

A functional logic for neurotransmitter co-release in the cholinergic forebrain pathway

Aditya Nair^{1,4}, Martin Graf¹, Yue Yang Teo^{1,2}, George J. Augustine^{1,3}

¹Lee Kong Chian School of Medicine, Nanyang Technological University

²National University of Singapore

³Institute of Molecular and Cellular Biology, Agency of Science, Technology and Research

⁴Current Address: Computation and Neural Systems, California Institute of Technology, Pasadena, CA, USA

Corresponding author: George Augustine

E-mail: george.augustine@ntu.edu.sg

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22 **Abstract**

23 The forebrain cholinergic system has recently been shown to co-release both
24 acetylcholine and GABA. We have discovered that such co-release by cholinergic
25 inputs to the claustrum differentially affects neurons that project to cortical versus
26 subcortical targets. The resulting changes in neuronal gain toggles network
27 efficiency and discriminability of output between two different projection subcircuits.
28 Our results provide a potential logic for neurotransmitter co-release in cholinergic
29 systems.

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40 **Main Text**

41 The cholinergic system of the basal forebrain is a crucial pathway that modulates
42 attention, arousal and learning¹⁻³. Such actions arise from the ability of the
43 cholinergic system to alter neuronal excitability and shape the correlational structure
44 of neural populations⁴⁻⁶. The prevailing view that the cholinergic system implements
45 such computations solely by releasing acetylcholine (ACh) has been challenged by
46 the recent discovery that nearly all forebrain cholinergic neurons co-release the
47 inhibitory transmitter, GABA, along with ACh^{7,8}. While such co-release has been
48 observed in multiple brain areas, a functional logic for it is missing: does co-release
49 happen in a target-specific manner and how does it impact cholinergic computations?
50 We have addressed these questions by analyzing cholinergic modulation of the
51 claustrum. The claustrum receives input from basal forebrain cholinergic neurons¹¹⁻
52 ¹³ and has been implicated in attention, perhaps by altering cortical gain^{11,14}.
53 Cholinergic modulation of claustrum neurons was examined by whole-cell patch
54 clamp recordings in brain slices from a ChAT-Cre mouse line crossed with another
55 line with Cre-dependent expression of ChR2-YFP¹⁵, thereby targeting ChR2
56 exclusively to cholinergic neurons. To isolate cholinergic responses, recordings
57 were performed in the presence of a glutamate receptor blocker (kynurenic acid,
58 KYN; 1 μ M) and GABA receptor blocker (Gabazine, GBZ; 10 μ M). The claustrum
59 consists of multiple types of projection neurons and interneurons¹⁶. A trained
60 classifier was applied to whole-cell patch clamp measurements of intrinsic electrical
61 properties to identify neurons that project to cortical or non-cortical targets, as well
62 as to identify the three known types of local interneurons¹⁶.

63 ChR2-mediated photostimulation of cholinergic input elicited responses in claustror-
64 cortical (CC), claustror-subcortical (CS) projection neurons and VIP interneurons
65 (VIP-IN; Fig 1a). Only a small fraction (5%, $n = 5/97$ neurons) of CC neurons
66 received cholinergic excitatory input, while five times more CS neurons (25%, $n =$
67 $16/64$ neurons) and even more VIP-IN (44%, $n = 4/9$ neurons) received such
68 excitation (Fig 1b, EPSC amplitude: CC: 14 ± 1.2 pA, CS: 35.3 ± 5 pA, VIP: $64.7 \pm$
69 5.5 pA). These were monosynaptic inputs, because they persisted after tetrodotoxin
70 (TTX; $1 \mu\text{M}$) was used to block action potentials and 4-AP was applied to enhance
71 ChR2-mediated depolarization¹⁷⁻¹⁹ (Fig 1c). These excitatory responses were
72 mediated by nicotinic ACh receptors, because they were blocked by a nicotinic
73 receptor blocker (mecamylamine, MECA; $10 \mu\text{M}$; Fig 1c) and had reversal potentials
74 near zero (Fig S1a), typical of responses mediated by nicotinic receptors²⁰. SST and
75 PV interneurons never responded to cholinergic photostimulation (Fig 1b). These
76 results reveal cell-type specific modulation of claustrum neurons and are consistent
77 with reports that VIP interneurons are a critical target for cholinergic modulation in
78 other parts of the brain^{21,22}.

79 Because the inhibitory neurotransmitter, GABA, can be co-released by cholinergic
80 neurons⁷, we asked whether claustrum neurons receive GABAergic inhibition by
81 photostimulating cholinergic neurons after blocking excitatory responses with KYN,
82 MECA and atropine (ATR; $10 \mu\text{M}$). Under such conditions, monosynaptic outward
83 currents were observed (Fig 1d, IPSC amplitude: 49.3 ± 6.2 pA), that persisted in
84 the in the presence of TTX and 4-AP. These responses were mediated by GABA_A
85 receptors, because they were blocked by GBZ (Fig 1d) and were inhibitory because

86 their reversal potential of -70 mV was more negative than action potential threshold
87 of CC neurons (-33.8 ± 0.2 mV, Fig S1b). Remarkably, we only found inhibitory
88 inputs to CC neurons (Fig 1e). Indeed, CC neurons were much more likely to exhibit
89 inhibitory responses to cholinergic input (21%, $n = 6/28$ neurons) than excitatory
90 responses (5%). These results reveal a logic for cholinergic co-release of GABA:
91 while claustral neurons projecting to subcortical structures, as well as VIP-IN, are
92 excited via cholinergic activation of nicotinic receptors, neurons projecting to cortical
93 structures are more likely to be inhibited by co-released GABA. Such opposing
94 regulation by cholinergic input may also be present in cortical circuits²³.

