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Neuropathy Phentoyping Protocols - Streptozotocin Treatment For Rats

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1 Works for me dx.doi.org/10.17504/protocols.io.3njgmcn

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ABSTRACT

Summary:

Phenotyping of Rodents for the Presence of Diabetic Neuropathy
In man, the development of diabetic neuropathy is dependent on both the degree of glycemic control and the duration of diabetes. Diabetic neuropathy is a progressive disorder, with signs and symptoms that parallel the loss of nerve fibers over time. Consequently, assessments of neuropathy in mice are not performed at one time point, but are characterized at multiple time points during a 6 month period of diabetes. The degree of diabetes is evaluated in 2 ways: tail blood glucose measured following a 6 hour fast and glycated hemoglobin levels. The initial degree of neuropathy is screened using the methods discussed below. Detailed measures of neuropathy are employed when the initial screening instruments indicate a profound or unique phenotypic difference. This document contains protocols used by the DiaComp staff to examine and measure diabetic neuropathy at the whole animal, tissue and cellular levels.

Diabetic Complication:

EXTERNAL LINK

https://www.diacomp.org/shared/document.aspx?id=54&docType=Protocol

MATERIALS TEXT

Reagents:

Sodium citrate MW = 296.10
Citric Acid MW = 210.1
Streptozotocin (Sigma S-0130)
Sucrose

Solutions:

10 mM Sodium citrate
10 mM Citric acid
Citrate buffer; Sodium citrate + Citric acid pH 5.5
50% Sucrose water (regular tap water)

Supplies and Equipment:

ULAM Water bottles, sippers, cage clips, "Do Not Feed" and "Do Not Water" tape
1 ml syringes

5 ml tubes, plastic or glass
Aluminum foil
25 gauge 0.5 inch needles
Glucometer
Glucose test strips
Pipeter and tips
Graduate cylinder

Bring to animal room:

Bottles, Bottle Clips and Stoppers
Syringes and Needles
Tubes of Streptozotocin covered with parafilm, wrapped in foil
Citrate buffer
Pipeter for measuring citrate buffer
Sugar water
Graduate cylinder for diluting sugar water

1 Order animals to arrive several days before the planned injection date. This allows them to acclimatize to their new surroundings.

2 The day before STZ injection, remove the food from the feeders. Leave a clear note saying, "**Do Not Feed**" (specify appropriate rats) from (time, date) to (time, date). **INFORM ULAM**; be prepared to give them your animal protocol number. If there is evidence that the rats have been fed, contact ULAM to clarify and postpone STZ injection to the next day.

3 Order water bottles stopper/sippers, and clips the morning before STZ injection. Following STZ injection, be sure to put "**Do Not Water**" tape on the cages, or a sign to that effect on the rack.

4 Make Citrate Buffer.

5 Make 50 or 100% sugar water either the day before or the morning of the STZ injection.

6 Weigh rats. Rats must be weighed on the day of injection.

7 Calculate dose and weigh out streptozotocin. (see chart at the end of this document). 4 rats/ml, no more than 2ml/tube. Wrap the tube in foil to protect from light.

8 In the animal room, add citrate buffer to a tube, mix quickly and thoroughly.

9 Inject appropriate dose based on weight (see chart) using a 1 ml syringe and 25 gauge 0.5" needle, intraperitoneally, i.p. Use all of the STZ in the tube prior to mixing the next dose.

10 Following STZ injection, the rats are given 10% sucrose water to drink to protect them hypoglycemia. Alert the animal facility caretakers to the fact that the animals have been STZ injected so they can monitor for signs of distress. Hypoglycemia can be expected to occur anywhere from 8 to 24 hours after injection. If the 10% sucrose water is not sufficient to cover the hypoglycemia, any animal with signs of hypoglycemia can be given a 1ml bolus of 10% sucrose by gavage as a further effort to protect them. The animals are also given back their rat chow following STZ injection.

11 After 24 hours, the animals are placed on regular water. The next morning, the animals are checked for the induction of diabetes by measuring tail blood glucose. Using a standard glucometer and glucose test strips. Blood glucoses are recorded at onset and once every week or two weeks according to experimental design. Time line example, Monday fast, Tuesday inject, Wednesday evening put on regular water, Thursday check blood glucose.

12 **Chart**

Weight (g)	mls STZ	For Dose, use	mg/ml STZ in	citrate buffer
173-186	0.12			
187-201	0.13	55 mg/kg	82.5	
201-217	0.14	50 mg/kg	70	
218-232	0.15	45 mg/kg	67.5	
233-247	0.16	40 mg/kg	60	
248-262	0.17			
263-277	0.18			
278-292	0.19			
293-307	0.2			
308-322	0.21			
323-337	0.22			
338-352	0.23			
353-367	0.24			
368-382	0.25			
383-397	0.26			
398-412	0.27			
413-427	0.28			