Acetylcholine controls input selectivity in CA1 of the hippocampus by muscarinic regulation of feedforward excitatory-inhibitory balance

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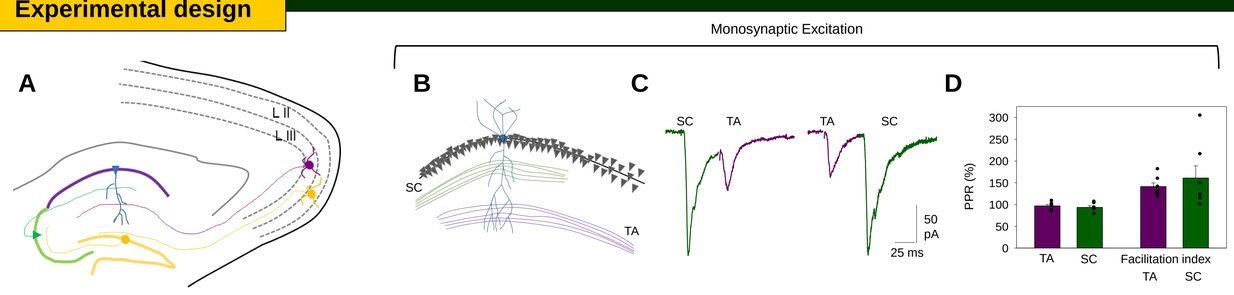
Summary

Acetylcholine fundamentally reconfigures cortical circuits to switch their function. In the hippocampus acetylcholine is thought to prioritise input to CA3 and CA1 circuits from sensory modalities containing new information about the environment and away from internally held representations. This reconfiguration enables acetylcholine to signal that previously held representations require updating with new information. However, the mechanism by which acetylcholine enables this critical switch in function is unclear. In the CA1 region, internally held representations are proposed to enter via the Schaffer collateral (SC) pathway from CA3 whereas new information enters via the temporoammonic (TA) pathway direct from the entorhinal cortex. Therefore, it is predicted that acetylcholine will reduce SC input whilst enhancing TA input to CA1. Previous studies have reported that acetylcholine reduces excitatory synaptic transmission in both SC and TA pathways apparently contradicting this prediction. However, these studies did not measure the effects of acetylcholine on feedforward inhibition which have a major role in determining the CA1 response to SC or TA input. Therefore, our goal was to test the core hypothesis that acetylcholine prioritises TA input over SC input. We used electrical stimulation to obtain monosynaptic excitatory or disynaptic inhibitory postsynaptic currents ($_m$EPSC or $_d$IPSC respectively) from SC and TA pathways on the same CA1 pyramidal neuron. The acetylcholine receptor agonist carbachol (CCh) reduced both $_m$EPSC and $_d$IPSC synaptic responses for the SC input which resulted in no change to excitatory-inhibitory balance and indeed a decrease in postsynaptic spiking. In contrast, although CCh also reduced both $_m$EPSC and $_d$IPSC synaptic responses for the TA input the $_d$IPSC was reduced much less resulting in an increase in excitatory-inhibitory balance. This was particularly evident after repetitive high frequency stimulation resulting in an increase in postsynaptic spiking. Pharmacological interventions revealed that presynaptic muscarinic M3 receptors mediated the CCh induced facilitation of excitatory-inhibitory balance on TA pathway, in contrast to the SC pathway where muscarinic M4 receptors are believed to mediate presynaptic inhibition.

