

ORIGINAL ARTICLE

Contribution of hippocampal area CA1 to acetone cyanohydrin-induced loss of motor coordination in rats[☆]



E. Rivadeneyra-Domínguez^{a,*}, A. Vázquez-Luna^{a,b}, R. Díaz-Sobac^{a,b},
E.E. Briones-Céspedes^a, J.F. Rodríguez-Landa^{a,c}

^a Facultad de Química Farmacéutica Biológica, Universidad Veracruzana, Xalapa, Veracruz, Mexico

^b Instituto de Ciencias Básicas, Universidad Veracruzana, Xalapa, Veracruz, Mexico

^c Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, Mexico

Received 20 August 2015; accepted 9 November 2015

Available online 30 March 2017

KEYWORDS

Cassava;
Acetone cyanohydrin;
Neuropathy;
Rotational behaviour;
Motor impairment

Abstract

Introduction: Some vegetable foodstuffs contain toxic compounds that, when consumed, favour the development of certain diseases. Cassava (*Manihot esculenta* Crantz) is an important food source, but it contains cyanogenic glucosides (linamarin and lotaustralin) that have been associated with the development of tropical ataxic neuropathy and konzo. In rats, intraperitoneal administration of acetone cyanohydrin (a metabolite of linamarin) produces neurological disorders and neuronal damage in the hippocampus. However, it is unknown whether hippocampal area CA1 plays a role in neurological disorders associated with acetone cyanohydrin.

Method: A total of 32 male Wistar rats 3 months old were assigned to 4 groups ($n = 8$ per group) as follows: vehicle (1 μ l physiological saline), and 3 groups with acetone cyanohydrin (1 μ l of 10, 15, and 20 mM solution, respectively). The substances were microinjected intrahippocampally every 24 hours for 7 consecutive days, and their effects on locomotor activity, rota-rod and swim tests were assessed daily. On the fifth day post-treatment, rats underwent further assessment with behavioural tests to identify or rule out permanent damage induced by acetone cyanohydrin.

Results: Microinjection of acetone cyanohydrin 20 mM resulted in hyperactivity, motor impairment, and reduced exploration from the third day of treatment. All concentrations of acetone cyanohydrin produced rotational behaviour in the swim test from the first day of microinjection.

[☆] Please cite this article as: Rivadeneyra-Domínguez E, Vázquez-Luna A, Díaz-Sobac R, Briones-Céspedes EE, Rodríguez-Landa JF. Participación del área CA1 del hipocampo en la incoordinación motora inducida por acetocianohidrina en la rata. Neurología. 2017;32:230–235.

* Corresponding author.

E-mail address: edrivadeneyra@uv.mx (E. Rivadeneyra-Domínguez).

PALABRAS CLAVE

Yuca;
Acetonacianohidrina;
Neuropatía;
Conducta de giro;
Incoordinación motriz

Conclusion: The hippocampal area CA1 is involved in motor alterations induced by microinjection of acetone cyanohydrin, as has been reported for other cassava compounds.

© 2015 Sociedad Española de Neurología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Participación del área CA1 del hipocampo en la incoordinación motora inducida por acetonacianohidrina en la rata

Resumen

Introducción: Algunos vegetales destinados a la alimentación contienen compuestos tóxicos que, al ser consumidos, predisponen al desarrollo de algunas enfermedades. La yuca (*Manihot esculenta* Crantz) es una fuente importante de alimento, pero contiene glucósidos cianogénicos (linamarina y lotaustralina) que han sido asociados con el desarrollo de la neuropatía atáxica tropical y el konzo. En la rata, la administración intraperitoneal de acetonacianohidrina (un metabolito de la linamarina) produce alteraciones neurológicas y daño neuronal en el hipocampo. No obstante, se desconoce si el área CA1 del hipocampo participa en las alteraciones neurológicas asociadas a la acetonacianohidrina.

Método: Treinta y dos ratas macho Wistar de 3 meses de edad fueron destinadas a 4 grupos (n = 8 cada grupo): vehículo (1 de solución salina fisiológica) y 3 grupos con acetonacianohidrina (1 de solución 10, 15 y 20 mM). Las sustancias fueron microinyectadas intrahipocampalmente durante 7 días consecutivos (cada 24 h); los efectos fueron evaluados diariamente en las pruebas de actividad locomotora, rota-rod y nado. Al quinto día postratamiento se evaluaron nuevamente en las pruebas conductuales para identificar o descartar la permanencia del daño inducido por la acetonacianohidrina.

