

Canadian Council on Animal Care
Conseil canadien de protection des animaux

Good Animal Practice in Science Bonnes pratiques animales en science



Three Rs Search Guide

Gilly Griffin, PhD & Marc Avey, PhD
Canadian Council on Animal Care

Animal use protocol worksheet

This animal use protocol worksheet was created by the CCAC to assist investigators in compiling Three Rs-related information when they prepare animal use protocols. Please note the following:

- Use of the worksheet is intended to be voluntary – it does not supplant the completion of an animal use protocol. There is no CCAC requirement for investigators to complete this form.
- The worksheet is for personal use – there is no CCAC requirement for this worksheet to be submitted to local animal care committees.

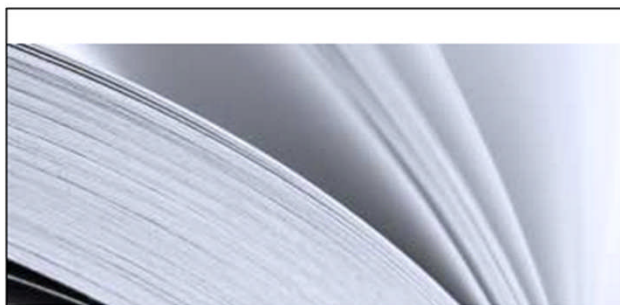


Pre-search information

Type of information	Description
Working title of the project and the scientific objective(s)	<p>Dehydration and Rehydration impacts on vasopressinergic supraoptic neurons after water deprivation</p> <p>To test the role of oropharyngeal and gastric afferents on hypothalamic activation in the lamina terminus and perinuclear zone..</p>
Proposed animal model	Dehydrated rats instrumented with gastric fistulas and allowed to drink water or isotonic saline compared with euhydrated controls
Proposed procedures on animals	Water Deprivation, Gastrointestinal Fistulation, Anesthesia (isoflurane, thiobutabarbital), Cardiac Puncture, Perfusion
Potential causes of pain and distress in the animals	Water Deprivation, Gastrointestinal Fistulation, Anesthesia (isoflurane, thiobutabarbital), Cardiac Puncture, Perfusion
Any known species-specific considerations	None
Expected Category of Invasiveness	<p><input type="checkbox"/> A. Experiments on most invertebrates or on live isolates</p> <p><input type="checkbox"/> B. Experiments which cause little or no discomfort or stress</p> <p><input type="checkbox"/> C. Experiments which cause minor stress or pain of short duration</p> <p><input checked="" type="checkbox"/> D. Experiments which cause moderate to severe distress or discomfort</p> <p><input type="checkbox"/> E. Procedures which cause severe pain near, at, or above the pain tolerance threshold of unanesthetized conscious animals</p>

Type of information	Description
Any known potential Replacement alternatives	None
Any known potential Reduction alternatives	None
Any known potential Refinement alternatives	None





PubMed

PubMed comprises more than 21 million citations for biomedical literature from MEDLINE, life science journals, and online books. Citations may include links to full-text content from PubMed Central and publisher web sites.

Using PubMed

[PubMed Quick Start Guide](#)

[Full Text Articles](#)

[PubMed FAQs](#)

[PubMed Tutorials](#)

[New and Noteworthy](#) 

PubMed Tools

[PubMed Mobile](#)

[Single Citation Matcher](#)

[Batch Citation Matcher](#)

[Clinical Queries](#)

[Topic-Specific Queries](#)

More Resources

[MeSH Database](#)

[Journals in NCBI Databases](#)

[Clinical Trials](#)

[E-Utilities](#)

[LinkOut](#)

GETTING STARTED

[NCBI Education](#)
[NCBI Help Manual](#)
[NCBI Handbook](#)
[Training & Tutorials](#)

RESOURCES

[Chemicals & Bioassays](#)
[Data & Software](#)
[DNA & RNA](#)
[Domains & Structures](#)

POPULAR

[PubMed](#)
[Nucleotide](#)
[BLAST](#)
[PubMed Central](#)

FEATURED

[Genetic Testing Registry](#)
[PubMed Health](#)
[GenBank](#)
[Reference Sequences](#)