95 To understand the functional consequences of the dual modulation produced by co-
96 transmitter release, we determined the effects of cholinergic input on action
97 potentials (APs) evoked by depolarizing current pulses using a 1-second long train
98 of blue light (488 nm) pulses delivered at 10Hz. In CC neurons, cholinergic input
99 reduced the frequency of APs evoked by a 100 pA depolarizing current pulse (Fig
100 2a, left, $n = 12$ neurons), presumably due to the inhibitory action of GABA. In contrast,
101 this input increased AP firing in both CS neurons and VIP neurons, presumably due
102 to ACh excitation (Fig 2b,c, left, $n = 10$ CS neurons, 5 VIP-IN). The slope of the
103 relationship between current magnitude and AP frequency, the input-output (IO)
104 curve, reveals neuronal gain²⁴; changes in gain are a characteristic feature of
105 cholinergic modulation of the cortex²⁵. While inhibitory input decreased the gain of
106 CC neurons (Fig 2a, right), excitatory input increased the gain of CS neurons and
107 VIP-IN (Fig 2b,c, right). Increased modulation of CS neuron gain was also
108 accompanied by a secondary decrease in AP frequency at higher current intensities

109 (Fig 2b, right). Thus, the co-transmitters released by cholinergic input produces
110 opposing, cell-type specific gain modulation of claustrum neurons (Fig 2d).

111 In the cortex, optogenetic activation of forebrain cholinergic input improves neuronal
112 signal-to-noise ratio²⁶. We used our results to predict how opposing cholinergic gain
113 control alters this cholinergic computation by simulating how responses to weak (X_A)
114 and strong (X_B) inputs would be transformed by the empirically measured IO
115 functions of single CC and CS neurons (Fig 3a). Such analyses have been used to
116 demonstrate the ability of norepinephrine to alter neuronal signal-to-noise ratio²⁷. In
117 CC neurons, for two inputs centred around 100 and 200 pA (with noise of 100 pA;
118 Fig. 3a1), cholinergic action (Fig. 3a2) improved the separation of output
119 distributions (Y_{GA} , Y_{GB} ; Gain modulated output distributions) compared to basal
120 conditions (Y_A , Y_B) by 56% (Fig 3a3, 3a4). However, for CS neurons the situation
121 was reversed (Fig 3b): cholinergic input reduced the separation of output
122 distributions by 35% (Fig 3b3, 3b4). This simulation indicates that cholinergic input
123 improves the signal-to-noise ratio for CC neurons while reducing it for CS neurons;
124 thus, co-release of ACh and GABA acts as a toggle to switch both the gain (Fig 2)
125 and the signal-to-noise ratio of these projection neuron subpopulations. This effect
126 occurs across a wide range of inputs for both CC and CS neurons (Fig S2a, S2b).

127 Recent studies demonstrate that cholinergic input improves network encoding
128 efficiency by altering the relationship between signal and noise correlations in the
129 neuronal activity of cortical networks^{4,28}. A strong relationship between these is
130 harmful for encoding a given signal, because it reduces discrimination of this signal
131 from noise; in the cortex, cholinergic input - as well as attention - weakens this

132 relationship^{4,29,30}. We predicted the impact of cholinergic co-release on claustrum
133 network correlation structure by using a recurrent circuit model based on an
134 inhibition-stabilized network³¹. This model contained 300 neurons, including
135 excitatory CC and CS projection neurons and the three inhibitory interneuron types;
136 the IO function of each neuron type was defined by experimental measurements
137 (See Methods, Fig S3). This network was driven by two types of stimuli similar to
138 those previously applied *in vivo*⁴: (1) a signal with Gaussian amplitude distribution,
139 with a SD of 1/10th of the mean; and (2) Poisson distributed noisy input with a SD is
140 equal to its mean (Fig 3c). For both stimuli, we examined the impact of cholinergic
141 input on signal correlations and noise correlations between neurons. Remarkably,
142 changing neuronal gain, by replacing native IO functions with IO functions measured
143 during cholinergic photostimulation, decreased the slope of the signal correlation–
144 noise correlation plot for CS neurons across a range of signal input sizes above 140
145 pA ($\Delta Slope = -15\%$, Fig 3d, Fig S2c), thus weakening the relationship between
146 signal and noise correlations, as predicted from theory and *in vivo* experiments^{4,32}.
147 Because weakening this relationship leads to better signal discrimination, the
148 reduction in correlation slope is associated with greater encoding capacity in
149 networks^{4,29,32}. In contrast, for CC neurons the slope increased for a range of signal
150 input sizes above 140 pA⁴ ($\Delta Slope = +16\%$, Fig 3e, Fig S2d), thus strengthening
151 the relationship between signal and noise correlations and reducing the encoding
152 capacity of this population. Hence, we also observe a toggle in encoding efficiency
153 between the CC and CS populations.

154 For signal inputs smaller than 100 pA, we observed a toggle in the opposite direction,
155 with efficiency increasing for CC neurons while decreasing for CS neurons (Fig S2
156 c, S2d). Our model indicates that the opposing cholinergic effects on gain due to co-
157 release of ACh and GABA toggles network encoding efficiency in an input-
158 dependent manner between distinct subpopulations of projection neurons,
159 increasing efficiency for one population while reducing it for the other (Fig 3f).