Resultados: La microinyección de acetonacianohidrina 20 mM produjo hiperactividad, incoordinación motora y reducción de la exploración a partir del tercer día del tratamiento. En la prueba de nado, todas las concentraciones de acetonacianohidrina produjeron la conducta de giro desde el primer día de microinyección.

Conclusión: El área CA1 del hipocampo participa en las alteraciones motoras inducidas por la microinyección de acetonacianohidrina, como ha sido reportado para otros compuestos de la yuca.

© 2015 Sociedad Española de Neurología. Publicado por Elsevier España, S.L.U. Este es un artículo Open Access bajo la licencia CC BY-NC-ND (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Various plant foods regarded as daily staples in some parts of the world contain compounds that can have a toxic effect under certain conditions. Under normal conditions, these foodstuffs are processed properly and are safe to consume; however, when they have not been prepared correctly, the toxic compounds that remain may cause illness, including different central nervous system diseases.¹ One of these foodstuffs is manioc root or cassava (*Manihot esculenta* Crantz), which is a good source of energy. The root and the above-ground parts of the cassava plant contain cyanogenic compounds (linamarin and lotaustralin); hydrolysis of these compounds via the linamarase enzyme produces glucose and acetone cyanohydrin, which in turn decomposes into acetone and hydrogen cyanide, a neurotoxin.²

Earlier studies reported that rats treated with juice from cassava root (equivalent to 0.30 mg/2 mL of linamarin)

developed hyperactivity and loss of motor coordination,³ as well as a reduction in the number of neurons in hippocampal area CA1.⁴ These studies point to the role of the hippocampus in the motor impairment caused by cassava consumption, and probably also in the aetiology of such neurological diseases as konzo and tropical ataxic neuropathy among cassava consumers.⁴ Linamarin microinjections to the hippocampus (CA1) elicit hyperactivity and loss of motor coordination in rats, manifesting as higher numbers of crossed squares in the open field test, shorter latency to fall on the rotarod test, and spinning behaviour on the swimming test.⁵ Aside from these findings, we do not yet know whether other compounds in cassava, such as acetone cyanohydrin, may also impair coordination and motor activity when delivered to the hippocampus by microinjection. We attempt to answer this question by studying how intrahippocampal microinjections of acetone cyanohydrin affect behaviour in Wistar rats, evaluated using locomotor function, rotarod, and swimming tests.

Methods

Subjects

We used 32 three-month-old male Wistar rats weighing 250 to 300 g at the beginning of the study. Rats were housed in transparent acrylic cages in a vivarium kept at room temperature ($25 \pm 2^\circ\text{C}$) with a 12:12 light-dark cycle (lights on at 7:00 am). Rats had ad libitum access to water and food. Rats were handled in accordance with the international ethical standards listed in the Guide for care and use of laboratory animals⁶ and official Mexican guidelines for the breeding, care, and use of laboratory animals.⁷

Stereotactic surgery

As described in previous studies, animals were deeply anaesthetised during the unilateral implantation of a guide cannula.⁸ Researchers used a stereotactic apparatus (Stoelting, Wood Dale, IL, USA) to immobilise the rats' heads before exposing the skull. A dental lab drill (Saeshin Dental Lab 35000 RPM, South Korea) was used to trepan the skull and implant a cannula in the dorsal hippocampal area CA1. The stereotactic coordinates from bregma⁹ were AP = -3.8 mm; L = -2 mm; H = -2 mm. A stainless steel guide cannula (8 mm long, 0.7 mm in diameter) was subsequently implanted and secured to the skull with dental acrylic (Arias Distribuidora Dental; Tlalnepantla, Mexico). Four days after implantation of the cannula, microinjections were started.

Experimental groups and treatment

Rats were randomly assigned to 4 groups ($n=8$ rats per group): one group received the vehicle (physiological saline) and the remaining 3 groups received acetone cyanohydrin dosed at different concentrations (10, 15, and 20 mM) that were calculated based on earlier studies.¹⁰ Microinjections of either the vehicle or acetone cyanohydrin were delivered every 24 hours for 7 consecutive days. Five minutes after the microinjection, rats were evaluated using the behavioural tests to determine the effect of treatment; on the fifth day post-treatment, the behavioural tests were repeated without treatment to detect or rule out any permanent damage caused by acetone cyanohydrin.

