AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training

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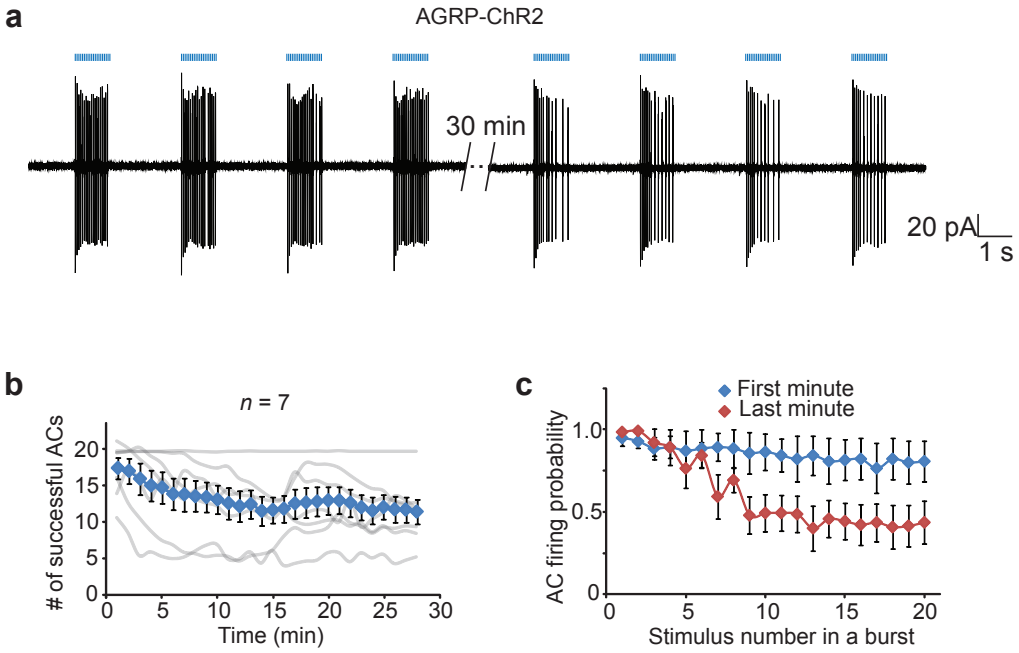
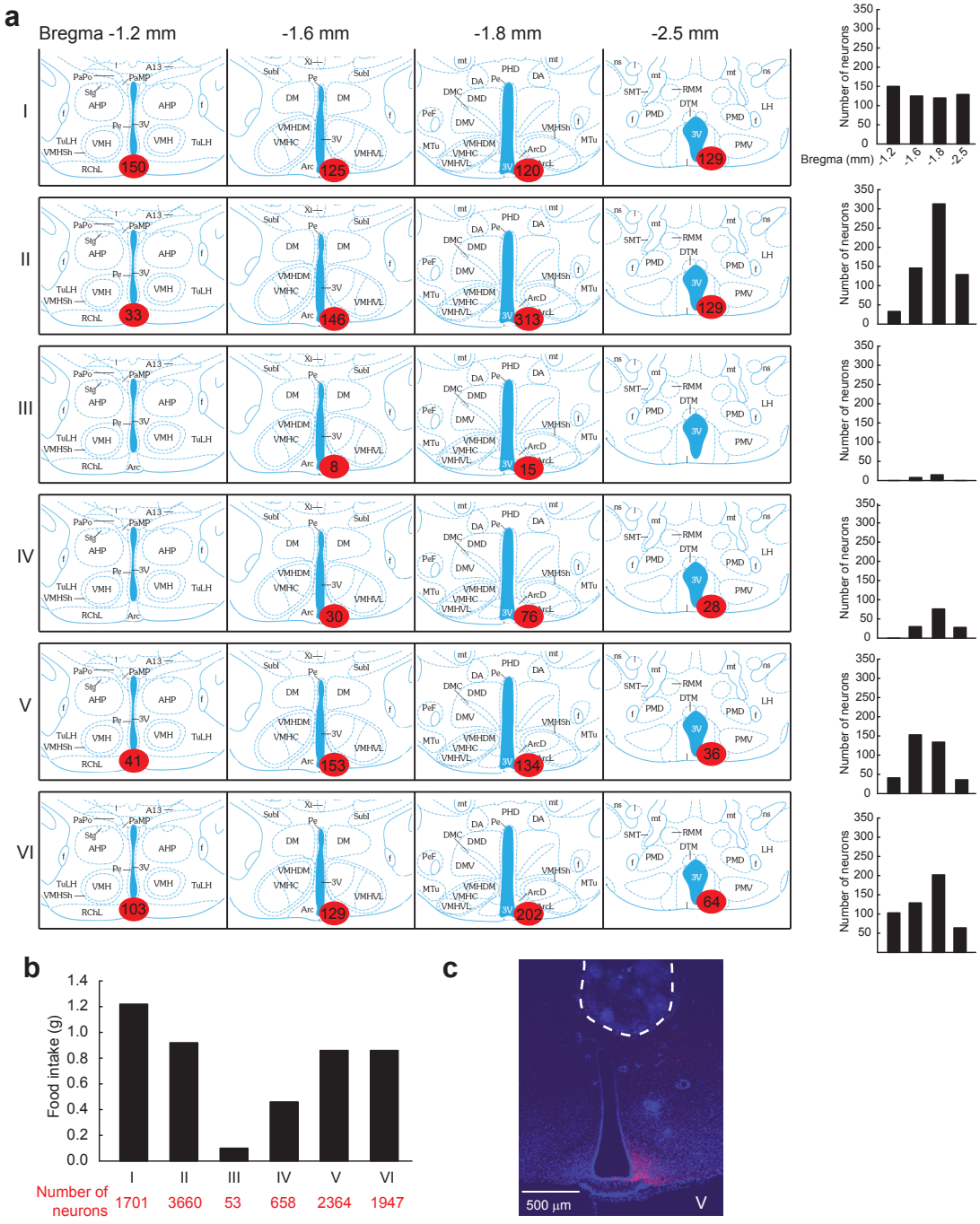
Supplementary Figures 1-7



