A Protocol to Perform Systemic Lipopolysaccharide (LPS) Challenge in Rats

Protocolo para realizar reto sistémico con lipopolisacárido (LPS) en ratas

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ABSTRACT

Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria. In animals, intraperitoneal administration of LPS, stimulates innate immunity and the production of pro-inflammatory cytokines. LPS provides an inflammatory stimulus that activates the neuroimmune and neuroendocrine systems resulting in a set of responses termed sickness behavior. The purpose of this protocol is to describe step-by-step the preparation and procedure of application of intraperitoneal injection of LPS in rats, as a guide for those researchers that want to use this assay to mount an inflammatory response. LPS intraperitoneal challenge in rats has been widely used to evaluate anti-inflammatory reagents and to address basic scientific questions.

KEYWORDS

Lipopolysaccharide; LPS challenge; LPS stimulation; Endotoxin; Inflammation.

RESUMEN

El lipopolisacárido (LPS) es un componente de la membrana externa de las bacterias Gram negativas. En animales, la administración intraperitoneal de LPS estimula la inmunidad innata y la producción de citoquinas proinflamatorias. El LPS proporciona un estímulo inflamatorio que activa el sistema neuroinmunológico y el sistema neuroendocrino, lo que da como resultado un conjunto de respuestas denominadas conductas de enfermedad. El propósito de este protocolo es describir paso a paso la preparación y el procedimiento de aplicación de la inyección intraperitoneal de LPS en ratas, como una guía para aquellos investigadores que desean utilizar este método para estimular una respuesta inflamatoria en el animal. La estimulación con LPS en ratas, aplicada intraperitonealmente, se ha utilizado ampliamente para evaluar reactivos antiinflamatorios y para abordar preguntas básicas de investigación científica.

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PALABRAS CLAVE

Lipopolisacárido; Reto con lipopolisacárido; Estimulación con lipopolisacárido; Endotoxina; Inflamación.

INTRODUCTION

Lipopolysaccharide (LPS) is a component of the outer membrane of Gram-negative bacteria and its major virulence factor (Figure 1 A, B). It is a potent endotoxin that provides a persistent inflammatory stimulus (1). Systemic administration of LPS, even at low doses, induces the production of pro-inflammatory cytokines that activate the neuroimmune and neuroendocrine systems, resulting in sickness behavior (2). Specifically in rats, the acute administration of LPS induces symptoms of depression such as anhedonia, diminished cognitive function, retarded motor activity, reduction in exploration, decreased social interaction, fever, hypersomnia, activation of the hypothalamic-pituitary-adrenal axis, and increased sympathetic activation (3-6).

It has been shown that acute administration of LPS, disrupts the consolidation of memory processes. For example, if it is administered acutely, prior to training, it impairs cue-fear conditioning, whereas chronic administration of LPS, has been found to impair spatial memory and promote memory and learning deficits (7-9). A single intraperitoneal injection of LPS in a dose of 100 micrograms per kilogram in adult male Wistar rats, impaired memory object recognition (10). Shaw *et al.*, 2005, reported that a single intraperitoneal injection of LPS in a dose of 250 micrograms per kilogram impaired hippocampal dependent spatial learning in the Morris water maze behavioral test. In another study, in adult male Wistar rats, a single injection of LPS in a dose of 1mg/kg, impaired cognitive performance in the Barnes Maze test and in the inhibitory avoidance test (12).

Peripheral administration of LPS, emulates a systemic infection, therefore this immune challenge has been widely used to increase neuroinflammatory signaling. In addition, LPS is used as a tool to activate microglia and cells of the peripheral immune system (13). It has been shown that peripheral administration of LPS induces the activation of microglia throughout the brain (14). This activation manifests itself in a matter of hours, observing a peak from 8 to 24 hours, depending on the concentration applied of the endotoxin (14).

LPS binds CD14 on microglia membranes forming the complex LPS-CD14 which then interacts with the Toll-like-receptor - (TLR-) 4 (15,16) (Figure 1 C). TLR-4 activates microglia by initiating signal transduction cascades to produce an early synthesis of pro-inflammatory cytokines in the brain, such as interleukin (IL) -1ß, IL-6 and tumor necrosis factor alpha (TNF-α), IL-12, IL-17A, IL-18, inducible nitric oxide synthase (17-19); chemokines such as CCL2, CCL5, and CXCL8; complement system proteins; and anti-inflammatory cytokines such as IL-10 and transforming growth factor- ß (TGF-ß) (20-22).

Cytokines are key modulators of the immune response and if pro-inflammatory cytokines are present chronically in the central nervous system, it can cause dyshomeostasis (23). A sustained release of pro-inflammatory mediators by the activation of microglia, enhances oxidative

stress and nitrosative stress that can perpetuate neuroinflammation, and may be the cause of neurodegenerative diseases (24).

LPS has become a very popular substance to investigate the responses of glial cells to neuroinflammatory stimuli, simulating the neuronal environment in different disorders of the central nervous system (25). For example, LPS-induced systemic inflammation has been used worldwide in experimental *in vivo* models of neurodegenerative diseases such as Alzheimer's disease (8, 26, 27), Parkinson disease (28,29), Amyotrophic Lateral Sclerosis (30), and Multiple Sclerosis (31). The LPS-induced animal model for these specific diseases, have been used to study the pathologies' mechanisms as well as to test therapeutic agents.

