Figure S1

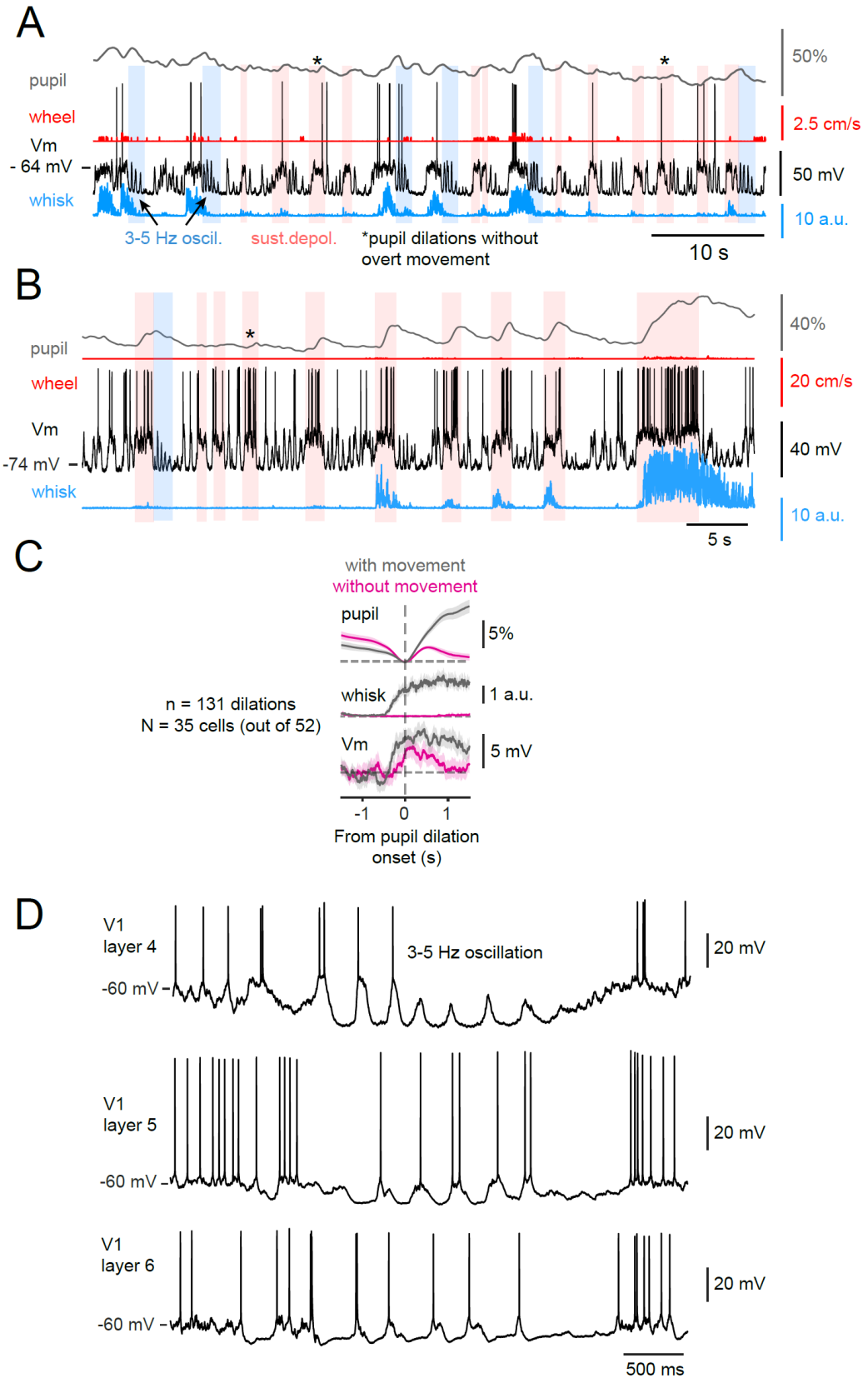
A
pupil
wheel
Vm
- 64 mV
whisk
50%
2.5 cm/s
50 mV
10 a.u.
3-5 Hz oscil.
sust.depol.
*pupil dilations without overt movement
10 s
B
pupil
wheel
Vm
-74 mV
whisk
40%
20 cm/s
40 mV
10 a.u.
5 s
C
with movement
without movement
pupil
5%
whisk
1 a.u.
Vm
5 mV
n = 131 dilations
N = 35 cells (out of 52)
-1
0
1
From pupil dilation onset (s)
D
V1 layer 4
-60 mV
3-5 Hz oscillation
20 mV
V1 layer 5
-60 mV
20 mV
V1 layer 6
-60 mV
20 mV
500 ms

Figure S1 (related to Figure 1). Pupil dilations in absence of overt movements of the whiskers or running wheel are associated with rapid depolarizations in L2,3 V1 neurons and 3-5 Hz oscillations can also be found in neurons of layers 4,5 and 6. (A) and (B) Example recordings of two different L2,3 V1 neurons in which pupil dilations occurred without overt movements of the whiskers or running wheel (indicated by asterisks; running wheel detects not only rotation, but also body postural changes; see Methods). (C) Examination of the membrane potential of 35 V1 L2,3 recordings (out of n = 52 cells) revealed the occurrence of a total of 131 pupil dilations without discernible movements. The average of these traces (shown in pink) is compared to the average response of another 131 pupil dilations in these cells in which whisker movements did occur (gray traces). Note that even in the absence of detectable movement of the whiskers, there is a significant depolarization of cortical membrane potential. In the presence of movement, this depolarization is slightly larger and longer lasting, as expected from the increased level of pupil dilation (arousal). (D) Example recordings of layer 4, 5 and 6 neurons illustrating the occurrence of the 3-5 Hz oscillation. Shaded regions in the graphs indicate the 95% bootstrapped confidence interval. A. is the same cell as shown in Figure S2A and S7F (top).