NCBI INFORMATION

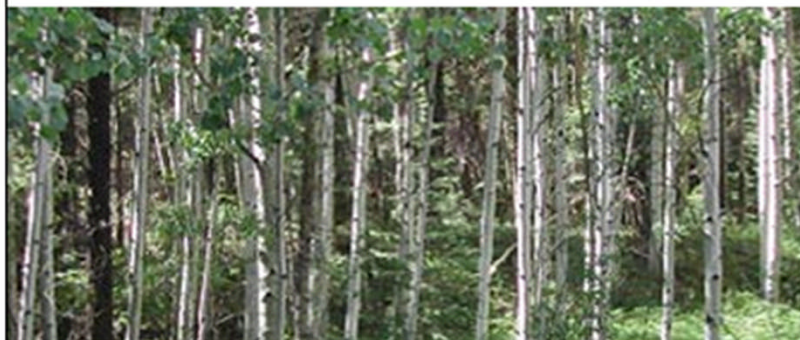
[About NCBI](#)
[Research at NCBI](#)
[NCBI Newsletter](#)
[NCBI FTP Site](#)

MeSH

MeSH

water deprivation

[Limits](#) [Advanced](#)



MeSH

MeSH (Medical Subject Headings) is the NLM controlled vocabulary used for indexing articles for PubMed.

Using MeSH

[Help](#)

[Tutorials](#)

More Resources

[E-Utilities](#)

[NLM MeSH Homepage](#)

You are here: [NCBI](#) > [Literature](#) > MeSH Database

GETTING STARTED

[NCBI Education](#)

RESOURCES

[Chemicals & Bioassays](#)

POPULAR

[PubMed](#)

FEATURED

[Genetic Testing Registry](#)

[Display Settings:](#) ☒ Full

Water Deprivation

The withholding of water in a structured experimental situation.

Year introduced: 1969

PubMed search builder options

[Subheadings:](#)

☐ complications

☐ physiology

☐ physiopathology

☐ drug effects

☐ Restrict to MeSH Major Topic.

☐ Do not include MeSH terms found below this term in the MeSH hierarchy.

Entry Terms:

- Deprivation, Water
- Deprivations, Water
- Water Deprivations

Previous Indexing:

- [Motivation \(1966-1968\)](#)
- [Thirst \(1966-1968\)](#)

See Also:

- [Thirst](#)

[All MeSH Categories](#)

[Psychiatry and Psychology Category](#)

[Behavior and Behavior Mechanisms](#)

[Motivation](#)

Water Deprivation

[Send to:](#) ☒

PubMed search builder

Add to search builder

AND

Search PubMed

Related information

[PubMed](#)

[PubMed - Major Topic](#)

[Clinical Queries](#)

[NLM MeSH Browser](#)

Search details

"water deprivation"
[MeSH Terms] OR water
deprivation[Text Word]

Search

[See more...](#)

Recent activity

[Turn Off](#) [Clear](#)

🔍 water deprivation (1)

MeSH

Information gathering

Keywords and concepts used in literature search	Databases searched
Replacement search	
rehydration and dehydration AND neuron	Pubmed
Reduction search	
rehydration AND dehydration AND brain AND cell	Pubmed
Refinement search	
("water deprivation" OR "water restriction" OR water schedule") AND (sprague-dawley OR rat OR rattus) AND (rehydration AND dehydration)	Pubmed

Other resources	Description
Three Rs websites	CCAC Three Rs Microsite
Experts consulted	<input type="checkbox"/> Laboratory animal veterinarian <input type="checkbox"/>
	<input type="checkbox"/> Animal welfare specialist <input type="checkbox"/>
	<input type="checkbox"/> Other investigator <input type="checkbox"/>
	<input type="checkbox"/> Statistician <input type="checkbox"/>
	<input type="checkbox"/> Other <input type="checkbox"/>
Other resources	<input type="checkbox"/>



Replacement Search

- Search for comparisons
 - ◆ **rehydration** and **dehydration**
- Search for what is measured
 - ◆ **neuron**



1. [Structural and neurochemical plasticity in both supraoptic and paraventricular nuclei of hypothalamus of a desert rodent Meriones Shawi after a severe dehydration versus opposite treatment by rehydration: GFAP and vasopressin immunohistochemical study.](#)

Elgot A, Hiba OE, Gamrani H.

Neurosci Lett. 2012 Apr 25;515(1):55-60. Epub 2012 Mar 16.

PMID: 22445884 [PubMed - in process]

[Related citations](#)

2. [Dehydration followed by sham rehydration contributes to reduced neuronal activation in vasopressinergic supraoptic neurons after water deprivation.](#)

Knight WD, Ji LL, Little JT, Cunningham JT.

