Original Paper

Investigating the Neuroprotective and Neuroregenerative Effect of Trazodone Regarding Behavioral Recovery in a BL6C57 Mice Stroke Model

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ABSTRACT: Stroke is a major cause of death and disability worldwide. Between 1990 and 2010, its global burden increased notably with reference to the absolute number of incident events, number of deaths, and disability-adjusted life-years lost. Trazodone is a triazolopyridine derivative that was approved for more than 40 years as monotherapy or in combination with other antidepressant drugs for the treatment of major depressive disorder in adult patients. The aim was investigated if trazodone can improve behavioural outcome after stroke in a mice model of middle cerebral artery occlusion (MCAo) due to the potential neuroprotective and neurodegenerative effects by using three behavioural tests: adhesive tape test, beam test and hole board test. Trazodone administration show modest improvements regarding the motor-sensorial function after stroke especially in the acute post-stroke phase in aged and young animals. The antidepressant effect of the drug was observed in the post-stroke period in aged animals and to a lesser extent in young animals. Future research is needed to evaluate the effects of trazodone at the cellular level to be sure that it has no benefit in stroke patients who do not suffer from depression.

KEYWORDS: *Stroke, behavioural test, trazodone, drug repurposing.*

Introduction

Stroke is a major cause of death and disability worldwide.

Its global burden increased notably with more than 1 million individuals having a stroke each year, in Europe alone [1,2].

Stroke involves multiple underlying pathological mechanisms, but ultimately it is caused by a sudden focal disruption of the cerebral blood flow, which is shortly fallowed by neurological deficits.

Approximately 80% of cases of cerebral infarction are attributed to ischemia, while the remaining 20% are classified as hemorrhagic [2].

At the cellular level, stroke primarily involves mechanisms such as hypoxia, edema, apoptosis, and infarction leading to cellular necrosis.

The pharmacological management of ischemic stroke, which accounts for 87% of all stroke cases, is currently inadequate.

Tissue-type plasminogen activator (tPA) is currently the sole authorized medication for stroke therapy.

However, its application is limited to only 3% of patients due to time constraints.

Furthermore, it does not possess any observable neuroprotective characteristics. [3,4].

Given the increasing life expectancy and the fact that stroke predominantly impacts the elderly population, there exists a pressing demand for the discovery of neuroprotective agents that could aid post-stroke recovery.

Despite the intensification of research efforts in this area, no pharmacological agent has yet received approval [5].

Trazodone, a derivative of triazolopyridine, has been authorized for over four decades as a monotherapy or in conjunction with other antidepressants for managing major depressive disorder in adult individuals.

However, trazodone exhibits a unique dual mechanism of action, whereby it functions as both a serotonin transporter inhibitor and a serotonin type 2 (5-HT2) receptor antagonist (specifically targeting 5-HT2A and 5-HT2C receptors) (Figure 1).

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With reports showing beneficial effects in treating post-stroke depression, this means that it may have a positive impact on rehabilitation outcomes by boosting patient motivation [6].

Another study also points out that trazodone administrated to individuals who have suffered a stroke and exhibit obstructive sleep apnea, reduces the severity of the condition without concomitantly increasing the occurrence of nocturnal hypoxia [7].

With that medication already given to stroke patients we wanted to investigate the potential neuroprotective effects of trazodone, as some reports have showered that it can reduce memory deficits, neuronal loss and hippocampal atrophy in prion-diseased and tauopathy mice [8].

Its neuroprotective effects were also proved in a cohort of Alzheimer's disease patients that followed long term trazodone treatment, delaying their progressing neurological deficits [9].

Furthermore, other experimental work has shown that a unique dose of trazodone temporarily slows down motricity recovery in rats [10] and reducing catalase concentration and restoring mitochondrial activity ultimately leading to lower neuron toxicity and oxidative stress [11].

Here we aim to investigate, if trazodone can improve recovery after stroke in a mice model of middle cerebral artery occlusion (MCAo) by using powerful automatic tools to assess behavioural outcomes [12].

