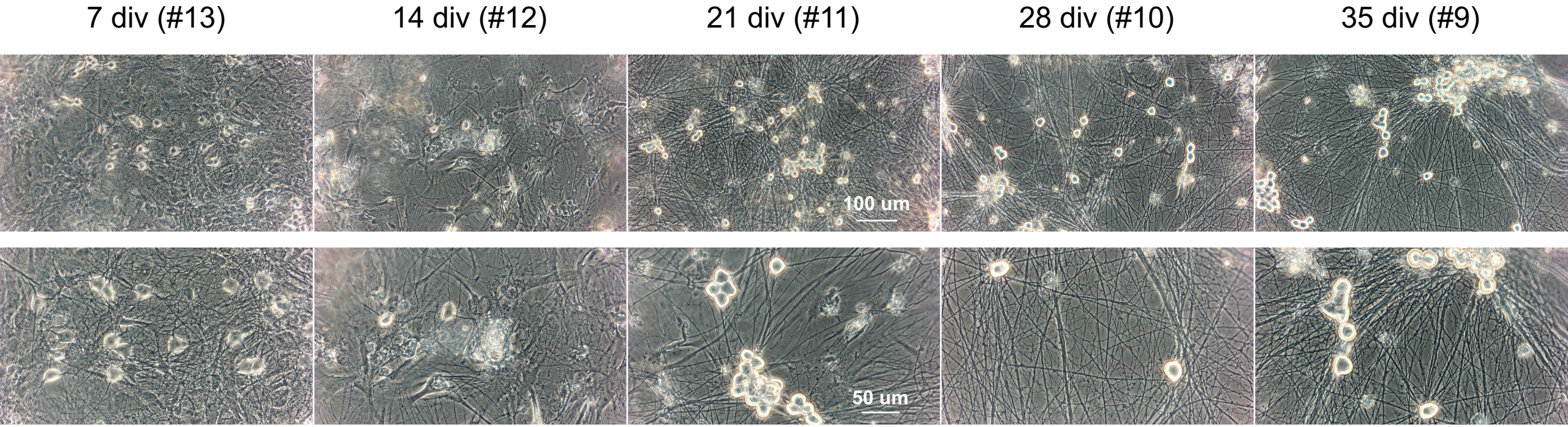
PATCH CLAMP

Characterization of Human iPSC-derived Neurons

neuroservices ALLIANCE

Axol sensory neurons plated at low density on astrocytes are improved

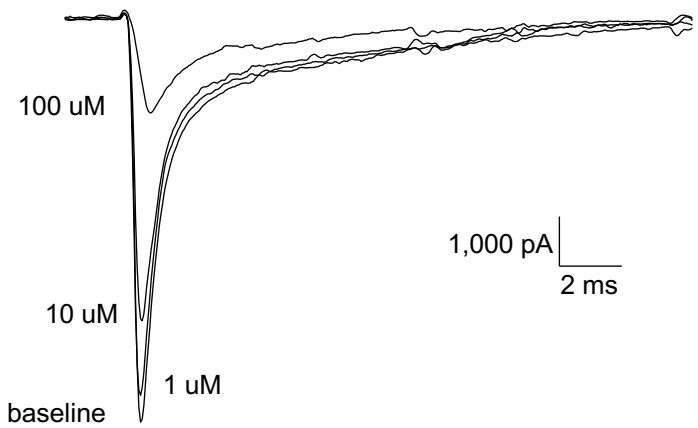


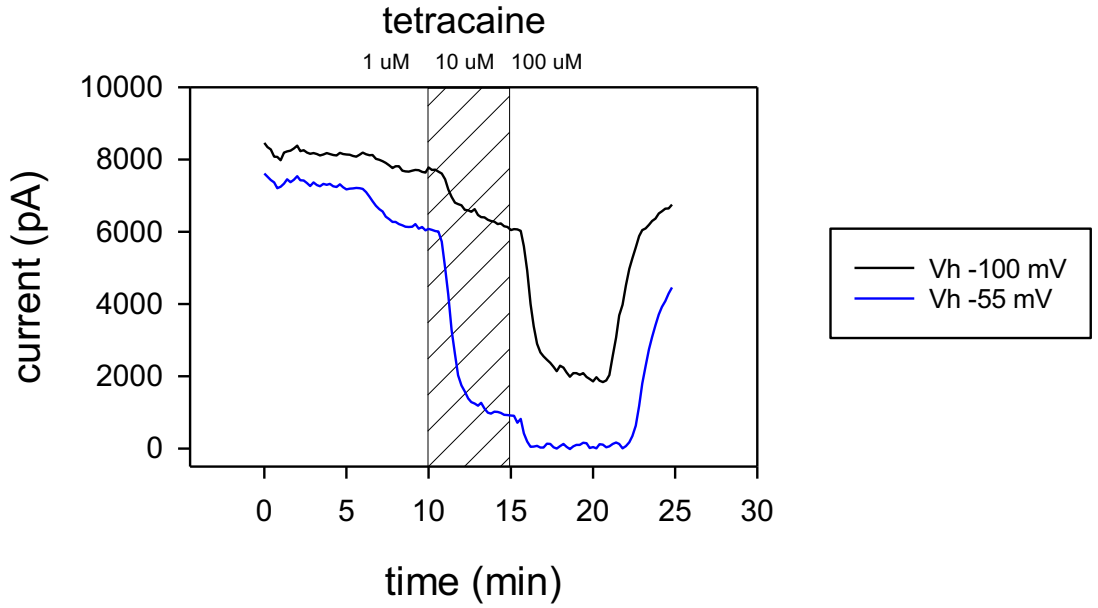
1. Axol sensory neurons plated at low density on astrocytes grew for >5 weeks without “balling up”.
2. Single cells could be readily recorded by patch clamp electrophysiology.

neuroservices ALLIANCE

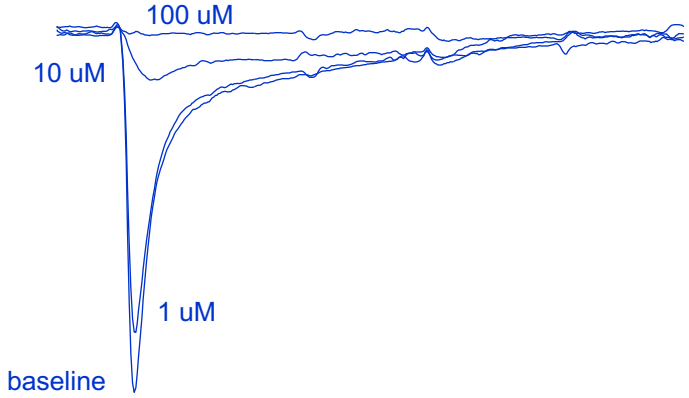
Tetracaine blocks Na currents in a voltage-dependent fashion

