SYMPOSIUM REVIEW

Cholinergic circuit modulation through differential recruitment of neocortical interneuron types during behaviour

Rogier B. Poorthuis, Leona Enke and Johannes J. Letzkus
Max Planck Institute for Brain Research, 60438 Frankfurt, Germany

Abstract Acetylcholine is a crucial neuromodulator for attention, learning and memory. Release of acetylcholine in primary sensory cortex enhances processing of sensory stimuli, and many in vitro studies have pinpointed cellular mechanisms that could mediate this effect. In contrast, how cholinergic modulation shapes the function of intact circuits during behaviour is only beginning to emerge. Here we review recent data on the recruitment of identified interneuron types in neocortex by cholinergic signalling, obtained with a combination of genetic targeting of cell types, two-photon imaging and optogenetics. These results suggest that acetylcholine release during basal forebrain stimulation, and during physiological recruitment of the basal forebrain, can strongly and rapidly influence the firing of neocortical interneurons. In contrast to the traditional view of neuromodulation as a relatively slow process, cholinergic signalling can thus rapidly convey time-locked information to neocortex about the behavioural state of the animal and the occurrence of salient sensory stimuli. Importantly, these effects strongly depend on interneuron type, and different interneuron types in turn control distinct aspects of circuit function. One prominent effect of phasic acetylcholine release is disinhibition of pyramidal neurons, which can facilitate sensory processing and associative learning.

(Received 4 March 2014; accepted after revision 19 May 2014; first published online 30 May 2014)

Corresponding author J. J. Letzkus: Max Planck Institute for Brain Research, 60438 Frankfurt, Germany.
Email: johannes.letzkus@brain.mpg.de

Abbreviations PV, parvalbumin; SOM, somatostatin; VIP, vasoactive intestinal polypeptide.

Introduction

The function of neuronal circuits is flexible and continuously adjusted to current behavioural requirements (Bargmann, 2012), a process in which neuromodulators are critically involved (Lee & Dan, 2012). Acetylcholine is an essential neuromodulator for the coordination of behavioural state, arousal, attention and learning and memory (Sarter & Bruno, 1997). The main source of neocortical acetylcholine derives from

Rogier B. Poorthuis obtained his PhD from the VU University Amsterdam, where he investigated modulation of prefrontal cortical circuits by nicotinic acetylcholine receptors and nicotine, and subsequently joined the Letzkus lab. During his PhD at the John Curtin School of Medical Research (Canberra, Australia), Johannes J. Letzkus examined dendritic and axonal mechanisms of neocortical information processing. As a postdoc and subsequently an independent fellow at the Friedrich Miescher Institute for Biomedical Research (Basel, Switzerland), he started to analyze the circuit mechanisms of associative learning in cortex. In 2013 he moved to the Max Planck Institute for Brain Research (Frankfurt, Germany), where he is currently an independent group leader. The lab focuses on the mechanisms and consequences of neocortical circuit modulation by behavioral context, combining cell-type specific recording and activity perturbation approaches with learning and attention tasks.

R. B. Poorthuis and L. Enke contributed equally to this work.

This review was presented at the symposium Synaptic properties and functional consequences of cholinergic transmission in the CNS, which took place at the annual meeting of the Society for Neuroscience, San Diego, CA, USA on 10 November 2013.

the basal forebrain, which sends diffuse projections throughout the cortical mantle (Woolf, 1991). Acetylcholine release from these projections over long timescales (minutes to hours) facilitates attentional processes and detection of rare stimuli (Parikh et al. 2007; Sarter et al. 2009; Paolone et al. 2012). On shorter timescales basal forebrain neurons are activated upon presentation of novel sensory information (Wilson & Rolls, 1990b; Miranda et al. 2000) and encode the salience of sensory stimuli (Richardson & DeLong, 1990; Wilson & Rolls, 1990a). In sensory cortex, acetylcholine enhances neuronal responses to relevant stimuli, decreases trial-to-trial variability and induces gamma oscillations (Reynolds & Chelazzi, 2004; Herrero et al. 2008; Fries, 2009; Goard & Dan, 2009; Harris & Thiele, 2011). However, how these effects are produced mechanistically in the local circuit is only beginning to emerge.

