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Cholinergic enhancement of perceptual learning: behavioral, physiological, and neuro-pharmacological study in the rat primary visual cortex

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Cholinergic enhancement of perceptual learning: behavioral, physiological, and neuro-pharmacological study in the rat primary visual cortex

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Résumé

Les cortices sensoriels sont des régions cérébrales essentielles pour la perception. En particulier, le cortex visuel traite l'information visuelle en provenance de la rétine qui transite par le thalamus. Les neurones sont les unités fonctionnelles qui transforment l'information sensorielle en signaux électriques, la transfèrent vers le cortex et l'intègrent. Les neurones du cortex visuel sont spécialisés et analysent différents aspects des stimuli visuels. La force des connections entre les neurones peut être modulée par la persistance de l'activité pré-synaptique et induit une augmentation ou une diminution du signal post-synaptique à long terme. Ces modifications de la connectivité synaptique peuvent induire la réorganisation de la carte corticale, c'est à dire la représentation de ce stimulus et la puissance de son traitement cortical. Cette réorganisation est connue sous le nom de plasticité corticale. Elle est particulièrement active durant la période de développement, mais elle s'observe aussi chez l'adulte, par exemple durant l'apprentissage. Le neurotransmetteur acétylcholine (ACh) est impliqué dans de nombreuses fonctions cognitives telles que l'apprentissage ou l'attention et il est important pour la plasticité corticale. En particulier, les récepteurs nicotiniques et muscariniques du sous-type M1 et M2 sont les récepteurs cholinergiques impliqués dans l'induction de la plasticité corticale.

L'objectif principal de la présente thèse est de déterminer les mécanismes de plasticité corticale induits par la stimulation du système cholinergique au niveau du télencéphale basal et de définir les effets sur l'amélioration de la perception sensorielle.

Afin d'induire la plasticité corticale, j'ai jumelé des stimulations visuelles à des injections intracorticales d'agoniste cholinergique (carbachol) ou à une stimulation du télencéphale basal (neurones cholinergiques qui innervent le cortex visuel primaire). J'ai analysé les potentiels évoqués visuels (PEVs) dans le cortex visuel primaire des rats pendant 4 à 8 heures après le couplage. Afin de préciser l'action de l'ACh sur l'activité des PEVs dans V1, j'ai injecté individuellement l'antagoniste des récepteurs muscariniques, nicotiniques, α7 ou NMDA avant l'infusion de carbachol. La stimulation du système cholinergique jumelée avec une stimulation visuelle augmente l'amplitude des PEVs durant plus de 8h. Le blocage des

récepteurs muscarinique, nicotinique et NMDA abolit complètement cette amélioration, tandis que l'inhibition des récepteurs α7 a induit une augmentation instantanée des PEVs. Ces résultats suggèrent que l'ACh facilite à long terme la réponse aux stimuli visuels et que cette facilitation implique les récepteurs nicotiniques, muscariniques et une interaction avec les récepteur NMDA dans le cortex visuel. Ces mécanismes sont semblables à la potentiation à long-terme, évènement physiologique lié à l'apprentissage.

L'étape suivante était d'évaluer si l'effet de l'amplification cholinergique de l'entrée de l'information visuelle résultait non seulement en une modification de l'activité corticale mais aussi de la perception visuelle. J'ai donc mesuré l'amélioration de l'acuité visuelle de rats adultes éveillés exposés durant 10 minutes par jour pendant deux semaines à un stimulus visuel de type «réseau *sinusoïdal*» couplé à une stimulation électrique du télencéphale basal. L'acuité visuelle a été mesurée avant et après le couplage des stimulations visuelle et cholinergique à l'aide d'une tâche de discrimination visuelle. L'acuité visuelle du rat pour le stimulus d'entrainement a été augmentée après la période d'entrainement. L'augmentation de l'acuité visuelle n'a pas été observée lorsque la stimulation visuelle seule ou celle du télencéphale basal seul, ni lorsque les fibres cholinergiques ont été lésées avant la stimulation visuelle. Une augmentation à long terme de la réactivité corticale du cortex visuel primaire des neurones pyramidaux et des interneurones GABAergiques a été montrée par l'immunoréactivité au c-Fos. Ainsi, lorsque couplé à un entrainement visuel, le système cholinergique améliore les performances visuelles pour l'orientation et ce probablement par l'optimisation du processus d'attention et de plasticité corticale dans l'aire V1.

Afin d'étudier les mécanismes pharmacologiques impliqués dans l'amélioration de la perception visuelle, j'ai comparé les PEVs avant et après le couplage de la stimulation visuelle/cholinergique en présence d'agonistes/antagonistes sélectifs. Les injections intracorticales des différents agents pharmacologiques pendant le couplage ont montré que les récepteurs nicotiniques et M1 muscariniques amplifient la réponse corticale tandis que les récepteurs M2 muscariniques inhibent les neurones GABAergiques induisant un effet excitateur. L'infusion d'antagoniste du GABA corrobore l'hypothèse que le système inhibiteur est essentiel pour induire la plasticité corticale. Ces résultats démontrent que l'entrainement

visuel jumelé avec la stimulation cholinergique améliore la plasticité corticale et qu'elle est contrôlée par les récepteurs nicotinique et muscariniques M1 et M2.

Mes résultats suggèrent que le système cholinergique est un système neuromodulateur qui peut améliorer la perception sensorielle lors d'un apprentissage perceptuel. Les mécanismes d'amélioration perceptuelle induits par l'acétylcholine sont liés aux processus d'attention, de potentialisation à long-terme et de modulation de la balance d'influx excitateur/inhibiteur. En particulier, le couplage de l'activité cholinergique avec une stimulation visuelle augmente le ratio de signal / bruit et ainsi la détection de cibles. L'augmentation de la concentration cholinergique corticale potentialise l'afférence thalamocorticale, ce qui facilite le traitement d'un nouveau stimulus et diminue la signalisation cortico-corticale minimisant ainsi la modulation latérale. Ceci est contrôlé par différents sous-types de récepteurs cholinergiques situés sur les neurones GABAergiques ou glutamatergiques des différentes couches corticales.

La présente thèse montre qu'une stimulation électrique dans le télencéphale basal a un effet similaire à l'infusion d'agoniste cholinergique et qu'un couplage de stimulations visuelle et cholinergique induit la plasticité corticale. Ce jumelage répété de stimulations visuelle/cholinergique augmente la capacité de discrimination visuelle et améliore la perception. Cette amélioration est corrélée à une amplification de l'activité neuronale démontrée par immunocytochimie du c-Fos. L'immunocytochimie montre aussi une différence entre l'activité des neurones glutamatergiques et GABAergiques dans les différentes couches corticales. L'injection pharmacologique pendant la stimulation visuelle/cholinergique suggère que les récepteurs nicotiniques, muscariniques M1 peuvent amplifier la réponse excitatrice tandis que les récepteurs M2 contrôlent l'activation GABAergique. Ainsi, le système cholinergique activé au cours du processus visuel induit des mécanismes de plasticité corticale et peut ainsi améliorer la capacité perceptive. De meilleures connaissances sur ces actions ouvrent la possibilité d'accélérer la restauration des fonctions visuelles lors d'un déficit ou d'amplifier la fonction cognitive.

Mots-clés : électrophysiologie, système cholinergique, amélioration cognitive, plasticité corticale, récepteur nicotinique, récepteur muscarinique, apprentissage perceptuel, cortex visuelle

Abstract

Sensory cortex is an essential area where sensory perception occurs. Especially visual cortex processes visual information transmitted from the retina through the thalamus. By different neuronal activation the information is segregated and sent to diverse visual area for interpretation. Neurons are the basic unit that transform sensory information into electrophysiological signal, transfer to the cortex and integrate it. Connection between neurons can be modulated depending on the persistent presynaptic activity inducing either a long-term increase or decrease of the post-synaptic activity. Modification in synaptic strength can affect large area and induce reorganization of cortical map (i.e. cortical plasticity) which changes the representation of the visual stimulus and its weight in visual processing. Cortical plasticity can occur during juvenile while forming developmental connection or in adult while acquiring novel information (i.e. learning). The neurotransmitter ACh is involved in many cognitive functions, such as learning or attention and it was demonstrated that lesioning or blocking cholinergic system diminishes cortical plasticity. It was shown that nicotinic, M1 subtype and M2 subtype muscarinic receptors are the major cholinergic receptors abundant in the cortex and implicated during cortical plasticity induction.

In a first part, I analyzed visual evoked potentials (VEPs) in V1 of rats during a 4-8h period after coupling visual stimulation to an intracortical injection of ACh agonist carbachol or stimulation of basal forebrain. To clarify the action of ACh on VEP activity in V1, we individually injected muscarinic, nicotinic, α7, and NMDA receptor antagonists just before carbachol infusion. Stimulation of the cholinergic system paired with visual stimulation significantly increased VEP amplitude for long-term. Pre-inhibition of muscarinic, nicotinic and NMDA receptor completely abolished this long-term enhancement, while α7 inhibition induced an instant increase of VEP amplitude. This suggests a role of ACh in facilitating visual stimuli responsiveness which involves nicotinic and muscarinic receptors with an interaction of NMDA transmission in the visual cortex. These mechanisms were similar to long-term potentiation, a neurobiological mechanism of learning.

In a second step, I evaluate whether cholinergic modulation of visual neurons results in cortical activity and visual perception changes. Awake adult rats were exposed repetitively for two weeks to an orientation-specific grating with coupling visual stimulation to an electrical stimulation of the basal forebrain. The visual acuity, as measured using a visual water maze before and after coupling visual/cholinergic stimulation was increased. The increase in visual acuity was not observed when visual or basal forebrain stimulation was performed separately nor when cholinergic fibers were selectively lesioned prior to the visual stimulation. There was a long-lasting increase in cortical reactivity of the primary visual cortex shown by c-Fos immunoreactivity of both pyramidal and GABAergic interneuron. These findings demonstrate that when coupled with visual training, the cholinergic system improves visual performance for the trained orientation probably through enhancement of attentional processes and cortical plasticity in V1 related to the ratio of excitatory/inhibitory inputs.

Finally, I also investigated the different pharmacological mechanisms involved in the visual enhancement. Pre- and post-pairing visual/cholinergic stimulation VEP were compared with selective administered agonist/antagonist during the pairing. Awaken adult rats were exposed during 10 minutes per day for 1 week to an orientation specific grating with an electrical stimulation of the basal forebrain. Intracortical injection of different pharmacological agents during pairing demonstrated that nicotinic and M1 muscarinic receptors are used to amplify cortical response while M2 muscarinic receptor suppresses GABAergic neurons to disinhibit excitatory neurons. Infusion of GABAergic antagonist supported that inhibitory system is crucial to induce cortical plasticity. These findings demonstrate that visual training coupled with the cholinergic stimulation enhances the cortical plasticity mediated by nicotinic, M1 and M2 muscarinic receptors, which the latter induces a disinhibition by suppressing GABAergic neuron.

The cholinergic system is a potent neuromodulatory system. Boosting this system during perceptual learning robustly enhances the sensory perception. Especially, pairing a cholinergic activation with a visual stimulation increases the signal-to-noise ratio, cue detection ability in the primary visual cortex. This cholinergic enhancement increases the strength of thalamocortical afferent to facilitate the treatment of a novel stimulus while decreasing the

cortico-cortical signaling to minimize recurrent or top-down modulation. This is mediated by different cholinergic receptor subtypes located in both glutamatergic and GABAergic neurons of the different cortical layers. The mechanisms of cholinergic enhancement are closely linked to attentional processes, long-term potentiation and modulation of the excitatory/inhibitory balance.

The present thesis shows that electrical stimulation of the basal forebrain has similar effect with cholinergic agonist release and pairing visual/cholinergic stimulation induces cortical plasticity. Repetitive pairing of visual/cholinergic increases visual discrimination capacity and enhances perceptual ability. This enhancement is followed by an augmentation of neuronal activity demonstrated by c-Fos immunohistochemistry. Immunoreactivity also shows difference in glutamatergic and GABAergic neurons activities between layers. Pharmacological injection during visual/cholinergic pairing suggests that nicotinic and M1 muscarinic receptor can amplify excitatory response while M2 receptor controls GABAergic activation. Altogether cholinergic system activated during visual process induces cortical plasticity and can enhance perceptual ability. Further understanding of this training has the potential to accelerate visual recovery or boost cognitive function.

Keywords: electrophysiology, cholinergic system, cognitive enhancement, cortical plasticity, nicotinic receptors, muscarinic receptors, perceptual learning, visual cortex

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Abbreviations

ACh	acetylcholine
AMPA	α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	brain-derived neurotropic factor
BF	basal forebrain
CaMKII	calcium/calmodulin-dependent protein kinases II
CCh	carbachol
ChAT	choline acetyltransferase
CNS	central nervous system
CPD	cycle per degree
CR	calretinin
CRE	cAMP response element
CREB	cAMP response element binding
E/I	excitation/inhibition ratio
ERK	extracellular-signal-regulated kinase
FP	field potential
GABA	γ-amino butyric acid
HDB	horizontal limb of the diagonal band of Broca
LFP	local field potential
LGN	lateral geniculate nucleus
LPZ	lesion projection zone
LTD	long-term depression

LTP	long-term potentiation
M1 mAChR	M1 subtype muscarinic receptor
M2 mAChR	M2 subtype muscarinic receptor
mAChR	muscarinic receptors
Mec	mecamylamine
MLA	methyllycaconitine
nAChR	nicotinic receptors
NMDA	N-methyl-D-aspartate
NSF	N-ethylmaleimide-sensitive factor
OD	ocular dominance
PK	protein kinase
PNS	peripheral nervous system
PSD	power spectral density
PTX	picrotoxin
PV	parvalbumin
PZP	pirenzepine
RBPC	rat brain pyramidal cell marker
RF	receptive field
Sch	Schaffer-commissural
sco	scopolamine
SOM	somatostatin
STDP	spike-timing dependent plasticity
TBS	theta-burst stimulation

V1	primary visual cortex
VEP	visual evoked potential
VS	visually stimulated rats
VS/HDB	visual stimulation paired with electric stimulation in HDB

I dedicate this thesis to GOD in heaven.

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CHAPTER I: General Introduction

I.1 Prologue

Pablo Picasso once said “If only we could pull out our brain and paint using only our eyes.” Although nowadays it is a common sense that without the brain we could not see anything, the famous painter probably wanted to describe the world without brain’s interpretation. As matter of fact, like a computer that transform electrical signal (e.g. from keyboard) into a letter, the brain interprets the outer information that is transferred from sensory organs so that our body could understand it. Sometimes the information is saved to be retrieved whenever needed or it could permanently affect the whole system. Based on previous and novel data, the process that our body understands the environment through the brain, we call it “perception”. For a living organism perception is critical to survive since based on the information it could make a decision and hence avoid dangerous situation.

The improvement of perception is nominated as perceptual learning since it appears only after repetitive training. This enhancement could occur through life time and recently it was observed that it is possible to accelerate the process by specific training. This study will be focused on the function and mechanism of the brain to increase its perceptual ability by presenting relevant concepts.

I.2 Introduction to the cortical function

I.2.1 Sensory perception

Perception of sensory information is analyzed in a hierarchical manner. Different organs convey different types of information and each sensory modality reaches different

cortical areas according to their specificity. The first cortical area where sensory signals are entered is called the primary sensory cortex. Fingers (somatosensory cortex), eyes (visual), ears (auditory) and nose (olfactory) send signals to their own primary cortex. As the raw information is transferred from lower to higher sensory cortex, it is segregated based on its property (e.g. shape for visual or frequency for auditory) with more precision (Kandel et al., 2013).

Compared to sensory cortices, associative areas process and integrate the information. For example in primate, the ventral intraparietal area in the parietal lobe receives input from visual, somatosensory and auditory senses (Avillac et al., 2005). Also the frontal lobe is suggested to have the ability of planning (Shallice and Burgess, 1991) or motivation (Eslinger and Damasio, 1985). Memory retrieval could occur during the perception by limbic system such as hippocampus and influence the interpretation of the stimulus. Cortical area which receives sensory information from primary sensory cortex and is involved in cognitive function (e.g. attention, learning or planning), it is called higher order sensory area.

The process of sensory perception is bidirectional and changeable. The flow of information from periphery to lower sensory cortex and to higher cortical area is called bottom-up. The reverse action from higher area modulating the response of lower area is called top-down. Top-down effect could influence the selectivity of stimulus in lower cortical area and facilitate the perception of a specific target. Bottom-up and top-down effect occur simultaneously and experience could refine the process. Repetitive exposures to similar environment reduce the process time by strengthening the cortical connection and increase the acuity by expanding the cortical area that treat the information. All those ameliorations of

perception is achieved through perceptual learning. Perceptual learning can influence in global, which means it affects both bottom-up and top-down process from neuronal to systematic change. To understand better how the brain perceive the world, it will be necessary to look into the function of its basic unit; the neuron.

I.2.2 Neurons

I.2.2.1 Neurons anatomy

Neuron is the basic unit of the brain composed of cell bodies (soma), axon and dendrite which transmit information through electrical and chemical signals. The neuron receives outer signal through their dendrites and transfer it to neighboring neuron by the axon (Figure I.1). The junctions between two neurons are called synapses. Generally synapses are established between axons to a dendrite, to another axons or a cell body. Sensory information is transported under form of electrical signal and at the end of the axon it is transferred by releasing chemical molecules (neurotransmitters) from presynaptic neuron delivered by the synaptic vesicles. At the dendritic membrane of neuron receiving neurotransmitters (postsynaptic neuron) there are proteins (receptors) which bind specifically the released neurotransmitters. Receptor, when coupled with ligand-gated channel, opens the ion gate to transfer the electrical signal to the soma.

There are numerous ways to classify neurons (e.g. shape, size, discharge pattern, or function) but probably the simplest way is its action on postsynaptic neurons. Neuron that release neurotransmitters permitting positive ion (Na^+, Ca^{2+}) entrance is called excitatory (e.g. glutamate). Neuron that release neurotransmitters permitting negative ion (Cl^-) (e.g. GABA: γ-

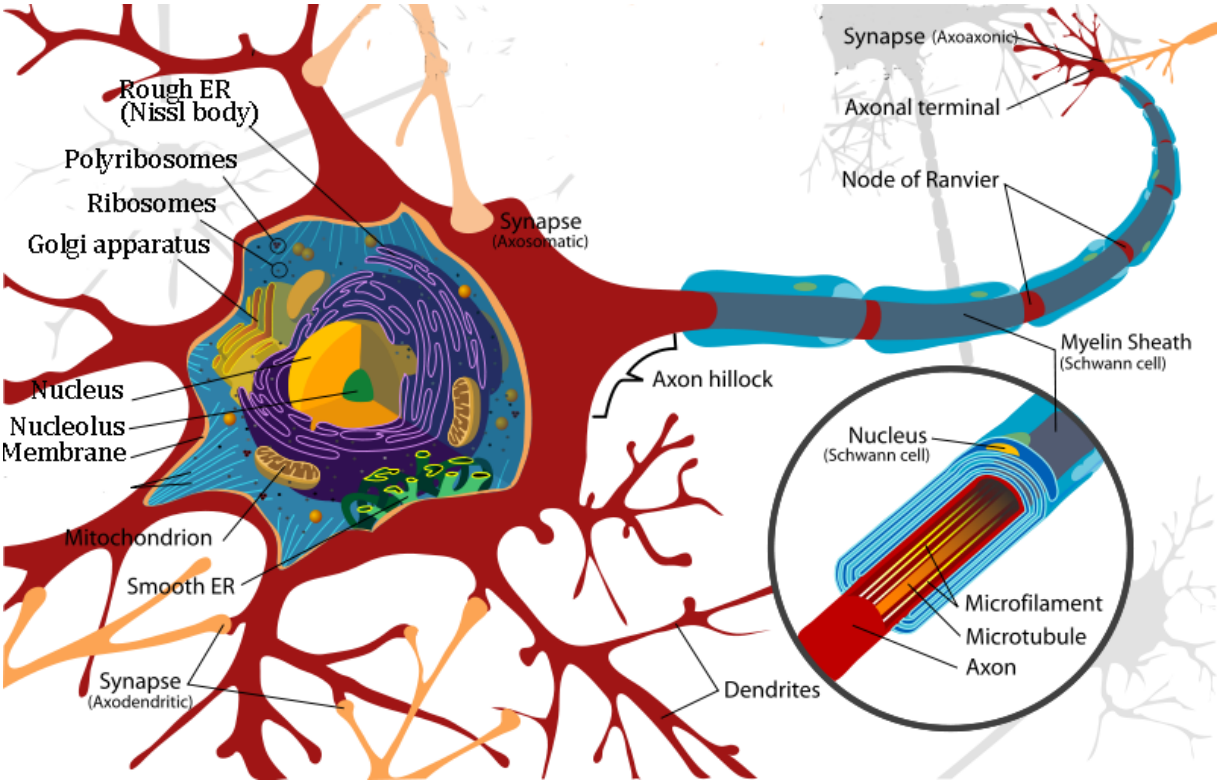

Figure I.1. Structure of a neuron.

The neuron is composed by a soma (cell body), axon and dendrite. Myelin sheath is a phospholipid layer surrounding axon in most of the neurons, although some short projecting or interneurons do not have myelin. Adapted from http://wikimedia.org

amino-butyric-acid) admission is called inhibitory and is generally released by interneuron although spiny stellate cell in sensory cortex is glutamatergic (Kandel et al., 2013). Pyramidal neuron is excitatory neuron that has long axons to send information from one cortical area to another and in contrast interneuron has short axons to locally process signals (Rockland and Lund, 1982).

I.2.2.2 Single Neuronal activity

In general, neurons communicate each other through synaptic transmission which evokes action potential. During the rest state, the extracellular fluid contains excessive positive ion and maintains -75mV of difference (resting potential) between the extracellular and intracellular compartment. This membrane potential is maintained by ionic pump which are constantly pumping out Na+ and intruding K+ ions. Binding of an excitatory neurotransmitter induces conformational change of ionic channel and allows positive ions to enter within the neuron and initiates action potential. An action potential is generally composed of three sequences of events. (1) A depolarization of membrane causes entrance of Na+ through voltage-gated-channel. (2) A repolarization, when the inward Na+ induces an outward of K+ current to repolarize the membrane. (3) And hyperpolarization state by overwhelmed entrance of K+ before voltage-gated K+ channels shut (Hille, 2001). To measure such electro-potential activation Huxley and Hodgkin used voltage clamp where an intracellular electrode measured transmembrane voltage while a current electrode maintained the cell at a constant voltage (Hodgkin and Huxley, 1952). The action potential is said to be all-or-none signal since either it occurs fully independent of the amount of stimulating current or it does not occur at all.

This change of polarity on the membrane by depolarization propagates along the axon until the axon terminal. When the action potential reaches axon terminal or en passant varicosities it opens the voltage-sensitive calcium channels in the presynaptic membrane. This triggers synaptic vesicles filled with neurotransmitter to migrate to the synaptic cleft and release their content (Rusakov, 2006, Suudhof, 2008).

Neuronal activation can also be measured extracellularly. Compared to intracellular method which inserts electrode through the cell membrane, extracellular recording places electrode close to the neuron. By inserting close enough so that a single trans-membrane current (associated with an action potential) dominates the signal, it is possible to isolate the neuronal activity. Although compared to intracellular method (e.g. voltage clamp or patch clamp) this cannot provide information about postsynaptic potentials. Extracellular method can detect neuronal activation without cell damage and for longer period (Boulton et al., 1990). For example, Hubel and Wiesel showed by this method how single unit activities respond to very specific aspects of a visual stimulus (Hubel and Wiesel, 1965).

I.2.2.3 Neuronal activity (Multiple neurons)

The sensation of an external stimulus generally induces a simultaneous activation of a massive number of neurons. Multiple neuronal activations (i.e. multi-unit activities) can be measured either by inserting multiple electrodes or by enlarging electrode tip. With a large tip electrode it is possible to record the sum of multiple action potentials in the range of 50-350μm (Legatt et al., 1980, Gray et al., 1995).

Another method to measure multiple actions potential is the use of local field potentials (LFP). LFP is obtained by recording signals using extracellular low impedance microelectrode

inserted in the cortex. This signal is then filtered at ~300 Hz. With such filtering the action potential near the electrode which activates at high frequency range has less contribution to LFP signal. Compared to the action potentials which are visible only for adjacent electrodes, the synaptic events may be recorded in a distant area through the extracellular space. Moreover, since LFP records multiple activities on a large range, it is less attenuated with a small positional change of the electrode compared to single-unit activity recording. The LFP also differs from electroencephalogram which is recorded at the surface of the scalp or electrocorticogram which is recorded from the surface of the brain. LFP is believed to represent the synchronized input such as the synaptic current within 0.5-3 mm from the electrode tip (Juergens et al., 1999)(but see also (Katzner et al., 2009)). Despite its utility, physiological origin of LFP is still incompletely understood. Many attempts were made to understand its source although the current view is that synchronized synaptic currents on cortical pyramidal neurons generate LFPs (Niedermeyer and Lopes da Silva, 2005, Nunez and Srinivasan, 2006). In sum, LFP is useful to record synchronized synaptic events in a large area.

I.2.3 The visual system

Among sensory systems, the visual system is probably the most studied and well determined. Vision is provided through highly complex and organized interconnected processes among various parts of the brain. Although this mechanism is still not completely revealed and differs from species to species the visual function in primate is introduced to accommodate a better comprehension and comparison.

I.2.3.1 Primate visual system

Neurons in sensory system have their own receptive field (RF), an area of space where stimulation will cause the firing of that neuron. Light projected in the RF triggers activation of two different classes of photoreceptor in the retina: the rods which are sensitive to light's intensity and the cones sensitive to the wavelength of the afferent light which allow color vision. In order to reach the photoreceptors the light must go through different layers in the retina: ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, and outer nuclear layer (Fig I.2). The visual information adapted by the photoreceptors is transformed into electrical signals, delivered to the bipolar cells and to the retinal ganglion cells. The axons from the retinal ganglion cells project to the optic chiasm where they are distributed to major subcortical targets: the superior colliculus, the pretectum and the Lateral Geniculate Nucleus (LGN). The visual information from the retina reaching LGN via optic nerves and optic tracts, are transferred via the optical radiations mainly to the cortex in the occipital lobe, called the primary visual cortex (V1). To the other hand, information is received by the superior colliculus and the pretectum which have an essential role in visual ability by controlling saccade movement and pupillary reflexes (Kandel et al., 2013).

