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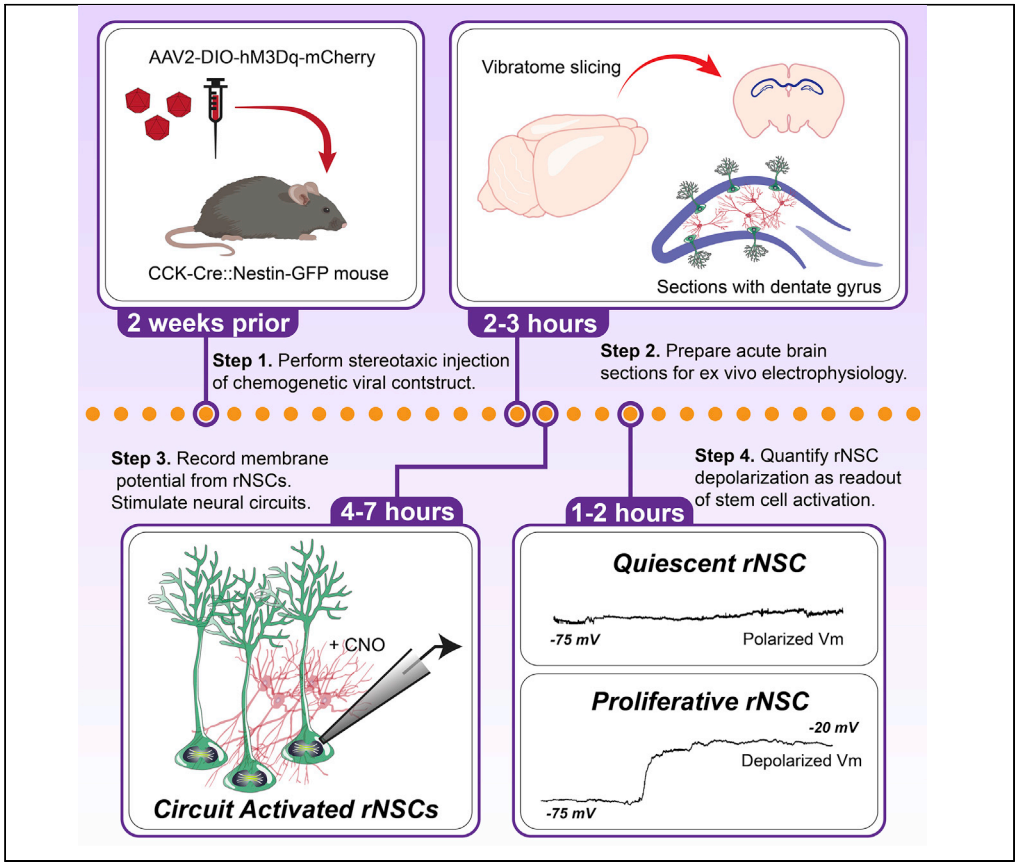
Recording membrane potential in adult neural stem cells as readout for stem cell activation following neural circuit stimulation in mouse hippocampal slices



The transition from quiescence to activation in radial neural stem cells (rNSCs) is a key first step in the process of neurogenesis and can be monitored in the context of circuit activation in live tissue by whole-cell patch-clamp electrophysiology in acute brain sections. However, membrane recordings in small non-neuronal cells such as rNSCs can be challenging. Here, we describe the preparation of materials, recording and stimulation protocols, and analysis procedures necessary to evaluate the efficacy of activity-dependent control of stem cell behavior.

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HIGHLIGHTS

In vivo delivery of AAV containing excitatory DREADD for circuit stimulation in mouse

Preparation of acute brain sections containing the hippocampal neurogenic niche

Electrophysiological recording of adult neural stem cells by whole-cell patch-clamp

Depolarization of membrane potential reflects proliferative state upon circuit activity

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Recording membrane potential in adult neural stem cells as readout for stem cell activation following neural circuit stimulation in mouse hippocampal slices

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SUMMARY

The transition from quiescence to activation in radial neural stem cells (rNSCs) is a key first step in the process of adult neurogenesis, which can be monitored in the context of circuit activation in live tissue by whole-cell patch-clamp recording of membrane potentials of rNSCs in acute brain slices. However, membrane recordings in small non-neuronal cells such as rNSCs can be challenging. Here, we describe the preparation of materials, recording and stimulation protocols, and analyses necessary to evaluate the efficacy of activity-dependent control of rNSC behavior.

For complete details on the use and execution of this protocol, please refer to Asrican et al. (2020).

BEFORE YOU BEGIN

Performing electrophysiological recordings from rNSCs is similar to standard techniques used for recording neurons. The protocol here makes some adjustments to typical electrophysiology methods, due to the smaller size and unusual membrane properties of rNSCs (such as low resistence, low capacitance, and non-spiking) as compared to typical mature neurons.

Preparation of laboratory animals, and viral reagents specific to the experiment should be planned in accordance with the research questions. The choice of an appropriate method of stimulating specific circuits is extensively discussed in the literature. Here, we describe use of a recombinant CCK-Cre mouse and viral delivery of floxed chemogenetic DREADD constructs (Armbruster et al., 2007), which we have found particularly useful, as fast time-locked stimulation protocols are not necessary nor recommended when recording non-neuronal cells that activate on much slower time scales.

Breeding of research animals

Timing: 2–6 months prior to rNSC recording

Most research laboratories interested in circuit-based regulation of neural stem cell activation likely already have ongoing breeding programs using transgenic mouse lines appropriate for their own research questions. We have described here one particular mouse line for Cre-dependent control of CCK interneurons, and one particular reporter line for visualizing the rNSCs and targeting for electrophysiology.