Challenge of LPS in rats can also be used as a model to study the pathophysiology of depression, since it has long been hypothesized that clinical depression may be a consequence of a dysregulated immune system with an increased production of pro-inflammatory cytokines, such as IL-1 ß, IL-6. And TNF-α (32-35). LPS administration to healthy study participants, caused an increase of these pro-inflammatory cytokines in circulation, accompanied by an elevation in serum cortisol levels and increased negative emotions such as sadness, anxiety, and depressed mood, all of these symptoms of major depression (36-41). In the same way, systemic LPS injection to animals causes effects comparable to those found in humans (42).

Investigations in rodents, using from low to high doses of LPS, induce "depressive-like" behavior and produce an effect on neuroendocrine and inflammatory pathways. For example, after 24 hours post-intraperitoneal injection, LPS (1 µg/kg to 250 µg/kg), significantly reduced body weight, increased corticotrophin hormone (ACTH) (doses ranging from 15 µg/kg to 250 µg/kg) and serum corticosterone levels (doses ranging from 5 µg/kg to 250 µg/kg) (Bison *et al.*, 2009). In addition, LPS raised serum interleukins, specifically IL-1ß and IL-6 (doses ranging from 5 µg/kg 250 µg/kg) and TNF-α (doses ranging from 1 µg/kg 250 µg/kg). Social interaction and preference for saccharin were significantly decreased at all doses of LPS tested (1 to 250 µg/kg) while a robust reduction in home-cage activity was observed starting at 15 µg/kg (42). Accordingly, studies have demonstrated that peripheral administration of LPS depresses social exploration and locomotor activity as well (43-47). The behavioral effects caused by LPS challenge at different doses tested, are associated with the stimulation of the hypothalamic-pituitary-adrenal axis and the immune system. Even at very low doses LPS can stimulate the immune system and cause behavioral alterations in rats. The LPS challenge provides a preclinical model for investigating potential therapeutics for inflammatory associated mood disorders.

Possible interactions between stress and immune-inflammatory pathways in the pathogenesis of depression have been proposed, and the LPS systemic challenge combined with stress paradigms, may address these pathways. For instance, when combined with a low dose of LPS challenge (0.1mg/kg to 0.5 mg/kg), chronic stress in rodents, exacerbate depressive-like behavior (48). The combination of stress with LPS elevated IL-1 ß levels in the brain (48). Repeated challenges of LPS when combined with chronic mild stress can induce as well, increases in plasma corticosterone and TNF-α in rats (49).

Inflammation is a pathogenic pathway to neuropsychiatric disorders, but also in a wide range of diseases including rheumatoid arthritis (50), Chron's disease (CD) (51), and cardiovascular disease (52), among others. The LPS peripheral challenge offers

researchers an experimental model to investigate specific targets of inflammatory pathways and responses under controlled conditions.

Rheumatoid arthritis is a chronic inflammatory autoimmune disease characterized by extensive synovitis resulting in erosions of marginal bone and articular cartilage that may lead to joint destruction (50). A popular experimental model of autoimmune arthritis is achieved by intraperitoneal immunization with type II collagen (CII) emulsified with complete Freund's adjuvant (mineral oil, and heat- killed mycobacteria) and LPS (53). This model results in signs of arthritis seen in humans, including joint inflammation. Histologically, an intense infiltration of inflammatory cells is seen, and anti-CII immunoglobulin G (IgG) and IgG2a antibodies are markedly produced. Also, secretion of cytokines, including interleukins-12 and-1ß, interferon-Y and TNF-α, is observed in rodents that receive this challenge compared to controls (53).

CD is a chronic inflammatory bowel disease characterized by mucosal ulceration and inflammation, and commonly affects the distal small intestine (51). Nonetheless, it can also occur anywhere among the gastrointestinal tract. The main factors that provoke the onset of this immune mediated condition are genetics, gut immune response, the microbiota, and triggering factors. LPS has been detected in the plasma of patients with CD, and an abnormal microflora and/or an increased permeability of the intestinal mucosa have been identified as cofactors causing endotoxemia (54). Phagocytosis and killing exerted by polymorphonuclear cells and monocytes and the T-cell dependent antibacterial activity are decreased in CD patients. This may also explain the origin of LPS in these diseases (54).

Clinical and epidemiological studies have demonstrated a strong link between markers of inflammation and risk of future cardiovascular disease (52). Inflammation in the vasculature may be a response to infectious agents. LPS, as an infective agent, activates the innate immune response cells with a plethoric production of cytotoxic mediators. Cardiac myocytes are cells that also express TLR-4, and are susceptible to direct damage by LPS. It has been reported, low levels of LPS depress cardiac myocyte contractility, weakens the response of the beta-adrenergic system, and induces cell death by apoptosis (55-56).

Since the LPS challenge can also be used to study sepsis in high doses, a dosing regimen is suggested that triggers a mild inflammatory response. Consulting the vast amount of literature is recommended to provide important information regarding the dose to be used depending on the effects that will be measured with the LPS challenge. It is also important to keep in mind the effects produced by LPS immune challenge depend on genetic background (strain) of the animal (58). Also, systemic administration of LPS induces time-dependent behavioral alterations in rodents, which are related to either sickness behavior or depression. Short term-behavioral alterations are related to sickness behavior during the peak secretion of cytokines, and long-term, are related to the pathophysiology linked to depression (59).

