Regulation of the temporoammonic pathway in the hippocampus by acetylcholine

Jon Palacios-Filardo & Jack R Mellor

School of Physiology and Pharmacology, University of Bristol, UK

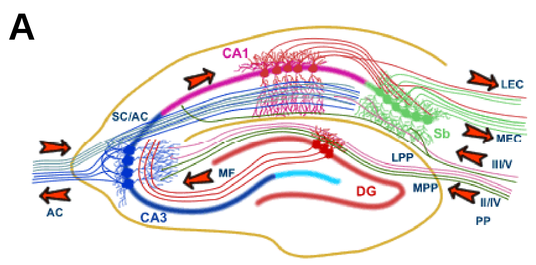
Supported by: wellcome trust

Summary

The release of acetylcholine in the hippocampus during awake behaviour is important for encoding memory. Within the hippocampal network, acetylcholine has diverse effects: it increases neuronal excitability, controls synaptic strength and regulates the induction of synaptic plasticity. However, these effects are not ubiquitous and instead are exhibited at individual neurons and synapses within the network with each effect mediated by specific subtypes of acetylcholine receptor. 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.

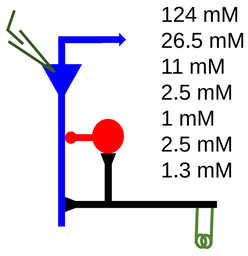
To determine how acetylcholine regulates the temporoammonic pathway we made whole cell patch clamp recordings from CA1 pyramidal neurons in acute hippocampal slices from adult mice. Electrical stimulation in the Stratum Lacunosum Moleculare elicited monosynaptic excitatory and polysynaptic inhibitory synaptic responses. The acetylcholine receptor agonist carbachol (10 µM) reduced both excitatory and inhibitory synaptic responses and increased paired-pulse ratio for excitatory responses, indicating a presynaptic locus of action. The reduction in synaptic response for excitatory and inhibitory responses 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. The reduction in synaptic responses caused by carbachol was blocked by atropine but not mecamylamine, indicating a role for presynaptic muscarinic receptors. However, the reduction was not replicated by a selective muscarinic M1 receptor agonist (GSK-5). Instead, the reduction in synaptic response induced by carbachol was blocked by a muscarinic M3 receptor antagonist (DAU5884 1µM). We conclude that acetylcholine modulates the temporoammonic pathway onto CA1 pyramidal neurons by presynaptically located M3 muscarinic receptors.

Experimental design



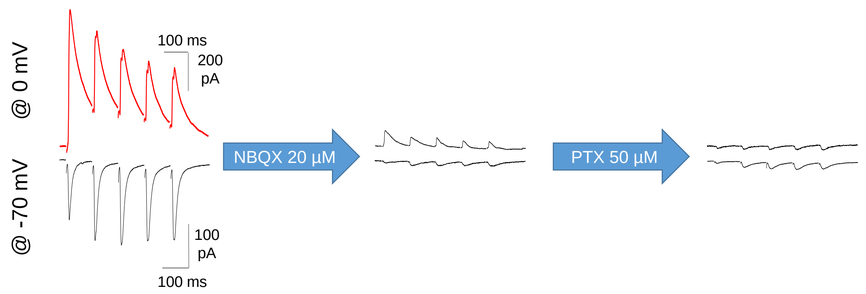
Internal solution:

130 mM	$CsMeSO_4$
4 mM	NaCl
10 mM	HEPES
10 mM	TEA
2 mM	ATP
0.5 mM	GTP
1 mM	QX 314
0.5 mM	EGTA



External solution:

124 mM	NaCl
26.5 mM	$NaHCO_3$
11 mM	Glucose
2.5 mM	KCl
1 mM	$NaHPO_4$
2.5 mM	$CaCl_2$
1.3 mM	$MgSO_4$