1. Obtain and cross stock mouse lines.

a. Obtain reporter mice for expressing GFP in rNSCs: such as Nestin-GFP (Jax).
b. Obtain recombinant mice for circuit-based manipulations.
    i. Several reliable Cre lines are available from Jax or other sources. CCK-Cre mice will be used in this example.

Alternatives: Alternative methods of circuit manipulation are available, including Flp lines as well as viral delivery systems utilizing specific promoters if that is preferred.

c. Cross Nestin-GFP mice with homozygous CCK-Cre breeders to get double transgenic CCK-Cre::Nestin-GFP pups.

2. Phenotyping or Genotyping
    a. Nestin-GFP pups may be phenotyped. Toe clippings at $p7^{+}$ can be placed under any upright or inverted microscope using a GFP filter set and identified by fluorescence intensity.
    b. CCK-Cre pups can be bred from homozygous parents. No genotyping is necessary. If homozygous parents are unavailable, standard PCR techniques will suffice.
    c. Animals ideally around 2–4 months of age of both sexes can be used for experiments. Earlier than 1 months of age, the Nestin-GFP^{+} cells are too dense to be selectively identifiable and may not be considered "adult" stem cells. Animals older than 6 months of age increases the difficulty of patching cells in acute brain slices but is not impossible.

Viral injection

Timing: 2 weeks prior to rNSC recording

3. AAVs are easily obtained from commercial sources and can be injected with high accuracy into brain regions of interest, such as into the hilus of the dentate gyrus.
    a. Obtain and dilute viral aliquots in PBS. In this protocol we used AAV2-hSyn-DIO-hM3Dq-mCherry (AddGene), which is a Cre-dependent version of an excitatory DREADD virus, which in our case will be expressed in CCK type interneurons.
    b. Inject viral constructs using stereotaxic coordinates AP −2.0 mm, ML ± 1.5 mm, DV −2.1 mm. If injecting into the dentate gyrus, it is important to use small volumes (~300 nL) and low titers (~1 × 1,011 viral genomes/mL) to avoid rNSC cell death and loss of neurogenic progeny (Johnston et al., 2020). See Figure 1A for a successful viral injection inducing selective expression of the DREADD construct in hilar CCK cells.

⚠ CRITICAL: Confirm accuracy of viral injection. Mistargeting of the hilus can easily result in unintended infection of CA3 pyramidal cells, or dentate granule cells. A practice injection is recommended in order to adjust the coordinates to ensure only hilar CCK cells become labeled. Injection of fluorescent beads, or a dye, such as Fast Green can quickly inform on anatomical localization. Infected cells can be seen after ~7–10 days post injection, and typically are used around 2 weeks post injection. Healthy cells should appear morphologically intact, with rounded soma. Expression of hM3Dq-mCherry can be punctate while still functional. Excessive blebbing may indicate toxicity.

Prepare stock solutions

Timing: 1 day prior to rNSC recording

4. Healthy brain slices require careful preparation of cutting solutions, recovery solutions, and recording solutions. These stocks can be prepared ahead of time, and then adjusted for osmolarity and pH on the day of recording.
    a. Prepare 200 mL of 1 molar stocks of:
        i. $MgSO_4$

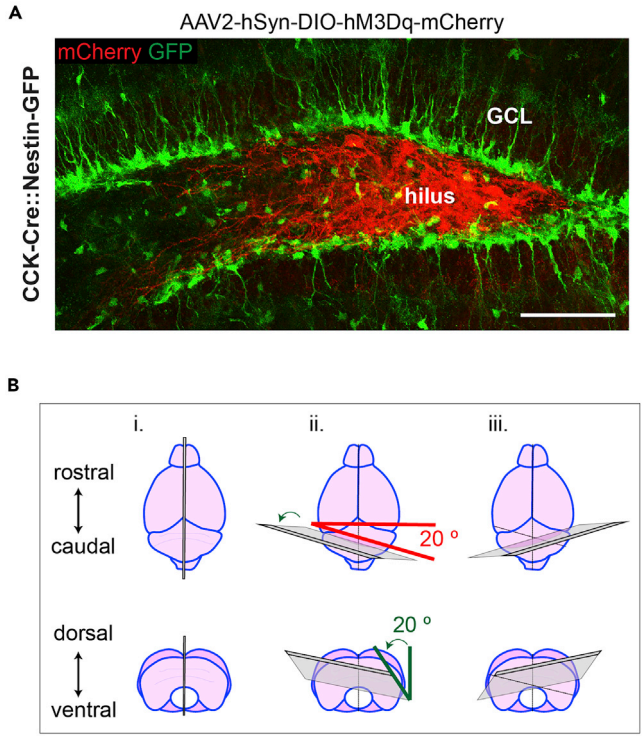

Figure 1. Successful viral expression and blocking technique to prepare brains for electrophysiological recordings of rNSCs

(A) Sample confocal image of an antibody-stained dentate gyrus expressing hM3Dq-mCherry (red) construct in the hilar CCK cells from a CCK-Cre::Nestin-GFP animal. rNSCs express GFP (green). GCL, granule cell layer. Scale bar, 100 μm. Image is reprinted with permission from Asrican et al. (2020).