Figure S2

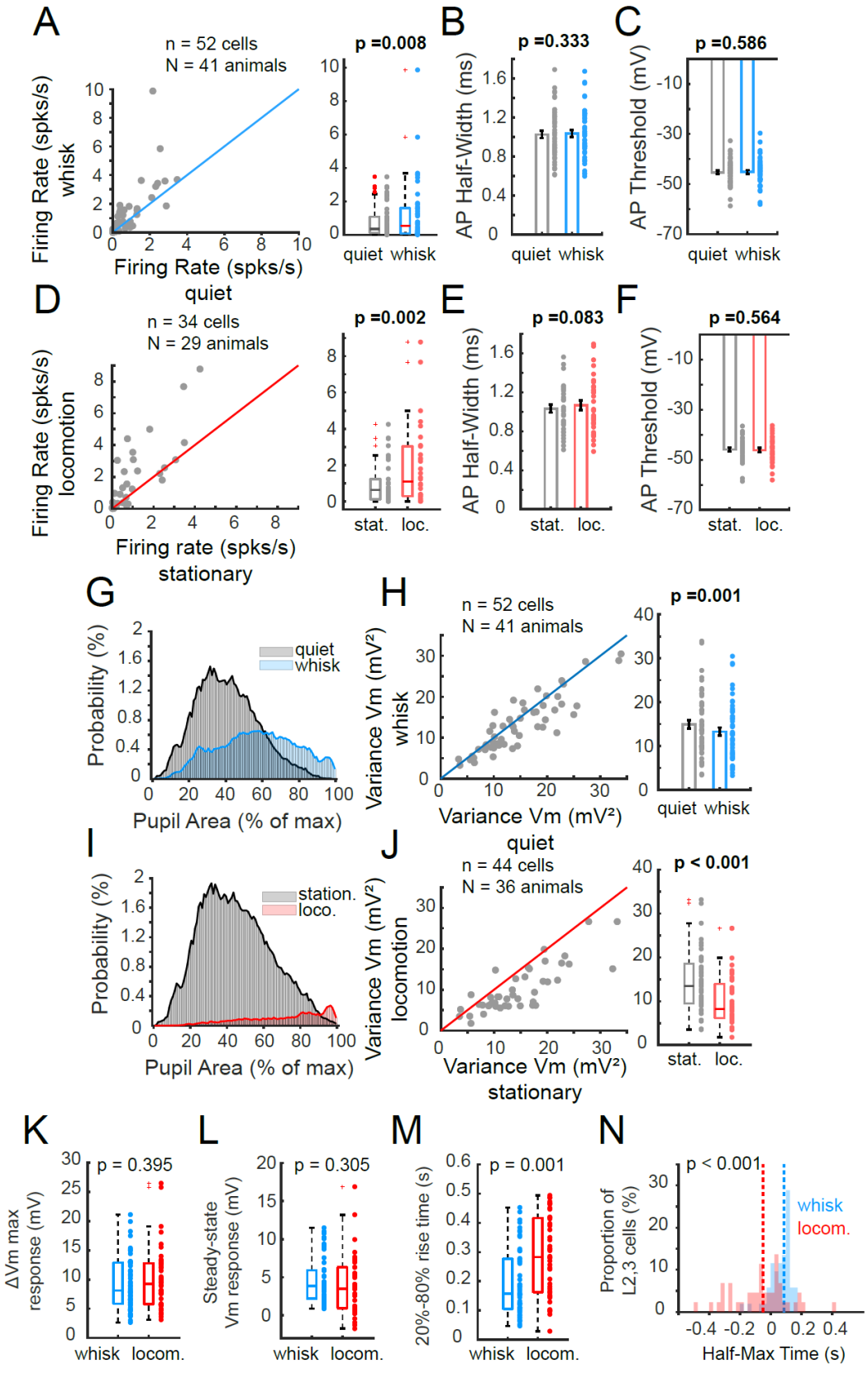
A
n = 52 cells
N = 41 animals
Firing Rate (spks/s)
whisk
Firing Rate (spks/s)
quiet
p =0.008
quiet whisk
B
p =0.333
AP Half-Width (ms)
quiet whisk
C
p =0.586
AP Threshold (mV)
quiet whisk
D
n = 34 cells
N = 29 animals
Firing Rate (spks/s)
locomotion
Firing rate (spks/s)
stationary
p =0.002
stat. loc.
E
p =0.083
AP Half-Width (ms)
stat. loc.
F
p =0.564
AP Threshold (mV)
stat. loc.
G
Probability (%)
quiet
whisk
Pupil Area (% of max)
H
n = 52 cells
N = 41 animals
Variance Vm (mV²)
whisk
Variance Vm (mV²)
p =0.001
quiet whisk
I
Probability (%)
station.
loco.
Pupil Area (% of max)
J
quiet
n = 44 cells
N = 36 animals
Variance Vm (mV²)
locomotion
Variance Vm (mV²)
stationary
p < 0.001
stat. loc.
K
ΔVm max response (mV)
p = 0.395
whisk locom.
L
Steady-state Vm response (mV)
p = 0.305
whisk locom.
M
20%-80% rise time (s)
p = 0.001
whisk locom.
N
p < 0.001
Proportion of L2,3 cells (%)
whisk
locom.
Half-Max Time (s)