Cortical circuits are composed of local inhibitory interneurons and excitatory pyramidal cells, which project to long-range target areas. The effects of acetylcholine on pyramidal neurons have been investigated in great detail, and include direct depolarization and reduction of cortico-cortical input by muscarinic acetylcholine receptor activation, as well as enhancement of thalamo-cortical input via nicotinic receptors (McCormick & Prince, 1985; Gil et al. 1997; Kimura & Baughman, 1997; Disney et al. 2007; Kawai et al. 2007). The combination of these effects is thought to increase the signal-to-noise ratio for feed-forward thalamic input, thereby enhancing sensory processing during learning and attention (Hasselmo & Giocomo, 2006).

In addition to these direct effects of acetylcholine, activity of pyramidal neurons is also under tight control of a diverse set of inhibitory interneurons. Different interneuron types control distinct aspects of pyramidal cell function, and thereby exert control over a broad repertoire of circuit functions (Isaacson & Scanziani, 2011; Kepecs & Fishell, 2014). Importantly, studies in brain slices have shown that different interneuron types also display differential responses to acetylcholine (Bacci et al. 2005). Fast-spiking parvalbumin-positive (PV) interneurons targeting the perisomatic region of pyramidal cells for the most part do not display cholinergic responses (Kawaguchi, 1997; Xiang et al. 1998; Gulledge et al. 2007; Kruglikov & Rudy, 2008). In contrast, dendrite-targeting interneurons identified by somatostatin (SOM) expression can be depolarized by activation of muscarinic receptors (Porter et al. 1999; Fanselow et al. 2008). In cortical layers 2–6, a group of interneurons expressing 5HT1A receptors is depolarized by acetylcholine acting on nicotinic receptors (Gulledge et al. 2007; Lee et al. 2010; Poorthuis et al. 2013). A subpopulation of these neurons co-expresses vasoactive intestinal polypeptide (VIP; Porter et al. 1999; Lee et al. 2010) and preferentially targets other interneurons (David et al. 2007; Pfeffer et al. 2013; Pi et al. 2013). Finally, all interneurons located in cortical layer 1 are depolarized from rest by nicotinic receptors (Christophe et al. 2002; Letzkus et al. 2011; Arroyo et al. 2012). This changes during ongoing firing, when acetylcholine leads to an inhibition in layer 1 neurogliaform cells that in turn target pyramidal neurons (Brombas et al. 2014). In contrast, single-bouquet cells which preferentially target deeper layer interneurons (Jiang et al. 2013) display activation by acetylcholine under these conditions.

These in vitro studies suggest that differential modulation of interneuron types may be an important mechanism by which acetylcholine affects cortical computation. While the sparseness and heterogeneity of interneurons has for a long time hampered their detailed investigation in vivo, recently developed genetic approaches for cell-type targeting (Taniuchi et al. 2011), combined with two-photon imaging (Kerr & Denk, 2008) and optogenetics (Zhang et al. 2007) now provide the requisite tools for these experiments. Here, we review the available recent findings on how different types of neocortical interneurons are recruited by the cholinergic basal forebrain in vivo, and on how these effects combine to affect information processing in pyramidal neurons during behaviour.

Differential recruitment of neocortical interneuron types by basal forebrain stimulation