Supplementary Figure 1. Extended photostimulation of AGRP neurons.
(a) Representative traces for a loose-seal, cell attached recording from a ChR2-expressing AGRP neuron in an acute brain slice, showing action currents generated upon repeated rounds of 20 Hz photostimulation for 1 s followed by a 2 s delay and repeated over 30 min. Traces are taken from t = 1 min and t = 30 min. 10 ms light pulses are indicated by blue bars above the traces.
(b) Number of successfully photostimulated action currents (AC) per burst averaged over 1 minute bins for individual AGRP neurons (grey lines) and averaged over all cells (blue diamonds, *n* = 7).
(c) Action current (AC) firing probability for each light pulse in a burst of 20 stimuli is plotted as a function of its position in the burst. The blue symbols indicate average success rate from the first 20 bursts (t = 0 - 1 min) and red symbols indicate average success rate from the last 20 bursts (t = 29 - 30 min). These data show that the first several stimuli in a burst are effective at reliably driving firing, even after 30 min, and that about half of the later light pulses in the burst activate firing in AGRP neurons. Error bars represent s.e.m.

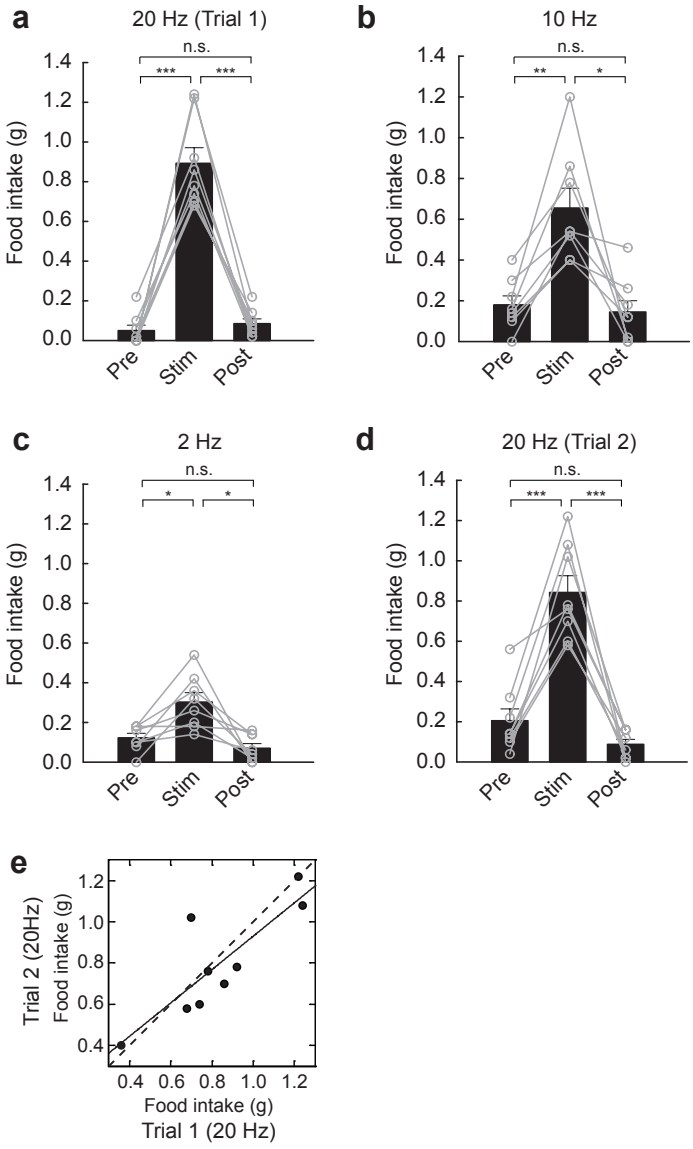


Supplementary Figure 2. Rostral-caudal distribution of ChR2-expressing AGRP neurons and food intake in representative *agrp-cre* mice.

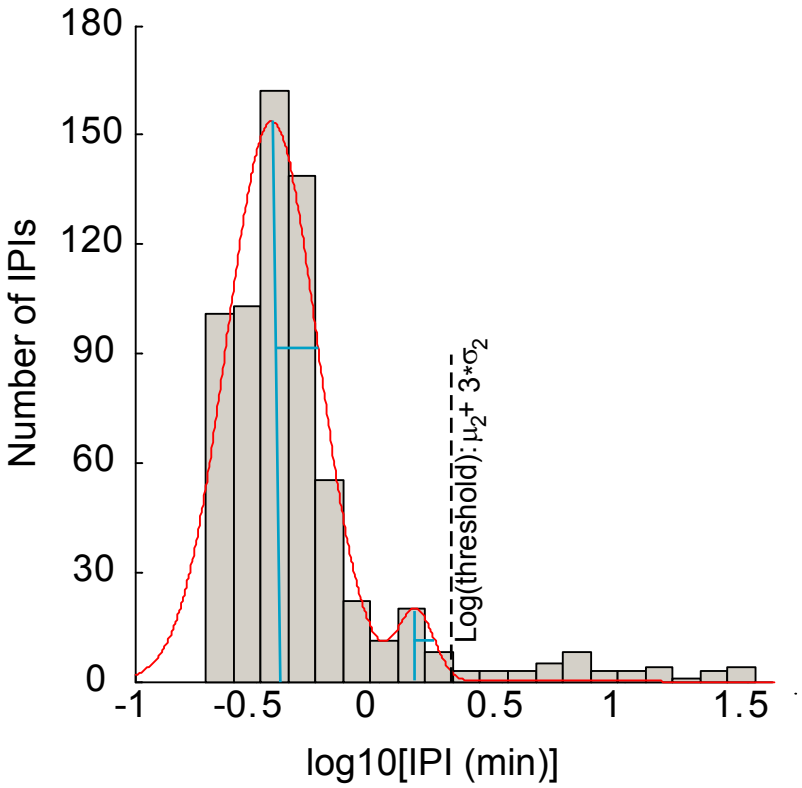
(a) Schematic showing the distribution of ChR2-positive AGRP neurons in the ARC from six representative mice at four rostral/caudal levels (number of neurons displayed in the red oval). Bar graphs on the right show the number of neurons at each position. Multiple distributions are represented which include an even distribution across the ARC (mouse I), central distribution within the ARC (mice III, V), caudally biased distribution (mice II, IV), and rostrally biased distribution (mouse VI)

