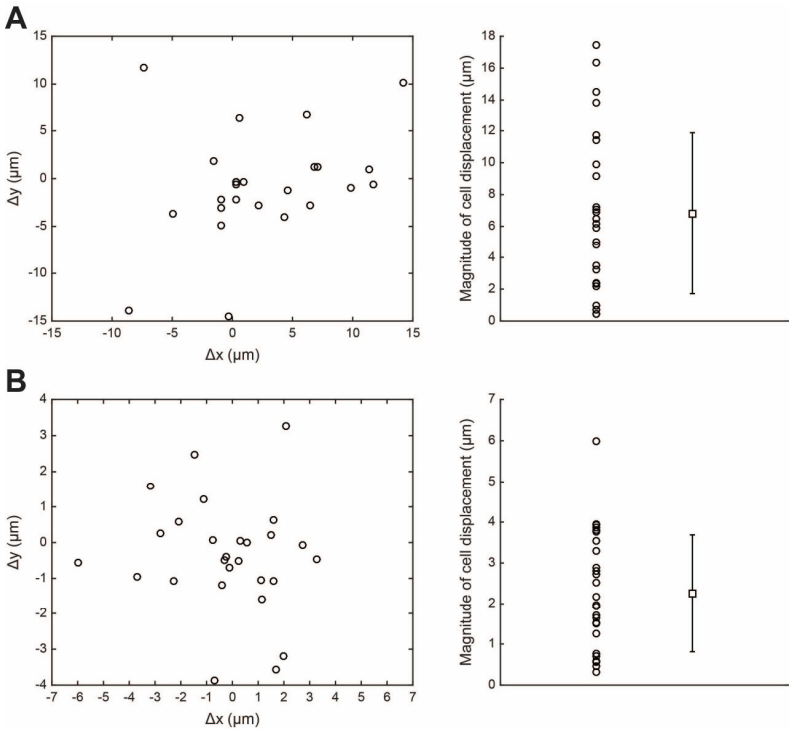

Figure S1, related to Figure 1. Cell movements following pipette navigations into the brain.
(A-B) Target cell displacements in the transverse plane (left) and their magnitude (right), following pipette navigations into the brain. Δx, change in the x coordinate of the target cell centroid, with a positive value corresponding to a cell movement to the right relative to the original location; Δy, change in the y coordinate of the target cell centroid, with a positive value corresponding to a cell movement in the anterior direction relative to the original location. Each circle represents a movement of a single cell, while squares and error bars are mean ± standard deviation.
(A) Target cell movements following pipette navigation along a linear trajectory parallel to the pipette axis (n = 25 cells in 6 anesthetized mice), with the pipette moving from above the brain surface to cortical layer 2/3.
(B) Target cell movements following pipette navigation in the x, y, and z directions (n = 27 cells in 17 anesthetized mice), with the pipette moving from a point 20 – 30 μm away from the target cell centroid to a point 10 – 20 μm directly above the target cell centroid.

