

Population imaging of neural activity in awake behaving mice

Supplementary Information

Supplementary Table 1. Characteristics of screened soma-localized Archon1 variants.

Full name of construct	How far from the soma, approximately, was the fluorescence detected using visual inspection, in this study?	Membrane/cytoplasmic localization detected using visual inspection in near-infrared channel, in this study	Voltage sensitivity*
Archon1-linker-KA2(1-150)-EGFP	50-100 μ m	Cytoplasmic with bright puncta in soma	Non-functional
Archon1-linker-KA2(1-100)-EGFP	50-100 μ m	Cytoplasmic with bright puncta in soma	Non-functional
KA2(1-150)-linker-Archon1-EGFP	50-100 μ m	Cytoplasmic with bright puncta in soma	Non-functional
Archon1-KGC-EGFP-Nav1.2(II-III)-ER2	80-100 μ m	Membrane with significant aggregation in soma	Not measured
Archon1-KGC-EGFP-Nav1.6(II-III)-ER2	30-60 μ m	Membrane with no aggregation	12% of $\Delta F/F$ per 100 mV voltage step
Archon1-KGC-EGFP-Kv2.1-motif-ER2 (SomArchon)	20-40 μ m	Membrane with no aggregation	30% of $\Delta F/F$ per 100 mV voltage step
Archon1-KGC-EGFP-AnkMB(270)-motif-ER2	>100 μ m	Cytoplasmic with bright puncta in soma	Non-functional
Archon1-KGC-EGFP-AnkMB(490)-motif-ER2	>100 μ m	Cytoplasmic with bright puncta in soma	Non-functional
Archon1-KGC-EGFP-AnkSB-motif-ER2	>100 μ m	Cytoplasmic with bright puncta in soma	Non-functional
Archon1-KGC-EGFP-AnkCt-motif-ER2	50-70 μ m	Membrane with minor aggregation in soma	15% of $\Delta F/F$ per 100 mV voltage step
Archon1-KGC-EGFP-AnkSR-motif-ER2	40-60 μ m	Membrane with minor aggregation in soma	15% of $\Delta F/F$ per 100 mV voltage step
Archon1-KGC-EGFP-AnkTail-motif-ER2	>100 μ m	Cytoplasmic with bright puncta in soma	Non-functional

*voltage sensitivity was quantified by whole-cell patch clamp in cultured neurons

Amino acid sequences:

Linker:

GGSGGTGGSGGT

KA2(1-150)

MPAELLLLLLIVAFANPSCQVLSSLRMAAILDDQTVCGRGERLALALAREQINGIIEVPAK
ARVEVDIFELQRDSQYETTTDTMCQILPKGVS SVLGPSSSPASASTVSHICGEKEIPHIKVG
PEETPRLQYLRFASVSLYPSNEDVSLAVS

KA2(1-100)

MPAELLLLLLIVAFANPSCQVLSSLRMAAILDDQTVCGRGERLALALAREQINGIIEVPAK
ARVEVDIFELQRDSQYETTTDTMCQILPKGVS SVLGPSSSP

KGC:

KSRTSEGEYIPLDQIDINV

ER2:

FCYENEV

Na_v1.2(II-III):

SSFSSDNLAATDDDNEMNNLQIAVGRMQKGIDFVKRKIREFIQKAFVRKQKALDEIKPL
EDLNNKKDSCISNHTTIEIGKDLNYLKDGNGTTSIGIGSSVEKYVVDESDYMSFINNPSLT
VTVPIALGESDFENLNTEEFSSSESDMEESKEKLNATSSSEGSTVDIGAPAEGEQPEAEPEE
SLEPEACFTEDCVRKFKCCQISIEEGKGKLWWNLRKTCYKIS

Na_v1.6(II-III)

TVRVPIAVGESDFENLNTEDVSSESDP

K_v2.1-motif:

QSQPILNTKEMAPQSKPPEELEMSSMPSPVAPLPARTEGVIDMRSMSSIDSFISCATDFPE
ATRF

AnkMB(270)-motif (270-kDa AnkyrinG (1-837aa)):