(B) Views from the dorsal surface (top) and caudal viewpoint (bottom) of brain blocking in preparation for semi-transverse vibratome sectioning. This method is useful for preserving the laminar orientation along the region in interest within the hippocampal curvature. Adjustments can be made to optimize slice angles to align best with whichever particular stereotaxis coordinates were used during viral injection. (i) Bisect the brain along the midline using a standard single edged safety-razor. (ii) Make an angled cut about 20 degrees off from coronal (red angle) through the cerebellum and outer tip of cortex, with the blade tilted "inward" about 20 degrees from vertical (green angle). (iii) Repeat on opposite hemisphere. Gently glue the cut (cerebellar) surface to the vibratome plate for sectioning.

ii. $CaCl_2$
iii. $MgCl_2$

Salt stocks can remain at 22°C for several months but check for cloudiness or contamination. Remake as necessary.

b. Prepare the following solutions listed in the "Materials and equipment" section.
   i. 10× HEPES stock
   ii. 1× NMDG solution
   iii. 1× HEPES Solution
   iv. 10× ACSF Stock
   v. K^+ internal solution

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
AAV2-hSyn-DIO-hM3Dq-mCherry	Addgene	RRID: AddGene_44361

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
CNO	NIH	Cat# C-929
HEPES	Sigma	Cat# H3375
N-Methyl-D-glucamine (NMDG)	Sigma	Cat# M2004
Sodium ascorbate	Sigma	Cat# A7631
Thiourea	Sigma	Cat# T8656
Sodium pyruvate	Sigma	Cat# P5280
K-gluconate	Sigma	Cat# P1847
Ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA)	Sigma	Cat# 03777
Na_2ATP	Sigma	Cat# A6419
NaGTP	Sigma	Cat# G8877
Isoflurane	Covetrus	Cat# 029404
Experimental models: organisms/strains		
Mouse: CCK-IRES-Cre	Jackson Laboratory	RRID: IMSR_JAX:012706
Mouse: Nestin-GFP	Jackson Laboratory	RRID: IMSR_JAX:033927
Software and algorithms		
pClamp 10	Molecular Devices	RRID: SCR_011323
Micro-Manager	Micro-manager.org	RRID: SCR_000415
Igor Pro	Wavemetrics	RRID: SCR_000325
Neuromatic	Thinkrandom.com	RRID: SCR_004186
Other		
Semiautomatic vibratome slicer	Leica	Cat# VT1200S
Osmometer	Wescor	Cat# Vapro 5520
SliceScope recording microscope	Scientifica	Cat# SliceScope Pro 1000
40× water fluorescence objective	Olympus	Cat# LUMPLFLN40XW
Multiclamp 700B amplifier	Molecular Devices	Cat# Multiclamp 700B
DigiData 1440A digitizer	Molecular Devices	Cat# DigiData 1440A
RC-27LD ultra quiet imaging chamber	Warner	Cat# 64-1532
Slice anchor	Warner	Cat# 64-1551
Microelectrodes 1.5 mm	WPI	Cat# 1B150F-4
Dual stage microelectrode puller	Narishige	Cat# PC-10
Accuflow flow regulator infusion set	EMED Technologies	Cat# FLOW-100
Optimos sCMOS camera	Q-Imaging	Cat# 01-OPTIMOS-R-M-16-C
Slice holding chamber*	N/A	N/A

**Note:* While commercial slice holding chambers exist (Automate Scientific or Warner Instruments), it is simple enough to construct one from a 250 mL beaker with cut 30 mL syringe tubes affixed with nylon netting. See Ting et al. (2018) for example.

MATERIALS AND EQUIPMENT

- 10× HEPES stock (store at 4°C, lasts about 1 month).

Reagent	Final (1×) concentration (mM)	10× concentration (mM)	Amount for 1 L
KCl	2.5	25	1.86 g
NaH_2PO_4	1.25	12.5	1.50 g
$NaHCO_3$	30	300	25.2 g
HEPES	20	200	47.6 g
ddH_2O	-	-	bring the volume to 1 L

- 1× NMDG solution (for cutting; store at 4°C; lasts about 1 week). NMDG solution reduces excitotoxicity during the slicing procedure by replacement of sodium ions (Ting et al., 2014).

Reagent	Stock (mM)	Final concentration (mM)	Amount for 1 L*
10× HEPES stock	10×	1×	100 mL
NMDG		92	17,960 mg
D-Glucose		25	4,505 mg
Sodium ascorbate		5	990 mg
Thiourea		2	152.2 mg
Sodium pyruvate		3	330 mg
$MgSO_4$	1,000	10	10 mL
$CaCl_2$	1,000	0.5	0.5 mL
ddH_2O	-	-	*bring the volume to ~900 mL

⚠ CRITICAL: NMDG solution comes out very alkaline. Adjust the stock to about pH 7.5–8 using HCl. This typically requires about 10–12 mL of 6 N HCl. Final adjustments to pH will be made on day of recording.

**Note:* Prepare NMDG solution slightly overconcentrated (bring ddH_2O to about 900 mL when making this stock). Final adjustments to volume will be made on the day of recording based on osmolarity.

- 1× HEPES holding (for recovery; store at 4°C; lasts about 1 week) (Ting et al., 2014).

Reagent	Stock (mM)	Final concentration (mM)	Amount for 1 L*
10× HEPES stock	10×	1×	100 mL
NaCl		92	5,372 mg
D-Glucose		25	4,505 mg
Sodium ascorbate		5	990 mg
Thiourea		2	152.2 mg
Sodium pyruvate		3	330 mg
$MgSO_4$	1,000	2	2 mL
$CaCl_2$	1,000	2	2 mL
ddH_2O	-	-	*bring the volume to ~900 mL

**Note:* Prepare HEPES holding slightly overconcentrated (bring ddH_2O volume to only 900 mL when making this stock). Final adjustments to volume will be made on the day of recording based on osmolarity.