160 Cholinergic modulation has been investigated in diverse experimental paradigms.
161 Our results connect these observations and provide a microcircuit basis for the
162 cholinergic control of signal-to-noise ratio and encoding capacity, based on opposing
163 gain control of specific cell types in the claustrum. Our results highlight that
164 cholinergic modulation does not affect networks uniformly: instead, it toggles
165 information between subpopulations, from a cortically projecting to a subcortically
166 projecting population in an input-dependent manner in the case of the claustrum (Fig
167 3f). This mechanism might also explain the ability of the claustrum to inhibit the
168 cortex during slow-wave sleep¹³, where a low cholinergic tone would switch the
169 discriminability of input towards the cortically-projecting claustral population. The
170 ability to switch information between subpopulations might constitute a network
171 mechanism to implement cholinergic control of attention⁵.

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240 **Figure Legends**

241 **Figure 1:** Cell-type specific direct cholinergic inputs in claustrum neurons. **a.** Direct
242 excitatory inputs to claustrum neurons measured in the presence of Kynurenic Acid (KYN,
243 1 μ M) and Gabazine (GBZ, 10 μ M) by photoactivating cholinergic terminals using 50ms
244 light pulses at 488 nm. **b.** Distribution of excitation probability in all cell-types tested for
245 direct excitation Cells held at -40 mV. **c.** A subset of neurons were tested for
246 monosynaptic connectivity using Tetrodotoxin (TTX, 1 μ M) and 4- Aminopyridine (4-AP,
247 500 μ M). **d.** CC neurons receive direct inhibition measured using KYNA, Atropine (ATR)
248 and Mecamylamine (MECA). Monosynaptic connectivity is tested using TTX and 4-AP.
249 **e.** Distribution of inhibition probability in all cell-types tested for direct inhibition.

250

251 **Figure 2:** Opposing gain control of claustrum cell-types. Left: Responses of claustrum
252 neurons to 100 pA current injection during control and cholinergic photostimulation: 20ms
253 pulses at 10 Hz for one second (**a:** Claustrrocortical, **b:** Claustrrosubcortical, **c:** Putative
254 VIP interneurons). Right: Quantification of changes on input-current output-frequency (IO)
255 curve in control and cholinergic photostimulation conditions. **d.** Quantification of changes
256 on neuronal gain (** $p < 0.01$, *** $p < 0.005$, Kruskal Wallis test with Dunn's post-hoc test for
257 multiple comparison).

258

259 **Figure 3:** Incorporation of opposing gain control toggles signal-to-noise ratio and alters
260 the correlation structure of model recurrent networks in a subpopulation specific manner.
261 **a.** Paradigm used for signal-noise experiment: **a1:** Two inputs distributions (X_A , X_B) are
262 transformed by empirical IO functions of CC (**a2**) to produce output distributions (Y_A , Y_B ,
263 **a3**). **a4.** Output distributions for CC neurons with IO functions with cholinergic

264 photostimulation (Y_{GA} , Y_{GB}). **b.** The same inputs (**b₁**) are transformed by the empirical IO
265 functions of CS neurons (**b₂**) to produce output distributions for with native IO functions
266 (Y_A , Y_B , **b₃**) compared to output distributions with IO functions with cholinergic
267 photostimulation (Y_{GA} , Y_{GB} , **b₄**) **c:** Paradigm used for modelling experiment: A signal
268 (Gaussian distributed; $\mu = 200$ pA, $\delta = 20$ pA) and noise (Poisson distributed; $\mu = 100$ pA,
269 $\delta = 100$ pA) are presented to an inhibition stabilized model of the claustrum. Right:
270 Outputs of individual neurons (grey) and population average (black). **d.** Quantification of
271 noise and signal correlations for CC neurons. (Linear fit, Ctrl: $R^2 = 0.55$, Cholinergic input:
272 $R^2 = 0.40$) (**B₂**). **e.** Quantification of noise and signal correlations for CS neurons. (Linear
273 fit, Ctrl: $R^2 = 0.50$, Cholinergic input: $R^2 = 0.57$). **f.** Cell-type specific cholinergic gain
274 modulation leads to a toggle between cortical and sub-cortical projections in the
275 claustrum.

276

277 **Supplementary Figure Legends**

278 **Supplementary Figure 1:** Current-Voltage relationship for direct cholinergic inputs. The
279 amplitude of voltage clamp responses is measured for a range of holding potentials to
280 determine the reversal potential of the response **a.** Current-Voltage plots for direct
281 excitatory input for CC, CS and putative VIP neurons. Direct excitatory inputs are nicotinic
282 receptor mediated as E_{rev} is closest to the reversal potential of the nicotinic receptor (0
283 mV). **b.** Current-Voltage plots for direct inhibitory inputs reveals E_{rev} closest to the reversal
284 potential of chloride in internal solution (-80 mV).

285

286 **Supplementary Figure 2:** Examining signal-to-noise ratio and encoding efficiency for a
287 range of inputs. **a.** Effect of varying input size (**a₁**) and input noise (**a₂**) of distributions X_A
288 and X_B in Fig 3 on SNR for CS neurons. **b.** Effect of varying input size (**b₁**) and input noise
289 (**b₂**) of distributions X_A and X_B on SNR for CC neurons. **c, d.** Dependence of correlations'
290 slope on input size, i.e mean of Gaussian-signal and mean of Poisson-noise for CS
291 neurons (**c**) and CC neurons (**d**). The grey dotted line shows that the toggle is dependent
292 on signal-input size and takes place in an opposite direction above 140 pA.