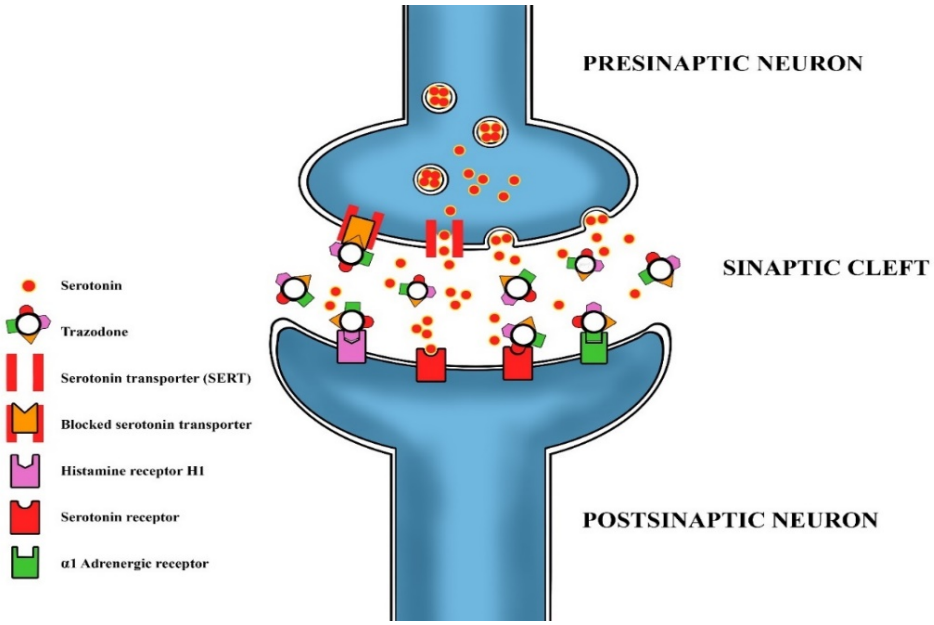

Figure 1. Trazodone mechanism of action. Inhibition of the serotonin transporter and serotonin type 2 (5-HT2) receptor antagonism (5-HT2A and 5-HT2C receptors); also acts as an antagonist on alpha 1-adrenergic receptors and histamine H1 receptors.

Material and Methods

The study was carried out within the Research Centre for Normal and Pathological Aging (ARES) of the University of Medicine and Pharmacy of Craiova.

The experiment received the approval of the Ethics Committee of the University of Medicine and Pharmacy of Craiova (No. 26/29.10.2020) and it was performed according with Directive 2010/63/EU, governing animal research in Europe (revising Directive 86/609/EEC).

Animals

We used 43 C57BL6 female mice, obtained from the Animal facility of the university.

Young (3 months, n=26) and aged animals (2 years, n=17) were taken out of their housing facilities and acclimated for 24 hours in the testing rooms.

The mice were randomly divided into groups and the behavioural assessment was done by the blinded investigators.

The animals received food and water ad libitum and were housed in rooms with the 12 hours day/night cycle from 07:00 to 19:00, the ambient temperature being 21°C and 60% air humidity.

Pharmacological treatment

Fresh trazodone solution was made every two days by dissolved trazodone hydrochloride

(Sigma-Aldrich, Germany) in sterile saline solution.

The remaining solution was stored at 4°C, away from light.

Intraperitoneal injections of 36mg trazodone/kg body weight were made daily, starting 24 hours after MCAo.

Middle cerebral artery occlusion surgery

Before surgery, mice were fasted for 24h in order to reduce the blood glucose level.

MCAo was induced as previously described [14].

Briefly, after anaesthesia (1.5% isoflurane in a mixture of 75% nitrous oxide and 25% oxygen), both common carotid arteries were isolated and prepared for occlusion.

A small craniotomy above the middle cerebral artery was made and an electro-thermal instrument was used to coagulated the base of the MCAo, after the blood flow through the common carotid arteries was stopped.

The local changes in blood flow were monitored using a laser Doppler device (Perimed, Stockholm, Sweden).

A decrease in laser Doppler signals to<20% of control values was considered to be successful MCAo.

After 90 minutes, blood flow was restored through the common carotid arteries and the muscle, soft tissue and skin above the craniotomy were sutured.

Pain relief medication (buprenorphine) was given subcutaneous twice, every 6h after surgery at a dose of 0.3mg/kg.

Behavioural assessment

Adhesive tape removal test

This is the most relevant method for identifying sensory and motor deficit in animals.

The test involves the use of 0.3x0.4cm adhesive tapes on the front limbs of the mouse.

The adhesive tape is applied on the palmar face of the forelimbs with the same pressure on each member.

The animal is then placed in the test cage and 4 different values are registered (the time to contact and the time needed to remove the tape, for each member).

The animals have two minutes to complete the test [15].

Animals are given two days for training, meant to familiarize the animal with the test, to reduce the anxiety which leads to the decrease of the defecation and urination frequency during the test, important to achieve an optimal performance and not least to reduce the inter-individual variability, homogenizing the performance of animals.

The tactile response is defined as the time needed by the animal to feel the adhesive tape and is represented by a shaking of the limb or by the mouth touching the adhesive tape.

The complete removal of the adhesive tape represents the completion of the test or exceeding the 2 minutes allocated to this test.

Usually, the animals remove the adhesive tape by mouth or by grooming [15].

Beam test

This test is widely used in experimental studies in order to assess motor coordination and balance of the animal.

The equipment consisted of wooden bars with a length of 1 meter having a flat surface of 12mm and 6mm respectively.

Two lines were drawn on these, the first representing the start line and the second, at a distance of 80 centimeters, representing the stop line.

The beam is placed at a height of 50cm.

Every mouse received a two consecutive days training.

The training consisted of crossing each bar 3 times, encouraging the animals to move every time it stopped.

These trainings are meant to familiarize the animal with the test and make its behaviour during the test more stable and accurately, better reflecting motor coordination.

For each trial, the time needed to travel each of the two bars and the number of missteps were recorded.

A slip is defined as one or more limbs do not reach the beam.

At first, the mice had to cross the wide bar followed by the narrow bar, after a 10 minutes resting period.

Each time the animal stopped (to sniff or to look around) it was encouraged forward with a gentle push.

After each animal, the bar was cleaned with 70% ethanol to eliminate the odour of the previous mouse as through as possible [16].