Reagent	Final (1×) concentration (mM)	10× concentration (mM)	Amount for 1 L
NaCl	125	1,250	73.05 g
KCl	2.5	25	1.863 g
NaH_2PO_4	1.25	12.5	1.499 g
$NaHCO_3$	26	260	21.84 g
D-Glucose	20	200	36.03 g
ddH_2O	-	-	bring the volume to 1 L

- 10× ACSF Stock (for recording; store at 4°C; lasts about 2 months).

Reagent	Final Concentration (mM)	Amount for 50 mL
K-gluconate	130	1,522.6 mg
NaCl	2	5.8 mg
$MgCl_2$	4	19.0 mg
HEPES	20	238.3 mg
Na_2ATP	4	110.2 mg
NaGTP	0.4	10.5 mg
EGTA	0.5	9.5 mg
ddH_2O	-	bring the volume to ~50 mL

- K^+ internal solution (for patch recording pipette)

Adjust pH to 7.24. Adjust osmolarity to 310 mOsm. It is recommended to bring the volume of water to slightly less than 50 mL at first, and adjust volume to necessary osmolarity. Aliquot 0.5 mL in centrifuge tubes and freeze (storage at −80°C is recommended for 1^+ years).

STEP-BY-STEP METHOD DETAILS

Acute brain slice preparation

Timing: 2.5–3 h

Preparation and careful sectioning of brain slices using a vibratome is key to preserving healthy tissue suitable for patching. Pay attention to the pH and osmolarity of solutions to avoid unnecessary cell death.

1. Final preparation of solutions. ~**25 min**
   a. Measure 180 mL of NMDG stock solution. Bubble with 95% O_2 and 5% CO_2 for > 10 min at 22°C.
   b. Measure 90 mL of HEPES holding solution. Bubble with 95% O_2 and 5% CO_2 for > 10 min at 22°C.
   c. pH the NMDG and HEPES solutions to 7.25 using HCl. Overshooting pH can be compensated with KOH (for NMDG) or NaOH (for HEPES holding). Avoid adding NaOH to NMDG solution to keep Na^+ concentration low.
   d. Adjust osmolarity to 300 mOsm.
      i. Measure the slightly overconcentrated solutions with an osmometer.
      ii. Reduce osmolarity by adding volumes of ddH_2O.
      For NMDG; volume needed (mL) ~= (Osm × 180/300 − 180) × 0.9
      For HEPES; volume needed (mL) ~= (Osm × 90/300 − 90) × 0.9
      iii. Typically, this will bring the total volume to 200 mL of NMDG, and 100 mL of HEPES.
   e. Place NMDG solution in −20°C or −80°C freezer until very cold (not frozen). This usually takes about 5–10 min.

2. Dissection and Sectioning ~**45 min**
   a. Setup dissection tools and vibratome and water bath.
      i. Bring water bath to 35°C.
      ii. Assemble useful tools including: large scissors (for removing head), small scissors (for cutting skin and skull), blunt forceps, spatula, single edge razor blade; tray and Styrofoam lid for pinning and dissection; syringe for cardiac perfusion.

iii. Pack vibratome outer-chamber with ice. Fill inner-chamber with 100 mL of the ice-cold NMDG solution. Bubble with 95% O_2 and 5% CO_2.
iv. Place remaining 100 mL of ice-cold NMDG in slice holding chamber and keep on ice. Bubble with 95% O_2 and 5% CO_2. Reserve a small amount (10 mL) for cardiac perfusion.

b. Anesthetize the research animal according to institutional requirements. Vaporized isoflurane will anesthetize the mice and slow the breathing rate. Animals should be alive, but non-responsive to serious toe pinch. An inverted 15 mL centrifuge tube containing a tissue moistened with additional isoflurane placed over the animal's snout will ensure a continued anesthetized state during cardiac perfusion procedure.

Alternatives: Sodium pentobarbital is a common alternative anesthetic.

c. Pin animal, expose heart, puncture right atrium and perfuse ~10 mL of ice-cold NMDG solution into left ventricle.
d. Quickly remove head, open skull, and remove brain directly into the ice-cold NMDG. Let sit for about 1 min.
e. Remove to a towel and bisect hemispheres. Block brain for semi-transverse sections (about 20 degrees from coronal and angled slightly off vertical, inward/downward) (see Figure 1B) with single edge razor blade.
f. Dab on towel and glue the cut cerebellar side to the vibratome plate. Insert plate into vibratome and orient the cortex toward the cutting blade to provide structural stability.
g. Slice at 280 μm thickness, using speed ~0.16–0.20 mm/s and amplitude of 1.6 mm. Slower sectioning while cutting through the hippocampus will preserve integrity of the hippocampal layers.
h. Gently collect sections containing hippocampus into the slice holder containing ice-cold NMDG using inverted/cut transfer pipette, or gentle paintbrush.

3. Warming ~10 min
   a. Transfer the NMDG containing slice holder with sections from the ice, into the 35 degree water bath.
   b. Continue bubbling in the water bath for 8–10 min. Do not over warm. It is recommended to shield from light to preserve fluorescence.
4. Recovery ~1.5 h
   a. Remove holder from water bath.
   b. Carefully discard the NMDG solution and replace with room-temperature (at 22°C), bubbled HEPES holding solution. Slices may either remain in the slice holding chamber during solution change or may be carefully transferred to a second container with HEPES. Ensure that sections are submerged and loosely separated. Continue bubbling at 22°C for 1–1.5 h. Take care that bubbling is not so vigorous as to cause turbulence/tumbling of the sections. Continue shielding from light with sheets of aluminum foil.