(b) Food intake for mice from each distribution of neurons was similar for mice with greater than 800 neurons. For other mice food intake was dependent on the number of ChR2-expressing AGRP neurons (red). Bars represent 1 hour food intake during photostimulation for individual mice.

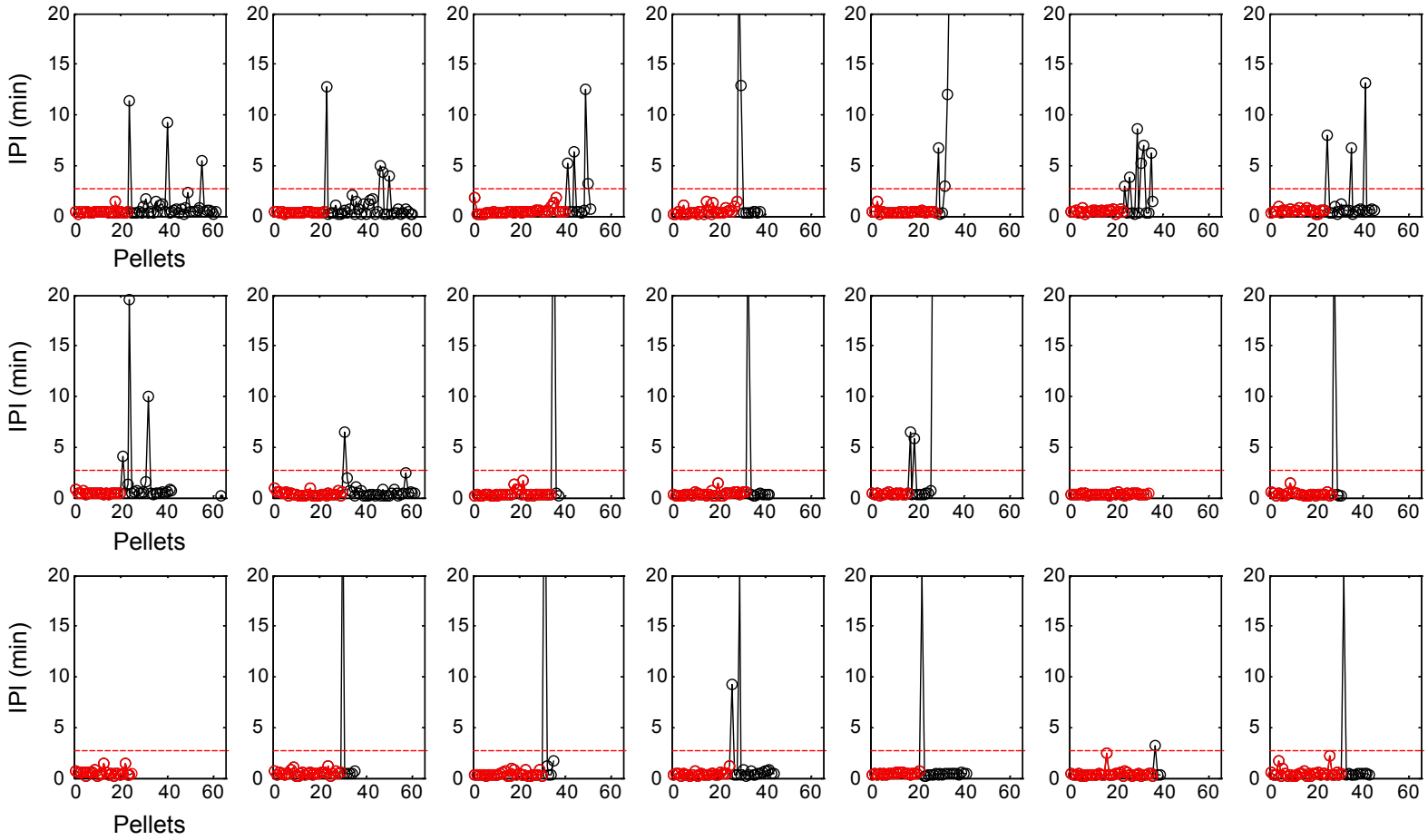
(c) Brain slice from mouse V, showing the guide cannula and optical fiber track (white outline) ~0.8 mm above the ARC as well as ChR2:tdtomato fluorescence in the ARC (red). The background image of the slice was obtained from 4',6'-diamidino-2-phenylindole fluorescence (blue).