To address the effects of acetylcholine release on different genetically identified neuron types, Alitto & Dan (2013) combined electrical stimulation of the basal forebrain with two-photon calcium imaging in the superficial layers of visual cortex in anaesthetized mice (Fig. 1A). This approach revealed time-locked responses in a subset of neurons, suggesting that acetylcholine is able to rapidly modulate the firing rate of cells in visual cortex (Fig. 1B–C). Importantly, both the incidence and the polarity of these responses depended on cell-type (Fig. 1D and E). Only a small fraction of pyramidal neurons displayed activation via muscarinic receptors in this study, which is likely to be a direct action of acetylcholine on these cells (cf. McCormick & Prince, 1985; Gulledge et al. 2007). In contrast, the vast majority of both VIP and layer 1 interneurons were strongly activated by acetylcholine (Fig. 1D). In agreement with previous observations, layer 1 interneuron responses were abolished by nicotinic receptor antagonists (Fig. 1E; Christophe et al. 2002; Letzkus et al. 2011), while VIP interneurons showed a mixed activation profile that was partially blocked by both nicotinic and muscarinic receptor antagonists (Kawaguchi, 1997; Porter et al. 1999). Importantly, both single-bouquet cells in layer 1 and VIP interneurons preferentially contact other interneurons (Christophe et al. 2002; David et al. 2007; Letzkus et al. 2011; Jiang et al. 2013; Pfeffer et al. 2013; Pi et al.
suggesting that the net effect of their activation by acetylcholine is a reduction of ongoing inhibition in the circuit. Consistent with this notion, the majority of PV interneurons that responded to basal forebrain stimulation were inhibited through a combination of nicotinic and muscarinic effects (Fig. 1E). Since these cells for the most part do not display direct responses to acetylcholine (Kawaguchi, 1997; Guldledge et al. 2007; Kruglikov & Rudy, 2008), the most parsimonious interpretation is indirect inhibition via single-bouquet cells in layer 1 and VIP interneurons (cf. Arroyo et al. 2012). A second response type in PV interneurons was excitation, which was abolished by muscarinic antagonists and was likely to be due to an increased drive from pyramidal neurons. Interestingly, excitation of PV interneurons by acetylcholine was converted to inhibition under block of muscarinic receptors, and inhibition of PV cells was converted to excitation when nicotinic receptors were blocked, suggesting that excitation and inhibition compete in the same population of PV interneurons (Fig. 1E). In contrast to these strong effects, only very few SOM interneurons displayed excitation or inhibition in response to basal forebrain stimulation. Based on recent connectivity data (Pfeffer et al. 2013; Pi et al. 2013), activation of VIP interneurons would be expected to cause inhibition of SOM cells. However, since calcium imaging predominantly captures suprathreshold effects (Kerr & Denk, 2008), it is likely that the low firing rate of SOM interneurons under these conditions may have prevented detection of this inhibition. Indeed, in awake conditions VIP interneurons have been found to inhibit SOM interneurons, a mechanism that is absent under anaesthesia (Lee et al. 2013; Fu et al. 2014).

In conclusion, these data demonstrate that neocortical acetylcholine release elicits rapid, reliable and distinct responses in several types of interneurons. Given that different interneuron types control distinct aspects of pyramidal cell function, this is likely to be an important
and computationally rich mechanism by which acetylcholine modulates information processing in neocortical circuits.

**Cholinergic recruitment of neocortical interneuron types during locomotion**

While these data suggest that acetylcholine can exert diverse and cell-type specific control over cortical circuits, only experiments in relation to behavioural functions can directly address whether and when these mechanisms are recruited during physiological activation of the basal forebrain. One factor that strongly influences sensory processing is the behavioural state of the animal, which spans a wide range from sleep and quiet wakefulness to active episodes (Niell & Stryker, 2010; Gentet et al. 2011). Recent evidence suggests that locomotion strongly enhances sensory responses in visual cortex relative to quiet wakefulness (Niell & Stryker, 2010), an effect accompanied by changes in the excitation–inhibition balance (Bennett et al. 2013). Fu et al. (2014) investigated the circuit mechanisms underlying this effect using two-photon calcium imaging of genetically identified interneurons in the superficial layers of visual cortex while the mouse was free to run on a trackball (Fig. 2A). They observed that running episodes caused a marked and sustained increase in VIP interneuron activity (Fig. 2B), whereas unidentified neurons displayed no such correlation. Interestingly, VIP interneurons in barrel and auditory cortex displayed a similar albeit less pronounced modulation. Blockade of nicotinic receptors strongly attenuated the increase in VIP interneuron activity in visual cortex during locomotion (cf. Porter et al. 1999), while block of AMPA receptors had no effect (Fig. 2C and D), indicating that the basal forebrain is a major source of locomotion-induced alterations in cortical state. Consistent with strong connectivity between VIP and SOM interneurons (Pfeffer et al. 2013; Pi et al. 2013), running was associated with a reduction in SOM interneuron activity, whereas PV interneurons displayed heterogeneous modulation. These results suggest that one prominent effect of locomotion could be disinhibition of pyramidal neurons.