MAHAASQLKKNRDLEINAEETEKKKKHRKRSRDRKKKSDANASYLRAARAGHLEKA
LDYIKNGVDVNICNQNLNALHLASKEGHVEVVSELLQREANVDAATKKGNTALHIAS
LAGQAEVVKVLVTNGANVNAAQSQNGFTPLYMAAQENHLEVVRFLLDNGASQSLATED
GFTPLAVALQQGHDQVVSLLLENDTKGKVRLPALHIAARKDDTKAAALLLQNDTNADI
ESKMVVNRATESGFTSLHIAAHYGNINVTALLNRAAAVDFTARNITPLHVASKRGNA
NMVKLLLDRGAKIDAKTRDGLTPLHCGARSGHEQVVEMLLDRAAPILSKTKNGLSPLH
MATQGDHLNVCVQLLLQHNVVPVDDVTNDYLTALHVAAHCGHYKVAKVLLDKKANPNA
KALNGFTPLHIACKKNRIRVMELLLKHGASIQAVTESGLTPIHVAAAFMGHVNIVSQLMH
HGASPNTTNVRGETALHMAARSGQAEVVRYLVQDGAQVEAKAKDDQTPLHISARLGK

ADIVQQLLQQGASPNAATTSGYTPLHLSAREGHEDVAAFLLDHGASLSITTKKGFTPLH
VAAKYGKLEVASLLLQKSASPDAAGKSGLTPLHVAAHYDNQKVALLLLDQGASPHAA
AKNGYTPLHIAAKKNQMDIATSLLEYGADANAVTRQGIASVHLAAQEGHVDMVSLLLS
RNANVNLSNKSGLTPLHLAAQEDRVNVAEVLVNQGAHVDAQTKMGYTPLHVGCHYG
NIKIVNFLQHS AKVNAKTKNGYTPLHQAAQQGHTHIINVLLQNNASPNELTVNGNTAL
AIARRLGYISVVDTLKVVTEEIMTTTTIT

AnkMB(490)-motif (490-kDa AnkyrinG (1-800aa))

MAHAASQLKKNRDLEINAEETEKKRKRHRKRSRDRKKKSDANASYLRAARAGHLEKA
LDYIKNGVDVNICNQNLNALHLASKEGHVEVVSSELLQREANVDAATKKGNTALHIAS
LAGQAEVVKVLVTNGANVNAQSQNGFTPLYMAAQENHLEVVRFLLDNGASQSLATED
GFTPLAVALQQGHDQVVSLLLENDTKGKVRLPALHIAARKDDTKAAALLLQNDTNAD
VESKSGFTPLHIAAHYGNINVATLLLNRAAAVDFTARNDITPLHVASKRGNANMVKLLL
DRGAKIDAKTRDGLTPLHCGARSGHEQVVEMLLDRSAPILSKTKNGLSPLHMATQGDH
LNCVQLLLQHNVPVDDVTNDYLTALHVAAHCGHYKVAKVLLDKKASPNAKALNGFTP
LHIACKKNRIRVMELLLKHGASIQAVTESGLTPIHVAAFMGHVNIVSQLMHHGASPNTT
NVRGETALHMAARSGQAEVVRYLVQDGAQVEAKAKDDQTPLHISARLGKADIVQQLL
QQGASPNAATTSGYTPLHLAAREGHEDVAAFLLDHGASLSITTKKGFTPLHVAAKYGKL
EVASLLLQKSASPDAAGKSGLTPLHVAAHYDNQKVALLLLDQGASPHAAAKNGYTPLH
IAAKKNQMDIATSLLEYGADANAVTRQGIASVHLAAQEGHVDMVSLLLSRNANVNLSN
KSGLTPLHLAAQEDRVNVAEVLVNQGAHVDAQTKMGYTPLHVGCHYGNIKIVNFLQ
HSAKVNAKTKNGYTALHQAAQQGHTHIINVLLQNNASPNELTVNGNTAL

AnkSB-motif (270-kDa AnkyrinG (801-1521aa)):

AIARRLGYISVVDTLKVVTEEIMTTTTITEKHKMNVPETMNEVLDMSDDEVKASAPEK
LSDGEYISDGEEGEDAITGDTDKYLGPQDLKELGDDSLPAEGYVGFSLGARSASLSRFSF
DRSYTLNRSSYARDSMMIEELLVPSKEQHLTFTREFDSDSLRHYSWAADTLDNVNLVSS
PVHSGFLVSFMVDARGGSMRGSRRHHGMRIIPPRKCTAPTRITCRLVKRHKLANPPPMVE
GEGLASRLVEMGPAGAQLGPVIVEIPHFGSMRGKERELIVLRSENGETWKEHQFDSKN
EDLAELLNGMDEELDSPEELGTKRICRIITKDFPQYFAVVSRIKQESNQIGPEGGILSSTTV
PLVQASFPEGALTKRIRVGLQAQPVPEETVKKILGNKATFSPIVTVEPRRRKFHKPITMTI
PVPPPSGEGVSNQYKGDATPNLRLLCITGGTSPAQWEDITGTTPLTFIKDCVSFTTNVSA
RFWLADCHQVLETVGLASQLYRELICVPYMAKFVVF AKTNDPVESSLRCFCMTDDRVD
KTLEQQENFEEVARSKDIEVLEGKPIYVDCYGNLAPLTKGGQQLVFNFYSFKENRLPFSI
KIRDTSQEPCGRSLFLKEPKTTKGLPQTAVCNLNITLPAHKKETESDQDDAEKADRRQSF
ASLALRKRYSYLTPSMKTVERSSGTARSLPTTYSHKPPFFSTRPYQSWTTAPITVPGPAKS
GSLSSSPSNTPSA