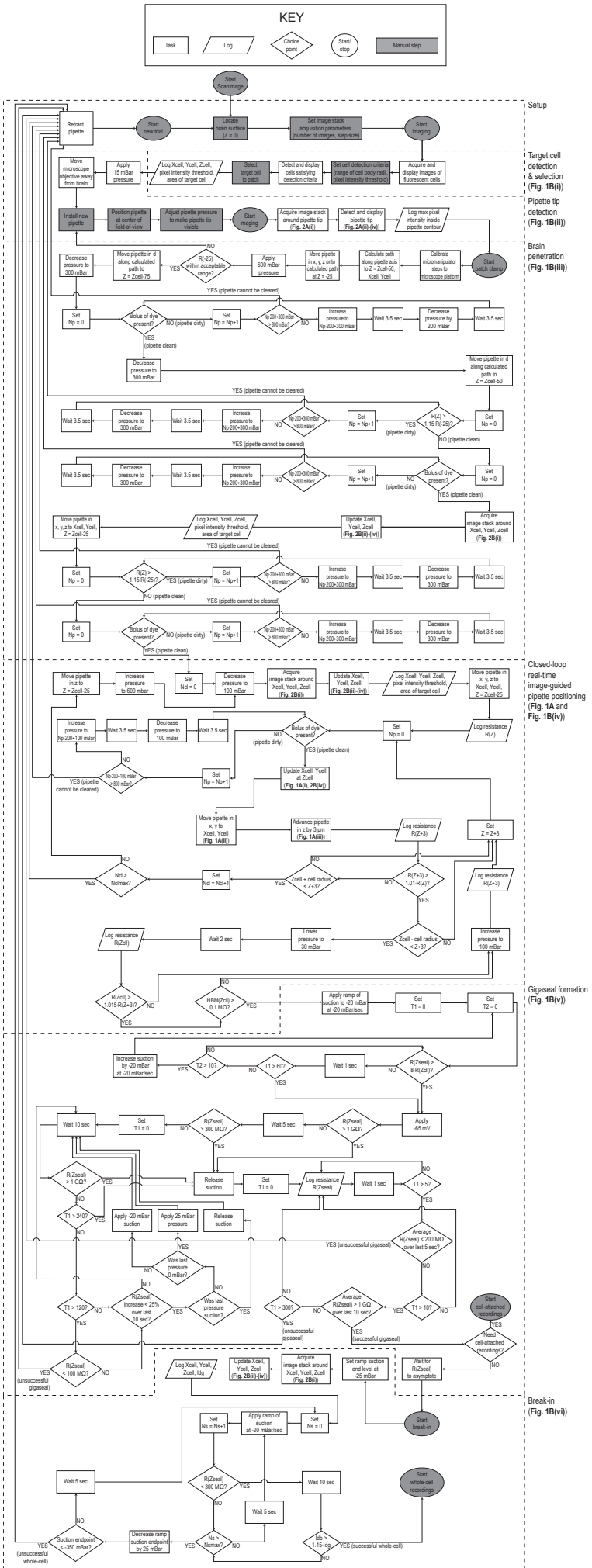
KEY
Task
Log
Choice point
Start/ stop
Manual step
Start Scan/Image
Setup
Retract pipette
Start new trial
Locate brain surface (Z = 0)
Set image stack acquisition parameters (number of images, step size)
Start imaging
Target cell detection & selection (Fig. 1B(i))
Move microscope objective away from brain
Apply 15 mBar pressure
Log Xcell, Ycell, Zcell, pixel intensity threshold, area of target cell
Select target cell to patch
Detect and display cells satisfying detection criteria
Set cell detection criteria (range of cell body radii, pixel intensity threshold)
Acquire and display images of fluorescent cells
Pipette tip detection (Fig. 1B(ii))
Install new pipette
Position pipette at center of field-of-view
Adjust pipette pressure to make pipette tip visible
Start imaging
Acquire image stack around pipette tip (Fig. 2A(i))
Detect and display pipette tip (Fig. 2A(ii)-(iv))
Log max pixel intensity inside pipette contour
Brain penetration (Fig. 1B(iii))
Decrease pressure to 300 mBar
Move pipette in d along calculated path to Z = Zcell-75
R(-25) within acceptable range?
Apply 600 mBar pressure
Move pipette in x, y, z onto calculated path at Z = -25
Calculate path along pipette axis to Z = Zcell-50, Xcell, Ycell
Calibrate micromanipulator steps to microscope platform
Start patch clamp
YES (pipette cannot be cleared)
Set Np = 0
Bolus of dye present?
NO (pipette dirty)
YES (pipette clean)
Set Np = Np+1
Np·200+300 mBar > 800 mBar?
Increase pressure to Np·200+300 mBar
Wait 3.5 sec
Decrease pressure by 200 mBar
Move pipette in d along calculated path to Z = Zcell-50
R(Z) > 1.15·R(-25)?
Move pipette in x, y, z to Xcell, Ycell, Z = Zcell-25
Log Xcell, Ycell, Zcell, pixel intensity threshold, area of target cell
Update Xcell, Ycell, Zcell (Fig. 2B(ii)-(iv))
Acquire image stack around Xcell, Ycell, Zcell (Fig. 2B(i))
Closed-loop real-time image-guided pipette positioning (Fig. 1A and Fig. 1B(iv))
Move pipette in z to Z = Zcell-25
Increase pressure to 600 mbar
Set Nd = 0
Decrease pressure to 100 mBar
Increase pressure to Np·200+100 mBar
Np·200+100 mBar > 800 mBar?
Set Np = Np+1
YES (pipette cannot be cleared)
Update Xcell, Ycell at Zcell (Fig. 1A(i), 2B(iv))
Log resistance R(Z)
Move pipette in x, y to Xcell, Ycell (Fig. 1A(ii))
Advance pipette in z by 3 µm (Fig. 1A(iii))
Log resistance R(Z+3)
Set Z = Z+3
Nd > Ndmax?
Set Nd = Nd+1
Zcell + cell radius < Z+37
R(Z+3) > 1.01·R(Z)?
Log resistance R(Zcell)
Wait 2 sec
Lower pressure to 30 mBar
Zcell - cell radius < Z+3?
Increase pressure to 100 mBar
R(Zcell) > 1.015·R(Z+3)?
HBM(Zcell) > 0.1 MΩ?
Gigaseal formation (Fig. 1B(v))
Apply ramp of suction to -20 mBar at -20 mBar/sec
Set T1 = 0
Set T2 = 0
Increase suction by -20 mBar at -20 mBar/sec
T2 > 10?
T1 > 60?
Wait 1 sec
R(Zseal) > 8·R(Zcell)?
Wait 10 sec
R(Zseal) > 300 MΩ?
Wait 5 sec
R(Zseal) > 1 GΩ?
Apply -65 mV
R(Zseal) > 1 GΩ?
Release suction
Log resistance R(Zseal)
T1 > 5?
T1 > 240?
Apply -20 mBar suction
Apply 25 mBar pressure
Release suction
Average R(Zseal) < 200 MΩ over last 5 sec?
YES (unsuccessful gigaseal)
Was last pressure 0 mBar?
T1 > 120?
R(Zseal) increase < 25% over last 10 sec?
Was last pressure suction?
T1 > 300?
Average R(Zseal) > 1 GΩ over last 10 sec?
T1 > 10?
Start cell-attached recordings
Need cell-attached recordings?
R(Zseal) < 100 MΩ?
YES (unsuccessful gigaseal)
Log Xcell, Ycell, Zcell, Idg
Update Xcell, Ycell, Zcell (Fig. 2B(ii)-(iv))
Acquire image stack around Xcell, Ycell, Zcell (Fig. 2B(i))
Set ramp suction end level at -25 mBar
Wait for R(Zseal) to asymptote
Break-in (Fig. 1B(vi))
Start break-in
Set Ns = Ns+1
Apply ramp of suction at -20 mBar/sec
Set Ns = 0
Wait 5 sec
R(Zseal) < 300 MΩ?
Wait 10 sec
Start whole-cell recordings
Wait 5 sec
Suction endpoint < -350 mBar?
Decrease ramp suction endpoint by 25 mBar
Ns > Nsmax?
Idb > 1.15·Idb
YES (successful whole-cell)
YES (unsuccessful whole-cell)