To directly address how VIP interneuron activation influences visual processing, the channelrhodopsin variant ChETA (Gunaydin et al. 2010) was introduced into these cells. In line with a disinhibitory role of VIP interneurons, responses of unidentified neurons to visual stimulation were enhanced during VIP interneuron activation in stationary animals (Fig. 2E and F). Moreover, optogenetic activation of VIP interneurons caused no change in orientation selectivity, similar to the effect of locomotion (Niell & Stryker, 2010). Ablation of local VIP interneurons, on the other hand, strongly attenuated circuit modulation by locomotion, indicating that VIP interneurons are both necessary and sufficient for regulating the gain of visual responses during running (Fu et al. 2014).

These data demonstrate that acetylcholine release during running rapidly recruits VIP interneurons to increase the gain of sensory responses in pyramidal neurons through disinhibition, which in turn might underlie enhanced behavioural performance in a visual detection task during locomotion (Bennett et al. 2013). This interpretation is also in line with a recent study showing that rapid and local enhancement of cholinergic signalling by stimulating basal forebrain fibres in the visual cortex improves visual perception (Pinto et al. 2013).

**Cholinergic recruitment of neocortical interneuron types during learning**

In addition to behavioural state, several forms of associative memory critically depend on the cholinergic system (e.g. Hasselmo, 2006; Weinberger, 2007). Recent research has addressed the circuit mechanisms of fear learning in auditory cortex (Letzkus et al. 2011). Auditory fear conditioning is a simple form of associative learning acquired by temporal coincidence of a neutral auditory stimulus with a mild foot-shock (LeDoux, 2000). To understand the mechanisms mediating convergence of these two stimuli, Letzkus and colleagues asked whether the foot-shock alone elicits responses in auditory cortex (Fig. 3). Two-photon calcium imaging revealed that while, on average, foot-shocks caused no response in the neuronal population in layer 2/3, many layer 1 interneurons were strongly activated. Targeted cell-attached recordings showed that foot-shocks elicit a strong increase in firing in approximately 75% of layer 1 interneurons, whereas the remainder displayed long-lasting inhibition (Fig. 3A–C). Surprisingly, the excitatory foot-shock response was virtually independent of glutamatergic transmission, but completely blocked by nicotinic antagonists (Fig. 3D). In agreement with this pharmacology, microstimulation of the cholinergic basal forebrain evoked strong excitation of layer 1 interneurons similar to foot-shocks (Fig. 3E). Finally, excitatory foot-shock responses were also observed in layer 1 interneurons in primary visual cortex (cf. Alitto & Dan, 2013), a finding paralleled by the observation that locomotion leads to cholinergic activation of VIP interneurons in several cortical areas (see above, Fu et al. 2014), and consistent with the diffuse nature of projections from the basal forebrain. These data indicate that foot-shocks rapidly recruit the basal forebrain to activate a large percentage of neocortical layer 1 interneurons through nicotinic receptors. The latency of cholinergic activation (50–60 ms for foot-shocks; 10–20 ms for basal forebrain microstimulation) approaches the speed of conventional synaptic transmission, consistent with recent experiments
Figure 2. Cholinergic recruitment of VIP interneurons during locomotion

A, diagram displaying the experimental setup. A head-fixed mouse is free to run on a Styrofoam ball floating on air, while two-photon calcium imaging is performed in primary visual cortex. 

B, example traces showing running speed of the animal (bottom) and correlated activity of a VIP interneuron (top).

C, cross-correlation (mean ± SEM) between running speed and the calcium response of VIP interneurons in control (red), and after blockade of nicotinic (orange) and AMPA receptors (blue). 

D, the zero-time cross-correlation (mean ± SEM) between running and VIP interneuron activity is strongly reduced by block of nicotinic acetylcholine receptors, but not by block of glutamatergic receptors.