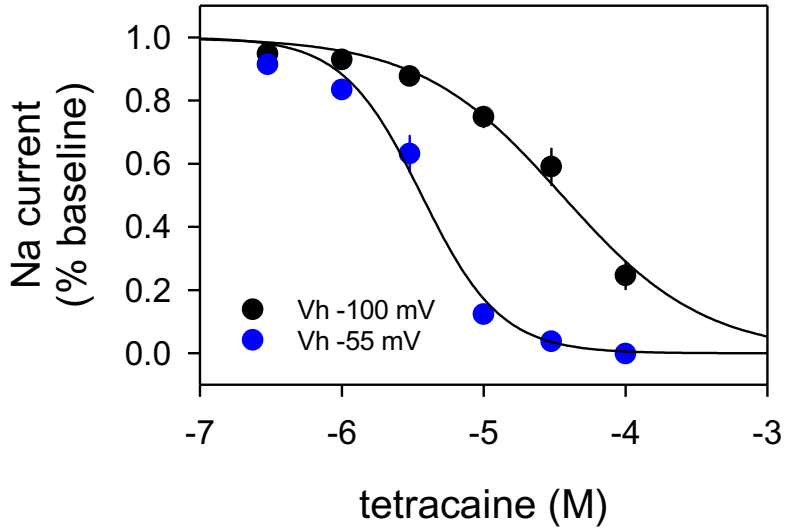
Vhold -100 mV





Vhold -55 mV

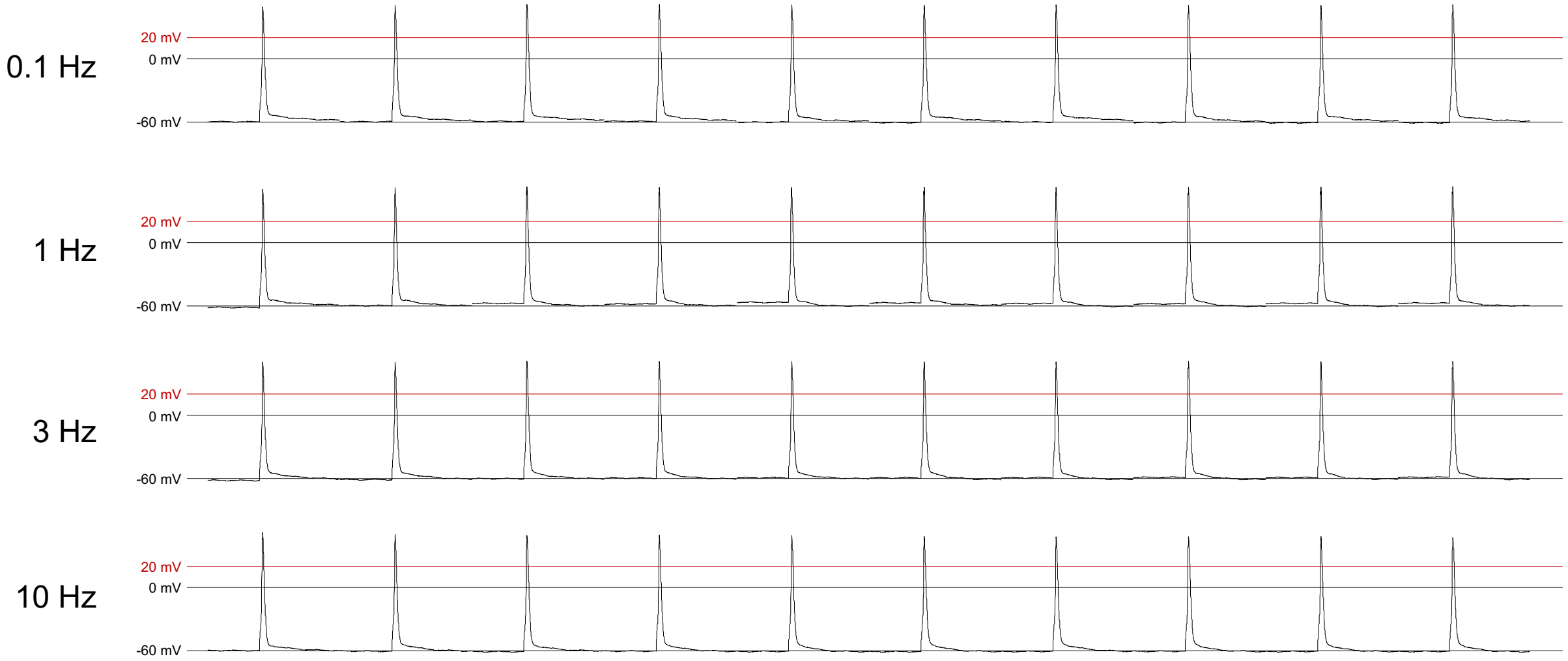




	IC50
Vh -100 mV	36 uM
Vh -55 mV	3.6 uM

A-2020-117-106

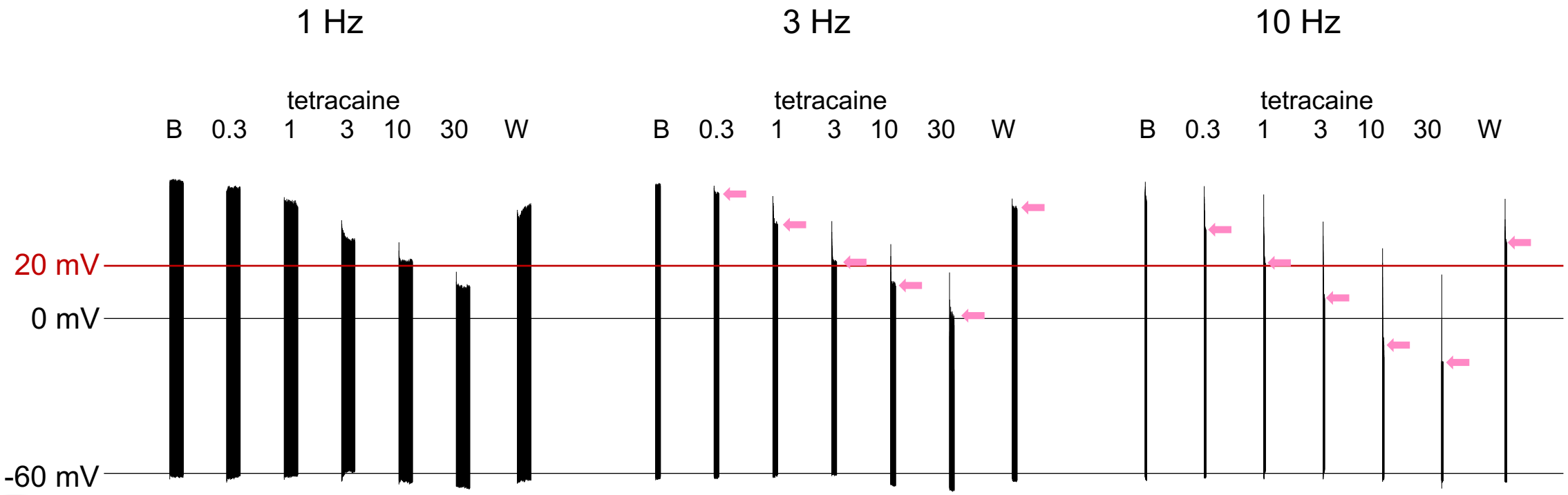
Axol hiPSC sensory neuron action potentials faithfully follow stimulation



A-2020-117-106

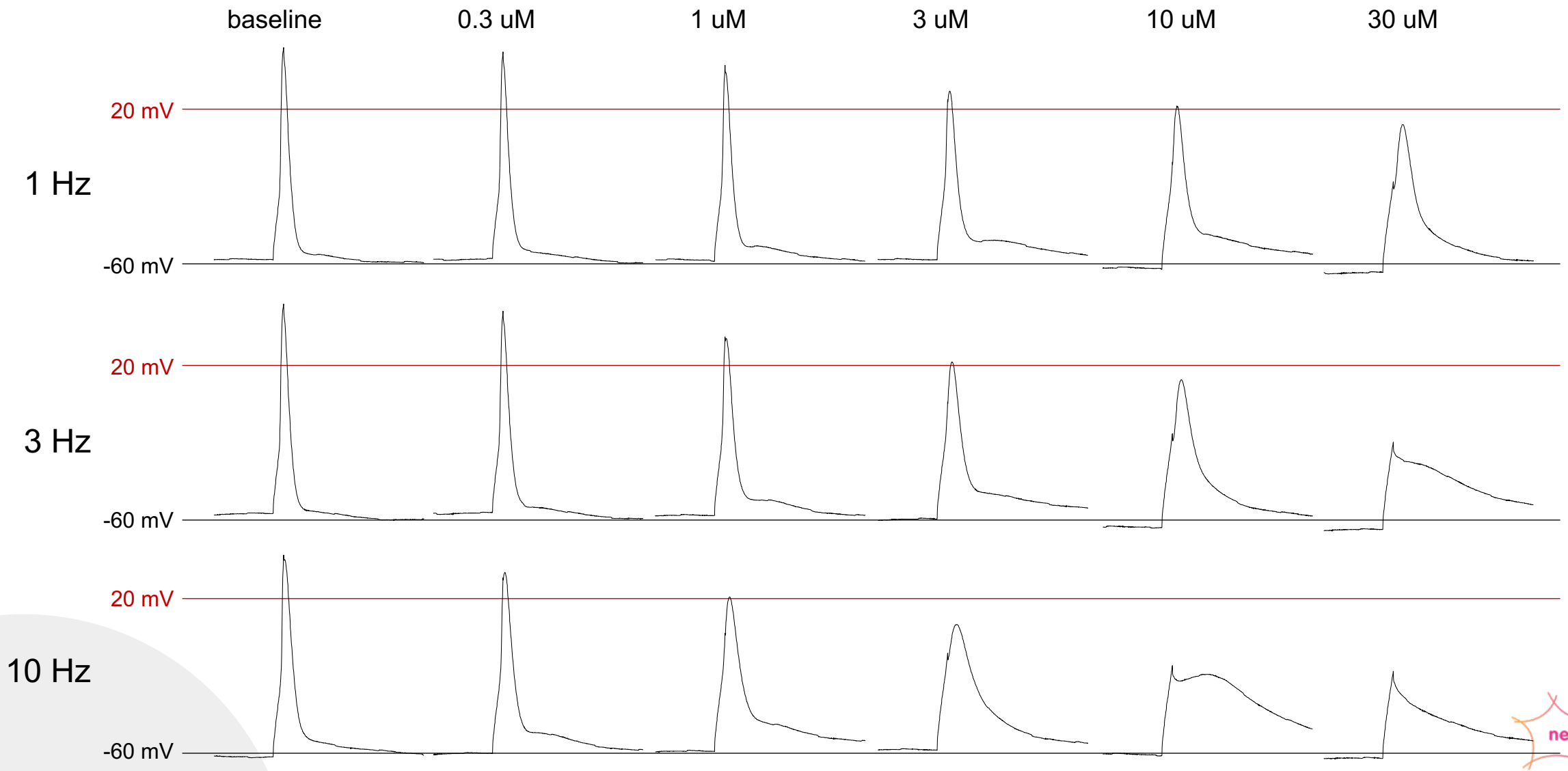
Tetracaine block of action potentials is frequency-dependent

