Regulation of the temporoammonic pathway in the hippocampus by acetylcholine.

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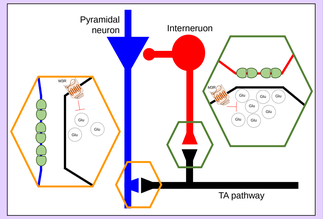
Summary

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 or PV$^+$ interneurons in acute hippocampal slices from adult mice. Electrical stimulation in the Stratum Lacunosum Moleculare was used to isolate excitatory postsynaptic currents (EPSC) recorded at -70 mV in the presence of Picrotoxin or disynaptic inhibitory postsynaptic currents (IPSC) recorded at 0 mV. 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 showed that neither M1 agonist was able to reproduce CCh induced synaptic currents reduction, nor did M1 antagonist block the effect. In contrast, M3 receptor antagonist or genetic deletion of M3 receptors, blocked CCh induced reduction of synaptic probability of release to the same extent for EPSC and IPSC. Furthermore, we revealed that PV$^+$ Interneurons are feedforward upon TA pathway stimulation, whose excitatory inputs are inhibited by the activation of M3 receptors. The reduction in synaptic response for excitatory and inhibitory responses at pyramidal neurons 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 produced an increase in the number of spikes in the CA1 pyramidal neurons when TA synapses were repeatedly stimulated over a range of frequencies. This increase was principally mediated by 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.

Conclusions

- TA stimulation yielded monosynaptic facilitating EPSC and disynaptic depressing IPSC responses on CA1 pyramidal neurons.
- Both excitatory and inhibitory synaptic responses from TA pathway were decreased by presynaptically located M3 muscarinic receptors, identified by pharmacological inhibition or genetic deletion.
- PV$^+$ IN in the hippocampus are feedforward interneuron in the TA pathway and their excitatory inputs are depressed by M3 muscarinic receptors.
- Repetitive stimulation of TA axons enhanced EPSCs more than IPSCs in CA1 pyramidal cells resulting in an increase of excitatory to inhibitory balance.
- Cholinergic receptor activation also enhanced excitatory to inhibitory balance in response to repetitive stimulation of the TA pathway and also depolarized CA1 pyramidal cells causing an increase in spike generation.



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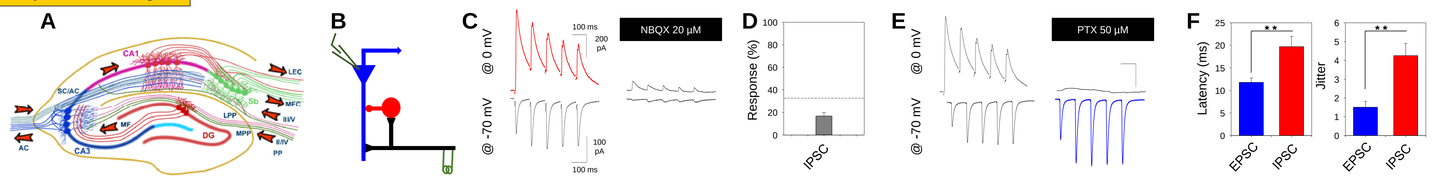
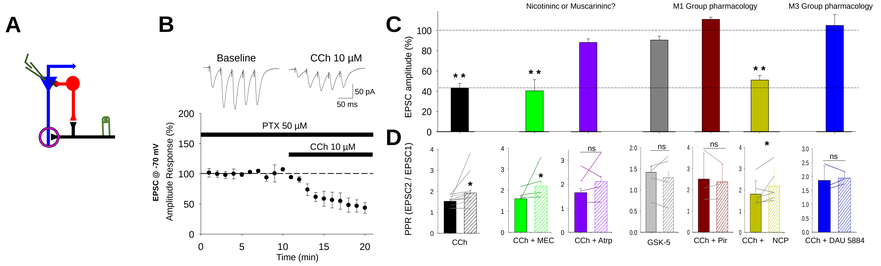
Experimental design