AnkCt-motif (270-kDa AnkyrinG (2334-2622aa))

RTDIRMAIVADHLGLSWTELARELNFSVDEINQIRVENPNLSISQSFMLLKKWVTRDGKN
ATTDALTSVLTKINRIDIVTLLEGPIFDYGNISGTRSFADENNVFHDVPDVGWQNETPSGSL
ESPAQARRLTGGLLDRLDDSSDQARDSITSYLTGEPGKIEANGNHTAEVIPEAKAKPYFP
ESQNDIGKQSIKENLKP KTHGCGRTEEPVSPLTAYQKSLEETSKLVIEDAPKPCVPVGMK

KMTRTTADGKARLNLQEEEGSTRSEPKQGEgykvTKKEIRNVEKKTH

AnkSR-motif (270-kDa AnkyrinG (1534 -1933aa))

SPLKSIWSVSTPSPIKSTLGASTTSSVKSISDVASPIRSFRTVSSPIKTVVSPSPYNPQVASGT
LGRVPTITEATPIKGLAPNSTFSSRTSPVTTAGSLLERSSITMTPPASPKSNITMYSSSLPFK
SIITSATPLISSPLKSVVSPTKSAADVISTAKATMASSLSPLKQMSGHAEVALVNGSVSPL
KYPSSSALINGCKATATLQDKISTATNAVSSVVSAAASDTVEKALSTTTAMPFSPLRSYVS
AAPSAFQSLRTPSASALYTSLGSSIAATTSSVTSSIITVPVYSVVNVLPALKKLPDSNSF
TKSAAALLSPIKTLTTETRPQPHFNRTSSPVKSSLFLASSALKPSVPSSLSSSQEILKDVAE
MKEDLMRMTAILQTDVPEEKPFQTDLP

AnkTail-motif (270-kDa AnkyrinG (1934-2333aa)):

REGRIDDEEPFKIVEKVKEDLVKVSEILKKDVCVESKGPPKSPKSDKGHSPEDDWTEFSS
EEIREARQAAASHAPSLPERVHGKANLTRVIDYLTNDIGSSSLTNLKYKFEEAKKDGEER
QKRILKPAMALQEHKLKMPPASMRPSTSEKELCKMADSFFGADAILESPDDFSQHDQDK
SPLSDSGFETRSEKTPSAPQSAESTGPKPLFHEVPIPPVITETRTEVVHVIRSYEPSSGEIPQS
QPEDPVSPKPSPTFMELEPKPTTSSIKEKVKAQFMKASSEEDHSRVLSKGMRVKEETHI
TTTTTRMVYHSPPGGECASERIEETMSVHDIMKAFQSGRDPSKELAGLFEHKSAMSPDVA
KSAAETSAQHAEKDSQMKPKLERIIEVHIEKGPQSPCE

Supplementary Table 2. Statistical analyses for **Fig. 1e** **Fig. 3f**, **Fig. 4** and **Extended Data Figs. 1, 3, 5, 8, 10**.

Two-sided Wilcoxon Rank Sum test for **Fig. 1e** between electrical and optical FWHM (full width, half maximum) of SomArchon-expressing neurons in mouse brain slices

FWHM	Cortex
p-value	0.0010
Wilcoxon rank sum statistic	131

FWHM	Hippocampus
p-value	0.0019
Wilcoxon rank sum statistic	40

FWHM	Striatum
p-value	0.0411
Wilcoxon rank sum statistic	26

Two-sided Wilcoxon Rank Sum test for **Fig. 3f** comparing striatal neurons' firing rate during periods of low speed movement vs. high speed movement

Cell	p-value	Wilcoxon rank sum statistic	zval
1	0.583	698.5	0.5497
2	0.072	451	-1.7989
3	0.043	417.5	-2.0238
4	2.27E-07	3303	-5.176
5	7.23E-05	2359	3.9684
6	0.525	1071	-0.6359
7	0.655	1086	-0.4468
8	0.0012	865.5	-3.228
9	0.815	1084.5	0.2336
10	0.219	1418.5	1.228
11	0.144	1075	-1.4617
12	0.0015	7239	3.1753
13	0.782	1296.5	-0.2768
14	1.32E-04	-3.8227	920.5

χ^2 test for neurons included in **Fig. 4a-d** testing against the null hypothesis of a uniform distribution. DOF is degrees of freedom.

