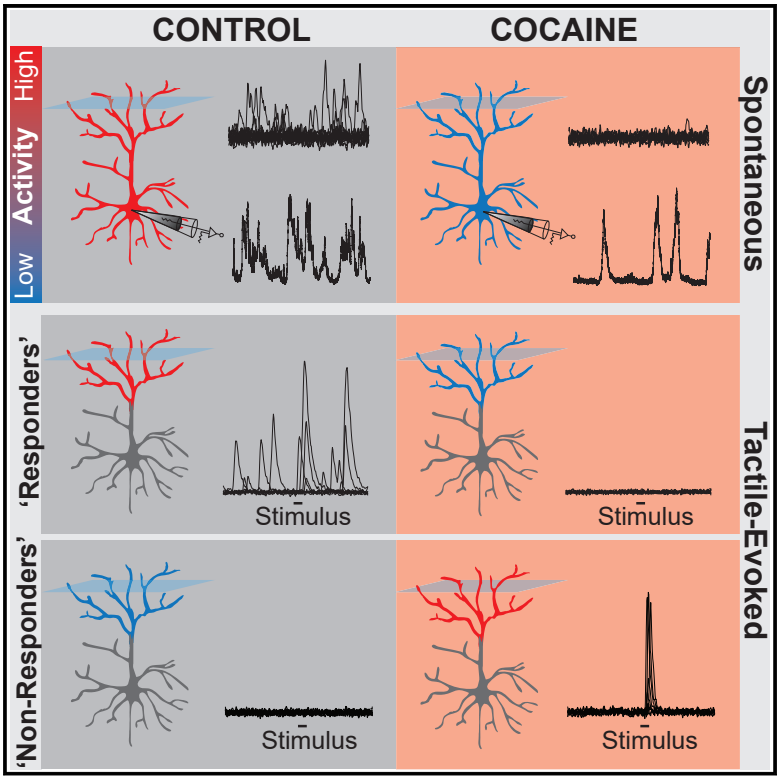
Cell Reports

Cocaine regulates sensory filtering in cortical pyramidal neurons

Graphical abstract



Highlights

- Cocaine dampens state transitions in layer 2/3 cortical neurons
- Spontaneous activity in cortical neurons is decreased during cocaine
- Cocaine has a heterogeneous influence on sensory encoding in cortical dendrites
- Cocaine influences sensory signaling in both anesthetized and awake states

Authors

Sean C. Murphy, Luca Godenzini, Robertas Guzulaitis, Andrew J. Lawrence, Lucy M. Palmer

Correspondence

lucy.palmer@florey.edu.au

In brief

Following cocaine administration, Murphy et al. observe a decrease in spontaneous activity and a heterogeneous influence on the encoding of sensory information in layer 2/3 pyramidal neurons within the somatosensory cortex. Therefore, cocaine acts as a filter that dampens background noise to selectively modulate sensory signaling in the cortex.

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Report

Cocaine regulates sensory filtering in cortical pyramidal neurons

Sean C. Murphy,[1,2] Luca Godenzini,[1,2] Robertas Guzulaitis,[1,3] Andrew J. Lawrence,[1,2] and Lucy M. Palmer[1,2,4,*]
[1]Florey Institute of Neuroscience and Mental Health, Parkville, VIC 3052, Australia
[2]Florey Department of Neuroscience and Mental Health, University of Melbourne, Parkville, VIC 3052, Australia
[3]Life Sciences Center, Vilnius University, 10257 Vilnius, Lithuania
[4]Lead contact
*Correspondence: lucy.palmer@florey.edu.au
https://doi.org/10.1016/j.celrep.2023.112122

SUMMARY

Exposure to cocaine leads to robust changes in the structure and function of neurons within the mesocorticolimbic pathway. However, little is known about how cocaine influences the processing of information within the sensory cortex. We address this by using patch-clamp and juxtacellular voltage recordings and two-photon Ca^{2+} imaging *in vivo* to investigate the influence of acute cocaine exposure on layer 2/3 (L2/3) pyramidal neurons within the primary somatosensory cortex (S1). Here, cocaine dampens membrane potential state transitions and decreases spontaneous somatic action potentials and Ca^{2+} transients. In contrast to the uniform decrease in background spontaneous activity, cocaine has a heterogeneous influence on sensory encoding, increasing tactile-evoked responses in dendrites that do not typically encode sensory information and decreasing responses in those dendrites that are more reliable sensory encoders. Combined, these findings suggest that cocaine acts as a filter that suppresses background noise to selectively modulate incoming sensory information.

INTRODUCTION

The cortex is central to sensory processing, contributing to sensory encoding,[1–5] perception,[6,7] and learning.[8–10] Receiving both external sensory[11,12] and internal contextual[13] information, cortical neurons dynamically integrate different information pathways in their elaborate dendritic arbors. Since dendrites can powerfully and dynamically drive neural output,[14,15] they are ideal substrates for rapidly altering the gain of cortical neurons.[16] Indeed, dendritic neuromodulation, that is, the alteration of dendritic signaling by neurons themselves or substances they release, can rapidly and flexibly change the efficacy of sensory processing.[17,18]

Sensory signals are typically embedded in varying levels of background spontaneous activity.[19,20] Competing with noisy spontaneous information,[21] sensory processing in the cortex is therefore an interplay between spontaneous activity and sensory information. To selectively amplify relevant sensory inputs, background noise needs to be effectively suppressed.[22] Such sensory filtering can be mediated by various input pathways, including the basal ganglia-to-thalamus[23] and motor cortex.[24] Chemical neuromodulation is also an ideal candidate to drive sensory filtering, as neuromodulators can have a contrasting influence on cellular response properties.[25]

As a potent stimulant that acts on endogenous neuromodulators, cocaine leads to synaptic re-activation, neuronal hyperexcitability, and plasticity.[26–29] Our current understanding of how cocaine influences cellular processes mostly stems from the mesocorticolimbic pathway, where cocaine administration can cause robust changes to the structure and function of neurons.[30,31] Within the cortex, cocaine exposure results in enhanced sensory responses[32]; however, much is still unknown about how cocaine influences signaling in sensory cortices, and even less is known about the influence of cocaine on cortical dendrites. This is surprising considering cocaine can alter cognition,[33–35] a process that is typically considered to have a cortical origin.[36,37]

Using whole-cell patch-clamp and juxtacellular electrophysiology and two-photon Ca^{2+} imaging before and after a single exposure to cocaine, we investigated the acute influence of cocaine on the encoding of spontaneous and sensory information in cortical neurons. Our findings illustrate that exposure to cocaine has a heterogeneous influence on cortical neurons, leading to the dampening of background spontaneous activity and selective filtering of sensory input.

