Neurotoxicology and Teratology 64 (2017) 63–72

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Behavioral impairments following repeated intranasal glyphosate-based herbicide administration in mice

Carlos Javier Baier[a,b,*], Cristina Eugenia Gallegos[b,c], Rita Raisman-Vozari[d], Alejandra Minetti[b,c]

[a] Instituto de Investigaciones Bioquímicas de Bahía Blanca, Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina
[b] Departamento de Biología, Bioquímica y Farmacia, UNS, Bahía Blanca, Argentina
[c] Laboratorio de Toxicología, Instituto de Ciencias Biológicas y Biomédicas del Sur (INBIOSUR), Universidad Nacional del Sur (UNS)-CONICET, Bahía Blanca, Argentina
[d] Institut National De La Santé Et De La Recherche Médicale, U 1127, CNRS, Unité Mixte De Recherche (UMR) 7225, Sorbonne Universités, UPMC Univ Paris 06, UMR S 1127, Institut Du Cerveau Et De La Moelle Epinière, ICM, Paris, France

ARTICLE INFO

Keywords:
Glyphosate-based herbicides
Intranasal administration
Locomotor activity
Anxiety
Memory
Mouse

ABSTRACT

Inhalation or intranasal (IN) administration of neurotoxicants could constitute a route of toxin delivery to the brain. Pesticides have been proposed as the main environmental factor associated with the etiology of neurodegenerative disorders. In Argentina, the area used for glyphosate (Gly)-resistant crops are sprayed annually with ~200 million liters of Gly-based herbicides (Gly-BHs). Gly residues are often found in the environment, and considering the frequency and amount of its applications, it is probable that the inhalation of Gly-BHs occurs. The present study investigates the neurobehavioral effects of repeated IN administration of Gly-BH in male CF-1 mice (~2 mg/nostrils/day) three days a week, during four weeks (50 mg/kg/day). Locomotor activity and anxiety levels were studied by the Open Field (OF) test. Anxiety was also analyzed through the plus maze (PM) test. Novel Object Recognition (NOR) test was used for recognition memory analysis. Repeated IN Gly-BH administration in mice decreased the ambulatory activity. Moreover, Gly-BH treated mice showed a pronounced increase in thigmotaxis, compared to control group, indicating higher anxiety levels. The anxiogenic behavior in Gly-BH treated mice was then confirmed by PM test. The recognition memory was significantly impaired after 6 h in the Gly-BH treated group. No differences were observed between both groups when the NOR test was performed 24 h after. The present study reveals that repeated IN exposure to Gly-BH in mice affects the central nervous system probably altering neurotransmission pathways that participate or regulate locomotor activity, anxiety and memory.

1. Introduction

Neurotoxicants, including agricultural chemicals, metals, viruses or toxins could access to the brain through inhalation or intranasal (IN) administration (Prediger et al., 2012). The IN pathway includes the olfactory and trigeminal pathways. The olfactory pathway arises in the upper portion of the nasal passages, where olfactory receptor neurons in the olfactory region are the externally exposed portions of the central nervous system (CNS). In this pathway the drug passes through the olfactory epithelium into the olfactory bulb and then to the brain or the cerebrospinal fluid (Dhuria et al., 2010; Mittal et al., 2014). The trigeminal pathway involves the trigeminal nerve, which innervates the respiratory and olfactory epithelium of the nasal passages and enters the CNS in the pons. In the trigeminal pathway the drug is transported via this nerve system (Dhuria et al., 2010; Mittal et al., 2014). The blood–brain barrier (BBB) is known to prevent most drugs to enter in the brain, while IN administration may bring absorption and onset of effects on the CNS (Wu et al., 2008). Alternatively, the drug could be absorbed directly into the systemic circulation across the nasal cavity and then across the BBB into the brain (Dhuria et al., 2010; Mittal et al., 2014).

Pesticides are used extensively throughout the world and abundant evidence make a link between pesticide exposure and health impairment. Pesticide exposure is associated with deficits in neurobehavioral performance (Kamel and Hoppin, 2004). Moreover, pesticides have been proposed as the main environmental factor associated with the etiology of neurodegenerative disorders, such as Parkinson's and Alzheimer's disease (Le Couteur et al., 1999; Richardson et al., 2014). The massive influx of genetically modified (GM) crops resistant to glyphosate (Gly) in Argentina is the main reason why the most widely marketed herbicides in this country are those containing Gly in their formulation. In 1996, Gly-resistant soybean became the first GM crop

* Corresponding author at: Instituto de Investigaciones Bioquímicas de Bahía Blanca, Camino La Carrindanga Km 7, B8000FWB Bahía Blanca, Argentina.
E-mail address: cjbaier@criba.edu.ar (C.J. Baier).

http://dx.doi.org/10.1016/j.ntt.2017.10.004
Received 11 July 2017; Received in revised form 12 October 2017; Accepted 18 October 2017
Available online 20 October 2017
0892-0362/

approved in Argentina, and since then, the area dedicated to GM crops has been growing steadily, reaching 22 million hectares nowadays (Gallegos et al., 2016; GRAIN, 2009; Trigo, 2011). As these crops are sprayed with 200 million liters of Gly per year (Aparicio et al., 2013; Gallegos et al., 2016; Teubal, 2009; Teubal et al., 2005) its residues are often found in the environment (soil, water and food) (Bohn et al., 2014; Peruzzo et al., 2008; Ronco et al., 2016; Sanchis et al., 2012; Van Stempvoort et al., 2014).

Little is known about the impact of sub-lethal doses of Gly on human health and the environment, and although Gly is considered to be safe for living beings, its safety has been questioned worldwide. Some clinical reports on human intoxication with commercial formulations of Gly described negative effects in the nervous system, related to motor (Barbosa et al., 2001; Wang et al., 2011), anxiety and short-term memory impairments (Nishiyori et al., 2014). In vivo studies in rodents have demonstrated neurotoxic effects of Gly and Gly-based herbicides (Gly-BHs). Intraperitoneal Gly administration decreased locomotor activity and brain dopaminergic markers in rats (Hernandez-Plata et al., 2015). Oral exposure to Gly-containing herbicide in pregnant rats altered the activity of enzymes involved in NADPH generation, both in the brain of the mothers and in the offspring (Daruich et al., 2001). Rat offspring exposed during pregnancy and lactation to Gly-BH in the drinking water showed lower locomotor activity and anxiety levels in the adulthood (Gallegos et al., 2016). Additionally, acute Gly-BH treatment in hippocampal slices of Gly-BH-exposed offspring decreased glutamate uptake, leading to glutamate excitotoxicity (Cattani et al., 2014). Subchronic and chronic oral Gly-BH exposure in mice decreased locomotor activity, increase anxiety and depression-like behavior levels, reduced tyrosine hydroxylase and serotonin-immunoreactivity in different brain areas (Ait Bali et al., 2017).

Since agricultural practices are based on Gly-formulations instead of pure Gly, for the present study it was decided to employ a marketed Gly product (i.e., a Gly-BH). As pesticides are used in formulations which combine an active ingredient with adjuvants, the toxicity exerted by Gly-BH cannot therefore be exclusively attributed to the active ingredient but either to the intrinsic toxicity of adjuvants or to the possible synergy between Gly and the other formulation ingredients (El-Shenawy, 2009; Gallegos et al., 2016; Mesnage et al., 2013; Richard et al., 2005). That is, in the case of a specific commercial formulation of Gly, as the Gly-BH used in the present study, the formulation is the responsible for the effects reported, and it is not possible to determine which component(s) of the mixture was (were) responsible for the toxicity. Even though the Gly-BH dose used in the present work was higher than the Gly-BH levels to which the population is normally exposed (Solomon, 2016), it was similar to other toxins and pesticides doses employed in intranasal administration studies (Prediger et al., 2012; Prediger et al., 2010; Rojo et al., 2007; Rojo et al., 2006; Tristao et al., 2014). In agreement with Ford et al. (2017), toxicological studies with pesticides are usually performed at higher doses in order to demonstrate a plausible drug-action mechanism.

While the aforementioned studies address the neurotoxic characteristics of Gly, or Gly-BH, administrated by oral or intraperitoneal routes, the IN administration pathway has not been explored. Given the frequency and amount of spray applications using Gly-BHs, the inhalation of the sprays is likely to occur (Xu et al., 2016). In this regard, a short-term health-related outcomes study reported that aerial spraying of Gly-BHs in Colombia increased the medical consultations related to dermatological and respiratory illnesses, as well as the number of miscarriages (Camacho and Mejia, 2017). In addition, IN Gly administration studies in mice showed that Gly induced pulmonary inflammation (Kumar et al., 2014). It has been reported that the amino acid transporters LAT1/2 are involved in the absorption of Gly across the epithelia in Caco-2 cells and nasal mucosal tissues, suggesting that Gly in the olfactory epithelium could be transferred through the nasal epithelium to the CNS using this pathway (Xu et al., 2016). The present work represents the first report about the neurobehavioral effects of repeated IN administration of Gly-BH in CF-1 mice (~2 mg/nostrils/day; 50 mg/kg/day), evaluated through a set of behavioral tests referred to locomotion, anxiety and memory.