Hole board test

The procedure is used to evaluate behavioural components in mice such as curiosity and the ability to explore.

These components provide information about anxiety or depression.

The equipment used for this test is automatic, represented by a 40x40cm grey plate with a thickness of 2.2cm.

10.12865/CHSJ.49.02.10

It is provided with 16 holes with a diameter of 3cm each, in which detectors are located (recessed in each hole, 1cm from the surface of the plate).

The board was located at a distance of 15cm from the ground and connected to a source that records the number of head dives in each hole.

Each animal was placed in the centre of the plate and for 5 minutes the number of head dives in the holes on the plate was recorded; after each animal the plate was cleaned with 70% ethanol [17,18].

Statistical Analysis

GraphPad 9.2 and Microsoft Excel 2016 were used for statistical analysis.

Differences in means among the groups were analysed using a two-stage step-up (Benjamini, Krieger, and Yekutieli) multiple unpaired t test, with a false discovery rate of 1%.

All figures show mean value and standard deviation (SD) and the statistical significance is displayed as follows: *, # $p<0.05$; **, ## $p<0.01$; ***, ### $p<0.001$ and ****, #### $p<0.0001$.

Results

Trazodone improves recovery outcome in acute and less in chronic post-stroke phase.

At the beginning of the adhesive tape test, both young and aged animals displayed similar detection and removal times for both tested front legs.

However, post-stroke, aged animals were slower in both detection and removal of the adhesive tape compared to their young counterparts (Figure 2).

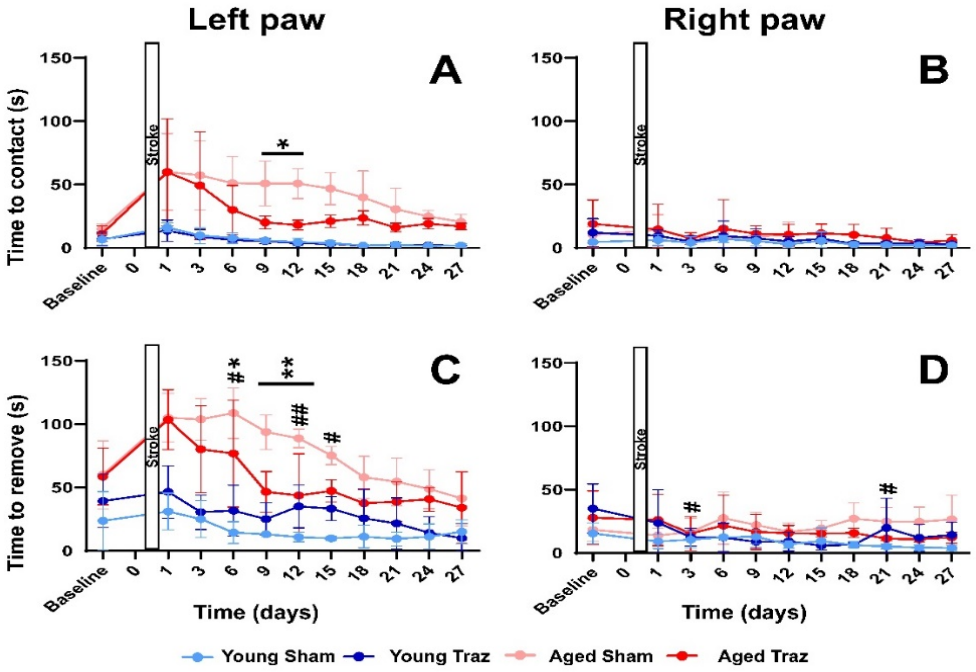

Figure 2. Adhesive tape test. A. Time needed by the animals to feel the tape on the left paw. B. Time needed by the animals to feel the tape on the right paw. C. Time needed by the animals to remove the tape on the left paw. D. Time needed by the animals to remove the tape on the right paw. Young Sham: young animals subjected to MCAo. Young Traz: young animals trated with trazodone, Aged Sham: aged animals subjected to MCAo. Aged Traz: aged animals trated with trazodone. * -statistical difference between the group of treated and control aged animals. # -statistical difference between the group of treated and control young animals. Data is presented as mean±SD. *, # p<0.05; **, ## p<0.01; ***, ### p<0.001 and *, #### p 0.0001.***

Trazodone administration to aged animals resulted in a significant reduction in the adhesive tape detection between day 9 to 12 post-stroke (Figure 2A).

On day 9, aged animals treated with trazodone feel the adhesive tape faster (20±5.07) compared with control (50.75±17.68), $p<0.05$.

The same is observed on day 12 post-stroke (18±3.89) vs. (50.75±11.87), $p<0.05$.

The observed dissimilarity between the detection times of untreated and treated aged animals was not sustained, as both groups began to display comparable detection times in the late acute and chronic phases (Figure 2A).

No difference was observed between control and treated animals, regardless of age when testing the ipsilateral (right) part of the body,

regarding the detection time of the adhesive tape (Figure 2B).

Concerning the time needed to remove the tape, statistically significant differences were found from day 6 to day 15 post- (Figure 2C).