The visual signals are normally processed in two pathways: the dorsal and the ventral pathways. The dorsal pathway begins in the retina with ganglion cells of M type (M for *Magnus*, meaning large because of the large RF of these cells). These cells respond transiently to sustained illumination. M cell projections going through magnocellular layers (I and II) of LGN reach the layer IV in the V1. Layer IV is divided into three sublayers nominated IV A, IV B, and IV C. Layer IV C is itself subdivided into IV Cα and IV Cβ, and axons in

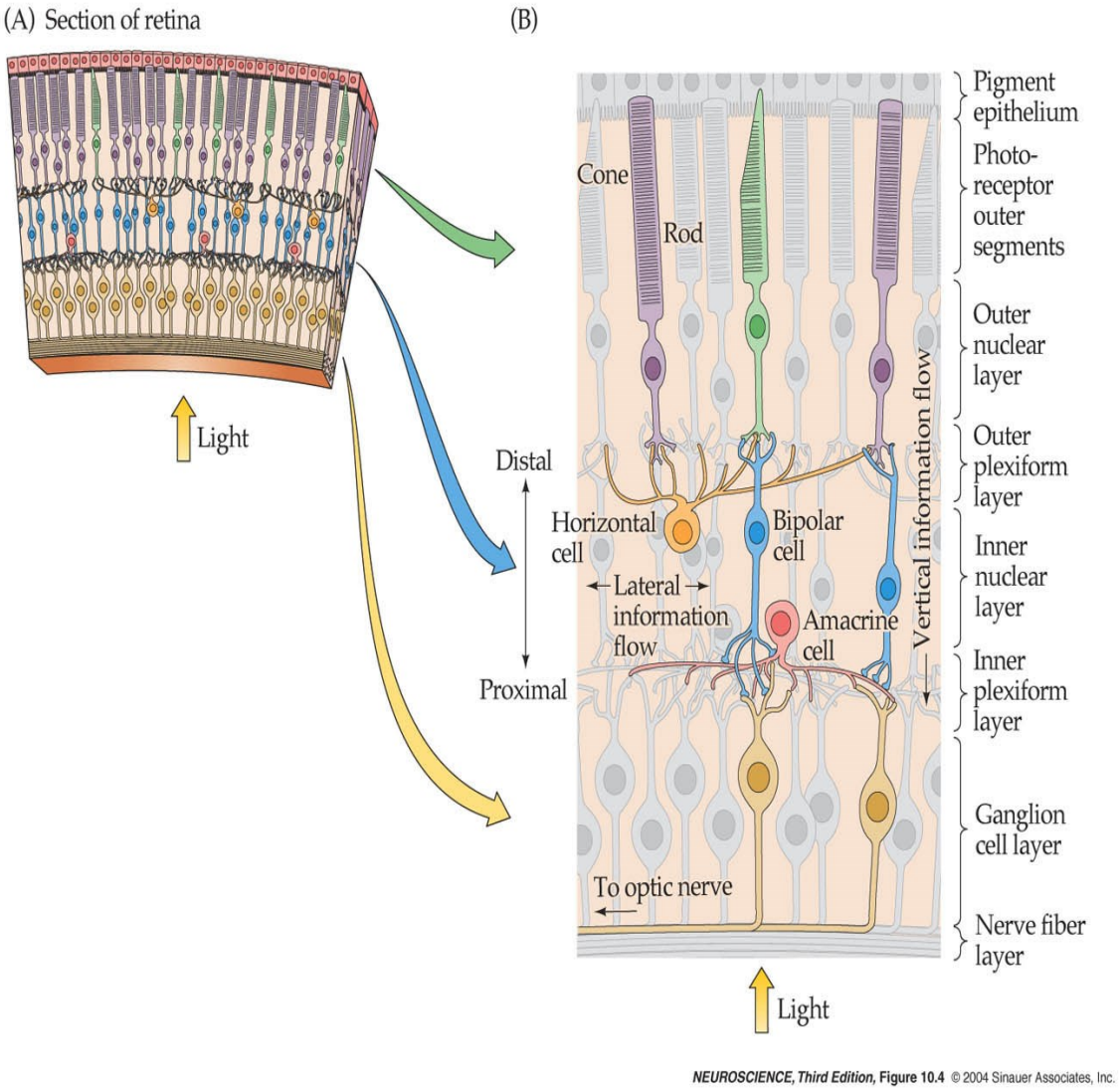

Figure I.2. Anatomy of the retina

Photoreceptors (rod and cone) are situated in the deep layer. Light must pass through ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer and outer nuclear layer to reach photoreceptors. (See text for details)

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magnocellular layers of LGN project into IV Cα (Fig I.3) (Callaway, 1998, Nassi and Callaway, 2009). The majority of neurons in the layer IV are spiny stellate cells. Their axons transfer information to the dendrites of the pyramidal cells in layers IV B and III, which are excitatory and use glutamate as their neurotransmitters. Pyramidal neuron projections in V1 reach the dorsal area of brain, the middle temporal area (also referred area V5) and extend to the posterior parietal cortex. Neurons in this area are relatively insensitive to color or to stationary objects (Corbetta et al., 1991).

For the ventral pathway, P cells (for *parvus* meaning small) in the retina send visual response to parvocellular layers in LGN (3 to 6) that project to layer IV Cβ and IV A of V1. This information is sent to V4 that is linked with the inferior temporal cortex where neurons are sensitive to the outline of images or orientation, color and shape. It is suggested that dorsal pathway is related with "where" objects are, and ventral pathway with "what" the objects are (Mishkin et al., 1983). Several studies suggest that each system is specialized for different visual functions (Zeki, 1978, DeYoe and Van Essen, 1988, Livingstone and Hubel, 1988, Zeki and Shipp, 1988). The visual information transferred in different area is used differently i.e. visuospatial recognition for dorsal pathway and recognition of complex objects for ventral pathway (Fig I.4).

Between layers of LGN there is koniocellular cell (K cell) located in koniocellular layer (Fig I.3). Koniocellular layers of LGN receive projection from bistratified retinal ganglion cells. It seems that koniocellular layer supplement the color information by transmitting to the blobs in layer II/III of V1 (Hendry and Yoshioka, 1994).

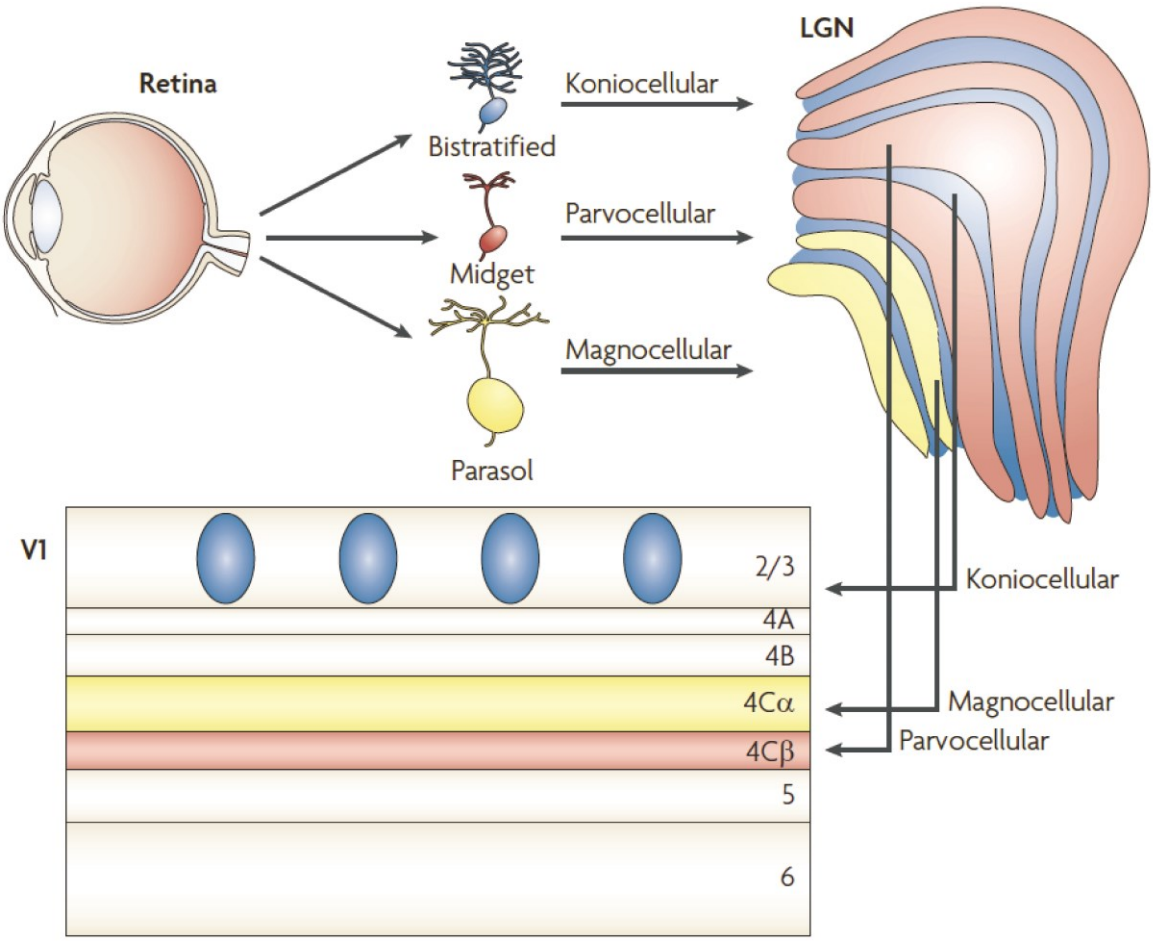

Figure I.3. Visual projection from the retina to the thalamus and visual cortex (V1)

The retinal ganglion cells, midget, parasol and bistratified remain separate through LGN and into V1. Parvocellular layers of the LGN receive projection from midget cells and project onto layer IV Cβ of V1 (red). Magnocellular layers of the LGN receive projection from parasol cells and project onto layer IV Cα of V1 (yellow). Koniocellular layers of the LGN receive projection from bistratified cells and project to the cytochrome oxidase-expressing batches (or blobs) of layer II/III (blue). See text for details (Abbreviations: LGN, lateral geniculate nucleus; V1, primary visual cortex) (Nassi and Callaway, 2009).

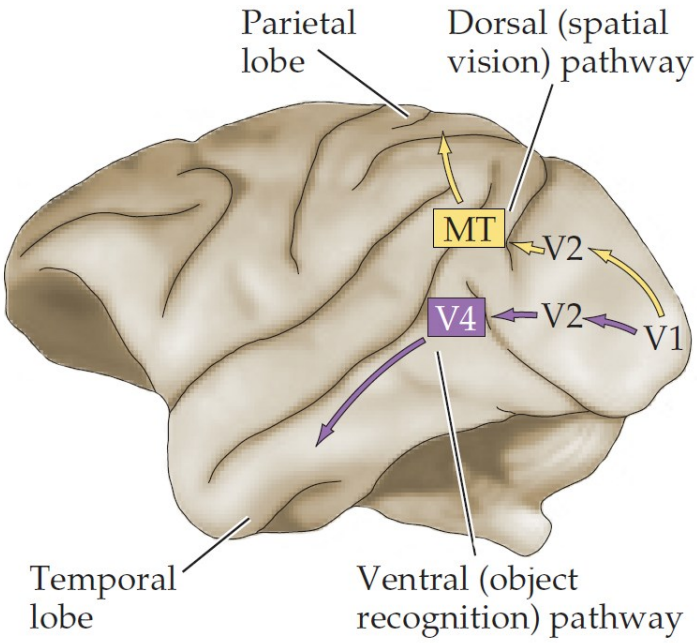

Figure I.4 Connections between visual cortical areas.

The visual areas are organized into two pathways: a ventral pathway that is important for object recognition heading toward temporal lobe, and a dorsal pathway important for spatial vision leading to the parietal lobe.

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In the primate's visual cortex, specific features of the organization of V1 are orientation columns, blobs and ocular dominance columns. Tangential penetrations with microelectrodes, neurons in the same orientation column usually respond to same oriented light bars (Hubel and Wiesel, 1968). Each column contains cells in layer IVC and permits cortical cells to produce linear RF properties from the information generated by cells of LGN. Blobs, mostly situated in layer II and III, respond to different color stimuli but have no preferred orientation. Transferring monocular visual information optic nerves cross each other in optic chiasm and most of them (~60%) reach contralateral visual cortex. In the result according to their input source those two separate tracts compose ocular dominance columns.

I.2.3.2 Rodents visual system

Comparing to primate, the rat's visual system has some distinctive features. First, laterally placed eyes provide a large panoramic visual field with a small binocular overlap. Moreover only a small proportion (~5%) of retinal ganglion cells project ipsilaterally. Secondly, the rat photoreceptors are different from those of primate. Not only has the density of cone in rat retina appeared to be lesser than other mammals but also the photoreceptor cones had shown to be sensitive at ultraviolet light (Jacobs et al., 1991).

Although in the rat system the visual pathway also includes LGN, superior colliculus and pretectum, our main interest is on LGN afferent pathway, so the physiological description in the present section will be focused on the structure of LGN. Precisely, the retinal ganglion inputs reach the dorsal area of LGN (dLGN). Despite the lack of lamination in dLGN of the rat, many studies have discriminated different regions in the distribution of cells of different sizes, in the composition of afferent axons and different patterns of degeneration after lesions

(Martin, 1986, Land, 1987, Reese, 1988). According to Reese (1988) discerning caudodorsally located nucleus as “outer shell” and “inner core” for ventromedial located nucleus, regions of dLGN were observed to be innervated by different classes of retinal ganglion cells. Variety in the regional distribution of inputs signifies that different cell types within dLGN are located in different broad regions. Cells in the outer shell mostly project to V1 and to cortical area Oc2L (occipital cortex; cytoarchitectonic area 18a). Even though the homology between Oc2L with V2 region of primate is still controversial.

In the visual cortex, most of the innervation from dLGN reaches the layer IV of V1. But dLGN projections were also observed in lower layer III and layer VI. It was observed that geniculocortical axons form asymmetrical synapses in layer IV (sparsely spined stellate cells, spiny nonpyramidal cells with perikarya and dendritic spines of pyramidal cells) and the lower part of layer III (dendritic spines of basal dendrites of pyramidal cells) (Peters et al., 1976, Peters and Feldman, 1976, 1977, Feldman and Peters, 1978). Prominent projection from layer IV cells exists in a precise manner to lower layers II/III while weaker projection extends laterally and diffusely in layer II/III. There are also a vertical projection toward the layers V and VI. The vertical intracortical connections convey the information to layers above and below to provide RF properties such as binocularity (Gilbert, 1983).

Pyramidal neurons in the layer V receive projections from the layer II/III and from the layer VI. Whereas the lower layer V make clustered projections in a diffuse manner to the layer I, the bottom of the layers II/III, and the top of the layer IV and V. Finally, neurons in the layer VI make clustered projections to the boarder of layer III and IV. Additional projections

were also observed from the layer VI to the boarder of layer V and VI and layer I/II (Burkhalter, 1989).

Nonpyramidal neurons, which are about 15% of the entire neuronal population in rat's visual cortex, are GABAergic. Three major families of GABAergic neurons are distinguished according to their immunoreactivity: parvalbumin (PV), calretinin (CR), and somatostatin (SOM). The PV-immunoreactive neurons are present in all layers except the layer I and constitute about 51% of GABAergic neurons. The SOM-immunoreactive neurons are also absent in the layer I but mainly located in the infragranular layers V and VI. Finally, the CR coexpressing neurons account for 17% of GABAergic neurons and they are abundant in the layer I (Gonchar and Burkhalter, 1997). The function of GABAergic neurons during visual stimulation will be discussed further.

Although the rodent functional organization is similar to that of primate, in the rat's visual cortex there is no evidence of orientation column (Girman et al., 1999). However, most neurons show a sharp adjusted selectivity about the direction of stimuli presented with a tendency for horizontal stimuli (Burne et al., 1984, Girman et al., 1999). This implies a distinctive mechanism for neurons in the visual cortex of the rat toward orientation selectivity.

I.3 Plasticity in the brain

In one of the episode of Sherlock Holmes, in a scene he said "I consider that a man's brain originally is like a little empty attic, … It is a mistake to think that that little room has elastic walls and can distend to any extent.". Sherlock Holmes was probably the greatest

detective character of the century, but obviously, he was not aware of plasticity of the brain. Plasticity is the ability of the brain to adapt to environmental change by reorganizing its neural circuits. Plasticity permits the brain to expand its capacity, to optimize its function or even to delete unused connection. It is estimated that instead of changing genetic code, as was chosen during evolution, our physiological system chose to change brain network to adapt to the environment. For this reason, plasticity is considered to occur as a result of long and continuous mechanism instead of spontaneous changes. Two kinds of plasticity are presented in this study: synaptic plasticity and cortical plasticity.

I.3.1 Synaptic plasticity

Synaptic plasticity is the ability of a synapse between presynaptic and postsynaptic neurons to modify its strength by changing the efficacy of receptor response and/or changing postsynaptic transduction. Earlier works in laboratories such as the one of Eric Kandel in aplysia had revealed part of the molecular mechanisms for synaptic plasticity (Castellucci et al., 1978).

Plasticity at the presynaptic level is likely to be a result of Ca2+ influx that activate calcium/calmodulin-dependent protein kinases II (CaMKII). These kinases phosphorylate synaptic vesicle associated proteins, synapsin and detach them from cytoskeleton. Direct entrance through voltage-gated Ca2+ channels or modulation of presynaptic K+ channels can both induce an increase of intracellular Ca2+. This facilitation can occur autonomously by a homosynaptical transmitter release from the terminal itself or heterosynaptically by a modulatory neuron at axo-axonic synapses.

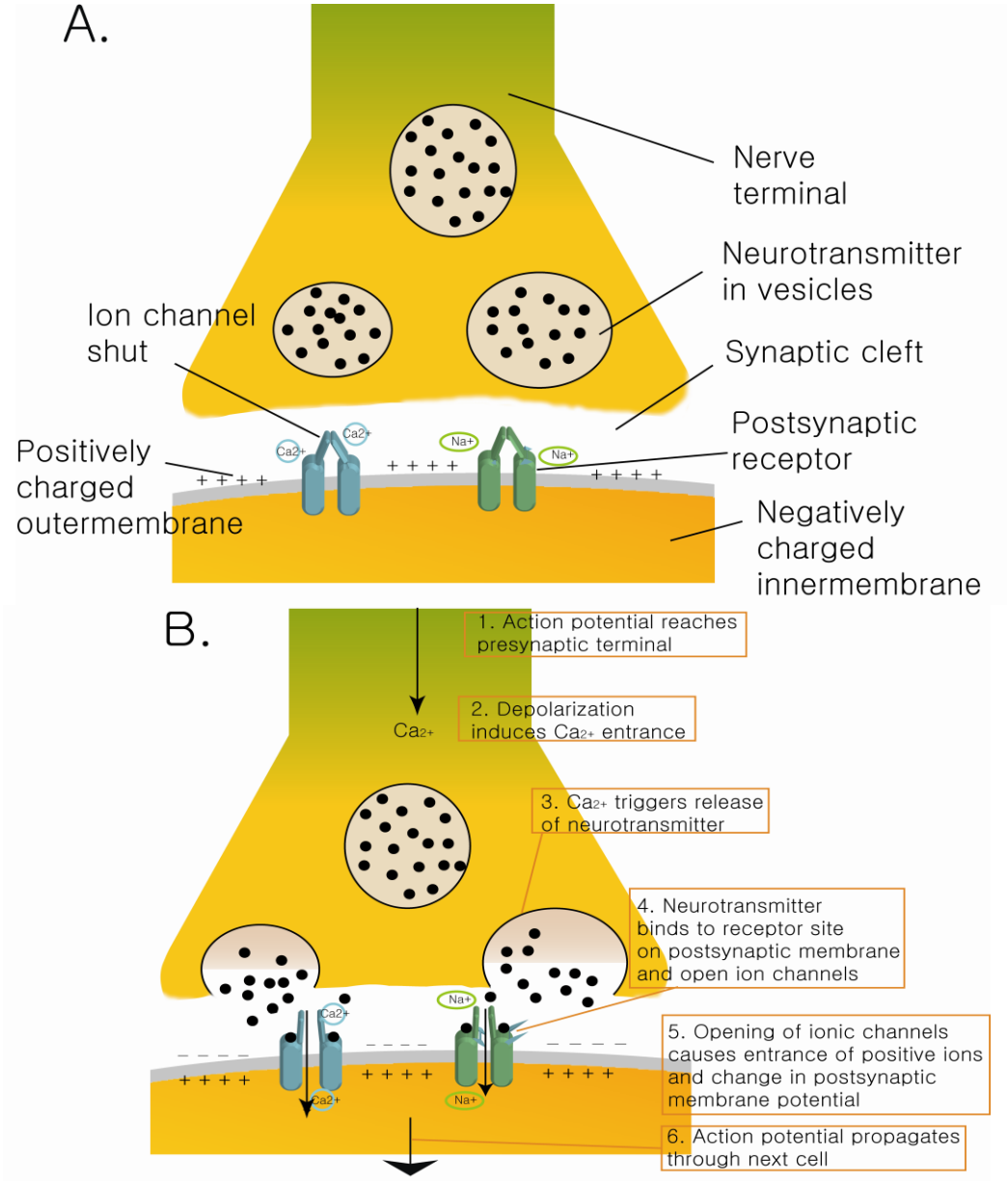

Figure I.5. Synapse and action potential.

(A) Resting potential. Neurotransmitters are synthesized at nerve terminals and transported to the synaptic cleft by vesicles. Outer membrane of neuron is positively charged due to the sodium and calcium ion. (B) Synaptic transmission. The action potential is propagated through synapse and delivered to next cell. When ionic channel is opened those ions enter in the neuron and convert the polarity. This signal is transmitted through the cell.

The most common mechanism of postsynaptic plasticity results from the direct phosphorylation of an ionotropic receptor by serine/threonine or tyrosine protein kinases. Typically when modification of existing synaptic proteins, mostly protein kinases (i.e. PKA, PKC), is involved, it alters the synaptic function (Shi et al., 1999). However a second long lasting mechanism which is triggered by protein phosphorylation depends on second messenger neurotransmitters and involves changes in the levels of key protein as well as gene transcription (Kaang et al., 1993). This second mechanism provides the mechanism for long-lasting memory storage.

I.3.1.1 Hebbian rule

Among many models of synaptic plasticity that were introduced, Hebbian rule is summarized as "Cells that fire together, wire together". Synaptic plasticity that follows Hebbian theory (or Hebbian plasticity) is induced by a continuous activation of presynaptic neuron stimulating postsynaptic cell. The repetitive and simultaneous activation increases the synaptic efficacy in the hippocampus. Representative aspects of Hebbian plasticity are long-term potentiation (LTP) and long-term depression (LTD).

I.3.1.1.a LTP

Discovered in the rabbit hippocampus (Andersen et al., 1966), LTP is the long-lasting enhancement of connection. Postsynaptic neuron shows a persistent increase in synaptic strength after high frequency stimulation of a chemical synapse. Since LTP and long-term memory possess common features it has been suggested as the most attractive candidate for cellular mechanism for learning.

LTP is usually induced with presynaptic tetanic stimulation (100 Hz during 1 sec) followed by an increase of excitatory postsynaptic potential (EPSP) lasting more than an hour (Fig I.6)(Huang and Kandel, 1994). Non-tetanic stimulation causes release of glutamate in presynaptic axon terminal which binds to AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) receptors embedded in the postsynaptic membrane. Glutamate binding causes EPSP and can results in depolarization. However, when repetitive stimuli at high frequency are given to the presynaptic fiber this can cause prolonged EPSP in postsynaptic cell. The expression of stronger EPSP will remove magnesium ion blocking NMDA (N-methyl-D-aspartate) receptors and allow calcium influx during glutamate binding. The rise of Ca2+ triggers the activation of several protein kinase enzymes, such as CaMKII, protein kinase C (PKC) and cAMP-dependent protein kinase A (PKA) (Sweatt, 1999).

I.3.1.1.b LTD

As the name imply, LTD shows a depression of EPSP after a prolonged presynaptic stimulation with low frequency (0.5-10 Hz) (Dudek and Bear, 1992). Comparable with LTP, induction of LTD through low frequency stimulation (LFS) could be blocked by NMDAR antagonists (Lee et al., 1998, Kamal et al., 1999).

LTD is correlated with dephosphorylation of an AMPAR subunit (GluR1) containing serine 831 and 845. Serine 831 in GluR1 can be phosphorylated by CaMKII and PKC while serine 845 is phosphorylated by PKA. The latter has higher basal phosphorylation rate than former. Compared to LTP which is induced by phosphorylation of the CaMKII-PKC site LTD is induced by dephosphorylation of the PKA site (Barria et al., 1997a, Lee et al., 2000).

Reversing the LTD process by phosphorylation of PKA complements that dephosphorylation of AMPAR is necessary for LTD in hippocampal CA1.

Besides the phosphorylation regulation, several lines of evidence suggest that AMPAR expression in the postsynaptic membrane is subject to mechanism in LTD; (1) Prior saturation of LTD yields the AMPARs at the synapse and insensitive to inhibitors of NSF-GluR2 interaction (Luthi et al., 1999). (2) A low-frequency presynaptic stimulation induced an NMDAR dependent depression of miniature excitatory postsynaptic current amplitude and a decrease of GluR1 expressed in surface (Carroll et al., 1999). Altogether these results suggest that AMPAR internalization is an intermediate mechanism for LTD. It was proposed that another subtype of AMPAR GluR2 binds with N-ethylmaleimide-sensitive factor (NSF), which is an important protein during membrane fusion events (Nishimune et al., 1998). Blocking this operation causes the process of rapid internalization of receptors and decrease of AMPAR currents. Since LFS had no effect after receptor internalization, it is estimated that LTD requires the pool of NSF-regulated AMPARs (Luscher et al., 1999, Luthi et al., 1999).

Recently another form of Hebbian synaptic plasticity theory is introduced: spike-timing dependent plasticity (STDP). This theory highlights the precise timing of firing between presynaptic and postsynaptic neurons. Since the depolarization is followed by a hyperpolarization due to K+ efflux, it is obvious that neuronal activation at the same moment could not induce synaptic plasticity. The precise timing of the perisomatic inhibition alter the backpropagation which is critical in STDP (Dan and Poo, 2004). Two connected neurons stimulated with varying interval confirmed the importance of precise timing to induce Hebbian plasticity (Caporale and Dan, 2008).

I.3.1.2 Homeostatic plasticity

Homeostatic plasticity refers to the compensatory mechanism of neuron to maintain its normal electrical activity. It is suggested that this self-adjustment is required to balance Hebbian plasticity. For instance, chronic neuronal firing could induce continuous LTP and quickly saturating plasticity. Instead of reaching an extreme level, the receptors are desensitized and the stability of network activity is preserved. On the other side, when a prolonged sensory deprivation occurs (e.g. light-deprivation or damage by stroke) the sensitivity of the synapses is increased to maintain the overall activity.

Homeostatic plasticity was observed in the rat's pyramidal neuron in the visual cortex after a blockade of GABAergic synapses to increase neuronal activity. Turrigiano et al. observed that such stimulation results in decrease of excitatory postsynaptic currents by down-regulating postsynaptic glutamate receptors (Turrigiano et al., 1998). Comparatively, the injection of TTX that decreases the neuronal activity induced (1) an increase of the intrinsic excitability (Gibson et al., 2006), (2) a spontaneous activity enhancement via synaptic plasticity (Maffei and Turrigiano, 2008), or (3) an up-regulation of presynaptic Ca2+ influx (Zhao et al., 2011). Homeostatic plasticity can occur either by modulating ion channel expression (intrinsic homeostasis) or by modulating the synaptic input (synaptic homeostasis). During the development of the postnatal visual cortex, for example, dark rearing reduces the ratio of NMDAR subunits NR2A/NR2B (Quinlan et al., 1999). Compared to NR2A the NR2B which subunit remains open longer and reduces the thresholds for LTP and LTD probably by facilitating Ca2+ influx (Erreger et al., 2005). On the other hand, blocking the excitatory synaptic transmission for several days increases the synapse size and thereby enhances the exocytosis rate of synaptic vesicles (Murthy et al., 2001). In sum, homeostatic plasticity is a

balance mechanism to maintain electrophysiological homeostasis in synapse allowing neuron to be modified by a strong activity exceeding the threshold.