Figure S2 (related to Figure 1). Both whisker movement, and walking with whisker movement, result in an overall increase in firing rate of V1 L2,3 neurons, without a significant change in AP half width or AP threshold. (A) Whisker movement increased the AP activity of a subset of L2,3 V1 neurons recorded intracellularly. Distribution of action potential firing rates in quiescence and whisker movement is shown on the right. Action Potential width at half maximum (B) and threshold (C) are not significantly affected by whisker movement. (D) Locomotion is associated with an increase in firing rate of intracellularly recorded visual cortical neurons. This increase in activity is not associated with a significant change in action potential width (E) or threshold (F). Both whisker movement (G) and locomotion (I) are associated with a shift of pupil diameter towards larger amplitudes. This is particularly true after 1-2 seconds from movement onset (not shown). Data shown here are not adjusted for pupil diameter lag. Both whisker movement (H) and locomotion (J) are associated with a significant decrease in membrane potential variance in V1 L2/3 neurons. (K-L) Peak (K) and steady state (L) depolarization of visual cortical neurons associated with whisking and walking. (M) 20-80% rise time of membrane potential depolarization with the onset of whisking and locomotion. (N)_Distribution of half-max time relative to onset of whisk or locomotion onset. Negative values indicate half-max time occurred prior to movement onset. Non-parametric datasets are depicted as boxplots which show the median, 25th and 75th percentiles and the whiskers indicate the most extreme data points that are not considered outliers. Normally distributed datasets are depicted as bars and the error bars show the standard error of the mean. The unpaired t-test (for normally distributed data), or the Wilcoxon rank sum test (non-parametric) were applied to test statistical significance.

Figure S3

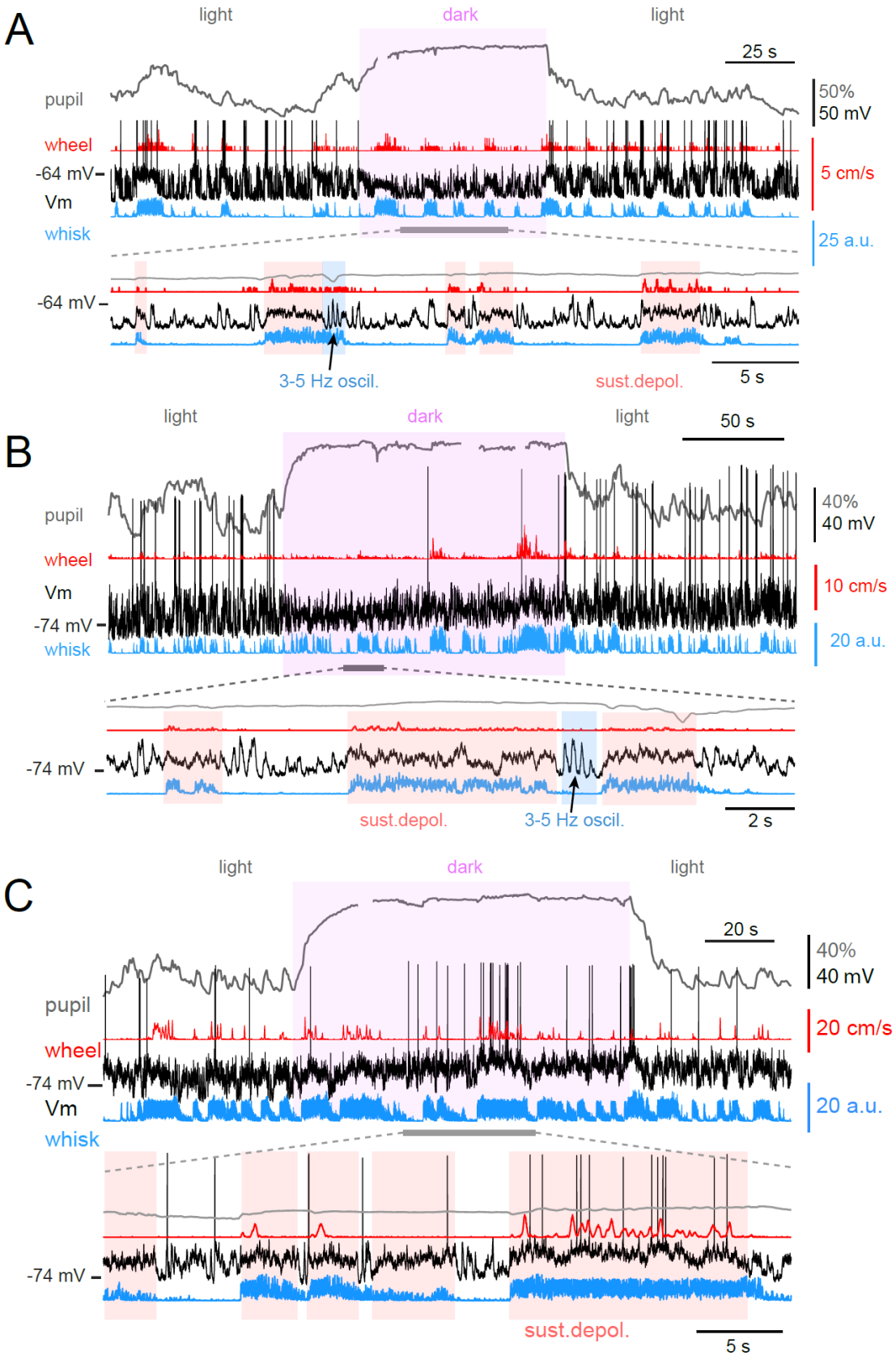
A
light
dark
light
25 s
pupil
50%
50 mV
wheel
5 cm/s
-64 mV
Vm
whisk
25 a.u.
-64 mV
3-5 Hz oscil.
sust.depol.
5 s
B
light
dark
light
50 s
pupil
40%
40 mV
wheel
10 cm/s
Vm
-74 mV
whisk
20 a.u.
-74 mV
sust.depol.
3-5 Hz oscil.
2 s
C
light
dark
light
20 s
pupil
40%
40 mV
wheel
20 cm/s
-74 mV
Vm
20 a.u.
whisk
-74 mV
sust.depol.
5 s