Cell #	LFP			V_{mo}		
	p-value	χ^2 test statistic	DOF	p-value	χ^2 test statistic	DOF
1	1.58E-07	64.925	17	1.10E-07	65.849	17
2	0.0557	27.168	17	6.23E-40	232.946	17
3	0.619	14.670	17	2.23E-29	180.564	17
4	0.00117	40.308	17	4.14E-171	856.475	17
5	0.12	23.983	17	1.67E-49	279.756	17
6	0.51	16.190	17	1.72E-31	191.132	17
7	0.271	20.064	17	3.66E-20	133.677	17
8	0.618	14.688	17	1.11E-04	47.280	17
9	0.643	14.340	17	7.73E-13	94.909	17
10	0.718	13.263	17	3.92E-02	28.526	17
11	0.0263	30.000	17	1.14E-08	71.596	17
12	4.54E-06	56.109	17	1.00E-10	83.263	17
13	2.23E-34	205.508	17	2.40E-165	829.457	17
14	0.0636	26.641	17	5.47E-02	27.237	17
15	2.87E-05	51.073	17	1.46E-22	146.035	17
16	0.891	10.283	17	4.57E-04	43.146	17

Two-sample Kolmogorov-Smirnov (K-S) tests comparing fluorescence intensity of Archon1 vs. SomArchon at different positions along neurites in cortex, hippocampus, and striatum for **Extended Data Fig. 1i-k**)

Cortex

Distance along neurite from soma (μm)	p-value
5	0.0006
10	0.1451
15	0.0973
20	1.43E-05
25	3.81E-08
30	1.54E-09
35	1.22E-13

Hippocampus

Distance along neurite from soma (μm)	p-value
5	0.1543
10	1.54E-05
15	0.0007
20	5.83E-05
25	3.74E-06
30	2.53E-07
35	6.37E-08

Striatum

Distance along neurite from soma (μm)	p-value
5	0.1103
10	0.1672
15	0.5598
20	0.1542
25	0.0011
30	8.34E-05
35	5.48E-07

40	5.06E-15
45	3.14E-16
50	7.69E-16
55	4.91E-18
60	6.44E-17
65	8.50E-18
70	3.83E-17
75	3.83E-17
80	2.38E-17
85	3.30E-16
90	1.08E-15
95	1.19E-13
100	2.75E-12

40	6.37E-08
45	1.16E-08
50	1.73E-08
55	2.97E-09
60	5.89E-11
65	1.26E-11
70	2.59E-12
75	2.59E-12
80	2.59E-12
85	2.78E-11
90	2.78E-11
95	1.42E-11
100	4.42E-11

40	5.48E-07
45	1.65E-09
50	5.48E-07
55	1.27E-08
60	1.65E-09
65	1.65E-09
70	8.12E-07
75	1.96E-07
80	3.80E-08
85	6.90E-08
90	1.08E-08
95	1.30E-07
100	4.56E-08

Two-sided Wilcoxon Rank Sum test for the membrane resistance of SomArchon-expressing vs. Archon-expressing or negative neurons in mouse brain slices for **Extended Data Fig. 3a-c**.

Membrane Resistance	Cortex
p-value	0.8026
Wilcoxon rank sum statistic	103

Membrane Resistance	Hippocampus
p-value	0.6294
Wilcoxon rank sum statistic	68.5

Membrane Resistance	Striatum
p-value	0.7308
Wilcoxon rank sum statistic	52

Two-sided Wilcoxon Rank Sum test for the membrane capacitance of SomArchon-expressing vs. Archon-expressing or negative neurons in mouse brain slices for **Extended Data Fig. 3a-c**.

Membrane Capacitance	Cortex
p-value	0.8895
Wilcoxon rank sum statistic	111

Membrane Capacitance	Hippocampus
p-value	0.9720
Wilcoxon rank sum statistic	52

Membrane Capacitance	Striatum
p-value	0.8357
Wilcoxon rank sum statistic	51

Two-sided Wilcoxon Rank Sum test for the resting potential of SomArchon-expressing vs. SomArchon-expressing vs. Archon-expressing or negative neurons in mouse brain slices for **Extended Fig. 3a-c.**