Microinjection

Researchers used an injection cannula (10 mm long, 0.7 mm in diameter) consisting of a stainless steel needle measuring $0.7\text{ mm} \times 32\text{ mm}$ ($22\text{ G} \times 1\frac{1}{4}''$) attached to a 10 L Hamilton syringe by means of a polyethylene tube. An automatic infusion pump (KD Scientific; Holliston, MA, USA) was used to microinject $1\text{ }\mu\text{L}$ of liquid at a constant rate of $0.1\text{ }\mu\text{L}/\text{min}$ for 10 minutes. During this procedure rats were able to move freely. After microinjection, the injection cannula was left in place for 5 additional minutes to allow diffusion of the injected substance and prevent it from returning by capillarity. We used behavioural testing to evaluate each rat immediately after treatment on the 7 consecutive days of treatment, and on day 5 post-treatment.

Locomotor activity test

Each rat was placed inside an opaque acrylic cage ($44 \times 33 \times 20\text{ cm}$) whose base was divided into squares measuring $11 \times 11\text{ cm}$. We assessed the following: (a) number of crossed squares (the rat was considered to have crossed a square when at least three-fourths of its body passed from one square to another); and (b) number of vertical behaviours (times standing on its hind legs). Crossed squares were an indicator of spontaneous motor activity and vertical behaviour was observed to detect any potential alterations in motor coordination.

Rotarod test

During the 5 days prior to beginning microinjections, rats were trained on a rotarod (LE 8300, LSI Letica, Panlab Scientific Instruments, Barcelona, Spain) at a speed of 18 rpm. After receiving microinjections, rats were placed on the rotarod for daily assessments of latency to fall, that is, the time it takes the rat to fall off the rod. This variable is used to identify any alterations in motor coordination and balance.

Forced swim test

We placed rats in a glass tank (base: $26 \times 29\text{ cm}$; height: 50 cm) filled with water at a temperature of $25 \pm 1^\circ\text{C}$. The water level was such that rats could touch the bottom of the tank with their hind feet and tails. This test was used to evaluate the number of spins, that is, periods during which the rat was moving in a circular direction rather than forward.^{11,12}

We recorded video feed from all sessions of the locomotor activity and swimming tests. Two independent observers quantified open field test variables until reaching a concordance of at least 95%. In the forced swimming test, the variable 'number of spins' was quantified by analysing video feeds with a software tool (ANY-maze 4.73; Stoelting, Wood Dale, IL, USA).

Verification of the microinjection site

After completing the behavioural tests, rats were euthanised with pentobarbital (PiSA Agropecuaria, Guadalajara, Mexico) and transcardially perfused with 100 mL of physiological saline (NaCl 0.9%) followed by 100 mL of formaldehyde 30% (J.T. Baker, Ecatepec, Mexico). The microinjection site was marked with Evans blue. Brains were removed and cut into thick slices to analyse the site of microinjection under a light microscope; Paxinos and Watson's rat brain atlas was used as a reference.⁹ The statistical analysis included data from only those rats in which cannulas were confirmed to have been implanted correctly in hippocampal area CA1.

Statistical analysis

Data were analysed with the 2-way repeated measures ANOVA; the 2 factors were treatment and days of

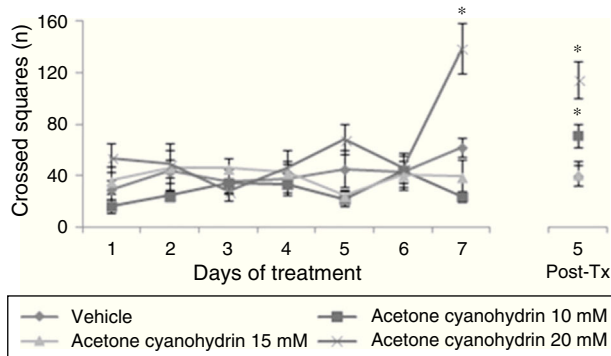


Figure 1 Number of squares crossed in the locomotor activity test. This number was higher in rats treated with 20 mM acetone cyanohydrin on day 7 of treatment compared to results from other groups on the same day.

