Regulation of the temporoammonic pathway in the hippocampus by acetylcholine

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Supported by: wellcome trust

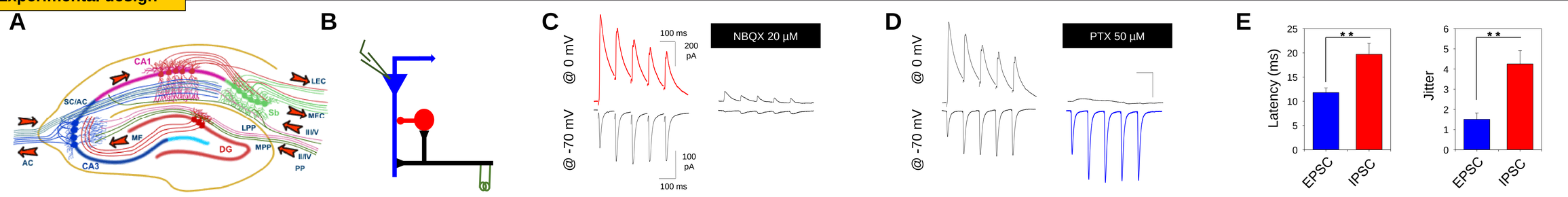
Summary

The release of acetylcholine in the hippocampus during awake behaviour is important for encoding memory. Within the hippocampal network, acetylcholine has diverse effects which are not ubiquitous and instead are exhibited at individual neurons and synapses. The Temporoammonic (TA) pathway carries spatial information from grid cells in entorhinal cortex layer III (EC LIII) to CA1 hippocampal place cells, synapsing onto the distal dendrites. It is not currently known how acetylcholine regulates synaptic transmission in the temporoammonic pathway or which acetylcholine receptors mediate this regulation.

To determine how acetylcholine regulates the temporoammonic pathway we made whole cell patch clamp recordings from CA1 pyramidal neurons in acute hippocampal slices from adult mice. Electrical stimulation in the Stratum Lacunosum Moleculare elicited monosynaptic excitatory and polysynaptic inhibitory synaptic responses. The acetylcholine receptor agonist carbachol (CCh 10 µM) reduced both excitatory and inhibitory synaptic responses and increased paired-pulse ratio for excitatory responses, indicating a presynaptic locus of action. Specific pharmacological intervention shows the participation of M3 receptors. The reduction in synaptic response for excitatory and inhibitory synapses was similar for both but the increase in paired pulse ratio for excitatory responses, produced a facilitation of excitatory-inhibitory balance in response to repetitive stimulation. In addition, CCh produces an increase in the number of spikes when TA synapses were repeatedly stimulated at different frequencies. This increase responded to a membrane potential depolarization, rather than a synaptic effect.

We conclude that acetylcholine modulates the temporoammonic pathway onto CA1 pyramidal neurons by presynaptically located M3 muscarinic receptors.

