like behavior in DABI rats, we used the mean ± 2 SD method, which revealed that 35% of the rats developed post-traumatic anxiety-like behavior compared to sham rats within a month after injury (7 out of 20 DABI rats vs. 0 out of 20 sham rats; $p < 0.004$, according to a chi-square, Fisher's exact test).

Calculation of the correlation between depressive and anxiety-like behaviors comorbid. A weak correlation was found between the development of depression and anxiety in 40 experimental rats ($\varphi = 0.359$; $n = 40$; $p < 0.05$). Depression was found in 42.9% of rats with anxiety symptoms (3 of 7), and anxiety was found in 50% of rats with depression (3 of 6). When calculating the correlation between the quantitative results (Pearson coefficient) of the sucrose test and the time spent in the open arms of the elevated plus maze, no significant correlation was found.

DISCUSSION

The objectives of this study were to investigate the role of DABI in the occurrence of post-TBI depressive- and anxiety-like behaviors in a rat model, and to establish diagnostic criterion cut-offs for these behavioral pathologies. Due to the complexities of TBI and its many associated diseases, it remains a very difficult condition for which to provide an accurate model and effective treatments. Rodent models must consider the heterogenous nature of TBI in order to yield results that best replicate the human condition [56]. Similar to the high rates of depression and anxiety observed in people who suffer from TBI, rodent models of TBI have also shown increased depressive-like and anxiety-like behavior [40].

Previous studies have shown a correlation between depression and anxiety in people who have suffered from TBI [57]. Depression and anxiety after TBI presented high within-domain persistency and cross-domain concurrent associations [58]. Comorbid anxiety and depression after TBI prevalence range from 7% [59] to 20.7% [60] compared to the general population, which has a 20–70% risk of depression comorbid with social anxiety disorder [61]. In addition, 41.6% of individuals in the general population with major depression over the course of one year had one or more anxiety disorders in the same timeframe [61]. Although TBI is a contributing factor to both depression and anxiety, and comorbidity exists between depression and anxiety in both TBI patients and the general population, it does not appear that TBI increases the comorbidity between anxiety and depression.

The rodent model shows a clear advantage as a potential model for TBI in humans. However, although many neuroprotective agents have been identified in TBI studies, none has resulted in significant improvement in long-term outcomes in clinical trials [62]. This is due in part to the limitations of preclinical testing in general, and also to the diverse conditions of TBI. Currently, the majority of potential pharmacological treatments for neuropsychiatric and cognitive complications of TBI have been tested in weight-drop, fluid percussion injury, and controlled cortical impact models [38, 39, 63–67]. This may limit the treatments' ability to be effective in patients with other types of TBI, such as diffuse TBI [62]. Based on our results, it can be concluded that the occurrence of depressive-anxious behavior after traumatic brain injury is mainly modulated by the diffuse axonal component of traumatic brain injury, an isolated model of which we presented in this study. It is therefore useful to test potential drug treatment approaches for depression and anxiety specifically in a DABI model or in combination with the focal TBI models, not exclusively in the focal TBI models.

A suitable model is especially important given the considerable variability of the development of psychiatric illnesses among TBI patients. Post-TBI depressive symptoms affect 10–77% of individuals [68–70], while anxiety reaches up to 70% [19]. Defining clinical diagnostic criteria for mental disorders in humans is a lengthy and rigorous process, regularly reviewed by the American Psychiatric Association and World Health Organization to enhance validity. In contrast, animal behavioral studies often overlook these stringent criteria and typically treat the entire group of animals subjected to specific study conditions as homogeneous [71]. This approach is particularly problematic in studies that test a new drug administered to all animals in the group, including those that do not develop psychiatric pathology. In clinical practice, various popular statistical techniques for the identification of the cut-off values include mean ± 2 SD (95% CI for mean), logistic regression, receiver operating characteristic curves and discriminant analysis [54, 72, 73].

To validate DABI induction, neurological assessment and MRI were performed at 48 h after brain injury. The main conclusion of this study was that isolated DABI may cause post-traumatic depression and anxiety in 30 and 35% of cases, respectively. Using the "mean ± 2 SD" statistical method, we established diagnostic criterion cut-offs for depressive-like and anxiety-like behavior in injured rats, highlighting the role of DABI in these post-traumatic conditions.

Neurological regulation is crucial for motor function, managed by a complex neural system originating in the cortex [74]. Brain trauma disrupts the normal communication between brain areas and the spinal cord needed for movement [74]. These deficits result from the disruption of complex motor pathways and sensorimotor integration caused by brain trauma [49]. Therefore, the majority of tests commonly used to evaluate the effects of such injuries in animal models are sensorimotor assessments [16, 41, 49].

As we previously observed, the overall extent of neurological impairment was significantly lower in rats who underwent the DABI model compared to the rats who were included in established models of TBI, stroke, and subarachnoid hemorrhage [16]. This can be explained by the fact that only the beam walking and beam balance categories are useful for neurological assessments in the DABI model [16]. Thus, in this study, the neurological scale was adapted accordingly, and the maximum number of points was reduced from 25–15 (for a description of the methodology for assessing neurological deficits, see the "Methods" section). The NSS was significantly different between study groups in our experiment and is, as we have previously noted, a very sensitive test for determining

post-TBI severity in both the TBI model [49] and the isolated DABI model [16].