Post-Tx: 5 days post-treatment.

* $P < .001$ vs all groups on day 7 treatment and day 5 post-treatment.

treatment. Given P -values $\leq .05$, we applied the post hoc Student–Newman–Keuls test. Results are expressed as means $\pm$ standard deviation.

Results

Locomotor activity test

We found significant differences in the number of crossed squares for treatment type ($F_{[3,177]} = 15.906$, $P < .001$), days of treatment ($F_{[7,177]} = 5.878$, $P < .001$), and the interaction between factors ($F_{[21,177]} = 3.578$, $P < .001$). The post hoc test indicated that the group treated with acetone cyanohydrin 20 mM crossed a higher number of squares on day 7 of treatment, and that this difference remained on day 5 post-treatment, with respect to the vehicle and acetone cyanohydrin 10 mM and 15 mM groups measured on the same day (Fig. 1).

Analysis of the number of vertical behaviours did not reveal significant differences linked to treatment type ($F_{[3,177]} = 0.645$, $P = .578$) or to interaction between factors ($F_{[21,177]} = 0.796$, $P = .723$). Nevertheless, the analysis revealed significant differences with respect to the day of treatment ($F_{[7,177]} = 3.437$, $P = .002$). The post hoc test showed that the number of vertical behaviours decreased beginning on day 3 of treatment; the difference was significant with respect to days 1 and 2, and it was still present at 5 days post-treatment (Fig. 2).

Rotarod test

Analysis of time to fall on the rotarod test found significant differences among treatment groups ($F_{[3,192]} = 6.382$, $P < .001$); the post hoc test showed that groups treated with 10 mM, 15 mM, and 20 mM of the substance had shorter times to fall from the rotarod compared to the vehicle group. The group treated with 20 mM displayed the shortest latencies on the rotarod test (Fig. 3). Analyses by day of treatment

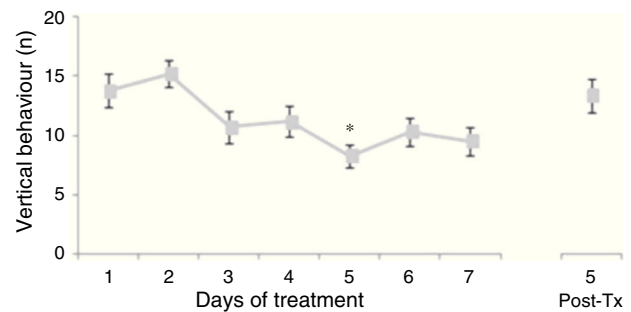


Figure 2 Number of vertical behaviours on locomotor activity test. The number of vertical behaviours decreased significantly beginning on day 3 of treatment, with the lowest number of vertical behaviours observed on day 5 of treatment.

* $P < .002$ vs days 1 and 2 of treatment.

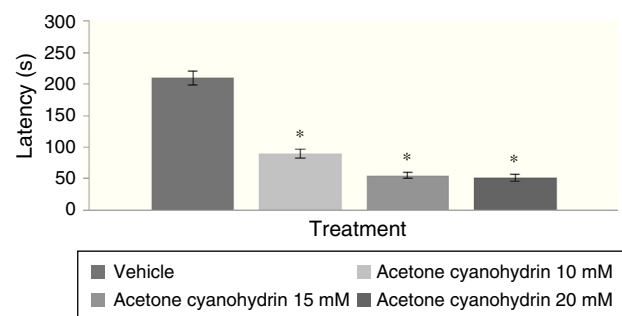


Figure 3 Latency to fall on the rotarod test. Rats treated with acetone cyanohydrin at 10, 15, and 20 mM showed a shorter latency to fall on the rotarod test than did rats treated with vehicle.

* $P < .001$ vs vehicle.

($F_{[7,192]} = 1.307$, $P = .249$) or by interaction between factors ($F_{[21,192]} = 0.342$, $P = .997$) did not reveal significant differences.

Forced swim test

Since the vehicle group did not display spinning behaviour, only the groups treated with acetone cyanohydrin (10, 15, and 20 mM) were included in the statistical analysis.