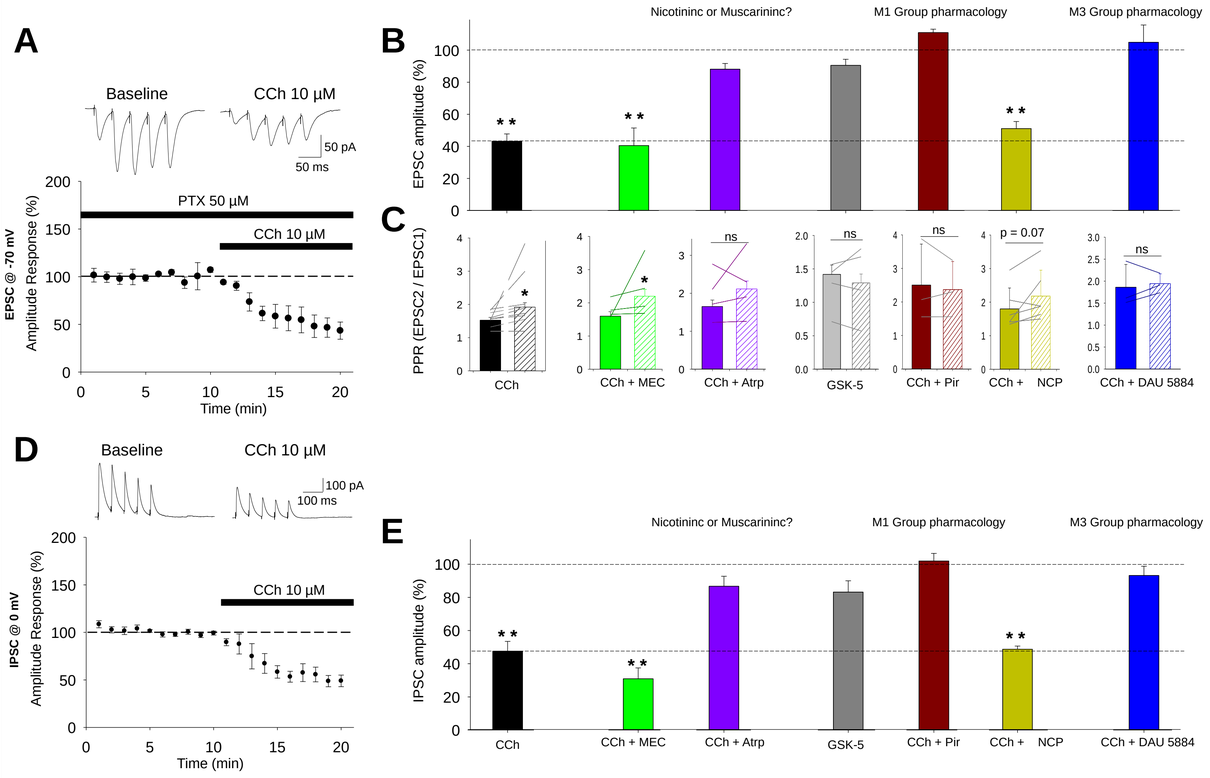
Experimental design



A, Schematic diagram of the hippocampus, where the main input and output 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 TA axons (black), where the stimulation electrode is located. This axons innervate interneurons (red) and pyramidal neurons (blue), where recordings are made. C, Experimental approach to separate disynaptic IPSC and monosynaptic EPSC. Recording at 0 mV (reversal potential for glutamate receptors) showed the inhibitory transmission (red), which is partially blocked (>70 %) by glutamate receptors antagonist (NBQX 20 µM), arguing in favour of a synaptic activation of interneurons. Recording at -70 mV under $GABA_A$ receptor blockage (PTX 50 µM) showed the excitatory synaptic transmission (blue). E, quantification of latency (measured as the time between the stimulus and the peak of the response) and jitter (measured as the variability of the latency). Data are mean ± SEM; One-ANOVA followed by Bonferroni comparison test. $^{**}P< 0.01$. n = 10.

Results

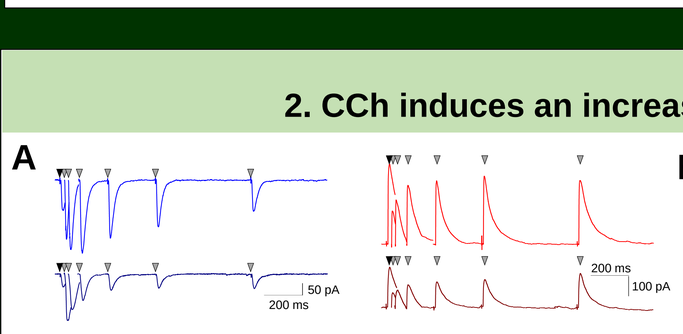
1. TA to CA1 evoked EPSC & IPSC are reduced by 10 µM CCh



A, evoked EPSC recorded examples from pyramidal neurons under Picrotoxin at -70 mV (up) and time course of EPSC reduction after CCh 10 µM application (bottom). B, EPSC amplitude reduction was quantified after different drug combination application. C, pair-pulse ratio was evaluated as the ratio between the second and the first response, which indicates a relative measurement of release probability, . D, evoked IPSC recorded examples from pyramidal neurons at 0 mV (up) and time course reduction of IPSC after CCh 10 µM application (bottom). E, IPSC amplitude reduction was quantified after different drug combination application.

Colour code: CCh 10 µM (black), CCh + mecamylamine 25 µM (green), CCh + atropine 10 µM (purple), GSK-5 500 nM (grey), CCh + Pirenzepine 1 µM (dark red), CCh + Nitrocaramiphen 1 µM (dark yellow) and CCh + DAU 5884 1 µM (blue). Data are mean ± SEM; One-way ANOVA followed by Bonferroni comparison test (C) and paired T-Test (D). $^{*}P> 0.05$; $^{**}P< 0.01$; $^{***}P<0.005$. n ≥ 4 per condition.