The main objective of this protocol is to provide a guide to researchers, and describe step-by step on how to prepare a solution of LPS on saline vehicle, and how to manipulate a rat when placing an intraperitoneal injection. As stated before, the dose of LPS depends on the variables and outcomes that will be measured in each experiment performed. This protocol follows

guidelines for acceptable injection volumes in rats. The procedure must be done by trained persons and approved by an Animal Care Committee.

MATERIALS AND METHODS

EQUIPMENT

- Analytical balance.
- Vortex mixer.
- Magnetic mixer.

MATERIALS

- One vial to store saline solution or an Eppendorf tube. The size will depend on the amount of LPS solution that will be prepared and the quantity of saline solution to be stored.
- A multi-dose amber or opaque vial to store LPS.
- Weighing boat.
- Weighing spoon or spatula.
- Magnetic stirring bar.
- (N) 1 milliliter volume syringes (N stands for the quantity of rats to be injected).
- (N) 23-25 gauge X 1 inch hypodermic needles (N stands for the quantity of rats to be injected).
- Small towel or piece of cloth to cover half of the animal's body at the time of injection (if it is applied by one person).
- Sharps bin to dispose used needles.
- Sterile gauze pads.

CHEMICAL REAGENTS

- Sterile saline solution containing 0.9% sodium chloride (NaCl) in water.
- Ethanol (70%) to disinfect the cap of the vials.

BIOLOGICAL REAGENTS

- Lipopolysaccharide from Escherichia coli 0127: B8 (Sigma, code: L3129).
- Rats of any age according to the aims of the project (Sprague Dawley, Wistar or any other strain).

HUMAN RESOURCES

Table 1. Personnel requirements for the execution of this protocol

Optimal		**Minimum**
Person	**Tasks**	**Person**
A	Weighing of the animals to be injected, preparation of saline solution and LPS solution	A
B	Transport of rats from the vivarium to the laboratory or room where the injection is made and returned to the vivarium	
C	Intraperitoneal injection of the animal	
3	Total people required according to condition: ←Optimal Minimum→	1

PROCEDURE

Important: Before starting the execution of any procedure, read this section and the following one (Considerations) completely, evacuate doubts and make sure you have understood all the necessary information.

LPS PREPARATION

The best thing to do would be to prepare it on the same day that it is going to be injected.

However, under minimal conditions (that is, they are not the most desired, but are acceptable), you can work with LPS from previous days (especially if it was a left over), as long as it has been kept in refrigeration (2-8°C) and shake very well before using it. This is the recommendation given by the commercial provider.

- To prepare a dose of 0.5 milligrams (mg) per kilogram (kg) of weight of the rat and obtain an injection volume of 0.5 milliliters (ml) per kg of weight, calculate the mass of reagent and the

total volume of solution for injection to prepare, taking into account the total weight of the rats (see Table 2). Note in Table 2 that a mass in excess of the LPS is always weighed in order to prepare a surplus of the solution, foreseeing that there may be volume loss in the walls of the syringes and/or needles ("dead volume").

Table 2. Data to calculate the amount of reagents that must be prepared.

Value A	Value B	Value C	Value D
Total mass of animals (kg)	Minimum mass of LPS required (mg)	Mass of LPS to be measured (mg)	Volume of saline solution to dissolve the LPS (mL)
	[0.5 mg/kg * Value A]=	[Value B* 1.75]=	[Value C / 1 mg/mL] =

- Once the amount of LPS to prepare has been calculated, start the mass measurement. Since very small masses will normally be measured, the best measure is the mass difference, using at once the container that will contain the final solution (thus avoiding loss of reagent during transfer from one container to another). To do this, open one of the side gates of the analytical balance and place in the center of the plate the opaque vial in which the LPS will be prepared and close all the gates of the weighing chamber. Use the tare function ("Tare" or "0" button) to return the balance reading to zero with the mass of the vial included.
- Once the reading of the scale indicates 0.0 mg, take the amount of milligrams of LPS to be diluted (Value C, Table 2) in saline solution with the stainless steel spoon spatula and place them in the vial.
- Remove the vial from the weighing chamber of the balance, close the gates, tare the balance again and, once it has returned to 0.0 mg, turn it off.
- To the mass of reagent measured in the vial, add the volume of corresponding saline solution to reach a solution of concentration 1 mg LPS / mL (Value D, Table 2). This is to inject 0.5 mL per kg of the weight of the rat with this dose (0.5 mg LPS / kg). For example, if you calculate that the total weight of the rats to be injected is 2545 grams (g), dilute 2545 mg of LPS in 2545 mL of saline.
- Once the saline solution is added, introduce the agitation tablet and place the vial in the magnetic stirrer for 15 minutes at medium speed to mix the solution well.
- If the LPS solution is not to be used immediately, store the refrigerator at 4 degrees Celsius.
- When it is to be used, remove from the refrigerator and shake on the vortex shaker for 30 seconds before placing the injections (or on a magnetic stirrer for 1 minute, if the magnetic stirring bar has not been removed).
- If it has been more than 24 hours since it was placed in the refrigerator (check storage conditions in Table 3) and it is going to be used to inject the rats, it is advisable to put it back in the magnetic stirrer for 15 minutes since it is normal for the solution to sediment.