Spinning behaviour

An analysis of the number of spins per treatment group revealed statistically significant differences ($F_{[3,177]} = 8.840$; $P < .001$). The post hoc test indicated that the group treated with 20 mM acetone cyanohydrin displayed more spins than did the 10 mM and 15 mM treatment groups (Fig. 4). The analysis did not detect any significant differences regarding days of treatment ($F_{[7,177]} = 0.736$; $P = .642$). An analysis of the interaction of factors did not reveal any significant differences ($F_{[21,177]} = 0.560$, $P = .940$).

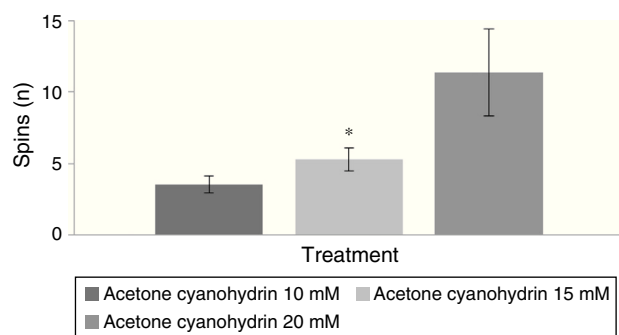


Figure 4 Spinning behaviour in forced swim test. The group treated with 20 mM acetone cyanohydrin displayed the most spinning behaviour during the swimming test.

* $P < .001$ vs acetone cyanohydrin 10 mM.

Discussion

In this study, interhippocampal delivery of acetone cyanohydrin in rats resulted in decreased coordination and motor activity. These findings indicate that the metabolite, like other compounds present in cassava, may form a part of the neurotoxic substrate associated with the development of neuropathies in humans who consume cassava and its derivatives under certain conditions. Locomotor activity testing allows us to identify changes in the rats' motricity (hypoactivity or hyperactivity) and exploratory behaviour, which in turn may point to potential neurological damage (peripheral neuropathies) caused when certain drugs or toxic substances affect the central nervous system.^{13,14} The locomotor activity test employed in our study helps us identify changes in rats' motor function by analysing their exploratory patterns and the coordination of their movements, as other authors have observed.¹⁵ Hippocampal lesions in adult rats elicit behavioural changes related to the dopaminergic system; these include increased locomotor activity in new environments, as well as learning and memory deficiencies.^{16,17} This study reports that the group receiving 20 mM acetone cyanohydrin displayed increased spontaneous motor activity on the open field test. In this case, hyperactivity may have been associated with the neurotoxic effects of acetone cyanohydrin on the hippocampus, which would prevent the animal from forming memories. This was observed with linamarin in an earlier study.⁵ Under natural conditions, a rat undergoing repeated locomotor activity evaluations would demonstrate a reduction in spontaneous motor activity (crossed squares, vertical behaviours, and grooming); this decrease indicates familiarity with the experimental setting.¹⁸ In contrast, an animal subjected to neural damage in the hippocampus and then evaluated repeatedly on the open field test would maintain or even increase the level of motor activity. This behaviour may indicate decreased consolidation of memory resulting in continued exploration of what is viewed as a novel setting.¹⁸ Humans with neuropathies associated with cassava consumption develop motor coordination problems.^{19,20} This study found that treatment with acetone cyanohydrin (10, 15, or 20 mM) promotes loss of motor coordination and balance on the rotarod test, in line with findings for other neurotoxic compounds.²¹

Additionally, intrahippocampal microinjection of acetone cyanohydrin led to the appearance of spinning motions during the forced swim test, but this behaviour decreased as the experiment progressed. It is important to point out that spinning motions have been linked to alterations in limb motor coordination in rats. These changes may be similar to behaviours observed in patients with tropical ataxic neuropathy and konzo, specifically, the development and presence of slow, progressive paralysis of the upper and lower limbs.²² We emphasise that certain compounds present in cassava can cause demyelination of the spinal cord, resulting in ataxia with decreased limb coordination.²⁰ Acetone cyanohydrin may also contribute to the motor impairment associated with long-term consumption of cassava, including the alterations occurring in tropical ataxic neuropathy and konzo.³