RESULTS

Cocaine dampens state transitions in cortical pyramidal neurons

To assess the influence of single exposure of cocaine on cortical neurons, we first characterized membrane potential changes in layer 2/3 (L2/3) pyramidal neurons within the forepaw area of the primary somatosensory cortex (S1). Using whole-cell patch-clamp electrophysiology in anesthetized mice (postnatal days 33–62), voltage recordings were performed pre- and

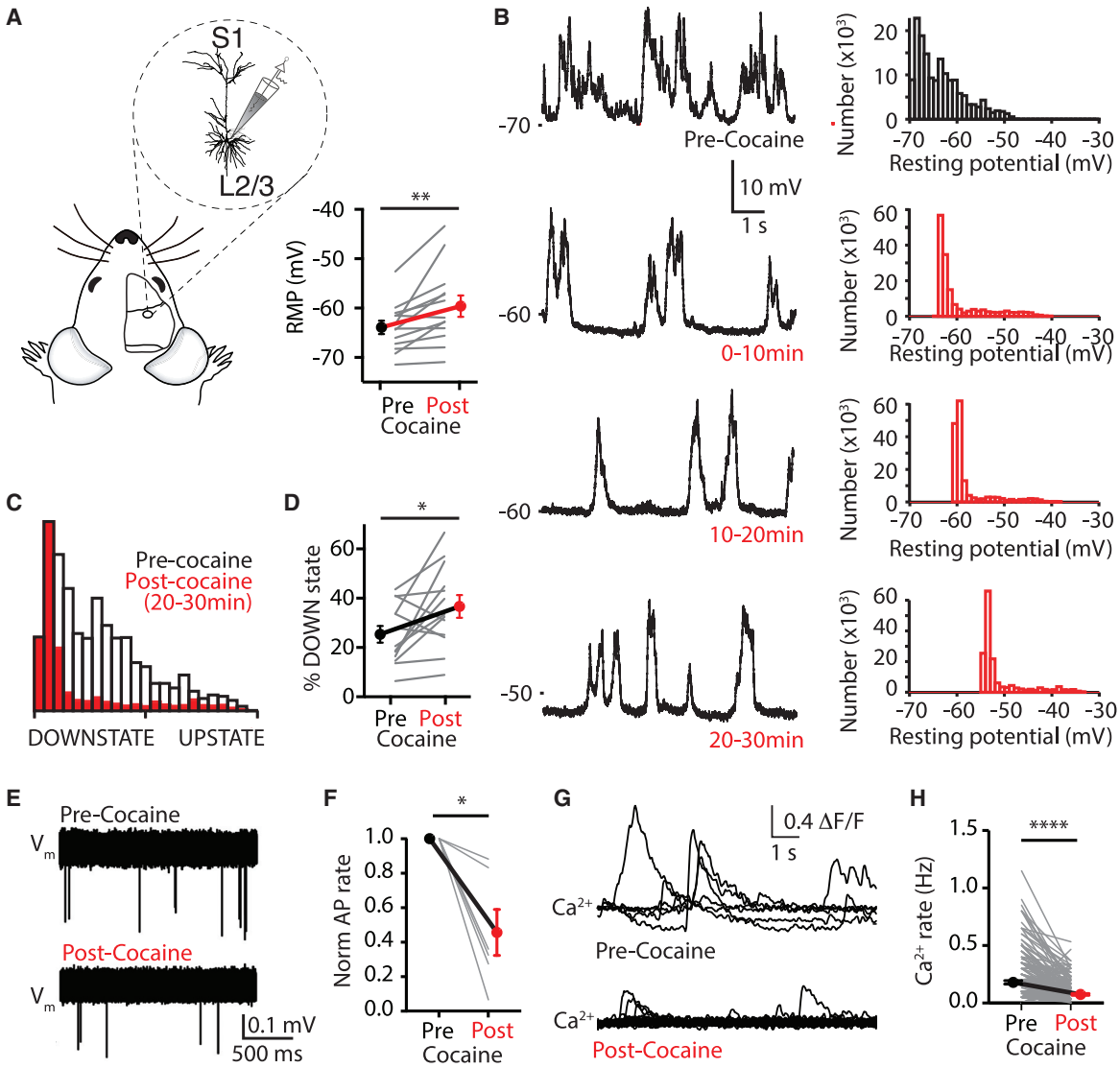

Figure 1. Cocaine dampens spontaneous state transitions and action potentials

(A) Schematic of experimental design. Whole-cell patch-clamp recordings were performed from L2/3 pyramidal neurons in anesthetized mice. Inset, resting membrane potential pre- and 20 min post-cocaine injection (n = 13 neurons).

(B) Membrane potential (left) and histogram (right) from example L2/3 pyramidal neuron following cocaine administration.

(C) Overlay of membrane potentials for example shown in (B) (normalized to maximum).

(D) Average percentage of time in down state (within 2 mV of the most hyperpolarized potential; n = 13 neurons).

(E) (Top) Example juxtacellular recording pre- (top) and post- (bottom; 20 min) cocaine injection.

(F) Average action potential (AP) rate following cocaine (0–30 min) normalized to pre-cocaine rates (n = 6 neurons).

(G) Spontaneous somatic Ca^{2+} transients pre- and post-cocaine injection (0–30 min) for an example L2/3 pyramidal neuron.

(H) Average spontaneous Ca^{2+} transients rate for all recorded neurons (n = 201 somas, 6 mice).

*p < 0.05, **p < 0.01; ****p < 0.0001. Error bars, SEM.

post-intraperitoneal injection of 20 mg/kg (intraperitoneal [i.p.]) cocaine (Figure 1A), which we have previously used in mouse studies involving both behavior and electrophysiology.[38] At rest, the membrane potential of layer 2/3 (L2/3) pyramidal neurons was −63.9 ± 1.3 mV, and following cocaine administration, it was depolarized by 5.6 ± 1.8 mV (to −59.6 ± 2.2 mV; 20 min post-cocaine exposure; p = 0.001; Wilcoxon matched-pairs signed rank test; n = 13 neurons; Figure 1A, inset). Under anesthesia[39,40] and sleep,[41,42] the membrane potential of neurons fluctuates between depolarized "up" and hyperpolarized "down" states. Cocaine influenced these membrane potential fluctuations in L2/3 pyramidal neurons, increasing the time the membrane potential was in the down state and decreasing up states (Figure 1B). On average, the membrane potential was in

the down state for significantly longer following cocaine exposure (25.9% ± 3.3% vs. 36.7% ± 4.6%; p = 0.03; Wilcoxon matched-pairs signed rank test; n = 13 neurons; Figure 1C). Compared with cocaine, saline did not significantly influence state transitions (p = 0.25; Wilcoxon matched-pairs signed rank test; n = 3 neurons; Figure S1), and there was no effect of mouse age on the influence of cocaine on neural biophysical properties (Figure S2). Despite changes in state transitions following cocaine exposure, there was no significant difference in the amplitude of up states (18.3 ± 1.9 vs. 18.6 ± 2 mV; p = 0.79; Wilcoxon matched-pairs signed rank test; n = 13 neurons). Likewise, there was no significant difference in the amplitude of action potentials following cocaine exposure (p = 0.75; Kruskal-Wallis test; Figure S3), suggesting that cocaine does not primarily influence post-synaptic voltage-gated ion channels.