2. Materials and methods

2.1. Animals

Twenty mature male CF-1 mice (65 days old), weighing ~37 g, from our own breeding center were used in this study. They were maintained under constant temperature (22 ± 1 °C) and humidity (50–60%) conditions in a 12 h light-dark cycle, with food (Ganave®, Alimentos Pilar S.A., Argentina) and water ad libitum. Both, animal care and handling procedures were in agreement with the standards for the care of laboratory animals as outlined in the NIH Guide for the Care and Use of Laboratory Animals (Garber et al., 2011) and approved by the Institutional Animal Care and Use Committee (CICUAE 089/2016, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Argentina). Special care was taken as well to minimize the number of mice used.

2.2. Materials

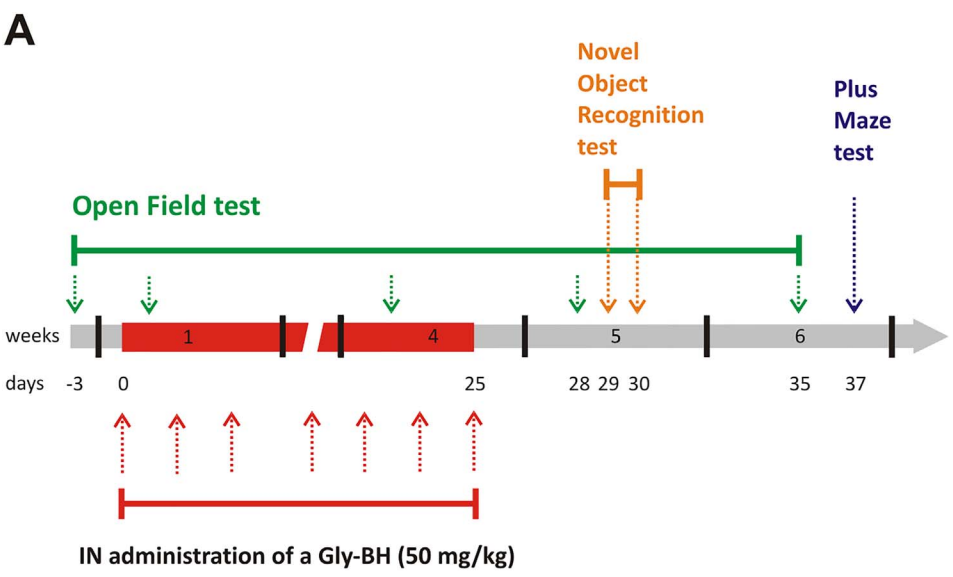
The pesticide used in this study is a commercial formulation marketed in Argentina as Glifloglex® from Gleba S.R.L., which contains 48 g of Gly isopropylamine salt per 100 cm^3 product (equivalent to 35.6% w/v of Gly acid) (Gallegos et al., 2016) together with an unspecified mix of inerts and adjuvants. Gly-BH was dissolved in saline at a concentration of 96 mg Gly acid/ml, after which it was administrated by the IN route. The pH value of saline and Gly-BH-saline solution was ~5–5.5.

2.3. Experimental design

Mice were weighed (Section 2.4) and randomly assigned to the control group (n = 10), administrated with 0.9% NaCl (saline) solution, or Gly-BH-treated group (n = 10), respectively (Section 2.4, see scheme Fig. 1A). The battery of behavioral tests included: Open field (OF) test (Section 2.5), Novel Object Recognition (NOR) test (Section 2.7) and Plus Maze (PM) test (Section 2.6), which were conducted in the sequence described in Fig. 1A, in the same groups of mice enumerated above. Each behavioral test was separated at least by 1 day (Shibata et al., 2007; Wahlsten, 2010).

2.4. Intranasal administration of a Gly-BH solution

In the present work we used the frequency of administration and Gly-BH doses in the range previously described in the bibliography (Astiz et al., 2009; Cattani et al., 2014; Gress et al., 2016; Hernandez-Plata et al., 2015). This protocol is similar to that employed for IN administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a widely used dopaminergic neurotoxin (Prediger et al., 2012; Prediger et al., 2010; Rojo et al., 2007; Rojo et al., 2006; Tristao et al., 2014). Mice were weighed and randomly assigned to the control group (n = 10), administrated with 0.9% NaCl (saline) solution, or Gly-BH-treated group (n = 10), respectively. Mice were adapted to handling daily for one week before the onset of IN administration. The IN administration of Gly-BH was performed as described previously (Rojo et al., 2007; Rojo et al., 2006; Tristao et al., 2014). Briefly, non-anesthetized CF-1 mice received the equivalent to ~2 mg of Gly acid through the nostrils using a micropipette (10 µl solution/nostril/day; ~1 mg Gly acid/nostril/day; i.e. total/day (both nostrils) ~2 mg Gly acid/day), 3 times per week during 4 weeks, amounting to 50 mg/kg/day of Gly acid (see scheme Fig. 1A). Similarly to the procedure described by Rojo et al.(2006), animals were held by the neck and were laid upside down to limit liquid flow down the trachea. Control mice



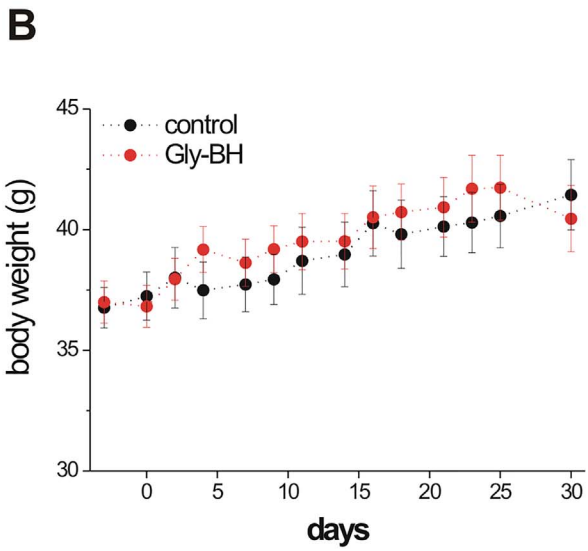


Fig. 1. A) Diagram of the experimental design. B) Mice body weights in control and Gly-BH-exposed groups in the course of the experiment.

were similarly administrated with saline. Animals were given a 3-min interval to regain normal respiratory function, and then this procedure was repeated through the contralateral nostril. Body weight was recorded before the beginning of the treatment, during IN Gly-BH administration, and 5 days after the last IN administration (Fig. 1B).

2.5. Open field (OF) test

Motor activity, which is considered to be a test of nervous system function, shows the integrated output of the sensory, motor and associative processes of the nervous system in case of absence of systemic toxicity (Hubler et al., 2005). Behavior in the OF test is used to assess locomotor activity as well as emotionality (Walsh and Cummins, 1976). Individual mice were released in the corner of a square (50 cm × 50 cm) OF arena. Mice were left undisturbed and recorded with a camera mounted above the center of the OF arena for 15 min. At the end of the test, mice were returned to their home cage. After each animal was removed from the test area, its floor was carefully cleaned with a piece of cloth soaked with a 10% ethanol solution. The locomotor activity and thigmotaxis (see below) were analyzed with the OF at 3 time points (Fig. 1A): i) before the beginning of the treatment (day − 3; we assumed as day 0 the day when we started the IN administration of the Gly-BH), ii) during Gly-BH IN administration (once a week during 4 weeks), and iii) after the last IN administration (3 and 10 days later). To avoid interference with the possible short-term effects of the herbicide, motor activity was analyzed a day after the first weekly IN administration (Rojo et al., 2007). Typically, when mice are introduced into an OF, they explore mainly the peripheral zone of the OF. This tendency to remain close to the walls, called thigmotaxis, decreases gradually during the first minutes of exploration, entering to the central zone of the arena during the following time intervals. The preferential exploration of the peripheral zone in the OF is considered an index of anxiety (Simon et al., 1994). Thigmotaxis was determined in the OF, virtually divided in a peripheral and a central zone, determining the ratio of time spent along the periphery relative to time spent in the center over each 5-min interval (Simon et al., 1994; Patel et al., 2014). The analysis of distance travelled was performed with the automated software program recently described in Patel et al. (2014) and with ETHOWATCHER® (Crispim Junior et al., 2012). For analysis of thigmotaxis we used the software developed by Patel et al. (2014). Grooming episodes (face washing, forepaw licking and head stroking) and rearing episodes (the mouse lifts both of its forefeet off the floor)