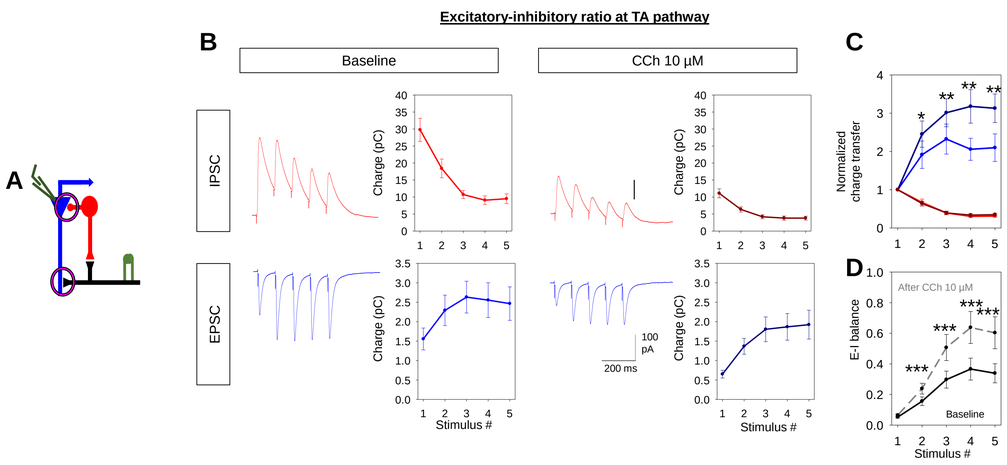
Experimental design



A, Schematic diagram of the hippocampus, where the main input pathways are high lightened. Briefly, EC LII projects to dentate gyrus to start the trisynaptic pathway (DG → CA3 → CA1), while EC LIII directly stimulates CA1 sub region of the hippocampus. B, schematic drawing of the CA1 region of the hippocampus showing the two major excitatory input pathways (green for SC and purple for TA pathway) onto CA1 pyramidal neurons. C-D, two pathway independence test. CA1 pyramidal neuron was recorded at -60 mV (reversal potential for $GABA_A$ receptors) in whole cell voltage clamp configuration to isolate excitatory responses. While both pathways facilitate independently, consecutive stimulation (50 ms apart) of the pathways did not facilitate, suggesting independent responses between them. E, schematic drawing of CA1 region showing different interneurons linked to separate excitatory pathways. F, CA1 pyramidal neuron recorded at 0 mV (reversal potential for glutamate receptors) showed the inhibitory transmission (green for SC and purple for TA pathway), which is partially blocked (>70 %) by glutamate receptors antagonist (NBQX 20 µM), arguing in favour of a synaptic activation of interneurons.

Results

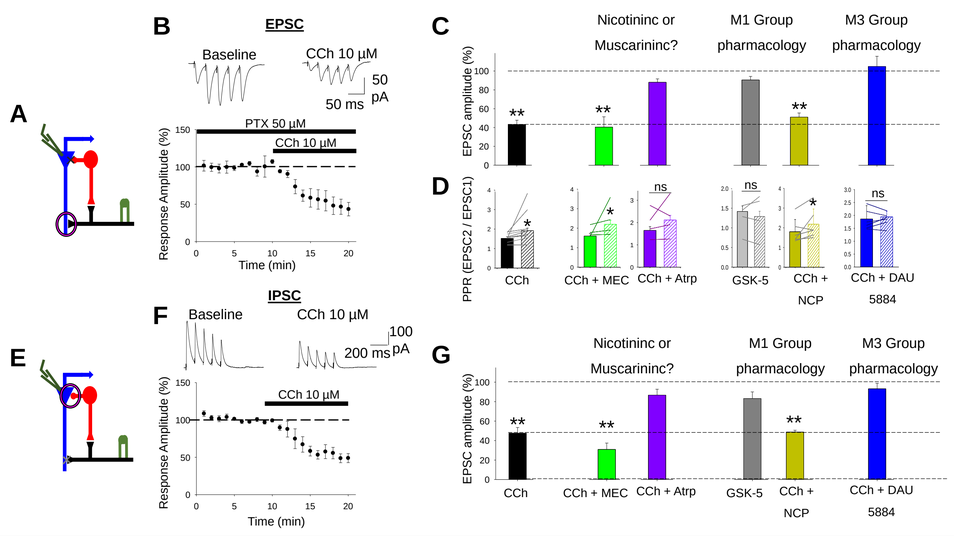
1. Differential modulation of SC and TA pathway excitatory-inhibitory ratio upon cholinergic receptor activation