100 action potentials elicited at each test concentration at each frequency



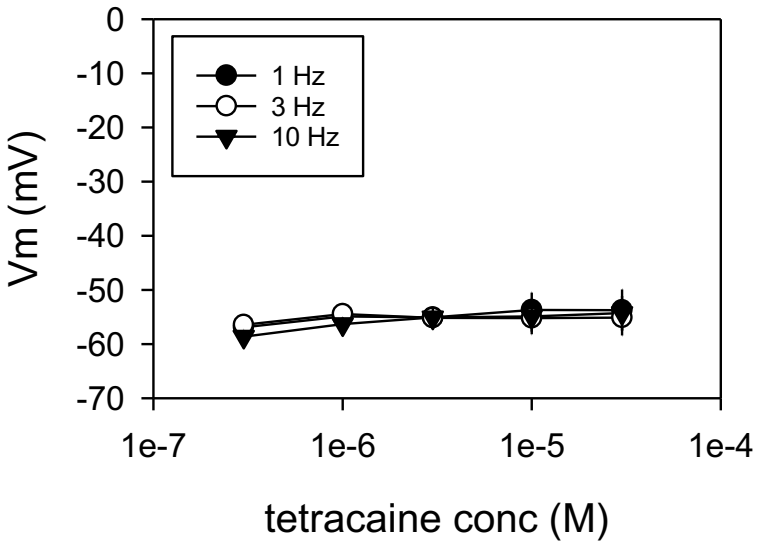
A-2020-117-106

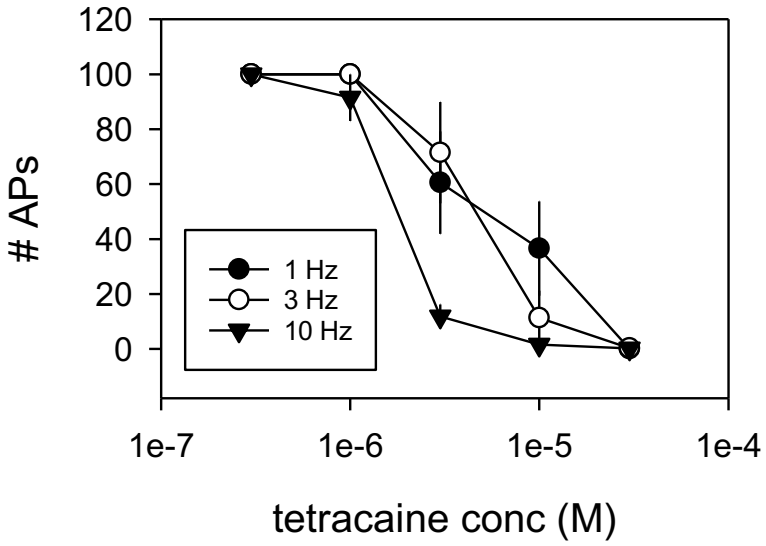
Tetracaine block of action potentials is frequency-dependent