INTRAPERITONEAL INJECTION OF LPS

- Disinfect the lid of the multiple dose vial with gauze and 70% ethanol.
- Determine the amount of volume per rat to be injected from the LPS solution.
- Raise the plunger of the syringe to be used to fill the syringe to the amount of LPS solution that will be administered to the rat. In this step it is recommended to place the bevel of the needle up and that the syringe be positioned in such a way that we can read the numbering of the syringe.
- With care and delicacy the animal is removed from its cage and its movement is restricted appropriately, the head of the animal is positioned towards the floor. A small towel or piece of cloth is placed to cover the animal from the head to the middle of its body. It should not be too tight. Rotate the animal and place the head and back

of the body on the arm and elbow on the arm with less force. This arm will help to immobilize the animal. The tail is placed between the thumb and ring fingers of that same hand. With the other hand inject.

- Identify the anatomical points of the rat to inject into the appropriate area of the abdomen.
- Generally, the site to be injected into the animal is the lower right quadrant of the abdomen to avoid damaging the surrounding abdominal organs, the urinary bladder and the cecum which is the first portion of the large intestine. If you are going to inject for several days you can alternate the injection site to the lower left quadrant of the abdomen (Figure 2).
- Insert the needle with the bevel of the needle facing up into the lower quadrant of the abdomen at an angle of 30-40 degrees. Insert the needle until the entire bevel enters the abdominal cavity. In middle-aged and elderly rats it is necessary to insert almost the entire length of the needle since these animals have more fat in that abdominal area than the young.
- You should pull the plunger before placing the injection to ensure negative pressure, if any, proceed with the injection.
- Push the plunger until the entire solution is administered. This can last from one to two seconds.
- Once the solution is administered, place the animal back into the cage.
- Remove the needle from the syringe and discard the syringe in the sharps bin and discard the syringe in the normal trash. If the syringe becomes contaminated with fluids from the rat, it should be discarded in a biohazard waste disposable bag.

CONSIDERATIONS

Table 3. Critical points in the protocol, justification and care to ensure adequate execution.

Critical Points	Why is it a critical point?	Related care
Storage of the LPS solution	Once prepared, the LPS solution can only be stored for a certain time and under certain conditions to ensure its integrity at the time of injection.	To ensure the stability of the LPS solution, use the following storage conditions, depending on the supplier of the reagent (Sigma): • Cooling (2-8 ° C): stability up to 1 month. • Freezing (-20 ° C): stability up to 2 years (store in aliquots to avoid repeated freeze / thaw cycles).
Rat injection	When injecting the LPS solution, care must be taken so that the needle does not move inside the abdomen, to avoid lacerating surrounding organs or blood vessels.	Avoid causing harm to the animal: • Carrying out a good immobilization of it. • Making sure to puncture with the needle at the anatomical site indicated.
	When injecting, if a green solution is aspirated, it may be a sign that the intestine has been penetrated, if a yellow liquid comes out it may indicate that the bladder was punctured, and if blood comes out, it may indicate that a blood vessel was perforated.	Puncture of these sites should be avoided, however, if any of these substances are present, it is better to remove the needle from the animal, evaluate the puncture wound, evaluate if the animal requires some treatment and, if feasible, redo the puncture. If the syringe got contaminated, discard the needle and syringe into the sharps bin and biohazard waste bin, respectively, and use a new syringe and a new needle in the next injection.
After the intraperitoneal injection	Complications may arise that affect the well-being of the animal such as bleeding at the injection site or even more serious ones such as peritonitis, laceration of internal organs, and infection.	The animal should be monitored immediately after the injection, about 10 minutes later and the next day. • If bleeding occurs, place gauze and apply pressure. Once bleeding stops, clean the site with gauze and water. • In case of peritonitis, laceration of internal organs and / or infection, consult a veterinarian to assess if the animal can continue in the experiment.