Does the depolarized resting membrane potential and dampened state transitions following cocaine exposure also influence the rate of spontaneous action potentials? To investigate this, juxtacellular voltage recordings were performed from L2/3 pyramidal neurons within S1 (Figure 1E). Here, the spontaneous firing rate was dampened following single exposure to cocaine, decreasing by 55% ± 13% from pre-cocaine firing rates (0–30 min post-cocaine; p = 0.03; n = 6 neurons; Wilcoxon matched-pairs signed rank test; Figure 1F). In contrast, saline did not significantly alter the spontaneous firing rate (0–30 min post-cocaine; p = 0.469; n = 7 neurons). The influence of cocaine on somatic activity was also investigated using two-photon Ca^{2+} imaging from the somata of L2/3 pyramidal neurons (Figure 1G). Similar to voltage recordings, spontaneous somatic Ca^{2+} activity was dampened following cocaine administration, with a significant decrease in the average peak amplitude (0.47 ± 0.04 vs. 0.27 ± 0.05 ΔF/F; p < 0.0001; Mann Whitney test) and rate (0.18 ± 0.02 vs. 0.08 ± 0.01; p < 0.0001; Wilcoxon matched-pairs signed rank test; n = 201 somas, 6 mice; Figure 1H). Taken together, in agreement with the dampening of state transitions, cocaine decreases spontaneous activity in L2/3 pyramidal neurons.

Cocaine dampens spontaneous activity in tuft dendrites of L2/3 pyramidal neurons

To test the influence of cocaine at the dendritic site of feedback input,[11] we next assessed the activity of tuft dendrites of L2/3 pyramidal neurons (<80 μm below pia) that were sparsely transfected with the genetic Ca^{2+} indicator GCaMP6f (Figure 2A). Following expression, two-photon Ca^{2+} imaging was performed in anesthetized mice both pre- and post-cocaine injection (Figure 2B). On average, tuft dendrites of L2/3 pyramidal neurons were spontaneously active at a rate of 0.19 ± 0.03 Hz (n = 235 dendrites, 11 mice). Following cocaine, this rate decreased to 0.04 ± 0.007 Hz and remained significantly dampened throughout the recording period (up to 50 min post-cocaine injection; p = 0.0001; Kruskal-Wallis test; Figures 2C and 2D). In contrast to cocaine, injection of saline did not significantly influence the average spontaneous rate of Ca^{2+} transients (p = 0.31; Wilcoxon test; 108 dendrites, Figure 2E). Despite the overall dampening of spontaneous activity, the amplitude of spontaneous Ca^{2+} transients were not significantly influenced by cocaine (p = 0.47; Mann Whitney test) or saline (p = 0.44; Mann-Whitney test). Therefore, cocaine dampens the rate of spontaneous dendritic activity, leading to prolonged silencing of tuft dendrites in L2/3 pyramidal neurons within the S1.

The influence of cocaine on sensory-evoked Ca^{2+} responses in tuft dendrites

Given cocaine leads to changes in sensory perception,[43,44] we next investigated the influence of cocaine exposure on dendritic processing of sensory information. A tactile stimulus (500 ms, 200 Hz) was delivered to the contralateral forepaw in anesthetized mice, and the sensory-evoked Ca^{2+} responses were measured in tuft dendrites for up to 50 min post-cocaine administration. As previously reported,[45] tuft dendrites of L2/3 pyramidal neurons within S1 sparsely encode sensory information, with tactile stimulation evoking Ca^{2+} responses above baseline in 33% of dendrites (n = 55/165 dendrites). Following cocaine administration, tactile-evoked activity in tuft dendrites was altered according to prior rates of sensory encoding. Dendrites that encoded the tactile stimulus with a stimulus-evoked rate that was above average ("responders") were dampened following cocaine administration. On average, these dendrites had a significant decrease in the maximum sensory-evoked activity following cocaine exposure (0.15 ± 0.007 vs. 0.07 ± 0.02 Hz, p = 0.0001; n = 55/165 dendrites; Figures 3A–3C). This decrease in activity typically remained throughout the recording period (50 min post-cocaine injection). In contrast, those dendrites that weakly encoded the tactile stimulus with a stimulus-evoked rate that was less than average ("non-responders") showed significantly increased sensory-evoked activity following cocaine administration (pre, 0.03 ± 0.002 Hz; post, 0.05 ± 0.01 Hz, p = 0.03; n = 110/165 dendrites; Figures 3D–3F). Remarkably, a subset of dendrites that did not respond to the tactile stimulus prior to cocaine had tactile-evoked Ca^{2+} responses following cocaine exposure. Despite these differences in sensory encoding, responder and non-responder dendrites had similar amplitudes of baseline spontaneous activity (0.80 ± 0.12 vs. 0.73 ± 0.10 ΔF/F; p = 0.495), which was uniformly dampened following cocaine administration. Taken together, cocaine acts as a filter for sensory encoding in the tuft dendrites of L2/3 pyramidal neurons, abolishing sensory-evoked activity in some dendrites while converting other non-responders into encoders of tactile stimulation.

Cocaine increases sensory-evoked Ca^{2+} activity in tuft dendrites in the awake state

Since anesthesia alters feedback activity, which targets distal dendrites,[46] we next investigated whether sensory-evoked responses in tuft dendrites were also influenced by cocaine in the awake state. First, we measured mouse movement before and after cocaine administration via tracking face motion[47] (Figure 4A). Following cocaine administration and throughout the duration of the recordings (up to 30 min), overall spontaneous mouse movement significantly increased from 0.68 ± 0.07 × 10^5 to 1.14 ± 0.14 × 10^5 motion energy (p = 0.032; n = 5 mice; Figures 4B and 4C). This dramatic increase in movement following cocaine contrasts with saline (0.54 ± 0.06 × 10^5 motion energy; p = 0.19; Wilcoxon matched-pairs signed rank test; n = 5