Regarding the post-treatment effect, that is, results gathered 5 days after the last intrahippocampal microinjection, rats receiving 20 mM acetone cyanohydrin continued to show motor hyperactivity in the open field test (increased number of crossed squares and vertical behaviours). In this case, the rat's hyperactive exploratory behaviour might be linked to the neurotoxic effect of acetone cyanohydrin microinjections to hippocampal area CA1, and it might indicate a permanent effect on exploratory behaviour. Studies by other teams working with young rodents have shown that damage to pyramidal neurons in hippocampal area CA1 elicits changes in learning and spatial memory,²³ and in locomotor ability and exploration and searching strategies.^{24,25} Other studies indicate that these alterations may correspond to functional and morphological changes in the hippocampus,²⁶ and they link them to decreased neurogenesis in this region.²⁵ This mechanism may explain how acetone cyanohydrin would prevent the consolidation of memory in rats. Furthermore, the rotarod test completed 5 days after the last intrahippocampal microinjection demonstrated that the lesion caused by acetone cyanohydrin (10, 15, and 20 mM) had caused lasting impairment to the rats' coordination and motor equilibrium²⁷; results on day 5 post-treatment indicated a significant decrease in time to fall. Lastly, animals in the 15 mM and 20 mM treatment groups evaluated with the forced swim test on day 5 post-treatment, with no new microinjections, continued to display spinning motions. This behaviour indicates persistent motor deterioration and loss of coordination and balance responding to the neural damage caused by acetone cyanohydrin microinjections to the hippocampus.^{3,4,11} Lastly, although this study did not identify the mechanism underlying the motor impairment observed following acetone cyanohydrin microinjections to area CA1, we are able to offer a plausible explanation. A variety of neurological diseases share pathological features indicative of gradual and selective neuron loss, and many of these diseases are related to hippocampal alterations. By forming part of the limbic system and providing part of the glutamatergic excitatory pathway reaching the nucleus accumbens, the hippocampus has a direct role in motivating the execution of motor responses associated with emotionally arousing events. Based on the above, we can suppose that the sideways and spinning motions occurring in the forced swim test result from overactivation of glutamatergic receptors in the hippocampus subjected to chemical lesion with acetone

cyanohydrin, while recognising that other brain structures may also have been damaged. Loss of neuronal membrane integrity results in loss of glutamate, which in turn contributes to prolonged activation of glutamatergic receptors in neighbouring cells.²⁸ Furthermore, an influx of calcium will also provoke glutamate release by exocytosis, and this process establishes a positive feedback system that gives rise to prolonged neuronal overexcitation, followed by neuronal apoptosis.²⁹ This mechanism provides an explanation for the motor alterations that may be caused by delivery of acetone cyanohydrin, a cassava derivative, to the hippocampus.

Funding

This study was partially supported by the study group for the biology, chemistry, and molecular functionality of vegetable metabolites (UV-GC-368) at Universidad Veracruzana.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