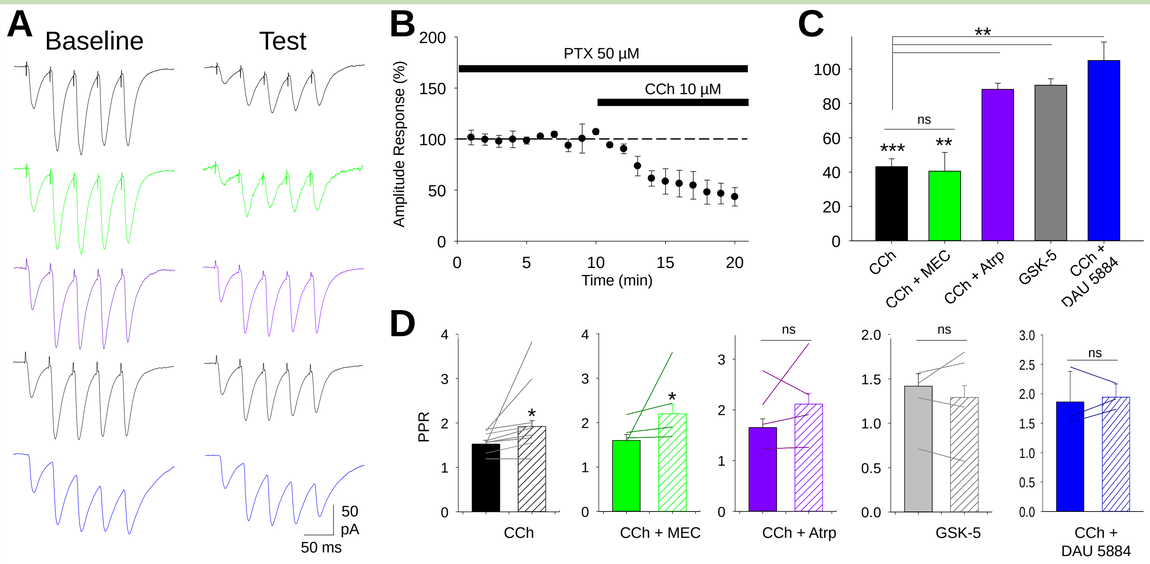


A, Comparison of the trisynaptic organization versus direct cortical innervation of the hippocampus. Briefly, EC LII projects to dentate gyrus to start the trisynaptic pathway (DG → CA3 → CA1), while EC LIII directly stimulates CA1 sub region of the hippocampus (top). Schematic drawing illustrating the TA axons stimulation which contact interneurons (red) and pyramidal neurons (blue) in the CA1 sub region. Experimentally used internal and external recording solutions are shown (bottom).

B, 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 disynaptic activation of interneurons. Subsequent blockage of GABA receptors (Picrotoxin – PTX 50 µM) further reduced IPSC, confirming the inhibitory component of the synaptic responses. Recording at -70 mV under GABA receptor blockage (PTX 50 µM) showed the excitatory synaptic transmission (blue), which is blocked after glutamate receptor antagonist application (NBQX 20 µM).

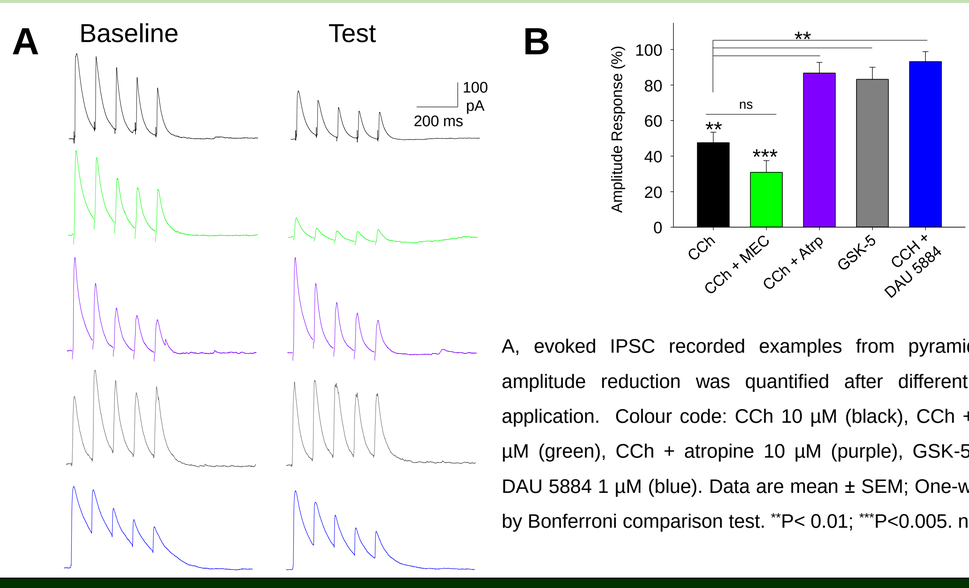
Results

1. TA to CA1 evoked EPSC is reduced by 10 µM CCh



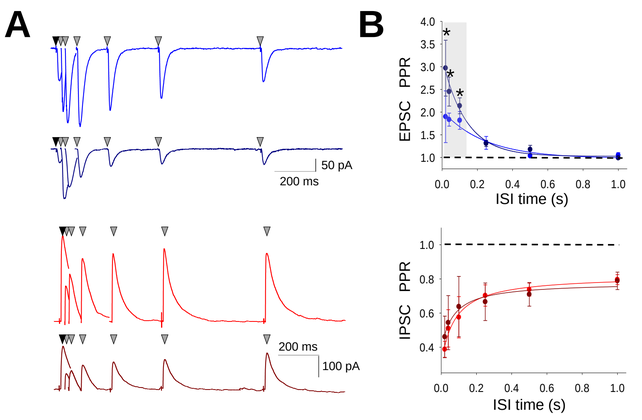
A, evoked EPSC recorded examples from pyramidal cells. B, time course of EPSC reduction after CCh 10 µM application. C, EPSC amplitude reduction was quantified after different drug combination application. D, pair-pulse ratio was evaluated as a relative measure of release probability. Colour code: CCh 10 µM (black), CCh + mecamylamine 25 µM (green), CCh + atropine 10 µM (purple), GSK-5 (grey) 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 > 3 per condition.