⚠ CRITICAL: All materials and equipment involved in the preparation of acute brain slices must not have come in contact with fixative (paraformaldehyde or equivalent). Reserve glassware, dissection tools, and vibratome for "live-tissue only." Do not share equipment with lab-mates that use fixative or other harsh chemicals, as it will seriously compromise your tissue. It is recommended not to use detergents or use department dishwashers. Thorough rinsing in water and scrubbing with reserved glassware brush will suffice. Brief application of 70% ethanol may be used to keep bacteria from growing on tools or slice chambers.

Patch recording

Timing: 4–7 h

The bioelectric state of the cellular membrane has been shown to control cell behavior and proliferation in many cell types through regulation of ion channels or pumps in the plasma membrane (Aprea and Calegari, 2012; Levin, 2014). Cells undergoing division events require depolarization for the division process and will otherwise remain hyperpolarized during the non-division parts of the cell cycle. In Nestin-GFP$^+$ rNSCs, electrophysiological patch recordings during chemogenetic stimulation allows monitoring of the membrane potential (Vm) as a readout of the stem cell activation state. This section assumes readers have some general knowledge of the patch-clamp technique and will therefore highlight specificities for recording rNSCs.

5. Final preparation of 1× ACSF recording solution
   a. Combine in ddH_2O.

Reagent	Stock (mM)	Final concentration (mM)	Amount for 500 mL
10× ACSF stock	10×	1×	50 mL
$CaCl_2$	1,000	2	1,000 µL
$MgCl_2$	1,000	1.3	650 µL
ddH_2O	-	-	bring the volume to 500 mL

   b. Bubble for at least 10 min at 22°C with 95% O_2 and 5% CO_2.
   c. pH to 7.24.
   d. Adjust osmolarity to ~305 mOsm.

6. Identify cells for recording.
   a. Place a single slice in upright microscope recording chamber (actively perfused with bubbled ACSF). Secure with a slice anchor.
   b. Locate dentate gyrus and confirm presence of GFP$^+$ rNSCs and mCherry$^+$ virally targeted CCK neurons containing DREADD construct. See Figure 1A.
   c. Using a 40× water dipping objective, identify the small GFP$^+$ cells that contain radial processes that extend into the granule cell layer. Avoid enlarged and overtly rounded cells which may be proliferating progenitor cells, rather than rNSCs. Cells closest to the interface of hilus and granule cell layer are best.
   d. Avoid cells directly on the surface of the slice, which may be damaged or cut.
   e. Switching back and forth from fluorescence to DIC imaging modalities will help locate the soma of a rNSC that can be patched.

7. Patch a rNSC. See Figure 2A.
   a. Pull 1.5 mm capillary glass to 4.5–6.5 Mohm resistance when filled with the K-gluconate based internal recording solution.

   Note: The rNSCs are small, and therefore the user will need to pull microelectrode tips slightly smaller than typically used for neuronal recordings.
   b. Approach cells under DIC illumination with moderate positive electrode pressure to pass through the granule cell layer, and then reduce to weak positive pressure when in proximity to the cell of interest. rNSCs do not always form an obvious dimple in the membrane as a neuron might, but they are somewhat hardy, and will withstand slight touches from the pipette tip itself. Cells that shift entirely when nudged are probably dead and have overly hardened membranes or shriveled appearance.
   c. Zero the offset, apply very weak suction for 2–4 s while adjusting the holding potential in order to form a seal. Gohm seals should form quickly in most circumstances. Do not over suction, as the cellular material is very limited.
   d. Set holding potential to -75 mV.

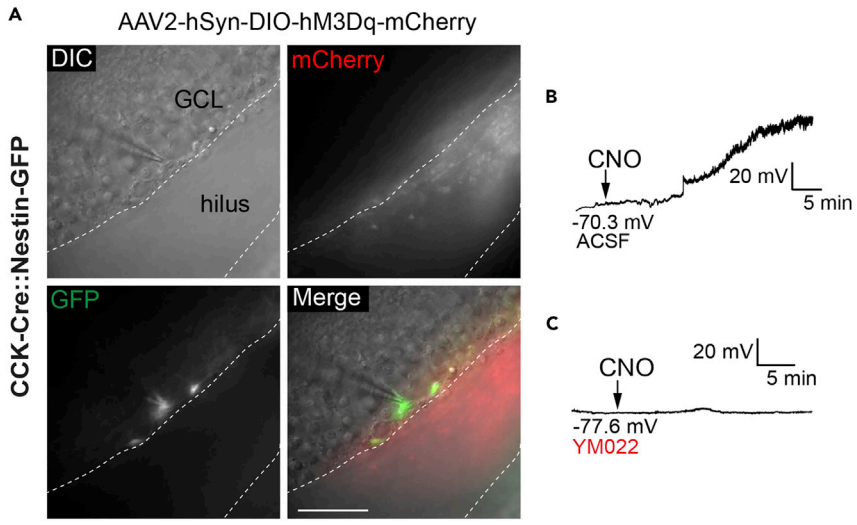

Figure 2. Sample recordings from rNSC during chemogenetic stimulation of virally targeted CCK interneurons in the dentate gyrus of the hippocampus

(A) Differential interference contrast (DIC) and fluorescent images from live tissue during targeted recording of a GFP$^+$ rNSC in a CCK-Cre::Nestin-GFP animal. Scale bar, 50 μm.