A, schematic drawing of the experimental approach. B & E (applies for TA and SC pathway respectively), IPSC (red) and EPSC (blue) recorded example traces and charge transfer quantification before (left) and after CCh 10 µM right) application from the same neuron. C & F (applies for TA and SC pathway respectively), charge transfer normalization to each first response before (red or blue light colour) and after CCh (red or blue dark colour) application. D & G (applies for TA and SC pathway respectively), relationship between excitatory and inhibitory charge transfer responses, plotted for the five evoked stimulation before (black) and after CCh (grey) application. Data are mean ± SEM; paired T-test. n = 20, from 11 mice. *P> 0.05; **P< 0.01; ***P<0.005.

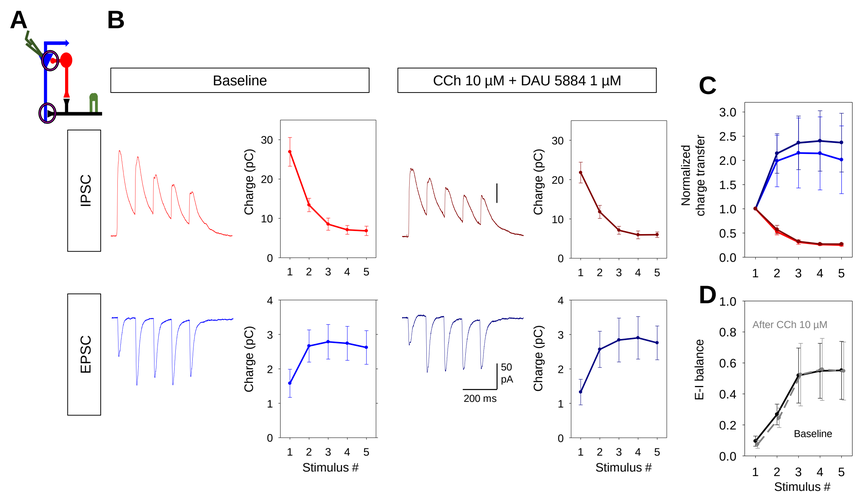
H, EPSC (left) and IPSC (right) charge transfer change quantification for TA and SC pathways (purple and green respectively) after CCh application. I, TA over SC excitatory-inhibitory ratio relationship plotted for five evoked stimulation before (black) and after CCh application (grey). Data are mean ± SEM; paired T-test. n =20, from 11 mice. *P> 0.05; **P< 0.01; ***P<0.005.