I.3.2 Cortical plasticity

While synaptic plasticity occurs between two neurons, the cortical plasticity refers to the changes occurring in the organization of the cortex according to the experience. Brain activity transferring from a given function to a different location which results from a normal experience or a brain damage is the remarkable consequence of cortical plasticity. The cortical plasticity involves changes in multiple neuronal connections that are represented by alternation of broad range cortical response.

There are many similarities between synaptic plasticity and cortical plasticity. For example when a rat whisker of postnatal day 12-14 is stimulated it expresses recombinant AMPAR subunit GluR1 into synapses of the somatosensory cortex (Takahashi et al., 2003). In the visual cortex, similarly with LTD, monocular deprivation shows alternation in the GluR1 phosphorylation level (Heynen and Bear, 2001). An LTP is also induced in V1 after tetanic stimulation in the LGN (Heynen and Bear, 2001). The cortical plasticity is also shown to be NMDAR dependent. The inhibition of NMDAR in developing visual cortex blocks the effects of monocular deprivation suggesting a crucial role of NMDAR (Bear et al., 1990). Involvement of NMDAR implies that Ca2+ dependent enzymes are implicated in cortical plasticity. Indeed, similarly with synaptic plasticity PKA (Beaver et al., 2001), extracellular-signal-regulated kinase (ERK (Di Cristo et al., 2001)) and αCaMKII (Taha et al., 2002) are active during cortical plasticity process. The role of those kinases are to phosphorylate substrates like synapsin (Hosaka et al., 1999), AMPAR (Barria et al., 1997a, Barria et al.,

1997b, Benke et al., 1998), GABAR (Brandon et al., 2003), or actin (Matus, 2000). Those molecules are used in synaptic transmission, neuronal excitability and morphological stabilization.

Changes in synaptic molecules can activate transcription factor, such as CREB (cAMP response element binding) (Liao et al., 2002). CREB protein that binds to a specific DNA sequence called cAMP response elements (CRE) is also involved in LTP (Martin and Kandel, 1996). Starting by the postsynaptic receptor activation, the production of a second messenger such as cAMP or Ca2+ activates in turn the protein kinase which then induces CREB protein to bind to a CRE region. With successive binding of CREB-binding protein, CREB regulates other transcription factors such as c-fos, c-jun or egr-1 (Boutillier et al., 1992, Masquilier and Sassone-Corsi, 1992). Gene transcription synthesizes new proteins, a process critical for both ocular dominance plasticity (Taha et al., 2002) and long-term changes in synaptic strength (Silva et al., 1998).

Although cortical and synaptic plasticity share numerous common aspects there is no direct evidence that they are correlated. For example, some essential molecules that are used in synaptic plasticity (e.g. BDNF or type 2 metabotropic glutamate receptor) were shown to be unnecessary to induce a shift in ocular dominance (Bartoletti et al., 2002, Renger et al., 2002). Moreover, the continuous induction of LTP in synapse fails to render in cortical plasticity (Hensch, 2003).

Although many models of cortical plasticity in V1 were proposed, two classes of cortical plasticity are well documented: ocular dominance shift and lesion-induced plasticity.

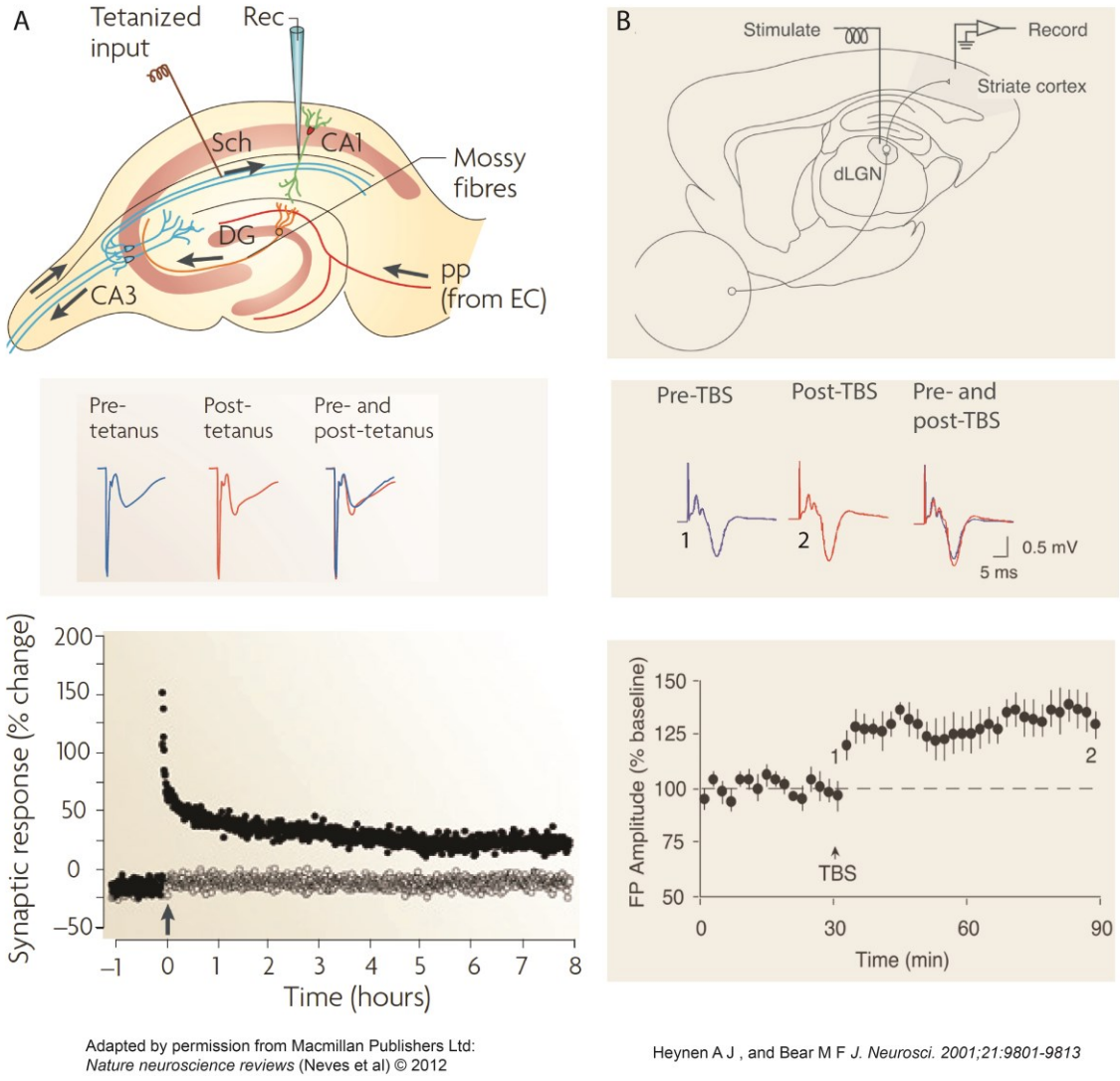

Figure I.6 Comparison of LTP in hippocampus and LTP in V1.

(A) Schematic illustration showing extracellular recording and tetanic stimulation on the Sch projection to CA1 (Top). Tetanus stimulation (a brief, high-frequency train of electrical stimuli) induces an enhanced synaptic response for many hours (i.e. LTP) (Bottom). (Neves et al., 2008)(B) Schematic illustration showing the position of stimulating electrode in the LGN and recording electrode in V1 (Top). TBS in the LGN evoked LTP of FPs in V1. After 30 minutes of baseline recording, application of TBS to the LGN elicits LTP of FPs in the V1 (Bottom). (Abbreviations: Sch, Schaffer-commissural; TBS, theta-burst stimulation; LGN, lateral geniculate nucleus; V1, primary visual cortex; FP, field potential)

Different models of cortical plasticity which are neurotransmitter-dependent and learning-induced plasticity will be introduced later.

I.3.2.1 Cortical plasticity in juvenile: ocular dominance shift during critical period

Few decades ago when the neocortex was considered unmodifiable, Hubel and Wiesel had demonstrated that during development and before the critical period, ocular dominance (OD) columns in V1 were highly plastic (Wiesel and Hubel, 1963). Exclusively, monocular vision during the critical period results in an expansion of the columns serving the open eye and the columns that were responding to the deprived eye becomes reduced in size and afferent connectivity. Following this result of Hubel and Wiesel, the cortical plasticity had been shown to occur in the somatosensory cortex (Van der Loos and Woolsey, 1973), the auditory cortex (Moore, 1985) and in diverse experimental and natural conditions.

It is suggested that GABAergic system is essential for the initiation of critical period. In mice with genetically deprived GABA-synthesizing enzyme (glutamic acid decarboxylase: GAD65), the critical period was delayed until treatment with the GABAA receptor agonist diazepam (Hensch et al., 1998, Fagiolini and Hensch, 2000). Also overexpression of brain-derived neurotropic factor (BDNF) shows a premature onset of the critical period. This is probably because BDNF promotes the maturation of GABAergic system in the visual cortex (Hanover et al., 1999, Huang et al., 1999).

On the other hand, recent study suggests that inhibitory system maturation closes the critical period. It was found that the disruption of the perineuronal nets can re-induce OD plasticity during adulthood (Carulli et al., 2010). This extracellular matrix (ECM) structure is known to surround densely the PV interneurons (Carulli et al., 2006). Also, it was

demonstrated that restoration of deprived eye vision or OD plasticity in adult rat can be induced by a reduction of GABAergic inhibition (Baroncelli et al., 2010, Harauzov et al., 2010). Those studies suggest that inhibitory system maturation affects the potential of OD plasticity.

Among theories about how inhibitory innervation can regulate plasticity of excitatory connections (e.g. glutamate and AMPAR), one convincing mechanism is by altering STDP. Parvalbumin (PV) expressing GABAergic interneurons innervate excitatory neurons and other interneurons on the soma and dendrites. Such connection allows PV neurons to modulate backpropagation (Tsubokawa and Ross, 1996) which is crucial for STDP. Indeed, increased perisomatic inhibition can disrupt STDP while blocking it facilitates (Pouille and Scanziani, 2001). In a broader range, PV interneurons can form groups of 30-50 cells and synchronize their activations through gap junctions (Galarreta and Hestrin, 1999, 2002). Such coordination alters the firing timing of excitatory neurons and hence PV interneurons can control large sets of excitatory neurons. This functioning also supplies the underlying mechanism of gamma-band oscillations (30-90 Hz) (Tamas et al., 2000). The gamma-band oscillation is suggested to be involved in sensory input processing and reflects the synchronized neural activity (Cardin et al., 2009). Overall excitatory system is affected by inhibitory input via STDP and PV interneurons create network to organize coherent firing of neurons in the visual cortex.

In summary, the initiation or the closure of critical period is affected by GABAergic system. It seems that the maturation of the GABAergic system regulate the cortical plasticity. The regulation on excitatory synapse is achieved through STDP rule and PV interneurons

network can synchronize neuronal firing. Long-term alternation of synaptic strength can shift OD response.

I.3.2.2 Cortical plasticity in adult: lesion-induced plasticity

Under normal conditions in the adult primary sensory cortex, the gross structure of neurons and their circuitry cannot be modified dramatically (Grutzendler et al., 2002, Trachtenberg et al., 2002). However, following a partial retinal lesions structural plasticity was found in the adult primary visual cortex (Darian-Smith and Gilbert, 1994, 1995). A binocular lesion at the retinal level produces a silenced region in the corresponding retinotopic zone (the lesion projection zone: LPZ). During restoration, neurons in the LPZ recover their sensitivities to visual input from the undamaged retinal regions surrounding LPZ (Darian-Smith and Gilbert, 1995, Eysel et al., 1999).

Reorganization of the cortical topography was proposed to explain the changes in RF properties, circuitry and molecular mechanism during restoration. It was shown with fMRI that reorganization of V1 retinotopic map occurs after a partial damage of the input fibers caused by macular degeneration (Baker et al., 2005) or stroke (Dilks et al., 2007). Rearrangement of cortical map is mediated by the long-range horizontal connections (Rockland and Lund, 1982). Normally, these connections are used for propagation of information and to integrate information of large area on the visual field (Albright and Stoner, 2002). Such a stimuli placed outside of RF can still influence neuronal response by the global context (Hubel and Wiesel, 1965, Crist et al., 2001). Strengthening of these horizontal connections enables neurons surrounding LPZ to innervate lesion-affected neurons and thereby shifting RFs.

It is documented that cortical reorganization in LPZ is accompanied by growth or degradation of synaptic branches. With the use of *in vivo* two-photon imaging Yamahachi et al. observed that the horizontal axons in V1 rapidly sprout their branches while removing older connections (Yamahachi et al., 2009). Also, retinal lesions induce an upregulation in the rate of turnover of dendritic spines (Keck et al., 2011) which is considered to be a sign of cortical plasticity. These changes of connections are observed both in excitatory and inhibitory system (Keck et al., 2011, Marik et al., 2014) probably to balance inputs in reorganizing cortex (Priebe and Ferster, 2012). Altogether those studies indicate that sprouting and pruning of synaptic branches is the underlying mechanism of the remapping of cortical topography following retinal lesions.

The cortical map reorganization after a partial retinal lesion opens the possibility that the cortical plasticity could also occur in mature cortex. However, the distinction in properties and connections should be made from that of juvenile cortex (e.g. OD plasticity). The nature of the cortical plasticity after lesion is similar with the mechanism of experience-dependent plasticity in adulthood. Especially, it was proposed that cortical plasticity during perceptual learning is mediated by strengthening horizontal connections and change of cortical topography (Recanzone et al., 1992, Ramalingam et al., 2013). It is possible that both changes use the same cortical circuits (Gilbert and Li, 2012).

I.4 Cholinergic system

The cholinergic system is widely distributed in the cortex and carries out the complex function with region specific manner. The two main intrinsic systems in the cortex are glutamatergic and GABAergic systems which have excitatory and inhibitory action,

respectively (DeFelipe et al., 2002). Compared to those two transmitters which roles are mainly to transfer information, other transmitters such as acetylcholine, norepinephrine, serotonin, dopamine, histamine, or adenosine have modulatory effect and arise from neurons located outside of the cortex. A single modulator may be coupled to diverse postsynaptic receptors and produce distinct postsynaptic effect and hence launching different second messenger cascade (McCormick, 1992). Such functional diversities allowed neuromodulator to be involved during control of cortical state (e.g. arousal, attention, slow-wave sleep, etc) and its network (McCormick, 1992, Briand et al., 2007). Among various neuromodulator, recently acetylcholine (ACh) attracted high interest for its implication in attention and learning (Sarter et al., 2005).

I.4.1 Cholinergic pathways in the brain

Cholinergic forebrain innervations are divided into six distribution pathways; Ch1-Ch6 (Mesulam et al., 1983). Cholinergic nuclei from the medial septum (Ch1), the vertical and horizontal limb of the diagonal band (Ch2 and Ch3), project to the hippocampus and prefrontal and occipital cortex, the nucleus basalis of Meynert (Ch4) project to the entire cerebral cortex, and the cholinergic neurons in the pedunculopontine tegmental nucleus (Ch5) and laterodorsal tegmental nucleus (Ch6) project to superior colliculus, thalamus, basal forebrain and substantia nigra. According to their activating agonist cholinergic receptors are categorized as muscarinic (activated by muscarine) and nicotinic (activated by nicotine) receptors.

I.4.2 Nicotinic system

Nicotinic cholinergic receptors (nAChR) are ionotropic and are found both in peripheral and central nervous system (PNS and CNS). It is assumed that neuronal nAChR structure is pentameric and composed of two subunit types generally 2α subunits for 3β subunits. However diversity of nicotinic receptors through various combinations between subunits (nine α: α2 to α10, three β: β2 to β4, 1728 possible receptors) (Steinlein, 1998) allows difference in their selectivity for and sensitivity to nicotinic agonists and antagonists which results in a difference in the permeability of their cationic channel (Changeux et al., 1998).

Activation of the nicotinic receptors opens Na+, K+ and Ca2+ channels. Comparing to muscle nAChRs which are more permeable to Na+ ion, neuronal nAChR are highly permeable to Ca2+. A general consent is that neuronal nAChRs are located in a presynaptic element (Wonnacott, 1997, Dani, 2001) to modulate neurotransmitter, for example glutamate (Radcliffe and Dani, 1998). The Ca2+ permeability of nAChR influences intracellular Ca2+ dependent mechanisms and may act on synaptic plasticity (McGehee, 2002). In the central nervous system, α4β2 and α7 are dominant subtypes of nAChR. Those receptors possess some distinct properties. While α4β2 excites neuron by increasing sodium and potassium permeability α7 acts through calcium channel. In the hippocampus, α4β2 and α7 are both widely distributed but it is observed that α4β2 is dominantly involved in the modulation of GABAergic inhibition to the human cerebral cortical interneurons compared to α7 (Alkondon et al., 2000). Furthermore, it is known that injection of nicotine enhance the attention in human and rodents (Levin et al., 1998, Mirza and Stolerman, 1998). Deletion of α7 nicotinic

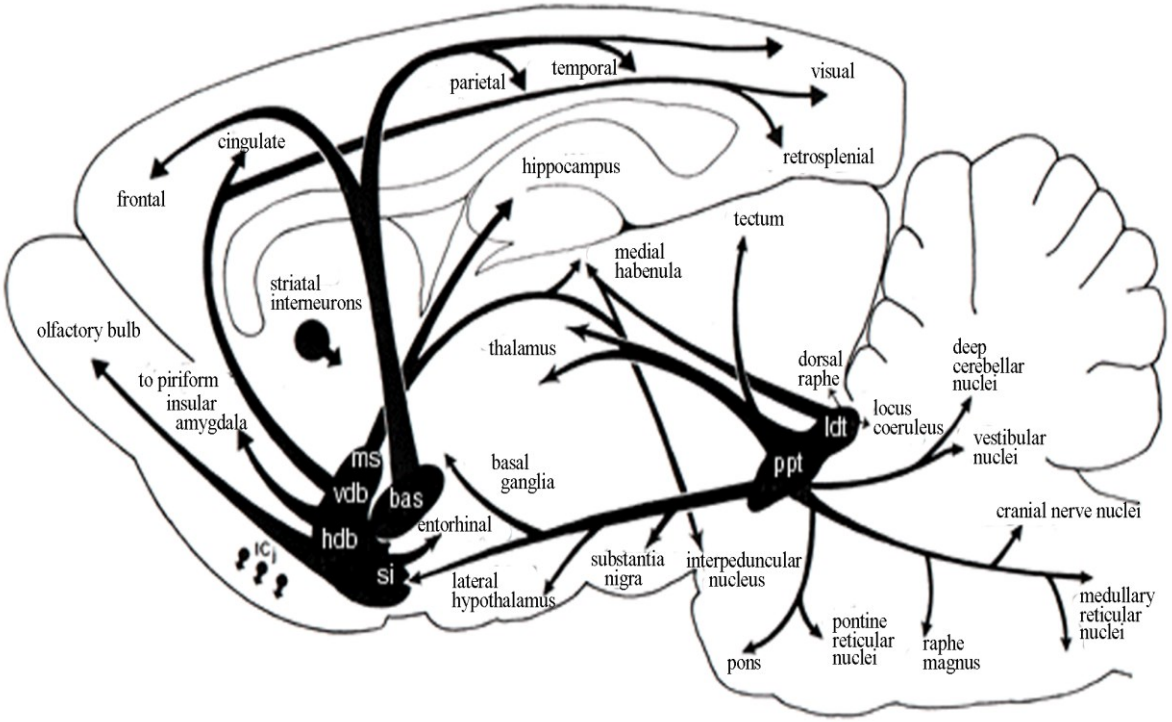

Figure I.7. Rat cholinergic central pathway.

Among all the nuclei of the basal forebrain, the horizontal limb of the diagonal band of Broca (hdb) and the substantia innominata is the principal source of the cholinergic innervation of the occipital cortex (Gaykema et al., 1990, Zaborszky et al., 1999, Laplante et al., 2005). Abbreviation; ms: medial septum, vdb: vertical diagonal band of broca, hdb: horizontal diagonal band of Broca, bas: nucleus basalis, si: substantia innominata, ppt: pedunculopontine tegmentum, ldt: laterodorsal tegmentum (Woolf, 1991)

receptor showed impairing of attention in a 5-choice serial reaction-time task (Hoyle et al., 2006).

I.4.3 Muscarinic system

Muscarinic receptors are known to be metabotropic. Hammer et al distinguished strong pirenzepine affinity receptors as M1 and intermediate or low affinity receptors as M2 (Hammer et al., 1980). Later on five types of subunit genes are characterized from m1 to m5 and their expression types are named M1 to M5. The family of M1 receptors comprising M1, M3 and M5, depolarize via G-protein (Gq/11) leading to closure of K+ channels by the phosphoinositol pathway (Nathanson, 2000). On the contrary the M2-like receptors (M2 and M4) inhibit voltage-gated Ca2+ channel by deactivating adenylate cyclase via G-protein (Gi) (Egan and North, 1986).

A preliminary study demonstrated that M1 subtype is widely spread in all cortical layers while M2 and M4 are less abundant (Levey et al., 1991). A stimulation of postsynaptic muscarinic receptors induces a neuron depolarization by inhibiting K+ efflux, usually Ca2+ independent (McCormick and Prince, 1985). M2/M4 receptors are traditionally considered to be situated at presynaptic area for an autoreceptor as a negative feedback implication (Douglas et al., 2001).

I.4.4 Cholinergic modulation of cortical plasticity

It has been proposed that modulation in receptive field properties contribute to the memory coding of a stimulus referring its importance (Weinberger, 2003). A study demonstrating that basal forebrain cholinergic lesions inhibit the learning process but not the performance suggests that cholinergic activity is required to mediate learning associated expansion and retuning of cortical receptive fields (Conner et al., 2003).

The relation between vision and ACh has yet many undiscovered mysteries. Direct application of ACh to visual cortex modifies neuronal responses showing an increase of the spontaneous activity, facilitation of the evoked responses, or suppression of the evoked responses. It has been demonstrated that visual response can be enhanced by stimulating the mesencephalic reticular nuclei (Singer 1977) which is presumed to be an action of arousal state or attention. Another observation is that during sensory stimulation, ACh is released in the sensory cortex (Inglis and Fibiger, 1995, Kilgard and Merzenich, 1998, Verdier and Dykes, 2001, Laplante et al., 2005). As mentioned previously, when kitten cortex has impaired innervation of the cholinergic basal forebrain and the dorsal noradrenaline bundle, its ocular dominance shift was blocked despite a monocular eyelid suture (Bear and Singer, 1986). Following experiments by blocking muscarinic but not the nicotinic receptors, and muscarinic M1 but not M2, demonstrated that it can prevent the ocular dominance shift in kitten visual cortex (Gu and Singer, 1989, 1993). Studies using selective cholinergic immunolesion by cholinergic toxin 192 IgG saporin confirmed that M1 and M2 lesioned juvenile mouse the synaptic plasticity was severely affected (Kuczewski et al., 2005). These studies indicate that muscarinic M1 receptors may have a critical role during cortical plasticity.

Cholinergic modulation effect is also shown in orientation dominance column shift. In normal condition, neurons do not alternate its preferred orientation simply by continual exposure to another. However, when this repetitive visual stimulus of sub-optimal orientation is paired with the application of ACh, the responses of neurons become stronger at the expense of diminishing response to the previous optimal orientation and remained long lasting (Greuel et al., 1988).

Cortical plasticity induced by ACh is also found in different location. For example, in rodent somatosensory cortex unilateral removal of a digit (e.g. a whisker) followed by neighboring digit stimulation results an expansion of the adjacent digit responding neurons. However, with cholinergic deficiency caused by basal forebrain damage no propagation of receptive field was observed (Juliano et al., 1991). In addition, a stimulation of basal forebrain paired with whisker showed a long-term enhanced somatosensory response (Verdier and Dykes, 2001). Additionally in the auditory cortex, combining the nucleus basalis stimulation and a tone emission induced changes of receptive field (Ma and Suga, 2005). On the contrary, this effect was not observed when muscarinic receptors were blocked with an antagonist (Miasnikov et al., 2001).

Although the exact mechanism of how ACh application can induce an increase of the cortical response still request a lot of studies, two possible pathways can be estimated. First, ACh could directly interfere with intracellular second messenger. It has been shown that M1 receptors stimulation leads to an increase of inositol 1, 4, 5-triphosphate (Hamilton and Nathanson, 2001) and this change results in augmentation of intracellular Ca2+ level (Yamamoto et al., 2000) which will promote the plasticity in the visual cortex (Kato et al., 2000). This pre-increased Ca2+ level can activate intracellular protein kinases (Hamilton and Nathanson, 2001) which may facilitate responses induced by NMDA receptor (Aramakis et al., 1999). Second possibility, the cholinergic contribution is to reduce membrane K+ conductance. The activation of muscarinic receptor increasing the depolarization of the cortical pyramidal cells associated with NMDA receptor-gated conductance is possible (Kirkwood et al., 1999). This effect will facilitate depolarization in response to visual input which is transmitted through glutamatergic neurons. With a direct contact in visual cortex, ACh can regulate

GABAergic neuronal inhibition (Xiang et al., 1998, Erisir et al., 2001). Since GABAergic interneurons have crucial role in cortical plasticity (Fagiolini and Hensch, 2000), ACh can influence modification threshold of cortical plasticity.

I.5 Objectives of the study

Sensory cortex is an essential area where perception initiates. Sensory information is transmitted by neurons and persistent activity of presynaptic neuron modulates the connection between neurons. Such long-term modification can result in learning which involves reorganization of cortical map (i.e. cortical plasticity) and amelioration of behavioral performance. The neurotransmitter ACh is implicated in many cognitive function, such as learning or attention, and it was demonstrated that lesioning or blocking cholinergic system diminishes cortical plasticity. It was shown that nAChR, M1 mAChR and M2 mAChR are implicated in cortical plasticity induction.

Despite the cholinergic function is well documented, it still remains to discover the pharmacological mechanism how ACh induces cortical plasticity. Recent studies confirm that cholinergic activation during visual process can trigger cortical plasticity. Including my laboratory colleague, many researchers investigated the role of M1, M2 mAChR as well as nAChR during cortical plasticity but at the moment I started this study, such functioning was not revealed. Furthermore, the cognitive effect after cholinergic-dependent cortical plasticity is still not clearly understood.

For these reasons, we investigated the effect of cortical plasticity induced by the cholinergic stimulation in the V1. We focused on the cholinergic effect on

electrophysiological response and its association with behavioral change. We used the visual acuity assessment task newly developed by Prusky and colleagues to evaluate the improvement of visual perception of the rats. Electrophysiology was chosen over other methods due to its excellent temporal resolution. This method allowed me to observe the instant effect of cholinergic stimulation and synchronized neuronal activities. Immunocytochemistry was used to determine the neuronal activation pattern in the different cortical layers and the chemical nature of neurons involved in these processes.

In order to induce cortical plasticity and behavioral change we developed a novel method, visual training. Visual training consists of repetitive simultaneous stimulations in the cholinergic system and visual system in an awake rat. This setup was designed to boost cholinergic system activation and evaluate its effect.