Figure S2, related to Figure 1. **Step-by-step flowchart, showing the entire imagepatching process.**

Dotted lines frame each of the stages of the algorithm; within the dotted line frames, symbols represent task, logging, and choice points, along with text explaining the individual steps and consequences of each decision (see "**KEY**" for definition of symbols). Abbreviations: Z, depth inside the brain, in microns (with more positive Z values indicating deeper positions inside the brain); Zcell, depth of the target cell, Xcell, x-coordinate of the target cell centroid at Zcell; Ycell, y-coordinate of the target cell centroid at Zcell; x, movement direction along the x-axis of the 4-axis manipulator; y, movement direction along the y-axis of the 4-axis manipulator; z, movement direction along the z-axis of the 4-axis manipulator; d, movement direction along the diagonal axis (i.e., axis parallel to the pipette) of the 4-axis manipulator; R(Z), pipette resistance at depth Z; Np, counter for the pipette clearing pressure pulse; Ncl, total number of times the closed-loop is run; Nclmax, limit on the number of times the closed-loop is run; Zcll, depth at which pipette pressure is lowered to 30 mBar; R(Zcll), pipette resistance at Zcll; HBM(Zcll), amount of heartbeat modulation at Zcll; R(Zseal), pipette resistance at the depth at which a gigaseal and the whole-cell state are being achieved, which will vary over time as the algorithm progresses; T1, time, in seconds; T2, time, in seconds; Ns, total number times a suction pulse is applied for break-in; Nsmax, limit on the number times a suction pulse is applied for break-in; Id, mean pixel intensity inside the target cell contour, in the microscope channel corresponding to the pipette dye; Idg, Id when R(Zseal) is higher than 1 GΩ; Idb, Id when R(Zseal) is less than 300 MΩ.