293

294 **Supplementary Figure 3:** Optimization of inhibition stabilized claustrum model. The
295 excitatory connectivity is specific by in-vitro experiments and previous findings¹⁸ while
296 inhibitory weights are modified by removing the unstable eigenvalues of the weight matrix
297 **W** towards stability³³. **a.** Optimization of the model by refining the spectral abscissa
298 (largest real part of the eigenvalues in **W**) over multiple iterations. **b.** Network weight
299 matrix **W** before and after optimization process for all 300 neurons.

300

301 **Methods**

302 **Animals**

303 All animal experiments were performed according to the Guidelines of the Institutional
304 Animal Care and Use Committee of Nanyang Technological University, Singapore
305 (Protocol number: 151075). 35 adult ChAT-Cre x floxed ChR2-YFP (B6;129S6-
306 *Chat^{tm2(cre)Low}* /J; # 006410) mice of both sexes were used to study cholinergic input to
307 CLA cells. The average age of mice used in our experiments was postnatal day 65 \pm 0.6.

308

309 **Brain slice recording**

310 Acute brain slices were prepared according to the general procedures described in Graf
311 et al. (2020). Mice were deeply anesthetized with isoflurane and euthanized via
312 decapitation. The brains were isolated and transferred into ice-cold sucrose solution
313 containing the following: 250 mM sucrose, 26 mM NaHCO₃, 10 mM glucose, 4 mM MgCl₂,
314 3 mM myo-inositol, 2.5 mM KCl, 2 mM sodium pyruvate, 1.25 mM NaH₂PO₄, 0.5 mM
315 ascorbic acid, 0.1 mM CaCl₂, and 1 mM kynurenic acid, with an osmolality of 350–360
316 mOsm and a pH of 7.4. Coronal brain slices (250 μm) were cut with a Leica VT 1000S
317 vibratome. Slices were kept for 0.5 h at 34°C in artificial CSF (ACSF) containing the
318 following: 126 mM NaCl, 24 mM NaHCO₃, 1 mM NaH₂PO₄, 2.5 mM KCl, 2 mM CaCl₂, 2
319 mM MgCl₂, 10 mM glucose, and 0.4 mM ascorbic acid; 300–310 mOsm, pH 7.4, and
320 gassed with a 95% O₂/5% CO₂ mixture before transfer to ACSF at room temperature for
321 recordings.

322

323 Whole-cell patch clamp recordings were performed using Borosilicate glass pipettes (5-9
324 MΩ) filled with internal solution containing the following: 130 mM K-gluconate, 10
325 mM KOH, 2.5 mM MgCl₂, 10 mM HEPES, 4 mM Na₂ATP, 0.4 mM Na₃GTP, 5 mM EGTA,
326 5 mM Na₂ phosphocreatinin, and 0.2% neurobiotin (290–295 mOsm, pH 7.4).
327 Recordings were performed at 24°C with a MultiClamp 700B amplifier (Molecular
328 Devices) and a Digidata 1440 interface (Molecular Devices). Signals were acquired at 50
329 kHz and filtered at 10 kHz. Access resistance (R_a) was measured and only cells with R_a
330 < 30 MΩ were used for further analysis. Cell-type identity was determined using an
331 automated classifier using electrical properties described in Graf et al., 2020. Fourteen

332 electrophysiological properties described in Graf et al., 2020 are extracted using software
333 made available by the authors at <https://github.com/adityanairneuro/claustrom>. A trained
334 classifier is used to distinguish between the two subtypes of claustral projection neurons
335 and three subtypes of claustral interneurons.

336

337 For optogenetic photoactivation of cholinergic terminals, slices were illuminated by a
338 130W short arc mercury lamp (Olympus) passed through an EYFP filter set and a x25
339 water-immersion objective. For voltage clamp experiments, we delivered 50 ms light
340 pulses while clamping neurons at -40 mV. For current clamp experiments, we delivered
341 blue light at 10Hz in 20 ms light pulses for a duration of one second. We choose this
342 stimulation protocol to mimic average firing rates of basal forebrain cholinergic neurons
343 which range from 7-14 Hz during wakefulness and REM sleep^{34,35}.

344

345 **Analysis of neuronal gain**

346 In current clamp experiments, we constructed input-output (IO) curves for each neuron
347 by injecting depolarizing current pulses in the range 0-400 pA in 20 pA steps and
348 measuring output firing frequency. Empirically determined input current-output frequency
349 curves were fit with sigmoidal tanh functions of the form:

350

$$351 \quad y = (r_{max} - r_0) \times \tanh(g_k x_i / (r_{max} - r_0)) \quad (1)$$

352

353 Where r_{max} is the maximum observed firing frequency, r_0 is the baseline firing frequency,
354 x is the input current and g_k is the gain or slope of the function at baseline and thus
355 represents the input-output sensitivity of the neuron k .

356

357 **Analysis of signal-to-noise ratio with simulations using empirical IO functions**

358 Given that cholinergic input can alter gain, we analyzed whether these empirically
359 observed differences in IO curves of CC and CS neurons might be sufficient for these
360 projections to process input differently. We modelled this possibility by considering how
361 two input probability distribution functions (PDF: X_A , X_B) would be transformed by IO
362 functions of neurons with and without cholinergic photostimulation (Fig 3a). To quantify
363 the amount of separation between output PDFs, we compared the change in the ratio of
364 their means with and without cholinergic gain control as this reflects the SNR of the two
365 signals X_A and X_B ²⁷. Formally:

366

$$367 \quad \Delta SNR = \left\{ \frac{\mu(Y_{GB})}{\mu(Y_{GA})} - \frac{\mu(Y_B)}{\mu(Y_A)} \right\} / \frac{\mu(Y_B)}{\mu(Y_A)} \quad (2)$$

368 Where Y_{GB} and Y_{GA} are output PDFs in the presence of cholinergic gain control whereas
369 Y_B and Y_A are outputs PDFs in its absence. $\mu(Y)$ indicates the mean of distribution Y .