2. Pharmacological dissection of cholinergic effect on TA to CA1 evoked EPSC & IPSC



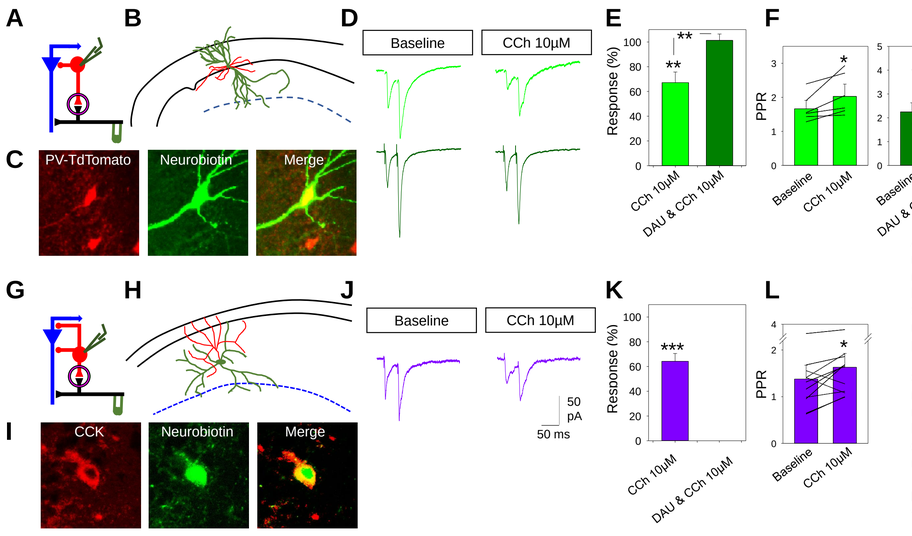
A, schematic drawing of the experimental approach. Isolation of excitatory synaptic transmission in TA pathway. B, evoked EPSC recorded from pyramidal neurons under Picrotoxin at -70 mV (up) and time course of EPSC reduction after CCh 10 µM application (bottom). C, Quantification of EPSC amplitude reduction after drug application. D, paired-pulse ratio was evaluated as the ratio between the second and the first response. Colour code: CCh 10 µM (black), CCh + mecamylamine 25 µM (green), CCh + atropine 10 µM (purple), GSK-5 [M1 receptor agonist] 500 nM (grey), CCh + Nitrocaramiphen 1 µM (dark yellow) and CCh + DAU 5884 1 µM (blue). Data are mean ± SEM; One-way ANOVA followed by Bonferroni correction (C) and paired T-Test (D). *P> 0.05; **P< 0.01; ***P<0.005. n ≥ 4 per condition.
E, schematic drawing of the experimental approach. Isolation of inhibitory synaptic transmission in TA pathway. F, evoked IPSC recorded from pyramidal neurons at 0 mV (up) and time course reduction of IPSC after CCh 10 µM application (bottom). G, Quantification of IPSC amplitude reduction after drug application. Colour code, same as above.

3. Muscarinic M3 receptor antagonist blocks the increase in excitatory-inhibitory ratio produced by cholinergic receptor activation



A, schematic drawing of the experimental approach. B, IPSC (red) and EPSC (blue) recorded example traces and charge transfer quantification before (left) and after CCh 10 µM + DAU 5884 1 µM (muscarinic M3 receptor antagonist; right) application from the same neuron. C, charge transfer normalization to each first response before (red or blue light colour) and after CCh + DAU 5884 (red or blue dark colour) application. D, relationship between excitatory and inhibitory charge transfer responses, plotted for the five evoked stimulation before (black) and after CCh + DAU 5884 (grey) application. Data are mean ± SEM; paired T-test. n ≥ 7.