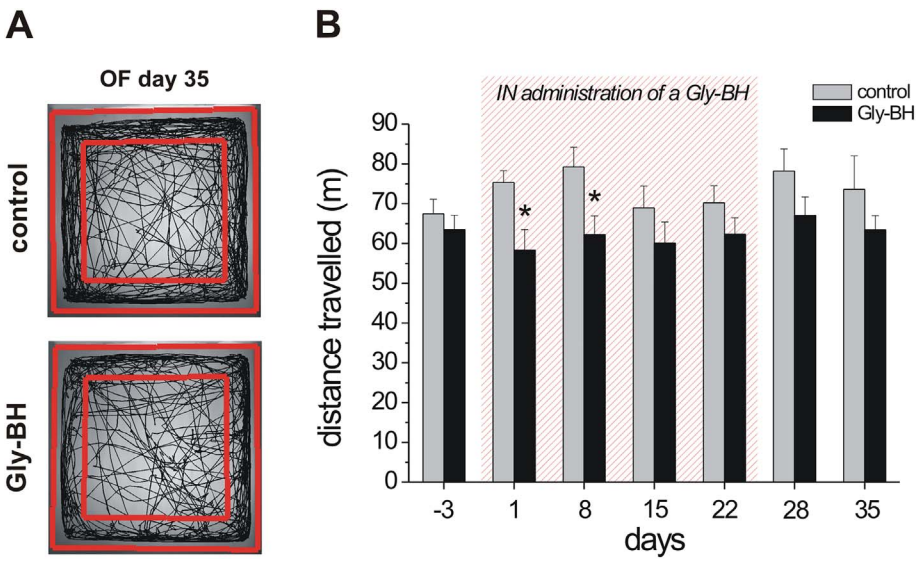


Fig. 2. *Locomotor activity in the OF.* A) Cumulative trajectories of a representative control and Gly-BH treated mouse, respectively, over 15 min in the OF at day 35. Outer and inner rectangles in the OF arena denotes peripheral and central zone, respectively. B) Distance travelled (in meters) for control and Gly-BH treated mice, respectively, during the 15 min test. Results are expressed as mean ± SEM. n = 9–10 mice per group. $^{*}p < 0.05$ compared to control group at the indicated day.

were analyzed manually off line (Choleris et al., 2001). The test was always carried out between 09:00 am and 03:00 pm in a quiet room intended only for this purpose.

2.6. Plus maze (PM) test

Anxiety levels in mice intranasally exposed to a Gly-BH were analyzed using the PM test, which represents a valid behavioral model to study the emotional response of animals (Pellow et al., 1985). The PM apparatus was made up of wood and consisted of four arms, all of them having the same dimensions (50 cm × 10 cm), which were elevated 50 cm above the floor. Two of these arms were enclosed by 40 cm high lateral walls with an open roof and were located perpendicularly to the other two opposed open arms. The four arms delimited a central area of 10 cm^2. This test exploits a rodent's natural conflict between avoidance and exploration of open and elevated areas. Mice were placed in the centre of the maze, facing an enclosed arm and were allowed to explore the maze freely for 5 min. The following parameters were assessed: i) percentage of time spent in open arms, ii) percentage of entries to open arms, and (iii) total number of entries in open and closed arms. A decrease in parameters i) and/or ii) are consistent with an increase in anxiety behavior, whereas parameter (iii) is indicative of locomotor activity (Pellow et al., 1985). The floor of the maze was wiped out thoroughly with a piece of cloth soaked with a 10% ethanol solution after each test. The test was carried out in a quiet room from 09:00 am to 03:00 pm.

2.7. Novel Object Recognition (NOR) test

The NOR test is a learning test for animals. In this test, the mouse is presented with two similar objects during the first session, and then one of the two objects is replaced by a new one during a second session (Leger et al., 2013). The time spent exploring the novel object, relative to the familiar object, is an estimation of episodic recognition memory. The objects selected to the NOR test were: Falcon tissue culture flasks (50 ml) filled with sand; a glass bottle filled with green colored water; and a tower of toy bricks. These objects differ in shape, color and texture, whereas the size and volume of them were similar. The NOR test was performed according to the guidelines of Leger et al. (2013) with minor modifications (Barbieri et al., 2016; Kubota et al., 2016). The study consisted of three trials: a sample (familiarization) trial and two test trials carried out at 6 h and 24 h after the familiarization trial, respectively. In the familiarization trial, two identical 50 ml tissue culture flasks filled with sand and parafilm-sealed were placed at opposite ends of the OF (~5 cm from the corner). A mouse was then allowed to explore the OF for 10 min while being video recorded (referred to as the 'familiarization' session). In the test sessions, 6 h and 24 h later, one of the tissue culture flasks was replaced with a glass bottle filled with green colored water, or with a tower of toy bricks, respectively, and the same mouse was allowed to explore the OF for 5 min whilst being video recorded. Object exploration was defined as sniffing or touching the object with either the nose or forepaw, but not sitting or standing on the objects (Patel et al., 2014). The mouse's preference for the novel object over the familiar object was measured with the automated software program developed by Patel et al. (2014) mentioned above.

2.8. Statistical analysis

A two way (treatment x experimental day) repeated-measures ANOVA was performed for a comparative analysis of the OF parameters evaluated (distance and thigmotaxis). Differences between groups for each experimental day were assessed using Bonferroni post hoc test. Paired samples *t*-test was used for comparative analysis in the NOR test. Student's *t*-test was used for PM test analysis. Results were expressed as mean ± S.E.M. A value of $p < 0.05$ was considered statistically significant. All statistical analyses were carried out using the software SPSS Statistics for Windows.

3. Results

3.1. Repeated IN Gly-BH administration decreased locomotor activity in mice

Mice's body weight was not affected by the IN Gly-BH administration (Fig. 1B). To assess whether repeated subacute IN administration of Gly-BH alters locomotor activity over time, we analyzed control and Gly-BH treated mice through the OF test. Fig. 2 summarizes the effects of IN administration of Gly-BH on the ambulatory activity of treated mice. No habituation to the open field after re-exposures was observed for both mice's groups (Fig. 2B). As shown in Fig. 2B, the total distance travelled (i.e., the ambulatory capacity) was significantly lower in Gly-BH treated mice, ~20%, respect to control mice [$F(1,17) = 5.326$ $p < 0.05$ for treatment effect]. No differences were found for day effect.

The time spent immobile was longer in intranasally Gly-BH treated mice (Fig. 3A) [$F(1,17) = 8.484$ $p < 0.05$ for treatment effect; $F(6,102) = 4.844$ $p < 0.001$ for day effect]. Besides, the mean speed of ambulation (Fig. 3B) decreased in intranasally Gly-BH treated mice [F