Figure S3 (related to Figure 1). Rapid movement related depolarizations in L2,3 V1 neurons persist in the absence of visible light. (A-C). Example recordings of three different L2,3 V1 neurons with and without visible light. The infrared light was not switched off during these recordings to allow the quantification of whisker pad movements and change in pupil area. Note that in all three cells, depolarizations still occur in the absence of visible light. The neurons shown in A and B exhibited a pronounced reduction in AP firing when the visible light was switched off (purple window).

Figure S4

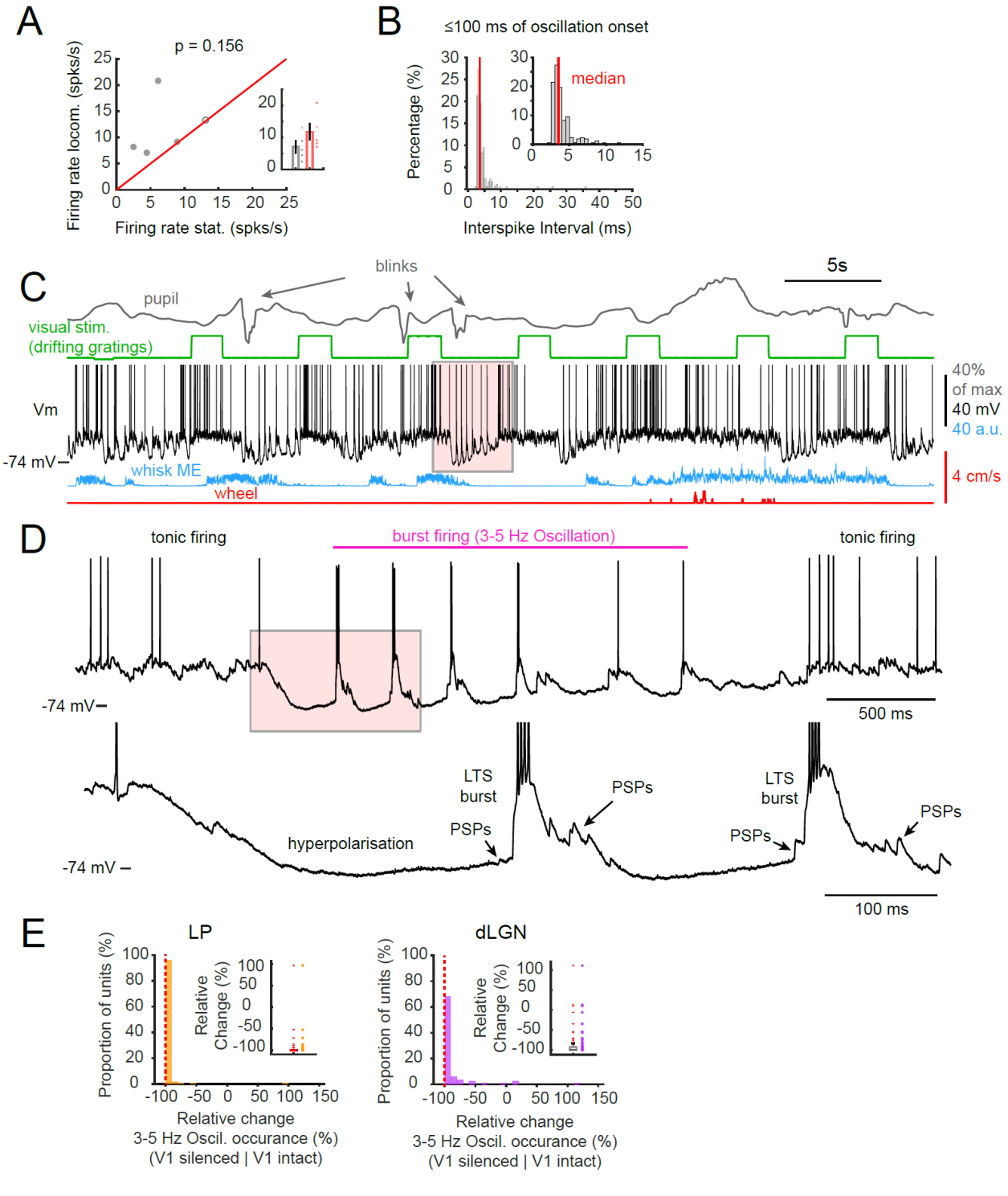
A
p = 0.156
Firing rate locom. (spks/s)
Firing rate stat. (spks/s)
B
≤100 ms of oscillation onset
median
Percentage (%)
Interspike Interval (ms)
C
blinks
5s
pupil
visual stim.
(drifting gratings)
Vm
-74 mV
whisk ME
wheel
40%
of max
40 mV
40 a.u.
4 cm/s
D
tonic firing
burst firing (3-5 Hz Oscillation)
tonic firing
-74 mV
500 ms
LTS
burst
PSPs
hyperpolarisation
-74 mV
100 ms
E
LP
dLGN
Proportion of units (%)
Relative
Change (%)
Relative change
3-5 Hz Oscil. occurance (%)
(V1 silenced | V1 intact)