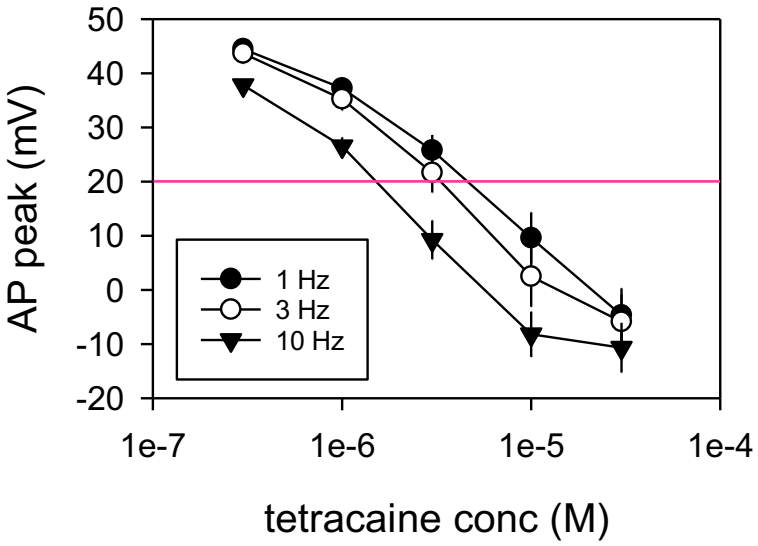


neuroservices ALLIANCE

Tetracaine block of action potentials is frequency-dependent







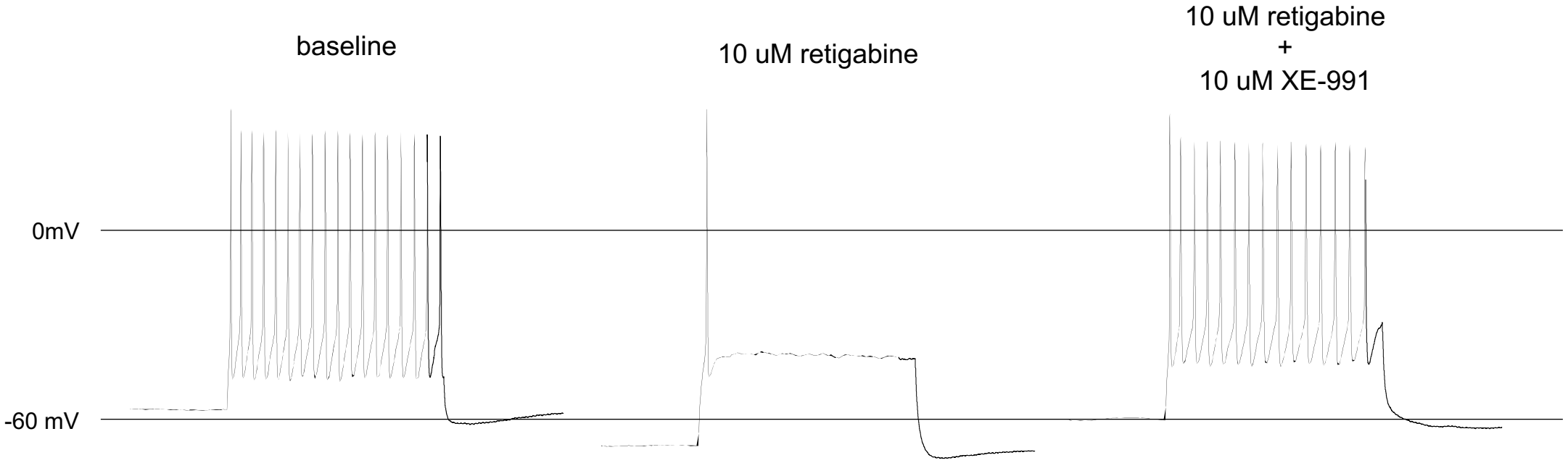
No effect on resting membrane potential

Reduced excitability at 10 Hz > 3 Hz > 1 Hz seen in 2 endpoints

1. Number of action potentials >+20 mV
2. Peak amplitude of action potentials

neuroservices ALLIANCE

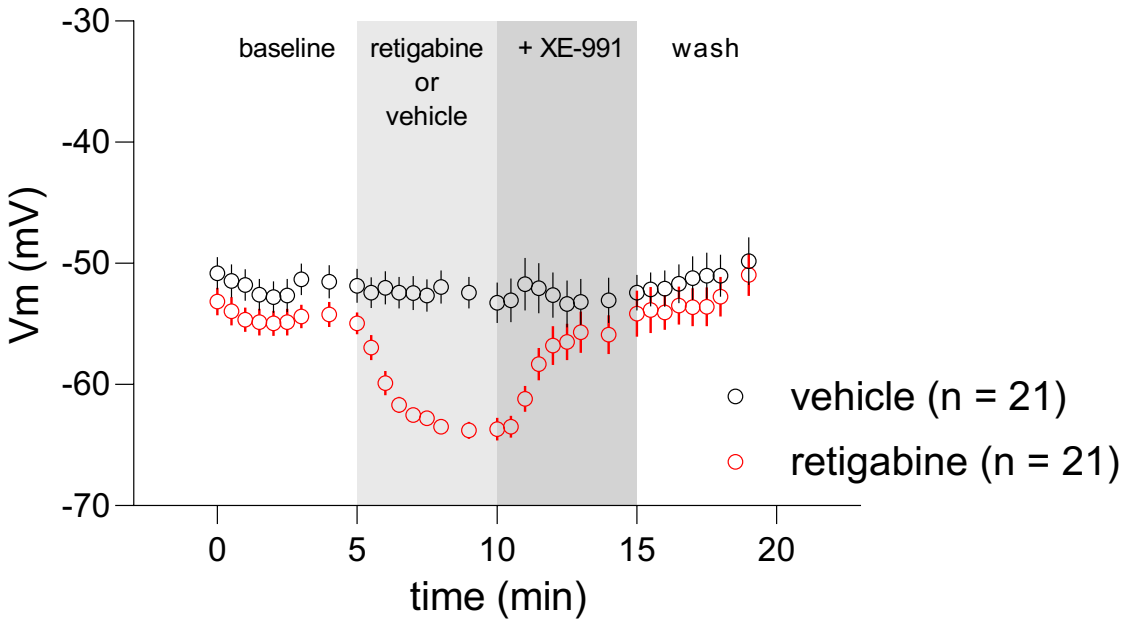
Retigabine hyperpolarized the resting membrane potential and decreased action potential firing in human iPSC-derived sensory neurons



A-2021-0316-103

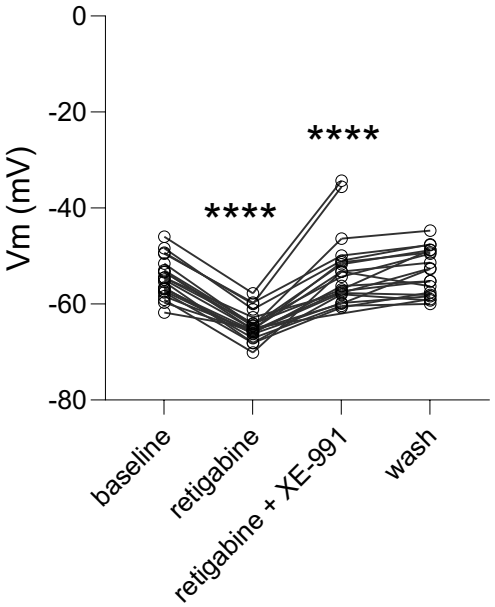
neuroservices ALLIANCE

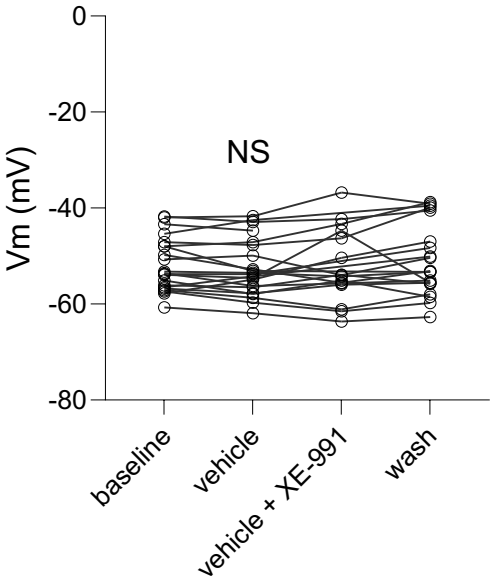
Retigabine hyperpolarized the resting membrane potential in human iPSC-derived sensory neurons



neuroservices ALLIANCE

Retigabine hyperpolarized the resting membrane potential in human iPSC-derived sensory neurons



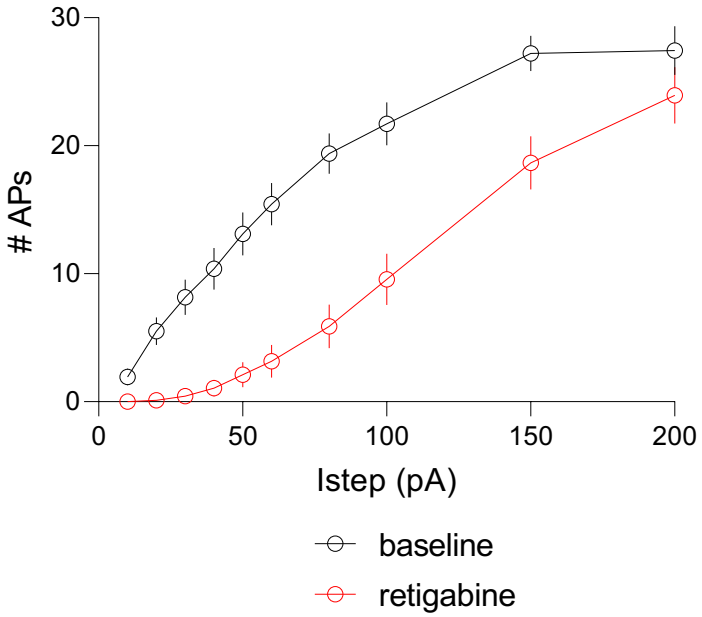


	baseline	retigabine	retigabine + XE-991	wash
n	21	21	19	19
average ± sem	-54.7 ± 0.9 mV	-64.7 ± 0.7 mV	-53.1 ± 1.7 mV	-53.3 ± 1.1 mV
p-value (paired t-test)		<0.0001 (vs baseline)	<0.0001 (vs retigabine)	