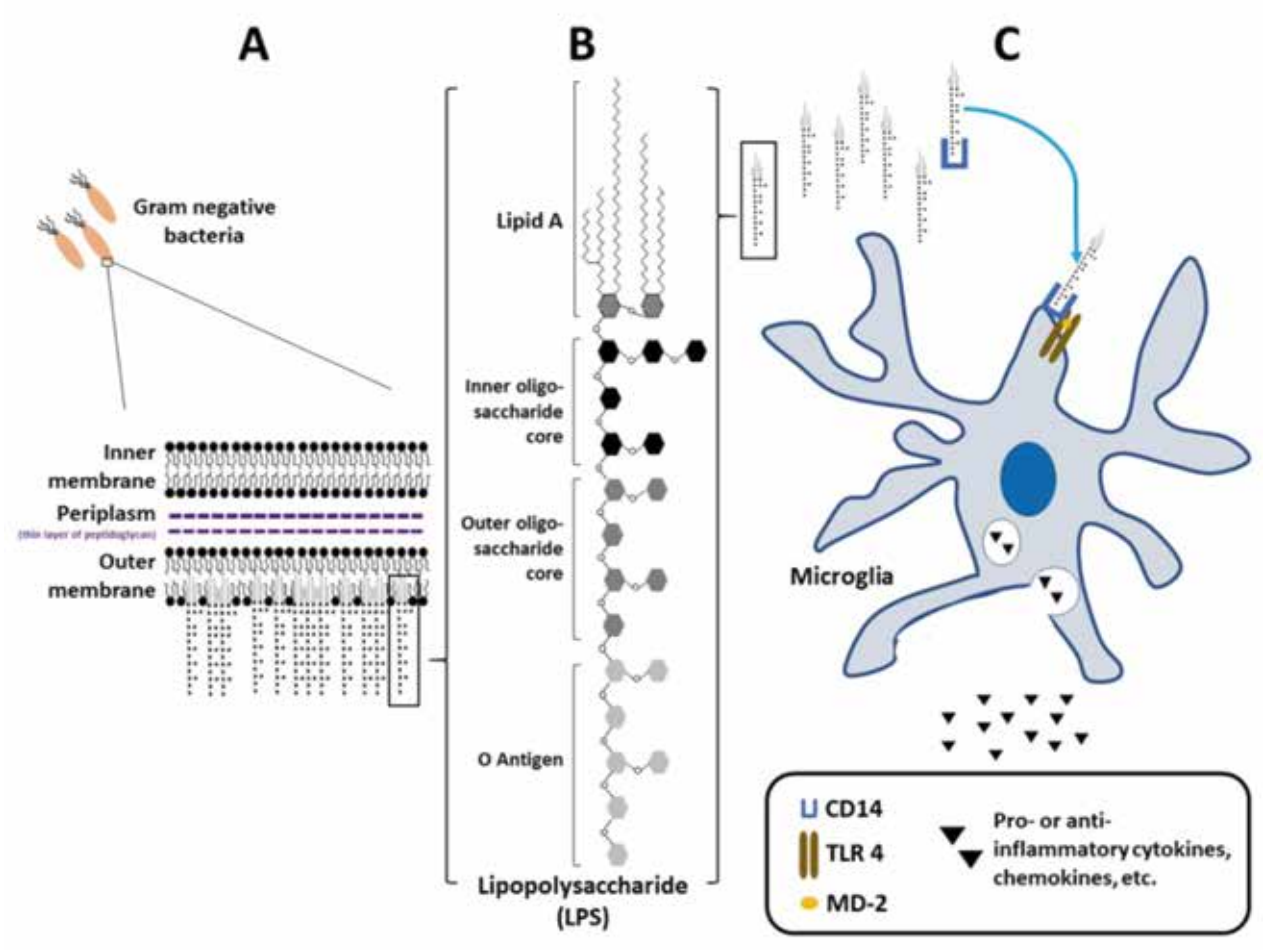

Figure 1. Lipopolysaccharide (LPS) (A) location, (B) molecular structure and (C) effects on immune cell signaling. (A) The gram-negative bacteria cell envelope is composed of a phospholipid inner membrane, followed by a small periplasmic space with little amounts of peptidoglycan cell wall and is topped by an outer membrane which contains the LPS among other structures such as membrane proteins. (B) At the molecular level, LPS can be divided in four main parts: the lipid A, made up by fatty acid chains attached to a glucosamine disaccharide; an inner and an outer oligosaccharide core; and a final section called O antigen, consisting of a repetitive polysaccharide which varies between bacterial species and strains. (C) Upon contact with its corresponding receptor molecules -the CD14-TLR 4-MD-2 system- the LPS elicits a signaling cascade within the immune cell (e.g. microglia), which may culminate in the synthesis and/or release of pro- and anti-inflammatory cytokines, as well as complement proteins and the inducible nitric oxide synthase.

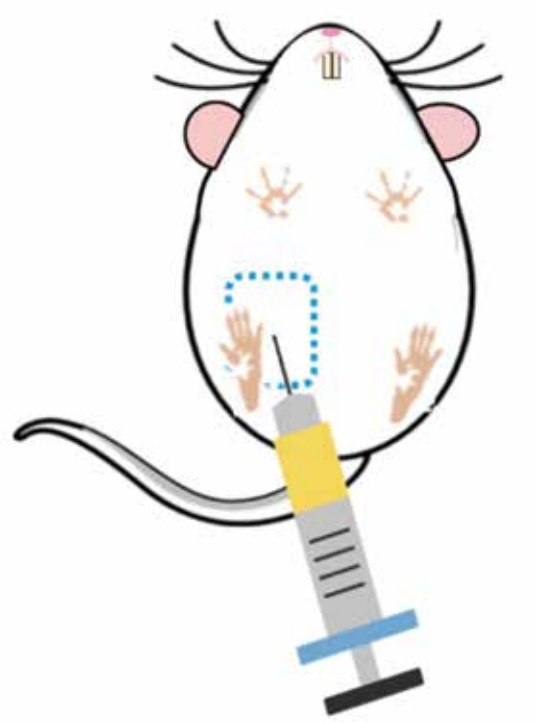

Figure 2. Injection site. For an adequate intraperitoneal injection, the ventral lower right quadrant of the animal is the preferred site. If the animal must be injected several times, injections may be applied alternating between the left and right lower quadrants.