- Adamolekun B. Neurological disorders associated with cassava diet: a review of putative etiological mechanisms. *Metab Brain Dis.* 2011;26:79–85.
- Ceballos H, de la Cruz GA. Taxonomía y morfología de la yuca. In: Opsina B, Ceballos H, compiladores. *La yuca en el tercer milenio. Sistemas modernos de producción, procesamiento, utilización y comercialización.* Cali: CIAT; 2002. p. 17–33.
- Rivadeneira-Domínguez E, Vázquez-Luna A, Rodríguez-Landa JF, Díaz-Sobac R. Neurotoxic effect of linamarin in rats associated with cassava (*Manihot esculenta* Crantz) consumption. *Food Chem Toxicol.* 2013;59:230–5.
- Rivadeneira-Domínguez E, Vázquez-Luna A, Rodríguez-Landa JF, Díaz-Sobac R. A standardized extract of *Ginkgo biloba* prevents locomotion impairment induced by cassava juice in Wistar rats. *Front Pharmacol.* 2014;5:213.
- Rivadeneira-Domínguez E, Rodríguez-Landa JF. Alteraciones motoras inducidas por la microinyección de linamarina en el hipocampo dorsal de la rata Wistar. *Neurología.* 2014;1–7.
- National Research Council Guide for the Care and Use of Laboratory Animals. A report of the Institute of Laboratory Animal Resource Committee on the care and Use of Laboratory Animals. NIH Publication no. 85-23. Washington, D.C.: U.S. Department of Health and Human Services; 1996.
- Norma Oficial Mexicana NOM-062-ZOO-1999. Especificaciones técnicas para la producción, cuidado y uso de animales de laboratorio. México D.F.: Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación; 1999.
- Rodríguez-Landa JF, Contreras CM, García-Ríos RI. Allopregnanolone microinjected into the lateral septum or dorsal hippocampus reduces immobility in the forced swim test: participation of the GABAA receptor. *Behav Pharmacol.* 2009;20:614–22.
- Paxinos G, Watson C. *The rat brain in stereotaxic coordinates.* San Diego: Academic Press; 1998.
- Soler-Martin C, Riera J, Seoane A, Cutillas B, Ambrosio S, Boadas-Vaello P, et al. The targets of acetone cyanohydrin neurotoxicity in the rat not the ones expected in an animal model Konzo. *Neurotoxicol Teratol.* 2010;2:289–94.
- Rivadeneira-Domínguez E, Saavedra M, Rodríguez-Landa JF. El tratamiento con progesterona previene las alteraciones motoras inducidas por la intoxicación con semillas de cicada (*Dioon spinulosum*) en la rata macho. *Rev Toxicol.* 2009;26:36–40.
- Saavedra M, Rivadeneira-Domínguez E, Rodríguez-Landa JF. Alteraciones motoras inducidas por la microinyección intrahipocámpal de metilazoximetanol en ratas macho forzadas a nadar. *Arch Neurociencia (Méx).* 2011;16:186–92.
- Brudzynski SM, Krol S. Analysis of locomotor activity in the rat: parallelism index, a new measure of locomotor exploratory pattern. *Physiol Behav.* 1997;62:635–42.
- Díaz-Véliz G, Mora S. Uso de modelos animales en el estudio de plantas medicinales con propiedades ansiolíticas y antidepresivas. *Rev Farmacol Chile.* 2012;5:21.
- Evangelista de Duffard AM. Neurotoxicidad y comportamiento del sistema nervioso. *Ciencia Hoy.* 1997;7:1–8.
- Flores G, Barbeau D, Quirion R, Srivastava LK. Decreased binding of dopamine D3 receptors in limbic subregions after neonatal bilateral lesion of rat hippocampus. *J Neurosci.* 1996;16:2020–6.
- Silva-Gomez AB, Rojas D, Juárez I, Flores G. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Res.* 2003;983:128–36.
- Clelland CD, Choi M, Romberg C, Clemenson GD, Fragniere A, Tyers P, et al. A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science.* 2009;323:210–3.
- Llorens J. Enfermedades neurológicas asociadas al consumo de variedades de mandioca con alto contenido en gluconitrilos. *Endocrinol Nutr.* 2004;51:418–25.
- Ernesto M, Cardoso AP, Nicala D, Mirione E, Massaza F, Cliff J, et al. Persistent konzo and cyanogens toxicity from cassava in northern Mozambique. *Acta Trop.* 2002;82:357–62.
- Carter RJ, Morton J, Dunnet SB. Motor coordination and balance in rodents. *Curr Protoc Neurosci.* 2001;8:8–12.
- Hirono A, Malamud N, Kurkland LT. Parkinsonism-dementia complex, an endemic disease on the island of Guam II. Pathological features. *Brain.* 1961;84:662–79.
- Block F. Global ischemia and behavioural deficits. *Prog Neurobiol.* 1999;58:279–95.
- D'Hooze R, de Deyn PP. Applications of the Morris water maze in the study of learning and memory. *Brain Res Brain Res Rev.* 2001;36:60–90.
- Gil-Mohapel J, Brocardo PS, Choquette W, Gothard R, Simpson JM, Christie BR. Hippocampal neurogenesis levels predict WATERMAZE search strategies in the aging brain. *PLoS One.* 2013;8:e75125.
- Driscoll I, Howard SR, Stone JC, Monfils MH, Tomanek B, Brooks WM, et al. The aging hippocampus: a multi-level analysis in the rat. *Neuroscience.* 2006;139:1173–85.
- Crawley JN. *What's wrong with my mouse? Behavioral phenotyping of transgenic and knockout mice.* 2nd ed. New Jersey: John Wiley and Sons, Inc; 2007. p. 69–71.
- Schwartz R, Foster AC, French ED, Whetsell C. Excitotoxic models for neurodegenerative disorders. *Life Sci.* 1984;35:19–32.
- Shaw PJ. Excitatory amino acid receptors excitotoxicity, and the human nervous system. *Curr Opin Neurol Neurosurg.* 1993;6:414–22.