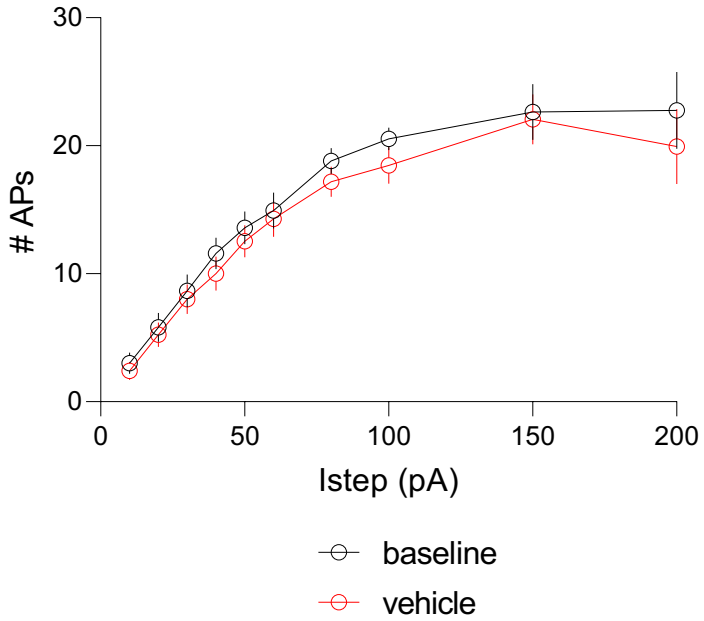
	baseline	vehicle	vehicle + XE-991	wash
n	21	21	16	20
average ± sem	-51.8 ± 1.2 mV	-52.5 ± 1.3 mV	-52.2 ± 1.9 mV	-50.7 ± 1.7 mV
p-value (paired t-test)		0.1411 (vs baseline)	0.1962 (vs vehicle)	

neuroservices ALLIANCE

Retigabine decreased action potential firing in human iPSC-derived sensory neurons



n = 18

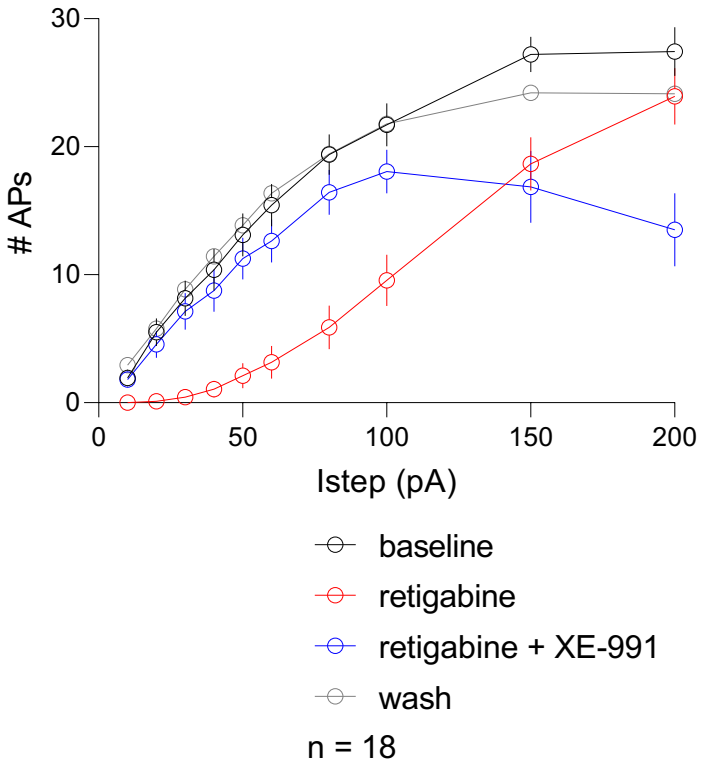


n = 17

neuroservices ALLIANCE

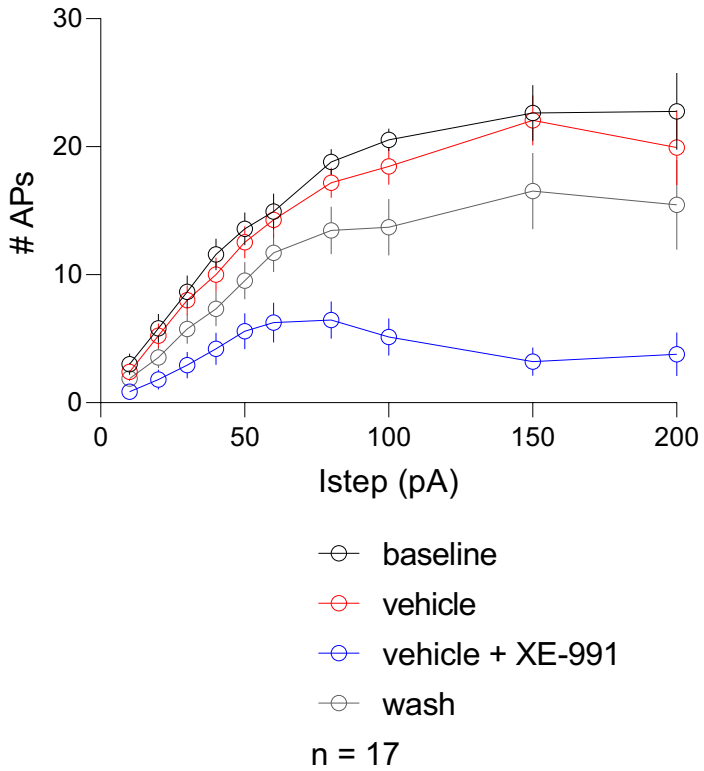
Retigabine decreased action potential firing in human iPSC-derived sensory neurons

XE-991 reversed the effect of retigabine



n = 18

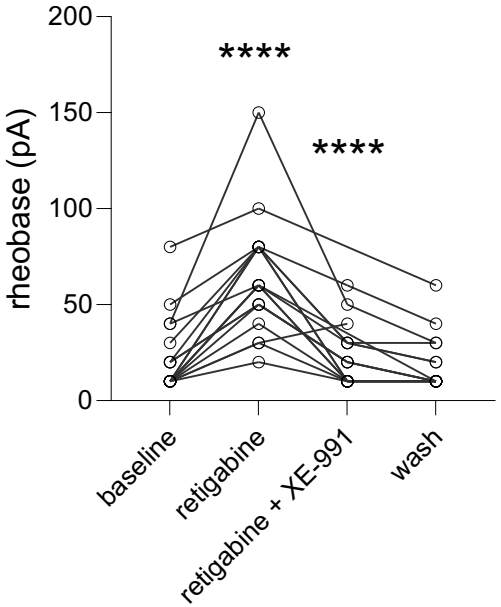
XE-991 “reduced” APs in vehicle treated cells?

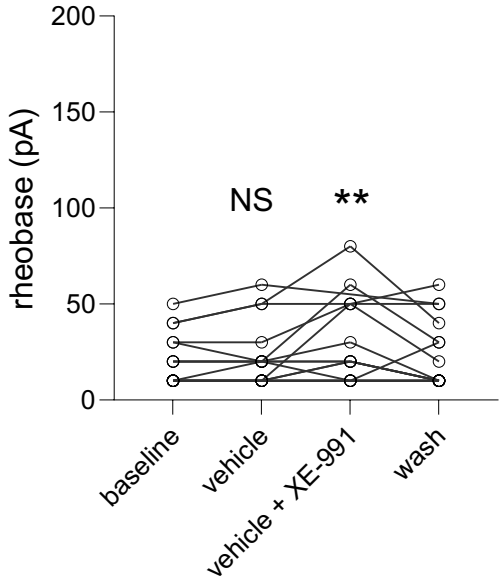


n = 17

neuroservices ALLIANCE

Retigabine increased the rheobase (current to elicit 1st action potential) in human iPSC-derived sensory neurons



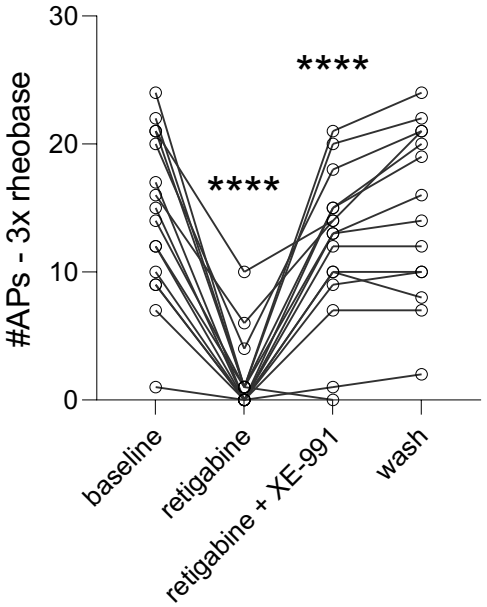


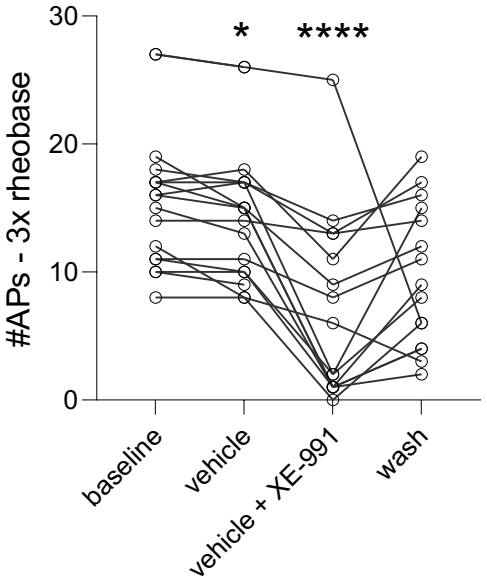
	baseline	retigabine	retigabine + XE-991	wash
n	18	18	16	14
average ± sem	22.2 ± 4.5 pA	64.4 ± 7.1 pA	23.1 ± 4.0 pA	18.8 ± 3.6 pA
p-value (paired t-test)		<0.0001 (vs baseline)	<0.0001 (vs retigabine)	