Am J Physiol Regul Integr Comp Physiol. 2010 Nov;299(5):R1232-40. Epub 2010 Sep 15.

PMID: 20844266 [PubMed - indexed for MEDLINE] **Free PMC Article**

[Related citations](#)

3. [Reflex inhibition of electrically induced muscle cramps in hypohydrated humans.](#)

Miller KC, Mack GW, Knight KL, Hopkins JT, Draper DO, Fields PJ, Hunter I.

Med Sci Sports Exerc. 2010 May;42(5):953-61.

PMID: 19997012 [PubMed - indexed for MEDLINE]

[Related citations](#)

4. [Subdiaphragmatic vagotomy prevents drinking-induced reduction in plasma corticosterone in water-restricted rats.](#)



Information review

Did the Three Rs search determine any possible Replacement alternatives?

Replacement alternative category	No	Yes	If yes, describe and/or note citation
Absolute replacement	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Relative replacement	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Other:	<input checked="" type="checkbox"/>	<input type="checkbox"/>	

Example proposal is duplicative
but...

Can we find Reduction or Refinements?



Reduction Search

- Search for papers that have done sample size calculations
- Consult statistician to calculate sample size



Reduction Search – Sample Size

- Could number of animals per group be reduced?
- Is number of animals per group sufficient?



Dehydration followed by sham rehydration contributes to reduced neuronal activation in vasopressinergic supraoptic neurons after water deprivation.

Knight WD, Ji LL, Little JT, Cunningham JT.

METHODS

Sample size?

All experiments were conducted on adult male Sprague-Dawley rats (200–300 g body wt; Charles River, Germantown, MD). Prior to surgery, rats were individually housed in a temperature-controlled room on a 14:10-h light-dark cycle with light onset at 0700. Food and water were available ad libitum, except during the dehydration experiments; food was not resupplied during rehydration periods. Experiments involving rehydration were conducted and terminated within the early portion of the light phase. Water and saline were provided at room temperature. All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio according to National Institutes of Health guidelines.

Gastrointestinal fistulation. Each rat was anesthetized with isoflurane (drop jar) and maintained with 2% isoflurane delivered by an atomizer with O₂. An abdominal midline incision was made, and rats

Sample size?

returned to presurgical levels (2-wk minimum).

Protocol. Fistulated rats were randomly divided into six treatment groups: Control (CON), 48-h water-deprived (48 WD), 46-h water-deprived followed by 2 h of rehydration with fistula closed (46+WC), 46 h water-deprived followed by 2 h sham rehydration with fistula open (46+WO), 46 h water-deprived followed by 2 h rehydration with isotonic (0.9%; wt:vol) saline with fistula closed (46+SC), and 46 h deprived followed by 2 h sham rehydration with isotonic saline with fistula open (46+SO). All rats were preacclimated and housed in individual metabolic cages that allowed for the collection of recovered fluids and measurement of fluid intake. To ensure the patency of open fistulas, animals were periodically monitored during rehydration to allow for adequate shunting of ingested fluid. During the 2-h rehydration period, recovered fluid and fluid intake were also measured and compared with the determined efficacy of the sham rehydration; sham-rehydrated (46+WO, 46+SO) rats that ingested 3 ml greater than the volume of the recovered fluid were eliminated. To control for unrecovered fluid, a separate group of unoperated rats were dehydrated for 46 h and given 2 h of access to 3 ml of water (46+3 ml). Because of the preceding 46-h deprivation period, the presence of urine in the recovered fluid was negligible.

Immediately following the experiment, rats were anesthetized with

ren
gre
col
is a
The
ere
pre
loc
#to
Ne
(Si
can

RE

de
oci
dra
and
Pla

ride and
ons were
inea pig
ila Labo-
i-Guinea
h, West
air dried
nount.
ontaining
roscope
epifluo-
l camera
tal Photo
for uni-
ied using
e to three
nPO, and
each rat
ction and

in the dorsal cap and lateral margin subregions of the CVLT

Table 1. *Effects of rehydration or sham rehydration on hemodynamics and water intake*