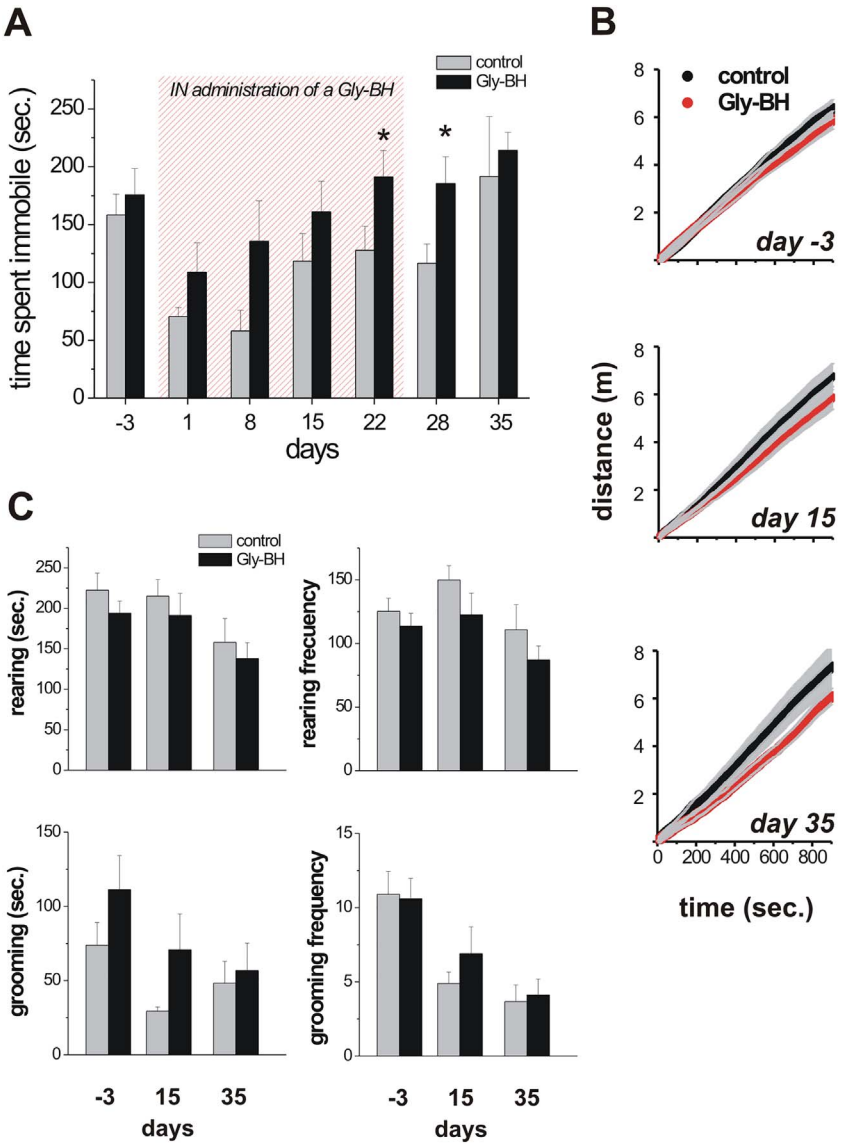


Fig. 3. *Locomotor activity and emotionality in the OF.* A) Time spent immobile (s) for control and Gly-BH treated mice, respectively, during the 15 min in the OF test. B) Plot of distance travelled over time for control and Gly-BH treated mice, respectively, during the 15 min test in the OF at the indicated experimental day. C) Duration (s) and frequency of rearing and grooming, for control and Gly-BH treated mice, respectively, placed in the OF for 15 min at the indicated experimental day. Results are expressed as mean ± SEM. n = 9–10 mice per group. *$p < 0.05$ compared to control group at the indicated day.

(1,17) = 6.921 $p < 0.05$ for treatment effect; F(6,102) = 2.846 $p < 0.05$ for day effect].

The duration and frequency of rearing were not affected by the Gly-BH treatment (Fig. 3C). However there was a day effect [rearing duration: F(2,32) = 7.528 $p < 0.01$; rearing frequency: F(2,32) = 5.230 $p < 0.05$ for day effect]. Similarly, the duration and frequency of grooming were not affected by the Gly-BH treatment (Fig. 3C), although there was a day effect for grooming frequency [F(2,32) = 11.971 $p < 0.001$ for day effect].

3.2. IN Gly-BH administration increased thigmotaxis in mice

Thigmotaxis is considered as an index of anxiety (Choleris et al., 2001; Simon et al., 1994). In Fig. 4 we show the thigmotaxis results divided in three 5 min-intervals (Fig. 4A–D). Gly-BH treated mice showed a pronounced increase in thigmotaxis when compared to control mice during the first two 5 min intervals in the OF [*thigmotaxis* 0–5 min: F(1,16) = 7.043 $p < 0.05$ for treatment effect, F(6,96) = 6.422 $p < 0.001$ for day effect; *thigmotaxis* 5–10 min: F(1,16) = 6.281 $p < 0.05$ for treatment effect; F(6,96) = 3.086 $p < 0.01$ for day effect]. These results indicate an increased state of anxiety in intranasally Gly-BH treated mice respect to controls.

3.3. Effects of repeated IN administration of Gly-BH on anxiety

In order to confirm the thigmotaxis results we performed the PM test at day 37 (12 days after the last IN administration of Gly-BH). As shown in Fig. 5 A, the percentage of time spent in open arms was lower for Gly-BH treated mice compared to control mice [$p < 0.05$]. Similarly, the percentage of entries to the open arms decreased in Gly-BH treated mice compared to control group [$p < 0.05$] (Fig. 5B). These results were in agreement with the thigmotaxis data, indicating that IN administration of Gly-BH induced an anxiogenic behavior. The total number of arm entries was lower in Gly-BH treated mice, compared to control group [$p < 0.05$] (Fig. 5C), in agreement with the lower locomotor activity observed in Gly-BH treated group described above (Figs. 2 and 3).

3.4. Effects of repeated IN administration of Gly-BH on recognition memory

To investigate whether repeated IN administration of Gly-BH affects memory retention in mice we performed the NOR test at 4–5 days after the last Gly-BH administration. As shown in Fig. 6A–B, there was a significant increased tendency of control mice to explore the novel object 6 h after familiarization trial [$p < 0.05$], whereas the IN Gly-BH administration induced a significant impairment of the recognition

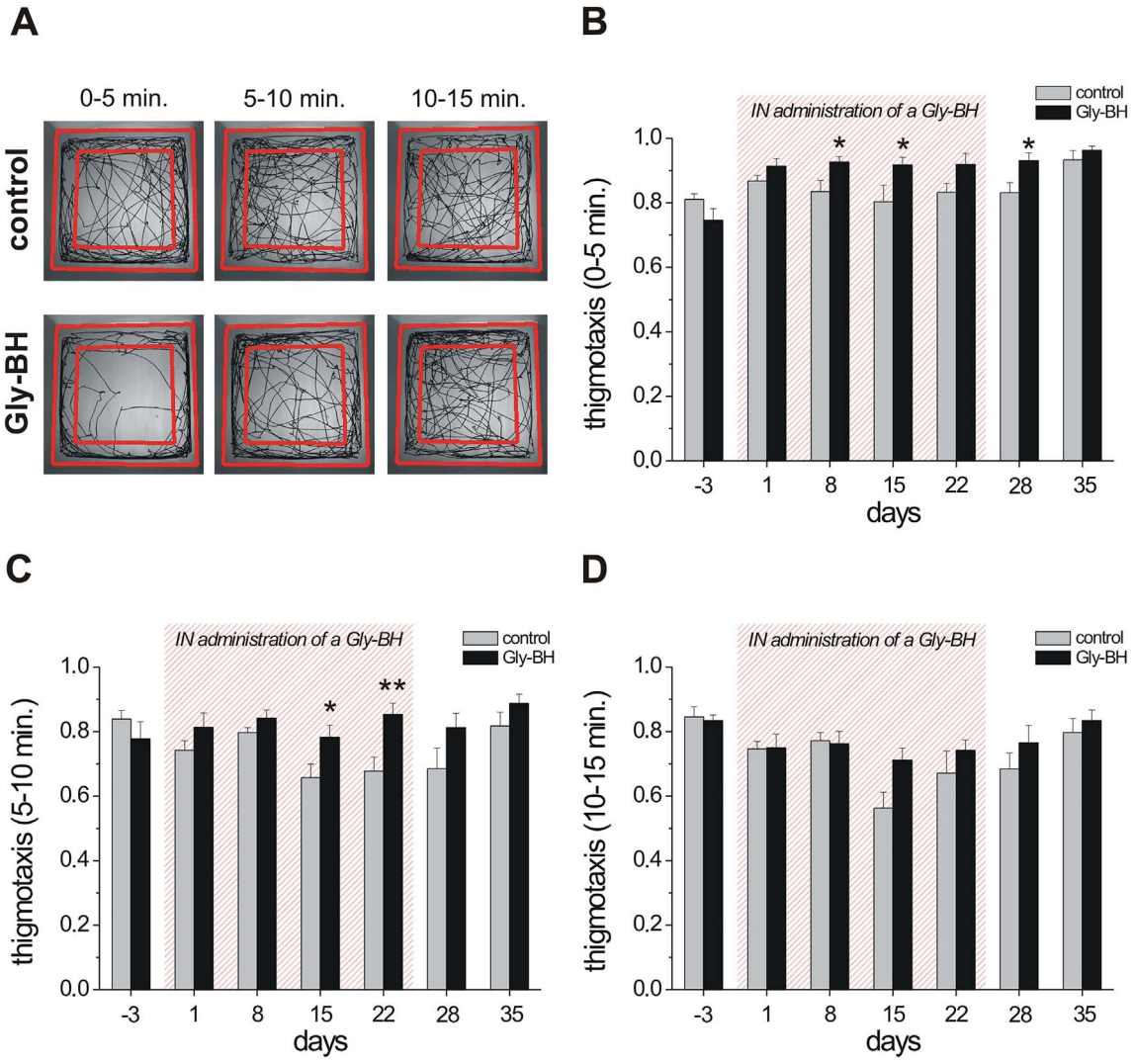


Fig. 4. *Thigmotaxis in the OF.* A) Cumulative trajectories of a representative control and Gly-BH treated mouse, respectively, over 5 min intervals in the OF at day 15. B), C), D) Thigmotaxis values for control and Gly-BH treated mice, respectively, over 5 min intervals in the OF. Results are expressed as mean ± SEM. n = 9–10 mice per group. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to control group at the indicated day.