4. Feedforward PV⁺ and CCK⁺ IN are modulated by cholinergic presynaptic receptor activation

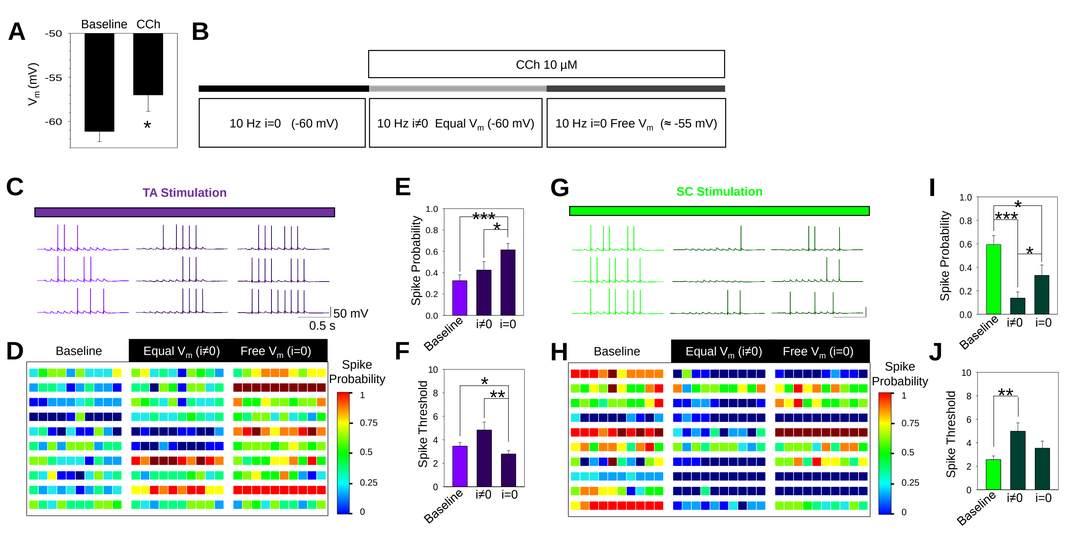


A, schematic drawing of the experimental approach. B, reconstruction of a successfully recorded PV⁺ IN. C, validation of recording target IN subtype. D, EPSC traces recorded from PV⁺ IN before and after carbachol application, compare with those where M3 antagonist (DAU 5584 1 µM) was infused (dark green). E, amplitude reduction was quantified after carbachol application. F, evaluation of paired-pulse ratio.

G, schematic drawing of the experimental approach. H, reconstruction of a successfully recorded CCK⁺ IN. I, validation of recording target IN subtype. J, EPSC traces recorded from CCK⁺ IN before and after carbachol application. K, amplitude reduction was quantified after carbachol application. L, evaluation of paired-pulse ratio.

Data are mean ± SEM; paired T-test. *P< 0.05; **P< 0.01. ***P< 0.005. n≥10.

5. Carbachol increases CA1 spike generation in response to TA pathway stimulation



A, CCh application depolarizes membrane potential. B, schematic diagram showing the experimental procedure. A baseline for SC or TA stimulation is recorded, then during CCh application current is injected to maintain membrane potential stable and last injected current is removed. C, repetitive 10 Hz TA pathway stimulation produces spike generation on CA1 pyramidal neurons. D, colour coded spike probability representing average data from 10 different cells for 10 recordings in each condition. E-F, spike probability and spike threshold quantification during previously described three experimental conditions. F-J, same as C-F in SC pathway. Data are mean ± SEM; paired T-test. *P< 0.05; **P< 0.01. ***P< 0.005. n≥14.

Conclusions

- TA and SC stimulation yielded independent monosynaptic facilitating EPSC and disynaptic depressing IPSC responses on the same CA1 pyramidal neuron.
- Cholinergic receptor activation, by its broad spectrum agonist carbachol, reduces EPSC and IPSC amplitude and charge transfer in TA and SC pathway.
- Repetitive stimulation of TA axons produces a facilitation of EPSC which is not reproduced by IPSC, producing an increase in the excitatory-inhibitory ratio by the activation of cholinergic receptors.
- Repetitive stimulation of SC axons produces a similar increase in the EPSC and IPSC facilitation, producing a slightly increase in the excitatory-inhibitory ratio by the activation of cholinergic receptors.
- Inhibitory drive shapes excitatory neurotransmission, affecting cholinergic modulatory outcome at feedforward hippocampal microcircuit.
- Both excitatory and inhibitory synaptic responses from TA pathway were decreased by presynaptically located M3 muscarinic receptors, identified by pharmacological inhibition.
- PV⁺ and CCK⁺ IN in the hippocampus are feedforward interneuron in the TA pathway and their excitatory inputs are depressed by cholinergic receptors activation.
- Cholinergic receptor activation reduces SC pathway spike generation while causes an increment in TA pathway spike generation of CA1 pyramidal cells, therefore making CA1 pyramidal cells more responsive to TA pathway in comparison to SC pathway.

- Differential expression of presynaptic muscarinic receptors on SC and TA inputs to CA1 enable acetylcholine to reconfigure the network to favour new information over internally held representations.

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Working model