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. These axons innervate feedforward 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 >70 % blocked by glutamate receptors antagonist (NBQX 20 µM), arguing in favour of a synaptic activation of interneurons. D, quantification of IPSC responses upon NBQX infusion; Successfully stimulated interneurons present disynaptic IPSC responses minor to 33% after NBQX. E, Recording at -70 mV under $GABA_A$ receptor blockage (PTX 50 µM) showed the excitatory synaptic transmission (blue). F, 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-way ANOVA followed by Bonferroni correction. **P< 0.01. n = 10.

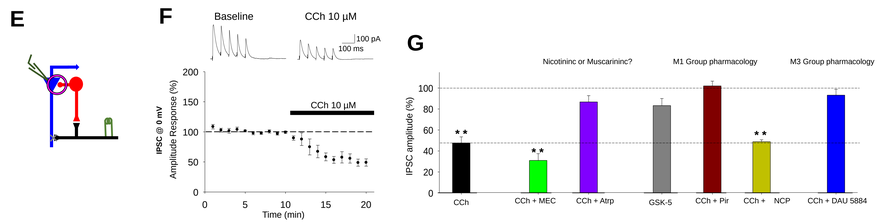
Results

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



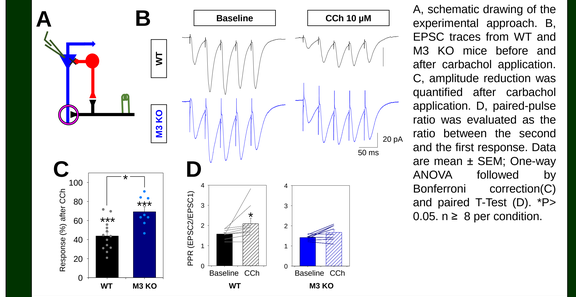
A, schematic drawing of the experimental approach. Isolation of excitatory synaptic transmission in TA pathway.

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, which indicates a relative measurement of release probability. Colour code: CCh 10 µM (black), CCh + mecamylamine [Nicotinic receptor antagonist] 25 µM (green), CCh + atropine [Muscarinic receptor antagonist;] 10 µM (purple), GSK-5 [M1 receptor agonist] 500 nM (grey), CCh + Pirenzepine [M1 + M3 receptor antagonist] 1 µM (dark red), CCh + Nitrocaramiphen [M1 receptor antagonist] 1 µM (dark yellow) and CCh + DAU 5884 [M3 receptor antagonist] 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.



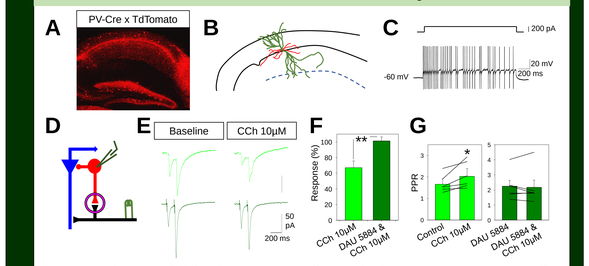
E, schematic drawing of the experimental approach. Isolation of inhibitory synaptic transmission in TA pathway. F, evoked IPSC recorded from pyramidal neurons at 0 mV (up) and time course reduction of IPSC after CCh 10 µM application (bottom). G, Quantification of IPSC amplitude reduction after drug application. Colour code, same as above.