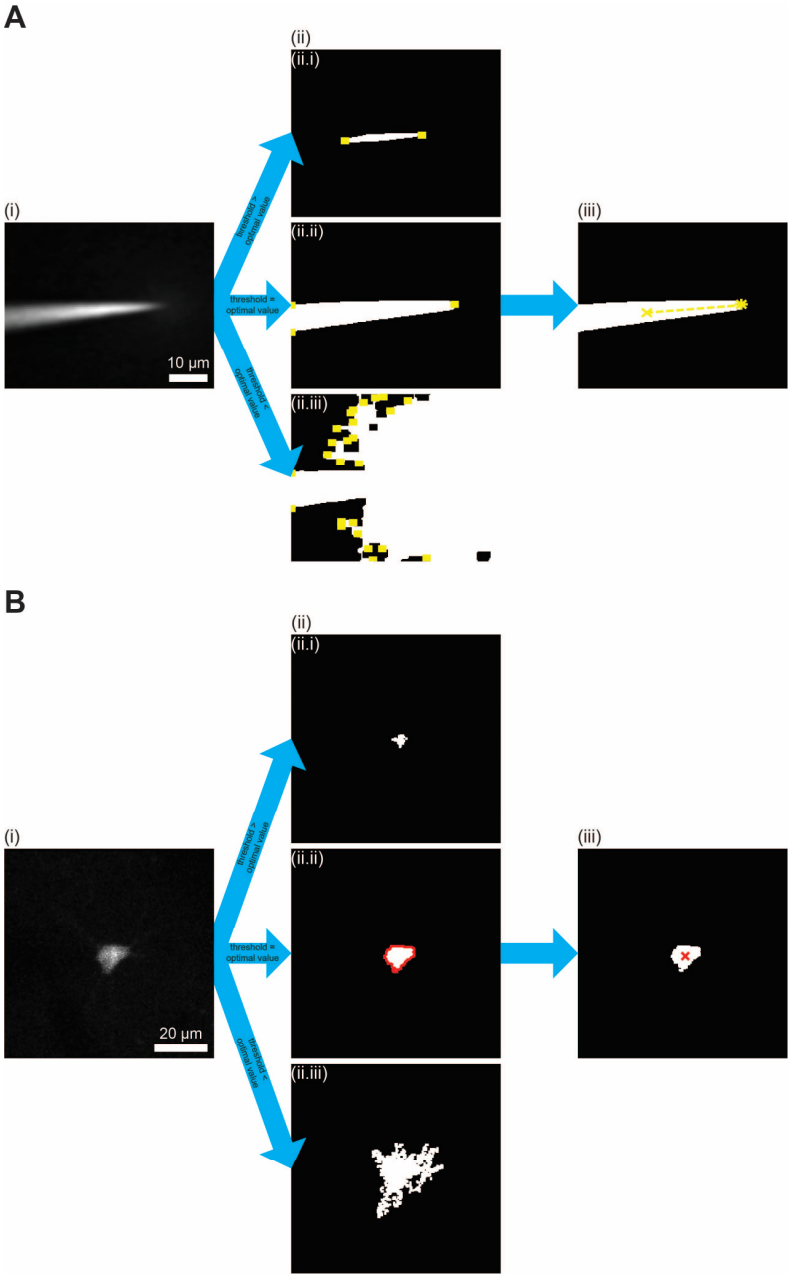

Figure S3, related to Figure 2. 2D image analysis algorithms for detecting pipette tips and cell centroids.