From day 6 to day 12 post-stroke, treated aged animals managed to remove the adhesive tape faster compared to aged controls, as follows: day 6 (76.83±42.25 vs. 108.75±19.92, $p<0.05$), day 9 (46.50±16.04 vs. 93.75±13.75, $p<0.01$), day 12 (43.62±32.91 vs. 88.75±7.32, $p<0.01$.

Regarding young animals, statistically significant differences were observed between young treated animals and young controls on day 6 (31.66±19.99 vs. 14.40±8.56, $p<0.05$), 12 (35.08±17.03 vs. 10.80±3.76, $p<0.01$) and 15 (33.33±9.56 vs. 9.80±1.48, $p<0.05$) post-stroke.

However, motor recovery seems to influence only acute recovery, as both young and aged treated animals lose their advantage starting 18 and respectively 15 days post-stroke (Figure 2C).

Also, no difference was observed between control and treated young animals, when testing the ipsilateral (right) part of the body, regarding the motor ability.

However, at day 3 and day 21 post-stroke, aged animals treated with trazodone showed superior motor skills compared to the untreated animals (Figure 2D).

Young control animals show a lack of dexterity when removing the tape on day 3 (9.52±5.52 vs. 23.66±26.33, $p<0.05$) and day 21 (5.20±1.92 vs. 19.83±23.31, $p<0.05$) post-stroke, compared with the group treated with trazodone (Figure 2D).

Young treated animals did not exhibit any significant improvement in their detection time compared to their control group, during both the acute and chronic phases of the experiment (Table 1).

Table 1. Mean values of parameters obtained by adhesive tape test for all animal groups at each analysed time point.

	Contact left										Remove left									
	Young					Aged					Young					Aged				
	Control		Treated		P	Control		Treated		P	Control		Treated		P	Control		Treated		P
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Baseline	6.533	4.227	6.952	5.335	0.833	11.078	6.172	15.000	4.082	0.778	23.667	23.046	39.278	21.129	0.164	60.000	26.771	58.608	22.340	0.922
1	16.200	3.493	13.500	8.410	0.175	60.000	30.177	59.765	42.286	0.986	31.000	14.560	46.583	20.791	0.065	105.000	19.149	103.412	23.725	0.911
3	9.800	6.419	8.857	5.682	0.635	57.250	27.060	49.200	42.518	0.560	25.000	14.595	30.500	13.681	0.512	103.750	16.520	80.133	34.473	0.101
6	7.800	4.087	6.143	2.476	0.405	51.000	21.245	30.091	19.269	0.146	14.400	8.562	31.667	19.992	0.041	108.750	19.923	76.833	42.254	0.031
9	5.800	2.775	5.286	2.016	0.796	50.750	17.689	20.000	5.071	0.042	13.000	2.236	25.083	12.774	0.151	93.750	13.745	46.500	16.045	0.003
12	4.600	2.966	3.714	1.204	0.656	50.750	11.871	18.000	3.899	0.040	10.800	3.768	35.083	17.037	0.004	88.750	7.320	43.625	32.911	0.004
15	3.800	2.168	3.314	1.347	0.807	46.750	12.633	21.000	4.830	0.139	9.800	1.483	33.333	9.564	0.015	75.250	7.182	47.250	8.770	0.122
18	1.800	0.837	1.500	0.535	0.890	39.750	21.093	23.500	5.745	0.349	11.200	8.927	25.667	23.261	0.131	58.000	16.693	37.500	10.847	0.256
21	2.400	1.949	2.500	1.309	0.963	30.500	16.862	16.000	3.464	0.403	9.400	5.595	21.500	20.276	0.206	54.500	18.806	38.750	15.650	0.382
24	1.600	0.548	2.000	0.535	0.854	24.750	4.646	19.000	4.243	0.740	11.200	9.935	14.167	12.952	0.756	48.750	15.435	40.750	9.430	0.657
27	1.800	1.304	1.750	0.463	0.982	20.500	5.972	17.000	2.449	0.840	15.000	9.539	10.000	11.314	0.616	41.250	21.407	34.000	28.249	0.687
	Contact right										Remove right									
	Young					Aged					Young					Aged				
	Control		Treated		P	Control		Treated		P	Control		Treated		P	Control		Treated		P
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Baseline	4.600	1.362	12.056	11.004	0.194	10.333	6.628	18.922	18.866	0.250	**15.467**	8.305	35.111	19.421	0.271	18.250	8.995	27.800	21.264	0.274
1	6.200	4.438	9.500	4.602	0.297	14.250	11.899	14.588	19.758	0.964	9.000	5.523	23.667	26.331	0.024	13.750	5.852	26.067	20.296	0.159
3	4.000	4.637	4.917	4.441	0.772	8.000	4.320	7.400	4.469	0.937	10.600	6.465	12.583	7.103	0.758	16.500	10.847	15.385	13.389	0.900
6	7.000	2.646	9.417	11.720	0.445	8.000	3.559	15.000	22.907	0.366	12.400	2.191	12.167	11.224	0.971	27.500	20.599	21.727	24.030	0.523
9	5.400	3.912	7.583	7.255	0.490	9.000	4.082	11.125	6.198	0.795	12.800	5.630	8.833	4.707	0.538	22.000	10.132	16.625	13.773	0.571
12	2.800	1.789	5.000	3.838	0.486	10.250	10.178	10.375	8.366	0.988	6.800	2.864	9.000	7.422	0.733	16.750	6.994	15.500	6.234	0.895
15	5.600	7.092	7.167	3.869	0.663	11.500	7.326	11.500	7.326	0.970	9.400	10.455	5.833	3.251	0.627	19.250	6.397	15.250	2.500	0.715
18	2.600	1.817	3.200	2.168	0.873	10.333	8.386	10.333	8.386	0.980	6.200	2.683	6.500	1.643	0.967	27.000	12.463	15.750	3.594	0.305
21	2.200	1.095	3.333	1.862	0.752	7.750	7.805	7.750	7.805	0.990	5.200	1.924	19.833	23.310	0.047	24.750	11.295	11.250	1.500	0.219
24	2.000	1.732	3.667	2.582	0.643	4.250	0.500	4.250	0.500	0.990	4.200	2.168	12.000	10.583	0.288	24.500	11.818	10.750	2.217	0.210
27	1.800	1.095	2.833	1.329	0.774	5.750	4.856	5.750	4.856	0.990	4.000	2.000	14.167	10.245	0.167	26.500	19.140	12.250	4.193	0.194