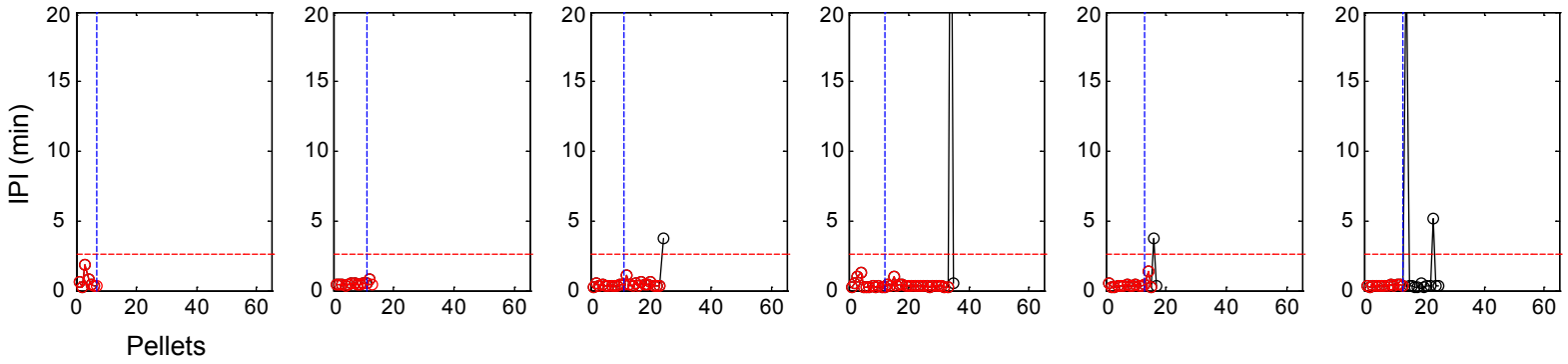
Supplementary Figure 3. Food intake before, during, and after photostimulation at different frequencies.
(a-d) For protocol 1, at each stimulus frequency, food intake was significantly increased during photostimulation relative to pre- or post-stimulus period (*n* = 8).
(e) Food intake for 20 Hz stimulus trial 1 plotted against consumption from trial 2 was similar. Solid black line: linear least squares fit; dashed line: reference function for intake(Trial 1) = intake(Trial 2); black circles are 1 hour food intake for individual mice. n.s., not significant; * P < 0.05; ** P < 0.01; *** P < 0.001. Error bars represent s.e.m.