	Hematocrit, %	Osmolality, mOsm	Reh. Fluid Intake, ml
CON	45.4 ± 0.2 ^a	296 ± 2 ^a	
48 WD	49.0 ± 0.6 ^b	307 ± 2 ^b	
46 + 3 ml	50.7 ± 0.9 ^b	308 ± 1 ^b	
46+WC	45.3 ± 0.5 ^a	278 ± 2 ^c	26 ± 3 ^a
46+WO	49.7 ± 0.6 ^b	306 ± 1 ^b	82 ± 10 ^b
46+SC	46.0 ± 0.9 ^a	301 ± 3 ^a	36 ± 3 ^a
46+SO	47.8 ± 0.6 ^b	308 ± 2 ^b	56 ± 3 ^c

Data are expressed as means ± SE. ^{a,b,c}Values with unique superscripted letters are significantly different ($P < 0.05$). CON, control; 48 WD, 48-h water-deprived; 46 + 3 ml, 46-h water-deprived + 2 h H₂O access to 3 ml of H₂O; 46+WC = 46 h water-deprived + 2 h H₂O access with fistulae closed; 46+WO, 46 h water-deprived + 2 h H₂O access with fistulae open; 46+SC, 46 h water-deprived + 2 h 0.9% SAL access with fistulae closed; 46+SO = 46 h water deprived + 2 h 0.9% SAL access with fistulae open *n* = 6 or 7.

Reduction Search - Sample Size

- No justification for number of animals per group in protocol (paper)
- Limited searching found no paper that did sample size calculation (e.g. power analysis)





[Am J Physiol Regul Integr Comp Physiol](#). 2010 Nov;299(5):R1232-40. Epub 2010 Sep 15.

Dehydration followed by sham rehydration contributes to reduced neuronal activation in vasopressinergic supraoptic neurons after water deprivation.

[Knight WD](#), [Ji LL](#), [Little JT](#), [Cunningham JT](#).

Department of Integrative Physiology and Cardiovascular Research Institute, University of North Texas Health Science Center at Fort Worth, Fort Worth, Texas 76107, USA. tom.cunningham@unthsc.edu

Abstract

This experiment tested the role of oropharyngeal and gastric afferents on hypothalamic activation in dehydrated rats instrumented with gastric fistulas and allowed to drink water or isotonic saline compared with euhydrated controls (CON). Rats were water-deprived for 48 h (48 WD) or 46 h WD with 2 h rehydration with water (46+W) or isotonic saline (46+S). 46+W and 46+S rats were given water with fistulas open (46+WO/46+SO, sham) or closed (46+WC/46+SC). Compared with CON, water deprivation increased and water rehydration decreased plasma osmolality, while sham rehydration had no effect. Water deprivation increased c-Fos staining in the lamina terminalis. However, none of the sham or rehydration treatments normalized c-Fos staining in the lamina terminalis. Analysis of AVP and c-Fos-positive neurons in the supraoptic nucleus (SON) revealed reduced colocalization in 46+WO and 46+SC rats compared with 48 WD and 46+SO rats. However, 46+WO and 46+SC rats had higher c-Fos staining in the SON than 46+WC or CON rats. Examination of c-Fos in the perinuclear zone (PNZ) revealed that sham and rehydrated rats had increased c-Fos staining to CON, while 48 WD and 46+SO rats had little or no c-Fos staining in this region. Thus, preabsorptive reflexes contribute to the regulation of AVP neurons in a manner independent of c-Fos expression in the lamina terminalis. Further, this reflex pathway may include inhibitory interneurons in

Sa



Re
Pu

Dif
m

Eff
m

Ro
an

An
(A)

Dif
de

Re

Refinement Search – Water Deprivation

- Search methods to refine
 - ◆ **water deprivation or water restriction or water schedule**
- Search for species/type
 - ◆ **sprague-dawley or rat or rattus**
- Search for comparisons
 - ◆ **rehydration and dehydration**



Differential regulation of parvocellular neuronal activity in the paraventricular nucleus of the hypothalamus following single versus repeated episodes of water restriction-induced drinking

Michelle M. Arnhold, Cheryl Wotus, and William C. Engeland

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (175-200 g; Charles River, Wilmington, MA) were housed two per cage under a 12-h light, 12-h dark cycle (lights on at 0530h) with food and water available ad libitum prior to initiation of the water restriction schedule. Experiments were initiated at least 2 to 3 days after arrival. Animals were maintained and cared for in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Experimental procedures were approved by the University of Minnesota Animal Care and Use Committee.