Figure S4 (related to Figure 6). Whole-cell recordings in visual thalamic neurons reveal enhanced AP firing during whisker movements in some visual thalamic neurons and 3-5 Hz oscillations to also occur often after visual stimulation. (A) The AP firing rate is enhanced in 3 out of 5 intracellularly recorded visual thalamic neurons during locomotion (no locomotion bouts occurred in 3 out of the 8 recordings; LP neurons are shown as filled circles; the dLGN neuron is shown as unfilled circle). (B) Distribution of interspike interval of 8 visual thalamic neurons (same cells as Fig. 3E and Figure 6D-F) during the first cycle of the 3-5 Hz oscillation (<100 ms after oscillation onset) exhibits a preponderance of short intervals (e.g. < 5 msec), indicative of burst firing. (C) Example recording of a visual thalamic neuron during visual stimulation (moving gratings). Previous studies showed that the likelihood of the 3-5 Hz rhythm is enhanced with visual stimulation (Einstein et al., 2017). Note that the example visual thalamic neuron also exhibited pronounced LTS mediated burst firing at a 3-5 Hz rhythm, which indicates that the cell intrinsic mechanism for the generation of the 3-5 Hz rhythm is similar between those occurring after visual stimulation offset and those occurring preferentially after enhanced movement activity. (D) Expansion of a 3-5 Hz oscillation occurring after the offset of visual stimulation (red box in C). Note the prominent EPSP shaped events arriving in the neuron with each depolarizing wave of the oscillation. (E) Relative change in rate of occurrence of 3-5 Hz oscillation in LP and dLGN with visual cortical silencing. Note that visual cortical silencing nearly completely abolishes the occurrence of 3-5 Hz oscillations.

Figure S5

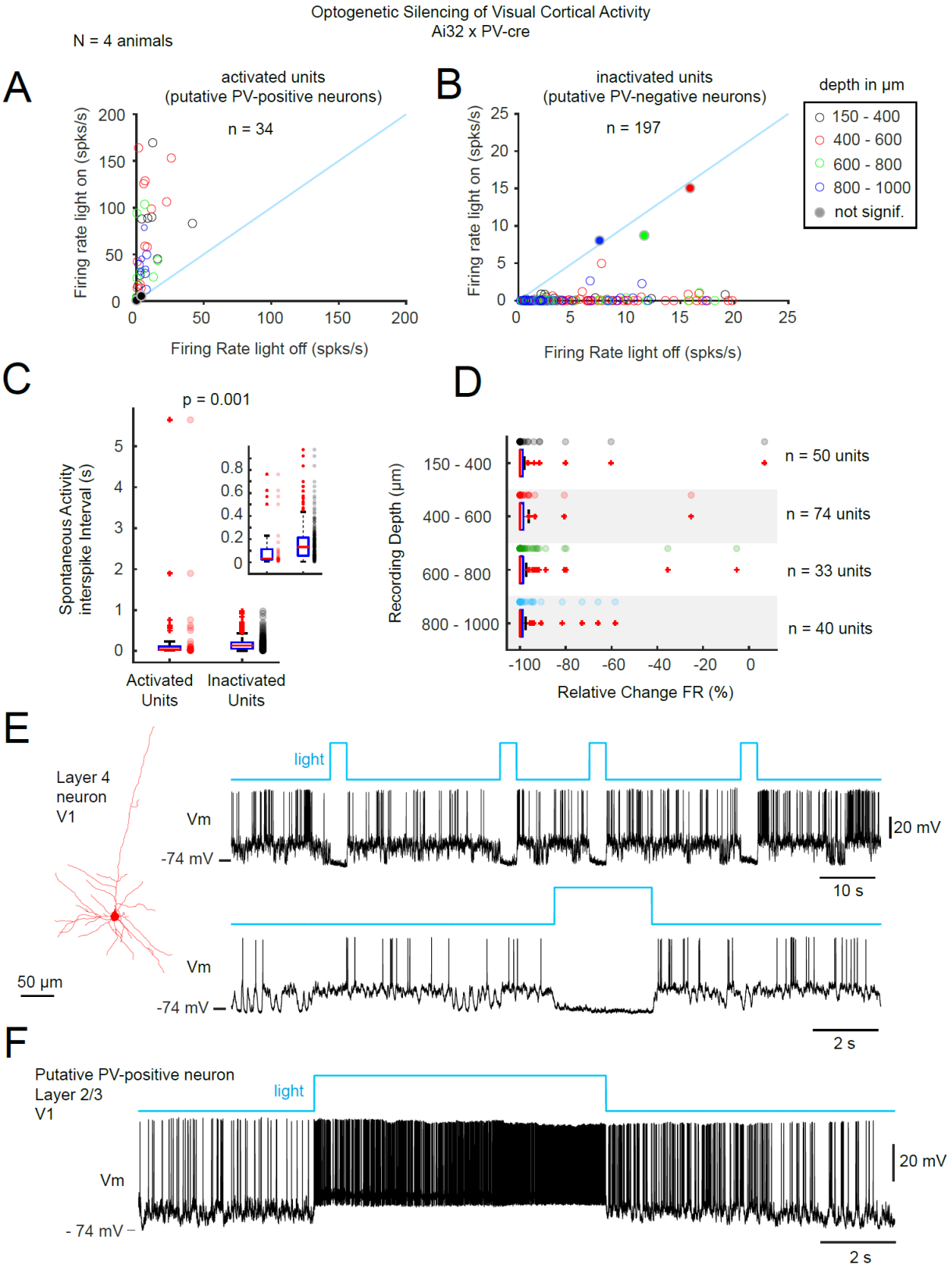
Optogenetic Silencing of Visual Cortical Activity
Ai32 x PV-cre
N = 4 animals
A
activated units
(putative PV-positive neurons)
n = 34
Firing rate light on (spks/s)
Firing Rate light off (spks/s)
B
inactivated units
(putative PV-negative neurons)
n = 197
depth in µm
150 - 400
400 - 600
600 - 800
800 - 1000
not signif.
C
p = 0.001
Spontaneous Activity
interspike Interval (s)
Activated Units
Inactivated Units
D
Recording Depth (µm)
n = 50 units
n = 74 units
n = 33 units
n = 40 units
Relative Change FR (%)
E
Layer 4
neuron
V1
light
Vm
-74 mV
20 mV
10 s
50 µm
2 s
F
Putative PV-positive neuron
Layer 2/3
V1
light
Vm
- 74 mV
20 mV
2 s