370

371 We verified that the SNR results we observed generalized for a range of input (PDF: X_A ,
372 X_B) means and SDs by systematically varying either the mean of X_A , X_B (Fig S2a₁, S2b₁)
373 or the SD of X_A , X_B (Fig S2a₂, S2b₂).

374

375

376 **Analysis of signal and noise correlations in model CLA-like networks**

377 To understand the role of cholinergic gain control in CLA-like networks, we constructed a
378 recurrently connected network model using stability optimised circuits (SOCs), a class of
379 networks where inhibition stabilises the network to create a non-chaotic network with
380 transient dynamics. Below we briefly describe this model.

381

382 We first generate synaptic weight matrices W with $N = 300$ neurons (with excitatory and
383 inhibitory neurons in the ratio 9:1 as empirically determined³⁶) as detailed in Hennequin
384 et al., 2014³¹.

385

386 We begin with set of sparse weights with non-zero elements set to w_o/\sqrt{N} for excitatory
387 neurons and $-\gamma w_o/\sqrt{N}$ for inhibitory neurons, where $w_o^2 = 2\rho^2/(p(1-p)(1+\gamma^2))$ with
388 connection probability p being 0.03 for excitatory neurons and 0.4 for interneurons as
389 empirically determined³⁷. We construct W with an approximately circular spectrum (i.e
390 set of eigenvalues) of radius $\rho = 10$ and inhibition/excitation ratio $\gamma = 3$ in line with
391 Hennequin et al., 2014 (Fig S3b, Left).

392

393 Following construction of W , we never change the excitatory weight, but refine the
394 inhibitory connections to minimize the ‘spectral abscissa’ of W , which is the largest real
395 part among the eigenvalues of W (Fig S3a). This optimization is performed according to
396 Stroud et al., 2018 and the resulting matrix, referred to as a stability optimized circuit or
397 SOC is non-chaotic³⁸ (Fig S3b, Right).

398

399 The use of SOCs is an approximation used due to the lack of precise cell-type specific
400 connectivity for the CLA. SOCs have been used to study the effect of gain modulation in
401 motor cortex circuits³⁸. Since we were interested in gain control of the CLA network, we
402 used SOCs to obtain a non-chaotic network with CLA-like connectivity for PN-PN and IN-
403 IN connections and empirically determined IO curves for each cell type.

404

405 Our model is governed by a differential equation which controls neuronal activity
406 (Equation 3) using the gain function (Equation 1) and the synaptic connectivity matrix W .

407

$$408 \quad \tau \frac{dx(t)}{dt} = -\mathbf{x}(t) + \mathbf{W} f(x(t); g) + I \quad (3)$$

409

410 We integrate Equation 1 using the ODE45 function in Matlab using default parameters.

411

412 The initial condition x_0 was chosen among the “most observable” modes which elicit the
413 strongest transient dynamics according to Hennequin et al., 2014³¹.

414

415 To mimic in-vivo experiments which examined the effect of cholinergic input on signal and
416 noise correlations in networks³⁹, we delivered time varying inputs I as shown in Fig 3 C.

417 We delivered two sets of inputs, a signal which consists of gaussian distributed input
418 where the SD of input is 1/10th of its mean. In a different set of trials, we provided a second

419 noisy input to the network which was Poisson distributed with SD equal to the mean of
420 the signal. Signal correlation – Noise correlation graphs are obtained by plotting the

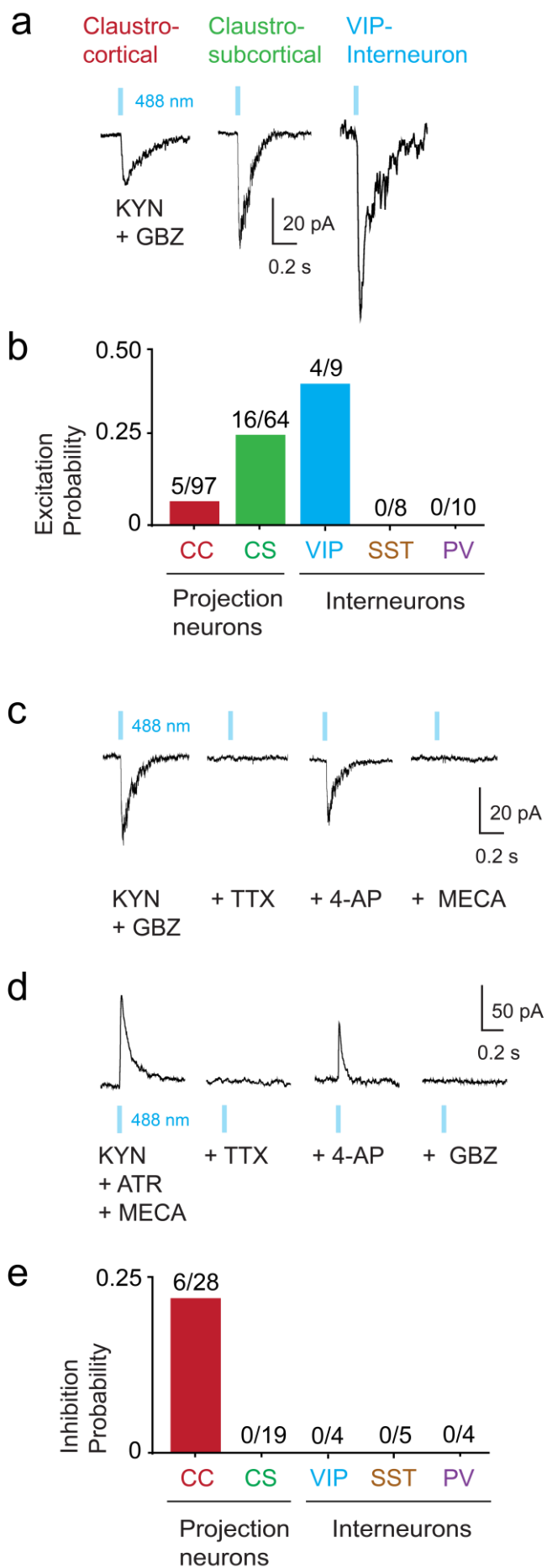
421 Pearson's correlations coefficient (PCC) between every pair of excitatory neurons during
422 the presentation of the Gaussian signal vs during the presentation of Poisson noise.