(A) Steps used to detect the tip of a fluorescent dye filled patch pipette from an image focused on the pipette tip. (i) An image of the pipette at the z-coordinate of the pipette tip (i.e., an image focused on the pipette tip) is filtered using a 2D Gaussian filter. (ii) Threshold values corresponding to 5 – 75% of the maximum pixel intensity of the filtered image are applied to the filtered image, and the resulting clusters (white) are subjected to MATLAB's endpoint detection function (bwmorph function with 'endpoints' operation) to identify the endpoints (yellow boxes). Clusters and endpoints resulting from threshold values corresponding to 75 (ii.i), 13 (i.e., optimal threshold value; ii.ii), and 3% (ii.iii) of the filtered image's maximum pixel intensity are shown as examples. (iii) The cluster resulting from the optimal threshold value is analyzed to locate its

centroid (yellow x) and extrapolate a line from the centroid in the direction of the pipette angle (yellow dotted line). The pixel in the cluster that is closest to the endpoint of the extrapolated line (i.e., endpoint opposite to the centroid) is designated as the pipette tip (yellow star).
(B) Steps used to detect the cell boundary and centroid from each image in a z-stack acquired around a fluorescent cell. (i) An image in a z-stack acquired around a tdTomato-expressing cell is filtered using a 2D Wiener filter. (ii) Threshold values corresponding to 5 – 95% of the maximum pixel intensity of the filtered image are applied to the filtered image, and the resulting clusters (white) are analyzed to determine their areas (i.e., numbers of pixels in the clusters). The cluster whose area is the closest to that of the cell chosen by the user during the target cell detection and selection stage ((ii.ii), white) is further processed to determine its boundary ((ii.ii), red outline). Clusters resulting from threshold values corresponding to 50 (ii.i), 20 (i.e., optimal threshold value; ii.ii), and 3.5% (ii.iii) of the filtered image's maximum pixel intensity are shown as examples. (iii) The cluster obtained by applying the optimal threshold value to the image at the z-coordinate of the cell centroid is processed to yield its centroid (red x), designated as the centroid of the cell.

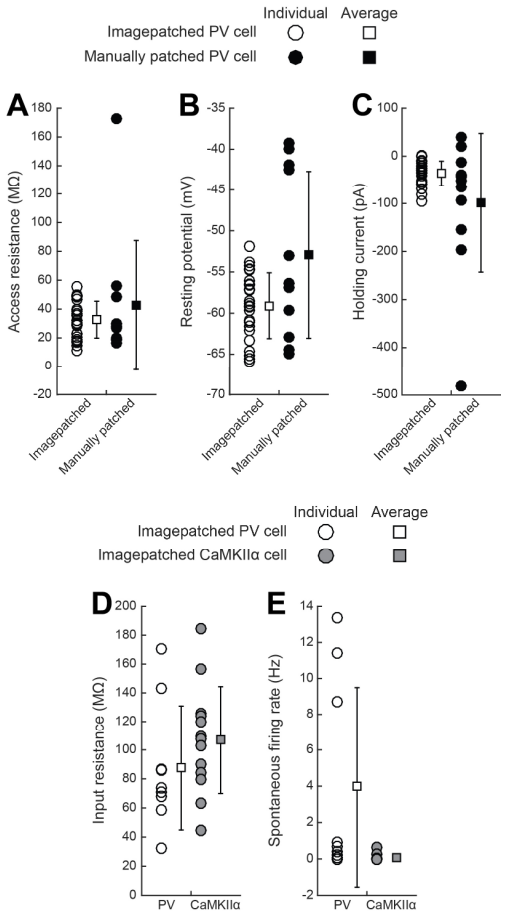

Figure S4, related to Figure 4. Recording quality and electrophysiological properties of imagepatched neurons.

(A-C) Recording quality of imagepatched (white symbols; n = 24 cells from 14 mice) vs manually patched (black symbols; n = 11 cells from 8 mice) tdTomato-expressing PV-positive interneurons in somatosensory and motor cortices of isoflurane-anesthetized PV-Cre x Ai14 mice. Squares and error bars are mean ± standard deviation.

(A) Access resistance.

(B) Resting potential.

(C) Holding current.

