Dynamic encoding of perception, memory and movement in a *C. elegans* chemotaxis circuit

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Supplemental Figure Legends

Supplemental Figure 1. Procedures for making a stable and linear salt gradient in agar (related to Figure 1).
(A) Procedures for making a stable linear salt gradient.

(1) Prepare a 25cm x 25cm Petri plate and melt agar with 50mM of NaCl.

(2) Elevate one side of the plate and fill half of the plate with the melted agar. It creates a triangle wedge once it solidifies.

(3) Lay the plate flat and fill the other half of the plate with melted agar of 0mM NaCl. (4) Allow NaCl diffuse for 24 hours.

(**B-D**) Numerical simulation of diffusion of NaCl as it generates a linear gradient over the first 14 hours (**B**), 48 hours (**C**) and 1-7 days (**D**) after casting.

Supplemental Figure 2. Calcium imaging in ASE sensory neurons in response to step changes (Related to Figure 3 and Figure 4)
(A, B) Traces of GCaMP3 fluorescence intensity in ASEL (upper panels) and ASER (lower panels) in *Pflp-6*::GCaMP3 transgenic animals (wild-type N2 background) grown at 50 mM NaCl and exposed to steps between 25 mM and 30 mM NaCl **(A)** or steps between 70 and 75 mM NaCl **(B)**. Solid lines and shading indicates mean ± SEM.
(C, D) Maximal ASE step responses as shown in **(A, B)**. Measurements indicate mean ± SEM. + denotes significant differences between pre-step change calcium measurements and the maximal response after step change (+++ or ***, $p<0.0001$; ++ or **, $p<0.001$; + or *, $p<0.01$; Student's paired *t* test).
(**E**) Optogenetic activation of ASER exhibited opposite behavioral response above and below the setpoint (50 mM NaCl). When worms were swimming in 25 mM NaCl dextran solution, they increase turning rate during 6s light stimulation of ASER (Student's *t*-test, $**p < 0.005$, 77 trials from $n = 8$ animals). However, when worms were swimming in 75 mM NaCl solution, they reduce the turning rate during the light stimulation (Student's *t*-test, ***p < 0. 0001, 236 trials from $n >10$ animals).

Supplemental Figure 3. Making linear NaCl gradients for calcium imaging (Related to Figure 3)
Schematic of an inline mixer to produce gradients (Please also see **Experimental Procedures**).
Inset, Samples of calibration traces with fluorescein showing the shape of increasing and decreasing gradients produced by the mixer.

Supplemental Figure 4. ASER calcium imaging in response to salt gradients (Related to Figure 3)
(A-D) Heat maps of GCaMP3 signals in ASER in mutants raised at 50 mM and responding to increasing or decreasing salt gradient starting at 75mM or 25 mM NaCl, showing similar dynamics as wild-type animals subjected to the same conditions in Figure 3A-3D. To calculate $\Delta F/\Delta F_{max}$, we subtracted the calcium intensity of each time point (F) by the minimal calcium intensity of the time series (F_{min}) and divided the difference by the difference between the maximal (F_{max}) and minimal (F_{min}) calcium intensity of the time series ($\Delta F/\Delta F_{max}= (F-F_{min})/(F_{max}-F_{min})$).

Supplemental Experimental Procedures

Quantification of Behavioral Assay

Data collected on salt chemotaxis was analyzed using customized particle-tracking and shape analysis algorithms written in Labview (National Instruments, Austin, TX) and MATLAB (Mathworks, Natick, MA). To automatically flag reorientations and differentiate sharp turns and reversal-turns, we considered both the posture of the animal and the movement of its center of mass. Rapid reorientations (sharp turns or reversal-turns) were flagged when the heading change of the center of mass trajectory was > 60° over 1 second. When the worm executes a sharp turn, the aspect ratio of the worm image decreases before the worm resumes forward movement in a new direction. When a worm executes a reversal, the aspect ratio remains high and the center-of-mass reverses its direction, backtracking along the original path. To quantify the aspect ratio, we took the ratio of the two eigenvalues of the covariance matrix of the pixel intensity recorded by the camera (http://en.wikipedia.org/wiki/Image_moment). A larger ratio indicated that the worm was elongated and a smaller ratio indicated that the worm had assumed a more circular shape. Reversals were flagged as sudden changes in the direction of the center-of-mass motion that exceeded 160° while maintaining an eigenvalue ratio of > 1.8. Otherwise, reorientations were flagged as sharp turns. Automated flagging of reorientation events along trajectories was proofread by hand using a graphical user interface that replayed the image of each worm as it moved along its trajectory.