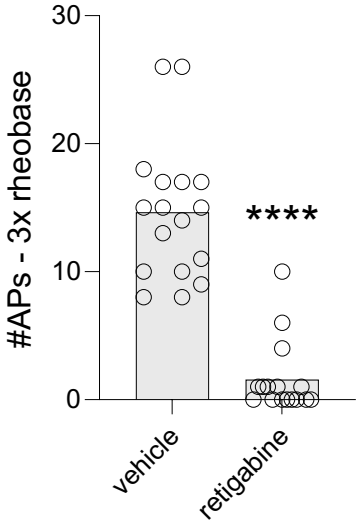
	baseline	vehicle	vehicle + XE-991	wash
n	17	17	15	17
average ± sem	20.0 ± 3.2 pA	21.8 ± 4.0 pA	30.0 ± 5.8 pA	22.4 ± 4.3 pA
p-value (paired t-test)		0.1876 (vs baseline)	0.0262 (vs vehicle)	

neuroservices ALLIANCE

Retigabine decreased the number of action potentials elicited by 3X rheobase in human iPSC-derived sensory neurons







	baseline	retigabine	retigabine + XE-991	wash
n	16	16	16	14
average ± sem	14.4 ± 1.6	1.6 ± 0.7	12.0 ± 1.6	14.7 ± 1.8
p-value (paired t-test)		<0.0001 (vs baseline)	<0.0001 (vs retigabine)	

	baseline	vehicle	vehicle + XE-991	wash
n	17	17	15	15
average ± sem	15.6 ± 1.3	14.7 ± 1.3	7.1 ± 1.8	9.7 ± 1.4
p-value (paired t-test)		0.0156 (vs baseline)	<0.0001 (vs vehicle)	

neuroservices ALLIANCE

Summary / Conclusions

1. Axol sensory neurons grow best when plated at low density on a monolayer of supportive astrocytes.
2. Axol sensory neurons are excitable (evoked action potentials) but exhibit little spontaneous activity (like human DRG sensory neurons).
3. Axol sensory neurons express sufficient TTX-S and TTX-R Na currents to support drug discovery of Nav1.7 and Nav1.8 inhibitors.
4. Axol sensory neurons were pharmacologically validated using:
   a) TTX
   b) tetracaine
   c) retigabine
5. **Human iPSC-derived sensory neurons from Axol reproduce many properties expected from bona fide DRG sensory neurons and may be useful for supporting drug discovery programs for pain.**

neuroservices ALLIANCE

Contact us

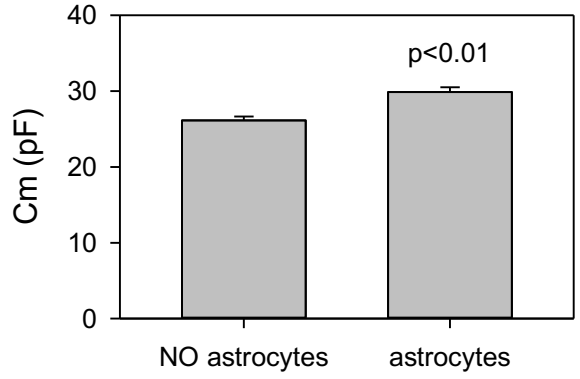
Raymond Price, PhD, EMBA
Chief Business Officer
Raymond.price@neuroservices-alliance.com
Mobile - +1 (858) 649 9403

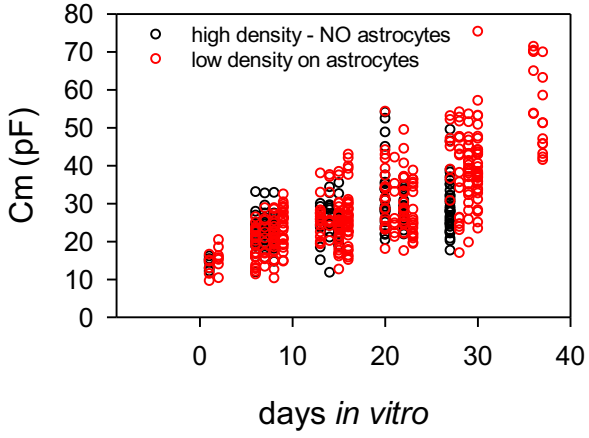
Bob Petroski, PhD
CSO, Cell Electrophysiology
Bob.petroski@neuroservices-alliance.com
Mobile - +1 (858) 774 4485

Annex

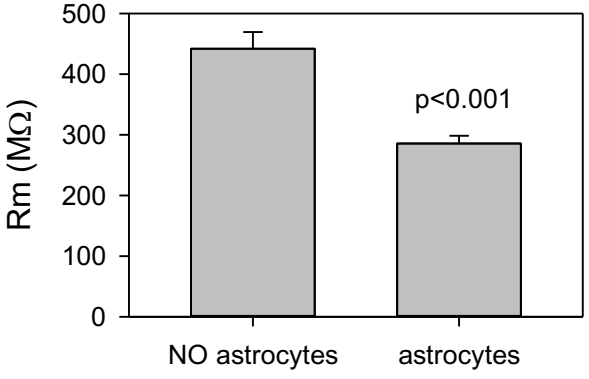
Additional Data

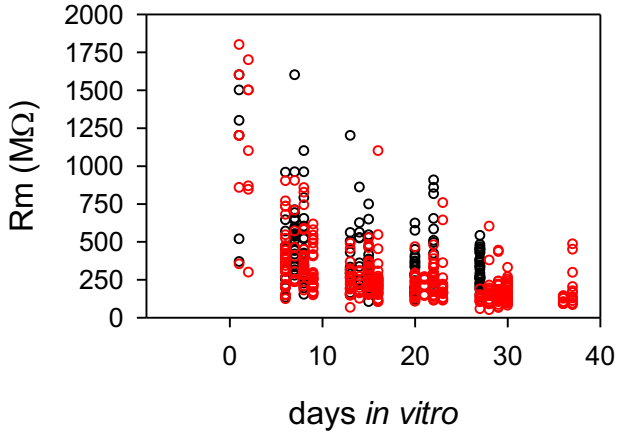
Cell Capacitance and Membrane Resistance





	Cm	Rm
Hi density w/o astrocytes	26.1 ± 0.5 pF (n=188)	442 ± 28 MΩ (n=187)
Low density on astrocytes	29.9 ± 0.6 pF (n=370)	286 ± 13 MΩ (n=370)



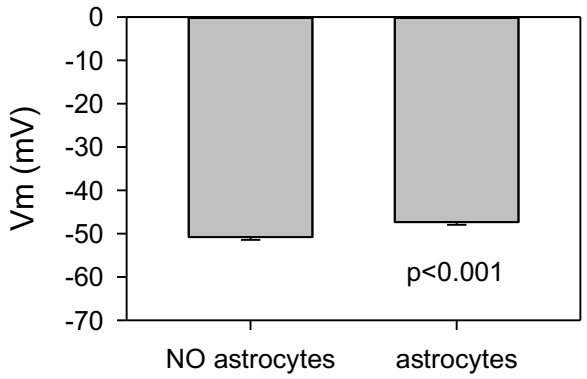


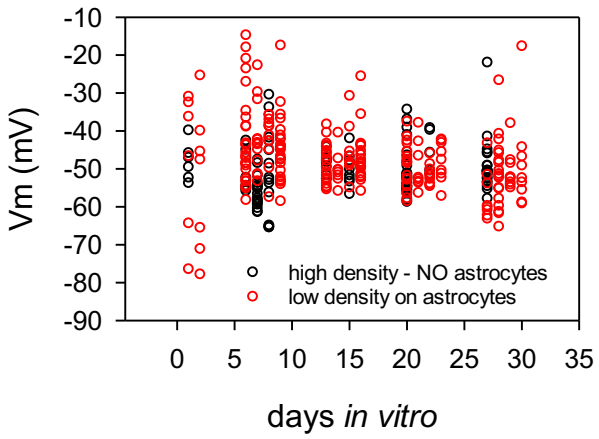
1. Axol sensory neurons grew larger with time in culture.
   - Increased Cm
   - Decreased Rm

