

Supplementary information

Acute head-fixed recordings in awake mice with multiple Neuropixels probes

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Item	Product used in this protocol	Approximate cost	Possible alternatives	Approximate cost of alternatives	Notes
Adhesive for animal skin	Vetbond	\$/mouse	Ivoclar Adhese Universal, cyanoacrylate (super glue)	\$/mouse	Please test alternatives carefully before using
Cement for headframe attachment	C&B Metabond	\$\$/mouse	Ivoclar Tetric EvoFlow	\$/mouse	Please test alternatives carefully before using
Headframe	Machined titanium with 30 degree bend (cite Groblewski et al.)	\$\$\$ /mouse	Straight piece of steel	\$/mouse	Successful application of Procedure 2 requires repeatable headframe alignment, which may be difficult to achieve with alternative designs
Headframe well	Custom 3D-printed design	\$\$/mouse	Rubber "O" ring	\$/mouse	An alternative well will make it more difficult to align the insertion window – only use this option if you don't require precise targeting
Glass coverslip	Custom milled from TowerOptical	\$\$\$ /mouse	2-3 round coverslips with optical glue	\$/mouse	The less expensive alternative is described in this protocol
Coverslip coating	Sylgard 184 Silicone	\$/mouse	No coating	Free	The small bleeds caused using uncoated glass can be stopped quickly by an experienced surgeon
Adhesive for glass coverslip	Vetbond	\$\$/mouse	Cyanoacrylate adhesives (super glue)	\$/mouse	Please test alternatives carefully before using
Polymer to cover edges of window and exposed glue	Kwik-Cast	\$\$/mouse	Smooth-On Body Double "Fast Set"	\$/mouse	Please test alternatives carefully before using
Adhesive for plastic window	Metabond	\$\$/mouse	Many alternative glues, cements and polymers	\$/mouse	
Brain stabilization compound	Mix of low melting temp agarose from BioRad and high EEO agarose from Sigma	\$\$\$ /mouse	Standard agar powder	\$/mouse	Alternatives we tested all had decreased clarity and/or stability
Cap for protecting the coverslip or brain between experiments	Custom 3D printed design	\$\$/mouse	Recycled plastic and tape	Free	

Supplementary Table 1: Approximate cost and possible alternatives for key consumable supplies. Note that the use of alternatives may require slight modifications to the exact steps presented in this protocol. Any substitutions should be made with caution including adequate preparation and testing. Also note that the cost scale is different from Supplementary Table 2, as the items in this table typically cannot be reused for multiple experiments. In both cases, costs are relative, ballpark values and will inevitably change over time.

Item	Product used in this protocol	Approximate cost	Possible alternatives	Approximate cost of alternatives	Notes
Stereotaxic frame	Kopf Model 1900	\$\$\$\$	Kopf Model 900, or purchase used	\$\$\$	Stereotaxic frames are a standard surgical tool and can be shared across multiple labs
Manual manipulator for surgery	Kopf Model 1760	\$\$	Place headframe and glass coverslip by hand	Free	Many surgical stations already include a manual manipulator for performing injections
Headframe holder	Custom machined 27.5 degree angled holder (cite Groblewski)	\$\$\$	Thorlabs parts	\$	Successful application of Procedure 2 requires repeatable headframe alignment, which may be difficult to achieve with alternative designs
Parts for precise head post placement	Custom machined design (cite Groblewski)	\$\$\$	Place headframe by hand	Free	
Silicon probes	Neuropixels	\$\$\$	NeuroNexus, Cambridge NeuroTech, Atlas Neuroengineering, others	\$\$\$	The cost of alternative probes is similar, but may not require a new purchase
Manipulators for probes	New Scale Linear Smart Stages	\$\$\$\$	Manual manipulators	\$\$	Manual manipulators make it difficult to insert probes slowly
Rig structure and manipulator mounts	Custom machined metal components	\$\$\$\$	Thorlabs parts/3d printed mounts, e.g. International Brain Laboratory rig design (https://elifsciences.org/articles/63711)	\$\$	
Microscope	Leica M80	\$\$\$	AmScope 3.5X-90X Stereo Zoom Microscope	\$\$	
Tail guard	Custom 3D-printed design	\$\$	Recycled plastic and tape	Free	

Supplementary Table 2: Approximate cost and possible alternatives for key non-consumable supplies. Note that alternatives may require slight modifications to the exact steps presented here. Any substitutions should be made with caution including adequate preparation and testing. Please note that the cost scale is different from Supplementary Table 1, as these items can be reused across many experiments. In both cases, costs are relative, ballpark values and will inevitably change over time.