Resting Potential	Cortex
p-value	0.8236
Wilcoxon rank sum statistic	103.5

Resting Potential	Hippocampus
p-value	0.8880
Wilcoxon rank sum statistic	65.5

Resting Potential	Striatum
p-value	0.7751
Wilcoxon rank sum statistic	39.5

Two-sided Wilcoxon Rank Sum test for the full width half maximum (FWHM) per action potential of SomArchon-expressing vs. non-expressing neurons in mouse brain slices for **Extended Data Fig. 3b,c.**

FWHM	Hippocampus
p-value	0.0037
Wilcoxon rank sum statistic	32

FWHM	Striatum
p-value	0.0931
Wilcoxon rank sum statistic	28

Two-sided Wilcoxon Rank Sum test for **Extended Data Fig. 5c**, the $\Delta F/F$ of SomArchon versus other voltage sensors (ASAP3-Kv, Voltron-ST, QuasAr3-s, and paQuasAr3-s) in mouse brain slices

$\Delta F/F$	ASAP3-Kv
p-value	6.75×10^{-6}
Wilcoxon rank sum statistic	178

$\Delta F/F$	Voltron-ST
p-value	9.25×10^{-6}
Wilcoxon rank sum statistic	106

$\Delta F/F$	QuasAr3-s
p-value	0.0022
Wilcoxon rank sum statistic	59

$\Delta F/F$	paQuasAr3-s
p-value	0.0045
Wilcoxon rank sum statistic	123

Two-sided Wilcoxon Rank Sum test for **Extended Data Fig. 5d**, SNR tested in mouse brain slices, between SomArchon and other voltage sensors (ASAP3-Kv, Voltron-ST, QuasAr3-s, and paQuasAr3-s).

SNR	ASAP3-Kv
p-value	0.0029
Wilcoxon rank sum statistic	218

SNR	Voltron-ST
p-value	0.1753

Wilcoxon rank sum statistic	173
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SNR	QuasAr3-s
p-value	1.07×10^{-4}
Wilcoxon rank sum statistic	46

SNR	paQuasAr3-s
p-value	0.1667
Wilcoxon rank sum statistic	153

Repeated measures ANOVA for Extended Data Fig. 8

Striatum Intensity	
p-value	0.023217
F	3.5861

post hoc Tukey's HSD test					
Trial	1	2	3	4	5
1		0.979088	0.600956	0.515978	0.36467
2	0.979088		0.440704	0.401273	0.214062
3	0.600956	0.440704		0.901844	0.003871
4	0.515978	0.401273	0.901844		0.1066
5	0.36467	0.214062	0.003871	0.1066	

Striatum SNR	
p-value	0.63726
F	0.64431

post hoc Tukey's HSD test					
Trial	1	2	3	4	5
1		0.907207	0.958312	0.999869	0.982537
2	0.907207		0.576767	0.705478	0.617842
3	0.958312	0.576767		0.831883	0.997305
4	0.999869	0.705478	0.831883		0.931505
5	0.982537	0.617842	0.997305	0.931505	

Striatum Firing Rate	
p-value	0.83742
F	0.35511

post hoc Tukey's HSD test					
Trial	1	2	3	4	5
1		0.055919	0.882805	1	0.98906
2	0.055919		0.999996	0.991153	0.944225
3	0.882805	0.999996		0.996383	0.95811
4	1	0.991153	0.996383		0.804642
5	0.98906	0.944225	0.95811	0.804642	

Hippocampus Intensity	
p-value	3.44E-22
F	21.491

post hoc Tukey's HSD test										
Trial	1	2	3	4	5	6	7	8	9	10

1		0.001593	0.04591	0.574795	0.688343	0.999087	1	1	0.93142	0.580117
2	0.001593		0.012262	2.57E-06	9.58E-05	9.54E-05	1.50E-05	0.000104	0.000122	0.000486
3	0.04591	0.012262		0.061443	0.000648	0.001063	8.37E-06	0.000308	0.000151	0.000585
4	0.574795	2.57E-06	0.061443		0.999964	0.923573	0.144261	0.223195	0.053445	0.055324
5	0.688343	9.58E-05	0.000648	0.999964		0.860278	0.004366	0.028524	0.004819	0.009799
6	0.999087	9.54E-05	0.001063	0.923573	0.860278		0.556941	0.270504	0.017902	0.040012
7	1	1.50E-05	8.37E-06	0.144261	0.004366	0.556941		0.95185	0.038815	0.102821
8	1	0.000104	0.000308	0.223195	0.028524	0.270504	0.95185		0.007861	0.14383
9	0.93142	0.000122	0.000151	0.053445	0.004819	0.017902	0.038815	0.007861		0.684809
10	0.580117	0.000486	0.000585	0.055324	0.009799	0.040012	0.102821	0.14383	0.684809	