2. Axol sensory neurons growing at low density on astrocytes were slightly, but significantly larger that cells plated at high density without astrocytes.

neuroservices ALLIANCE

Resting membrane potential (K internal)



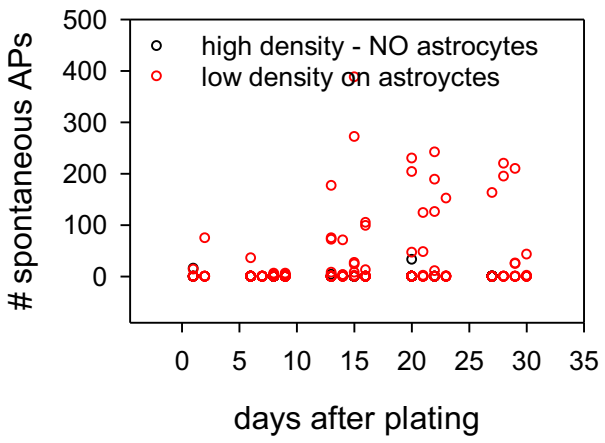


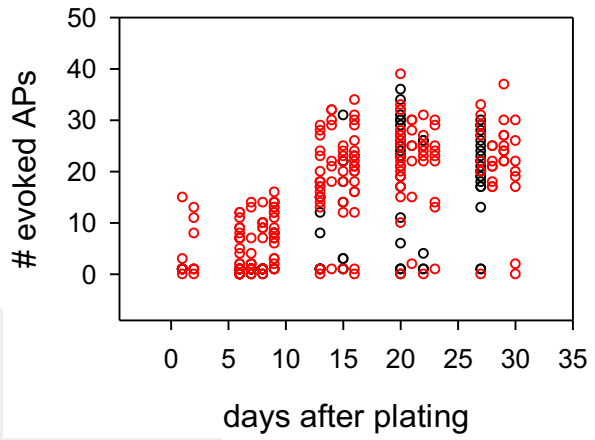
	Vm
Hi density w/o astrocytes	-50.8 ± 0.6 mV (n=119)
Low density on astrocytes	-47.4 ± 0.6 mV (n=236)

1. Axol sensory neurons exhibited a negative resting membrane potential (RMP) early after plating.
2. This suggests that the Na-K ATPase and K leak channels responsible for establishing the RMP are already expressed by neural precursors.
3. Further differentiation in culture did not result in more negative RMP.
4. There was no difference in Vm between cells plated at high density and cells plated at low density on astrocytes.

neuroservices ALLIANCE

Spontaneous and evoked action potentials (K internal)





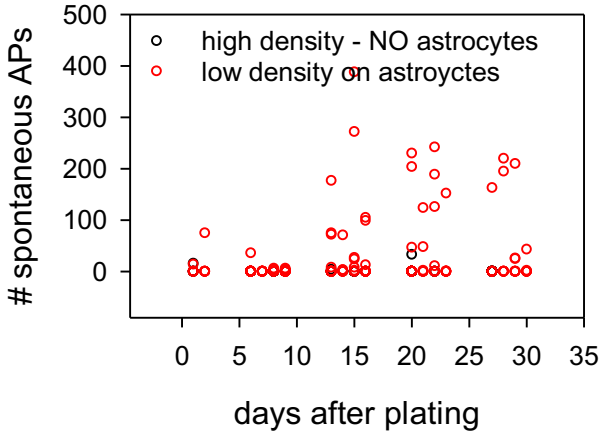
	# spontaneous APs in 1 min	# evoked APs in 1 sec
Hi density w/o astrocytes	0.7 ± 0.5 (n=80)	12.0 ± 1.1 (n=106)
Low density on astrocytes	17.2 ± 4.1 (n=178)	16.6 ± 0.7 (n=185)

INCLUSION CRITERIA: Vm <-40 mV

1. Axol sensory neurons exhibited very little spontaneous action potential firing.
2. More spontaneous activity was observed in cells plated on astrocytes.
3. Depolarizing current injection for 1 sec elicited only 1 action potential in most neurons in the first week in vitro.
4. However, the maximum number of action potentials elicited increased with time in culture.

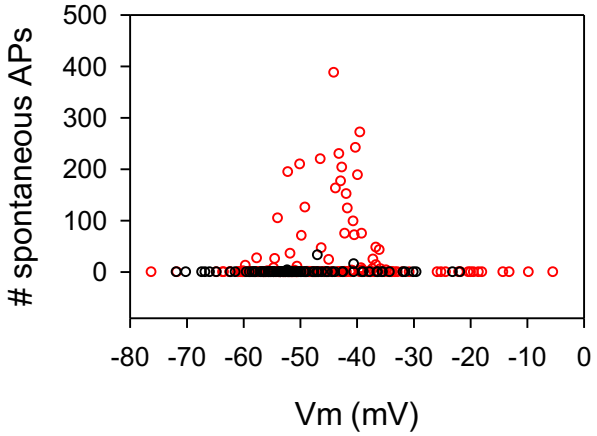
neuroservices ALLIANCE

Spontaneous action potentials (K internal)



	# cells NO spontaneous APs	# cells ≥1 spontaneous APs
Hi density w/o astrocytes	77	3
Low density on astrocytes	140	38

INCLUSION CRITERIA: Vm <-40 mV

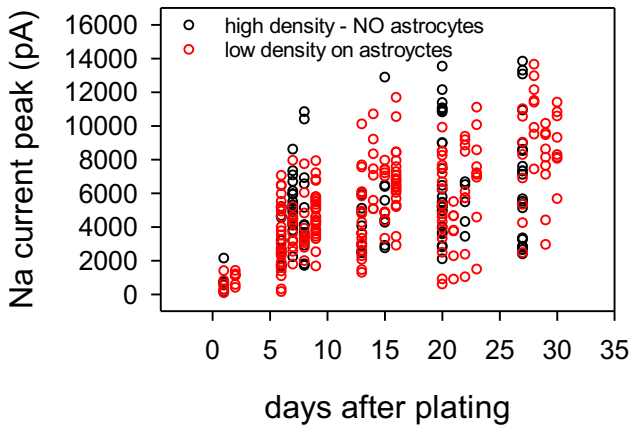


1. Axol sensory neurons exhibited very little spontaneous action potential firing.

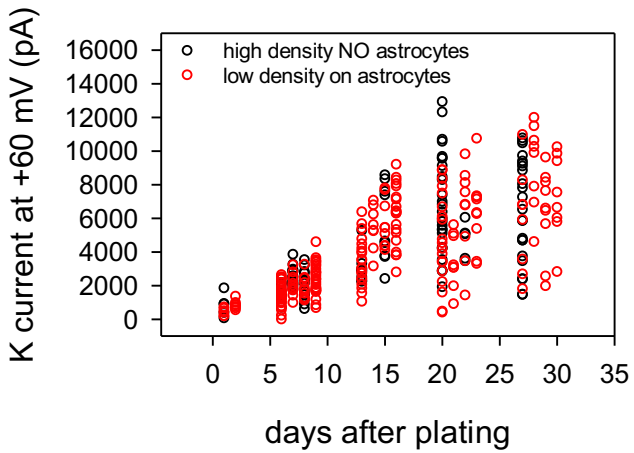
2. More spontaneous activity was observed in cells plated on astrocytes (21% vs 4%).