Figure S5 (related to Figures 6 and 7) Effects of optical silencing of visual cortex through channel-rhodopsin activation of PV neurons. (A) Distribution of firing rate of activated neurons with and without application of blue light. These activated neurons (n=34/231 cells in N = 4 animals) are presumably PV-containing interneurons. (B) Distribution of firing rates of inactivated neurons (n=197/231 cells). The depth of the cells recorded in A and B are color coded and the large majority of cells at all depths from layer 2 to 6 are inhibited by application of the blue light. (C). Examination of the interspike intervals during spontaneous activity in the blue light activated and inactivated groups reveal that the activated group have a preponderance of short interspike intervals – consistent with this group being composed of PV-containing interneurons. (D) Depth distribution of cells inactivated by blue light. Activation of PV interneurons resulted in the suppression of activity in neurons throughout the depth of the cortex. (E) Intracellular recording from a layer 4 excitatory neuron during the application of blue light reveals a strong hyperpolarization and suppression of activity. (F) Intracellular recording from a putative PV-positive interneuron during application of blue light exhibits a strong depolarization and activation. This cell exhibited interspike intervals of less than 3 msec, suggesting it was a fast-spiking interneuron. The Wilcoxon rank sum test (non-parametric) was applied to test significance in C.

Figure S6

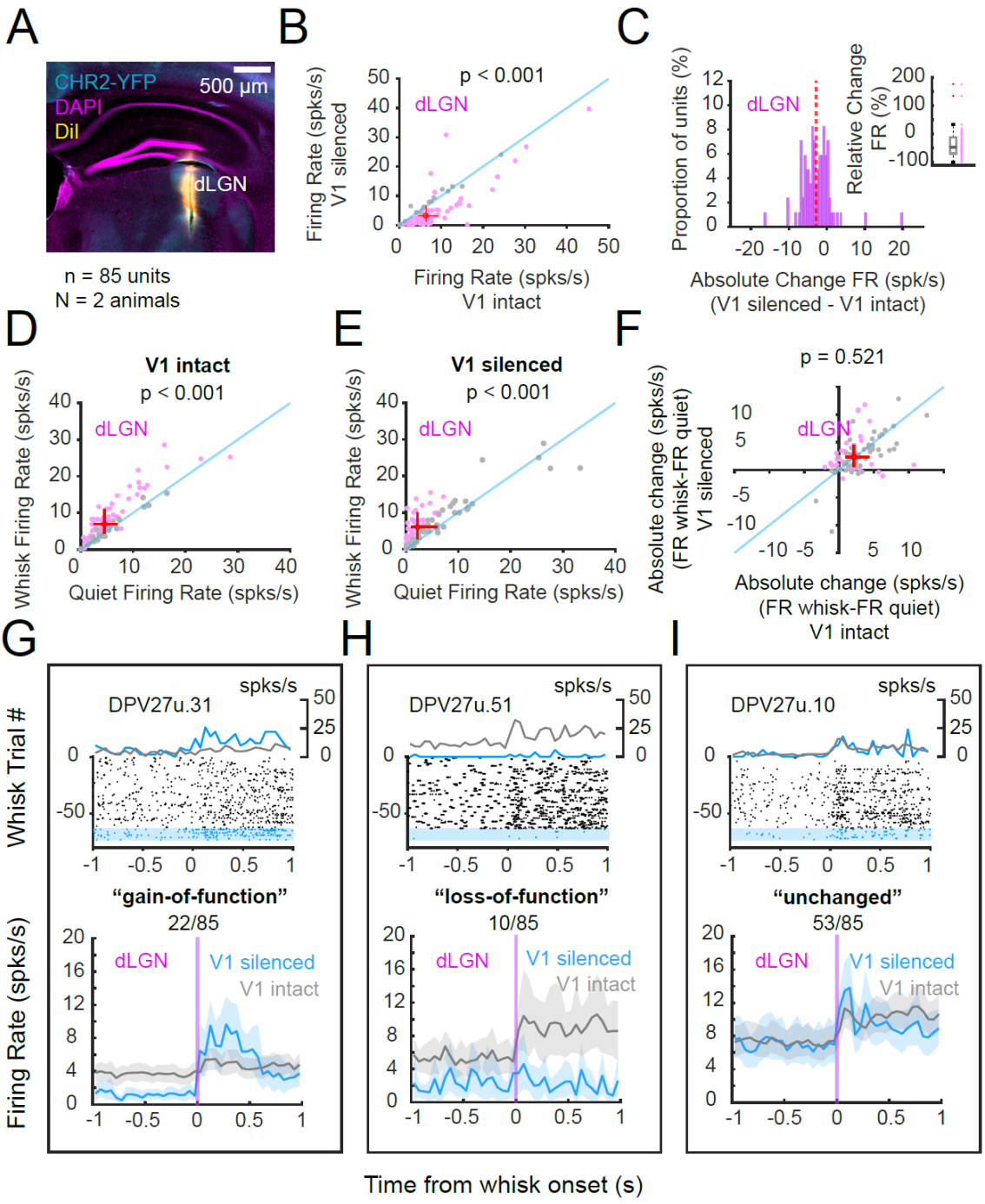
A
CHR2-YFP
DAPI
DiI
500 µm
dLGN
n = 85 units
N = 2 animals
B
p < 0.001
dLGN
Firing Rate (spks/s)
V1 silenced
Firing Rate (spks/s)
V1 intact
C
dLGN
Proportion of units (%)
Relative Change FR (%)
Absolute Change FR (spk/s)
(V1 silenced - V1 intact)
D
V1 intact
p < 0.001
dLGN
Whisk Firing Rate (spks/s)
Quiet Firing Rate (spks/s)
E
V1 silenced