Contact left / right: the time the animal felt the adhesive tape placed on the left / right paw, Remove left / right: the time the animal removed the adhesive tape placed on the left / right paw, Young: young animals, Aged: aged animals, Control: control animals groups, Treated: trazodone treated groups, Baseline: the test performed before the stroke, 1, 3, 6, 9, 12, 15, 18, 21, 24, 27: the days when the test was performed after the stroke.

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Although trazodone appears to have enhanced the acute detection time solely in aged animals, it augmented dexterity, as evaluated by the duration required for adhesive tape removal, in both aged groups relative to their respective controls (Table 1).

A similar trend was observed when evaluating the differences between untreated and treatment animals using the beam crossing test (Table 2).

Table 2. Mean values of parameters obtained by beam test for all animal groups at each analysed time point.

	Velocity Wide Beam										Velocity Narrow Beam									
	Young					Aged					Young					Aged				
	Control		Treated		P	Control		Treated		P	Control		Treated		P	Control		Treated		P
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Baseline	15.200	3.211	13.944	4.773	0.629	19.583	3.360	18.333	4.182	0.746	15.000	2.121	18.583	8.677	0.317	25.167	5.821	26.241	8.433	0.774
1	10.000	1.225	13.083	7.549	0.236	30.500	12.234	21.200	7.636	0.017	33.600	7.635	26.333	9.069	0.043	37.500	4.041	34.500	9.359	0.429
3	8.400	4.827	11.917	5.664	0.177	30.250	7.890	18.533	6.567	0.003	23.600	3.578	17.167	11.668	0.073	37.000	6.325	28.000	7.465	0.019
6	7.600	2.302	12.000	6.135	0.092	30.250	10.210	20.167	5.718	0.012	10.400	3.912	15.167	8.993	0.184	33.500	7.594	25.800	4.195	0.045
9	7.000	2.449	9.417	5.485	0.353	25.750	8.617	16.125	6.058	0.023	9.400	0.894	12.750	4.615	0.349	33.500	6.557	27.333	8.307	0.131
12	6.600	2.302	11.083	6.598	0.086	24.500	8.426	15.500	5.976	0.034	12.000	4.183	13.833	7.146	0.608	31.250	5.058	25.444	6.023	0.155
15	6.400	1.517	10.583	6.374	0.109	25.000	11.518	14.500	5.260	0.032	10.000	1.871	12.500	6.856	0.484	23.750	6.652	20.500	3.873	0.498
18	6.200	2.049	10.667	3.559	0.132	27.250	8.302	17.500	1.732	0.046	11.800	2.280	12.667	4.227	0.831	26.000	2.449	21.250	3.304	0.322
21	5.400	2.074	9.500	3.271	0.167	24.500	10.083	14.250	3.403	0.036	13.400	5.128	13.000	2.366	0.922	25.250	4.113	25.250	4.113	>0.999999
24	5.600	2.191	8.333	1.366	0.356	20.500	8.583	14.250	4.113	0.199	12.200	4.266	14.833	3.061	0.517	22.250	3.775	22.250	3.775	>0.999999
27	4.800	2.280	6.167	2.317	0.644	16.500	4.796	14.250	4.573	0.643	12.000	1.871	13.667	3.724	0.682	26.000	6.377	20.500	4.933	0.252
	Missteps Wide Beam										Missteps Narrow Beam									
	Young					Aged					Young					Aged				
	Control		Treated		P	Control		Treated		P	Control		Treated		P	Control		Treated		P
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Baseline	2.733	2.127	1.944	1.516	0.338	4.333	3.139	7.604	8.630	0.316	5.867	1.773	6.500	2.619	0.741	8.250	3.726	8.822	3.297	0.906
1	1.600	1.140	2.250	1.712	0.429	29.250	4.992	14.083	7.204	0.00002	6.400	2.702	4.917	3.777	0.439	47.000	17.263	25.867	11.338	0.00003
3	1.000	1.732	0.583	0.996	0.612	23.000	9.345	11.167	4.951	0.0006	5.400	3.209	3.917	4.209	0.439	30.750	14.477	18.200	10.910	0.0104
6	2.400	1.342	1.250	1.215	0.163	12.000	8.602	5.900	5.195	0.079	4.400	1.342	4.917	4.379	0.787	27.250	15.327	16.667	8.988	0.0343
9	1.200	1.643	1.000	0.953	0.808	10.500	7.234	7.143	4.914	0.359	5.200	2.775	3.917	3.728	0.503	31.750	13.150	24.500	9.754	0.169
12	2.800	1.924	0.833	0.718	0.018	10.500	5.568	6.143	3.848	0.234	6.000	2.345	4.333	4.539	0.385	25.500	4.509	20.500	4.408	0.342
15	2.000	2.000	0.750	0.965	0.130	14.000	2.449	11.750	3.500	0.585	5.000	2.345	7.333	5.382	0.224	25.500	2.887	21.750	2.500	0.537
18	2.000	1.000	0.833	1.169	0.213	12.000	5.228	9.250	2.500	0.505	7.200	1.789	4.333	3.386	0.190	22.500	1.732	20.000	2.309	0.680
21	1.000	1.732	0.500	0.548	0.593	8.000	4.320	5.500	1.915	0.544	5.200	2.168	4.333	4.033	0.691	20.250	1.500	19.250	1.258	0.869
24	3.000	2.550	1.000	1.673	0.034	9.000	2.708	7.250	3.202	0.671	4.600	1.673	7.167	4.875	0.240	20.250	2.630	18.500	1.291	0.773
27	3.200	3.962	0.833	1.602	0.012	5.250	2.217	5.250	2.217	>0.999999	3.800	1.095	2.667	1.633	0.603	20.750	7.136	15.750	0.957	0.410