For MRI-detected injury, we used diffusion-weighted imaging (DWI) with relative anisotropy and fractional anisotropy mapping. As shown in our earlier study, DWI is as a robust and sensitive tool for the determining the area of damage following DABI [16] and has been used extensively in detecting many neurologic conditions such as stoke [75], TBI [49], and brain tumors [76]. Previously, we found that MRI in the context of DABI assessment is a very sensitive method, even more sensitive than histology [16]. Fractional anisotropy and relative anisotropy maps in the thalamus and cerebral cortex regions were the most sensitive in diagnosing DABI pathology at 48 h after injury. Therefore, to confirm DABI pathology in this study, we used the above-mentioned maps (FA and RA) in the thalamus and cerebral cortex regions.

It is well-known that depression and anxiety assessments in both humans and rats can be affected by various factors, such as stress, emotional instability, physical condition, and others [77–79]. Stress, illness or physical condition can cause overdiagnosis. The general recommendation is to assess depression or anxiety no sooner than 3 months after injury in humans [80, 81] and 2–4 weeks in rats [38, 40]. To exclude false-positive assessments of depression and anxiety, our study conducted behavioral tests to assess depressive-like behavior and anxiety-like behavior 30 days after DABI. Despite the small sample size in our study, we noted comorbidity, with a weak correlation, between depressive- and anxiety-like behaviors among the 40 rats included in our study.

To study the depressive-like state in rats exposed to DABI, we used two behavioral tests. The first test was the sucrose preference test, which measures the level of anhedonia, a common symptom of depressive disorders [16]. In our study, rats with DABI developed anhedonia more often than sham rats at 1 month after brain trauma.

The second behavioral test we used was the open field test, a common method for detecting high emotionality and locomotor hyperactivity following TBI [82–84]. These behaviors are linked to depressive states and respond well to antidepressant drugs [84]. The observed hyperlocomotion in the DABI group during the open field test, although not statistically significant (reaching $p < 0.05$ only in the t-test, 1-sided), can be attributed to brain damage affecting the cerebral cortex, striatum, and olfactory bulbs [85]. Increased locomotion in the olfactory bulbectomy model of depression is well-documented and hyperlocomotion is also used to validate new TBI models [86, 87].

In our previous studies, we associated hyperlocomotion after TBI with dysregulation of the glutamatergic system [38], which we believe is a key mechanism in the development of depression [88, 89], anxiety [39], and other behavioral disorders [90, 91] following TBI. This is due to chronic destruction of BBB [92] and chronic glutamate neurotoxicity [93].

The results of the plus maze test (time in the open arms) showed that DABI rats develop post-DABI anxiety-like behavior in contrast to control rats. Thus, in this experiment, the induction of DABI clearly demonstrated the development of anxiety-like behavior in both males and females 1 month after brain injury.

In our study, we used a mean ± 2 SD method establishing diagnostic criteria cut-off for anxiety behavior in DABI rats. In the DABI group, we recorded depressive-like behavior in 30% and anxiety-like behavior in 35% compared with the control group. These results were significant according to a Fisher's exact test. Behavioral consequences after TBI have been well documented, and a number of hypotheses have been proposed that are potentially involved in the development of depression and anxiety. DABI is always accompanied by general TBI; however, the involvement of DABI in the mechanism of depression, anxiety, or other behavioral disorders has never been studied in the context of isolated DABI. Our study provides an opportunity to examine the contribution of DABI to depression and anxiety in the context of TBI.

CONCLUSIONS

Our main finding was that isolated DABI may cause post-traumatic depression-like behaviors in 30% of cases and anxiety-like behaviors in 35% of cases. Additionally, 20% of rats showed changes in locomotor and exploratory activity indicative of depressive-anxious behavior, though this was not statistically significant in a sample of 20 injured versus 20 control rats. Using the "mean ± 2 SD" statistical method, we established diagnostic cut-off criteria for depressive-like and anxiety-like behaviors in injured rats. We documented comorbidity between the development of depression and anxiety in DABI-exposed rats. This model helps clarify the role of DABI in developing post-traumatic depression and anxiety. We expect future TBI research to consider the long-term behavioral effects of DABI.

DATA AVAILABILITY

Data is available upon reasonable request.

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AUTHOR CONTRIBUTIONS

AO: Study conception, data collection, data analysis, manuscript writing/editing, final approval of manuscript. BFG: Study conception, data analysis, manuscript writing/editing, final approval of manuscript. VZ: Data collection, data analysis, manuscript editing, final approval of manuscript. IS: Data collection, data analysis, manuscript editing, final approval of manuscript. SN: Data collection, data analysis, manuscript editing, final approval of manuscript. IgM: Data collection, data analysis, manuscript editing, final approval of manuscript. IsM: Data collection, data analysis, manuscript editing, final approval of manuscript. AZ: Data collection, data analysis, manuscript editing, final approval of manuscript. AF: Study conception, data collection, data analysis, manuscript writing/editing, final approval of manuscript. MB: Study conception, data collection, data analysis, manuscript writing/editing, final approval of manuscript.

ETHICS APPROVAL

All methods in this study were performed in accordance with the relevant guidelines and regulations. Ethics approval for the use of animals in this research was obtained from The University Committee for the Ethical Care and Use of Animals in Experiments, with Authorization Number: BGU 317 03 2024 C. This study did not involve human participants; therefore, informed consent was not required.

COMPETING INTERESTS

The manuscript has been read and approved by all authors, and the authors state that no competing financial or other conflicts of interests exist. The authors have read and have abided by the statement of ethical standards for manuscripts submitted to *Translational Psychiatry*. The data that support the findings of this study are available from the corresponding author, [MB], upon reasonable request. The reported research, partially or in its entirety, is unpublished and not under consideration for publication elsewhere.

ADDITIONAL INFORMATION

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