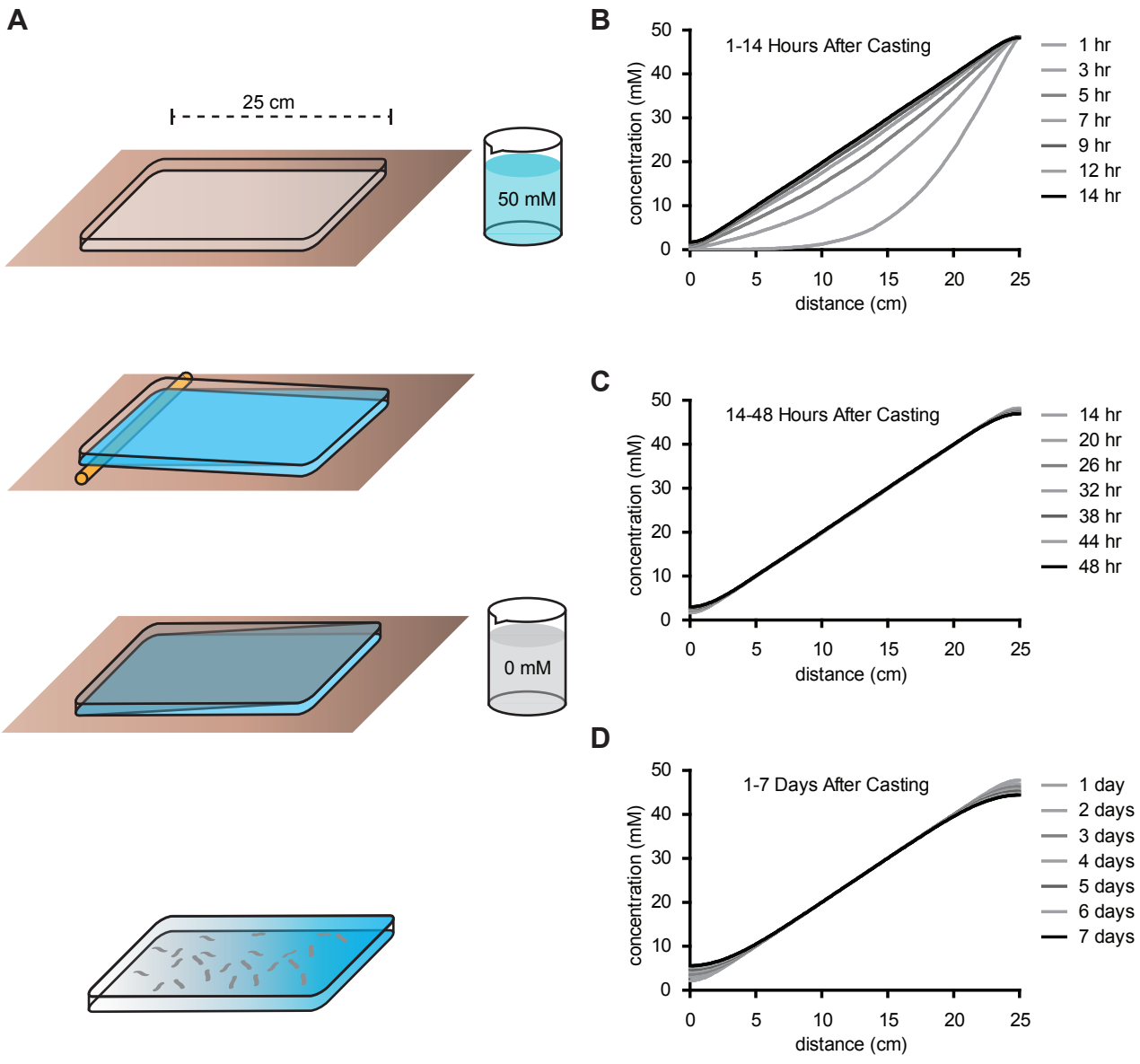
To quantify chemotactic rate and direction (see Figure 1), we use a non-dimensional index based on individual animal trajectories on linear gradients: the mean velocity of the trajectories along the gradient direction, $\langle v_g \rangle$, divided by the mean crawling speed along the trajectory, $\langle s \rangle$. Thus, if all worms crawled straight up or down gradients, the index would be +1 or -1, respectively. If worms moved randomly, the index would be near 0. To quantify the biased random walk index (see Figure 7), we used the mean run durations up or down the gradients based on the trajectories of individual animals (see Figure 2B and D), and calculated the fractional difference according to the formula $(\langle r_{up} \rangle - \langle r_{down} \rangle)/(\langle r_{up} \rangle + \langle r_{down} \rangle)$. The index approaches +1 or -1 if runs down or up the gradient, respectively, approach zero duration. If runs are equal durations in both directions, the index would be near zero. To quantify the klinotaxis index (see Figure 7), we used the probabilities of sharp turns reorienting the animal up or down the gradient based on individual trajectories (see Figure 2C and E) and calculated the fractional difference according to the formula $(\langle p_{up} \rangle - \langle p_{down} \rangle)/(\langle p_{up} \rangle + \langle p_{down} \rangle)$. The index approaches +1 or -1 if worms deterministically turn up or down the gradient. If there is no bias in turning probabilities, the index is near zero.