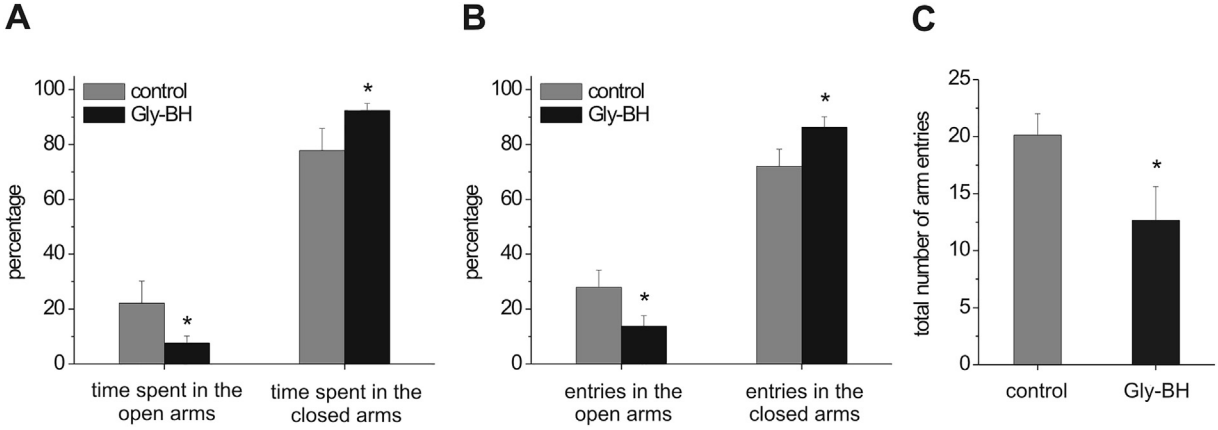


Fig. 5. *Plus maze test.* A) Percentage of time spent in open and in closed arms, for control and Gly-BH treated mice, respectively. B) Percentage of entries to open and to closed arms, for control and Gly-BH treated mice, respectively. C) Total number of arm entries, for control and Gly-BH treated mice. Data are expressed as mean ± SEM. n = 9–10 per group. $^{*}p < 0.05$ compared to control group.

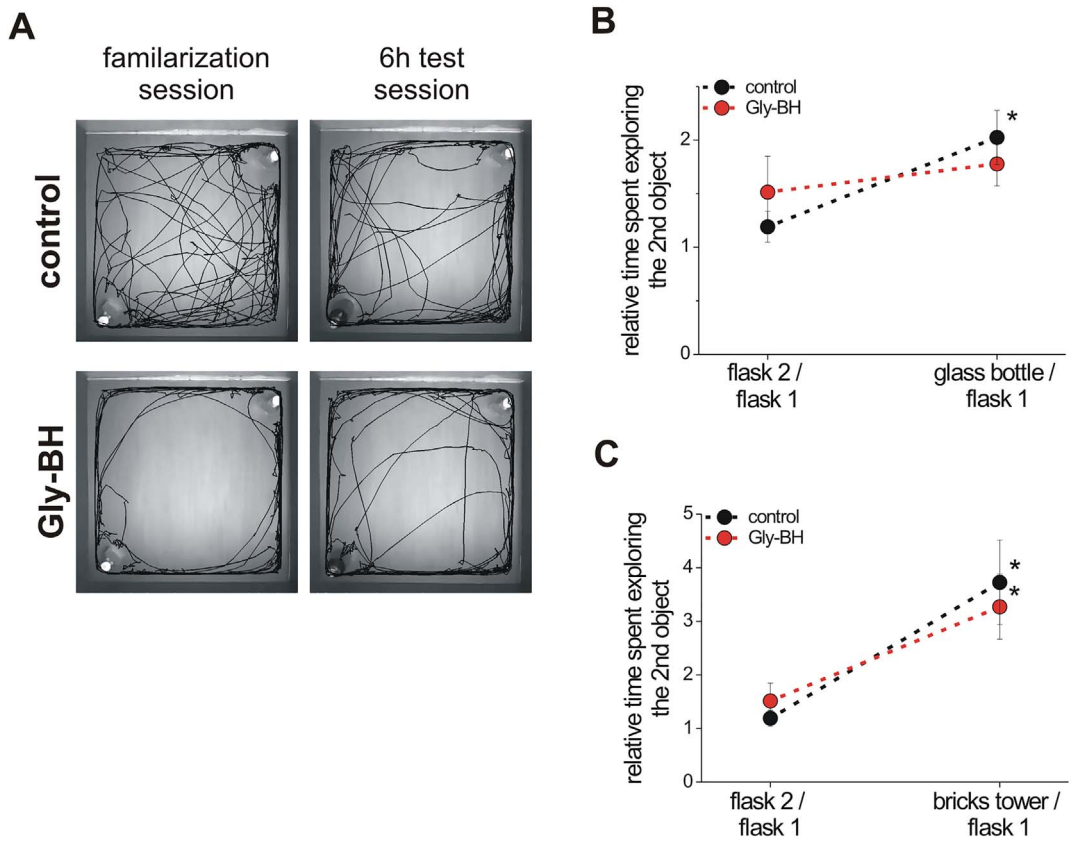


Fig. 6. Recognition memory in the *Novel Object Recognition* (*NOR*) *test.* A) Cumulative trajectories of a representative control and Gly-BH treated mice, respectively, during familiarization and 6 h test, over 5 min. B), C) Relative time spent exploring the 2nd object (i.e. flask 2 in the familiarization session; the glass bottle in the 6 h test session; or a tower of toy bricks in the 24 h test (C)) versus the 1st object (i.e. flask 1 in both test and familiarization sessions). Dot points represent the mean value for control and Gly-BH treated mice, respectively. Lines connect the values for the same mice group for both NOR test sessions. Data are expressed as mean ± SEM. n = 9–10 per group. $^{*}p < 0.05$ statistically different from familiarization session in the same group.

memory. That is, Gly-BH treated mice showed no differences in the time that they expend recognizing the new objet respect to the familiar objet (Fig. 6A–B) after 6 h from the familiarization trial. When we performed the NOR test after 24 h, both groups of mice showed an increased tendency to explore the novel object (Fig. 6C) [control, $p < 0.05$; Gly-BH treated mice, $p < 0.05$]. Total exploration time (total time spend by the mice exploring the familiar and the novel objects) was not affected in both groups after 6 h and 24 h.

4. Discussion

The present study demonstrates that repeated IN administration of a Gly-BH in adult mice produced neurobehavioral alterations, involving impairment in locomotion, anxiety and memory, indicating to the IN route as a possible pathway for Gly-BH access to the nervous system.

As described in Section 2.2, in our experiments we used a commercial formulation containing the active ingredient, i.e. Gly isopropylamine salt, together with undeclared adjuvants and inert compounds. In this regard, is important to emphasize that the specific formulation was responsible for the reported behavioral effects enumerated above. Considering that the formulation is a mixture of components, it is not possible to determine which component(s) of the formulation was (were) responsible for the toxicity. For this reason, the described results cannot be attributed solely to Gly, but it could be triggered by other components of the formulation, or to interactions between Gly and formulation ingredients (El-Shenawy, 2009; Gallegos et al., 2016; Mesnage et al., 2013; Richard et al., 2005).

Whereas some studies address the neurotoxic effect of Gly, or Gly-BH, administrated by oral or intraperitoneal routes, the IN administration pathway has not yet been explored. Studies on oral exposure to Gly-BH in pregnant rats described alterations in the activity of several brain enzymes both, in mothers and offspring (Daruich et al., 2001); decreased glutamate uptake, increasing glutamate excitotoxicity, in the hippocampus of exposed offspring (Cattani et al., 2014) and decreased locomotor activity and anxiety levels in adult rats perinatally exposed to Gly-BH (Gallegos et al., 2016). In addition, intraperitoneal Gly administration (doses 50, 100, and 150 mg/kg; 3 times per week, during 2 weeks) decreased locomotor activity and brain dopaminergic markers in rats (Hernandez-Plata et al., 2015). In agreement with our results, Ait Bali et al. (2017) observed that subchronic (daily for 6 weeks) and chronic (daily for 12 weeks) oral Gly-BH exposure (oral gavages, 250 or 500 mg/kg/day) decreased locomotor activity and increased anxiety in treated mice. Additionally, subchronic and chronic Gly-BH administration increase depression-like behavior levels and reduced tyrosine hydroxylase and serotonin immunoreactivity in different brain areas (Ait Bali et al., 2017). The aforementioned animal studies, in addition to reports from human-Gly-BH exposure (Barbosa et al., 2001; Menkes et al., 1991; Sato et al., 2011; Wang et al., 2011) revealed that Gly, and Gly-BH, are able to cross the BBB and affect the CNS.