p < 0.001
dLGN
Whisk Firing Rate (spks/s)
Quiet Firing Rate (spks/s)
F
p = 0.521
dLGN
Absolute change (spks/s)
(FR whisk-FR quiet)
V1 silenced
Absolute change (spks/s)
(FR whisk-FR quiet)
V1 intact
G
DPV27u.31
spks/s
Whisk Trial #
"gain-of-function"
22/85
dLGN
V1 silenced
V1 intact
Firing Rate (spks/s)
H
DPV27u.51
spks/s
"loss-of-function"
10/85
dLGN
V1 silenced
V1 intact
I
DPV27u.10
spks/s
"unchanged"
53/85
dLGN
V1 silenced
V1 intact
Time from whisk onset (s)

Figure S6 (related to Figure 7). Effects of movement on dLGN thalamic activity with and without silencing of visual cortex. (A) Illustration of the Neuropixels recording track demonstrating the recording sites in the medial portion of the dLGN. (B) and (C) Silencing of activity in V1 significantly reduces dLGN activity. Whisker movement is associated with increased dLGN activity whether the visual cortex is intact (D) or inhibited (E). (F) The effects of V1 inactivation on the ability of whisker movement to modulate dLGN activity varied between neurons (pink dots represent units with statistically significant difference between V1 intact and silent). (G-I) Examples and group averages of dLGN cells that increase (G), decrease (H) or have no significant change (I) in their whisker-related activity with visual cortical inactivation (bin size for spike rates = 50 ms). The paired t-test (for normally distributed data), or the Wilcoxon signed rank sum test (non-parametric) were applied to test statistical significance in D-F. The crosses in scatter plots show the 25th and 75th percentiles with the center representing the overall medians. Data points in F represent the average of bootstrapped means. Statistical significance for each of the data points in F was assessed by testing whether the bootstrapped 95% confidence intervals (not shown) crossed the reference line (shown in blue). Shaded regions in G-I indicate 95% bootstrapped confidence interval.