(B and C) Representative whole-cell recordings of membrane potential in rNSCs when CCK cells are activated by bath application of 10 μM CNO. Depolarization begins within approximately 10 min of CNO addition and reaches steady state in about 30 min. Depolarizations are blocked in the presence of CCK2 receptor antagonist, YM022. Traces reprinted with permission from Asrican et al. (2020).

e. Use slight but quick suction in conjunction with the zap function (1 V pulse) to break in. Use the smallest duration zap possible (typically around 0.5 ms), and progressively increase it until break in has been accomplished. rNSCs have very little capacitance, and low resistance, and therefore look very square upon break in. Whole-cell parameters typically obtained by a square voltage deflection (for example, using "cell" mode in the seal-test window of pClamp) will not able to provide capacitance and time constant measurements.
f. Switch to I=0 mode and monitor resting membrane potential. An average of 10 runs of a 200 ms sweep will suffice. Vm should be between -70 and -90 mV.
g. Change to voltage-clamp mode and apply a series of holding pulses (100 ms pulse starting from -95 mV and increasing at 5 mV steps) to monitor current responses. They should be large and very linear. No sodium currents typical of immature action potentials should be present.
h. Change to current clamp mode, and apply a series of current pulses (100 ms pulse starting at -30 pA and increasing by 5 pA steps) to monitor voltage responses. These should be small and no rectifying voltage deflection at higher current steps should be visible.

8. Record membrane potential. See Figures 2B and 2C.
   a. Remain in current clamp mode with no applied current (Ih = 0).
   b. Use the following protocol to record 10 min of baseline Vm. Breaking the 10-min recording into 30-s sweeps followed by brief test pulses allows for periodic monitoring of the seal integrity during recording.
      i. Use current clamp mode with the holding current set to 0 pA.
      ii. Enable comments in the data file, and use them to keep track of experimental conditions, such as when CNO or other pharmacology has been added.
      iii. Record 20 sweeps of 31.2 s long (totaling slightly longer than 10 min).
      iv. Of the 31.2 s sweep, 30 s will be allocated to data recording, and the remainder will be used for a small current pulse to monitor seal integrity.
      v. Apply a -100 pA, 100 ms monitoring pulse at time = 30.01 s.

vi. Sudden changes in the response to this monitoring pulse during the experiment may indicate seal failure.
vii. Typical experimental paradigms will include 1 run (10 min) of stable baseline recording, followed by several 10-min runs in the presence of chemogenetic stimulation by introducing 10 μM CNO into the bath perfusate.
viii. Slices should be changed between recordings to avoid residual effects of previous pharmacology applications.

Optional: Include various pharmacological agents such as glutamate receptor antagonists in the bath starting at baseline conditions to help determine mechanisms of action on the rNSCs. For examples, see (Asrican et al., 2020).

Note: Membrane responses in rNSCs are slow, and may take up to 30 min to develop, and typically are on the order of several tens of millivolts. For example, rNSCs may depolarize from -80 mV to -20 mV when CCK interneurons are activated chemogenetically. See Figures 2B and 2C. Depolarizations should be smooth and not abrupt.

⚠ CRITICAL: Electrode position must be constantly monitored via camera to avoid loss of the rNSC during the recording due to drift. Small microelectrode position adjustments are typically needed throughout the recording. As these cells are small, they are less tolerant than neurons to positional drift of the patch pipette.

EXPECTED OUTCOMES

Successful injection of virus containing hM3Dq excitatory DREADD will specifically label CCK cells in the hilus of the dentate gyrus in red, while the stem cells in the layer bordering the granule cells express GFP and have long processes that extend through the granule cell layer (Figure 2A). Membrane potential recordings from these cells should be about -70 to -80 mV at baseline and will begin to slowly depolarize within 8–10 min in response to activation of CCK neurons (Figures 2B, 2C, and 3).

Multiple cells can be recorded and then analyzed using commercial or customized analysis routines. Here, custom routines for Igor Pro are used. Alternatives do exist, such as using ClampFit, which comes with pClamp software (Molecular Devices). After discarding recordings considered unstable or unfit for analysis, summary data and plots can be assembled. It is typical of rNSCs to exhibit some diversity in responsiveness and many may display no change in membrane potential at all. With enough cells, it is useful to report the percent of cells that respond to stimulation.

QUANTIFICATION AND STATISTICAL ANALYSIS

1. Import ABF2 files.
   a. pClamp data files are written using the ABF2 data format, which can be read by a variety of software platforms, or one can be developed independently using published header and data structure information from Molecular Devices.
   b. Igor Pro is a highly customizable data processing and data presentation software similar in some respects to MATLAB and is highly recommended.
   c. Neuromatic is a collection of publicly available software tools built for Igor Pro by ThinkRandom.com (Rothman and Silver, 2018), and allows loading and browsing electrophysiology datasets, and can provide most of the necessary analysis routines. Some additional programming may still be necessary and can be accomplished using the commands found in the Igor help files.
2. Criteria for Inclusion/Exclusion