The research investigated the following objectives:

(Objective 1) To evaluate whether visual stimulation paired with HDB stimulation (VS/HDB) induce cortical plasticity, showed by long-term enhancement of cortical response. I compared electrophysiological response of V1 to visual stimulation before and after pairing. (Chapter 2)

(Objective 2) To determine whether HDB electrical stimulation has similar effect to ACh within V1. To assess this question I injected an ACh analog, carbachol, and compared its effect on cortical response with basal forebrain (HDB) electrical stimulation. (Chapter 2)

(Objective 3) To analyze whether repetitive VS/HDB pairing improves behavioral performance similar to perceptual learning would do. I used the visual water maze and measure rats' visual acuity. (Chapter 3)

(Objective 4) To examine the effect of VS/HDB pairing on excitatory and inhibitory neuron, I used immunohistochemistry double staining method (pyramidal/c-fos and parvalbumin or calretinin/c-fos) and compared the ratio of neuronal activation. (Chapter 3)

(Objective 5) To determine what mechanism contributes to VS/HDB pairing, I injected different kinds of pharmacological agents during repetitive VS/HDB pairing. Amplitude of electrophysiological response was measured before and after pairing. (Chapter 4)

(Objective 6) To discuss whether cholinergic activation during visual stimulation can boost visual perception. I introduced critical studies showing relation between cholinergic system and perceptual learning.

Each following chapter described the articles published or submitted to answer those questions.

CHAPTER II: Article 1

Cholinergic pairing with visual activation results in long-term enhancement of visual evoked potentials

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Contributions:

I contributed to the design, experiment, statistical analysis, production and revision of this article.

Dr. Elvire Vaucher supervised, and contributed to the design, experiment, statistical analysis, production and revision of this article.

Abstract

Acetylcholine (ACh) contributes to learning processes by modulating cortical plasticity in terms of intensity of neuronal activity and selectivity properties of cortical neurons. However, it is not known if ACh induces long term effects within the primary visual cortex (V1) that could sustain visual learning mechanisms. In the present study we analyzed visual evoked potentials (VEPs) in V1 of rats during a 4-8h period after coupling visual stimulation to an intracortical injection of ACh analog carbachol or stimulation of basal forebrain. To clarify the action of ACh on VEP activity in V1, we individually pre-injected muscarinic (scopolamine), nicotinic (mecamylamine), α7 (methyllycaconitine), and NMDA (CPP) receptor antagonists before carbachol infusion. Stimulation of the cholinergic system paired with visual stimulation significantly increased VEP amplitude (56%) during a 6h period. Pre-treatment with scopolamine, mecamylamine and CPP completely abolished this long-term enhancement, while α7 inhibition induced an instant increase of VEP amplitude. This suggests a role of ACh in facilitating visual stimuli responsiveness through mechanisms comparable to LTP which involve nicotinic and muscarinic receptors with an interaction of NMDA transmission in the visual cortex.

Introduction

Modulation of visual responses in the primary visual area (V1) by acetylcholine (ACh) contributes to visual attention (Herrero et al., 2008) and learning (Dotigny et al., 2008). In V1, ACh augments cortical plasticity in terms of intensity of neuronal activity (Brocher et al., 1992, Gil et al., 1997, Kimura et al., 1999, Rodriguez et al., 2004, Kuczewski et al., 2005, Zinke et al., 2006, Dotigny et al., 2008), preferred responses of visual neurons (Roberts et al., 2005, Zinke et al., 2006), receptive field properties (Greuel et al., 1988, Zinke et al., 2006) and performance in visual learning in the visual water maze (Dotigny et al., 2008). Neuronal effects of ACh vary from activation to inhibition (McCormick and Prince, 1985, Zinke et al., 2006) depending on the type of muscarinic or nicotinic cholinergic receptors (mAChR and nAChR) activated and location. Overall, the majority of anatomical and physiological data in V1 to date suggests that ACh primarily enhances thalamocortical inputs through the α4β2 nAChR located on the thalamocortical fibres and M1 mAChRs on glutamatergic cells of layer IV (Gil et al., 1997, Sarter et al., 2005, Zinke et al., 2006). Alternatively, ACh has been shown to decrease the strength of corticocortical input through M2 and M4 mAChRs located on corticocortical fibres (Gil et al., 1997, Mrzljak et al., 1998). ACh interaction with GABAergic interneurons through α7 nAChRs (Christophe et al., 2002, Albuquerque et al., 2009) also contributes to the modulation of sensory responses. The rapid desensitization and high calcium permeability properties of α7 nAChRs could also play a key role in cortical synaptic plasticity, although this action has not been investigated in V1 (Metherate, 2004, Levy et al., 2006).

Long-term modification of cortical responsiveness such as long-term potentiation (LTP) or depression (LTD) has been proposed as a necessary correlate of learning. The cholinergic

system has been shown to enhance long-term activation in certain cortical areas (Greuel et al., 1988, Rodriguez et al., 2004). Repetitive pairing of cholinergic and auditory stimulation over a period of two weeks results in long-term cortical map reorganization (Kilgard and Merzenich, 1998). Furthermore, pairing cholinergic activation with somatosensory stimulation (Verdier and Dykes, 2001) induces a long-term (≥ 1 h) increase of cortical electrophysiological responses. The involvement of ACh in pure LTP or LTD mechanisms, which involves NMDA receptors (NMDAR), has also been demonstrated in the hippocampus and cortex, including V1. Electrophysiologically induced LTP (Heynen and Bear, 2001, Dringenberg et al., 2007) or LTD (Kirkwood et al., 1999, McCoy and McMahon, 2007) in V1 or V1 slices (Brocher et al., 1992) is dependent on cholinergic component. Moreover, LTP and LTD are diminished in V1 of M2/M4 and M1/M3 double knock out mice, respectively (Origlia et al., 2006). This further indicates a role of ACh in cortical synaptic plasticity through an integrated action of different mAChR subtypes.

These data suggest that ACh may contribute to cortical LTP in V1, similar to other cortical areas (Kilgard and Merzenich, 1998, Verdier and Dykes, 2001). The present study was designed to test the hypothesis that pairing of external stimuli with cholinergic activation induces a long-term enhancement of integrated cortical responsiveness in V1. For this purpose, visual evoked field potentials (VEP) were measured over the course of 4-8 h in V1 after a transient pairing of patterned visual stimulation with local administration of the ACh analog carbachol (CCh) or electrical stimulation of the cholinergic projections to V1. In an attempt to clarify the underlying mechanisms and a possible link with classical LTP mechanisms, the involvement of mAChRs, nAChRs or NMDARs in these responses were

tested using scopolamine (a non-selective mAChR antagonist), mecamylamine (non-selective nicotinic receptors antagonist), or -3-(2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid (CPP, NMDAR antagonist). Moreover, the specific role of α7R was tested using methyllycaconitine (MLA, a α7 nAChR selective antagonist) to evaluate the influence of this receptor which has recently been recognized for its involvement in cortical plasticity (Metherate, 2004, Albuquerque et al., 2009).

Materials and methods

Animal preparation

Adult Long-Evans rats (n = 60, 250-300g) were obtained from Charles River Canada (St-Constant, Quebec, Canada) and maintained in a 12 h light/dark cycle with free access of food during both the pre- and post-implantation period. Two sets of experiments were performed to evaluate the long-term effects of cholinergic activation paired with visual stimulation on VEPs, i.e. the effects sustained more than 1 h following transient cholinergic stimulation. First, CCh intracortical (i.c.) injections (n = 10) were compared to vehicle injections (n = 11) in order to establish the effects of cholinergic activation on VEPs in V1. To verify the extent of the long-term effects of CCh, 3 animals were tested for an 8 h period. To verify that CCh intracortical infusion mimicked the activation of cholinergic basalo-cortical projections, an electrical stimulation (Vaucher et al., 1997) of the V1 projecting cholinergic neurons from the horizontal limb of the diagonal band of Broca (HDB) was performed on another set of animals (n = 4). Second, CCh was used to elucidate the receptors involved in this process. For this purpose 5 different groups in which the following antagonists were injected 1 h prior to CCh were examined: scopolamine (Sco+CCh, n = 4), mecamylamine (MEC+CCh, n = 5), MLA+CCh (n = 6), CPP+CCh (n = 6) and the control group, aCSF+CCh (n = 8). Complementary experiments to better evaluate the involvement of muscarinic receptors included a group of scopolamine i.p. injection 30 min before CCh (Sco i.p.+CCh, n = 5) or simultaneously with CCh (CCh+Sco i.p., n = 2, control group). The antagonistic effect of scopolamine occurs 30 min after it is injected i.p. and persists for around 120 min(Pfister et al., 1994). These two groups corresponded to inhibition of brain mAChR at the time of or just following CCh injection, respectively. Guidelines set out by the Canadian Council for the

Protection of Animals were followed for all procedures and approved by the local Animal Care Committee, "Comité de Déontologie de l'Expérimentation sur les Animaux" at the University of Montreal.

Surgery

Animals were anaesthetized with isoflurane (induction 5%, maintain 1.5%) and placed in a stereotaxic apparatus. Throughout the experiment, the rectal temperature was maintained at 37°C using a thermostatically controlled heating pad (FHC, Bowdoinham, ME, USA). A dental drill was used to make a hole (3.0 mm diameter) in the skull above the left visual cortex. A tungsten electrode (conductance < 0.8 MΩ; FHC, Bowdoinham, ME) along with an electrode guide (polyurethane tubing) was then inserted in V1 (mm from Bregma: AP -7.5, ML +4.0, DV -0.5 from dura mater surface) and tested for VEP response. The electrode was removed but the electrode guide was left in place at the surface of the skull. A push-pull cannula guide (Plastics1, Roanoke, VA) was placed adjacent to the electrode tip (mm from Bregma: AP -7.5, ML +3.6, DV -0.7 mm, 30° angle from verticality) (Fig. 1). The stimulating tungsten electrode denuded at each tip was implanted in the HDB ipsilateral to the recording cortical site (mm from Bregma: AP -0.3, L +2.0, DV -9.0). Two nylon screws (Small parts, Miami Lakes, FL, USA) were screwed into the skull, then the guides and the HDB implanted electrode were secured with dental cement. After suturing the incised skin, local anaesthesia (xylocaine 2%, Astra Zeneca, Mississauga, Canada) was topically administered to the wound and animals were returned to their cages.

Drug infusion

All drugs were obtained from Sigma Chemical Co and dissolved in a freshly made artificial cerebrospinal fluid (aCSF: NaCl, 1.0 M; $NaHCO_3$, 0.5 M; KCl, 1.47 M; $MgSO_4$, 1.25 M; KH_2PO_4, 0.25 M; $C_6H_{12}O_6$, 0.01 M; $CaCl_2$, 1.73 M pH 7.4). Drugs (CCh, 5 mM; scopolamine, 3 μM; mecamylamine, 10 μM; CPP, 20 μM; MLA, 50 nM) or vehicle (aCSF) were injected once intracortically (i.c., 1 μl/min, 10 min, simultaneously to one session of VEP recording) using an injection pump (Harvard Apparatus, Holliston, MA, USA). The push-pull cannula allowed for excess fluids at the injection site to be discarded and limited the accumulation of the drug within the cortex. Intraperitoneal injection of scopolamine (i.p., 10 mg/kg) simultaneously or 30 min prior CCh injection was also performed to compare i.p and i.c. injection regimens and match previous experiments (Laplante et al., 2005, Dringenberg et al., 2007).

HDB electrical stimulation

Electrical stimulation was performed over 10 min period using pulses (100 Hz, 0.5 ms, 50 μA, 1 sec on/1 sec off) generated (Pulsemaster A300, WPI, Sarasota, FL) and delivered through an isolation unit (WPI 365, WPI, Sarasota, FL)(Vaucher et al., 1997).

Visual stimulation paradigm

VEPs were elicited by a patterned visual stimulation provided by trains of sinusoidal gratings displayed on a computer screen in the dark. The computer monitor (30x25 cm, Titanium; luminance 21 cd/m^2; Apple Computer Inc., Cupertino, CA, USA) was placed parallel to the midline of the rat at a distance of 30 cm (Girman et al., 1999, Porciatti et al., 1999). Trains (100 ms on/ 30 sec off, 10 min) of horizontal sinusoidal grating (contrast 100%, 0.12

cycle/deg) were produced by Vpixx software (v 8.5; Sentinel Medical Research Corp., Quebec, Canada). Selected orientation and spatial frequency of the grating were based on previous studies (Girman et al., 1999, Porciatti et al., 1999, Laplante et al., 2005). Between each grating and during the rest period, the computer screen displayed a neutral grey stimulus with the same mean luminance as the gratings.

Visual evoked potentials recording procedure

Two days after implantation, rats were placed in the stereotaxic frame under anaesthesia (isoflurane, 1.5%) for VEP recording. The polyurethane tubing (electrode guide) was removed, leaving a hole through the dental cement over V1 through which the electrode was inserted. The electrode was placed 0.5 mm below the dura mater. The penetration of the electrode through the dura mater was identified by the 50% reduction of the mean amplitude of the noise signal without visual stimulation monitored by the audio monitor (AM10, Grass Technologies, Astro-Med, West Warwick, RI, USA) and data acquisition program. The cannula was also inserted within the cortex through the implanted guide. VEPs were calculated by averaging 20 electrical responses of extracellular field potentials over the 10 min stimulation period (trains of 100 msec visual stimuli, 0.03 Hz, Fig. 1). Evoked responses were amplified (5000X) and filtered at 3 Hz ~ 1 kHz (Grass Inc, West Warwick, RI, USA) and collected with the data acquisition system MP100 and Acknowledge software (v 3.8; Biopac system Inc, Goleta, CA, USA). The amplitude (difference between negative peak and positive peak) and latency (time spent between the artefact of stimulation and the first negative peak) of the VEPs were calculated using this software.

Figure 1

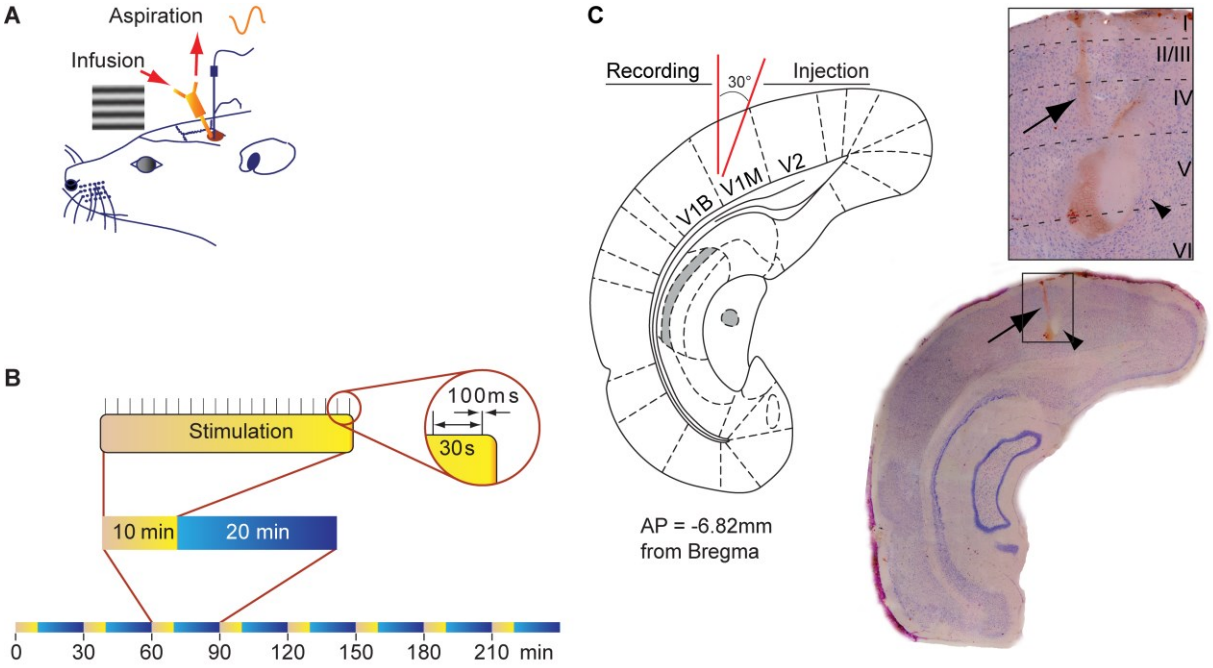

Figure II.1. Design of the experiment.

(A) Schematic diagram illustrating the chronic implantation of the recoding electrode in V1 and the push-pull cannula guide as well as the lateral stimulation of the retina with a horizontal grating displayed on a computer screen. The push-pull cannula guide and the recording electrode guide were implanted in visual cortex 2 days before VEP recording. (B) Visual stimulation. Rats were stimulated by displaying trains of sinusoidal horizontal grating (100 ms, 0.033 Hz, contrast 100%) for 8 cycles. Each cycle consisted of 10 min visual stimulation every 30 min. The VEP was obtained by averaging the 20 single electrophysiological signals evoked by the 20 presentations of the grating during the stimulation period. (C) Histology of the injection and recording sites. Schematic coronal section at the site of recording and cresyl violet-stained coronal section showing electrolytic lesion indicating position of electrode tip (arrow) and location of the infusion cannula (arrow head). Electrode and cannula tips are adjacent.

Repetitions of VEP recording were performed every 30 minutes during a 4 h period (Fig. 1). To verify the extent of the long-term effects of CCh, 3 additional animals were tested for an additional 4 h period with the same frequency of VEP recording (sixteen repetitions of VEP recording per animal). Sequence of drug injections were as follows: 1) two baseline VEPs were obtained; 2) then antagonists were injected during the next VEP recording session; 3) then one further VEP was recorded to verify that antagonists or vehicle had no effect by themselves on VEP amplitude; 4) then CCh was injected during the next VEP recording and VEP were recorded for 4 additional periods.

Histology

At the end of the experiment, an electrolytic lesion was performed to verify the recording site. The animal was then sacrificed by the administration of pentobarbital (30 mg/kg i.p.), the brain was removed, frozen at -50ºC in isopentane, and sectioned at 20 µm through the visual cortex using a cryostat (Microm, ESBE, Markham, ON). The sections were then stained with cresyl violet and electrode placement verified.

Statistical analysis

All quantitative data and the significance of difference in the amplitude of the VEPs between each group and each time point were tested by a mixed model ANOVA with a repetitive factor (time) and a non-repetitive factor (group). The mixed model ANOVA was used for the 2 sets of experiments, i.e. 4 groups (control, CCh, HDB stimulation, Sco(i.p.) +CCh), or 5 groups (mec+CCh, MLA+CCh, CPP+CCh, Sco+CCh and aCSF+CCh). In case of a significant ($P< 0.05$) interaction between these factors, a one-way ANOVA followed by the post-hoc LSD test

was performed for each time point in order to evaluate drug effects. The same analytical method was applied for the latency. All statistical analyses were carried out with SPSS 16.0 for Windows XP (SPSS Inc., Chicago, IL, USA) with a significance level of $p < 0.05$.

Results

Cholinergic stimulation induces a long-term increase of VEP amplitude

In our experimental conditions, the VEP was recorded as a wave composed of a negative peak followed by a positive deviation (Fig. 2) corresponding to electrophysiological signals recorded in cortical layer IV (Verdier and Dykes, 2001) . Mean amplitude difference between negative and positive peaks of the baseline VEP recorded was 0.965 ± 0.08mV. The amplitude ($F_{(7,70)} = 1.915$, $p = 0.080$) and the latency ($F_{(7,70)} = 1.275$, $p = 0.113$) of the VEP in the control animals did not change during the extent of the recording session (eight stimulations, 4 h, Fig. 2 and Table 1).

The mixed model ANOVA revealed a significant interaction of time and group in the amplitude ($F_{(21,182)} = 10.505$, $p < 0.001$) between the control group and CCh injected, scopolamine injected (i.p.) and HDB stimulated group. One-way ANOVA at each time point revealed that a single injection of the cholinergic agent CCh paired with visual stimulation after stabilisation of the VEP (at t = 60 min), induced an increase (range 27-56%) in VEP amplitude that lasted for the whole period of stimulation (4 h) (LSD test, $p < 0.0001$ compared to sham animals, Fig. 2). In the animals tested for a longer period of time (8 h), the enhanced effects were sustained for 6 h after which (remaining 2 h) there was variability amongst rats, probably due to the long-term isoflurane anaesthesia. The electrical stimulation of HDB paired with visual stimulation induced a long-term amplitude elevation of VEP (Fig. 2; Table 1), which was maintained during the whole period of time and was as great as CCh induced VEPs (compared to control group, $p < 0.001$). There was no difference between the amplitude of

Figure 2

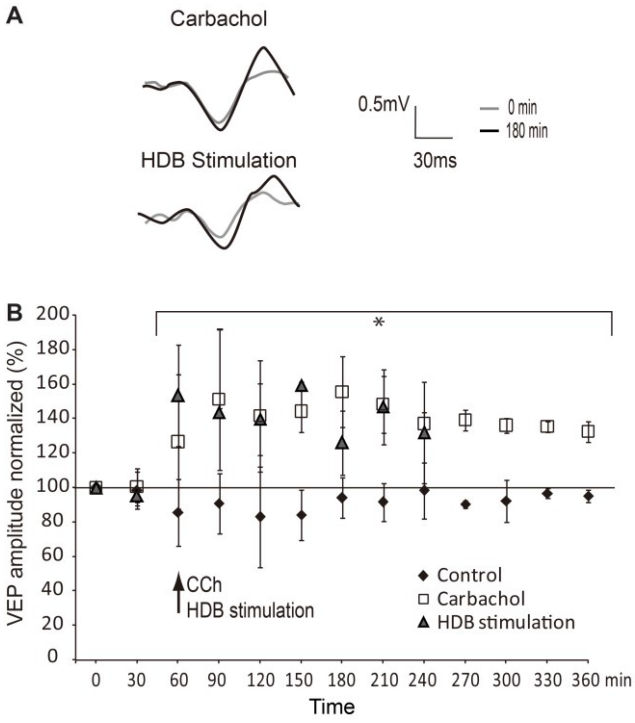

Figure II.2. Effects of cholinergic system activation through pharmacological injection and electrical stimulation paired with visual stimuli at different time points.

(A) Representative wave of the VEP recorded before (grey line) and after (black line) CCh injection or HDB stimulation. The recorded wave was composed of a negative peak followed by a positive deviation representative of layer 4 field potentials trace. (B) Long-term effect on VEP amplitude of CCh infused in V1 (open square) or of HDB stimulation (triangle). After 2 periods of baseline recording (0 and 30 min), application of CCh or HDB stimulation (indicated by arrow) produces an increase of VEP amplitude observed for several hours after CCh infusion or HDB stimulation. Error bars indicate the SD values.

Table1.

Amplitude (%)	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Cholinergic enhancement								
Control	100	99±11	86±19	91±17	83±29	84±15	94±12	92±11
Carbachol (CCh)	100	101±11	127±39*	151±41*	142±32*	144±12*	156±21*	149±16*
HDB stimulation	100	95±04	154±03*	144±03*	140±21*	159±02*	126±19*	147±22*
Sco (i.p.) + CCh	100	90±14	54±02*	89±14	78±15	86±19	95±18	114±08
CCh + Sco (i.p.)	100	95±08	130±45*	182±40*	165±17*	148±24*	162±04*	153±25*
Pharmacological treatment								
aCSF+CCh	100	95±15	105±13	92±12	115±18	122±14	130±18	112±13
Sco (i.c.) + CCh	100	78±26	108±34	93±24	64±05#	72±16#	84±29#	85±22#
Mec + CCh	100	99±10	115±10	101±10	77±21#	97±08#	90±09#	91±11#
MLA + CCh	100	102±06	94±07	83±15	152±29#	100±22#	86±06#	131±08#
CPP + CCh	100	106±22	91±08	111±16	88±14#	108±18	100±12#	81±15#

Table II.1 Amplitude of VEP normalized after CCh injection or HDB stimulation and drug treatment.

Values are expressed in mean ± SD. For the first set of experiment (cholinergic enhancement) CCh infusion and HDB stimulation were administrated at t=60min. For pharmacological treatment, antagonists were injected at t=60min followed by CCh at t=120min.

*: $p<0.05$, compared to control, ANOVA and LSD post-hoc

#: $p<0.05$, compared to aCSF+CCh, ANOVA and LSD post-hoc

VEPs induced by the CCh as compared to stimulation of HDB. Latency of VEP across the groups did not differ (Table 2, mixed model ANOVA, $F_{(21,182)} = 1.429$, $p = 0.143$).

Effects of muscarinic, nicotinic and NMDA receptor inhibition on the amplitude enhancement of the VEPs

The injection of inhibitors before the induction of CCh enhancement effect showed a significant interaction in amplitude between time and injected drugs ($F_{(28,168)} = 7.979$, $p < 0.001$) but not in the latency ($F_{(28,168)} = 1.105$, $p = 0.338$). The amplitude of the basal VEPs (before infusion of CCh) was not affected by muscarinic (one-way ANOVA, $p = 0.726$), nicotinic ($p = 0.236$) and NMDA receptor inhibition ($p = 0.115$) during this administration nor 30 minutes after compared to the aCSF injected group. This suggests that none of the drugs injected contributed significantly to the baseline electrophysiological response to visual stimulation before CCh injection. Scopolamine ($p < 0.001$), mecamylamine ($p = 0.024$) and CPP ($p = 0.046$) pre-treatment prevented the CCh-induced long-term enhancement of the amplitude of the VEPs (Fig. 3, Table 1). MLA showed fluctuating results (compared to the aCSF group values), that is, an increased VEP amplitude during CCh infusion ($p = 0.003$) and 2h after CCh infusion ($p = 0.02$), but a decreased amplitude in between these two time points. The latency was unchanged for each group (Table 2, $p = 0.086$). VEP amplitude was however reduced (up to 32% decrease compared to control) at t=120min when CCh was infused in the scopolamine group ($p = 0.028$ i.c. and $p = 0.048$ i.p). Moreover, there was no effect of scopolamine (i.p.) when it was injected simultaneously with CCh (Table 1), suggesting that mAChRs do not contribute directly to the enhanced VEPs of CCh.

Figure 3

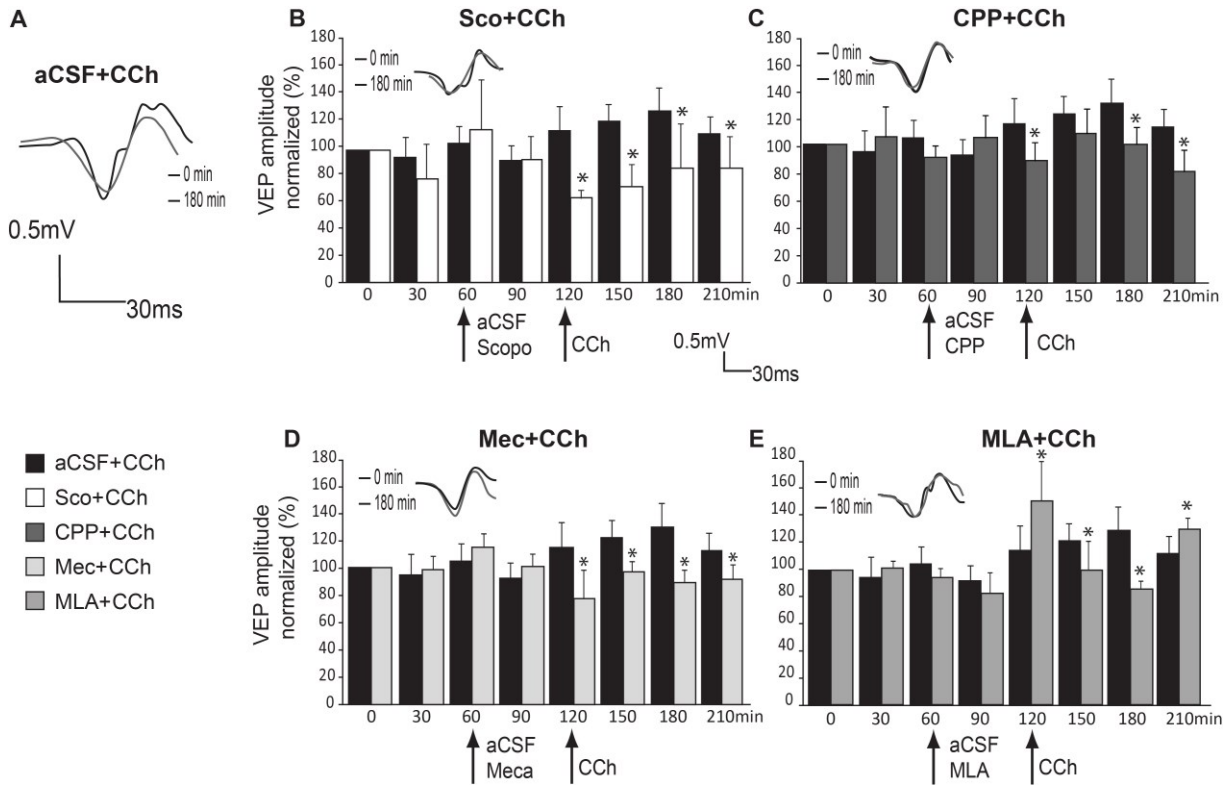

Figure II.3. VEP amplitude changes after pharmacological infusion of different drugs in the V1 with aCSF+CCh injected animals as control group.