Experimental evidence suggesting that the nasal route may be used by neurotoxins to reach the brain comes from studies on IN administration of MPTP (Prediger et al., 2012; Rojo et al., 2007; Rojo et al., 2006). IN MPTP administration in mice (30 and 60 mg/kg daily for 30 days) produced motor deficits in the OF that are correlated with depletion of striatal dopamine (DA) levels, loss of tyrosine hydroxylase and DA transporter as well as strong astrogliosis and microgliosis in substantia nigra and striatum (Rojo et al., 2006). These data demonstrated that MPTP, through the IN pathway, reached the basal ganglia and developed impairments related to Parkinson's disease (PD) neurodegeneration. Similar results were obtained with single (1 mg/nostril,

~60 mg/kg) (Prediger et al., 2010) or repeated (1 mg/nostril, ~60 mg/kg, 4 consecutive days) (Tristao et al., 2014) IN MPTP administrations, respectively. On the other hand, IN administration of two pesticides, rotenone and paraquat, positively associated with PD (Tanner et al., 2011), exhibited different results from those obtained with MPTP in mice: treatment with rotenone (IN dose of 2.5 mg/kg, daily for 30 days) were asymptomatic, whereas paraquat (IN doses of 10, 20, and 30 mg/kg; daily for 30 days) induced severe hypokinesia and vestibular damage but it did not produce alterations in the nigrostriatal system (Rojo et al., 2007). In agreement to Hernandez-Plata et al. (2015), Gress et al. (2016), Gallegos et al. (2016) and Ait Bali et al. (2017), but using the IN administration pathway, we observed locomotor hypoactivity in Gly-BH treated mice. The nigrostriatal pathway plays an essential role in the control of voluntary motor movement. This tract degenerates in PD, a syndrome characterized by a profound depletion of DA in the striatum, responsible for the observed motor alterations (Baier et al., 2012; Kuhar et al., 1999). Locomotor activity in the OF correlates positively with DA and DA receptors levels (Bano et al., 2014; Gallo et al., 2015; Kim et al., 2013).

IN Gly-BH administration produced an increase in thigmotaxis, reflecting an increase in anxiety levels (Choleris et al., 2001; Simon et al., 1994). This result was also confirmed through the PM test. Similarly, oral Gly-BH administration increase anxiety levels in treated mice (Ait Bali et al., 2017). Interestingly, Gallegos et al. (2016) reported that the offspring of rats orally exposed to Gly-BH during gestation and lactation, exhibit in adulthood lower anxiety levels that untreated rats. Animal strains and administration pathways as well as exposure-time window should be considered in order to evaluate the Gly-BH effects on the nervous system. Intraperitoneal paraquat administrations (10 mg/kg, 3 times a week during 3 consecutive weeks) develop an anxiety-like state in mice lacking the enzyme cyclooxygenase-2 (Litteljohn et al., 2008). Regarding the IN administration of toxins, single IN MPTP administration in mice didn't induce significant alterations in anxiety-related parameters (Prediger et al., 2010). Activity in the amygdala, bed nucleus of the stria terminalis, the ventral hippocampus and prefrontal cortex are involved in the anxiety response (Calhoon and Tye, 2015). Regarding to the neurotransmitters that participates in anxiety modulation, the influence of 5-hydroxytryptamine (serotonin, 5-HT), γ-aminobutyric acid (GABA), DA, glutamate and acetylcholine, as well as its receptors have been documented (Cortese and Phan, 2005; Liu et al., 2013; Martin et al., 2009; Olivier et al., 2013; Picciotto et al., 2015; Simon et al., 1994). Subchronic and chronic exposure to oral Gly-BH in mice produced a reduction of 5-HT-immunoreactivity in the dorsal raphe nucleus, basolateral amygdala and ventral medial prefrontal cortex (Ait Bali et al., 2017). Exposure to Gly-BH in the drinking water affects the glutamatergic neurotransmission in the hippocampus of rats exposed during both, prenatal and postnatal periods to herbicide formulation (Cattani et al., 2017; Cattani et al., 2014). Also, Cattani et al. (2017) described a decreased acetylcholinesterase activity in the hippocampus of Gly-BH- exposed rat. In this regard, Larsen et al. (2016) found that Gly is a weak inhibitor of acetylcholinesterase in samples of rat brain homogenates. However, with exception of the study of Ait Bali et al. (2017), those experimental data were not directly related with anxiety. The information about the effects of Gly or Gly-BH on the anxiety mechanisms is partial and fragmented, and future research will be necessary to understand the effects of Gly or Gly-BH in anxiety modulation.

IN Gly-BH administration induced a significant impairment of recognition memory in treated mice when the NOR test was performed 6 h after the familiarization trial. In line with our findings, Nishiyori et al. (2014) reported the case of a woman who after attempted suicide by Gly-BH ingestion showed anxiety, hippocampal lesion and short-term memory impairments. In rodents, different brain regions interact in recognition memory for the association of objects and places: perirhinal cortex, hippocampus, medial prefrontal cortex and medial dorsal thalamus (Warburton and Brown, 2015). Classical neurotransmitter systems participate in learning and memory of rodents: glutamate, GABA, DA, acetylcholine, serotonin, and norepinephrine (Calhoon and Tye, 2015). Intraperitoneal administration of low doses of Gly to rats (10 mg/kg, three times a week for 5 weeks) provoked severe oxidative stress in substantia nigra, cortex and hippocampus (Astiz et al., 2009). Exposure of hippocampal slices to Gly-BH produced calcium influx, activation of NMDA receptors and voltage-dependent calcium channels, leading to oxidative stress and neural cell death (Cattani et al., 2014). Moreover, Gly affected dopaminergic markers in rats (Hernandez-Plata et al., 2015), could act as a weak inhibitor of brain acetylcholinesterase (Cattani et al., 2017; Larsen et al., 2016), and affected 5-HT levels (Ait Bali et al., 2017). In reference to IN administration of neurotoxics, IN MPTP administration produced memory impairments in mice (Prediger et al., 2010).

A reason that limits the interpretation of the described experimental result is the fact that a specific commercial Gly-BH formulation was used instead of pure Gly. Agricultural practices are based on Gly-formulations and in vitro studies in different cell lines demonstrated that Gly-BH were more toxic that Gly alone (Mesnage et al., 2013; Mesnage et al., 2014). Pesticide formulations are mixtures of an active ingredient along with inert and adjuvants, many of which have intrinsic toxic effects. For this reason, the described results cannot be attributed solely to Gly, but it could be triggered by other components of the formulation, or to interactions between Gly and formulation's ingredients (El-Shenawy, 2009; Gallegos et al., 2016; Mesnage et al., 2013; Richard et al., 2005). Therefore, the behavioral alterations observed in mice after IN administration of a specific commercial formulation Gly-BH prevent to know which specific component(s) of the mixture was (were) responsible(s) for the neurobehavioral alterations reported in Gly-BH treated mice (Mesnage et al., 2013; Mesnage et al., 2014). Besides, a single IN Gly-BH dose was used by us, which was selected in the basis of previous reports for Gly and Gly-BHs administration (Astiz et al., 2009; Cattani et al., 2014; Gress et al., 2016; Hernandez-Plata et al., 2015). Although the selected dose in the present study was higher than the expected Gly exposure levels (Solomon, 2016), it was chosen in order to demonstrate a plausible drug-action mechanism as in Ford et al. (2017). Further studies involving doses-response analysis and time-course assessment of Gly distribution in the brain after IN administration will be necessary to completely understand the observations described above.

Not every substance can gain access to the brain via the nose, and its transport capacity is affected by several factors: nasal physiology, physicochemical properties of the compound and its formulation (Wu et al., 2008). The precise mechanisms of IN substance delivery to the CNS are not completely understood. Pathways involving nerves connecting the nasal passages to the brain and spinal cord, vasculature and cerebrospinal fluid have been implicated in the transport of molecules from the nasal cavity to the CNS (widely reviewed in Dhuria et al. (2010) and Prediger et al. (2012)). Considering that Gly is a glycine analog, Xu et al. (2016) recently proposed that Gly could be a substrate for the glycine-uptake pathways through the amino acid transporters LAT1/2. Therefore, Gly in the olfactory epithelium could be transferred through the nose to the CNS employing one of both of these transporters (Xu et al., 2016). Once in the brain, Gly could produce neurotoxicity by oxidative stress and glutamate excitotoxicity in specific brain areas (Astiz et al., 2009; Cattani et al., 2014), or could be biotransformed to reactive metabolites and react with several proteins causing protein-dysfunction and physiological impairments (Ford et al., 2017), with the consequent neurobehavioral alterations described in this report.

5. Conclusion

Taken together, our findings demonstrate that IN exposure to commercial Gly-BH produces alterations in locomotor activity, anxiety and memory in adult mice. These observations could be a consequence of alterations in neurotransmission systems comprising the GABAergic,

dopaminergic, serotoninergic and/or cholinergic systems. More experimental research including measurements of neurotransmitters, its receptors, cell damage and inflammatory processes in specific brain areas will be necessary to completely understand the mechanism subjacent after IN Gly-BH administration.

Declaration of interest

Research described in this article was supported by grant PGI 24/B224 (Universidad Nacional del Sur, Bahía Blanca, Argentina) to AM. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgements

The authors wish to thank Dr. Eugenio Aztiria (INIBIBB, Bahía Blanca, Argentina) for his helpful reading of the manuscript. We also want to thank the sworn translator Natalia Difilippo for helping us with the use of English in this paper (INBIOSUR, Bahía Blanca, Argentina).