Data Analysis on ASE Calcium Imaging

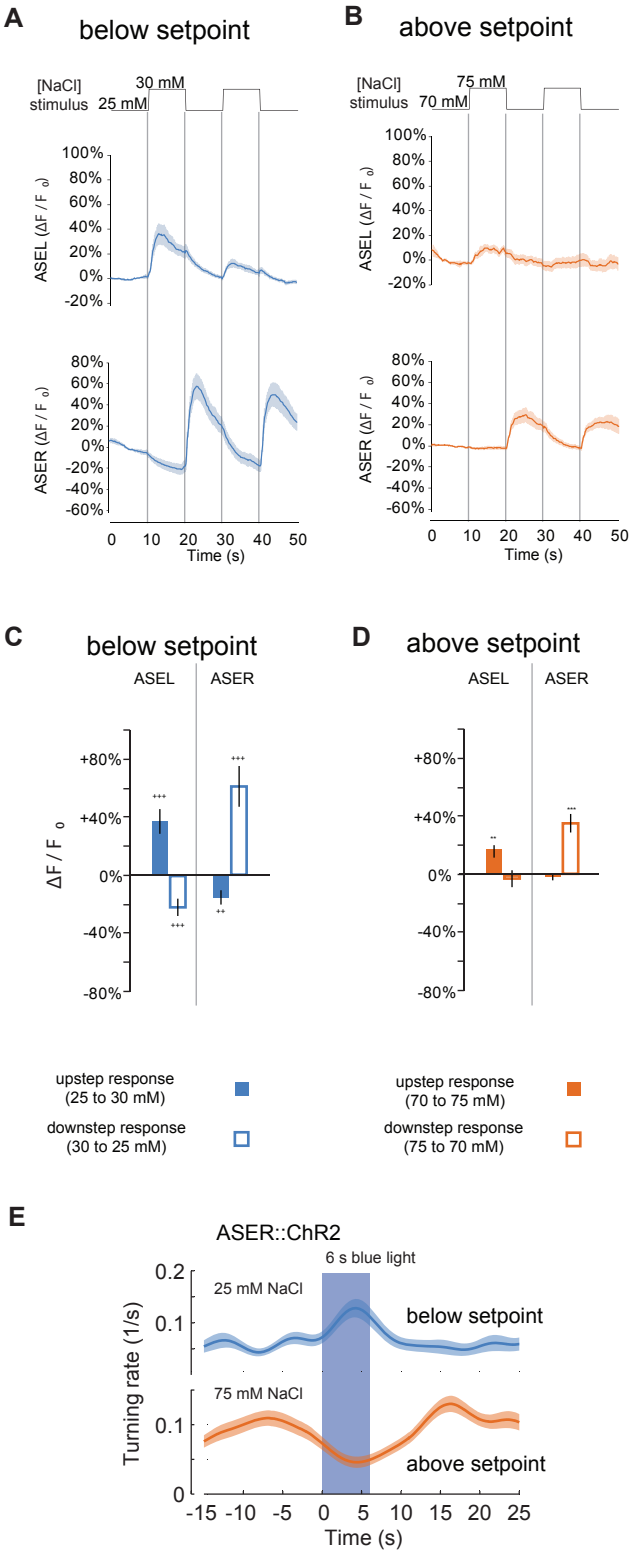
To calculate $\Delta F/\Delta F_{max}$ (Figure 3A-E and Figure S4), we subtracted the calcium intensity of each time point (F) by the minimal calcium intensity of the time series (F_{min}) and divided the difference by the difference between the maximal (F_{max}) and minimal (F_{min}) calcium intensity of the time series ($\Delta F/\Delta F_{max} = (F-F_{min})/(F_{max}-F_{min})$). To calculate the cumulative calcium intensity for each time point in Figure 3F, we divided the sum of the calcium intensity from time zero to the current time point by the average calcium intensity during the baseline period (*i.e.* the first 30