Effects of scopolamine (B, Sco+CCh), CPP (C, CPP+CCh), mecamylamine (D, Mec+CCh) or MLA (E, MLA+CCh) infusion prior to CCh administration are shown. The long term enhancement of VEP amplitude is abolished in an identical manner by scopolamine, CPP and mecamylamine, suggesting that mAChR and nAChR could act upstream of NMDAR intracellular pathways. Drug infusion time points are indicated by black arrows.

Table2.

Latency (ms)	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Cholinergic enhancement								
Control	36±3	35±3	37±3	35±4	35±2	33±4	34±3	35±4
Carbachol (CCh)	37±3	37±2	36±4	34±3	29±3	26±5	31±3	31±3
HDB stimulation	35±4	32±2	33±3	33±3	35±3	33±4	36±1	35±3
Sco (i.p.) + CCh	37±1	41±1	40±2	40±2	35±4	38±3	39±5	37±2
CCh + Sco (i.p.)	38±1	38±3	35±1	33±1	33±1	35±5	33±3	36±1
Pharmacological treatment								
aCSF + CCh	38±2	37±4	35±4	33±3	36±3	32±3	32±3	35±2
Sco(i.c.)+CCh	38±5	37±2	41±1	39±3	43±5	40±6	44±5	39±6
Mec + CCh	39±1	38±3	38±2	37±6	46±3	45±4	30±4	38±4
MLA + CCh	40±2	41±4	39±2	37±4	36±4	37±3	39±3	37±2
CPP + CCh	39±3	36±4	36±2	34±3	38±2	38±5	31±4	30±6

Table II.2. Latency of VEP after CCh injection or HDB stimulation and drug treatment.

Values are expressed in mean ± SD of ms after the stimulation artefact. There was no effect of the treatment on latency.

Discussion

The principal objective of this study was to pharmacologically analyze the long-term effect of transient pairing of visual stimulation with cholinergic activation on cortical neuronal functioning. This was achieved by measuring changes in evoked potentials in V1 as a function of time. The results showed a long-term enhancement in the amplitude of the VEPs for at least 6 h when the cholinergic system was stimulated either from the cortex or the basal forebrain. This effect was mediated by different types of receptors, i.e. mAChRs and nAChRs as well as NMDARs but not α7 nAChRs. It is concluded that cholinergic agents induced LTP-like events in the cortex by triggering intracellular NMDAR pathways in glutamatergic cells. We discuss below the role of the cholinergic system in modulating cortical response to visual stimulation, its possible intracellular pathways and its relation to attention and learning processes.

Acetylcholine modulates cortical responses in adult visual cortex

The results presented here demonstrate that a single synchronization between visual stimulation and cholinergic activation by CCh or electrical stimulation of the HDB was sufficient to induce a persistent increase of VEP amplitude lasting for several hours. Similar results were obtained by combining CCh injection and direct dorsal lateral geniculate nucleus tetanic stimulation in an LTP paradigm (Dringenberg et al., 2007) , and electrical basal forebrain stimulation combined with tactile stimulation (Verdier and Dykes, 2001, Penschuck et al., 2002, Alenda and Nunez, 2007). As well, these results corroborate data obtained in cats, showing a long lasting response synchronization (Rodriguez et al., 2004) or receptive field modification(Greuel et al., 1988) of cortical visual cells after co-application of cholinergic agonists and light stimuli. These results confirm *in vivo* that long-term effects of visual

stimulation are dependent on cholinergic activation. Interestingly, there was no spontaneous enhancement of VEPs amplitude in control conditions. This suggests that the low frequency visual stimulation in our experimental conditions did not increase ACh extracellular levels enough for inducing long-term effects in the control conditions. In agreement with this, it has been shown that visual stimulation with low frequency (0.067 Hz) checkerboards does not induce cortical long-term changes (Clapp et al., 2006). High frequency (9 Hz) stimulation does induce long-term changes in an effect termed sensory LTP (Clapp et al., 2006), suggesting different neurobiological mechanisms involved in high and low frequency sensory stimulation.

Involvement of NMDA receptors

The cessation of CCh-induced long-term enhancement of cortical response to visual stimulation during NMDAR inhibition supports the involvement of an interaction between cholinergic stimulation and NMDAR transmission (Sur et al., 2003, Yamazaki et al., 2005, Li et al., 2007). The long-term enhancement of VEP reported here is similar to LTP mechanisms whereby synaptic strength is increased by the opening of NMDAR which launches Ca^{2}+ influx followed by an upregulation of glutamatergic receptors (Yoshimura et al., 2003). LTP occurrence is accompanied by an amplification of VEP (Heynen and Bear, 2001), suggesting that the changes seen in the present study could reflect LTP.

The involvement of NMDAR is implicated in plasticity in the juvenile and adult visual cortex (Quinlan et al., 1999, Sawtell et al., 2003) suggesting that NMDAR is a key factor in the plasticity induced by thalamocortical inputs. Although the occurrence of LTP peaks during the development period and drastically drops in the adult cortex, our results indicates that LTP-

like mechanisms could participate in cortical plasticity in adult rats similar to what is reported in cat (Creutzfeldt and Heggelund, 1975) and mouse (Sawtell et al., 2003). Our results further implicate that these mechanisms are dependent on cholinergic mechanisms.

Involvement of muscarinic receptors

Given that mAChRs are widely expressed in the visual cortex - the predominant postsynaptic mAChR being M1 subtype and the presynaptic mAChR being M2 (Levey et al., 1991) - and that M1 and M3 receptors are involved in hippocampal LTP (Colgin et al., 2003), it was expected that inhibition of these receptors would abolish long-term enhancement of VEP. The present results of scopolamine administration verified this hypothesis since no long-term changes in VEP amplitude were seen after scopolamine infusion prior to CCh. This effect was robust and stable. Interestingly, i.p. infusion of scopolamine prior to CCh led to the same results as cortical infusion confirming that scopolamine i.p. could act at a local cortical target (Laplante et al., 2005, Dringenberg et al., 2007, Miasnikov et al., 2008). However, three findings suggest that mAChRs are involved in the induction of pathways generating long-term enhancement of electrophysiological responses, acting as a trigger mechanism rather than directly enhancing the ongoing neuronal excitability. First, the VEP amplitude was significantly decreased compared to baseline at the time of CCh infusion under scopolamine conditions which suggests that CCh may have a depressing effect during mAChRs antagonism. This effect might be mediated by nAChRs (Oldford and Castro-Alamancos, 2003, Levy et al., 2006), which could inhibit glutamatergic neurons through 1) activation of α4β2 or α7 nAChRs located on GABAergic neurons (Albuquerque et al., 2009) or 2) disinhibition of inhibitory interneurons by blocking of M2 mAChR expressed by the GABAergic interneurons

(Erisir et al., 2001, Salgado et al., 2007). Second, when mAChRs were fully inhibited secondary to CCh action (simultaneous scopolamine i.p. injection and CCh i.c. injection group, see methods), the enhanced long-term effects of CCh were not affected. This result suggests that mAChR activation is required for priming long-term enhancement of VEP but not directly for enhancing neuronal activity that contributes to the increase in amplitude of subsequent VEPs. This result contrasts with a recent study showing impairment of auditory memory when scopolamine was administered immediately after the cholinergic-paired training of the animal (Miasnikov et al., 2008). However, the electrical cortical responses were not recorded in this study, making it difficult to compare with our results. Finally, there was no significant difference between the effect of CPP and the one of i.c. scopolamine in terms of VEP amplitude. This might indicate an all-or-none effect on VEP enhancement, suggesting common intracellular pathways leading to LTP.

We propose that activation of mAChRs interact with intracellular NMDAR pathways to induce cholinergic-induced long-term effects on VEPs. It has been shown that M1 and M3 interact with NMDAR pathways in the hippocampus by elevating intracellular Ca^{2+} level and thereby enhancing the AMPA receptor currents (Markram and Segal, 1990). Post-synaptic mAChRs on pyramidal or spiny stellate cells are able to induce PKC or AKT (Sur et al., 2003, Li et al., 2007), which could be a mechanism of such intracellular interaction. Moreover, *in vitro* induction of LTP in V1 slices is impaired in M2/M4 mAChRs double knock-out mice (Origlia et al., 2006) , suggesting that inhibition of M2/M4 mAChRs impaired LTP. Alternatively, the long-term enhancement of VEP could result from an increase in VEP amplitude most likely due to the number and nature of cells involved or a change in the balance between LTP/LTD mechanisms induced. In this case, the inhibition of the different

subtypes of mAChRs located on different cell types (GABA interneurons, pyramidal or spiny stellate cells) could result in a decreased number of excitatory cells activated by the paired visual stimulation and CCh infusion.

Involvement of nicotinic receptors

Mecamylamine, a non-selective nAChRs antagonist, and MLA a selective α7 nAChR antagonist, were used to investigate the potential involvement of nAChRs in the long-term enhancement of VEPs. The α7 subtype of nAChRs is considered a key participant in cortical plasticity (Albuquerque et al., 2009), but its potential role in the visual cortex has not been elucidated. Mecamylamine, but not MLA, showed an impairment of long-term increases of VEP. Results obtained with mecamylamine treatment were expected since it has been shown that its administration abolished LTP induced by tetanic stimulation of the dorsal geniculate nucleus in V1 (Dringenberg et al., 2007, McCoy and McMahon, 2007) and in V1 slice preparation (Brocher et al., 1992). These results have also been observed in sound-evoked cortical response in the auditory cortex (Kawai et al., 2007). Mecamylamine inhibits both α4β2 and α7 nAChRs that are located on thalamocortical terminals and cortical GABAergic neurons (Christophe et al., 2002, Disney et al., 2007, Albuquerque et al., 2009). Activation of nAChRs located on the thalamocortical afferents increase thalamic input (Gil et al., 1997). Inhibition of these receptors should result in the reduction of incoming signals from the thalamus which is in agreement with the abolishment of VEP amplitude enhancement under mecamylamine conditions in the current study. Inhibition of nAChRs located on the GABAergic cells may not be sufficient to explain these results since inhibition of these receptors should also result in reducing the inhibitory drive within the intracortical network,

thereby lowering the threshold for eliciting a cortical response (Gil et al., 1997, Metherate, 2004, Zinke et al., 2006). In addition, it has been shown that mecamylamine could transiently inhibit the NMDAR *in vitro* at the concentration used in the present study (Papke et al., 2001). It is possible that the blockade of CCh-induced long-term effect on VEPs by mecamylamine in our study could result from an inhibition of the NMDAR located on the glutamatergic cells.

α7 nAChRs have been proposed to participate in cortical plasticity by activating silent AMPA receptors on glutamatergic neurons in the somatosensory cortex (Metherate, 2004). The blockade of α7 nAChRs in the present study did not consistently abolished the long-term enhancement of VEP induced by concomitant thalamocortical and cholinergic activation. The amplitude of the VEP response under MLA condition fluctuated, showing strong increases or decreases depending on the time point. This effect could be explained by an inactivation of GABAergic interneurons rather than glutamatergic cells during the α7 nAChRs blockade. Activation of α7 nAChRs of layer 1 interneurons has been shown to mediate disinhibition of cortical networks (Christophe et al., 2002), which can result in increased VEP response. Consequently, inactivation of these receptors could generate decreases in VEP amplitude, whereas, increases in VEP amplitude could be induced by inhibition of GABAergic cells from layer 4. Such blockade of α7 nAChRs has been shown to induce LTP in the hippocampus (Ge and Dani, 2005, Wang et al., 2006) due to their location on inhibitory interneurons (Yamazaki et al., 2005).

Functional implication of the cholinergic modulation of visual cortex

The permissive role by ACh shown here suggests that ACh is a key factor in experience-dependent plasticity allowing cholinergic enhanced stimuli to take over stimuli not associated with cholinergic reinforcement and modifying both cortical processing and representation of these stimuli. Our results bridge studies showing the role of the cholinergic system in selective attention (cholinergic reinforcement of visual stimuli) in V1 (Herrero et al., 2008) and visual learning (long-term modification of synaptic responses and connections in V1)(Ahissar and Hochstein, 1993, Sarter et al., 2005, Dotigny et al., 2008). Our results imply that the cholinergic reinforcement of visual stimuli 1) would be provided by the adequately-timed cortical release of ACh from the basal forebrain terminals (Laplante et al., 2005, Miasnikov et al., 2008) and 2) would be sufficient for visual learning (Miasnikov et al., 2008). These implications are further supported by previous work that ACh is released in cortex during numerous learning paradigms (Sarter et al., 2005, Miasnikov et al., 2008), or visual stimulation (Laplante et al., 2005, Origlia et al., 2008). This release might be induced by sensory feed-forward influxes (Laplante et al., 2005) or by top-down control, in which ACh mediates top-down attention mechanisms (Ahissar and Hochstein, 1993, Herrero et al., 2008) elicited by higher cognitive areas through basal forebrain activation (Golmayo et al., 2003, Sarter et al., 2005). This interplay between stimulus driven and top-down input to modulate neuronal activity has been addressed by computational neurosciences (Roelfsema and van Ooyen, 2005). In the computational model, the authors suggest that a reinforcement signal combined by an attention feedback signal, called attention-gated reinforcement learning, could model the cortical integration and mapping of sensory stimuli. The long-term mechanisms involving NMDAR and probably LTP pathways shown in the present study, suggest a

modification of synaptic functioning by the cholinergic system, which would give a neurobiological basis to this attention-gated reinforcement learning. It would also suggest that attention and visual stimuli elicit ACh release in V1, which modifies synaptic functioning by eliciting LTP-like mechanisms at an early level of cortical processing.

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CHAPTER III: Article 2

Visual training paired with electrical stimulation of the basal forebrain improves orientation-selective visual acuity in the rat.

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I contributed to the design, experiment, statistical analysis, production and revision of this article.

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Ms. Florence Dotigny contributed to the immunohistochemistry experiment and production.

Mr. Hugo Giguere contributed to the behavioral test (water maze) and visual training experiment.

Abstract

The cholinergic afferents from the basal forebrain to the primary visual cortex play a key role in visual attention and cortical plasticity. These afferent fibers modulate acute and long-term responses of visual neurons to specific stimuli. The present study evaluates whether this cholinergic modulation of visual neurons results in cortical activity and visual perception changes. Awake adult rats were exposed repetitively for two weeks to an orientation-specific grating with or without coupling this visual stimulation to an electrical stimulation of the basal forebrain. The visual acuity, as measured using a visual water maze before and after the exposure to the orientation-specific grating, was increased in the group of trained rats with simultaneous basal forebrain/visual stimulation. The increase in visual acuity was not observed when visual training or basal forebrain stimulation were performed separately nor when cholinergic fibers were selectively lesioned prior to the visual stimulation. The visual evoked potentials show a long-lasting increase in cortical reactivity of the primary visual cortex after coupled visual/cholinergic stimulation, as well as c-Fos immunoreactivity of both pyramidal and GABAergic interneuron. These findings demonstrate that when coupled with visual training, the cholinergic system improves visual performance for the trained orientation probably through enhancement of attentional processes and cortical plasticity in V1 related to the ratio of excitatory/inhibitory inputs. This study opens the possibility of establishing efficient rehabilitation strategies for facilitating visual capacity.

Keywords: acetylcholine; attention; cholinergic system; cortical plasticity; perceptual learning.

Abbreviations:

BF basal forebrain

V1 primary visual cortex

ACh acetylcholine

nAChR nicotinic receptors

mAChR muscarinic receptors

LTP long-term potentiation

VEP visual evoked potentials

HDB horizontal limb of the diagonal band of Broca

CPD cycle per degree

VS visually stimulated rats

ChAT choline acetyltransferase

PV parvalbumin

CR calretinin

RBPC rat brain pyramidal cell marker

E/I excitation/inhibition ratio

Introduction

Improving visual capacity through visual training is a promising treatment for visual impairment in adults. Visual training results in acquired experience-dependent plasticity that involves attentional mechanisms and cortical plasticity related to perceptual learning (Ahissar and Hochstein, 1993, Seitz and Watanabe, 2005, Sabel et al., 2011). Visual training is relatively long and results in small increments of visual function. Since the basal forebrain (BF) cholinergic afferents in the primary visual cortex (V1) play a key role in visual attention (Herrero et al., 2008) and cortical plasticity (Bear and Singer, 1986, Greuel et al., 1988, Kang and Vaucher, 2009), it is most probable that activation of these cholinergic afferents could potentiate visual training effects and results in improvement of visual capacity in adults. Acetylcholine (ACh) facilitates the processing of novel/relevant stimuli by the modulation of the efficiency of thalamo-cortical and cortico-cortical inputs in V1. First, glutamatergic transmission of thalamo-cortical synapses is facilitated by the nicotinic receptors (nAChR) (Gil et al., 1997). Second, glutamatergic transmission of cortico-cortical connections is inhibited by the muscarinic receptors (mAChR) (Gu, 2003, Roberts et al., 2005, Amar et al., 2010). This reduces both feedback control from higher cortical areas and lateral spread of activation (Kimura et al., 1999, Silver et al., 2008, Kosovicheva et al., 2012). This muscarinic effect might also be mediated via GABAergic interneurons in V1 (Zinke et al., 2006, Amar et al., 2010, Disney et al., 2012). Consequently, feed-forward processing of specific stimuli is prioritized and more efficient under cholinergic activation (Newman et al., 2012). In addition, cholinergic activation elicits long-term increase in V1 neuronal responses when transiently associated with visual stimulation (Kuczewski et al., 2005, Dringenberg et al., 2007, Kang and Vaucher, 2009). These effects associated with long-term potentiation (LTP)-like mechanisms

and synaptic plasticity, result in cortical plasticity and learning (Rokem and Silver, 2010). Finally, it has been shown that ACh is involved in cortical plasticity through its ability to control the balance between excitatory and inhibitory transmission in V1 (Lucas-Meunier et al., 2009, Amar et al., 2010) and the regulation of the plasticity brake, *lynx1* (Morishita et al., 2010). Together, these results suggest that the cholinergic system induces a long-lasting enhancement in the efficacy of processing selected visual stimuli.

To determine if the activation of the cholinergic system could result in improvement of visual perception, a 14-day visual training to a weak visual stimulus was coupled with electrical stimulation of the BF in awake adult rats. A 30º sine-wave grating was selected as the visual stimulus because it elicits a moderate electrophysiological response in V1 (Girman et al., 1999). The enhancement of the visual acuity to this specific orientation was tested using the visual water maze (Prusky et al., 2000, Dotigny et al., 2008). Long-term cortical responsiveness for this stimulus was measured by *in-vivo* field potential recordings in V1. Changes in the activation pattern of the visual cortex were quantified by counting the number and layer specificity of activated neurons immunoreactive for c-Fos. Finally, the neurochemical nature of these activated neurons was identified to investigate whether excitatory (i.e., glutamatergic) or inhibitory (i.e., GABAergic) neurons were involved in this response. Our data suggest that repetitive concomitant stimulation of the visual and cholinergic systems, a paradigm that could be used for vision recovery strategies, induced a persistent upregulation of cortical responses in V1 leading to improved visual performance.

Materials and Methods

Animal preparation

Adult Long-Evans rats (n=84, 200-225 g) were obtained from Charles River Canada (St-Constant, Quebec, Canada) and were maintained in a 12-h light/dark normal daylight cycle with *ad libitum* access to food and water. The guidelines set by the Canadian Council for the Protection of Animals were followed for all procedures and approved by the local Animal Care Committee, "Comité de Déontologie de l'Expérimentation sur les Animaux" at the University of Montreal (protocol # 10-133). All efforts were made to minimize suffering and the number of animals used for these experiments.

Experimental design

Awake rats were repetitively exposed to a visual/ stimulus (this procedure is called "visual training" for sake of simplicity) coupled or not with BF stimulation. The parameters assessed after the visual training were the visual acuity of the rat, visual evoked potentials (VEP) and V1 neuronal activity. Each experiment had seven components: a procedural learning of the visual discrimination test, a pre-training visual acuity behavioral test, electrode implantation/lesion of cholinergic fibers, a visual/sham training that was either coupled or uncoupled to BF stimulation, a post-training visual acuity behavioral test, VEP recordings, and perfusion/immunostaining (Fig. 1A). BF stimulation was performed in the horizontal limb of the diagonal band of Broca (HDB) because this nucleus provides the majority of the cholinergic innervation of V1 (Gaykema et al., 1990, Laplante et al., 2005). The behavioral test consisted of measuring the visual acuity of the rat for a 30º, 150º or 0º sine-wave grating compared with a gray screen using the visual water maze (Prusky et al., 2000, Dotigny et al.,

2008). The visual/sham training lasted for 14 days and consisted of presenting the rat with equal luminance images of a 30º orientation, 0.12 cycle per degree (CPD) sine-wave grating or the gray screen (control animals) for 10 min/day. We assumed and tested (see results section) that the visual acuity for a 30º orientation is not optimal and could be improved by training whereas detection of 0.12 CPD is optimal and discrimination performance could not be increased at this spatial frequency. Thus, only one feature of the stimulus (orientation) was tested for its potency to improve perception after training. After the pre-training behavioral test and electrode implantation, the rats were divided randomly into eight groups (Fig. 1B): control (CTL, n=7), sham training/no HDB stimulation; VS (n=9), 30º sine-wave grating presentation training/no HDB stimulation; HDB (n=8), sham training/HDB stimulation; VS/HDB (n=8), 30º visual training/HDB stimulation; VS/HDB/SAP (n=4), 30º visual training/HDB stimulation/192-IgG saporin injection in HDB prior to training; VS/HDB/SAPV1 (n=6), 30º visual training/HDB stimulation/192-IgG saporin injection in V1 prior to training; and VS/HDB/Sco (n=4), 30º visual training/HDB stimulation/scopolamine i.p. injection during the post-training behavioral test; VS/HDB/150 (n=7), 30º visual training/HDB stimulation but pre- and post-training visual acuity test made using a 150º orientation- equivalent in terms of salience and efficacy to 30º; VS/HDB/0 (n=4), 0º visual training/HDB stimulation and post-training visual acuity test made using a 0º orientation (see Fig. 1). The two latter groups were aimed at evaluating the orientation specificity of the perceptual changes. Additional VS/HDB (n=4), VS/HDB/SAPV1 (n=6) and CTL (n=5) rats were tested for VEP using a high spatial frequency paradigm (see below).

Figure 1

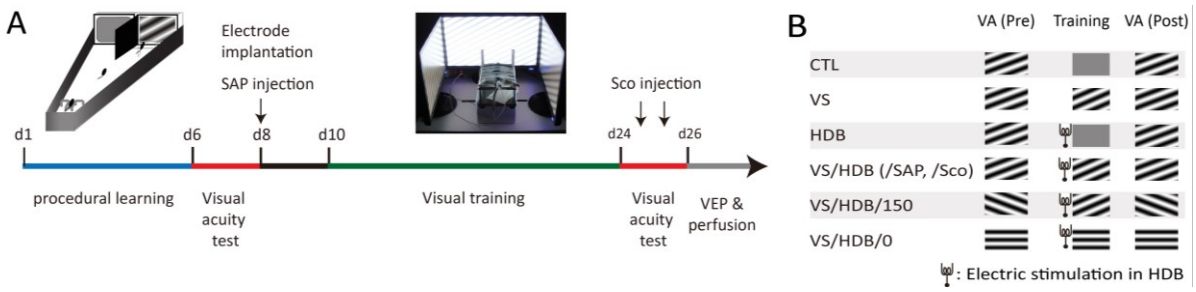

Figure III.1. Timeline of the experimental procedure and stimulus parameter for the different groups.

A) The visual acuity was measured using a visual water maze (upper left schematic). The pre-training value of visual acuity was taken after 6 days of procedural learning before 14 days of visual/sham training in the presence or absence of pairing with HDB stimulation. Visual/sham training was provided 10 min/day for 14 days to awake rats restrained in front of 3 screens (photograph). Electrode implantation and 192-IgG-saporin injections occurred at day 8, and scopolamine was injected in the VS/HDB/Sco group 30 min before the post-training acuity test. Post-training visual acuity was measured after training (d24), and then VEPs were recorded. B) To delineate the function of cholinergic activation during visual stimulation, six paradigms were administered, depending on the experimental group: CTL, sham training/no HDB stimulation; VS, visual training (sine-wave grating presentation training)/no HDB stimulation; HDB, sham training/HDB stimulation; VS/HDB, visual training/HDB stimulation. VS/HDB regimen was also administered in VS/HDB/SAP and VS/HDB/Sco. Additional groups (VS/HDB/150 and VS/HDB/0) were added to determine whether the training was orientation specific.

Visual water maze testing

In order to test the visual acuity and the discrimination ability, the rats were trained and tested in a two alternative forced choice visual discrimination water maze task (Fig. 2A) (Prusky et al., 2000, Dotigny et al., 2008). The task consisted of two visual stimuli; a sine-wave grating and a gray screen. The animal learn to associate a sine-wave grating (0.12 CPD, 90% contrast) with a positive stimulus (submerged platform) and the equiluminance gray screen to the absence of the positive stimulus. Given the larger number of cells in V1 with a preference for a 0º orientation compared to a 30º orientation stimulus (Girman et al., 1999), pilot experiments were performed to determine whether visual acuity was better at discriminating a horizontal (0º) grating from a gray screen compared to discriminating an oblique grating (30º) from a gray screen. This was tested in additional groups of rats (n= 6 / group). All the other rats (except VS/HDB/150) were tested for their ability to discriminate a 30º orientation sine-wave grating from a gray screen during the pre- and post-training periods. The orientation noted as 0º corresponds to the horizontal bar and the angle increases counterclockwise to 90º as represented by the vertical bar.