Velocity wide / narrow beam: the time that animals travelled the wide / narrow beam, Missteps wide / narrow beam: missteps that animals made during the crossing on the wide / narrow beam, Young: young animals, Aged: aged animals, Control: control animals groups, Treated: trazodone treated groups, Baseline: the test performed before the stroke, 1, 3, 6, 9, 12, 15, 18, 21, 24, 27: the days when the test was performed after the stroke.

Regardless of the treatment administered, young animals exhibited a comparable frequency of errors in both the narrow and wide beam assessments (Figure 3A,3B).

At the beginning of the experiment, aged treated animals tend to have less missteps compared with aged control animals on day 1 (25.86±11.33) vs. (47.0±17.26) $p<0.0001$, 3 (18.20±10.91) vs. (30.75±14.477) $p<0.05$ and day 6 (16.66±8.98) vs. (27.25±15.32) $p>0.05$, post-stroke (Figure 3A).

Trazodone administration had beneficial effect in aged animals in the acute post-stroke phase on day 1 (14.08±7.20) vs. (29.25±4.99), $p<0.0001$ and 3 (11.16±4.95) vs. (23.0±9.34) $p<0.001$ compared with aged controls (Figure 3B).

Regarding young animals, trazodone administration seems to have beneficial neuroprotective role more in the chronic post-stroke phases, statistically significant differences being observed on day 24 (1±1.67) vs. (3±2.55) $p<0.05$ and day 27 (0.83±1.60) vs. (3.2±3.95), $p<0.05$ (Figure 3B).

Aged trazodone treated animals had higher speeds between day 3 and 6 post-stroke, when crossing the narrow beam (Figure 3C).

However, for both tasks, these differences were not observed in late acute and chronic phases of stroke, as natural recovery of the untreated animals matched the accelerated recovery of treated animals (Figure 3C,3D).

Similarly, trazodone reduced the number of missteps of aged animals, in the acute post-stroke, with no impact on the chronic recovery.

The velocity in which aged treated animals were able to finish the crossing of the wide beam, in the first 21 days post-stroke, was significantly higher compared to their aged controls (Figure 3D).