CONCLUSIONS AND OUTLOOK

LPS challenge has been used in animal models for decades and even humans as an aid to elucidate the pathophysiology of immune system-related diseases and affective disorders. It is also a tool to investigate the epigenome that is controlled by social environment and immune system activity (57,58). There are numerous data on LPS applications. All tissues/organs and systems are affected by LPS. However, the dynamics of responses are different; some cell signaling systems (e.g., TLRs) respond within few minutes, while response of others may require hours.

The range of the applied LPS doses in rats reported in literature varies from 0.05 nanograms / kg to up to 5 mg/kg (60). The doses of administration should be optimized for the experiment that will be performed. As stated in the introduction, the best thing to do is to review the literature and find out the exact dose proven to get the effects wanted in each aim of the experiments performed.

Our main goal was to provide a guide to prepare LPS in saline solution and basic steps on how to perform an intraperitoneal injection. Each investigator should adapt the protocol according to his/her research proposal. The authors hope this guide is informative.

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CONFLICTS OF INTEREST

Nothing to Declare.

REFERENCES

1. Ohanian S. H., Schwab J. H. 1967. "Persistence of group a streptococcal cell walls related to chronic inflammation of rabbit dermal connective tissue". The Journal of Experimental Medicine 125 (6): 1137-48.
2. Maitra U., Deng H., Glaros T., Baker B., Capelluto D. G., Li Z., Li L. 2012. "Molecular mechanisms responsible for the selective and low-grade induction of proinflammatory mediators in murine macrophages by lipopolysaccharide". Journal of Immunology 189 (2): 1014-23.
3. Borowski T., Kokkinidis L., Merali Z., Anisman H. 1998. "Lipopolysaccharide, central in vivo biogenic amine variations, and anhedonia". Neuroreport 9 (17): 3797-802.
4. Fishkin R. J., Winslow J. T. 1997. "Endotoxin-induced reduction of social investigation by mice: interaction with amphetamine and anti-inflammatory drugs". Psychopharmacology (Berl) 132 (4): 335-41.
5. Shen Y., Connor T. J., Nolan Y., Kelly J. P., Leonard B. E. 1999. "Differential effect of chronic antidepressant treatments on lipopolysaccharide-induced depressive-like behavioural symptoms in the rat". Life Sciences 65 (17): 1773-86.
6. Dantzer R., Bluthé R. M., Layé S., Bret-Dibat J. L., Parnet P., Kelley K. W. 1998. "Cytokines and sickness behavior". Annals of the New York Academy of Sciences 840: 586-90.
7. Pugh C. R., Kumagawa K., Fleshner M., Watkins L. R., Maier S. F., Rudy J. W. 1998. "Selective effects of peripheral lipopolysaccharide administration on contextual and auditory-cue fear conditioning". Brain Behavior and Immunity (3): 212-29.
8. Hauss-Wegrzyniak B., Dobrzanski P., Stoehr J. D., Wenk G. L. 1998. "Chronic neuroinflammation in rats reproduces components of the neurobiology of Alzheimer's disease". Brain Research 780 (2): 294-303.
9. Hauss-Wegrzyniak B., Vannucchi M. G., Wenk GL. 2000. "Behavioral and ultrastructural changes induced by chronic neuroinflammation in young rats". Brain Research 859 (1): 157-66.
10. Hennigan A., Trotter C., Kelly A. M. 2007. "Lipopolysaccharide impairs long-term potentiation and recognition memory and increases p75NTR expression in the rat dentate gyrus". Brain Research 1130 (1): 158-66.
11. Shaw K. N., Commins S., O'Mara S. M. 2005. "Cyclooxygenase inhibition attenuates endotoxin-induced spatial learning deficits, but not an endotoxin-induced blockade of long-term potentiation". Brain Research 1038 (2): 231-7.
12. Vasconcelos A. R., Yshii L. M., Viel T. A., Buck H. S., Mattson M. P., Scavone C., Kawamoto EM. 2014. "Intermittent fasting attenuates lipopolysaccharide-induced neuroinflammation and memory impairment". Journal of Neuroinflammation. 11:85.
13. Chen Z., Jalabi W., Shpargel K. B., Farabaugh K. T., Dutta R., Yin X., Kidd G. J., Bergmann C. C., Stohlman S. A., Trapp B. D. 2012. "Lipopolysaccharide-induced microglial activation and neuroprotection against experimental brain injury is independent of hematogenous TLR4". Journal of Neuroscience 32 (34): 11706-15.
14. Buttini M., Limonta S., Boddeke H. W. 1996. "Peripheral administration of lipopolysaccharide induces activation of microglial cells in rat brain". Neurochemistry International (1): 25-35.
15. Hailman E., Lichenstein H. S., Wurfel M. M., Miller D. S., Johnson D. A., Kelley M., Busse L. A., Zukowski M. M., Wright S. D. 1994. "Lipopolysaccharide (LPS)-binding protein