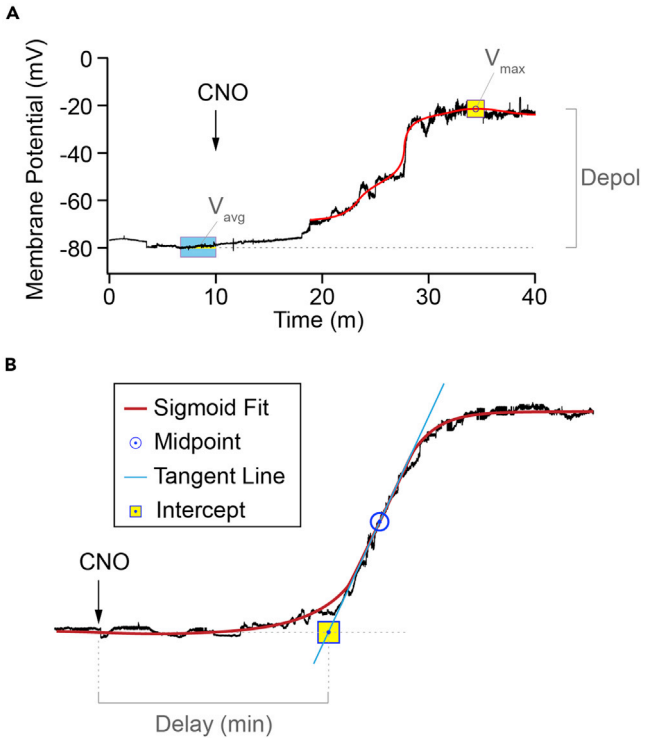

Figure 3. Quantification schemes for analyzing rNSC membrane potential responses

(A) Calculation of depolarization magnitude. Baseline level is defined as the mean of the last third of the data points during unstimulated recording. Depolarized level is the maximum of the smoothed response (red line) after chemogenetic stimulation. The amplitude of response is then determined by taking the difference between baseline and depolarized levels.

(B) Schematic for determining the delay time for rNSCs to respond to chemogenetic stimulation of neuronal circuitry. Data are fit with a Sigmoid function, and the fit parameter for the midpoint of inflection is obtained. Taking the first derivative of the Sigmoid provides the slope for a tangent line through the midpoint, which is then used to calculate the intercept point where the membrane potential deflects from baseline. Time from CNO application until deflection provides the delay parameter.

a. Check the initial membrane potential of the cells immediately following break in. Cells that are already depolarized beyond -65 mV may have a leaky seal and should be discarded.
b. Determine from the current/voltage step data whether the electrophysiology parameters are appropriate for stem cells (low resistance, low capacitance, non-rectifying, no voltage-dependent currents). Presence of a sodium spike indicates a neuron or neuroblast recording. Presence of an outward rectifying voltage deflection at high current pulses indicates recording from transient progenitor cell. Discard as necessary.
c. Determine stability of baseline. Typically, small membrane fluctuations of ~5 mV are acceptable. Drifting baselines can sometimes be corrected post-hoc if not too severe. Rapidly fluctuating baseline recordings indicate poor seals, and data from these cells should be discarded.
d. Membrane resistance can be calculated by analyzing the voltage response to the monitoring pulse found at the end of the 30 s data sweep. Sudden increase in the voltage response to the pulse is considered blockage of the electrode and should be discarded. Slow increases in resistance during the depolarization event are acceptable, as some membrane channels (presumably potassium channels) close as the rNSCs become activated.

3. Concatenate data sweeps.
a. Data files of membrane potential were collected as 30 s sweeps plus a small monitoring pulse, for a total length of 31.2 s.

b. A simple analysis routine to strip the last 1.2 s of data from each sweep will result in 30 s pieces of data.
c. Concatenate all 20 of the trimmed data files to get a full 10 min of data in that run.
d. Repeat for the additional runs, and then create a final concatenation that encompasses the entire dataset. Plotting this as a graph is useful to see the time-course of depolarization. Make note of the experimental conditions such as addition of pharmacological agents and indicate application times on the graph. Discard the dataset if the depolarization is too sudden (within 10 s), or if the membrane potential fluctuates too erratically.

4. Quantify depolarization (in mV). See Figure 3A.
   a. Determine baseline Vm level.
      i. The first 10 min of recording should have been baseline recording (in ACSF, or in receptor antagonist condition). Occasionally, there is some drift at the beginning of the recording as the cell stabilizes, so avoid analyzing data values at the beginning of the run.
      ii. Average all data points from the last 30% of the baseline run to determine Vm_baseline. The built in Igor Pro function, "Mean," can provide this value.
      iii. Measure response Vm level.
      iv. Typical recording have about 30 min of data in the CNO condition. The precise time point at which cells start to respond is variable, so therefore it is suggested to find the peak response instead of averaging at a particular timepoint.
      v. Apply a box car smoothing (running average) of 20,000 points (~20 s) to remove high frequency recording noise without distorting the shape of the slow depolarization waveform. The built in Igor Pro function, "Smooth," can perform this operation.
      vi. Take the maximum response of the smoothed dataset as Vm_max. The built in Igor Pro function, "Max," can provide this value.

   ⚠ CRITICAL: occasional recording artifacts can cause erroneous readings of the maximum. Visual confirmation of the detected level must be performed, and masking of the dataset during such incidents may be required.
   b. Calculate depolarization amount, Depol = Vm_max − Vm_baseline.

5. Quantify the delay to response. See Figure 3B.
   a. If a rNSC responds to circuit stimulation, the time-course of the response follows a typical "S" shape that can then be used to quantify the time it takes a cell to begin responding after CNO application.
   b. Use Igor Pro's built in curve-fitting procedures to fit the dataset to a sigmoid function. Take care to include a data mask to exclude parts of the dataset that may be causing erroneous fits to the data. The sigmoid function is defined as: y = K0+K1/(1+exp(-(x-K2)/K3)).
   c. Use the outputted Fitting Coefficient K2 as the halfway time point (Xhalf) of the sigmoid inflection.
   d. Take the derivative of the sigmoid fit to get a slope curve of the sigmoid. The built in Igor Pro function, "Differentiate," can provide this operation. The slope (Tan_slope) at point Xhalf is the slope of a tangent line to the sigmoid at the inflection point.
   e. Use the formula for a line, Y = mX + b, with Y = fit[Xhalf], X = Xhalf, and m = Tan_slope, to find the "b" value corresponding to the Y-intercept of the tangent line through the inflection point.
   f. Again, use the formula for a line Y = mX + b, with Y = Vm_baseline, m = Tan_slope, and b = Y_intercept to calculate the X_initiation time point. (The time at which the membrane potential begins to change).
   g. Use Time_delay = X_initiation − Time_CNO to calculate the delay time.
   h. *Optional:* Coefficient K3 corresponds to a "rate" parameter and may be useful for quantifying how fast the cell depolarizes.
6. Determine responder fraction.