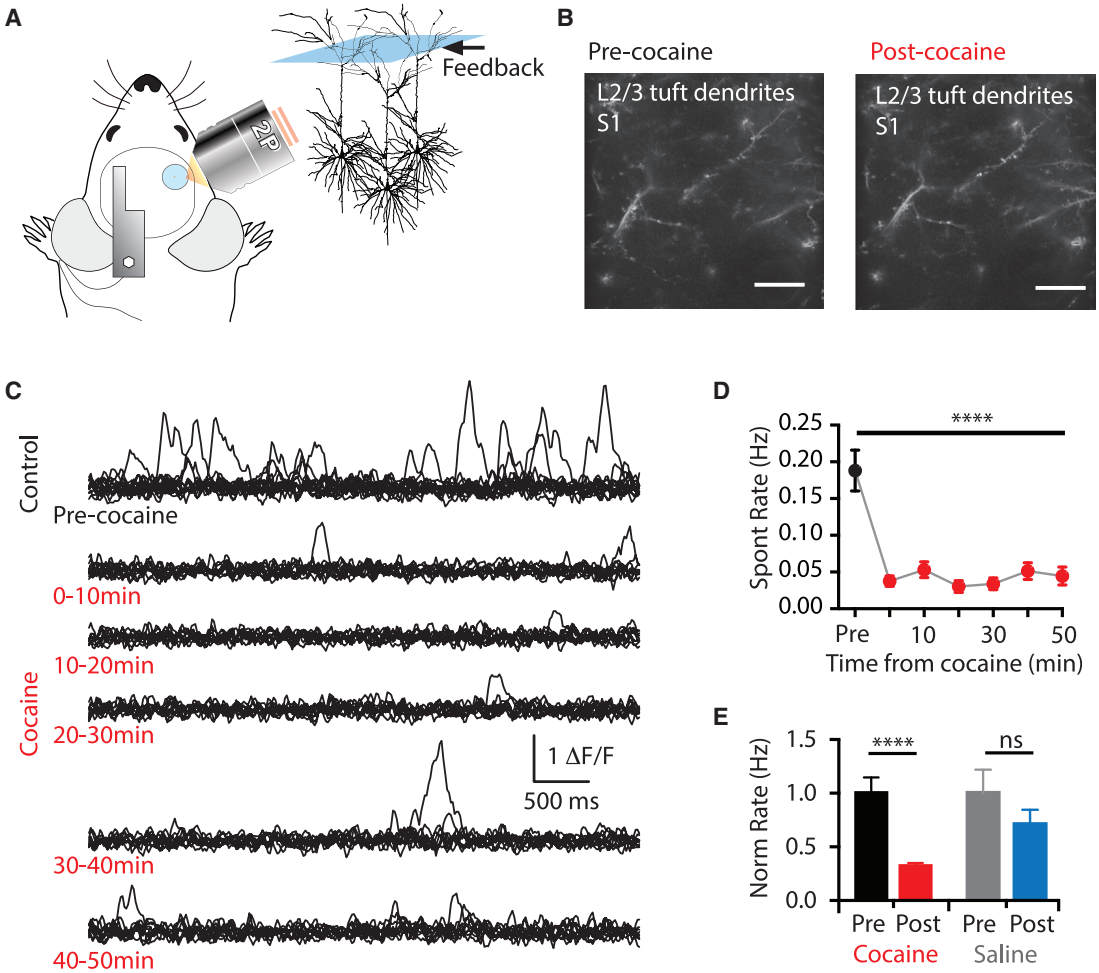

Figure 2. Spontaneous activity is decreased in tuft dendrites following cocaine

(A) Schematic of experimental design. Two-photon Ca^{2+} imaging was performed in tuft dendrites of L2/3 pyramidal neurons before (pre-) and after (post-) cocaine.

(B) Example illustrating the similar fields of view following cocaine injection. Scale bar, 20 μm.

(C) Overlay of spontaneous Ca^{2+} activity from a single tuft dendrite pre- and post-cocaine.

(D) Average rate of spontaneous Ca^{2+} activity pre- (black) and post- (red) cocaine (n = 235 dendrites, 11 mice).

(E) Spontaneous Ca^{2+} activity normalized to the average pre-injection rate, following injection of cocaine (red) and saline (blue).

*p < 0.05; ****p < 0.0001. Error bars, SEM.

mice), which had similar motion energy to pre-cocaine (Figure 4C). Despite this increase in baseline movement during cocaine, there was no difference in the movement response to the tactile stimulation (normalized to baseline; 1.15 ± 0.09 vs. 1.07 ± 0.05; p = 0.315; n = 5 mice; Wilcoxon matched-pairs signed rank test; Figure 4D). Given the influence of cocaine on movement behavior, we next assessed how cocaine affects Ca^{2+} activity in tuft dendrites of L2/3 pyramidal neurons within S1 in the awake mouse. Similar to the anesthetized state, there was an overall dampening of spontaneous Ca^{2+} transients following cocaine administration (0.19 ± 0.02 vs. 0.15 ± 0.01 Hz, p = 0.02; n = 100 dendrites, 5 mice).

Does cocaine influence the dendritic processing of sensory information in the awake state? To address this, dendritic Ca^{2+} responses were recorded in tuft dendrites in response to the same tactile stimulation delivered to the contralateral forepaw as the anesthetized state (200 Hz, 500 ms). Here, following cocaine administration, the amplitude of the Ca^{2+} response to the tactile stimulus in active tuft dendrites was significantly increased (2.54 ± 0.22 vs. 3.13 ± 0.24 ΔF/F, p = 0.016; Wilcoxon matched-pairs signed rank test; Figures 4E and 4F). In contrast, saline did not significantly influence the tactile-evoked response in awake mice (2.63 ± 0.21 vs. 2.68 ± 0.24 ΔF/F, p = 0.886; n = 38 dendrites, 4 mice; Wilcoxon matched-pairs signed rank test; Figures 4G and S4). Since synchronous activity is of great importance in cortical signaling,[48,49] we also assessed the synchrony of dendritic activity both before and after cocaine administration in the awake state. Overall, dendritic activity was significantly more correlated following cocaine administration (cocaine, 0.06 ± 0.002; saline, 0.05 ± 0.001;

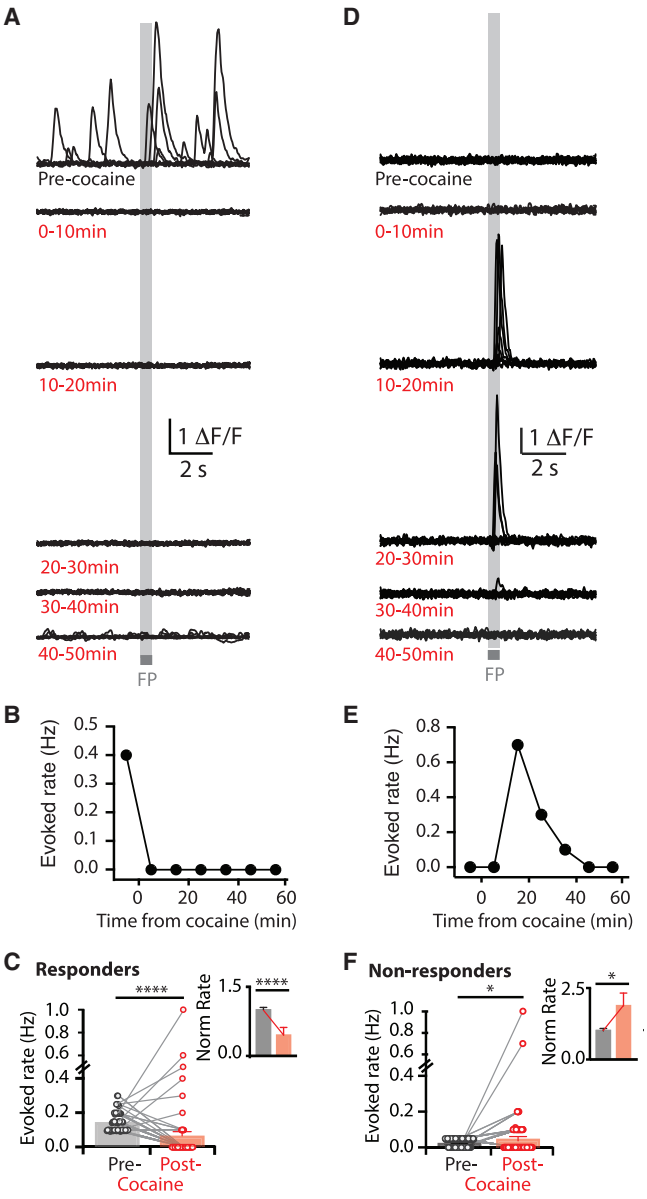

Figure 3. Cocaine alters sensory encoding in tuft dendrites

(A) Example tuft dendrite where cocaine dampened tactile-evoked activity. Ca^{2+} responses to tactile stimulation delivered to the forepaw (FP) pre- and post- (0–50 min) cocaine injection.