Hippocampus SNR	
p-value	2.74E-17
F	15.65

post hoc Tukey's HSD test										
Trial	1	2	3	4	5	6	7	8	9	10
1		0.000291	0.000278	0.002971	1.02E-05	0.002021	0.000753	0.000878	0.000441	0.000109
2	0.000291		1	0.97404	0.999693	0.999988	0.999997	1	0.999996	0.947458
3	0.000278	1		0.455752	0.999813	1	0.999999	1	0.999994	0.878177
4	0.002971	0.97404	0.455752		0.343328	0.872355	0.453222	0.304404	0.176835	0.179375
5	1.02E-05	0.999693	0.999813	0.343328		1	1	0.999487	1	0.762035
6	0.002021	0.999988	1	0.872355	1		1	1	1	0.981192
7	0.000753	0.999997	0.999999	0.453222	1	1		0.999909	1	0.936697
8	0.000878	1	1	0.304404	0.999487	1	0.999909		0.99915	0.581095
9	0.000441	0.999996	0.999994	0.176835	1	1	1	0.99915		0.584717
10		0.000291	0.000278	0.002971	1.02E-05	0.002021	0.000753	0.000878	0.000441	0.000109

Hippocampus firing rate	
p-value	0.38894
F	1.07

post hoc Tukey's HSD test										
Trial	1	2	3	4	5	6	7	8	9	10
1		0.921965	0.944598	0.996829	0.70055	0.907368	0.992801	0.944117	0.959096	0.999999
2	0.921965		0.999999	1	0.994524	1	0.999985	1	1	0.973125
3	0.944598	0.999999		0.999876	0.999961	1	0.995943	0.999998	0.999875	0.896394
4	0.996829	1	0.999876		0.946252	0.99998	1	1	1	0.883532
5	0.70055	0.994524	0.999961	0.946252		0.991271	0.883536	0.986476	0.874771	0.601617
6	0.907368	1	1	0.99998	0.991271		0.995514	0.999999	0.999564	0.940647
7	0.992801	0.999985	0.995943	1	0.883536	0.995514		0.999995	0.999999	0.995133
8	0.944117	1	0.999998	1	0.986476	0.999999	0.999995		0.999999	0.951565
9	0.959096	1	0.999875	1	0.874771	0.999564	0.999999	0.999999		0.936649
10	0.999999	0.973125	0.896394	0.883532	0.601617	0.940647	0.995133	0.951565	0.936649	

Hippocampus spike amplitude	
p-value	9.9361e-07
F	5.7382

post hoc Tukey's HSD test										
Trial	1	2	3	4	5	6	7	8	9	10
1		0.28304	0.68783	0.68004	0.99142	1	0.99999	0.99998	0.99643	0.99777
2	0.28304		0.46636	0.99993	0.049266	0.020181	0.00056424	0.00079058	4.7664e-05	0.973
3	0.68783	0.46636		0.98407	0.41356	0.071376	0.0015959	0.0010367	4.797e-05	0.99967
4	0.68004	0.99993	0.98407		0.031141	0.022343	0.0013281	0.00019081	2.2154e-05	0.9435
5	0.99142	0.049266	0.41356	0.031141		0.51441	0.019361	0.0057928	0.0012401	1

6	1	0.020181	0.071376	0.022343	0.51441		0.11384	0.05668	0.061031	0.98715
7	0.99999	0.00056424	0.0015959	0.0013281	0.019361	0.11384		1	0.87884	0.79075
8	0.99998	0.00079058	0.0010367	0.00019081	0.0057928	0.05668	1		0.68336	0.63414
9	0.99643	4.7664e-05	4.797e-05	2.2154e-05	0.0012401	0.061031	0.87884	0.68336		0.4607
10	0.99777	0.973	0.99967	0.9435	1	0.98715	0.79075	0.63414	0.4607	

Hippocampus noise	
p-value	3.1718e-08
F	6.9714

post hoc Tukey's HSD test										
Trial	1	2	3	4	5	6	7	8	9	10
1		0.00035545	0.010149	0.0058873	0.0020157	0.022518	0.31433	0.10414	0.098306	0.011564
2	0.00035545		0.46828	0.029797	0.2885	9.255e-05	0.9993	0.090932	0.030078	0.70542
3	0.010149	0.46828		0.99018	0.99993	0.9636	1	0.95964	0.75254	0.39425
4	0.0058873	0.029797	0.99018		0.99932	1	0.99893	1	0.99922	0.22413
5	0.0020157	0.2885	0.99993	0.99932		0.99972	0.99983	0.99927	0.86987	0.18828
6	0.022518	9.255e-05	0.9636	1	0.99972		0.99824	1	0.99486	0.28566
7	0.31433	0.9993	1	0.99893	0.99983	0.99824		0.97896	0.89708	0.60837
8	0.10414	0.090932	0.95964	1	0.99927	1	0.97896		0.99913	0.2488
9	0.098306	0.030078	0.75254	0.99922	0.86987	0.99486	0.89708	0.99913		0.22088
10	0.011564	0.70542	0.39425	0.22413	0.18828	0.28566	0.60837	0.2488	0.22088	

