

Materials List for:

# Perforated Patch-clamp Recording of Mouse Olfactory Sensory Neurons in Intact Neuroepithelium: Functional Analysis of Neurons Expressing an Identified Odorant Receptor

David Jarriault<sup>1</sup>, Xavier Grosmaître<sup>1</sup>

<sup>1</sup>UMR Centre des Sciences du Goût et de l'Alimentation, CNRS, INRA, Université de Bourgogne

Correspondence to: Xavier Grosmaître at [xavier.grosmaître@u-bourgogne.fr](mailto:xavier.grosmaître@u-bourgogne.fr)

URL: <https://www.jove.com/video/52652>

DOI: [doi:10.3791/52652](https://doi.org/10.3791/52652)

## Materials

Name	Company	Catalog Number	Comments
vibration table with Faraday cage	TMC	63-500 SERIES	required : isolates the recording system from vibrations induced by the environment (movements of experimenter, vibrations of equipment such as fans for cooling computers, etc); can also be purchased with a Faraday cage, or equipped by a custom made Faraday cage; this cage is recommended to avoid electric noise from the environment
optics			
microscope	Olympus	BX51WI	upright microscope equipped with epifluorescence; fixed or moving stage depending on the user's preference
objectives	Olympus	LUMPLFL40XW	at least 2 objectives required: a 4X or 10X for coarse approach to the cell; and a 40X immersion long distance example Olympus LUMPLFL40XW / IR /0,8 / WD:3.3 MM
magnifier	Olympus	U-TVAC	ABSOLUTELY REQUIRED: placed in the light path between the objective and the camera; allows to magnify the image on the screen in order to reach precisely the knob with the recording electrode
camera	Olympus	DP72	a good camera is required to see the neurons in fluorescence as well as in bright field; the controlling software is simple and allows to take pictures and do live camera image to monitor the approach of the electrode to the cell. An ultrasensitive camera is not necessary
filters	Olympus/Chroma		depending on the fluorescent protein used in the mice; example for GFP: excitation : BP460-490: emission: HQ530/50m
amplifier	HEKA	EPC10 USB	monitors the currents flowing through the recording electrode and also controls the puffing by sending a TTL signal to the

			spritzer; the EPC10 setup is controlled by computer
software	HEKA	Patchmaster	controls the amplifier during the experiment
micromanipulator	Sutter	MP225	precision micromanipulator, allows precise movements down to 1/10th of a micrometer; this model is very stable; avoid hydraulic manipulators that may drift
electrode puller	Sutter	P97	with a FT345-B wide trough filament; to prepare recording pipets of about 2µm diameter with a long tip to reach the cells; the resistance should be 15 to 20Mohm with perforated patch clamp solution
glass	Sutter	BF120-69-10	in our recording conditions, this glass is ideal for recording pipets
recording chamber	Warner Instruments	RC-26G	a chamber is needed to set the preparation under the microscope. To maintain the preparation in the center of the chamber, a net/ anchor should be used.
stimulation			
glass	WPI	TW100F-4	attached in groups of 7, these pipettes are used to prepare prepulled stimulating pipettes
multibarrel puller	MDI	PMP-107-Z	by association of pull and twist, this puller allows us to prepare puffing electrodes with 7 barrels
precision pressure injector	Toohey Company	P/N T25-1-900 Single Channel	this precision pressure injector controls the pressure ejected in the multibarrel puller; it is controlled manually or by the amplifier by a 5V TTLs
micromanipulator	Narishige	YOU-1	a coarse manipulator is enough to bring the puffing electrode close to the recording site
tubings	N/A		tygon tubing to bring the pressure from the puffer to the puffing pipette
solutions/perfusion/chemicals			
vacuum pump	gardner denver	300 series	a vibrating membrane pump is more quiet and efficient than other types of pumps
perfusion system	N/A	N/A	gravity perfusion system with polyethylen tubing to bring in and out the external solution from the recording chamber
nystatin	Sigma-Aldrich	N3503	mandatory to perpare internal solution for perforated patch clamp
DIMETHYL SULFOXIDE	Sigma-Aldrich	D5879	used to dissolve nystatin for internal solution for perforated patch
Sodium chloride	Sigma-Aldrich	S9625	extracellular solution
Potassium chloride	Sigma-Aldrich	P4504	intracellular/extracellular solution
Calcium chloride dihydrate	Sigma-Aldrich	C7902	extracellular solution
Sodium phosphate monobasic monohydrate (NaH <sub>2</sub> PO <sub>4</sub> )	Sigma-Aldrich	S9638	extracellular solution

Magnesium sulfate heptahydrate (MgSO <sub>4</sub> 7H <sub>2</sub> O)	Sigma-Aldrich	63140	extracellular solution
Glucose	Sigma-Aldrich	G8270	extracellular solution
Sodium bicarbonate	Sigma-Aldrich	S6297	extracellular solution
EGTA (Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid)	Sigma-Aldrich	E3889	internal solution
Potassium hydroxyde	Sigma-Aldrich	P1767	internal solution
Methyl Sulfoxide	Sigma-Aldrich	W387509	intracellular solution
Hepes-Na	Sigma-Aldrich	H7006	intracellular solution
Sucrose	Sigma-Aldrich	S0389	intracellular solution