As described previously, behavioral analysis consisted of procedural learning and visual acuity testing (Prusky et al., 2000, Dotigny et al., 2008). During the visual acuity test, the spatial frequency of the stimulus (from 0.12–0.9 CPD) was increased between blocks of trials until the ability to distinguish the grating from gray screen was equal to 70% performance. The highest spatial frequency achieved consistently was recorded as the acuity threshold. Using the spatial frequency where 70% performance was last achieved is a reliable and effective method of quantifying the visual acuity of our animals. Even though, it may slightly underestimate

their acuity compared to the frequency expected by chance (50%) because the performance declined rapidly at the threshold (Prusky et al., 2000, Dotigny et al., 2008). Beyond the threshold of detectability of the grating, the percentage of success was occasionally under 50 %. This was due to the interruption of some trials because some rats stay in the middle of the water maze, instead of selecting one arm. For that purpose, the performance values below the chance level were considered not reliable and do not reflect any visual discrimination ability. A more detailed explanation about each phase may be found in our previously published study (Dotigny et al., 2008). Using these criterions (Prusky et al., 2000), the testing phase can be completed in 2–3 days (40 trials/days). The visual acuity was defined as the highest spatial frequency value the rat succeeded in discriminating.

Electrode implantation and lesion surgery

After the pre-training test, animals were unilaterally implanted with electrodes for subsequent electrical stimulation of the HDB and VEP recording, and 192-IgG saporin (SAP) injection was performed for cholinergic neuron specific lesions in the appropriate group. Animals were anesthetized with isoflurane (induction 5%, maintain 3%) and placed in a stereotaxic apparatus. Throughout the experiment, the rectal temperature was maintained at 37ºC using a thermostatically controlled heating pad (FHC, Bowdoinham, ME, USA). A dental drill was used to make 2 holes (2 mm in diameter) in the skull above the left visual cortex and adjacent to Bregma to access V1 and HDB, respectively. An electrode guide (polyurethane tubing) was inserted above V1 (mm from Bregma: AP -7.5, ML +4.0, DV 0). A tungsten-stimulating electrode denuded at each tip was implanted in the HDB ipsilateral to the cortical recording site (mm from Bregma: AP -0.3, L +2.0, DV -9.0). In the case of the cholinergic system lesion

groups, the animals received an unilateral intracerebral injection (0.5 µl) of 192-IgG saporin (Advanced Targeting System, San Diego, CA, USA; 0.075 µg/µl) at the location where the stimulating electrode was implanted (HDB) or into V1 (mm from Bregma: AP -7.5, ML +4.0, DV -0.5). Two stainless steel screws (Small parts, Miami Lakes, FL, USA) were installed in the skull, the guides and the HDB implanted electrode were secured with dental cement. After suturing the incised skin, local anesthesia (xylocaine 2%, Astra Zeneca, Mississauga, Canada) was topically administered to the wound, and the animals were returned to their cages. An anti-inflammation agent, carprofen (Rimadyl, 5 mg/kg), was injected s.c. The recording site was identified by electrical lesion after the last VEP recording and confirmed by cresyl violet staining of fixed brain sections. The location of the sites where the electrodes were implanted, was identified in coronal sections using a Leica DMR microscope and the rat brain atlas. The lesion of the cholinergic fibers was evaluated by choline acetyltransferase (ChAT) immunostaining (see below) on coronal brain sections.

Pairing visual/sham training with HDB electrical stimulation

The visual/sham training paradigm was designed to examine whether the selective orientation response could be modified through visual training of a specific pattern and/or through cholinergic neuron stimulation. The stimulus was either a sine-wave grating (0.12 cycle/degree, orientation 30º, phase converting at 1 Hz) for the VS, VS/HDB, VS/HDB/SAP and VS/HDB/Sco groups or a gray screen for the CTL and HDB groups. During daily training, awake rats were restrained for 10 min a day for 14 days with their heads fixed in a frame surrounded by two lateral monitors and an additional monitor facing the rat 21 cm away from its eyes (Fig. 1). The visual stimulus was generated using Vpixx software (v 2.79, VPixx

technologies Inc., Saint-Bruno, Quebec, Canada) and displayed on three monitors (LG, luminance 37 cd/m2). Training was performed daily at the same time in the morning for each rat.

HDB electrical stimulation

The electrical stimulation started at the beginning of the visual stimulation period and was delivered over the 10 min (train of pulses 100 Hz, 0.5 ms, 50 μA, 1 sec on/1 sec off, Pulsemaster A300, WPI, Sarasota, FL) through an isolation unit (WPI 365, WPI, Sarasota, FL). This paradigm of electrical stimulation in the HDB has been designed to activate cholinergic system preferentially to GABAergic system (Vaucher et al., 1997, Kang and Vaucher, 2009).

VEP recording procedure

VEPs were recorded to assess the effect of visual/sham training and unilateral HDB stimulation on field potential cortical responses in the hemisphere ipsilateral to HDB stimulation. Two days after the last session of the behavioral test, the rats were placed in the dark in the stereotaxic frame under anesthesia (isoflurane, induction 5%, maintenance 1.5%). The polyurethane tubing (electrode guide) was removed, leaving a hole in the dental cement over V1. The recording electrode was placed 0.5 mm below the dura. Visual stimuli were displayed on a computer monitor (30x25 cm, Titanium; luminance 21 cd/m2; Apple Computer Inc., Cupertino, CA, USA) placed 30 cm in front of the rat (left eye closed) and centered on the animal's midline (Girman et al., 1999). As described previously (Laplante et al., 2005, Kang and Vaucher, 2009), VEPs were calculated by averaging 20 electrical responses of extracellular field potentials elicited by visual stimuli (oblique sine-wave gratings, orientation

30º, contrast 90%, 0.12 cycle/deg, 100 msec, 0.03 Hz) or gray screen (0 CPD, baseline signal) recorded over 300ms. The signal-to-baseline ratio was obtained by dividing the amplitude (difference between the negative peak and the positive peak) of the VEPs (signal) by the amplitude of the baseline signal (baseline). In additional groups of rats, the evoked responses were elicited by high spatial frequency sine-wave gratings (orientation 30º, contrast 90%, 0.7 CPD). Between each grating, the computer screen displayed a neutral gray stimulus with the same mean luminance. Evoked responses were amplified (5,000X), filtered at 3 Hz ~ 1 kHz (Grass Inc, West Warwick, RI, USA) and collected with the MP100 data acquisition system and Acknowledge software (v 3.8; Biopac system Inc., Goleta, CA, USA). A further 10 min period of visual stimulation was performed to obtain adequate c-Fos expression for immunolabelling. Rats were restrained in the dark for 30 minutes before perfusion.

Immunohistochemistry

The animals were deeply anesthetized with pentobarbital (54 mg/kg body weight i.p.) and perfused transcardially with 4% paraformaldehyde at room temperature. The brains were collected and post-fixed for 2 h in fresh fixative then stored in 0.1 M phosphate buffer (PBS, pH 7.4) overnight. The brains were sliced into 35-μm sections using a vibratome (Leica microsystems). The brain sections at the level of the visual cortex/superior colliculus were used for c-Fos staining (mm from Bregma, AP -7.3 ± 0.5) (Paxinos and Watson, 1995). The sections were collected serially in 24-well plates and labeled accordingly to the antero-posterior level.

Two consecutive sections for each rat were selected according to anatomical features (apparition of the subiculum) and sections were pre-incubated for 20 min at room temperature in phosphate buffer (PBS, 0.1 M, pH 7.4) containing 0.3% hydrogen peroxide, followed by 30 min in PBS containing 0.25% triton X-100 and 0.2% gelatin. The sections were incubated overnight at room temperature with rabbit-anti-c-Fos primary antibody (1:10000, Oncogene Research Products, San Diego, CA, USA) in PBS-triton-0.2% gelatin. This was followed by a 2 h incubation in donkey-anti-rabbit secondary antibody (1:500, Jackson ImmunoResearch, Westgrove, PN, USA) and then for 1 hr in the avidin-biotin complex (ABC Elite kit, Vector laboratories, Burlingame, CA, USA). After each incubation step, rinses were carried out in PBS containing 0.25% triton. A peroxidase-substrate-kit Vector SG (Vector laboratories) was used to visualize the reaction product during a standardized period of 5 minutes. Sections were then mounted onto slides, dehydrated and coverslipped with permount. Quantitative examination of c-Fos immunoreactivity allowed a comparison of the different layers of the contralateral hemisphere with the stimulated eye in VS/HDB and HDB animals. Visualization of the cholinergic fibers loss was performed on coronal sections using ChAT (1:500, Chemicon, Temecula, CA, USA) as described above (Dotigny et al., 2008).

Double immunostaining

In order to determine the cell-specificity of the c-Fos immunoreactive cortical cells, we performed a double immunocytochemical study to examine whether c-Fos was expressed within (1) GABA cells labeled for parvalbumin (PV) and calretinin (CR), markers that covers the labeling of most GABA interneurons of the rat cortex (Gonchar and Burkhalter, 2003), or (2) glutamatergic neurons using rat brain pyramidal cell marker (RBPC), a marker of the

pyramidal cells. The antibodies were used at the following dilution: parvalbumin (1:10000, made in mouse, Sigma, Saint-Louis, MS, USA), Calretinin (1:5000, made in mouse, Chemicon, Temecula, CA, USA), RBPC (rat brain pyramidal cell antigen; 1:1000, made in mouse, Swant, Bellizona, Switzerland). Vibratome sections from the visual cortex were processed with the same protocol as that used for c-Fos immunostaining. Sections were first incubated in the anti-c-Fos antibody and revealed with Vector SG then sequentially incubated overnight in the other primary antibody and revealed with DAB (Vector laboratories). The number of GABA or glutamatergic cells expressing c-Fos was counted in the stimulated hemisphere according to the layer (layer II/III, layer IV and layer V/VI) in the monocular part of V1. The proportion of the double-labeled cells over total c-Fos cells, total GABA or total glutamatergic cells was also evaluated. Total number of cells counted over 7 animals in the VS/HDB and HDB stimulated group is shown in parentheses PV-CR (339), RBPC (2526), c-Fos (6607). The balance between excitation and inhibition system (E/I ratio; number of c-Fos-RBPC neurons/ number of c-Fos-PV-CR neurons) was calculated for each animal and averaged.

Statistical analysis

Pre-training and post-training visual acuity values were compared within each group using a paired Student's t-test. A non-parametric Wilcoxon test was performed for group which has less than 6 subjects (VS/HDB/Sco, VS/HDB/SAP, and VS/HDB/0). Between-group comparisons were computed using a one-way ANOVA followed by a post-hoc LSD test for visual acuity and VEPs. For immunostaining data and E/I ratio analysis, layer-specific comparisons were carried out using the Mann-Whitney U test. All statistical analyses were

carried out with SPSS 17.0 for Windows XP (SPSS Inc., Chicago, IL, USA) at a significance level of $p < 0.05$.

Results

Pairing visual training with HDB activation in awake rats increases visual discrimination performance in a water maze task

To evaluate the effect of pairing visual training with BF activation, visual acuity (the spatial frequency threshold value for discriminating a sine-wave grating from a gray screen) was measured using the visual water maze task before and after the training period (Fig. 1, 2). The procedure of the visual water task was learned prior to visual training, such that post-training visual discrimination task tested changes in visual discrimination rather than procedural learning. Following the results of Girman et al., (1999), we hypothesized the rat's visual acuity for the selected stimulus (30º) could be enhanced by training, since 0º but not 30º is the optimal orientation for eliciting the maximal cortical response in naïve rats (Girman et al., 1999). In agreement with the level of cortical response, the visual acuity for a 30º orientation sine-wave grating (0.70 ± 0.01 CPD) was lower than for a 0º orientation sine-wave grating (0.87 ± 0.02 CPD, paired Student's t-test, $P < 0.001$) in naïve rats (Fig. 2B). Moreover, a ceiling effect of the visual performance at the optimal stimulation was demonstrated, since visual acuity at 0º orientation was not improved after training to a 0º sine-wave grating (pre-training 0.86 ± 0.03 CPD, post-training 0.85 ± 0.03 CPD; $P = 0.87$, VS/HDB/0) (Fig. 2B).

The daily pairing visual training to 30º orientation 0.12 CPD sine-wave grating with HDB stimulation induced a significant increase in visual acuity measured with the 30º sine-wave grating (VS/HDB group, 0.89 ± 0.01 CPD; VS/HDB/Sco group, 0.89 ± 0.01 CPD) compared with pre-training values (0.71 ± 0.02, paired t-test, $P < 0.001$, Fig. 2B). This post-training

Figure 2

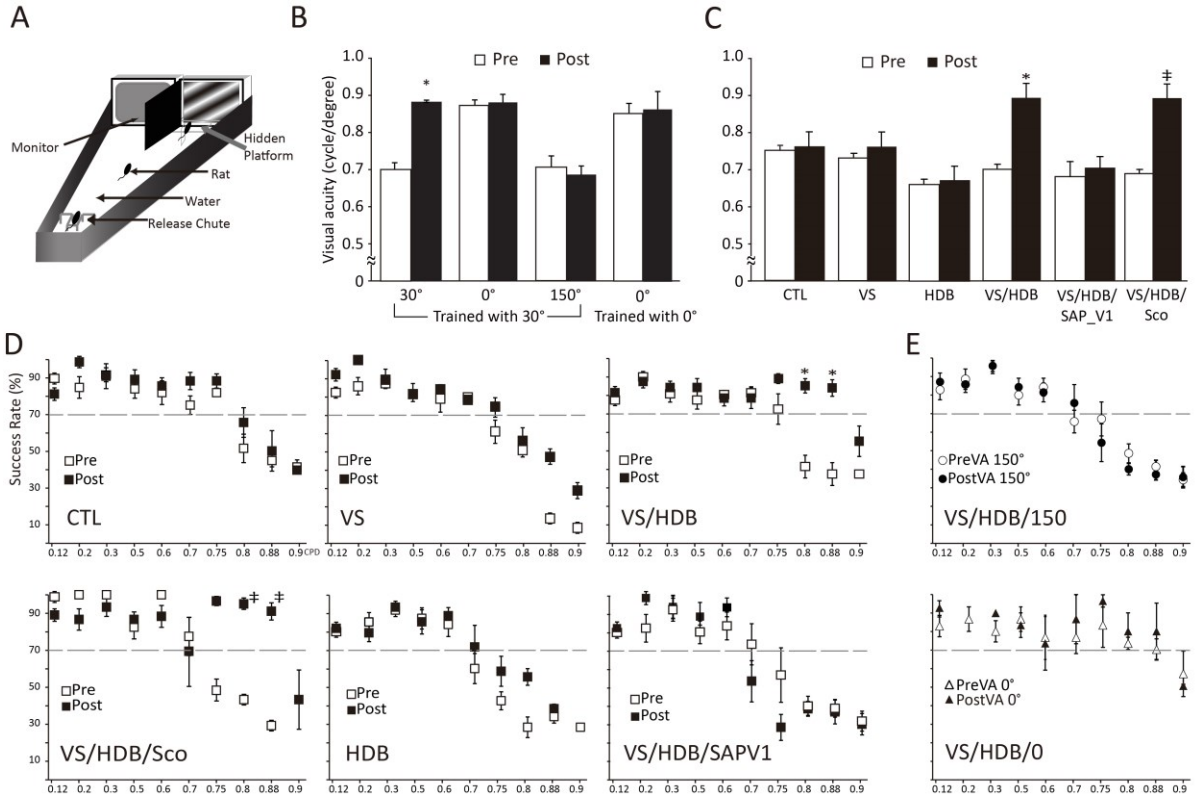

Figure III.2. Measurement of visual acuity in the different groups pre- and post- training.

A) Schematic representation of the visual water maze, (adapted from Prusky et al., 2000). B) 30°, 0°, 150° visual acuity measured after training with 30° stimulus and 0° visual acuity measured after training with 0° stimulus (right histogram). The pre-training (open symbols) visual acuity for a 30° sine-wave grating was lower than acuity for a 0° stimulus, but both values were identical after training in the VS/HDB group. Visual acuity for 150° or visual acuity for 0° was not changed after training with 30° stimulus. C) Pre- and post-training (filled symbols) values of the visual discrimination threshold for each group. Note that only the VS/HDB and VS/HDB/Sco groups showed better discrimination ability after visual training. D) Success rates (percentage of correct trials) for each group in response to each spatial frequency presented. Note that in the CTL, VS, HDB and VS/HDB/SAPV1 groups, the success rate after 0.75 CPD was not statistically different for pre- (open square) and post-training experiments (filled square). By contrast, in the VS/HDB and VS/HDB/Sco groups, the success rate significantly increased (see text for details) after training. (*, pre-post paired t-test, $p<0.05$; ‡, pre-post Wilcoxon test, $p<0.05$). E) Success rates for the discrimination of a sine-wave grating (150°, upper panel and 0°, lower panel) pre- and post-training with 30° stimulus. No statistical increase of visual acuity was found in both groups, suggesting that the improvement of visual acuity does not transfer to other orientations. Error bars represent ± s.e.m (Lack of error bars is because all rats at that point have same success rate).

visual acuity value (0.89 ± 0.01 CPD) reached the ceiling value, as measured with the 0º sine-wave grating in naïve animals (0.87 ± 0.02 CPD, t-test, P = 0.316, Fig. 2B), suggesting that V1 neuronal response to the 30º orientation improved to an extent equivalent to V1 response to the most efficient stimulus (0º). Visual acuity was not affected by sham treatment, by VS or HDB training alone (Fig. 2C, D). Intergroup comparisons showed that post-training visual acuity was greater in the VS/HDB and VS/HDB/Sco groups compared with any other group (one-way ANOVA, F[7,51] = 23.986, P < 0.001 both). As the visual discrimination of a 0.12 CPD grating is optimal and at the ceiling level, we did not observe any improvement of the success rate between groups in pre- vs post-training values at this spatial frequency (Fig. 2D). Thus, the behavioral outcome of the training, as tested with the visual water task, resulted in a shift of the discrimination threshold of the 30º sine-wave grating to high spatial frequency (0.7 to 0.89 CPD). Histological examination confirmed that all the stimulation sites were localized within HDB (Fig. 3).

Pairing visual training with HDB activation in awake rats is specific for cholinergic projections from HDB to V1

Injection of 192-IgG saporin in the HDB (VS/HDB/SAP, pre-post training Wilcoxon, P = 0.431) or in the V1 (VS/HDB/SAPV1, paired t-test, P = 0.771) prior to VS/HDB training significantly destroyed the cholinergic fibers in V1 (Fig. 4) and attenuated the enhancement of visual acuity for the trained stimulus (0.71±0.03 and 0.67±0.03 CPD, respectively) (Fig. 2C, D). The use of mAChR antagonist scopolamine during the post-training testing period (VS/HDB/Sco group) did not impair animal performance for the trained stimulus (ANOVA, post-hoc LSD, P = 0.929, Fig. 2C, D), indicating that improved visual acuity resulted from changes acquired during the training period, rather than from enhanced muscarinic

Figure 3

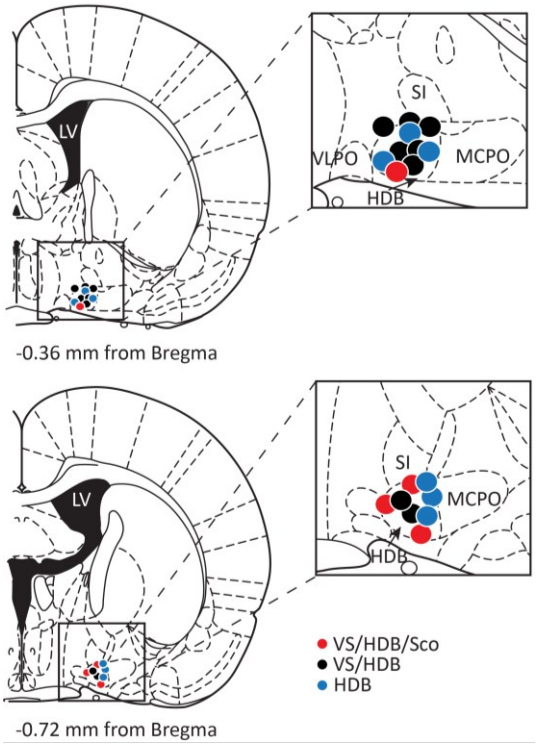

Figure III.3. Localization of the electrode implantation sites on coronal sections of the HDB.

Implanted electrode sites were localized following electrolytic lesion and examination on cresyl violet stained sections. The sites are drawn on the corresponding diagram from the rat brain atlas (Paxinos and Watson, 1995). HDB, horizontal diagonal band of Broca; LV, lateral ventricle; MCPO, magnocellular preoptic nucleus; SI, substantia innominate; VLPO, ventrolateral preoptic nucleus.

Figure 4

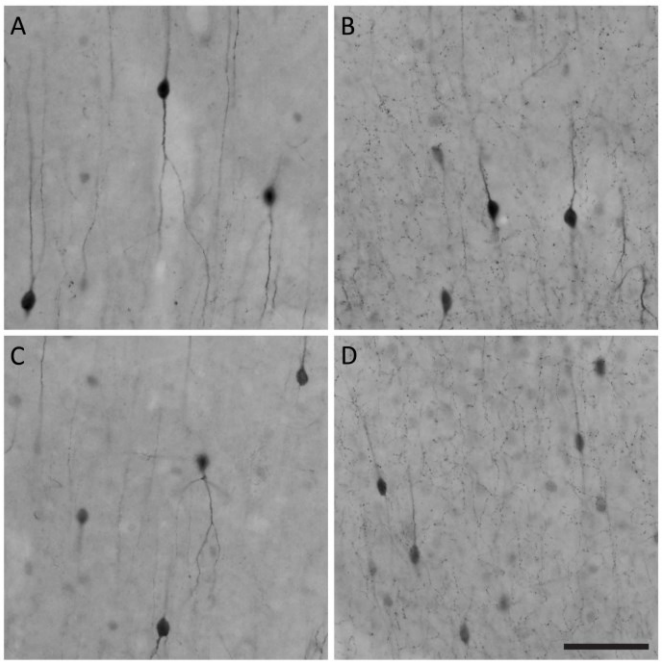

Figure III.4. Microphotographs of ChAT immunolabelling in V1.

A, B) effect of unilateral 192-IgG-saporin injection in HDB on the ipsilateral (A) or contralateral (B) cholinergic innervation in V1. C, D) effect of unilateral 192-IgG-saporin injection in V1 on the ipsilateral (C) or contralateral (D) cholinergic innervation in V1. Strong loss of cholinergic fibers (A, C) was observed in V1 of the injected hemisphere compared to non-injected hemisphere (B, D). Scale bar = 50 μm.

transmission during the test – although nAChR involvement has not been tested. Therefore, these experiments suggest that the effect of repetitive stimulation of the HDB is mainly mediated by the cholinergic neurons projecting to V1 and contribute to the enhancement of visual acuity.

Improvement of visual discrimination performance for a 30º grating by pairing visual training with HDB activation in awake rats does not occur at the expense of – or transfer to other orientations

Additional post-training visual acuity testing using a 0º orientation pattern in the VS/HDB rats was performed to determine whether the improvement of visual discrimination for a 30º orientation pattern was transferred to or did occur at the expense of the response for the 0º orientation. The performance for the 0º orientation was not altered by the daily visual training to 30º orientation 0.12 CPD sine-wave grating paired with HDB stimulation (0.873 ± 0.02 CPD vs 0.87 ± 0.02 CPD, paired t-test, P = 0.9, Fig. 2B). This suggests the 0º orientation selectivity of the V1 cells was preserved. This also suggests that visual discrimination performance improvement elicited by the VS/HDB training does not transfer to other orientations (Fig. 2B, see above). To avoid bias due to a ceiling effect at the 0º orientation, the visual performance for a stimulus with properties similar to 30º, i.e. symmetrical, was tested in VS/HDB/150 group. The visual acuity value for 150º (0.69 ± 0.02 CPD) was equivalent to the one for 30º (0.70 ± 0.01 CPD) in naïve animals. The performance for the 150º orientation was not altered by the daily visual training to the 30º orientation 0.12 CPD sine-wave grating paired with HDB stimulation in VS/HDB/150 animals (0.69 ± 0.02 vs 0.71 ± 0.03 CPD, P = 0.289, Fig. 2B). Thus, improvement in visual discrimination was orientation selective. This is consistent with previous studies showing that increased cortical response for a specific

orientation has not been proved transferable to another orientation (Fiorentini and Berardi, 1980, Cooke and Bear, 2010, 2012).

Pairing visual training with HDB activation in awake rats increases the amplitude of visual evoked potentials in V1

To determine whether the change in visual acuity correlated with a modification in cortical processing, VEPs were recorded in V1 after the training period. The signal-to-baseline ratio of averaged cortical responses to the presentation of the trained stimulus (sine-wave grating, 30º, 0.12 CPD) was significantly greater in the VS/HDB group compared with the CTL group (one-way ANOVA, $F[4,22]=3.977$, $P = 0.02$), the VS group ($P = 0.04$) or the HDB group ($P = 0.048$), but was not significantly different when compared with the VS/HDB/Sco group ($P = 0.368$) (Fig. 5). We further examined in independent experiments whether the repetitive VS/HDB pairing with a 30º, 0.12 CPD stimuli would also enhance cortical reactivity at the higher spatial frequency (0.7 CPD). The VEP amplitude for a 30°, 0.7 CPD stimulus was greater in VS/HDB group compared to the control (VS/HDB 2.37 ± 0.26 vs control 1.22 ± 0.15, one-way ANOVA, $F[2,14]=7.010$, $P = 0.003$) or VS/HDB/SAPV1 (1.7 ± 0.2, $P = 0.042$). This result indicates that the VS/HDB training effect was possibly transferred to higher spatial frequency than 0.12 CPD.

Pairing visual training with HDB activation in awake rats increases c-Fos immunoreactivity in V1

The number of c-Fos immunoreactive neurons was significantly increased in all cortical layers of the stimulated hemisphere of VS/HDB animals (Table 1) compared with the contralateral hemisphere, which did not show c-Fos immunoreactivity. This increase was consistently

Figure 5

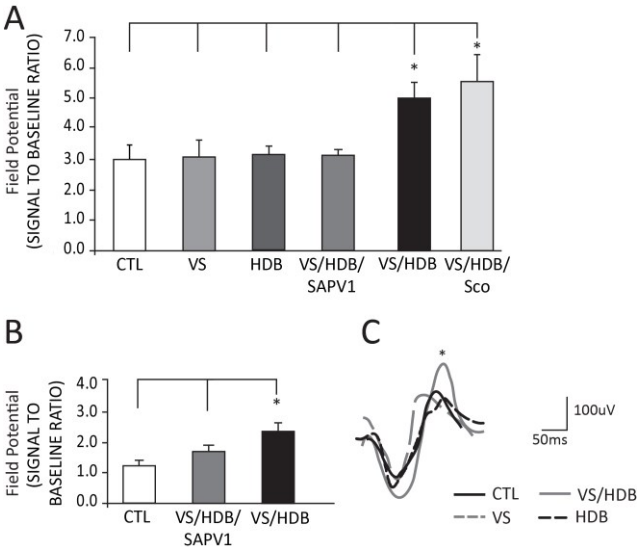

Figure III.5. VEP recording in the different groups after the training.

Amplitude of the VEP recorded during presentation of the 30º-0.12CPD (A) or 30º-0.7CPD (B) sine-wave grating. Neuronal activation was greater in VS/HDB and VS/HDB/Sco groups compared to other groups. (*, ANOVA post-hoc LSD, $P < 0.05$, error bars represent ± SEM). C) Representative traces of VEP recordings of CTL, VS, HDB and VS/HDB groups are shown.

Table 1.