2. TA to CA1 evoked IPSC is reduced by 10 µM CCh



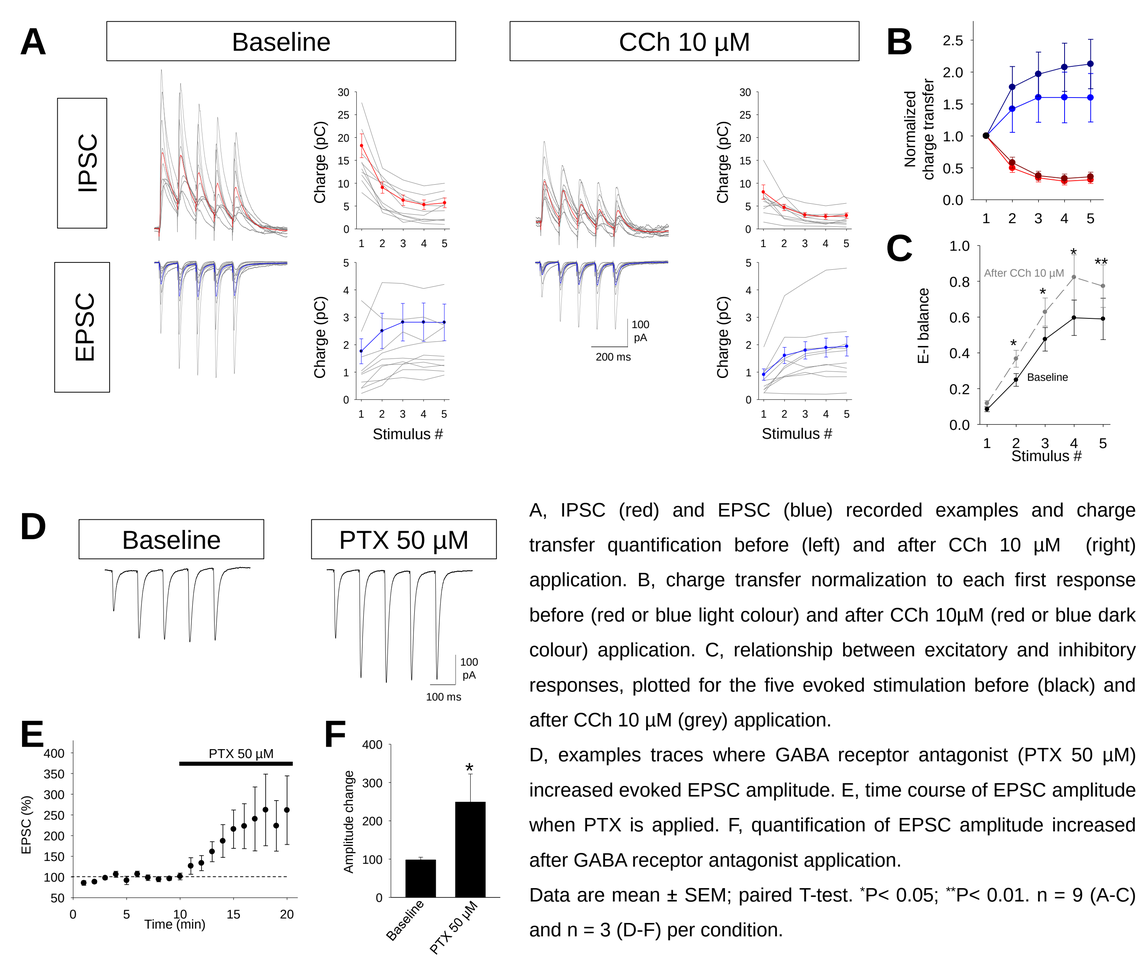
A, evoked IPSC recorded examples from pyramidal cells. B, IPSC amplitude reduction was quantified after different drug combination application. Colour code: CCh 10 µM (black), CCh + mecamylamine 25 µM (green), CCh + atropine 10 µM (purple), GSK-5 (grey) and CCh + DAU 5884 1 µM (blue). Data are mean ± SEM; One-way ANOVA followed by Bonferroni comparison test. **P< 0.01; ***P<0.005. n > 3 per condition.

3. CCh induces an increase of EPSC PPR



A, Composition of evoked EPSC (blue) and IPSC (red) recorded examples at different inter stimulus interval (ISI). B, quantification of pair-pulse ratio of EPSC ISI (top) and IPSC ISI (bottom), 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.

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



A, IPSC (red) and EPSC (blue) recorded 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 responses, plotted for the five evoked stimulation before (black) and after CCh 10 µM (grey) application.

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

Conclusions

- TA axons stimulation yielded monosynaptic EPSC and disynaptic IPSC responses.
- Both excitatory and inhibitory synaptic responses from TA pathway are decreased by presynaptically located M3 muscarinic receptors.
- TA axons repeated stimulation caused an increase of excitatory to inhibitory balance due to: I, an increase of EPSC PPR not replicated by IPSC and II, an increase of EPSC related with a reduction of the inhibitory neurotransmission.