3. The cells that did exhibit spontaneous activity, fired APs at a high rate (average 81 spikes per min).

Na currents and K currents (K internal)



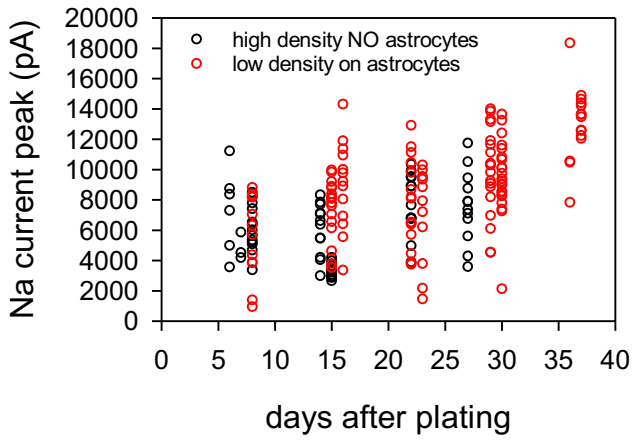
	Na current peak	K current at +60 mV
Hi density w/o astrocytes	5,482 ± 283 pA (n=116)	4,480 ± 283 pA (n=116)
Low density on astrocytes	5,347 ± 195 pA (n=224)	4,078 ± 187 pA (n=224)



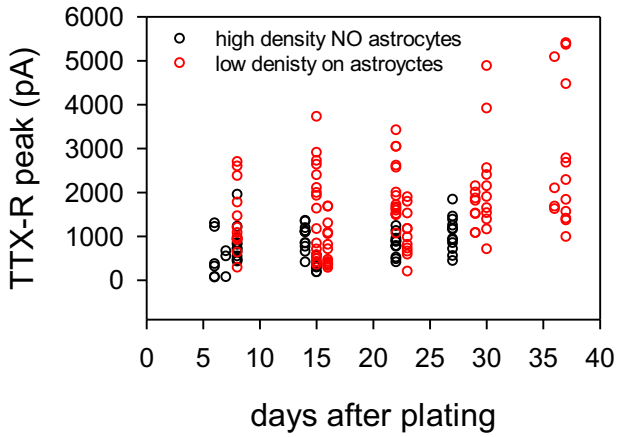
1. Axol sensory neurons exhibited large voltage gated Na and K currents.
2. Both Na current and K current amplitudes\s increased with time in culture.
3. There was no significant difference in the amplitudes of Na currents and K currents for cells plated at high density and cells plated at low density on astrocytes.

neuroservices ALLIANCE

Na currents (Cs internal)



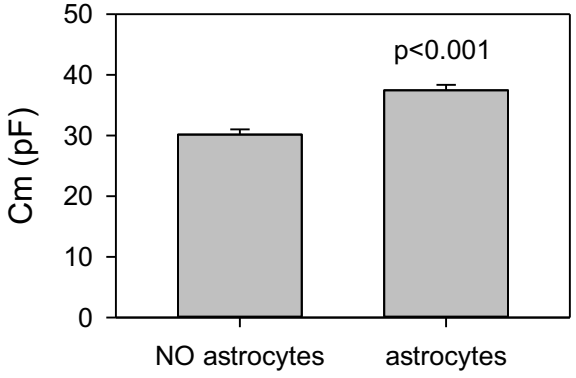
	Total Na current at -20 mV	TTX-R current at -20 mV
Hi density w/o astrocytes	6,292 ± 284 pA (n=67)	775 ± 51 pA (n=67)
Low density on astrocytes	8,630 ± 336 pA (n=104)	1,696 ± 109 (n=104)

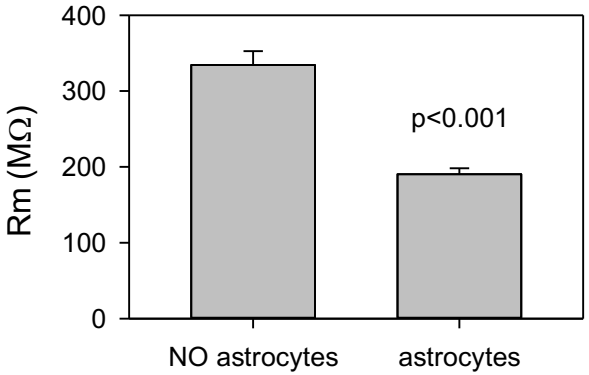


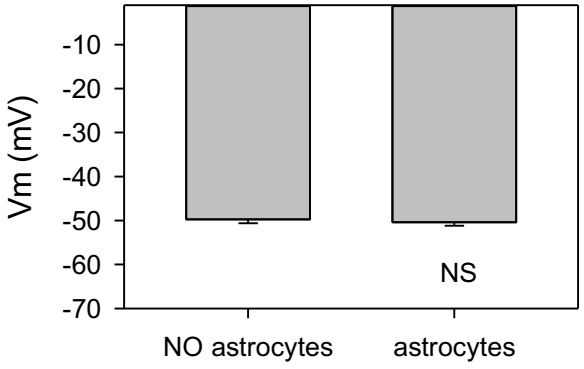
1. Axol sensory neurons exhibited large voltage gated Na currents.
2. 10-15% of the total Na current was resistant to TTX (0.5 uM).
3. Total and TTX-R Na currents amplitudes increased with time in culture.
4. Na and TTX-R Na currents amplitudes were larger in cells growing at low density on astrocytes.

neuroservices ALLIANCE

Axol cells >20 DIV: Capacitance and membrane resistance







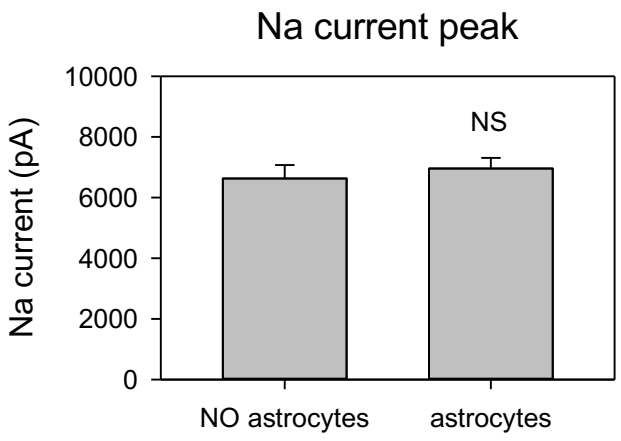
	Cm	Rm	Vm
Hi density w/o astrocytes	30.2 ± 0.8 pF (n=79)	335 ± 18 MΩ (n=78)	-49.7 ± 0.9 mV (n=55)
Low density on astrocytes	37.5 ± 0.9 pF (n=178)	190 ± 8 MΩ (n=178)	-50.4 ± 0.8 mV (n=89)

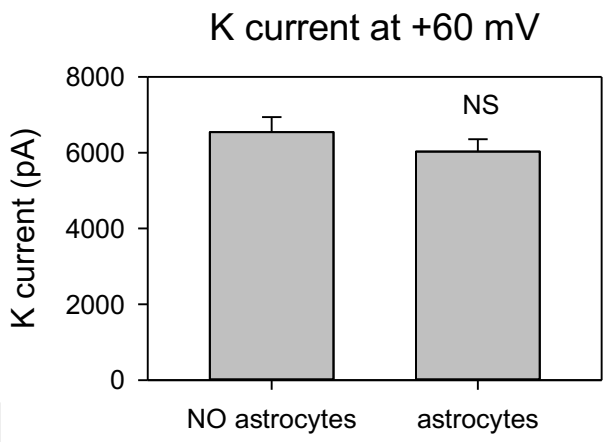
1. Axol sensory neurons growing at low density on astrocytes were significantly larger that cells plated at high density without astrocytes.

2. There was no difference in Vm between cells plated at high density and cells plated at low density on astrocytes.

neuroservices ALLIANCE

Axol cells >20 DIV: Na currents and K currents (K internal)





	Na current peak	K current at +60 mV
Hi density w/o astrocytes	6,629 ± 451 pA (n=54)	6,554 ± 398 pA (n-54)
Low density on astrocytes	6,961 ± 3561 Pa (n=78)	6,032 ± 324 pA (n=78)

1. Axol sensory neurons exhibited large voltage gated Na and K currents.

2. There was no significant difference in the amplitudes of Na currents and K currents for cells plated at high density and cells plated at low density on astrocytes.

neuroservices ALLIANCE