2. M3KO mice have reduced cholinergic modulation



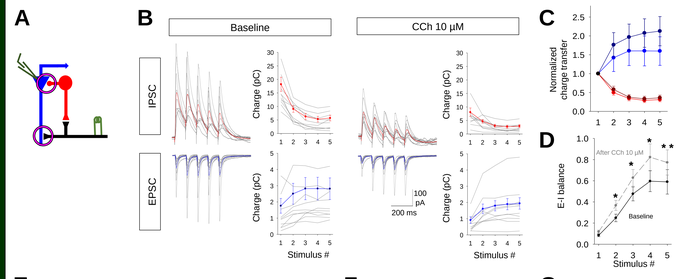
A, schematic drawing of the experimental approach. B, EPSC traces from WT and M3 KO mice before and after carbachol application. C, amplitude reduction was quantified after carbachol application. D, paired-pulse ratio was evaluated as the ratio between the second and the first response. Data are mean ± SEM; One-way ANOVA followed by Bonferroni correction(C) and paired T-Test (D). *P> 0.05. n ≥ 8 per condition.

3. Feedforward PV$^+$ IN are modulated by M3 activation

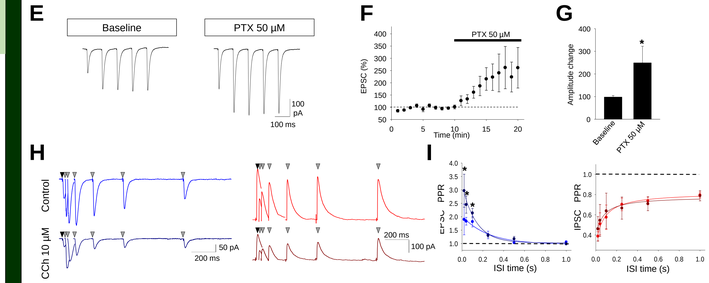


A, fluorescent image of PV$^+$ IN in the hippocampus. B, reconstruction of a successfully recorded PV$^+$ IN. C, current step application to a PV$^+$ IN shows multiple spikes. D, schematic drawing of the experimental approach. E, EPSC traces recorded from PV$^+$ IN before and after carbachol application, compare with those where M3 antagonist (DAU 5584 1 µM) was infused (dark green). F, amplitude reduction was quantified after carbachol application. D, paired-pulse ratio was evaluated as the ratio between the second and the first response. Data are mean ± SEM; One-way ANOVA followed by Bonferroni correction(F) and paired T-Test (G). *P> 0.05. n ≥ 6 per condition.

4. Carbachol produces an increase of excitatory-inhibitory ratio at CA1 pyramidal neurons



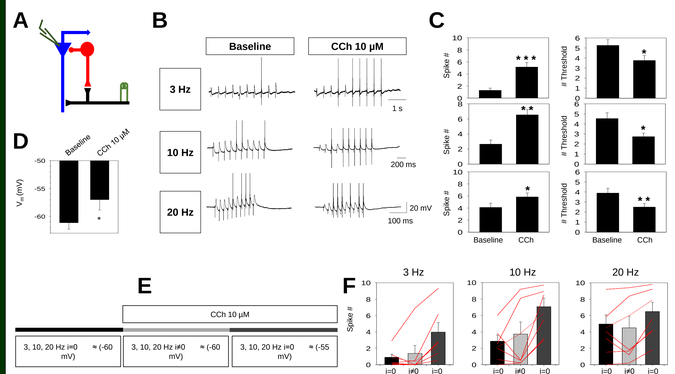
A, schematic drawing of the experimental approach. B, IPSC (red) and EPSC (blue) recorded example traces and charge transfer quantification before (left) and after CCh 10 µM (right) application from the same neuron. C, charge transfer normalization to each first response before (red or blue light colour) and after CCh 10µM (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 10 µM (grey) application.



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

H, Composition of evoked EPSC (blue, left) and IPSC (red, right) recorded at different inter stimulus interval (ISI). I, 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< 0.01; *P< 0.05. n > 5 per condition.

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



A, schematic drawing of the experimental approach. B, 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. C, quantification of spike number (spike #) and spike threshold (# Threshold).

D, CCh application depolarizes membrane potential.

E, schematic diagram showing the experimental procedure. 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. F, spike number quantification at different frequencies during previously described three experimental conditions. Paired 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.