(B) Evoked rate for example dendrite shown in (A).

(C) Rate of Ca^{2+} transients in "responder" dendrites. Inset, evoked rate normalized to average pre-cocaine rate for responder dendrites (n = 55 dendrites).

(D) Example tuft dendrite where cocaine increased tactile-evoked encoding. Ca^{2+} responses to tactile stimulation (FP) pre- and post- (0–50 min) cocaine injection.

(E) Evoked rate for example dendrite shown in (D).

(F) Rate of Ca^{2+} transients in "non-responder" dendrites. Inset, evoked rate normalized to average pre-cocaine rate for non-responder dendrites (n = 110 dendrites).

*p < 0.05; ****p < 0.0001. Error bars, SEM.

p < 0.0001; n = 171/179; Mann-Whitney test), suggesting that cocaine influences the overall pattern of signaling within the S1. We next assessed the influence of cocaine on the tactile-evoked rate of Ca^{2+} transients in the awake state. Here, only dendrites that had evoked Ca^{2+} transients were considered. Similar to anesthetized recordings, the influence of cocaine on tactile-evoked responses in tuft dendrites was correlated with the reliability of sensory encoding prior to cocaine exposure. In contrast to saline administration (Figure S4), dendrites that reliably responded during the sensory stimulus with a tactile-evoked rate that was above average (>0.17 ± 0.02 Hz, responders) were considerably dampened following cocaine administration. On average, these dendrites had a significant decrease in the sensory-evoked rate following cocaine exposure (0.32 ± 0.03 vs. 0.21 ± 0.02 Hz, p = 0.0001; n = 22 dendrites, 5 mice; Wilcoxon matched-pairs signed rank test; Figures 4H and 4I). In contrast, dendrites that weakly encoded the tactile stimulus with a stimulus-evoked rate that was less than average (non-responders) significantly increased their sensory-evoked activity following cocaine exposure (0.06 ± 0.008 vs. 0.13 ± 0.03 Hz, p = 0.0004; n = 32 dendrites, 5 mice; Wilcoxon matched-pairs signed rank test; Figures 4J and 4K). Therefore, in both the anesthetized and awake states, the influence of cocaine on sensory encoding within the S1 is dynamic, acting as a filter for encoding tactile information within cortical pyramidal neuron dendrites, uniformly decreasing spontaneous activity while specifically dampening dendrites that encoded the tactile-stimulus, and enhancing the sensory-evoked activity in dendrites that were unreliable sensory encoders. Together, these findings suggest that cocaine influences both the rate and pattern of signaling within the somatosensory cortex in the awake state.

DISCUSSION

Much of our knowledge about the influence of cocaine on cellular signaling has come from *in vitro* studies; however, since neurons are embedded in complex neural networks *in vivo*, this has limited our understanding of the acute influence of cocaine on the cellular processing of sensory information. Here, we used single-cell electrophysiology and two-photon Ca^{2+} imaging *in vivo* to measure the influence of cocaine on L2/3 pyramidal neurons within S1. Combining the dampening of spontaneous activity with changes in the processing of sensory input, our findings suggest that cocaine acts as a cortical filter that effectively suppresses background noise and selectively modulates incoming sensory information.

Similar to *in vitro* recordings from L5 pyramidal neurons following repeated cocaine exposure,[42] the resting membrane potential of L2/3 pyramidal neurons was depolarized during a single exposure to cocaine. However, counterintuitively, this depolarization was not accompanied by an increase in spontaneous activity. Instead, acute exposure to cocaine dampened spontaneous activity in both the somata and dendrites. Spontaneous activity serves an important role in cellular signaling as it provides a changing baseline that can dynamically alter the effectiveness of incoming information.[21,50] By dampening spontaneous activity, cocaine further increases sensory-evoked responses beyond increasing the Ca^{2+} transient itself, as the signal