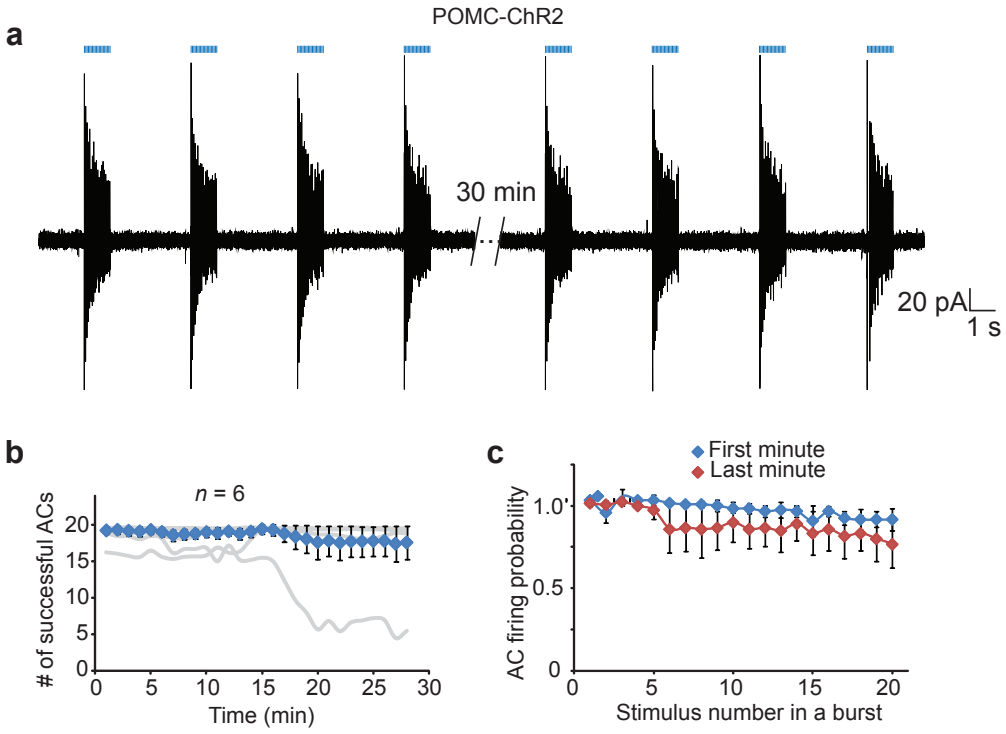
Supplementary Figure 4. Analysis of inter-pellet intervals for AGRP-ChR2 mice. Distribution of the log-transformed inter-pellet intervals (IPIs) during photostimulation for all AGRP-ChR2 mice with greater than 800 ChR2-expressing AGRP neurons (*n* = 21). The frequency distribution of log(IPI) was fit to a two component log-normal model (red curve). μ_1 and μ_2 are the calculated means resulting from the fit for the two components (vertical blue lines), and σ_1 and σ_2 are the resulting standard deviations (horizontal blue lines). The threshold value for the end of a bout was calculated from the mean and standard deviation of the second component; threshold = antilog(μ_2 + 3*σ_2).



Supplementary Figure 5. IPI threshold estimates feeding bouts.
IPIs for pellets consumed during continuous photostimulation for each animal (*n* = 21). Food consumption during the first bout (red) was determined from the calculated threshold (dashed red line).



Supplementary Figure 6. Feeding bouts resulting from 5 min photostimulation.
IPIs for each pellet consumed during photostimulation for each animal (*n* = 6). Photostimulation was terminated 5 min after consumption of the first pellet (dashed blue line). Food consumption during the first bout (red) was determined from the threshold (dashed red line) calculated from the 1 hour continuous stimulation data.



Supplementary Figure 7. Extended photostimulation of POMC neurons.
(a) Representative traces for a loose-seal, cell attached recording from a ChR2-expressing POMC neuron in an acute brain slice, showing action currents generated upon repeated rounds of 20 Hz photostimulation for 1 s followed by a 3 s delay and repeated over 30 min. Traces are taken from t = 1 min and t = 30 min. 10 ms light pulses are indicated by blue bars above the traces.
(b) Number of successfully photostimulated of action currents (AC) per burst averaged over 1 minute bins for individual POMC neurons (grey lines) and averaged over all cells (blue diamonds, *n* = 6). POMC neurons are reliably photoexcited over 30 min.
(c) Action current (AC) firing probability for each light pulse in a burst of 20 stimuli is plotted as a function of its position in the burst. The blue symbols indicate average success rate from the first 20 bursts (t = 0 - 1 min) and red symbols indicate average success rate from the last 20 bursts (t = 29 - 30 min).