Cortical area	c-Fos	c-Fos-RBPC		c-Fos-PV-CR	
	[6607]	[309]		[126]	
V1 HDB					
Layers II/III	129.0 ± 21.0	5.0 ± 2.8	(3.8%)	2.2 ± 1.2	(31.2%)
Layer IV	100.0 ± 10.2	3.3 ± 1.5	(2.6%)	1.7 ± 0.7	(8.5%)
Layers V/VI	110.0 ± 15.4	14.7 ± 2.8	(8.5%)	2.0 ± 0.3	(22.1%)
V1 VS/HDB					
Layers II/III	194.8 ± 29.3	32.5 ± 15.1 [a]	(25.1%)	4.4 ± 1.5	(41.3%)
Layer IV	201.2 ± 27.7 [a]	19.9 ± 8.6 [a]	(12.3%)	8.7 ± 2.0 [a]	(53.1%)
Layers V/VI	127.1 ± 22.9	13.7 ± 4.1	(10.5%)	5.7 ± 1.4 [a]	(41.1%)

Table III.1 Number and nature of activated neurons in monocular area of the activated visual cortex.

Values are number of cells/mm2 expressed as mean ± s.e.m. Numbers in brackets are total number of cell counted. Numbers in parentheses are the percentage of activated neurons (c-Fos-RBPC/RBPC or c-Fos-PV-CR/PV-CR ratio x 100).
[a], $P < 0.05$, compared to V1M HDB counterparts, Mann-Withney

observed in all of the VS/HDB rats. As previously shown (Dotigny et al., 2008), VS animals exhibited c-Fos labeling in layer IV (thalamus recipient) of the stimulated hemisphere but not in the other cortical layers or the non-stimulated contralateral hemisphere (data not shown). Consistently, c-Fos labeling in the layer IV was stronger in VS/HDB animals compared to HDB animals (Mann-Whitney, $P = 0.016$). Interestingly, as the c-Fos immunoreactivity experiment was performed one week after the last HDB stimulation session (allowing time for behavioral and electrophysiological testing), these results demonstrate that neuronal reactivity in V1 following these training conditions is long-lasting (at least for one week). It has to be noted that only half (4) of animals of the HDB group expressed c-Fos labeling in V1, showing variable labeling in this group. This suggests HDB stimulation enhanced visually-induced c-Fos expression one week after the last HDB stimulation only when the stimulus was paired with sensory stimulus.

Pairing visual training with HDB activation in awake rats activates both GABAergic and glutamatergic neurons in V1

A subpopulation of both pyramidal and GABAergic cells were immunoreactive for c-Fos, i.e. activated, in V1 of VS/HDB animals (Fig. 6A). No c-Fos labeling was detected in control animals (data not shown). The percentage of activated GABAergic and pyramidal cells related to total number of GABAergic and pyramidal cells, respectively, was elevated in all cortical layers of V1 VS/HDB animals (41-53% and 11-25%, respectively), suggesting the involvement of both inhibitory and excitatory cortical microcircuits in the training effect (Table 1). There were significantly more PV-CR or RBPC neurons immunoreactive for c-Fos in layer IV of VS/HDB animals compared to HDB (Mann-Whitney, $P = 0.027$ and 0.002, respectively). In addition, there were significantly more RBPC neurons immunoreactive for c-

Figure 6

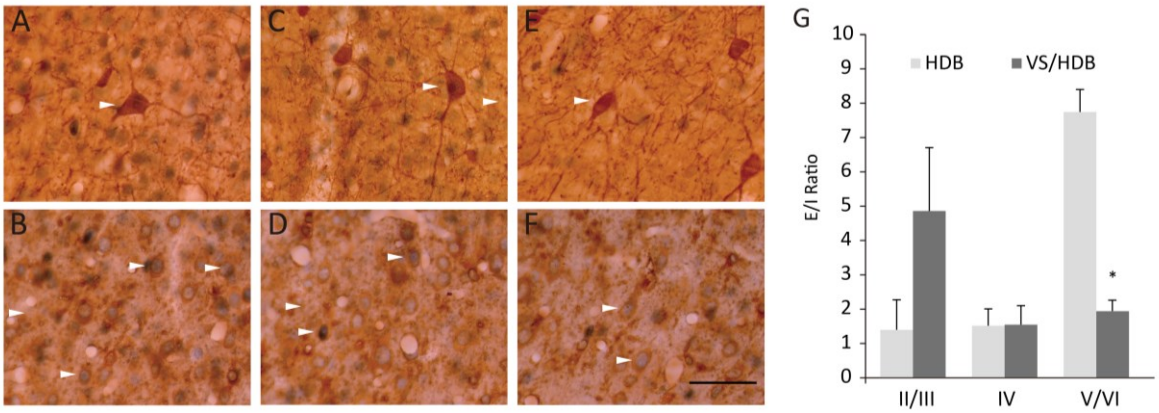

Figure III.6. Neurochemical phenotype of activated c-Fos neurons in V1.

A-F) Microphotographs (40x) of coronal brain sections, double-immunostained (arrow heads) for c-Fos (Vector SG, bluish-gray) and either GABAergic (DAB, brown, upper panel) or pyramidal (DAB, brown, lower panel) neurons (mm from Bregma, AP -7.3). A,C, E) c-Fos-PV-CR neurons in layers II/III (A), IV(C), or V/VI (E) of V1, and B, D, F) c-Fos-RBPC (rat brain pyramidal cell antigen) neurons in layers II/III (B), IV (D), or V/VI (F). The histograms in G) show the mean ± s.e.m of the excitation/inhibition (E/I) ratio – number of c-Fos- RBPC cells / number of c-Fos-PV-CR cells calculated for each rat in VS/HDB group compared to HDB group (taken as a control). Note the E/I ratio was significantly lower in layer V/VI of VS/HDB rats compared to HDB rats, reflecting a greater proportion of GABAergic–cFos activated neurons. Scale bar 50 µm

Fos in layer II/III (P = 0.049) and more PV-CR neurons immunoreactive for c-Fos in layer V/VI (P = 0.038) of VS/HDB animals compared to HDB group. This suggests an effect of VS/HDB training on pyramidal neuron activation, consistently with the flow of cortical activation from the thalamo-cortical recipient layer to superficial cortical layers.

The E/I ratio (average of the ratio of number of c-Fos-RBPC neurons / number of c-Fos-PV-CR neurons) was significantly lower in the layers V/VI of VS/HDB stimulated animals (1.92 ± 0.38) compared to HDB stimulated animals (7.72 ± 0.68, Mann-Whitney, P = 0.034, Fig. 6B). It was not significantly different in layer II/III or IV in VS/HDB compared to HDB group (Mann-Whitney, P = 0.248, P = 1.000, respectively) although there was a non-significant increase of E/I ratio in the layers II/III in VS/HDB.

Discussion

The present study demonstrates that repetitive pairing of a specific visual stimulation with stimulation of HDB cholinergic neurons in awake rats resulted in a selective increase of the visual acuity of the rats. The improvement was selective for the stimulus orientation which was initially non-optimal for eliciting the maximal visual acuity but reached a level of optimal detectability after the training. More precisely, the perception of the trained orientation was improved as shown by a shift of the discrimination threshold to higher spatial frequency, which suggests a transfer of the training effects to a higher spatial frequency than trained. The behavioral improvement was related to a long-term increase in neuronal activity in V1 as recorded by VEP and c-Fos labeling and to a change in the balance of excitatory/inhibitory activity. These results support a role for the cholinergic system in attention and perceptual learning, i.e. acquired experience-dependent plasticity, in V1. Moreover, it suggests that use of cholinergic enhanced visual training in the context of visual rehabilitation could improve visual recovery compared to visual training alone.

VS/HDB activation induces perceptual improvement due to cholinergic activity

This study shows better visual performance of the rats when VS was coupled with HDB stimulation. Although the effects of electrical HDB stimulation could not be attributed solely to the activation of cholinergic neurons, there is considerable evidence suggesting the plasticity observed in this study mainly results from ACh release. First, the HDB electrical stimulation paradigm has been designed to mimic the electrical properties of BF cholinergic cells rather than GABAergic neurons (see Kang and Vaucher, 2009). Second, improvement in

visual acuity and cortical reactivity is completely abolished by specific lesion of the cholinergic cells of the BF or cholinergic fibers in V1. This is in agreement with previous studies (Kocharyan et al., 2008).

VS/HDB activation induces long-term changes in V1 related to perceptual learning and attention

The present results show orientation specific perceptual improvement when VS was coupled with HDB stimulation. Improved visual acuity for the 30º orientation was long-lasting, orientation selective, and preserved the initial visual acuity for the optimal stimulus (0º orientation). The visual acuity for an untrained orientation (0º or 150º) was not affected. Perceptual improvement was associated with stronger long-term V1 reactivity, as shown by increased VEP amplitude and c-Fos expression. These features are similar to perceptual learning resulting from repetitive exposition to a specific stimulus, i.e orientation selective (Fiorentini and Berardi, 1980, Schoups et al., 2001) long-term change of perceptual capacity (Recanzone et al., 1993, Gilbert et al., 2001, Reed et al., 2011, Cooke and Bear, 2012) or performance in a learning task (Andersen et al., 2010, Sale et al., 2011) accompanied by cortical plasticity (Ahissar and Hochstein, 1993, Fahle, 2004). Moreover, our results showing discrete and selective, but not global, changes in stimuli processing are in agreement with improvement of visual capacity relative to perceptual learning (Fiorentini and Berardi, 1980, Gilbert et al., 2001, Li et al., 2004), although the present paradigm is not a pure perceptual learning task. In support of this, it has already been shown that 5-day training with a similar oblique, 0.12 CPD sine-wave grating in rodents is able to induce perceptual learning specific for this trained stimulus (Frenkel et al., 2006, Cooke and Bear, 2010), as measured with VEP recording. It is also possible that VS/HDB training reduced the load of attention required to

detect the 30º orientation, thus facilitating the cortical response to higher spatial frequency. This suggests that our paradigm induced not only perceptual learning-like effect for the 30º orientation grating, but also improved detectability of the pattern at higher spatial frequency, transfer which might be the contribution of the cholinergic activation.

This increase of salience of the 30º orientation pattern resulting in increased visual acuity, is likely due to reinforcement of the cortical microcircuitry by cholinergic HDB projections to V1 through mechanisms related to attention, cortical plasticity (NMDAR dependent-LTP) and perceptual learning (Vidnyanszky and Sohn, 2005, Roelfsema et al., 2010). Attentional processes are strongly regulated by ACh (Sarter et al., 2005), including visual attention. Scopolamine reduces spike firing induced by attentional demand in V1 (Herrero et al., 2008). Thus, in the present study, the cholinergic system might enhance attentional processes in V1 and consequently cortical reactivity. This effect most likely takes place during the VS/HDB training period, since scopolamine administration during the post-training task has no influence on rat performance – it is possible that nicotinic mechanisms could be involved during the post-training task (Bhattacharyya et al., 2012, Disney et al., 2012), which would probably be negligible since no significant ACh release increase is expected during this period. Consequently, the post-training performance of the rats is improved due to induction of long-term mechanisms during the training, i.e. learning. Accordingly, learning processes are blocked by cholinergic lesions or by antagonizing the cholinergic system pharmacologically (Conner et al., 2003), including in V1 (Dotigny et al., 2008). In addition, ACh provides long-term modulation of the electrophysiological properties of V1 neurons as well as LTP (Origlia et al., 2006, Dringenberg et al., 2007, Goard and Dan, 2009, Kang and Vaucher, 2009).

Therefore, ACh release during the training paradigm probably induced persistent change in the efficacy of the microcircuitry neurotransmission. This is evidenced here by the widespread overexpression of c-Fos in response to visual stimulation measured 5 days after the last HDB stimulation, which is not seen in sham or VS animals. At a cellular level, this long-term effect could be related to cortical LTP, i.e., increase in synaptic strength of recipient neurons initially elicited by heterosynaptic stimulation from both HDB and glutamatergic fibers. Accordingly, c-Fos expression is actually an index of LTP (Kaczmarek and Chaudhuri, 1997, Dotigny et al., 2008). It is therefore likely that repetitive HDB stimulation elicits a change in synaptic strength, only when the visual neurons are activated, similarly to what occurs during perceptual learning. Supporting this, VS or HDB stimulation alone does not result in performance improvement, and passive exposure to diverse sensory stimuli does not result in cortical map plasticity (Bao et al., 2001). It would have been expected that VS would also induce a slight improvement of visual acuity because it has been shown to induce perceptual learning (Sasaki et al., 2010) as well as spontaneous release of ACh in V1 (Laplante et al., 2005) . This was not seen in the training set-up tested in the present study probably because 1) the training period was too short, 2) it did not require attention demand or 3) VS-increased ACh extracellular concentration was too low. Usually, longer training periods and enhanced attention during perceptual learning tasks are necessary to elicit perception improvement. It also appears that spontaneous VS-increased ACh extracellular concentration in VS group was too low to induce enhanced performance. In contradistinction, the strong ACh release induced by HDB stimulation during the VS/HDB training might have induced attention-like and LTP-like mechanisms necessary for visual learning. This is in agreement with previous studies suggesting ACh has dose-dependent effects in the neocortex (Oldford and Castro-Alamancos,

2003, Deco and Thiele, 2011, Disney et al., 2012). It is also possible that VS/HDB-released ACh might allow a transfer to higher spatial frequencies by reducing the receptive field of V1 cells (Roberts et al., 2005) which would not be seen in classic perceptual learning tasks. Together, this suggests ACh might accelerate the process of spontaneous visual learning by maximizing the transfer of information for the thalamo-cortical afferent as attention would do and by changing the sensitivity of V1 neurons (Ahissar and Hochstein, 1993, Gilbert et al., 2001, Roelfsema et al., 2010) which results in memory traces.

Changes in responsiveness induced by cholinergic system activation implicates excitatory-inhibitory system

The present study demonstrates changes of excitatory and inhibitory drive against visually trained stimulus (30º, 0,12 CPD) with layer specificity. This result sheds some light on the mechanism of visual performance improvement of the rat. The cholinergic enhancement of feedforward drive might arise from nAChR activation of thalamocortical fibers in layer IV (Gil et al., 1997); from M1 mAChR excitation of glutamatergic neurons in layer II/III and layer IV by facilitating postsynaptic NMDAR opening (Calabresi et al., 1998, Gu, 2003); from disinhibition of intracortical network through M2 mAChR or nAChR receptors located on GABAergic cells (Salgado et al., 2007, Soma et al., 2012) or perisomatic GABAergic terminals (Kruglikov and Rudy, 2008); from disinhibition of feedback cortical control by mAChR located on corticocortical fibers (Gil et al., 1997, Oldford and Castro-Alamancos, 2003); and most likely from a combination of both these processes (Fig. 7). ACh may thus serve to maximize transfer of visual information from the periphery to the cortex by suppressing the dominant intracortical pathway (Oldford and Castro-Alamancos, 2003). After

Figure 7

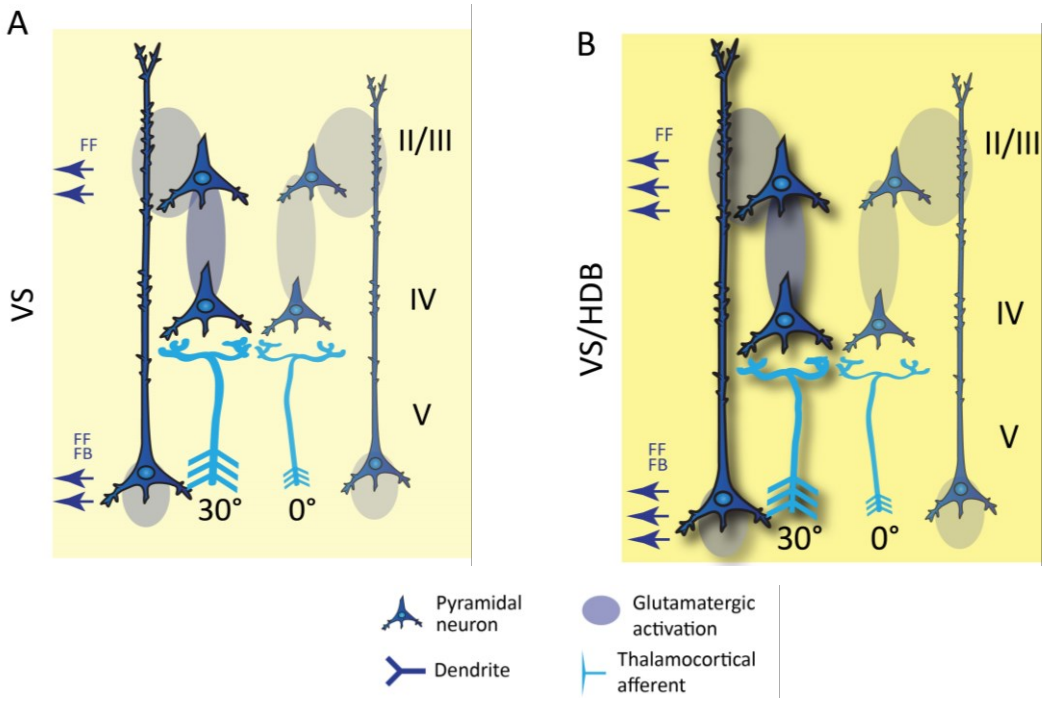

Figure III.7. Schematic representation of the effects after VS

(A) and VS/HDB (B) training on cortical processing of a 30º orientation stimulus. Thalamocortical inputs (light blue fibers) conveying 30º (left) and 0º (right) orientation provide selective bottom-up excitation of layer IV neurons transmitted to layer II/III neurons, then layer V and to higher visual area (FF, feed-forward connections; FB, feed-back connections). Excitatory influences are shown in blue. The larger size of the pyramidal neurons, the stronger neuronal activity. The darker the zone of glutamatergic activation (ovals), the stronger enhancement of the response. Acetylcholine modulation of the neuronal activity during the training is represented by the intensity of the yellow background. This modulation is mediated through muscarinic and nicotinic receptors located on different neuronal elements including glutamatergic and GABAergic neurons (not shown, see discussion for more details). A) Cortical processing for the 30º orientation grating after 2-week VS training is not strong enough to provide behavioral or VEP enhancement. 0º orientation processing is not affected. B) Cortical processing for the 30º orientation grating is significantly enhanced after VS/HDB training but 0º orientation processing is not affected. Note that the input from the thalamus is similar in VS training but the feedforward propagation is not increased nor reinforced.

14 days of training, there was an increase in activated GABAergic neurons relative to activated pyramidal neurons in layer V (reduced E/I ratio in VS/HDB trained group compared to HDB group) and an increase in activated pyramidal neurons relative to GABAergic neurons in layer II/III (increased E/I ratio in VS/HDB trained group compared to HDB group). These results suggest that pairing cholinergic fibers and visual stimulation modulate the activation of excitatory and inhibitory drive with layer specificity. The higher proportion of activated excitatory cells in layer II/III induced by VS/HDB might be due to stronger excitatory drive arising from the layer IV stimulated by VS and reinforced by HDB stimulation and from disinhibition of the GABAergic microcircuits through activation of nAChR in layer I neurons (Christophe et al., 2002). This increased number of activated layer II/III neurons most likely does not result from a spreading of the thalamic influx to adjacent layer IV neurons since ACh is presumed to reduce lateral spread of information. This is however related to visual stimulation since the E/I ratio is decreased compared to HDB stimulated animals. This result is in agreement with previous studies showing cholinergic facilitation of the thalamo-cortical connections and an increase in the firing capacity of layer II/III (Soma et al., 2012, Thiele et al., 2012), although a recent article show suppression mechanisms elicited by the mAChRs (Disney et al., 2012). The influence of the cholinergic system on the inhibitory drive of layer V is consistent with previous studies (Lucas-Meunier et al., 2009) and might be due to enhanced GABAergic activity. Reduced number of activated pyramidal cells in layer V might also result from less specific neuronal activation in layer II/III leading to a reduction of the number of upstream activated neurons. As E/I balance change has been suggested to participate in cortical plasticity (Gandhi et al., 2008, Lucas-Meunier et al., 2009, Yazaki-

Sugiyama et al., 2009, Morishita et al., 2010), the present change might contribute to facilitating cortical reorganization and increasing visual processing to a 30º sine-wave grating.

The improvement in discrimination ability may be attributed to an increase in the number or efficacy of neurons responsive to the 30º orientation. A previous study with VEP and single unit recording revealed that such modulation of orientation appeared not due to change of neurons' preferred orientation but rather to a shift in the orientation index, a relative measure between preferred and orthogonal orientation (Frenkel et al., 2006). According to the modulatory role of the cholinergic system and the absence of enhanced visual acuity in HDB groups, it could be suggested that the cholinergic activation does not change the properties of the cortical cells but reinforce the activity of cells responsive to the 30º orientation. Consequently, VS/cholinergic stimulation results in a change in vertical spread of thalamocortical activation, where a bottom-up excitation of layer IV is transmitted from layer II/III neurons and then to higher visual areas (Callaway, 2004), as shown by the c-Fos results. This is consistent with a recent proposal suggesting that sensory discrimination learning occurs through cortical processing using the most efficient neuronal circuitry rather than permanent wiring changes resulting in changes of selective properties of neurons (Kilgard and Merzenich, 1998, Reed et al., 2011). However, it is unclear whether lateral spreading is enhanced. According to previous studies, high extracellular levels of ACh reduce the lateral spreading of the thalamocortical activation, allowing finer control of the processing of a specific stimulus (Kimura et al., 1999, Silver et al., 2008, Kosovicheva et al., 2012). The improved selectivity for the 30º, but not the 0º or 150º orientation, as well as the excitation of

PV cells (most probably intralaminar basket cells) (Runyan et al., 2010) is consistent with a lack of excitation in neighboring cells with different preferred orientation selectivity.

Conclusion

This study demonstrated that visual training associated with cholinergic transmission builds strong 'memory traces' that result in cortical plasticity in the visual cortex and increased visual perception. These transient mechanisms might be related to attention and induce long-lasting changes in neuronal reactivity. Stimulation of BF afferents during visual training may induce an imbalance in excitatory-inhibitory systems by temporally tuning firing rate and subsequently releasing from a cortical plasticity 'brake' in V1 in the adult. The demonstration of the long-lasting enhancement of perceptual capacities and cortical efficiency following repetitive, coupled cholinergic and visual activation in awake rats provides hope to develop efficient rehabilitation strategies to improve plasticity in cortical areas with impaired sensory input and thus facilitate visual recovery.

Acknowledgments

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CHAPTER IV: Manuscript 3

Pharmacological mechanisms of cortical enhancement induced by repetitive pairing of visual/cholinergic stimulation

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Contributions:

I contributed to the design, experiment, statistical analysis, production and revision of this article.

Dr. Elvire Vaucher supervised, and contributed to the design, experiment, statistical analysis, production and revision of this article.

Dr. Frederic Huppe-Gourgues contributed to the design, Matlab programming, statistical analysis, and revision of this article.

Abstract

Repetitive visual training paired with activation of the cholinergic system that projects to the visual cortex induces an enhancement of the processing of the specific trained visual pattern. In the present study, we investigated whether nicotinic (nAChR) and muscarinic (mAChR) acetylcholine receptors and GABAergic receptors contribute to this long-term effect. Awake adult rats were exposed to an orientation-specific grating paired with an electrical stimulation of the basal forebrain for 10 minutes per day for 1 week. During these sessions of visual training, nAChR, mAChR, $GABA_A$ receptor ($GABA_AR$) antagonists or a $GABA_AR$ agonist were locally injected in the cortex. Pre- and post-training cortical visual evoked potential (VEPs) in the primary visual cortex (V1) were recorded to measure changes in visual acuity. The VEP recordings revealed a long-term increase of the cortical activity of V1 following the coupled visual/cholinergic stimulation, but this increase was blocked when nAChR, M1 subtype mAChR or $GABA_AR$ antagonists were administered. The injection of an M2 mAChR subtype antagonist or $GABA_AR$ agonist decreased the cortical responses to below the control VEPs. These findings demonstrate that visual training coupled with cholinergic stimulation improved perceptual ability by enhancing cortical plasticity in V1. This enhancement is mediated by nAChRs, M1 mAChRs and M2 mAChRs; the latter receptor induces a disinhibition by suppressing GABAergic drive.

Introduction

The cholinergic fibers projecting from the basal forebrain to the primary visual cortex contribute to modulate the integration of visual stimuli. It has been shown that repetitive cholinergic stimulation paired with visual stimulation induces increases in the visual acuity of the rats (Dotigny et al., 2008, Kang et al., 2013) and long-term improvements in the visual ability of humans (Furey et al., 2000, Bentley et al., 2004, Rokem and Silver, 2010). Boosting visual cortex functioning during rehabilitation paradigms via cholinergic enhancement might thus help individuals with cognitive or sensory deficits to better recover their sensory abilities and performance (Kang et al., 2014a).

The neuronal effect of acetylcholine (ACh) on the visual cortex differs depending on receptor subtypes involvement and the location of the receptors (Gil et al., 1997, Ji et al., 2001, Soma et al., 2013a, Groleau et al., 2014). For example, the acute effects of ACh have been shown to increase the thalamocortical signal in layer IV of V1 through nicotinic cholinergic receptor (nAChR)-mediated presynaptic mechanisms (Gil et al., 1997, Disney et al., 2007) and through M1 subtype muscarinic cholinergic receptors (M1 mAChR) that are located on the postsynaptic pyramidal neurons (Mrzljak et al., 1993, Gulledge et al., 2009). The cholinergic system also modulates inhibitory drive by activating GABAergic interneurons (Kocharyan et al., 2008) through nAChRs (Lucas-Meunier et al., 2009, Alitto and Dan, 2012) and M1 mAChRs (Salgado et al., 2007) or by suppressing GABA release through the M2 muscarinic cholinergic receptor (M2 mAChR) (Salgado et al., 2007, Nunez et al., 2012). Basal forebrain stimulation (Goard and Dan, 2009, Bhattacharyya et al., 2013) or the intracerebral injection of cholinergic agonists (Rodriguez et al., 2004) also produced gamma oscillations that reflected

the rhythmic firing of a large number of cortical cells. All of these mechanisms might be related to attention, cortical plasticity and perceptual learning, which respectively improve neuronal responses, neuronal communication and behavioral performance. Therefore, understanding the neuropharmacological mechanisms of the enhancement of visual processing enhancement by ACh might aid the identification of appropriate pharmacological targets for achieving selective effects that boost perceptual learning and visual performance.

Based on these data, the present study was designed to investigate the involvement of the different cholinergic receptor subtypes and the GABAergic system in the cholinergic-dependent enhancement of perceptual ability. The interaction between the cholinergic and the GABAergic systems was specifically investigated because the activation of GABAergic interneurons during sensory processing also induces oscillations in the gamma range (30-90 Hz) (Cardin et al., 2009). Moreover, the involvements of both acetylcholine and GABA in the structural and functional cortical plasticity that allow connectivity changes in perceptual learning processes have been demonstrated (Harauzov et al., 2010, Chattopadhyaya and Cristo, 2012, Lozada et al., 2012, Kang et al., 2013). Daily pairing of visual stimulation with basal forebrain stimulation (VS/HDB) was performed over one week with the simultaneous intracortical infusion of agonists of nAChRs (mecamylamine), M1 mAChRs (pirenzepine), M2 mAChRs (AF-DX116), $GABA_A$Rs (picrotoxin) or a $GABA_A$R agonist (muscimol). The effect of these treatments on visual acuity, cortical activity and neuronal synchronization were measured by comparing the visual evoked potential (VEP) responses in V1 to various spatial frequencies before and after VS/HDB training. Double immunohistochemistry was performed to visualize the relation between M2 mAChRs and parvalbumin (PV) expressing GABAergic

neurons in the rat. Parvalbumin (PV) is expressed in GABAergic neurons (Gonchar and Burkhalter, 1997) which participates in the processing of the visual information, cortical plasticity (Kuhlman et al., 2013) and learning (Donato et al., 2013). The results revealed an increase in the cortical response following repetitive VS/HDB stimulation that was mediated by nAChRs, M1 mAChRs and cortical microcircuit disinhibition via M2 mAChRs. Moreover, time-frequency analyses revealed an increase in neuronal synchronization in the gamma band following the VS/HDB training.