Figure S7

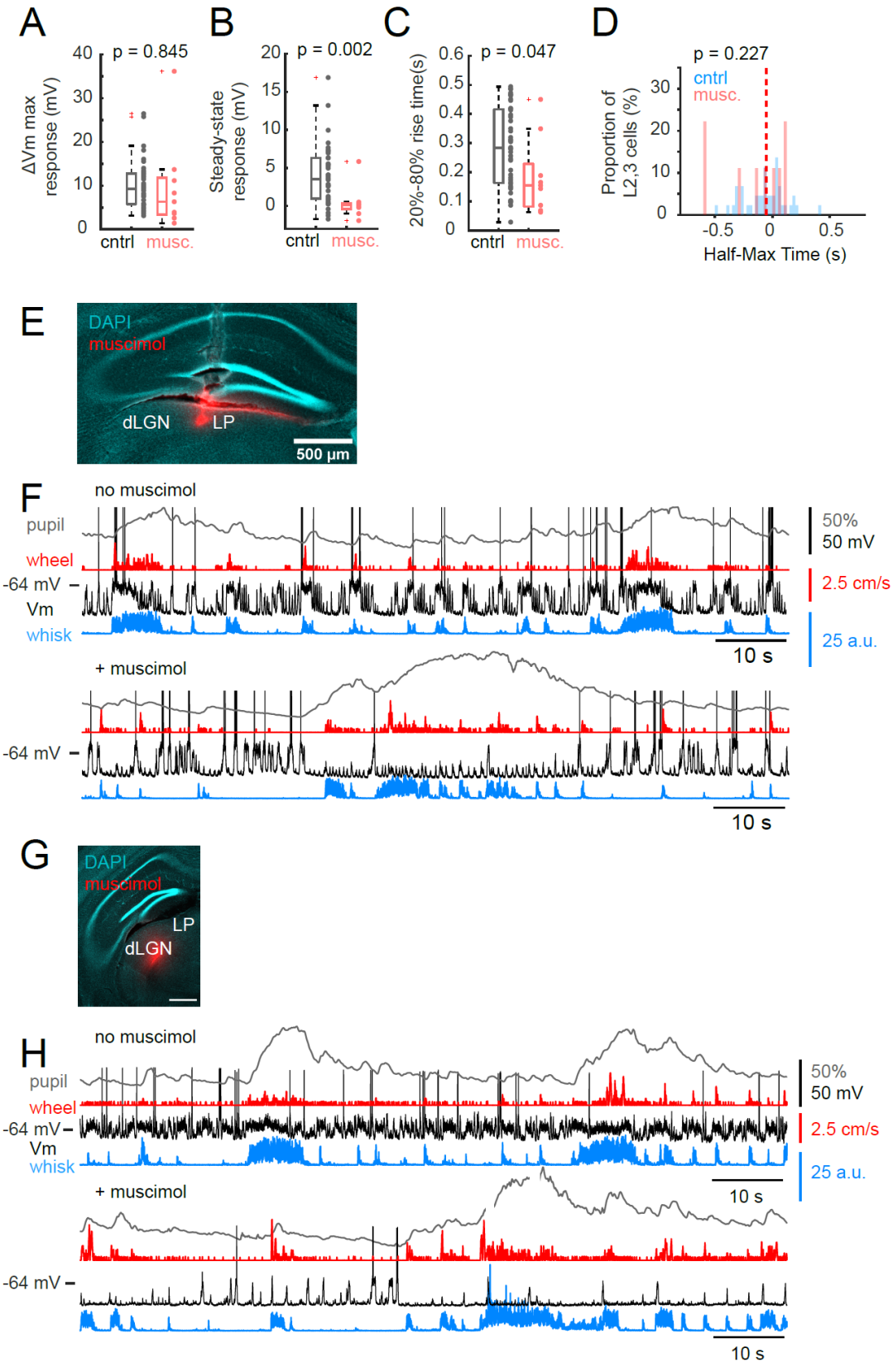
A
ΔVm max response (mV)
p = 0.845
cntrl
musc.
B
Steady-state response (mV)
p = 0.002
C
20%-80% rise time(s)
p = 0.047
D
Proportion of L2,3 cells (%)
p = 0.227
cntrl
musc.
Half-Max Time (s)
E
DAPI
muscimol
dLGN
LP
500 µm
F
no muscimol
pupil
wheel
-64 mV
Vm
whisk
50%
50 mV
2.5 cm/s
25 a.u.
10 s
+ muscimol
G
DAPI
muscimol
LP
dLGN
H
no muscimol
+ muscimol

Figure S7 (related to Figure 8). Effects of silencing of visual thalamus on movement/arousal related activity in V1. (A) The maximum response of the depolarization associated with locomotion in L2,3 V1 neurons is not significantly changed by the inactivation of the visual thalamus. (B) The steady-state response of the locomotion related response in L2,3 neurons is largely abolished by inactivation of the visual thalamus. (C) The 20%-80% rise time of these depolarizations is slightly changed but the (D) half max time remains unchanged by inactivation. (E) Example image of a muscimol injection that was centered around the LP nucleus. (F) The top trace shows a whole-cell recording of a L2,3 neuron before muscimol was injected into the visual thalamus. The bottom trace depicts a whole-cell recording of a L2,3 neuron from the same animal as in the top trace and from the brain shown in (E) after muscimol was injected. (G) Example image of a muscimol injection that was centered around the dLGN nucleus. (H) The top trace shows a whole-cell recording of a L2,3 neuron before muscimol was injected into the visual thalamus. The bottom trace depicts a whole-cell recording of a L2,3 neuron from the same animal as in the top trace and from the brain shown in (G) after muscimol was injected. Boxplots show the median, 25th and 75th percentiles and the whiskers indicate the most extreme data points that are not considered outliers. The unpaired t-test (for normally distributed data), or the Wilcoxon rank sum test (non-parametric) were applied to test statistical significance in panels A-D.