

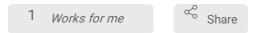


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## Viral-mediated short-hairpin RNA knockdown

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## **ABSTRACT**

Stereotaxic injection of viral vectors for gene knockdown experiments: The AAV vectors for knocking down the gene of interest were custom-made with Vector Biolabs using AAV-GFP-U6 vector for AAV1 packaging (Cat no, 7040, Vector Biolabs). They were then unilaterally injected into the striatum of mice as follows.

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Just before the	procedure
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1	The following	preparatory steps are followed:			
	1.1	Weigh the animals			
	1.2	Calibrate the David Kopf frame based on the manufacturer's instructions.			
	1.3	Clean the Hamilton Syringe with 70% ethanol and distilled water.			
	1.4	Shave the fur off the top of the animal's skull.			
Viral in	jections				
2	Anesthetize th	ne animal with 3-4% Isoflurane. Monitor closely the animal until it reaches pically 5–10 min) and does not respond to a light pinch to the hind paw or tail.			
3	Place the animal in the stereotaxic instrument by carefully placing one ear bar in the ear canal, securing the bar, and holding the animal in place as the other ear bar is placed and secured.				
4	Secure the mouth in the incisor adapter of the stereotaxic instrument. It is critical to ensure that the airway is not blocked.				
5	Cover the anesthetized animal's eyes with sterile ocular lubricant to keep them moist during the surgery.				

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Clean the skin's surface with alcohol and Iodine prep wipes.

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- 7 Make a midline incision to the skin's surface's skull with small surgical scissors or a scalpel.
- 8 Clean the skull with cotton swabs.
- 9 Identify the coordinates relative to bregma:
  - four sites in the striatum
  - anterior-posterior 0.8 mm,
  - mediolateral 2.4 mm
  - dorsoventral -2.8 and -3.6 mm
  - anterior-posterior 0.2 mm
  - mediolateral 1.8 mm,
  - dorsoventral -2.8 and -3.6 mm relative to bregma
- 10 Load the viral solution with Hamilton
- 11 Find bregma with the syringe needle and zero everything in the stereotactic frame
- 12 Determine your coordinates. Deliver virus by 100 nl/min speed (500-700 nl of AAV1-GFP-U6-shPKARIIβ or AAV1-GFP-U6-sh control viruses (5x10^12 genome copies/ml)
- 13 After injections, wait 10 minutes to allow the virus to diffuse
- 14 Remove the syringe very carefully and close the surgical sutures
- 15 Make a subcutaneous injection of Meloxicam right after the surgery and up to 48 hours.



16	Move the animal to the cage on the heat pad and monitor carefully its behavior.
17	Perform behavioral and biochemical experiments 4 weeks post operation.