seconds). To calculate the statistic values for Figure S2C and S2D, mean fluorescence intensity over a one second time window was compared to the maximal positive or negative response after the stimulus switch using a paired t-test.

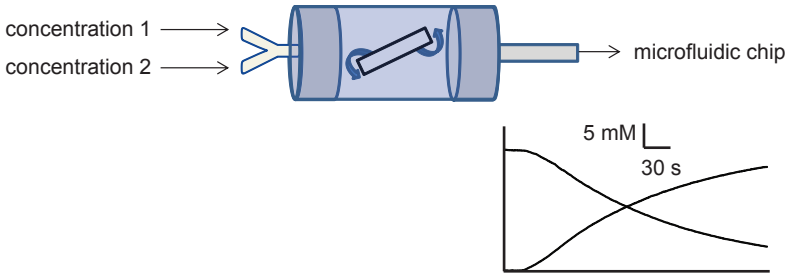
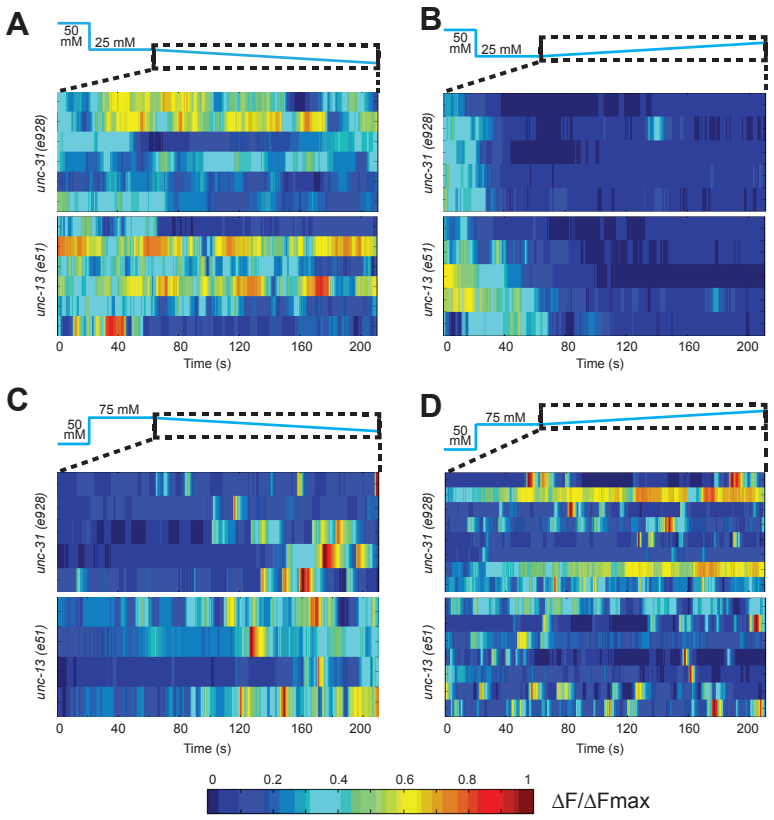
SUPPLEMENTAL FIGURE 1



SUPPLEMENTAL FIGURE 2



SUPPLEMENTAL FIGURE 3



SUPPLEMENTAL FIGURE 4