References

Ait Bali, Y., Ba-Mhamed, S., Bennis, M., 2017. Behavioral and immunohistochemical study of the effects of subchronic and chronic exposure to glyphosate in mice. Front. Behav. Neurosci. 11, 146.

Aparicio, V.C., De Geronimo, E., Marino, D., Primost, J., Carriquiriborde, P., Costa, J.L., 2013. Environmental fate of glyphosate and aminomethylphosphonic acid in surface waters and soil of agricultural basins. Chemosphere 93 (9), 1866–1873.

Astiz, M., de Alaniz, M.J., Marra, C.A., 2009. Effect of pesticides on cell survival in liver and brain rat tissues. Ecotoxicol. Environ. Saf. 72 (7), 2025–2032.

Baier, C.J., Katunar, M.R., Adrover, E., Pallares, M.E., Antonelli, M.C., 2012. Gestational restraint stress and the developing dopaminergic system: an overview. Neurotox. Res. 22 (1), 16–32.

Bano, F., Ahmed, A., Parveen, T., Haider, S., 2014. Anxiolytic and hyperlocomotive effects of aqueous extract of *Nigella sativa* L. seeds in rats. Pak. J. Pharm. Sci. 27(Spec no 5), 1547–1552.

Barbieri, M., Ossato, A., Canazza, I., Trapella, C., Borelli, A.C., Beggiato, S., Rimondo, C., Serpelloni, G., Ferraro, L., Marti, M., 2016. Synthetic cannabinoid JWH-018 and its halogenated derivatives JWH-018-Cl and JWH-018-Br impair Novel Object Recognition in mice: behavioral, electrophysiological and neurochemical evidence. Neuropharmacology 109, 254–269.

Barbosa, E.R., Leiros da Costa, M.D., Bacheschi, L.A., Scaff, M., Leite, C.C., 2001. Parkinsonism after glycine-derivate exposure. Mov. Disord. 16 (3), 565–568.

Bohn, T., Cuhra, M., Traavik, T., Sanden, M., Fagan, J., Primicerio, R., 2014. Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. Food Chem. 153, 207–215.

Calhoon, G.G., Tye, K.M., 2015. Resolving the neural circuits of anxiety. Nat. Neurosci. 18 (10), 1394–1404.

Camacho, A., Mejia, D., 2017. The health consequences of aerial spraying illicit crops: the case of Colombia. J. Health Econ. 54, 147–160.

Cattani, D., de Liz Oliveira Cavalli, V.L., Heinz Rieg, C.E., Domingues, J.T., Dal-Cim, T., Tasca, C.I., Mena Barreto Silva, F.R., Zamoner, A., 2014. Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. Toxicology 320, 34–45.

Cattani, D., Cesconetto, P.A., Tavares, M.K., Parisotto, E.B., De Oliveira, P.A., Rieg, C.E.H., Leite, M.C., Prediger, R.D.S., Wendt, N.C., Razzera, G., Filho, D.W., Zamoner, A., 2017. Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult offspring: implication of glutamate excitotoxicity and oxidative stress. Toxicology 387, 67–80.

Choleris, E., Thomas, A.W., Kavaliers, M., Prato, F.S., 2001. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci. Biobehav. Rev. 25 (3), 235–260.

Cortese, B.M., Phan, K.L., 2005. The role of glutamate in anxiety and related disorders. CNS Spectr. 10 (10), 820–830.

Crispim Junior, C.F., Pederiva, C.N., Bose, R.C., Garcia, V.A., Lino-de-Oliveira, C., Marino-Neto, J., 2012. ETHOWATCHER: validation of a tool for behavioral and video-tracking analysis in laboratory animals. Comput. Biol. Med. 42 (2), 257–264.

Daruich, J., Zirulnik, F., Gimenez, M.S., 2001. Effect of the herbicide glyphosate on enzymatic activity in pregnant rats and their fetuses. Environ. Res. 85 (3), 226–231.

Dhuria, S.V., Hanson, L.R., Frey 2nd, W.H., 2010. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J. Pharm. Sci. 99 (4), 1654–1673.

El-Shenawy, N.S., 2009. Oxidative stress responses of rats exposed to Roundup and its active ingredient glyphosate. Environ. Toxicol. Pharmacol. 28 (3), 379–385.

Ford, B., Bateman, L.A., Gutierrez-Palominos, L., Park, R., Nomura, D.K., 2017. Mapping proteome-wide targets of glyphosate in mice. Cell Chem. Biol. 24 (2), 133–140.

Gallegos, C.E., Bartos, M., Bras, C., Gumilar, F., Antonelli, M.C., Minetti, A., 2016. Exposure to a glyphosate-based herbicide during pregnancy and lactation induces neurobehavioral alterations in rat offspring. Neurotoxicology 53, 20–28.

Gallo, E.F., Salling, M.C., Feng, B., Moron, J.A., Harrison, N.L., Javitch, J.A., Kellendonk, C., 2015. Upregulation of dopamine D2 receptors in the nucleus accumbens indirect pathway increases locomotion but does not reduce alcohol consumption. Neuropsychopharmacology 40 (7), 1609–1618.

Garber, J.C., Barbee, R.W., Bielitzki, J.T., Clayton, L.A., Donovan, J.C., Hendriksen, C.F.M., Kohn, D.F., Lipman, N.S., Locke, P.A., Melcher, J., Quimby, F.W., Turner, P.V., Wood, G.A., Würbel, H., 2011. Guide for the Care and Use of Laboratory Animals, 8th ed. The National Academies Press, Washington, DC.

GRAIN, 2009. Twelve years of GM soya in Argentina — a disaster for people and the environment. In: Seedling, pp. 16–19.

Gress, S., Laurant, C., Defarge, N., Travert, C., Seralini, G.E., 2016. Dig1 protects against locomotor and biochemical dysfunctions provoked by Roundup. BMC Complement. Altern. Med. 16, 234.

Hernandez-Plata, I., Giordano, M., Diaz-Munoz, M., Rodriguez, V.M., 2015. The herbicide glyphosate causes behavioral changes and alterations in dopaminergic markers in male Sprague-Dawley rat. Neurotoxicology 46, 79–91.

Hubler, N., Gottschling, B., Jacobs, M., von Landenberg, F., Hewicker-Trautwein, M., 2005. Functional observational battery and motor activity in rats after single administration of two NHE 1 inhibitors. Toxicol. Appl. Pharmacol. 208 (3), 266–276.

Kamel, F., Hoppin, J.A., 2004. Association of pesticide exposure with neurologic dysfunction and disease. Environ. Health Perspect. 112 (9), 950–958.

Kim, H.G., Kim, T.M., Park, G., Lee, T.H., Oh, M.S., 2013. Repeated heat exposure impairs nigrostriatal dopaminergic neurons in mice. Biol. Pharm. Bull. 36 (10), 1556–1561.

Kubota, T., Matsumoto, H., Kirino, Y., 2016. Ameliorative effect of membrane-associated estrogen receptor G protein coupled receptor 30 activation on object recognition memory in mouse models of Alzheimer's disease. J. Pharmacol. Sci. 131 (3), 219–222.

Kuhar, M.J., Couceyro, P.R., Lambert, P.D., 1999. Catecholamines. In: Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., Uhler, M.D. (Eds.), Basic Neurochemistry: Molecular, Cellular, and Medical Aspects. Lippincott,Williams & Wilkins, Philadelphia.

Kumar, S., Khodoun, M., Kettleson, E.M., McKnight, C., Reponen, T., Grinshpun, S.A., Adhikari, A., 2014. Glyphosate-rich air samples induce IL-33, TSLP and generate IL-13 dependent airway inflammation. Toxicology 325, 42–51.

Larsen, K.E., Lifschitz, A.L., Lanusse, C.E., Virkel, G.L., 2016. The herbicide glyphosate is a weak inhibitor of acetylcholinesterase in rats. Environ. Toxicol. Pharmacol. 45, 41–44.

Le Couteur, D.G., McLean, A.J., Taylor, M.C., Woodham, B.L., Board, P.G., 1999. Pesticides and Parkinson's disease. Biomed Pharmacother 53 (3), 122–130.

Leger, M., Quiedeville, A., Bouet, V., Haelewyn, B., Boulouard, M., Schumann-Bard, P., Freret, T., 2013. Object recognition test in mice. Nat. Protoc. 8 (12), 2531–2537.

Litteljohn, D., Mangano, E.N., Hayley, S., 2008. Cyclooxygenase-2 deficiency modifies the neurochemical effects, motor impairment and co-morbid anxiety provoked by paraquat administration in mice. Eur. J. Neurosci. 28 (4), 707–716.

Liu, D., Xiao, B., Han, F., Luo, F., Wang, E., Shi, Y., 2013. Changes in 5-HT1A receptor expression in the oculomotor nucleus in a rat model of post-traumatic stress disorder. J. Mol. Neurosci. 49 (2), 360–368.