One-way ANOVA for Extended Data Fig. 10

Neuron pairs coherence at theta frequency	
p-value	0.2638
F	1.44

post hoc Tukey's HSD test		
Distance 1	Distance 2	p-value
10-20 μm	20-30 μm	0.6252
10-20 μm	30-40 μm	0.2059
10-20 μm	40-50 μm	0.8327
20-30 μm	30-40 μm	0.6801
20-30 μm	40-50 μm	0.9960
30-40 μm	40-50 μm	0.6801

Note: groups of 50-60 μm and 60-70 μm only contains one data point each and therefore were excluded.

Neuron pairs coherence at gamma frequency	
p-value	0.1338
F	2.1

post hoc Tukey's HSD test		
Distance 1	Distance 2	p-value
10-20 μm	20-30 μm	0.9766
10-20 μm	30-40 μm	0.2314
10-20 μm	40-50 μm	0.9997
20-30 μm	30-40 μm	0.2387
20-30 μm	40-50 μm	0.9558
30-40 μm	40-50 μm	0.1978

Note: groups of 50-60 μm and 60-70 μm only contains one data point each and therefore were excluded.

Neuron pairs correlation	
p-value	0.4351
F	1.00

post hoc Tukey's HSD test		
Distance 1	Distance 2	p-value
10-20 μm	20-30 μm	0.7305
10-20 μm	30-40 μm	0.4389
10-20 μm	40-50 μm	0.4670
20-30 μm	30-40 μm	0.8979
20-30 μm	40-50 μm	0.8930
30-40 μm	40-50 μm	0.9997

Note: groups of 50-60 μm and 60-70 μm only contains one data point each and therefore were excluded.

Donut pairs coherence at theta frequency	
p-value	0.592
F	0.65

post hoc Tukey's HSD test		
Distance 1	Distance 2	p-value
10-20 μm	20-30 μm	0.9777
10-20 μm	30-40 μm	0.9855
10-20 μm	40-50 μm	0.8993
20-30 μm	30-40 μm	0.7781
20-30 μm	40-50 μm	0.5735
30-40 μm	40-50 μm	0.9706

Note: groups of 50-60 μm and 60-70 μm only contains one data point each and therefore were excluded.

Donut pairs coherence at gamma frequency	
p-value	0.1632
F	1.93

post hoc Tukey's HSD test		
Distance 1	Distance 2	p-value
10-20 μm	20-30 μm	0.9834
10-20 μm	30-40 μm	0.3051
10-20 μm	40-50 μm	0.4829
20-30 μm	30-40 μm	0.2604
20-30 μm	40-50 μm	0.5048
30-40 μm	40-50 μm	0.9942

Note: groups of 50-60 μm and 60-70 μm only contains one data point each and therefore were excluded.

Donut pairs correlation	
p-value	0.4086
F	1.02

post hoc Tukey's HSD test		
Distance 1	Distance 2	p-value
10-20 μm	20-30 μm	0.9668
10-20 μm	30-40 μm	0.9775
10-20 μm	40-50 μm	0.7918
20-30 μm	30-40 μm	0.6934
20-30 μm	40-50 μm	0.3725
30-40 μm	40-50 μm	0.9121

Note: groups of 50-60 μm and 60-70 μm only contains one data point each and therefore were excluded.

Supplementary Table 3. Performance of genetically encoded fluorescent voltage sensors in mouse brain tissues and live mice.