423

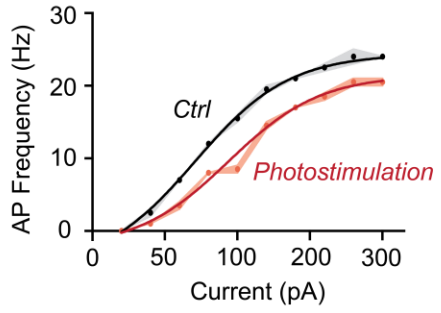
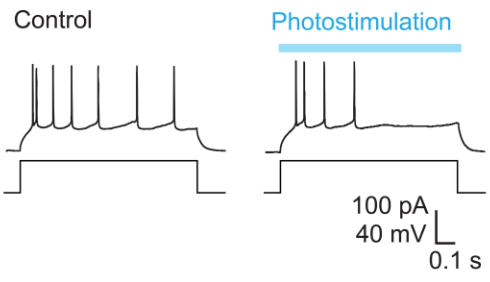
424 To ensure that the results we observed were robust for a range of input sizes, we
425 systematically varied the mean of either the Gaussian signal or Poisson noise and
426 examined the slope of the signal-noise correlation plot (Fig S2 c, S2d).

427

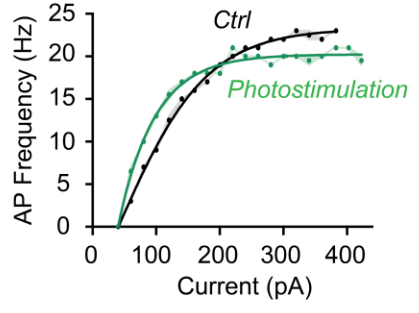
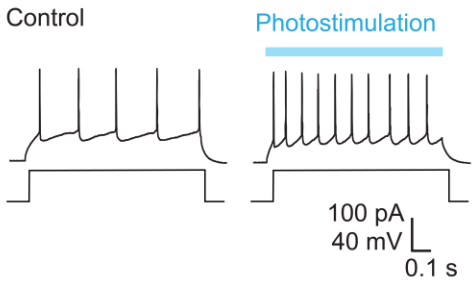
428 Code used for analysis of IO curves and the recurrent claustrum network is available at
429 <https://github.com/adityanairneuro/cholinergic>



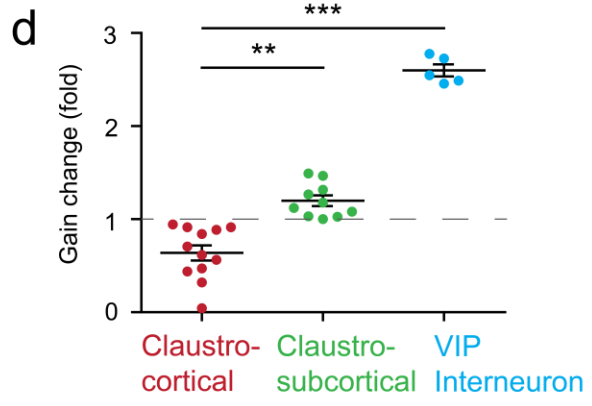
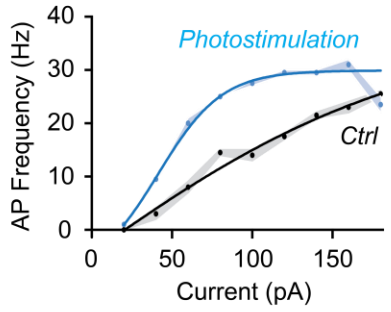
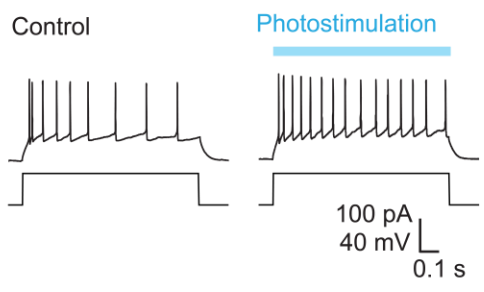
a Claustrocortical