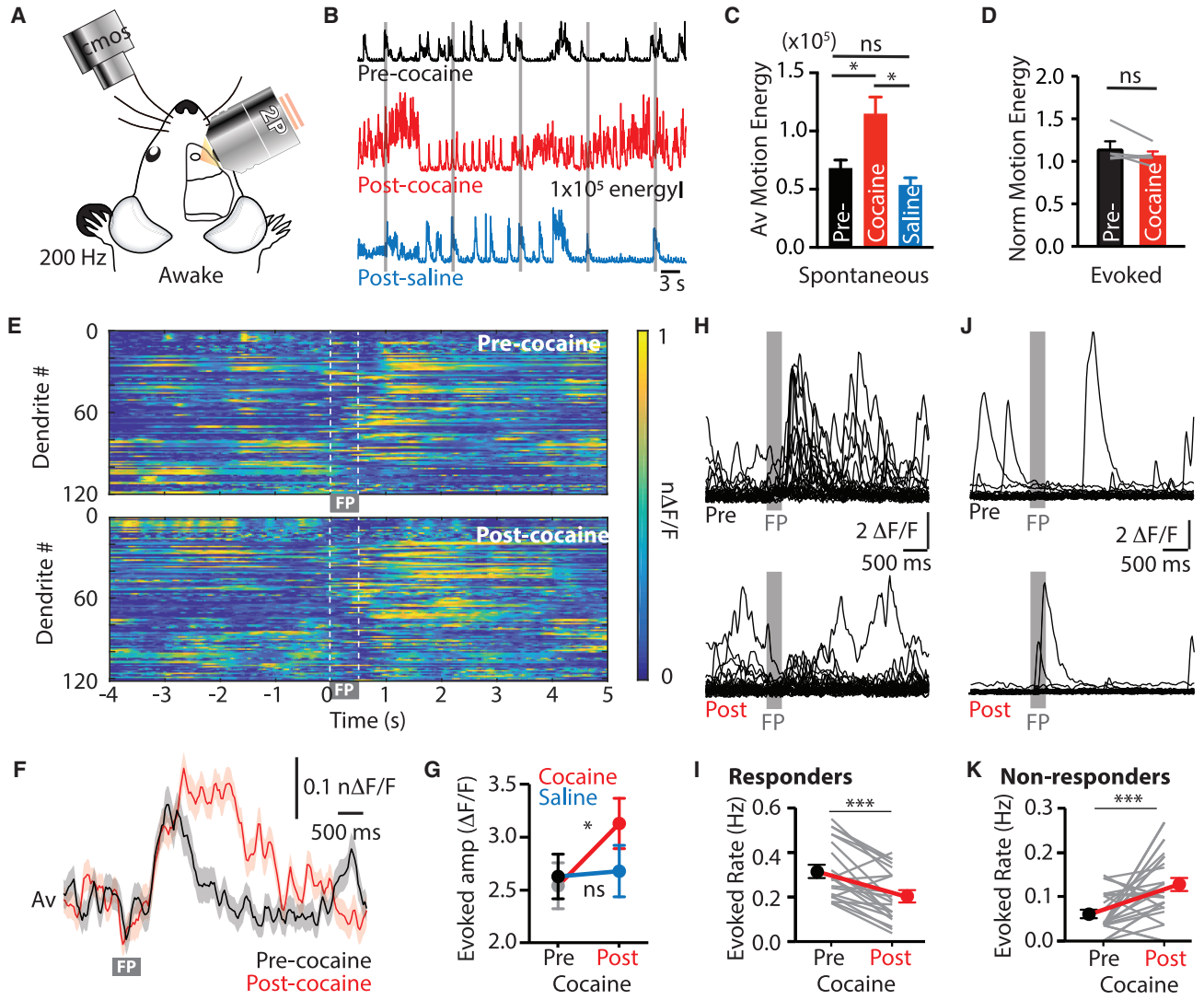

Figure 4. Cocaine increases sensory-evoked Ca^{2+} activity in tuft dendrites in the awake state

(A) Schematic of experimental design. Two-photon Ca^{2+} imaging was performed in tuft dendrites of L2/3 pyramidal neurons in the awake state pre- and post-cocaine. A CMOS camera was positioned to track facial motion.

(B) Motion energy measured in a typical mouse using facial tracking (facemap) measured pre-cocaine (black), post-cocaine (red), and post-saline (blue). Gray bars, tactile stimulus delivery.

(C) Average baseline motion energy (n = 5 mice).

(D) Average motion energy measured during tactile stimulus (n = 5 mice).

(E) Heatmap of Ca^{2+} activity in tuft dendrites in the awake state measured pre- (top) and post- (bottom) cocaine. Each row represents an individual dendrite, and responses are normalized to the maximum for each dendrite. Dashed line, tactile stimulus delivery.

(F) Overlay of average evoked Ca^{2+} responses for all dendrites illustrated in (E).

(G) Average amplitude of tactile-evoked Ca^{2+} responses following cocaine (red) and saline (blue) administration.

(H) Overlay of Ca^{2+} traces from an example responder tuft dendrite. Each trace is a single trial. Gray bar, stimulus delivery.

(I) Average evoked rate of responder dendrites (n = 22 dendrites, 5 mice).

(J) Overlay of Ca^{2+} traces from an example non-responder tuft dendrite. Each trace is a single trial. Gray bar, stimulus delivery.

(K) Average evoked rate of non-responder dendrites (n = 32 dendrites, 5 mice).

*p < 0.05; ***p < 0.001. Error bars, SEM.

is enhanced relative to background activity. Whether driven by the modulation of ionic conductances,[51,52] changes in synaptic input patterns,[53–56] or an imbalance between excitation and inhibition,[57,58] dampening state transitions and spontaneous activity may therefore act to influence the saliency of sensory signaling under the influence of cocaine.

The dendrites of cortical pyramidal neurons are renowned for sparse coding of sensory information.[59,60] Our findings illustrate

that a subset of dendrites changed from sparsely encoding tactile stimulation to reliably evoking Ca^{2+} transients in response to tactile stimulus following cocaine administration. In these dendrites, sensory encoding became more reliable following cocaine with large Ca^{2+} transients in response to all tactile stimuli. These findings further suggest that cocaine can have an opposing influence on different cellular processes within cortical neurons, dampening baseline spontaneous activity and enhancing sensory-evoked responses in a subset of dendrites. What are the possible cellular mechanisms contributing to the influence of cocaine on L2/3 pyramidal neurons within the S1? Previous studies have shown that cocaine can cause long-lasting changes in intrinsic membrane properties.[61] However, in contrast to ketamine, which has been shown to influence cortical activity by inhibiting NMDA receptors and HCN channels,[62] the acute effects of cocaine on cortical activity may not primarily be due to a direct influence on postsynaptic voltage-gated channels. We found no influence of cocaine on the voltage amplitude of either evoked action potentials or spontaneous state transitions, both of which are cellular events that involve voltage-gated ion channels. Instead, cocaine may, at least in part, modulate synaptic input to the S1. By targeting local excitatory and inhibitory neurons in different cortical layers, modulation of synaptic input can have a heterogeneous effect on cortical pyramidal neurons, leading to excitation and disinhibition in some dendrites and inhibition in others. Specifically, since cocaine can powerfully influence cellular activity within reward-related circuitry,[63] the acute influence of cocaine on dendritic activity may be due to changes in synaptic input from reward pathways to S1. Neuromodulation by the monoamines serotonin and norepinephrine have been shown to modulate cortical processing[64] and can have a contrasting influence on cellular response properties.[25] In particular, dopamine plays a critical role in the acute effects of cocaine in the reward pathway.[65] L2/3 pyramidal neurons within the S1 have both D1 class (D1 and D5) and D2 class (D2, D3, and D4) receptors[66]; however, since axonal projections from the ventral tegmental area within the mesocortical pathway almost exclusively target deep cortical L5 and L6,[67] direct dopamine projections from the ventral tegmental area (VTA) may not play a crucial role in the acute cocaine-mediated effects in L2/3 pyramidal neurons recorded in this study. Further, the influence of cocaine may not be from the direct action of cocaine on endogenous neuromodulators within the S1, per se, but instead from the indirect influence of cocaine on brain regions that directly target the somatosensory cortex. Here, acute first exposure to cocaine influenced sensory processing in both the anesthetized and awake states. This suggests that the influence of cocaine on cortical function does not explicitly require the attention or reward pathways that are not active in the anesthetized state. Instead, cocaine may act directly on either the cellular mechanisms required for processing sensory information or the sensory pathways that are activated during tactile stimulus.[68] Furthermore, the amplitude of the tactile-evoked Ca^{2+} responses were increased following cocaine administration in the awake, but not the anesthetized, state. This may be due to the increase in movement in the awake state; however, the change in motion energy in response to the tactile stimulus relative to baseline motion was similar in both states, suggesting that movement is not the primary contributing factor to this increased response. In addition, cocaine also increased the synchronous activity of overall dendritic activity. Since synchronous activity is of great importance in cortical signaling,[48,49] this may further enhance the influence of cocaine administration on output signaling in sensory cortices.

Limitations of the study

In this study, a single dose of cocaine was administered via i.p. injection. Future studies are needed to investigate the influence of repetitive cocaine administration on pyramidal neurons within the sensory cortex, as well as self-administration. Due to group housing for animal welfare, all experiments were performed on female mice. Therefore, findings reported in this study may not apply to male mice. Although previous studies have shown that there is no difference in the influence of acute cocaine on males and females,[69,70] a sex difference in cocaine self-administration emerged when the behavioral cost was great, with males responding more than females.[71] Furthermore, we found no effect of age on the influence of cocaine on L2/3 pyramidal neurons. However, a narrow age range was used, and future experiments may further investigate more broadly if age impacts the influence of cocaine on cortical neurons. Lastly, this study does not reveal the cellular mechanism(s) contributing to the regulation of sensory filtering following cocaine administration. Based on our findings, unraveling the cellular mechanism(s) underlying the influence of cocaine on cortical pyramidal neurons is an exciting area of future research.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead contact
  - Materials availability
  - Data and code availability
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Mice
- METHOD DETAILS
  - Virus injections
  - Craniotomy and surgical procedures
  - Whole-cell *in vivo* patch-clamp recordings
  - *In vivo* juxtacellular recordings
  - Cocaine delivery
  - Habituation for Ca^{2+} imaging in the awake state
  - Two-photon Ca^{2+} imaging
  - Video monitoring and analysis
  - Tactile-stimulus delivery
- QUANTIFICATION AND STATISTICAL ANALYSIS
  - Whole-cell recordings
  - Ca^{2+}imaging data
  - Dendrite classification
  - Face tracking
  - Statistics

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2023.112122.