Sensor	Ex (nm)	Em (nm)	$\Delta F/F$ per AP (%)		SNR per AP		On kinetics ^a (ms)	Off kinetics ^a (ms)	Reported maximum number of simultaneously imaged neurons <i>in vivo</i>	Ref
			Slice	<i>In vivo</i>	Slice	<i>In vivo</i>				
MacQ-Citrine	516	529	-2.5 in neocortical pyramidal neurons, -0.5 in PV interneurons	-1.5 Purkinje neurons	6 in neocortical pyramids, 2 in PV interneurons at 30mW/mm ² , 440Hz camera frame rate	5-10 ^b Purkinje neurons at 10mW/mm ² , 190Hz	2.8 (74)/71 measured in HEK cells at 22°C	5.4 (77)/67 measured in HEK cells at 22°C	2 (Cerebellar Purkinje neuron dendrites only)	⁶
Ace2N-4AA-mNeon	506	517	-6.5 in cortex L2/3	-3 in cortex L2/3	ND	5-10 ^b in visual cortex L2/3 at 25mW/mm ² , 1kHz	0.37 (58)/5.5 measured in HEK cells at 22°C	0.5 (60)/5.9 measured in HEK cells at 22°C	2 (L2/3 primary visual cortex)	⁷
QuasAr2	637	700	15 in hippocampus	ND	8.5 in hippocampal neurons at 8W/mm ² , 1kHz	ND	0.3 (62)/3.2 in HEK cells at 34 °C	0.3/(73) 4.0 in HEK cells at 34 °C	ND	^{8,9}
QuasAr3	637	700	14 in cortex L2/3 ^c	ND	6.4 in cortical neurons at 1.5W/mm ² , 500Hz ^c	ND	1.2 (77)/10 in HEK cells at 34°C	0.87 (91)/9 in HEK cells at 34°C	ND	¹⁰

paQuasAr3	637	700	23 in cortex L2/3 ^c	ND	28 in cortical neurons at 1.5W/mm ² , 500Hz ^c	ND	0.9 (57)/15 in HEK cells at 34°C	0.93 (79)/15 in HEK cells at 34°C	4 spiking, 6 non-spiking (pyramidal cell layer hippocampus),	¹⁰
ASAP3	900-920 (2P)		-10 in L2/3 cortex under 1P at 500Hz ^c ; -17 in hippocampus under 2P, -8.3 in cerebellar molecular layer interneurons, -37.3 in striatum under 2P and 1kHz	-9 in cortex L1 and L2/3 under 2P	2.6 in striatum under 2P and 1kHz; 19 in L2/3 cortex at 25mW/mm ² at 500Hz ^c	ND	0.94 (72)/7.2 measured in HEK cells at 33-35°C	3.8 (76)/16 measured in HEK cells at 33-35°C	1 (primary visual cortex L1 and L2/3), 3 (primary visual cortex L5 apical dendrites)	¹¹
Voltron ₅₂₅	525	549	-6.5 in L2/3 cortex ^c	~-5-10 in visual cortex and PV interneurons in hippocampus ^b , ~-0.5-3 in layer 1 visual cortex interneuron population ^b	27 in L2/3 of cortex at 29mW/mm ² 500 Hz ^c	4.4 at 3-20mW/mm ² 400Hz	0.64 (61)/4.1 neuronal culture temp not reported	0.78 (55)/3.9 neuronal culture at 34°C	46 (primary visual cortex)	¹²
SomArchon	637	700	53 in cortex L2/3; 37 in hippocampus; 26 in striatum;	ND	37 in cortex L2/3 at 1.5W/mm ² , 500Hz; 20 in hippocampus at 1.5W/mm ² , 1kHz 27 in striatum at 1.5W/mm ² at	7 in motor cortex at 1.6W/mm ² , 7 in visual cortex at 1.6W/mm ² , 12 in striatum at 4W/mm ² , 16 in hippocampus at	0.61 (88)/8.1 ^d neuronal culture at 34°C	1.1 (88)/13 ^d neuronal culture at 34°C	18 (CA1 hippocampus)	This study

					1kHz	4W/mm ²				
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Ex – excitation wavelength;

Em – emission wavelength;

ND – not determined;

^avoltage kinetics evaluated by bi-exponential fitting (in the format fast/slow), where the value in parentheses represents the % of current magnitude in the fast τ component; ^bestimated from traces presented in the indicated paper; ^cdata from this study measured for soma localized version of the sensors, QuasAr3, paQuasAr3, Voltron₅₂₅, ASAP3, see **Extended Data Fig. 5** for the details; ^ddata from ref¹³.

Supplementary Table 4. Number of manually selected neurons during *in vivo* population imaging in the hippocampus of awake mice.

FOV	Neuron #	Spiking Neuron #	Session duration (s)	Acquisition Freq. (Hz)
Mouse1_FOV1	15	8	27	741
Mouse1_FOV2	9	8	27	741
Mouse1_FOV3	18	10	27	741
Mouse1_FOV4	11	9	27	741
Mouse1_FOV5	15	11	27	741
Mouse1_FOV6	18	12	27	741
Mouse1_FOV7	11	6	13.5	741
Mouse2_FOV1	12	8	24.2	826
Mouse2_FOV2	6	4	24.2	826
Mouse2_FOV3	11	9	24.2	826
Mouse2_FOV4	15	9	24.2	826
Mouse2_FOV5	14	7	24.2	826
Mouse2_FOV6	15	6	24.2	826