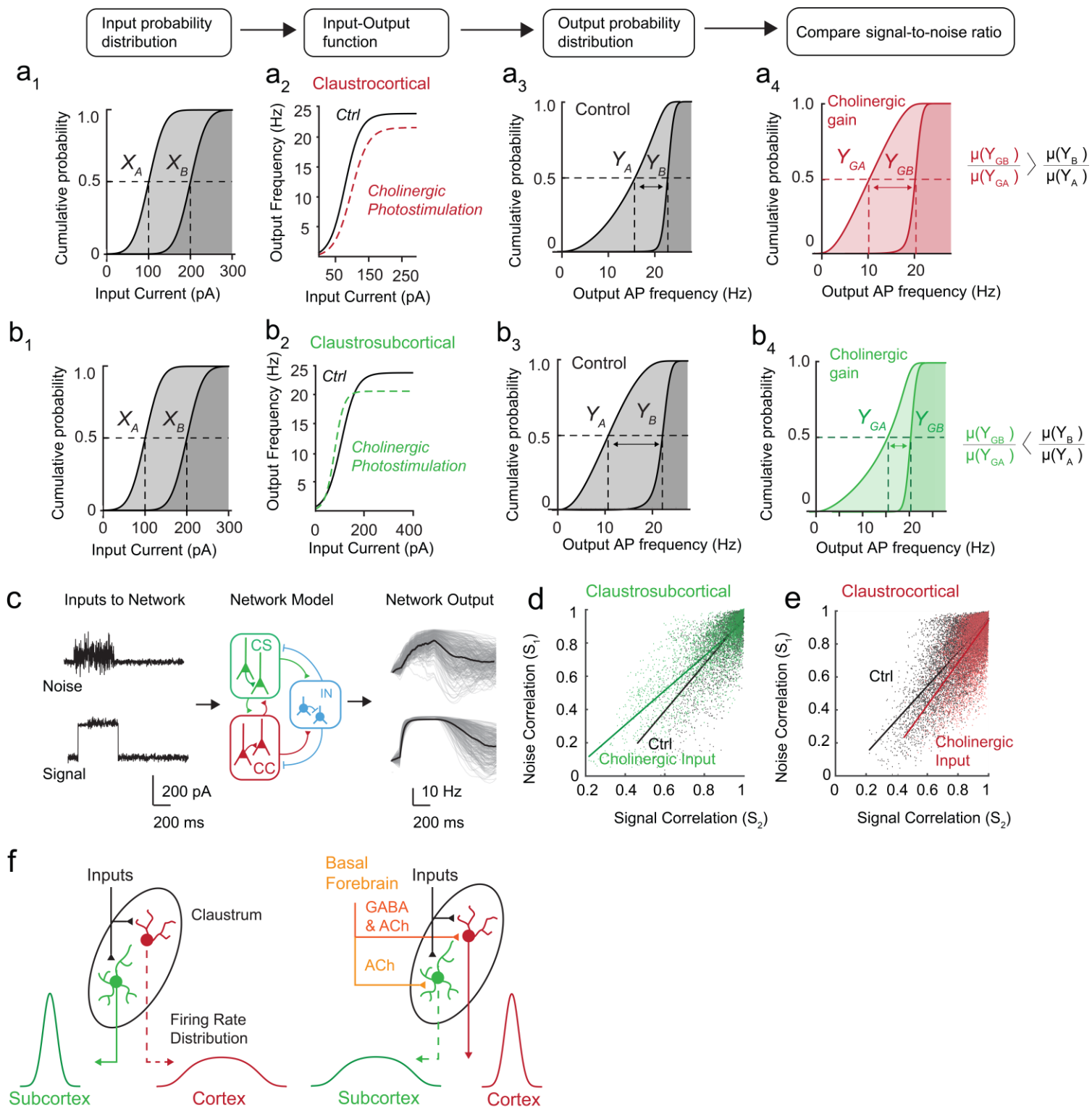


b Claustrosubcortical



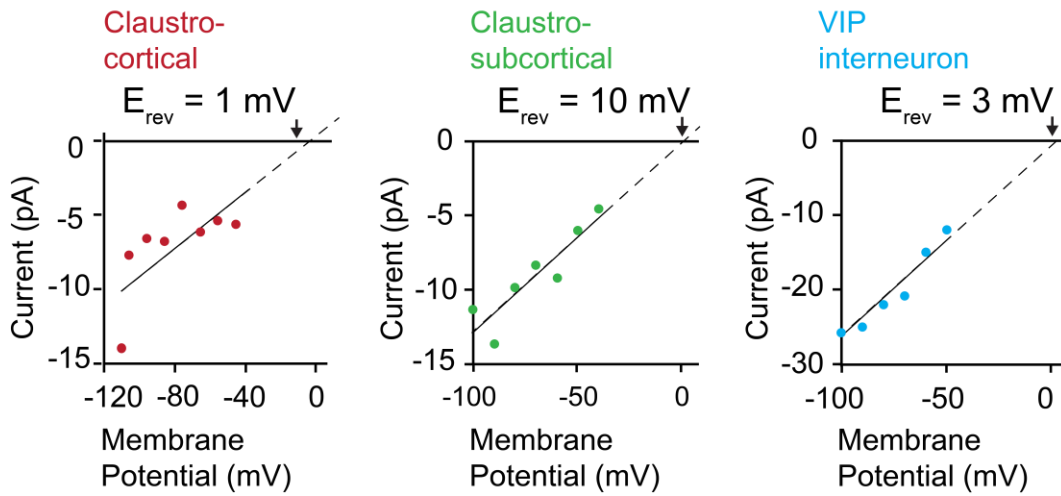
c VIP Interneuron





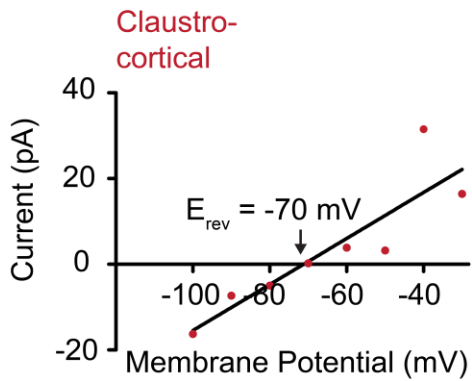
a

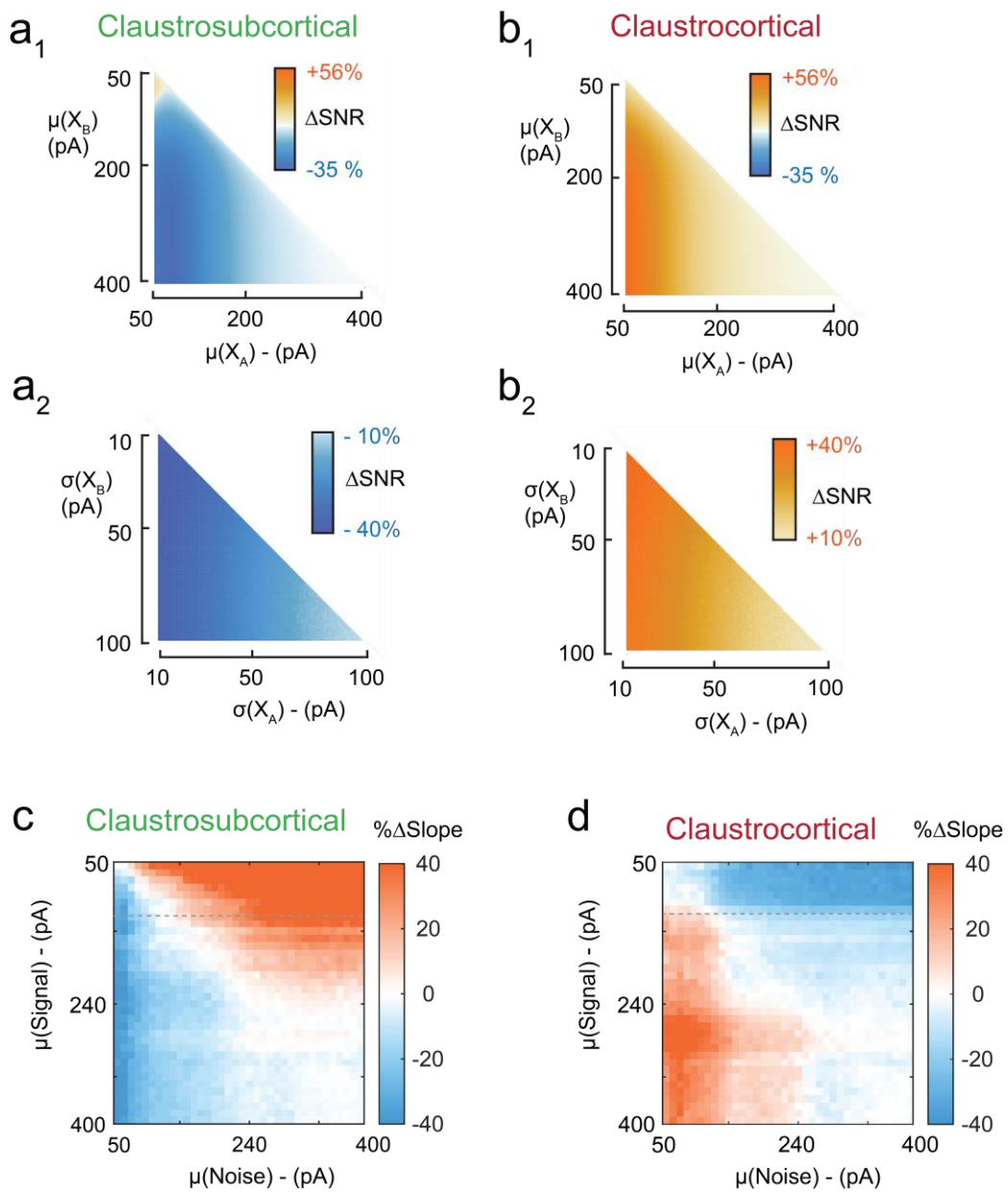