accelerates the binding of LPS to CD14" Journal of Experimental Medicine 179 (1): 269-77.
16. Lehnardt S., Massillon L., Follett P., Jensen F. E., Ratan R., Rosenberg P. A., Volpe J. J., Vartanian T. 2003 "Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway". Proceedings of the Nationall Academy of Sciences U S A 100 (14): 8514-9.
17. Gatti S., Bartfai T. 1993. "Induction of tumor necrosis factor-alpha mRNA in the brain after peripheral endotoxin treatment: comparison with interleukin-1 family and interleukin-6". Brain Research 624 (1-2): 291-4.
18. Layé S., Parnet P., Goujon E., Dantzer R. 1994. "Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice", Molecular Brain Research (1): 157-62.
19. Pitossi F., del Rey A., Kabiersch A., Besedovsky H. 1997. "Induction of cytokine transcripts in the central nervous system and pituitary following peripheral administration of endotoxin to mice", Journal of Neuroscience Research. 48 (4): 287-98.
20. Mizuno T., Sawada M., Marunouchi T., Suzumura A. 1994. "Production of interleukin-10 by mouse glial cells in culture", Biochemical and Biophysics Research Communication 205 (3): 1907-15.
21. Rivest S. 2009. "Regulation of innate immune responses in the brain". Nature Reviews Immunology 9 (6): 429-39.
22. Welser-Alves J. V., Milner R. 2013. "Microglia are the major source of TNF-α and TGF-β1 in postnatal glial cultures; regulation by cytokines, lipopolysaccharide, and vitronectin". Neurochemistry International 63 (1): 47-53.
23. Ghosh S., Lertwattanarak R., Garduño J., Galeana J., Li J., Zamarripa F., Lancaster J., Mohan S., Hussey S., Musi N. 2015. "Elevated muscle TLR4 expression and metabolic endotoxemia in human aging". Journal of Gerontology A Biological Science Medical Science 70: 232-246.
24. Block M., Hong J. 2005. "Microglia and inflammation-mediated neurodegeneration: Multiple triggers with a common mechanism" Progress in Neurobiology 76: 77-98.
25. Tarassishin L., Suh H. S., Lee S. C. 2014. "LPS and IL-1 differentially activate mouse and human astrocytes: role of CD14". Glia 62 (6): 999-1013.
26. Sheng J., Bora S., Xu G., Borchelt D., Price D., Koliatsos V. 2003 "Lipopolysaccharide induced- neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid beta peptide in APPswe transgenic mice". Neurobiology of Disease 14: 133-145.
27. Anaeigoudari A., Shafei M., Soukhtanloo M., Sadeghnia H., Reisi P., Beheshti F., Mohebbati R., Mousavi S., Hosseini M. 2015. "Lipopolysaccharide- induced memory impairment in rats is preventable using 7-nitroindazole". Arquivos Neuro-psiquiatria 73: 784-790.
28. Gao H., Jiang J., Wilson B., Zhang W., Hong J., Liu B. 2002. "Microglial activation´s mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease". Journal of Neurochemistry 81: 1285-1297.
29. Whitton P. 2007. "Inflammation as a causative factor in the aetiology of Parkinson's disease" British Journal of Pharmacology 150: 963-976.
30. Zhao W., Xie W., Le W., Beers D., He Y., Henkel J., Simpson E., Yen A., Xiao Q., Appel S. 2004. "Activated microglia initiate motor neuron injury by a nitric oxide and glutamate-mediated mechanism" J Neuropathol Exp Neurol 63: 964-97.
31. Walter S., Doering A., Letiembre M., Liu Y., Hao W., Diem R., Bernreuther C., Glatzel M., Engelhardt B., Fassbender K. 2006.

