

An Inescapable Cat Odor Exposure Protocol for Studying Innate and Contextual Threat Conditioning in Rats

María Rodríguez^{1,2}, Marco Contreras³, Marcos Domic-Siede⁴, Francisco Ceric⁵, Fernando Torrealba¹

¹Departamento de Fisiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile ²Instituto de Ciencias Sociales, Universidad de O'Higgins ³Department of Psychology, University of Arizona, Tucson ⁴Escuela de Psicología, Universidad Católica del Norte ⁵Laboratorio de Neurociencia Afectiva, Facultad de Psicología, Universidad del Desarrollo

Corresponding Author

María Rodríguez
maria.rodriguez@uoh.cl

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Materials

Name	Company	Catalog Number	Comments
3-3' diaminobenzidine hydrochloride (DAB)	Bio-Rad		Colorimetric blotting substrate; used with peroxidase antibody conjugate
Airtight plastic container	comercial		Used to store cat collars
Cat Collar	comercial		dimensions: width 1.5, thickness 0.5, length 30 cm
Cat Odor	domestic cat		Ovariectomized indoor-outdoor female cat fed with regular commercial cat food.
Enrofloxacin 5%	Bayer		Antibiotic (19mg/Kg i.p.). Used in the surgery protocol.
Ethanol	Sigma-Aldrich		5% v/v for cleaning testing box
Guide cannulae	Plastic One		26 gauge. Consists of a threaded cylindrical plastic pedestal molded around a piece of stainless steel tubing which is implanted into the specific target area of the brain following stereotaxic coordinates.
Hamilton syringe	Sigma-Aldrich		1 uL. Used in the inactivation protocol
Hydrogen Peroxide 30%	Merck Millipore		Used in immunohistochemical procedure
Injection cannula	Plastic One		33 gauge. This cannula is inserted into the guide cannula for fluids to be dosed into the specific target area of the brain.
Ketamine (Imalgene)	Rhodia Merieux		Sedative (100mg/Kg i.p.). Used in the surgery and immunohistochemical protocols
Ketoprofen 1%	Rhodia Merieux		Anti-inflammatory (0,2mg/Kg i.p.). Used in the surgery protocol.
Male rats	Universidad Católica de Chile		Sprague dawley strain (270–290 g)
Mechanical digit manual counter	Comercial		Used for cell counting

Muscimol	Sigma-Aldrich		0,5 ug/uL into the localized brain area
Normal goat serum	Life Technologies		Used in immunohistochemical procedure
Occluder cannulae	Plastic One		Inserted into guide cannula to seal it and prevent the outflow of tissue fluid after injection.
Paraformaldehyde powder	Sigma-Aldrich		Used to tissue fixation
PBS 10x, pH 7.4	Life Technologies		Used in immunohistochemical procedure
Primary antibody	Sigma, St Louis		Rabbit polyclonal F7799 used in immunohistochemical procedure
Red light bulb (80 watts)	Crompton		Used during the behavioral protocol
Screws	Plastic One		Used in the surgery protocol for anchoring guide cannulae in the skull
Secondary antibody	Jackson immunoresearch		Anti-rabbit IgG (H+L) used in immunohistochemical procedure
Single-dose sterile saline 0.9%	SalJet		0,5 ug/uL into the localized brain area
Sodium azide	Sigma-Aldrich		Used in immunohistochemical procedure
SPSS for windows	IBM		Version 20.0. Software used for statistical data analysis
Stereotaxis apparatus	Kopf		Used in the surgery protocol
Transparent Plexiglas rectangular chamber	assembled		60 x 40 x 40 cm, L, W, H); transparent poly(methyl methacrylate) rectangular chamber
Triton X-100	Merck Millipore		Used in immunohistochemical procedure
Vectastain ABC Elite kit	Vector Laboratories		avidin/biotin-based peroxidase system used for the detection of biotin-conjugated secondary antibodies
Videocamera	Sony		Prefer the use of a night vision camera
Xylazine (Rompun)	Bayer		Sedative (20mg/Kg i.p.). Used in the surgery and immunohistochemical protocols.