E, orientation tuning of an unidentified unit in control (No LED, green), and during optogenetic activation of VIP interneurons using ChETA (Gunaydin et al. 2010; With LED, blue). Responses were averaged over five presentations of moving bars.

F, average light-induced modulation of sensory responses in control mice (No Ch ETA), and in mice expressing Ch ETA in VIP interneurons. Adapted with permission from Fu et al. 2014.
Figure 3. Foot-shock modulation of auditory cortex circuits

A, schematic illustration of experimental setup. Foot-shock (yellow) responses are measured using two-photon targeted loose-seal cell-attached recordings. B, schematic of different cell types under investigation. Colour code corresponds to data in panel C. Ca, population response of layer 1 interneurons that are excited during foot-shocks (77%). Cb, inhibition of a smaller group of layer 1 interneurons (23%). Cc, firing of PV interneurons in layer 2/3 is inhibited by foot-shocks. Cd, whole-cell recordings of pyramidal neurons reveal a reduction of inhibitory postsynaptic currents (IPSCs) during foot-shock presentation. D, local block of nicotinic receptors (grey) strongly reduces foot-shock responses in layer 1 interneurons, while local block of AMPA receptors (black) leaves the response intact. E, foot-shock activation of layer 1 interneurons is mimicked by basal forebrain stimulation. F, two-photon population imaging shows that foot-shocks significantly boost sensory responses to tone presentation in layer 2/3 of auditory cortex. Adapted with permission from Letzkus et al. 2011.

© 2014 The Authors. The Journal of Physiology © 2014 The Physiological Society
employing optogenetic stimulation of basal forebrain axons in vitro (Bennett et al. 2012). Thus, on top of its well-established slow mode of action, acetylcholine can also convey rapid and time-locked information on the occurrence of salient sensory stimuli to neocortex (Parikh et al. 2007; Sarter et al. 2009; Paolone et al. 2012).

How do cholinergic responses in layer 1 interneurons in turn affect processing of sensory information in auditory cortex? Single bouquet cells in layer 1 preferentially target deeper layer interneurons, including PV interneurons (Christophe et al. 2002; Letzkus et al. 2011; Arroyo et al. 2012; Jiang et al. 2013; Lee et al. 2014). Consistent with this, we observed that foot-shocks cause a strong inhibition of firing in PV interneurons in both anaesthetized and freely behaving animals (Fig. 3C). In turn, this led to a marked disinhibition of pyramidal neurons during and after the foot-shock (Fig. 3D). A second source of disinhibition may derive from direct cholinergic inhibition of ongoing firing in layer 1 neurogliaform cells, which has recently been demonstrated in vitro (Brombas et al. 2014). Thus, the population of foot-shock inhibited layer 1 interneurons may correspond to neurogliaform cells, which provide strong direct inhibition to pyramidal neurons in layer 2/3 and layer 5 (Chu et al. 2003; Jiang et al. 2013; Brombas et al. 2014; Lee et al. 2014). Interestingly, rapid recruitment by aversive stimuli has also been observed for VIP interneurons in auditory cortex (Pi et al. 2013). Given that VIP interneurons express cholinergic receptors (Porter et al. 1999), and can be fired by acetylcholine in vivo (Alitto & Dan, 2013; Fu et al. 2014), it seems plausible that their activation during aversive stimulation is mediated by acetylcholine, although this was not assessed directly. Taken together, these data demonstrate that one prominent network effect of phasic acetylcholine release during aversive stimulation is disinhibition of pyramidal neurons via several parallel pathways.

Disinhibition of pyramidal neurons strongly boosts their responses to concomitantly presented auditory stimuli in both anaesthetized and freely behaving mice (Fig. 3F; cf. Froemke et al. 2007). This period of enhanced firing is likely to induce synaptic plasticity that may underlie the memory trace. A key prediction from this interpretation is that auditory cortex disinhibition during the foot-shock is required for learning at the behavioural level. To address this directly, we introduced channelrhodopsin-2 into PV interneurons, and used optogenetic activation of these neurons during the foot-shock to counteract the observed inhibition (Fig. 4A–C). This was performed in freely behaving mice during fear conditioning to complex auditory stimuli, which have been proposed to require auditory cortex for learning (LeDoux, 2000). When the memory of these animals was retrieved on the next day without optogenetic intervention, they displayed strongly reduced freezing in response to the conditioned stimulus (Fig. 4D), directly demonstrating that auditory cortex disinhibition during the foot-shock is crucial for fear learning.