2. CCh induces an increase of EPSC PPR

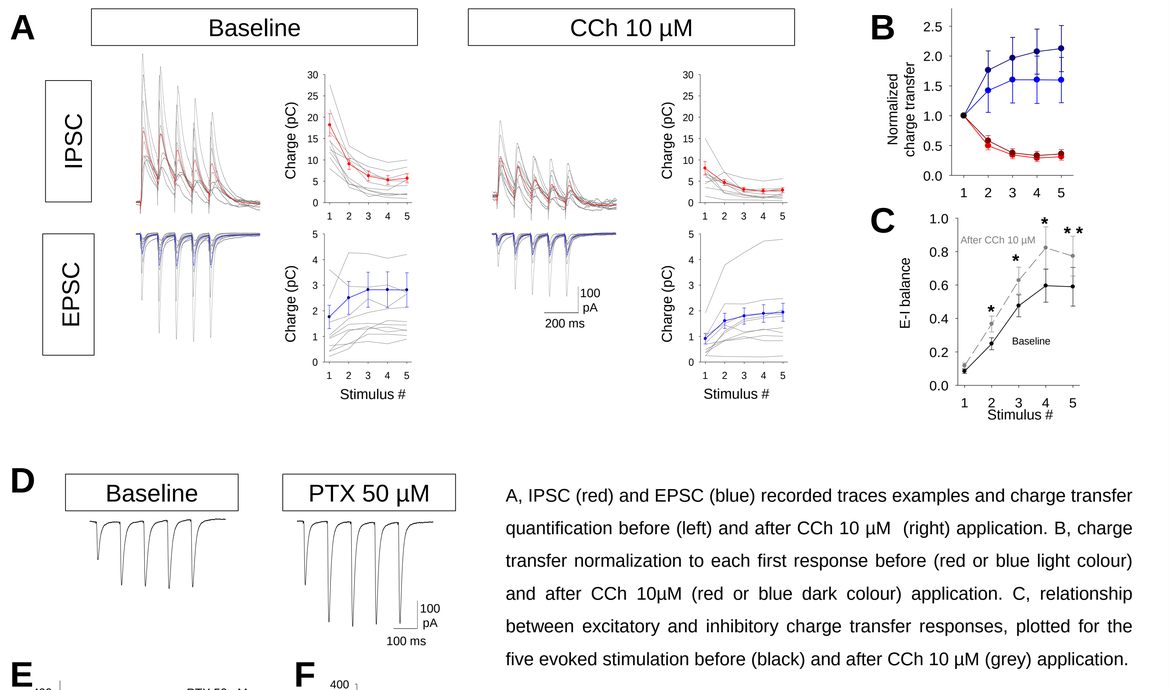


A, Composition of evoked EPSC (blue, left) and IPSC (red, right) recorded examples at different inter stimulus interval (ISI). B, quantification of pair-pulse ratio of EPSC ISI (blue, left) and IPSC ISI (red, right), before and after CCh 10 µM application (light and dark colour respectively). Data are mean ± SEM; paired T-test. $^{*}P< 0.05$. n > 5 per condition.

Conclusions

- TA axons stimulation yielded monosynaptic EPSC and disynaptic IPSC responses on CA1 pyramidal neurons.
- Both excitatory and inhibitory synaptic responses from TA pathway are decreased by presynaptically located M3 muscarinic receptors.
- Repeated stimulation of TA axons produce an increase of excitatory to inhibitory balance due to an increase of EPSC PPR not replicated by IPSC.
- Cholinergic agonist increases the spike generation of TA pathway due to increase of the membrane potential.

3. CCh produces an increase of excitatory-inhibitory ratio at CA1 pyramidal neuron



A, IPSC (red) and EPSC (blue) recorded traces examples and charge transfer quantification before (left) and after CCh 10 µM (right) application. B, charge transfer normalization to each first response before (red or blue light colour) and after CCh 10µM (red or blue dark colour) application. C, relationship between excitatory and inhibitory charge transfer responses, plotted for the five evoked stimulation before (black) and after CCh 10 µM (grey) application.

D, examples traces where $GABA_A$ receptor antagonist (PTX 50 µM) increased evoked EPSC amplitude. E, time course of EPSC amplitude when PTX is applied. F, quantification of EPSC amplitude increment after $GABA_A$ receptor antagonist application.

Data are mean ± SEM; paired T-test. $^{*}P< 0.05$; $^{**}P< 0.01$. n = 9 (A-C) and n = 3 (D-F) per condition.

4. Acetylcholine modules TA to CA1 spike generation

A, repetitive TA pathway stimulation produces spike generation on CA1 pyramidal neurons at different frequencies (3, 10 and 20 Hz), which increases after application of CCh. B, quantification of spike number (spike #) and spike threshold (# Threshold).

C, CCh application depolarizes membrane potential.

D, schematic diagram showing the time course procedure where a baseline for TA stimulation is recorded, then during CCh application current is injected to maintain membrane potential stable and last injected current is removed. E, spike number quantification at different frequencies during previously described three experimental conditions. Pair recordings are linked with a red line.

Data are mean ± SEM; paired T-test. $^{*}P< 0.05$; $^{**}P< 0.01$. $^{***}P< 0.005$. n≥7.