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AUTHOR CONTRIBUTIONS

S.C.M., A.J.L., and L.M.P. designed the study. Two-photon Ca^{2+} imaging and analysis were performed by S.C.M. and L.G. Electrophysiological recordings were performed by R.G. All authors contributed to figure formation and manuscript writing.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Bacterial and virus strains		
pAAV.syn.GCaMP6f.WRPE.SV40	Addgene	100,837-AAV1
pAAV.Syn.Flex.GcAMP6f.WRPRE.SV40	Addgene	100,833-AAV1
AAV1.hsyn.Cre.WPRE.hGH	Addgene	105,553-AAV1
Chemicals, peptides, and recombinant proteins		
Cocaine Hydrochloride	Johnson Matthey	37,906 (batch 14–00286)
Experimental models: Organisms/strains		
C57Bl/6	ARC colony	C57BL/6JArc
C57BL/6J-Tg(Thy1-GCaMP6s)	Jackson Laboratory	024,275
Software and algorithms		
Igor (6)	Wavemetrics	N/A
Prism (9)	Graphpad	N/A
Thorimage	Thorlabs	N/A
MATLAB (R2019b, R2021b)	MathWorks	N/A
Fiji	http://fiji.sc/	N/A

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Lucy Palmer (lucy.palmer@florey.edu.au).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.
- This paper does not report original code.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The experiments conducted in this paper strictly followed the Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council, Australia) and were approved by the veterinary office and Animal Ethics Committees of The Florey Institute of Neuroscience and Mental Health, University of Melbourne.

Mice

Wild type C57BL/6J (dendritic Ca^{2+} imaging, electrophysiology, somatic Ca^{2+} imaging) and GCaMP6s Thy1 transgenic (somatic Ca^{2+} imaging) female mice (P32-140) were used in this study. Mice were housed in groups of up to six individuals in a 12:12 natural light/dark cycle. All experiments were performed during the light phase.

METHOD DETAILS

Virus injections

Mice were anesthetized with isoflurane (1–3% in 0.75 L/min O_2) and body temperature was kept at 36–37°C. Dehydration of the eye was prevented using eye ointment, and meloxicam (1–3 mg/kg ip, Ilium) was intraperitoneally injected for peri- and post-operative care. The skin was disinfected with ethanol 70% and betadine, and lidocaine (20 mg/mL, Ilium) was applied subcutaneously for local

analgesia. A small craniotomy (0.7 × 0.7 mm) was made over the brain region of interest and the dura was left intact. To achieve sparse labeling of neurons within the forepaw area of S1, GCaMP6f (Cre dependent genetic Ca^{2+} indicator; AAV1.Syn.Flex.GCaMP6f.WPRE. SV40) and diluted Cre (1:6000; AAV1.hSyn.Cre.WPRE.hGH) were mixed and injected into layer 2/3 (at a depth of 200-400 μm) at the stereotaxic coordinates, 0 mm from bregma and 2 mm from midline. Layer specificity of the injection site and identification of the recorded cell-type was confirmed after each experiment by visualizing the entire dendritic arbor and location of the associated somata. After slowly retracting the microcapillary pipette, the skin was sutured, and mice were allowed to recover for at least 3 days prior to any further procedures.

Craniotomy and surgical procedures

For non-recovery surgery, mice were anesthetized with isoflurane (1–3% in 0.75 L/min O_2) before urethane anesthesia (intraperitoneal, 1.6 g/kg, Sigma) was administered. Anesthesia was monitored throughout the experiment, and a top-up dose of 10% of the initial urethane dose was administered when necessary. For recovery surgery, mice were anesthetized with isoflurane (1–3% in 0.75 L/min O_2) for the duration of the surgery and meloxicam (1–3 mg/kg ip, Ilium) was injected for analgesia and to reduce inflammation. Body temperature was maintained at 36–37°C and dehydration of the eye was prevented using eye ointment. Lidocaine (20 mg/mL, Ilium) was injected around the surgical site on the scalp and the head was stabilized in a stereotaxic frame by a head-plate attached to the skull with dental cement (paladur, Heraeus). A craniotomy was performed over the forepaw area of S1, centered at bregma and 2.2 mm lateral from midline. For whole cell patch clamp recordings, the craniotomy was ~1.5 × 1.5 mm^2 and the dura was surgically removed before normal ringer (135 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES) was used to bathe the craniotomy throughout the experiment. For Ca^{2+} imaging recordings, the craniotomy was 3mm in diameter and sealed with a 3mm diameter circular coverslip (chronic window) for the awake recordings. In the anesthetized state, one side of the craniotomy was unsealed to allow for pharmacological manipulations. For recovery recordings, mice were able to recover for at least 3 days prior to recordings.

Whole-cell *in vivo* patch-clamp recordings

Whole-cell recordings were obtained from L2/3 pyramidal neurons (~200 μm below pia) using a patch pipette (resistance 4–6 MΩ) filled with an intracellular solution containing 115 mM potassium gluconate, 20mM KCl, 10 mM sodium phosphocreatine, 10 mM HEPES, 4 mM Mg-ATP, 0.3 mM Na-GTP, adjusted to pH 7.3 - 7.4 with KOH. To assess the influence of cocaine on the activity of cortical L2/3 pyramidal neurons, whole-cell recordings were performed before (control) and after cocaine injection (20 mg/kg, intra-peritoneal). The patch pipette was inserted into the brain at an angle of 45° or 80° relative to the cortical surface, to a depth of ~100 μm and then advanced in steps of 2 μm until a neuron was encountered. All recordings were made in current-clamp configuration using a Dagan BVC-700A amplifier and sampled at 20 kHz with no bias current injected. Custom written Igor Pro (Wavemetrics) software was used for both acquisition and analysis and no correction was made for the junction potential. The identity of the recorded pyramidal cell in *in vivo* blind recordings was confirmed using the recording depth and voltage response to current steps (50 pA integrals, 1000 ms steps). Voltage measurements were taken at intervals of 5 min both pre (for 10 min) and post cocaine/saline injection until the recording was lost.