Summary and outlook

The studies reviewed here have advanced our understanding of the mechanisms by which acetylcholine modulates cortical circuit function during behaviour in several ways. First, they clearly demonstrate that interneurons are a prominent target of cholinergic modulation. The effects of acetylcholine, as predicted from slice work, strongly depend on interneuron type. In addition to the

---

**Figure 4. Auditory cortex disinhibition is required for fear learning**

*Panel A*: stimulation of ChR-2 expressing PV interneurons (green) in auditory cortex (red) via an optic fibre (blue). *Panel B*: schematic illustration of optogenetic manipulation in freely behaving mice. *Panel C*: differential fear conditioning protocol using frequency-modulated sweeps as conditioned stimuli (CS) with optogenetic stimulation during and for 5 s after every foot-shock. *Panel D*: freezing in a novel context without laser stimulation 1 day after conditioning. Compared to identically treated sham injected littermates (black), virus-injected mice (blue) exhibit drastically reduced freezing to the CS+. Reconditioning without optogenetic stimulation yielded strongly enhanced freezing (red) that was indistinguishable from sham. Grey background: CS–; green background: CS+. Adapted with permission from Letzkus et al. 2011.
direct actions of acetylcholine, these in vivo studies have also identified strong indirect effects, such as the inhibition of PV and SOM interneurons by layer 1 and VIP interneurons (Letzkus et al. 2011; Alitto & Dan, 2013; Pi et al. 2013; Fu et al. 2014). Second, in contrast to the traditional view of neuromodulation as a relatively slow process, these results show that cholinergic signalling can rapidly fire certain interneuron types, thereby conveying time-locked information to neocortex (Letzkus et al. 2011; Arroyo et al. 2012; Alitto & Dan, 2013; Fu et al. 2014). Very similar data have recently been reported from the hippocampus, where aversive air puffs rapidly fire SOM interneurons through acetylcholine release from medial septum afferents (Lovett-Barron et al. 2014). Third, the interneuron types displaying the strongest excitation by acetylcholine are layer 1 and VIP interneurons, which in turn preferentially contact other interneurons (Letzkus et al. 2011; Alitto & Dan, 2013; Pi et al. 2013; Fu et al. 2014). This suggests that one important network effect of phasic acetylcholine release is disinhibition of pyramidal neurons. A parallel disinhibitory mechanism that has been described in vitro is mediated by activation of muscarinic receptors on presynaptic PV interneuron terminals to directly inhibit GABA release (Fukudome et al. 2004; Kruglikov & Rudy, 2008; Szabo et al. 2010), and an interesting question for future experiments is under which conditions this occurs in vivo. Disinhibition is an attractive mechanism to increase sensory responses during learning and active behavioural states since it is permissive for strong activation of pyramidal neurons by sensory input, but does not cause firing in itself. In addition, disinhibition can increase the gain of pyramidal neuron responses without affecting stimulus selectivity (Fu et al. 2014).

In conclusion, differential recruitment of distinct interneuron types is emerging as an important mechanism through which acetylcholine can adjust the function of neocortical circuits according to current behavioural requirements. Interestingly, several of the interneuron types discussed here are also responsive to other neuromodulators such as serotonin (Ferezou et al. 2002; Foehring et al. 2002; Lee et al. 2010). Important open questions are therefore whether other neuromodulators are capable of mediating similarly rapid and specific recruitment of interneurons (cf. Varga et al. 2009), and how the effects of different modulators combine to shape the function of neocortical circuits.

References


© 2014 The Authors. The Journal of Physiology © 2014 The Physiological Society


Additional information

Competing interests
None declared.

Funding
Our research is supported by the Max Planck Society, the European Research Council, the Swiss National Science Foundation, the Netherlands Organization for Scientific Research (NWO Rubicon, 825.13.015) and the Boehringer Ingelheim Fonds.