Experiment 1: Effect of a single 23.5h episode of water restriction followed by rehydration versus repeated 23.5h episodes of water restriction and rehydration on HPA hormones The initial experiment was performed to compare a single episode versus a repeated episode of water restriction-induced drinking on inhibition of the HPA axis. Water restriction consisted of 23.5h water deprivation followed by 30 min access to water. Water was removed starting 3h after light onset (0830h; day 0) and was returned the following day 2.5h after light onset (0800h; day 1); control rats received water ad libitum. All rats had access to food at all times. To examine the effects of a single episode of restriction-induced drinking on HPA hormones, control rats (AL; n=6) and a group of water restricted rats (WR; n=6) were sacrificed by decapitation on day 1 at 0800h; another group of water restricted rats was given

Refinement Search

- Possible refinement with reduced water deprivation duration



Did Three Rs search determine any possible Reduction alternatives?

Reduction alternative category	No	Yes	If yes, describe and/or note citation
Experimental design	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Sample size calculation	<input type="checkbox"/>	<input checked="" type="checkbox"/>	No sample size calculation done
Animal model selection	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Telemetry	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Animal supply strategy	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Data sharing strategy	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Animal re-use strategy	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Other:	<input type="checkbox"/>	<input type="checkbox"/>	

Did Three Rs search determine any possible Refinement alternatives?

Refinement alternative category	No	Yes	If yes, describe and/or note citation
Animal handling	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Animal housing	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Anesthesia	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Analgesia/pain management	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Blood & tissue sampling	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Humane endpoints	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Welfare assessment	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Humane killing	<input checked="" type="checkbox"/>	<input type="checkbox"/>	
Other:	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Reduced water deprivation duration



Questions to assess if your Three Rs search is complete

To assist in deciding if your Three Rs search is complete, it may be useful to review the following questions:

- Is the proposed experiment or test duplicative?
- Are there any in vitro techniques that could replace use of animals?
- Have any computer simulations been developed that relate to the study?
- Are there any alternative animal models of lower sentience?
- Is there useful and current information about the proposed animal model?
- Has a particular strain of animal been shown to be more sensitive to the effects that will be studied?
- Is there information on the proposed model that might allow the use of fewer animals or might reduce any pain experienced by the animals?
- Could in vitro methods be incorporated into the protocol in any way to reduce the number of animals used (e.g., for early screening)?
- Have any statistical models been developed for use in this type of study, and would these affect the design of the experiment?
- Is there a way to decrease the level of invasiveness of the protocol without compromising the scientific objective?
- Could the proposed anesthetics, analgesics or other drugs pose a confounding influence on the experimental outcome?
- Is there information about assessing welfare and the level of pain of the animals?

- [CCAC guidelines and policies](#)
- [Where to Find Literature](#)
- [Questions to Assess if Your Three Rs Search is Complete](#)
- [Animal Use and Welfare Workshop](#)
- [Alternative Methods](#)



[About
the Three Rs](#)

[Care
& Techniques](#)

[Research](#)

[Testing
& Production](#)

[Teaching
& Training](#)

[Animal Care
Committees](#)

[Three Rs Searches
& Animal Index](#)

[Home](#) » [Three Rs Searches & Animal Index](#) » [Three Rs Search Guide](#) » [Step-by-Step Search Guide](#) » [Step-by-Step Search Guide](#)

[Animal Index](#) »

[Three Rs Search Guide](#) »

[Step-by-Step Search
Guide](#)

[Step 1](#)

[Step 2](#)

[Step 3](#)

[Step 4](#)

[Step 5](#)

[Step 6](#)

[Step 7](#)

[Three Rs Journals](#)

[Three Rs Centres](#)

[CCAC Reference Database](#)

Step-by-Step Search Guide

• Step 7: Finalize experimental plans and animal use protocol

Incorporate Three Rs alternatives into your animal-based procedures and document the Three Rs search in your animal use protocol. Double-check that your animal use protocol follows relevant **CCAC guidelines & policy statements on animal use protocols** and any policies or standard operating procedures (SOPs) at your home institution. Consider how a description of the Three Rs alternatives used could be included in any future publications about your work.

References used in the preparation of the Three Rs Search Guide: »

Quick Links

- [CCAC guidelines & policies on animal use protocols](#)
- [Where To Do A Literature Search](#)
- [Questions To Assess If Your Three Rs Search Is Complete](#)
- [Animal Use Protocol Worksheet](#)
- [Alternatives Test Methods](#)