In vivo juxtacellular recordings

Juxtacellular voltage recordings were obtained from L2/3 pyramidal neurons (~200 μm below pia) using *a* thick-wall glass filament pipette (4–10 MΩ) filled with normal ringer (in mM), 135 NaCl, 5.4 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES. The pipette was inserted into the brain at an angle of 45° or 80° relative to the cortical surface, to a depth of ~100 μm and then advanced in steps of 2 μm until a neuron was encountered. Test pulses (−10 mV, 20 ms) were applied to the pipette in voltage clamp mode while searching for neurons. Cell-attached juxtacellular recordings were performed using a differential amplifier (BVC-700A, Dagan). To assess the influence of cocaine on the somatic activity of cortical L2/3 pyramidal neurons, juxtacellular recordings were measured before (control) and after cocaine injection (20 mg/kg, intra-peritoneal). All recordings were made in current-clamp configuration using a Dagan BVC-700A amplifier and sampled at 20 kHz. Measurements were taken at intervals of 5 min post injection, and subsequently until the recording was lost. The effect of cocaine on firing rates are reported as the influence on the firing rate during the recorded period (0–30 min). Custom written Igor Pro (Wavemetrics) software was used for both acquisition and analysis.

Cocaine delivery

A single injection of cocaine (20 mg/kg, i.p) was administrated to the mouse *in situ* to enable recordings to be performed from the same neurons immediately after the injection (within 1 min). The same procedures were performed during the control administration of saline.

Habituation for Ca^{2+} imaging in the awake state

Mice which had previously undergone head-bar implantation surgery (see craniotomy and surgical procedures) were gradually habituated to head fixation and the microscope setup for up to 4 days. Once habituated, awake recordings were performed. Here, mice were head-fixed to the recording frame and their paws rested unaided on either an active (contralateral) or inactive

(ipsilateral) piezo-electric buzzer (Microdrive). Tactile stimulus was delivered to the contralateral forepaw (200 Hz; 500 ms). Online video of paw motion was obtained and trials were excluded if the mouse paw was displaced from the stimulator.

Two-photon Ca^{2+} imaging

Ca^{2+} imaging was performed through a cranial window implanted over S1 in mice either previously transfected with the Ca^{2+} indicator GCaMP6f (dendritic imaging; awake, anesthetized) or transgenic mice expressing GCaMP6s (soma). To assess the influence of cocaine on the Ca^{2+} activity of cortical L2/3 pyramidal neurons, spontaneous and evoked activity were measured before (control) and during cocaine exposure (20 mg/kg, intra-peritoneal) in L2/3 pyramidal neurons with apical tuft dendrites that extended into the upper layer in the cortex where dendritic recordings were performed. In the anesthetized state, measurements were taken at intervals of 5 min for up to 1 h post injection. In the awake state, measurements were taken at intervals of 5 min post injection, and subsequently for 30 min post injection. GCaMP6f was excited at 940 nm (~30 mW at the back aperture) with a titanium sapphire laser (140 fs pulse width; SpectraPhysics MaiTai Deepsee) and imaged on a Thorlabs or Sutter MoM two-photon microscope through a 16x Nikon objective (0.8 NA). Emitted light was passed through a dichroic filter (565dcxr, Chroma Technology) and short-pass filtered (ET525/70-2p, Chroma Technology) before being detected by a GaAsP photomultiplier tube (Hamamatsu). Images were acquired at a frequency of 30 Hz (512 × 512 pixels) using Thorimage or ScanImage software (Vidrio Technologies). The images were motion-corrected with a custom written MATLAB script. All imaging from tuft dendrites was performed in higher order branches beyond the bifurcation point. ROI was manually drawn in ImageJ and fluorescence signal extraction was performed using a custom MATLAB script. All trials with motion in the z axis were excluded from the analysis.

Video monitoring and analysis

In awake experiments, during the two-photon Ca^{2+} imaging recordings, the mice face and part of the body were monitored using a high-speed CMOS camera (Blaser aCA1300) mounted on a 50 mm lens (Kowa 50 mm/F2.8). The video were analyzed using FaceMap (https://github.com/MouseLand/facemap): an Roi was drawn to select the mouse face and the average motion energy across all pixels in the ROI were computed for each video before and after exposure to cocaine (or saline).

Tactile-stimulus delivery

Tactile stimulus was delivered to the contralateral forepaw (200 Hz; 500 ms) via a piezo-electric buzzer (Microdrive). The tactile stimuli were generated and delivered using Arduino micro-processing boards (Arduino Uno) and Bpod (Sanworks) with custom-written MATLAB (MathWorks) software. The tactile stimulation was delivered to the forepaw once every minute, for up to 1 h post cocaine.

QUANTIFICATION AND STATISTICAL ANALYSIS

Whole-cell recordings

Custom-written Igor Pro software (Wavemetrics) was used for the acquisition and analysis of whole-cell electrical recordings. Data is presented as mean ± SEM. UP- and DOWN- states were determined according to the membrane potential with DOWN state being defined as the minimum resting membrane potential. Percentage of DOWN state was calculated as the percentage of all membrane potential measurements that were within 2 mV of the minimum resting membrane potential reported.

Ca^{2+}imaging data

Custom-written MATLAB (MathWorks) software was used for analysis of Ca^{2+} imaging data. Ca^{2+} events were detected in a 1 s (anesthetized) or 3 s (awake) window from stimulus onset, when they crossed a threshold value of 2 standard deviations above baseline measured 3s from trial onset to stim onset. In ROIs with high baseline activity, Ca^{2+} events were detected when they crossed an absolute threshold. The amplitude of each Ca^{2+} transient were measured as the peak of the event which occurred during the detection window. Only recordings with at least one Ca^{2+} transient throughout recordings were considered for analysis. Evoked rates were only included from recordings with evoked activity (in either pre- or post-cocaine) whereas amplitudes were from all (unpaired) responses. Therefore, sample numbers might differ. The evoked rate of Ca^{2+} transients was measured as the number of events divided by the number of trials. Ca^{2+} responses were smoothed using a Savitzky–Golay filter with a second order polynomial. Only Ca^{2+} transients with a duration that was longer than 250 ms were included in analysis. The calcium activity is reported as the mean value of the detected events for each ROI. Unless specified, the influence of cocaine was calculated for the first 30 min post cocaine injection.

Dendrite classification

Dendrites were classified according to whether-or-not they encoded sensory information. Dendrites were considered 'responders' if they had a tactile-evoked rate that was greater than the average tactile-evoked rate for all recorded dendrites. Dendrites were considered 'non-responders' if they had a tactile-evoked rate that was less than the average tactile-evoked rate for all recorded dendrites. Based on the fluorescence morphology at the imaging plane, there was no evident difference in responder and non-responder dendrites.

Face tracking

Spontaneous motion energy is reported as the average motion energy recorded during baseline. Evoked motion energy is reported as the evoked motion energy normalized to the baseline motion energy measured 3s from stimulus onset.

Statistics

Measurements were taken from distinct samples and all numbers are indicated as mean ± SEM. N represents dendrites, mice. When comparing two populations of data, significance was determined using two-sided nonparametric tests (paired: Wilcoxon matched-pairs signed rank test; unpaired: Mann–Whitney test) at a significance level of 0.05. When multiple populations of data were compared, nonparametric one-way ANOVA Friedman or Kruskal–Wallis with Dunn post hoc tests were used for paired and unpaired comparison, respectively.