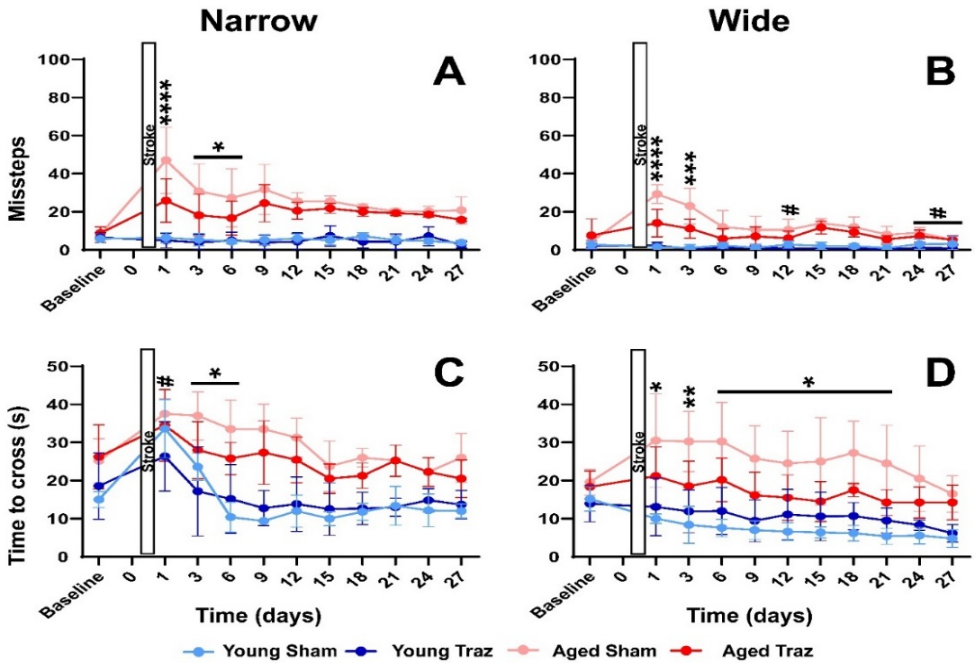

Figure 3. Beam test. A. Animals missteps on the narrow beam. B. Animals misstept on the wide beam. C. Crossing time on the narrow beam. D. Crossing time on the wide beam. Young Sham: young animals subjected to MCAo. Young Traz: young animals trated with trazodone, Aged Sham: aged animals subjected to MCAo. Aged Traz: aged animals trated with trazodone. * -statistical difference between the group of treated and control aged animals. # -statistical difference between the group of treated and control young animals. Data is presented as mean±SD. *, # p <0.05; **, ## p <0.01; ***, ### p <0.001 and *, #### p <0.0001.***

Trazodone administration showed limited post-stroke antidepressant effect in aged animals.

Hole board test showed that trazodone had no post-stroke antidepressant effect in treated young animals (Table 3).

Table 3. Mean values of parameters obtained by hole board test for all animal groups at each analysed time point.

	Hole Board									
	Young					Aged				
	Control		Treated		P	Control		Treated		P
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
Baseline	18.467	5.611	18.639	6.924	0.959	21.167	6.333	25.873	15.553	0.324
1	13.400	5.413	9.818	3.545	0.293	10.750	2.500	12.353	3.724	0.737
3	9.800	5.070	13.909	5.449	0.228	21.500	3.000	20.467	10.063	0.830
6	12.400	6.465	15.091	11.327	0.429	21.750	1.500	23.000	5.954	0.801
9	14.800	6.419	14.364	9.047	0.898	19.500	5.000	26.750	14.568	0.169
12	9.800	5.718	11.182	6.178	0.685	13.500	3.000	28.875	6.999	0.004
15	7.600	7.162	11.818	2.639	0.216	15.500	3.000	26.500	5.000	0.072
18	6.200	4.817	12.600	4.561	0.110	19.250	5.620	25.000	3.559	0.344
21	9.800	8.012	11.200	4.147	0.726	11.750	1.500	27.000	4.000	0.013
24	7.200	5.586	8.800	1.643	0.688	14.250	4.500	26.000	8.000	0.054
27	10.000	2.550	7.600	1.517	0.548	14.750	3.500	25.250	6.500	0.085

Hole Board: the number of head dippings, Young: young animals, Aged: aged animals, Control: control animals groups, Treated: trazodone treated groups, Baseline: the test performed before the stroke, 1, 3, 6, 9, 12, 15, 18, 21, 24, 27: the days when the test was performed after the stroke.

Although, testing periods, the p value was close to significance, significance was only reached for day 12 and 21, with treated animals dipping their head around 28 times compared with only 13 times at 12 days post-stroke and 11.7 at 21 days post-stroke (Figure 4 and Table 3).

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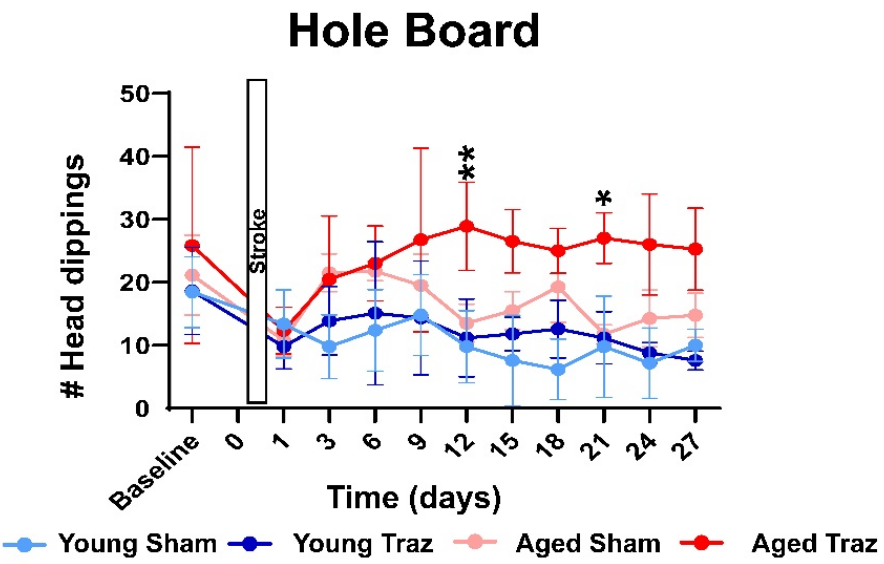

Figure 4. Hole board test. Number of head dippings. Young Sham: young animals subjected to MCAo. Young Traz: young animals trated with trazodone, Aged Sham: aged animals subjected to MCAo. Aged Traz: aged animals treated with trazodone. * -statistical difference between the group of treated and control aged animals. # -statistical difference between the group of treated and control young animals. Data is presented as mean±SD. *, # p <0.05; **, ## p <0.01; ***, ### p <0.001 and *, #### p <0.0001.***

Discussions

The increasing longevity gives rise to emerging health issues that pertain to the unique requirements of older demographics.