"The LPS receptor, CD14 in experimental autoimmune encephalomyelitis and multiple sclerosis" Cell Physiol Biochem 17: 167-172.
32. Connor T. J., Leonard BE.1998." Depression, stress and immunological activation: the role of cytokines in depressive disorders". Life Sciences 62: 583-606.
33. Maes M., Lin A. H., Delmeire L., et al. 1999." Elevated serum interleukin-6 (IL-6) and IL-6 receptor concentrations in posttraumatic stress disorder following accidental man-made traumatic events". Biological Psychiatry 45: 833-839.
34. Mikova O., Yakimova R., Bosmans E., Kenis G., Maes M. 2001. "Increased serum tumor necrosis factor alpha concentrations in major depression and multiple sclerosis". Eur Neuropsychopharmacology 11:203-208.
35. Thomas A. J., Davis S., Morris C., Jackson E., Harrison R., O'Brien J. T. 2005. "Increase in interleukin-1beta in late-life depression". American Journal of Psychiatry 162:175-177.
36. Raetz C. R., Whitfield C. 2002. "Lipopolysaccharide endotoxins". Annual Review of Biochemistry. 71:635-700.
37. Vedder H., Schreiber W., Yassouridis A., Gudewill S., Galanos C., Pollmacher T. 1999. "Dose-dependence of bacterial lipopolysaccharide (LPS) effects on peak response and time course of the immune-endocrine host response in humans". Inflammation Research 48: 67-74.
38. Martich G. D., Boujoukos A. J., Suffredini A. F. 1993. "Response of man to endotoxin". Immunobiology 187: 403-416.
39. Ottaway C. A., Fong I. W., Da Silva B., Singer W., Karras L. 1998." Integrative aspects of a human model of endotoxemia" Canadian Journal of Physiology Pharmacology 76: 473-478.
40. Schreiber W., Pollmacher T., Fassbender K., et al. 1993. "Endotoxin- and corticotropin-releasing hormone-induced release of ACTH and cortisol. A comparative study in men" Neuroendocrinology 58: 123-128.
41. Reichenberg A., Yirmiya R., Schuld A., et al. 2001. "Cytokine-associated emotional and cognitive disturbance in humans" Arch Gen Psychiatry 58: 445-452.
42. Bison S., Carboni L., Arban R., Bate S., Gerrard P. Razzoli M. 2009. "Differential behavioral. Physiological. And hormonal sensitivity to LPS challenge in rats". International Journal of Interferon, Cytokine and Mediator Research 1: 1-13.
43. Bluthe R. M., Dantzer R., Kelley K.W. 1992. "Effects of interleukin-1 receptor antagonist on the behavioral effects of lipopolysaccharide in rat". Brain Research 573: 318-320.
44. Yirmiya R., Rosen H., Donchin O., Ovadia H. 1994. "Behavioral effects of lipopolysaccharide in rats: involvement of endogenous opioids". Brain Research 648: 80-86.
45. Yirmiya R. 1996. "Endotoxin produces a depressive-like episode in rats". Brain Research 711: 163-174.
46. Hrupka B. J., Langhans W. 2001. "A role for serotonin in lipopolysac- charide-induced anorexia in rats". Pharmacology Biochemistry Behavior 68: 355-362.
47. Lugarini F., Hrupka B. J., Schwartz G. J., Plata-Salaman C. R., Langhans W. 2002. A role for cyclooxygenase-2 in lipopolysaccharide-induced anorexia in rats. American Journal of Physioogyl Regulalatory Integrative Comparative Physiology 283: R862-R868.
48. Yvonne Couch, Alexander Trofimov, Natalyia Markova, Vladimir Nikolenko, Harry W. Steinbusch, Vladimir Chekhonin, Careen Schroeter, Klaus-Peter Lesch, Daniel C. Anthony1 and Tatyana Strekalova. 2016. "Low-dose lipopolysaccharide (LPS) inhibits aggressive and augments depressive behaviours in a chronic mild stress model

in mice". Journal of Neuroinflammation 13 (1):108.
49. Elgarf A. S., Aboul-Fotouh S., Abd-Alkhalek H. A., El Tabbal M., Hassan A. N., Kassim S. K., Hammouda G. A., Farrag K. A., Abdel-tawab AM. 2014. Lipopolysaccharide repeated challenge followed by chronic mild stress protocol introduces a combined model of depression in rats: reversibility by imipramine and pentoxifylline, Pharmacol Biochem Behav 126: 152-62.
50. Chimenti M. S., Triggianese P., Conigliaro P., Candi E., Melino G., Perricone R. 2015. "The interplay between inflammation and metabolism in rheumatoid arthritis". Cell Death Dis 6: e1887.
51. Boyapati R., Satsangi J., Ho GT. 2015. "Pathogenesis of Crohn's disease". F1000Prime Rep 7:44.
52. Willerson J. T., Ridker P. M. 2004. "Inflammation as a cardiovascular risk factor, Circulation". Circulation 109 (21 Suppl 1): II2-10.
53. Yoshino S., Sasatomi E., Ohsawa, M. 2000. "Bacterial lipopolysaccharide acts as an adjuvant to induce autoimmune arthritis in mice" Immunology 99 (4), 607-14.
54. Caradonna L., Amati L., Magrone T., Pellegrino N. M., Jirillo E., Caccavo D. 2000. "Enteric bacteria, lipopolysaccharides and related cytokines in inflammatory bowel disease: biological and clinical significance". Journal of Endotoxin Research 6 (3): 205-14.
55. Yasuda S., Lew W. Y. W. 1997. "Lipopolysaccharide depresses cardiac contrac- tility and -adrenergic contractile response by decreasing myofilament response to Ca2 in cardiac myocytes". Circulation Research .1997; 81: 1011-20.
56. Li H. L., Suzuki J., Bayna E., Zhang F., Dalle Molle E., Clark A., Engler R. L., Lew W. Y. W. 2002. "Lipopolysaccharide induces apoptosis in adult rat ventricular myocytes via cardiac AT1 receptors". Am J Physiol Heart Circ Physiol 283: H461-7.
57. Szyf M., McGowan P., Meaney M. J. 2008. "The social environment and the epigenome". Environonmental and Molecular Mutagenesis 49 (1): 46-60
58. Evelin Painsipp, Martin J. Köfer, Frank Sinner, Peter Holzer. 2011. "Prolonged Depression-Like Behavior Caused by Immune Challenge: Influence of Mouse Strain and Social Environment". PLoS ONE 6(6): e20719.
59. Dantzer R., O'Connor J. C., Freund G. G., Johnson R. W., Kelley K. W. 2008. "From inflammation to sickness and depression: when the immune system subjugates the brain". Nature Reviews Neuroscience. 9 (1): 46-56.
60. Zakaria R., Wan Yaacob W. M., Othman Z., Long I., Ahmad A. H., Al-Rahbi B. 2017. "Lipopolysaccharide-induced memory impairment in rats: a model of Alzheimer's Disease", Physiological Research. 66 (4): 553-565.