Current-Voltage plots for direct excitatory responses



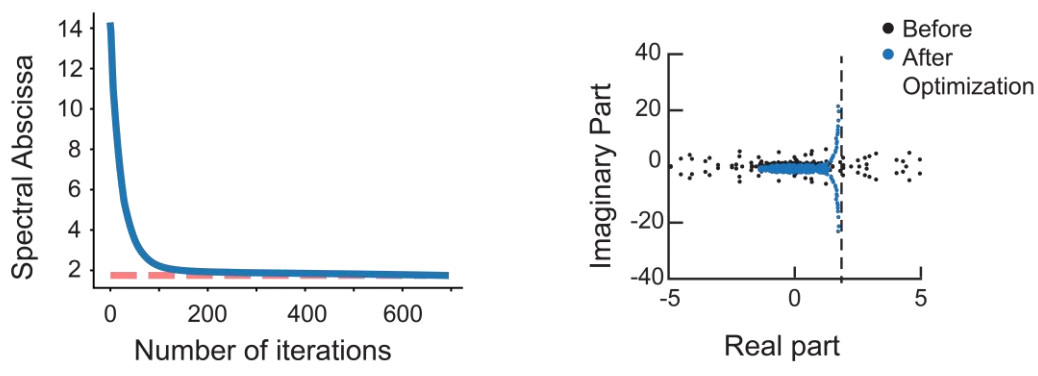
b

Current-Voltage plots for direct inhibitory responses





a Convergence of optimization



b Network weight matrix before and after optimization