Physiological aging is now widely acknowledged as encompassing all changes that transpire in various organs and systems throughout the aging process.

This phenomenon is distinguished by a diminishing capacity to cope with different forms of stress, disruption of homeostatic equilibrium, and susceptibility to diseases.

The process of brain ageing in particular, is characterized by a decline in both the structures and functions of neurons and glia.

This phenomenon is commonly known as neurodegeneration.

The phenomenon under consideration is a typical occurrence that has been observed to result in diminished communication abilities and impaired memory function [19].

Additionally, it has been found to be associated with suboptimal recovery following a stroke [20].

Neurodegeneration is a gradual phenomenon characterized by a reduction in the overall population of neurons.

This process is typically attributed to apoptosis and is accompanied by a deterioration in both the structural integrity and functional capabilities of cells in the cortex [21].

Here we investigated the neuroregenerative properties of trazodone in an animal stroke model.

Behavioural assessment showed that administration of trazodone in aged mice have limited beneficial effects in post-stroke recovery, with almost no impact or even a slight decrease in the motor recovery of young animals.

Adhesive tape test showed that trazodone had a limited positive impact regarding sensorial recovery after stroke in aged animals.

In the post-stroke period, trazodone treatment had a neuroprotective effect in aged animals while, regarding young animals, this seems to slow down the motor recovery.

Similar findings being highlighted in a study where trazodone in one single dose could temporarily slow down the motor rehabilitation in rat models of MCAo, even reinstating neurological deficits such as hemiparesis [10].

Regarding the right hemibody part, no statistical differences were found in the time needed to feel the tape, but when it comes to remove the tape, on day 3 and day 21 post-stroke, young control animals seem to have a greater deficit compared to the treated young group.

These outcomes are somewhat different compared to human trails where trazodone may have a better neuroprotective effect, proved in a cohort of Alzheimer's disease patients, that showed a slowdown in cognitive degeneration [9].

These findings were correlated with animal models, where the administration of trazodone restored memory deficits, prevented neurodegeneration, and prolonged survival in tau-pathology [8] and prion induced pathology in mice [22].

Beam narrow test showed that trazodone administration had modest beneficial impact regarding the coordination of aged and young animals in post-stroke period.

At the beginning of the experiment, aged treated animals tend to have less missteps compared with aged control animals.

Regarding the wide beam, due to the fact that the thickness of the bar is greater, both aged and young animals tend to make fewer missteps.

Trazodone administration had beneficial effect in aged animals in the acute post-stroke phase on day 1 and compared with aged controls.

Regarding young animals, trazodone administration seems to have beneficial neuroprotective role more in the chronic post-stroke phases on day 24 and day 27.

Concerning the time needed to cross the narrow beam, from day 1 post-stroke to the end of the experiment, aged animals treated with trazodone need less time to cross the beam compared with aged controls, although human studies suggest that the trazodone may have negative side effects, particularly in older adults such as dizziness, weight gain and hypotension [23].

The same thing can be observed in young animals treated with trazodone for the first 3 days post-stroke, after that, control animals seem to cross the beam in a shorter time.

These findings are consistent with a study conducted on human subjects that showed that trazodone improved self-care function, but not motor function [24].

Regarding the crossing time on the wide beam, trazodone seems to have a neuroprotective effect only on aged animals.

Moreover, trazodone administration in young animals seems to slow down the crossing.

These findings are consistent with a study evaluating the psychomotor effects of trazodone in humans, which found that trazodone produced small but significant impairments in balance and arm muscle endurance [25].

The well-known antidepressant and anxiolytic effects of trazodone in humans [26] that have also been highlighted in dogs and cats [27] were also pointed out in the present study only on aged animals.

The effect was visible starting from day 9 to the end of the experiment.

This increased curiosity behaviour proved maybe a grade of neuroprotection in aged animals, similar to different human or animal models, which proved some neuroprotection in Alzheimer patients [9] or tau-pathology/prion [8] induced pathologies in mice.

This effect was highlighted to a lesser extent in young animals.

Conclusions

Trazodone did not show any improvements regarding the motor-sensorial function after stroke in young animals, and only transient acute effect in aged animals.

Interestingly, the antidepressant effect manifests itself in the post-stroke phases of aged animals, with young treated animals having no anti-depressant benefit when receiving trazodone compared to controls.

These results could be due to some limited neuroprotective effects of trazodone, but future research seems to be needed in order to fully understand trazodone's cellular benefits in post-stroke patients who do not suffer from depression.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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