a. As rNSCs are diverse, not every cell responds by depolarization. The responding fraction of all recordings should be reported.
b. Gather all depolarization amounts, Depol, from all rNSCs for a given experimental condition.
c. Set a responder threshold at 10 mV.
d. Count all cells that have Depol values greater than the threshold.
e. Responding percent = responding cells/total cells × 100.
f. This value is useful when reported in bar graphs. Several experimental conditions can be combined to produce responder rates under different pharmacological antagonists.

7. Statistical analysis
   a. When reporting results, common practice is to report the mean depolarization obtained ± the standard error of the mean. These values are easily obtained by using the "WaveStats" function in Igor Pro.
   b. Construct a bar graph showing the mean depolarization and add error bars using the SEM values. Optional: append individual data points as a jittered scatter plot overlayed on the bar graph.
   c. When comparing experimental conditions for statistical analysis, compare the actual data points for the rNSCs (not the means) using the "Two Sample Tests…" menu in Igor Pro.
      i. Begin by selecting the datasets for comparison.
      ii. Perform the "Runs Test" to determine the data are random.
      iii. Perform the "Variance Test" to see if variances are equal.
      iv. Perform the T-Test for significance if these conditions are met.

   Alternatives: Igor Pro will prompt for alternative significance tests if the above conditions are unmet.
      v. Report the p-value.

LIMITATIONS

Electrophysiological recordings are a powerful quantitative measurement of rNSC activation. However, as it is only possible to record a few cells per day, it can be a somewhat low-throughput method. With multiple experimental conditions or pharmacological manipulations, achieving adequate number of cells for statistical analysis can be time-consuming. If experimental outcomes are particularly obvious, a minimum of 7 cells from 3 animals is recommended. More will certainly be needed if the effect is less obvious, and calculations of statistical power may be of assistance in determining what might be a necessary "n" value.

Reliability of quantifications depends on the health and quality of brain slices and minimization of electrical noise on the microscope rig. Drifting of the membrane potential can sometimes be compensated algorithmically, to a limited extent, but is usually avoided, and such data occasionally need to be excluded.

Ultimately, membrane potential is a proxy for stem cell activation and is not a direct measurement of cell cycle progression. While many publications have provided evidence that depolarization is important for proliferation (Aprea and Calegari, 2012; Levin, 2014), it would be the best if electrophysiological phenomena in rNSCs or other cell types are supported by standard proliferation assays, such as by immunohistochemical labeling. See Asrican et al. (2020) for examples.

TROUBLESHOOTING

Problem 1

Poor slice quality/health when attempting patch rNSCs (step 7 of Step-by-step method details).

Potential solution

Health of cut brain slices decreases with time after slicing on the vibratome. The best recordings (step 8) are typically made early, within the first 3–4 h after slicing, with decreasing chances of success as the day progresses. Having recording materials and reagents prepared ahead of time is beneficial.

Perfusion systems to provide adequate amounts of oxygenated ACSF are critical. Follow typical electrophysiology recording recommendations to ensure good conditions. If tubing is clogged, or suction fails, rNSCs will begin to die and depolarize artificially. Fresh IV drippers and drip-speed regulators are inexpensive (see Key resources table) and should be replaced weekly. Tubing and chambers should be cleaned and kept free of bacteria.

Problem 2

Perfusion artifacts are present during the analysis of the whole-cell electrophysiological recordings (step 4 of Quantification and analysis).

Potential solution

Suction systems can sometimes cause capacitance artifacts as the level of solution in the recording chamber is suddenly changed. Large transient artifacts can occasionally cause the analysis routines to pick up artificial maximums. Manual smoothing or artifact removal algorithms may be needed to correct this. Ideally, a quiet recording chamber (see Key resources table) and careful cleaning and placement of the suction apparatus during acquisition can minimize perfusion artifacts.

Problem 3

Loss of the rNSC during the recording due to positional drift of the patch pipette (step 8 of Step-by-step method details).

Potential solution

The rNSCs in the hippocampus are quite small, and drift in the slice tissue can pull cells off the recording pipette. A good slice anchor can help minimize drift (see Additional Laboratory Equipment Table), with any remaining motion corrected by micropipette position adjustments during the experiment.

Furthermore, solution changes can occasionally cause motion disturbances, or swelling/contraction of the tissue may occur if osmolarity is changed suddenly. Avoid excessive use of DMSO as a solvent, as it often disturbs cell membranes and causes tissue swelling. Limit the volumes of water added to the ACSF. Mix pharmacological agents at high stock concentrations (~1,000×) and use ACSF as the solvent if possible.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Juan Song, juansong@email.unc.edu.

Materials availability

No new mouse lines or viral strains were generated in this study. The particular animals and reagents used here are reported in the Key Resource table.

Data and code availability

Electrophysiology datasets from this study have not been deposited publicly, but samples may be requested from the lead contact or the technical contact. Further information on specific analysis code written in Igor Pro may be made from the technical contact, Brent Asrican, basrican@email.unc.edu.

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AUTHOR CONTRIBUTIONS

J.S. conceived and oversaw the original project published in Asrican et al. (2020). B.A. developed the protocols described here and authored this manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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