(D-E) Electrophysiological properties of imagepatched tdTomato-expressing PV-positive neurons (white symbols; 9 cells from 5 PV-Cre x Ai14 mice) and imagepatched tdTomato-expressing CaMKIIα-positive neurons (gray symbols; 13 cells from 7 CaMKIIα-Cre x Ai14 mice) in somatosensory and motor cortices of isoflurane-anesthetized mice. Squares and error bars are mean ± standard deviation.

(D) Input resistance.

(E) Spontaneous firing rate.

Failure Modes	PV	CaMKIIα
Pipette Blockage	20.4% (n = 22)	21.5% (n = 14)
Failed Gigaseal Formation	37.0% (n = 40)	43.1% (n = 28)
Loss of Seal During Break-in	16.7% (n = 18)	9.2% (n = 6)
Untargeted Patch	3.7% (n = 4)	6.2% (n = 4)

Table S1, related to Figure 4. Four failure modes of the imagepatcher.
Imagepatching attempts that did not result in successful whole-cell recordings (84 out of 108 attempts targeting PV-positive neurons, from 17 PV-Cre x Ai14 mice; 52 out of 65 attempts targeting CaMKIIα-positive neurons, from 10 CaMKIIα-Cre x Ai14 mice) can be grouped into 4 failure modes: (i) 'pipette blockage' includes imagepatching attempts in which a pipette failed to eject enough dye at its tip or experienced a significant rise in its resistance value after entering the brain or while approaching the target cell; (ii) 'failed gigaseal formation' includes imagepatching attempts in which contact between an uncontaminated pipette tip and the target cell membrane, followed by pipette pressure modulation and hyperpolarization, did not result in a gigaseal; (iii) 'loss of seal during break-in' includes imagepatching attempts in which application of suction pulses following successful gigaseal formation led to a loss of gigaseal or cell lysis; and (iv) 'untargeted patch' includes imagepatching attempts in which an unlabeled cell (i.e., a cell that was not fluorescently tagged), sitting right on top of a targeted cell, was patched instead of the target cell.

Target Cell Depths Below The Brain Surface	Success Rates			
	PV		CaMKIIα	
	Gigaseal	Whole-cell	Gigaseal	Whole-cell
≥100 and <200 µm	50.0% (n = 24 out of 48)	29.2% (n = 14 out of 48)	40.0% (n = 14 out of 35)	25.7% (n = 9 out of 35)
≥200 and <300 µm	48.6% (n = 18 out of 37)	27.0% (n = 10 out of 37)	31.3% (n = 5 out of 16)	25.0% (n = 4 out of 16)

Table S2, related to Figure 4. Success rates at different target cell depths, for trials that entered the closed-loop stage of imagepatching.
One unsuccessful attempt targeted a PV-positive cell at a depth below 300 µm and is not included in the table.

PV			CaMKIIα		
Number of Labeled Cells in 200 x 200 x 100 μm³ Volume	**Success Rates**		**Number of Labeled Cells in 200 x 200 x 100 μm³ Volume**	**Success Rates**	
	Gigaseal	**Whole-cell**		**Gigaseal**	**Whole-cell**
<6	42.1% (n = 8 out of 19)	31.6% (n = 6 out of 19)	<20	40.0% (n = 4 out of 10)	30.0% (n = 3 out of 10)
≥6 and <10	57.1% (n = 16 out of 28)	32.1% (n = 9 out of 28)	≥20 and <35	30.8% (n = 4 out of 13)	15.4% (n = 2 out of 13)
≥10 and <14	33.3% (n = 8 out of 24)	20.8% (n = 5 out of 24)	≥35 and <65	47.1% (n = 8 out of 17)	35.3% (n = 6 out of 17)
≥14	66.7% (n = 10 out of 15)	26.7% (n = 4 out of 15)	≥65	27.3% (n = 3 out of 11)	18.2% (n = 2 out of 11)

Table S3, related to Figure 4. Success rates for different labeling densities of cells around a target cell, for trials that entered the closed-loop stage of imagepatching.
The number of labeled cells around a target cell in a 200 x 200 x 100 μm^3 volume was determined by scaling the number of tdTomato-expressing cells in a z-stack (5 images, 10 μm step size, field of view of 223.5 x 223.5 μm^2) that captured the target cell near the center of its field of view.