Martin, E.I., Ressler, K.J., Binder, E., Nemeroff, C.B., 2009. The neurobiology of anxiety disorders: brain imaging, genetics, and psychoneuroendocrinology. Psychiatr. Clin. North Am. 32 (3), 549–575.

Menkes, D.B., Temple, W.A., Edwards, I.R., 1991. Intentional self-poisoning with glyphosate-containing herbicides. Hum. Exp. Toxicol. 10 (2), 103–107.

Mesnage, R., Bernay, B., Seralini, G.E., 2013. Ethoxylated adjuvants of glyphosate-based herbicides are active principles of human cell toxicity. Toxicology 313 (2–3), 122–128.

Mesnage, R., Defarge, N., Spiroux de Vendomois, J., Seralini, G.E., 2014. Major pesticides are more toxic to human cells than their declared active principles. Biomed. Res. Int. 2014, 179691.

Mittal, D., Ali, A., Md, S., Baboota, S., Sahni, J.K., Ali, J., 2014. Insights into direct nose to brain delivery: current status and future perspective. Drug Deliv. 21 (2), 75–86.

Nishiyori, Y., Nishida, M., Shioda, K., Suda, S., Kato, S., 2014. Unilateral hippocampal infarction associated with an attempted suicide: a case report. J. Med. Case Rep. 8, 219.

Olivier, J.D., Vinkers, C.H., Olivier, B., 2013. The role of the serotonergic and GABA system in translational approaches in drug discovery for anxiety disorders. Front. Pharmacol. 4, 74.

Patel, T.P., Gullotti, D.M., Hernandez, P., O'Brien, W.T., Capehart, B.P., Morrison 3rd, B., Bass, C., Eberwine, J.E., Abel, T., Meaney, D.F., 2014. An open-source toolbox for automated phenotyping of mice in behavioral tasks. Front. Behav. Neurosci. 8, 349.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14 (3), 149–167.

Peruzzo, P.J., Porta, A.A., Ronco, A.E., 2008. Levels of glyphosate in surface waters, sediments and soils associated with direct sowing soybean cultivation in north pampasic region of Argentina. Environ. Pollut. 156 (1), 61–66.

Picciotto, M.R., Lewis, A.S., van Schalkwyk, G.I., Mineur, Y.S., 2015. Mood and anxiety regulation by nicotinic acetylcholine receptors: a potential pathway to modulate aggression and related behavioral states. Neuropharmacology 96(Pt B), 235–243.

Prediger, R.D., Aguiar, A.S., Jr., Rojas-Mayorquin, A.E., Figueiredo, C.P., Matheus, F.C., Ginestet, L., Chevarin, C., Bel, E.D., Mongeau, R., Hamon, M., Lanfumey, L., Raisman-Vozari, R., 2010. Single intranasal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in C57BL/6 mice models early preclinical phase of Parkinson's disease. Neurotox. Res. 17(2), 114–129.

Prediger, R.D., Aguiar Jr., A.S., Matheus, F.C., Walz, R., Antoury, L., Raisman-Vozari, R., Doty, R.L., 2012. Intranasal administration of neurotoxicants in animals: support for the olfactory vector hypothesis of Parkinson's disease. Neurotox. Res. 21 (1), 90–116.

Richard, S., Moslemi, S., Sipahutar, H., Benachour, N., Seralini, G.E., 2005. Differential effects of glyphosate and roundup on human placental cells and aromatase. Environ. Health Perspect. 113 (6), 716–720.
Richardson, J.R., Roy, A., Shalat, S.L., von Stein, R.T., Hossain, M.M., Buckley, B., Gearing, M., Levey, A.I., German, D.C., 2014. Elevated serum pesticide levels and risk for Alzheimer disease. JAMA Neurol. 71 (3), 284–290.
Rojo, A.I., Montero, C., Salazar, M., Close, R.M., Fernandez-Ruiz, J., Sanchez-Gonzalez, M.A., de Sagarra, M.R., Jackson-Lewis, V., Cavada, C., Cuadrado, A., 2006. Persistent penetration of MPTP through the nasal route induces Parkinson's disease in mice. Eur. J. Neurosci. 24 (7), 1874–1884.
Rojo, A.I., Cavada, C., de Sagarra, M.R., Cuadrado, A., 2007. Chronic inhalation of rotenone or paraquat does not induce Parkinson's disease symptoms in mice or rats. Exp. Neurol. 208 (1), 120–126.
Ronco, A.E., Marino, D.J., Abelando, M., Almada, P., Apartin, C.D., 2016. Water quality of the main tributaries of the Parana Basin: glyphosate and AMPA in surface water and bottom sediments. Environ. Monit. Assess. 188 (8), 458.
Sanchis, J., Kantiani, L., Llorca, M., Rubio, F., Ginebreda, A., Fraile, J., Garrido, T., Farre, M., 2012. Determination of glyphosate in groundwater samples using an ultrasensitive immunoassay and confirmation by on-line solid-phase extraction followed by liquid chromatography coupled to tandem mass spectrometry. Anal. Bioanal. Chem. 402 (7), 2335–2345.
Sato, C., Kamijo, Y., Yoshimura, K., Ide, T., 2011. Aseptic meningitis in association with glyphosate-surfactant herbicide poisoning. Clin. Toxicol. (Phila.) 49 (2), 118–120.
Shibata, M., Yamasaki, N., Miyakawa, T., Kalaria, R.N., Fujita, Y., Ohtani, R., Ihara, M., Takahashi, R., Tomimoto, H., 2007. Selective impairment of working memory in a mouse model of chronic cerebral hypoperfusion. Stroke 38 (10), 2826–2832.
Simon, P., Dupuis, R., Costentin, J., 1994. Thigmotaxis as an index of anxiety in mice. Influence of dopaminergic transmissions. Behav. Brain Res. 61 (1), 59–64.
Solomon, K.R., 2016. Glyphosate in the general population and in applicators: a critical review of studies on exposures. Crit. Rev. Toxicol. 46 (sup1), 21–27.
Tanner, C.M., Kamel, F., Ross, G.W., Hoppin, J.A., Goldman, S.M., Korell, M., Marras, C., Bhudhikanok, G.S., Kasten, M., Chade, A.R., Comyns, K., Richards, M.B., Meng, C., Priestley, B., Fernandez, H.H., Cambi, F., Umbach, D.M., Blair, A., Sandler, D.P., Langston, J.W., 2011. Rotenone, paraquat, and Parkinson's disease. Environ. Health Perspect. 119 (6), 866–872.
Teubal, M., 2009. Expansión del modelo sojero en la Argentina. De la producción de alimentos a los commodities. In: Lizarraga, P., Vacaflores, C. (Eds.), La persistencia del campesinado en América Latina Comunidad de Estudios JAINA Tarija, Bolivia, pp. 161–197.
Teubal, M., Domínguez, D., Sabatino, P., 2005. Transformaciones agrarias en la Argentina. Agricultura industrial y sistema agroalimentario. In: Giarracca, N., Teubal, M. (Eds.), El campo argentino en la encrucijada. Estrategias y resistencias sociales, Ecos en la ciudad. Alianza Editorial, Buenos Aires, pp. 37–78.
Trigo, E.J., 2011. 15 Years of Genetically Modified Crops in Argentine Agriculture. Consejo Argentino para la Información y el Desarrollo de la Biotecnología (ArgenBio Report).
Tristao, F.S., Amar, M., Latrous, I., Del-Bel, E.A., Prediger, R.D., Raisman-Vozari, R., 2014. Evaluation of nigrostriatal neurodegeneration and neuroinflammation following repeated intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in mice, an experimental model of Parkinson's disease. Neurotox. Res. 25 (1), 24–32.
Van Stempvoort, D.R., Roy, J.W., Brown, S.J., Bickerton, G., 2014. Residues of the herbicide glyphosate in riparian groundwater in urban catchments. Chemosphere 95, 455–463.
Wahlsten, D., 2010. Mouse Behavioral Testing: How to Use Mice in Behavioral Neuroscience. Elsevier Science.
Walsh, R.N., Cummins, R.A., 1976. The Open-Field Test: a critical review. Psychol. Bull. 83 (3), 482–504.
Wang, G., Fan, X.N., Tan, Y.Y., Cheng, Q., Chen, S.D., 2011. Parkinsonism after chronic occupational exposure to glyphosate. Parkinsonism Relat. Disord. 17 (6), 486–487.
Warburton, E.C., Brown, M.W., 2015. Neural circuitry for rat recognition memory. Behav. Brain Res. 285, 131–139.
Wu, H., Hu, K., Jiang, X., 2008. From nose to brain: understanding transport capacity and transport rate of drugs. Expert Opin. Drug Deliv. 5 (10), 1159–1168.
Xu, J., Li, G., Wang, Z., Si, L., He, S., Cai, J., Huang, J., Donovan, M.D., 2016. The role of L-type amino acid transporters in the uptake of glyphosate across mammalian epithelial tissues. Chemosphere 145, 487–494.