Materials and Methods

Animal preparation

Adult Long-Evans rats (n = 51, 200-225 g) were obtained from Charles River Canada (St-Constant, Quebec, Canada) and were maintained in a 12-h light/dark normal daylight cycle with *ad libitum* access to food and water. The guidelines set by the Canadian Council for the Protection of Animals were followed for all procedures and approved by the local Animal Care Committee, "Comité de Déontologie de l'Expérimentation sur les Animaux" at the Université de Montréal (protocol # 12-172). All efforts were made to minimize suffering and the number of animals used for these experiments.

Experimental design

Recording and injection guide were implanted in the rat V1 prior to VEP recording (day 1). Pre-training VEPs were recorded (day 5) followed by 7 days of visual training (day 7-14). This result was compared to post-training VEPs (day 16) (Fig. 1A). After post-training, recorded rats were euthanized with an overdose of pentobarbiturate injection and perfused with paraformaldehyde 4%.

Implantation surgery

In order to record VEPs an electrode guide was implanted unilaterally in the horizontal limb of the diagonal band of Broca (HDB). For pharmacological injections, a push-pull cannula guide was implanted in the primary visual area ipsilaterally to the HDB stimulation electrode. Animals were first anesthetized with isoflurane (induction 5%, maintain 3%) and placed in a stereotaxic apparatus. Throughout the experiment, the rectal temperature was maintained at 37ºC using a thermostatically controlled heating pad (FHC, Bowdoinham, ME, USA).

Figure IV.1 . Design of the experimental procedure.

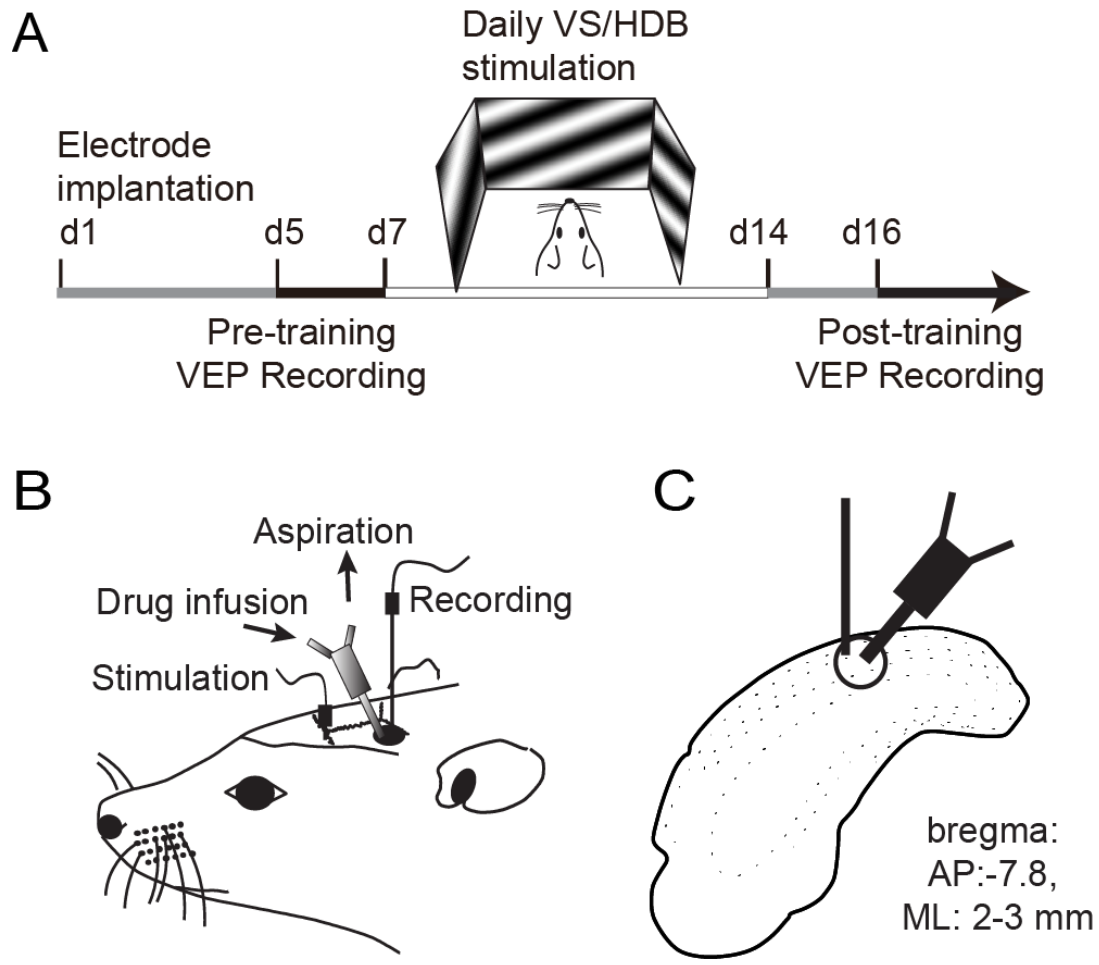

Figure 1. A) Timeline of the different experimental steps. The pre-training visual cortical responses to visual stimulation were recorded 4 days (d5) after the implantation of the electrodes and guide cannulas. Visual training was provided for 10 min/day for 7 days (d7-d14) and followed by the recording of the post-training VEPs (d16) (see text for details). B) Schematic diagram illustrating the chronic implantation of the recording electrode and the push-pull guide cannula in V1. The stimulating electrode was implanted in the HDB. C) Schematic representation of the areas of pharmacological agent injection and electrophysiological recording.

A dental drill was used to make 2 holes in the skull above the left visual cortex and adjacent to Bregma to access V1 and HDB, respectively. The electrode guide (polyurethane tubing) was placed above V1 (mm from Bregma: AP -7.5, ML +4.0, DV 0) and a push-pull cannula guide (Plastics1, Roanoke, VA) was inserted adjacent to the electrode guide (from Bregma: AP -7.5, ML +3.6, DV -0.7 mm, 30° angle from verticality). A tungsten-stimulating electrode denuded at each end was implanted in the HDB ipsilateral to the cortical recording site (mm from Bregma: AP -0.3, L +2.0, DV -9.0). The guides and the HDB implanted electrode were secured with dental cement, and two stainless steel screws (Small parts, Miami Lakes, FL, USA) were installed at the skull surface to hold the dental cement (Fig. 1B). After suturing the incised skin, local anesthesia (xylocaine 2%, Astra Zeneca, Mississauga, Canada) was topically administered to the wound, and the animals were returned to their cages. Prophylactically an anti-inflammation agent, carprofen (Rimadyl, 5 mg/kg s.c.), was administered to the animal. The recording site was identified by an electrolytic lesion made after the last VEP recording and then the electrode placement was confirmed by Cresyl violet staining of the fixed brain sections using a Leica DMR microscope and the rat brain atlas (Paxinos and Watson, 1995). This observation confirmed that the stimulating electrode was implanted in HDB and recording electrode was implanted in V1, respectively. We also verified by injecting Chicago sky blue through the push-pull cannula prior to the perfusion of the rat that the recording electrode in V1 was within the volume of vehicle infusion (Fig. 1C).

VEP recording procedure

The LFP recording method was chosen to observe cortical modification (Frenkel et al., 2006, Kang and Vaucher, 2009). VEPs were recorded ipsilaterally to HDB stimulation, as previously described (Kang and Vaucher, 2009, Kang et al., 2013). For VEP recording, the animal were

Table 1. Experimental groups

Name	Treatment	N
CTL	sham exposure/no HDB stimulation	8
VS/HDB	X° sine-wave grating presentation/HDB stimulation/saline injection	7
VS/HDB/PTX	visual exposure/HDB stimulation/picrotoxin injection	7
VS/HDB/muscimol	visual exposure/HDB stimulation/muscimol injection	9
VS/HDB/PZP	visual exposure/HDB stimulation/pirenzepine injection	8
VS/HDB/AFDX	visual exposure/HDB stimulation/AFDX-116 injection	6
VS/HDB/MEC	visual exposure/HDB stimulation/mecamylamine injection	6

anesthetized (isoflurane, induction 5%, maintenance 1.5%) then placed in a stereotaxic apparatus and kept in the dark. The electrode guide was removed, leaving a hole in the dental cement over V1 where the recording electrode was inserted 0.5 mm below the dura. Visual stimuli were displayed on a computer monitor placed 30 cm at the right side parallel to the animal's midline (left eye closed) and centered on the eye. As described previously (Kang and Vaucher, 2009), the visual stimulation consisted of a sine-wave grating with a 90 % contrast, phase converting at 0.25 Hz or of a baseline control gray screen of 0 cycle per degree (CPD) (Morishita et al., 2010). The electrical signal was recorded for 1500 ms. To avoid an orientation specific bias and verify that the cortical enhancement is not restricted to a specific orientation, we compared the VEP for 3 different orientations (X°: 30°, 45°, 60°) (Cooke and Bear, 2010; Frenkel et al., 2006). Based on the number of neurons responding (Girman et al., 1999), orientations which were shown to evoke a weak response (i.e. 30, 45 and 60, called unoptimal orientation) were selected as X. Since there is a ceiling effect (no improvement of visual acuity possible) for an optimal orientation (Kang et al., 2013) we excluded those orientations (0°: horizontal and 90°: vertical). We tested whether the signal (180° phase shift) to baseline (grey screen) ratio varies among orientations or differs at specific spatial frequency. Nine different spatial frequencies (0, 0.08, 0.12, 0.3, 0.5, 0.7, 0.8, 0.9, 1.0 CPD) of X° and X+90° orientation were presented in a pseudo-random manner. The same orientation (i.e. X°) with 0.12 CPD spatial frequency stimulus was used during visual training (7 days of visual/cholinergic stimulation pairing) and recording of VEP. Evoked responses were amplified (5,000X), filtered at 3 Hz ~ 1 kHz (Grass Inc, West Warwick, RI, USA) and collected with the MP100 data acquisition system and Acknowledge software (v 3.8; Biopac system Inc., Goleta, CA, USA).

VEP analysis

Mean amplitude (signal-to-baseline) of VEPs was calculated by measuring electrical responses of extracellular field potentials elicited by the visual stimuli presentation. Each analysis was performed between 0-500 ms after the stimulus onset. The signal-to-baseline ratio was measured

$$signal\ to\ baseline\ ratio = \frac{(signal\ amplitude - baseline\ amplitude)}{baseline\ amplitude} \times 100$$

and averaged for each orientation and spatial frequency. Signal amplitude was obtained by measuring the difference between the negative peak and the positive peak of the VEPs. Baseline was the mean response of 40 averaged cortical responses while showing grey screen.

Time-frequency analysis

To examine the evolution of VEP phase over time, a short-time Fourier transform spectrogram function was used in Matlab (Mathworks, Nattick, MA, USA). Power spectral density (PSD) matrix was obtained by $P(i,j) = k|S(i,j)|^2$ where k is a real-valued scalar defined as

$$k = \frac{1}{Fs \sum_{n=1}^{L} |w(n)|^2}$$

w(n) was 200, a rectangular window with 100 samples overlap and Fs was the sampling frequency which in this study was 2000 samples/s signal. The frequency resolution and time resolution was 1 Hz and 50 ms respectively. PSD between 30-90 Hz, which corresponds to gamma band, was summed and its expression was analyzed over 500 ms with a time window of 50 ms.

Drug infusion

All drugs were obtained from Sigma Chemical Co and dissolved in a saline solution. Muscimol (GABA agonist: 200 μM), Picrotoxin (GABA antagonist: 100 μM) (Kaur et al. (2004), Pirenzepine (M1 mAChR antagonist: 100 μM) (Sanz et al., 1997), AF-DX 116 (M2

mAChR antagonist: 8 nM) (Douglas et al., 2002), mecamylamine (nAChR antagonist: 10 μM) (Kang and Vaucher, 2009) or vehicle (saline) were freshly prepared and perfused intracortically (i.c., 1 μl/min, 10 min, simultaneously to visual training) using an injection pump (PHD, Harvard Apparatus, Holliston, MA, USA). The push-pull cannula allowed for excess fluids at the injection site to be discarded and limited the accumulation of the drug within the cortex (Figure 1B).

Repetitive visual/cholinergic stimulation pairing

The visual training paradigm was designed to examine whether the selective orientation response could be modified through visual training of a specific pattern and/or through cholinergic neuron stimulation. The stimulus was either a gray screen for the control group or a sine-wave grating (0.12 cycle/degree, orientation Xº, phase converting at 1 Hz) for other groups. Depending on the pharmacological agent injected during visual training rats were divided into seven groups: (Table 1). During daily training, the animals were restrained for 10 min a day for 7 days. The animals were awake with their heads fixed in a frame surrounded by three monitors (2 laterals and 1 frontal) placed at 21 cm away from their eyes (Fig. 1A). The visual stimulus was generated using VPixx software (v 2.79, VPixx technologies Inc., Saint-Bruno, Quebec, Canada) and displayed on the three monitors (LG, luminance 37 cd/m2). This training was performed daily at the same time in the morning for each rat while infusing drugs through push-pull cannula.

HDB electrical stimulation

The electrical stimulation started at the beginning of the visual stimulation period and was delivered for 10 min (train of pulses 100 Hz, 0.5 ms, 50 μA, 1 sec on/1 sec off, Pulsemaster A300, WPI, Sarasota, FL) through a current source (WPI 365, WPI, Sarasota, FL). This

paradigm of electrical stimulation in the HDB is known to activate cholinergic system preferentially to GABAergic system.

Immunohistochemistry

The animals were deeply anesthetized with pentobarbital (54 mg/kg body weight i.p.) and perfused transcardially with 4% paraformaldehyde at room temperature. The brains were collected and post-fixed for 2 h in fresh fixative then stored in 0.1 M saline phosphate buffer (pH 7.4) overnight. The brains were sliced into 35 μm sections using a vibratome (Leica microsystems). The brain sections at the level of the visual cortex were used for double immuno-staining (mm from Bregma, AP -7.3 ± 0.5)(Paxinos and Watson, 1995). The sections were collected serially in 24-well plates and labeled accordingly to the antero-posterior level.

The section were incubated overnight in rabbit-anti- PV (1:5000, Sigma, Saint-Louis, MS, USA) or rat-antiM2 mAChR (1:500, Millipore #MAB367). PV was revealed using an Alexa donkey anti-rabbit 488 (Molecular Probes, Eugene, OR, USA) econdary antibody and M2 mAChR with a donkey anti-rat (Jackson ImmunoResearch, West Grove, PA) antibody coupled with the Streptavidin rhodamine (Jackson).

Confocal microscopy

The fluorescent specimens were visualized using Leica TCS SP2 confocal laser-scanning microscope. Images were acquired using a 40x or 100x oil-immersion objective and were scanned at a 1024x1024 pixel resolution. The stack images were taken sequentially to avoid "bleed-through" effect. Images were captured and exported using Leica LCS software (v. 2.61). Offline processing was done with the ImageJ software (v. 1.48, NIH, USA).

Statistical analysis

Pre-training and post-training VEPs were compared within each group using a paired Student's t-test. The difference in the amplitude of the VEPs and PSD of time-frequency analysis between each group was tested by one-way ANOVA followed by post-hoc Dunnett test. All statistical analyses were carried out with SPSS 19.0 for Windows 7 (SPSS Inc., Chicago, IL, USA) at a significance level of $p < 0.05$.

Results

Equivalent VEPs between non-optimal orientations

To avoid an orientation-specific bias (Frenkel et al., 2006, Cooke and Bear, 2010), 3 different orientations (30°, 45°, and 60°, termed X°) were used for VEP recording. There were no differences in VEP amplitude in response to contrast reversion for any of these three selected orientations (Fig. 2A); thus, the individual VEP data were further pooled together into an X° orientation group. Moreover, there were also no significant differences in the VEP amplitudes for the X+90° stimuli (i.e., 120°, 135° and 150°; data not shown) that were used to test the orientation selectivity of the enhanced responses to the trained stimuli. These values were also further pooled together for the X+90° orientation group.

Increase in VEP amplitude following VS/HDB stimulation pairing

The optimal VEP responses in the pre-training recordings were obtained for repetitive 0.08 and 0.12 CPD (151–386 % greater than the baseline amplitudes; Fig. 2A, C). The VEP responses were not significantly different from the baseline level at > 0.7 CPD (2–55 % greater than the baseline amplitudes), which thus represents the visual acuity threshold in normal conditions. There were no post- vs. pre- sham-training changes in VEP amplitude in the CTL group for any of the spatial frequencies studied (Fig. 2B, C). However, the VEP response to X° was significantly increased (250–602 % greater than baseline) in the repetitive VS/HDB stimulation group in the post- vs. pre-training tests (86.5 – 392.5 %, paired t-test, $p = 0.026$; Fig. 2D), and the post-training responses of this group were also greater than those of the CTL group (135.0 – 399.8 %, one-way ANOVA, $F [6, 50] = 9.156$, post-hoc Dunnett's test, $p = 0.049$). This increase in VEP amplitude in the VS/HDB group was not only observed

Figure IV.2 Effects of repetitive VS/HDB stimulation on VEP amplitudes.

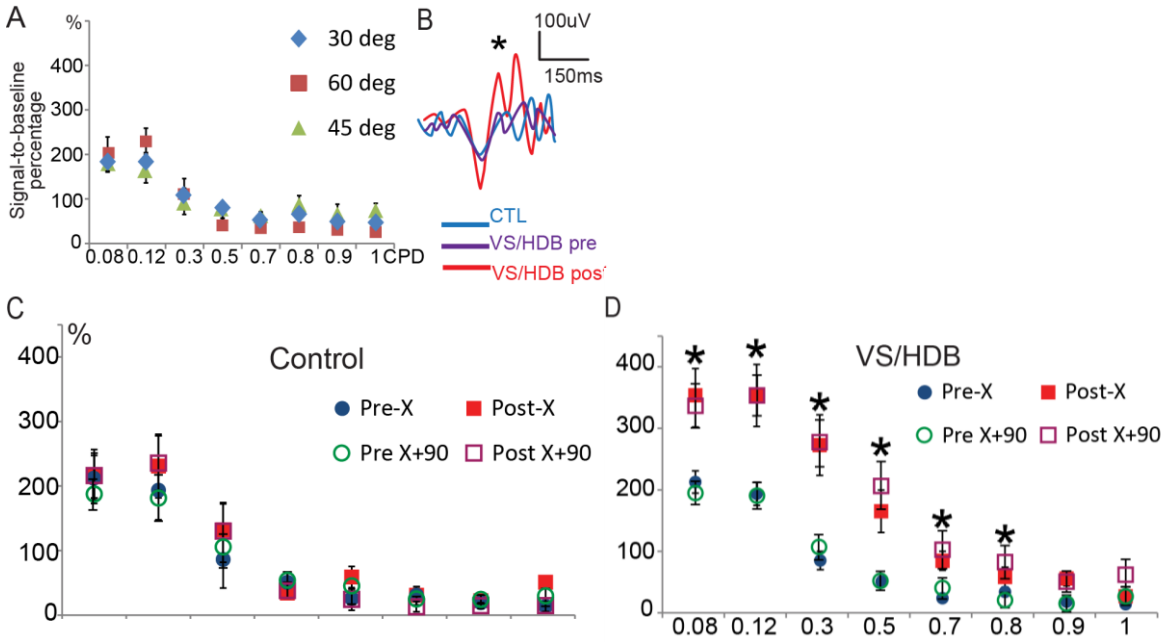

Figure 2. A) Basal VEPs (signal-to-baseline ratios) in response to 30°, 45° and 60° stimuli orientation recorded prior to any experimental procedure. There were no differences in VEP amplitudes between the orientations, which were subsequently pooled into the X° and X+90° groups. B) Representative VEP signal traces in response to a 0.12 CPD grating for the control, pre- and post-training VS/HDB groups. Note that the visual cortical response increased after the VS/HDB training. C) VEP amplitudes from the CTL (sham training) animals in response to different orientations and spatial frequencies. There were no significant differences between the pre- and post-training values. D) VEP amplitudes in the repetitive VS/HDB stimulation (training) animals in response to different orientations and spatial frequencies. Visual/cholinergic training induced increases in VEP amplitudes in response to the exposure of stimulus (0.12 CPD) and higher spatial frequency stimuli (0.3-0.8 CPD). This enhancement was also transferred to the un-exposed orientation (X+90°; *, pre-post paired t-test, $p < 0.05$). The error bars represent ± the s.e.m.

for the trained spatial frequency (0.12 cpd) but also for higher spatial frequencies (0.3, 0.5, 0.7 and 0.8 CPD) compared to pre-training results (Fig. 2D, paired t-test, $p = 0.021$, $p = 0.02$, $p = 0.021$ and $p = 0.016$, respectively) or the CTL group (Fig. 3A, one-way ANOVA, $p = 0.009$, $p < 0.001$, $p = 0.023$ and $p = 0.016$, respectively). These results are indicative of an increase in V1 responses to the exposed stimulus and a transfer of this enhancement to higher spatial frequencies following repetitive VS/HDB stimulation. We also observed that the VS/HDB stimulation effect was transferred to other orientations (i.e., X+90°; Fig. 2D). The increase was significant compared to the pre-training result (0.12 CPD, paired t-test, $p = 0.038$). The transfer also occurred at high spatial frequencies from 0.3 to 0.8 CPD ($p = 0.013$, $p = 0.005$, $p = 0.044$, and $p = 0.016$).

The VS/HDB stimulation effect is mediated by nAChR and mAChR action on the GABAergic system

To evaluate the different pharmacological players in the effect of VS/HDB stimulation in V1, we locally administered different agonists and antagonists of the cholinergic and GABAergic receptors into V1. To varying extents, all of these drugs blocked the enhancement of the VEP ratio that was induced by the repetitive VS/HDB stimulation.

Blockade of nAChRs (mecamylamine: VS/HDB/MEC) or M1 mAChRs (pirenzepine: VS/HDB/PZP) during each VS/HDB stimulation period disrupted the VEP amplitude enhancement for the trained spatial frequency such that these VEP amplitudes were not significantly different from the control level (ANOVA, post-hoc Dunnett's, compared to CTL, $p = 0.985$, $p = 0.965$, respectively) (Fig. 3). These findings suggest that nAChR and M1 mAChR stimulation probably mediate the effect of the cholinergic system on enhancing the VEP response to the trained stimulus in a long-term manner. The disruption of the

Figure IV.3 Changes in VEP amplitudes following pharmacological modulation during visual/cholinergic stimulation.

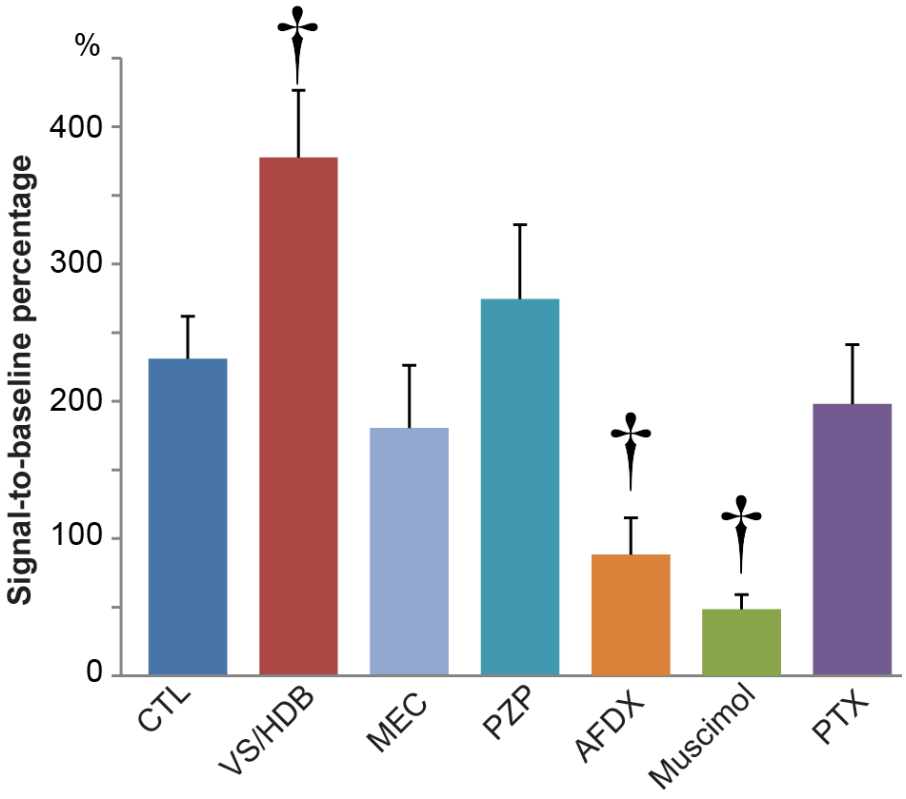

Figure 3. The histograms represent the VEP signal-to-baseline percentages for the different groups for the trained spatial frequency of 0.12 CPD. Note that the VEP amplitude enhancement following the visual/cholinergic training was blocked by PTX, PZP or MEC injection, while muscimol and AF-DX 116 decreased the VEP amplitudes. (†, $p < 0.05$, significantly different from control group, one-way ANOVA). The error bars represent ± the s.e.m. (Abbreviations; MEC: mecamylamine; PZP: pirenzepine; AFDX: AF-DX 116; PTX: picrotoxin)

enhancement of the VEP amplitude by nAChR or M1 mAChR antagonists was also observed for all of the tested spatial frequencies (>0.8 CPD). Antagonism of the M2 mAChR (AF-DX116) during each VS/HDB stimulation period not only disrupted the VEP amplitude enhancement for the trained spatial frequency but also significantly reduced the VEP amplitude compared to the basal level (CTL group; one-way ANOVA, post-hoc Dunnett's test compared to CTL, $p = 0.032$; Fig. 3). This reduction was also significant compared to the result prior to the administration of the drug (paired t-test, $p = 0.011$). These results suggest that the M2 mAChR likely mediates the effect of the cholinergic system on the enhancement of the response to the trained stimulus in a long-term manner and also contributes to the cortical response to the trained stimulus itself.

We further injected GABAergic agonists and antagonists to examine whether GABAergic neurons were involved in the VEP enhancement due to VS/HDB. Muscimol (a $GABA_AR$ agonist, VS/HDB/Muscimol group) injection during each VS/HDB stimulation period produced results that were similar to those of AF-DX116 injection (Fig. 3); i.e., not only was the VEP amplitude enhancement for the trained spatial frequency disrupted, but there was also a significant reduction in VEP amplitude compared to the basal level (CTL group, post-hoc Dunnett's test, $p = 0.003$). $GABA_AR$ activation also decreased the cortical response compared to the pre-training level (paired t-test, $p = 0.003$). The decrease in VEP was apparent only around the trained stimulus (i.e., at 0.08 and 0.12 CPD). In contrast, $GABA_AR$ inhibition via picrotoxin injection (VS/HDB/PTX) blocked the VEP enhancement such that the VEP response was not significantly different from the control level (paired t-test, $p = 0.343$).

Figure IV.4 Microphotographs of parvalbumin and M2 mAChR double-immunostaining of coronal brain sections.

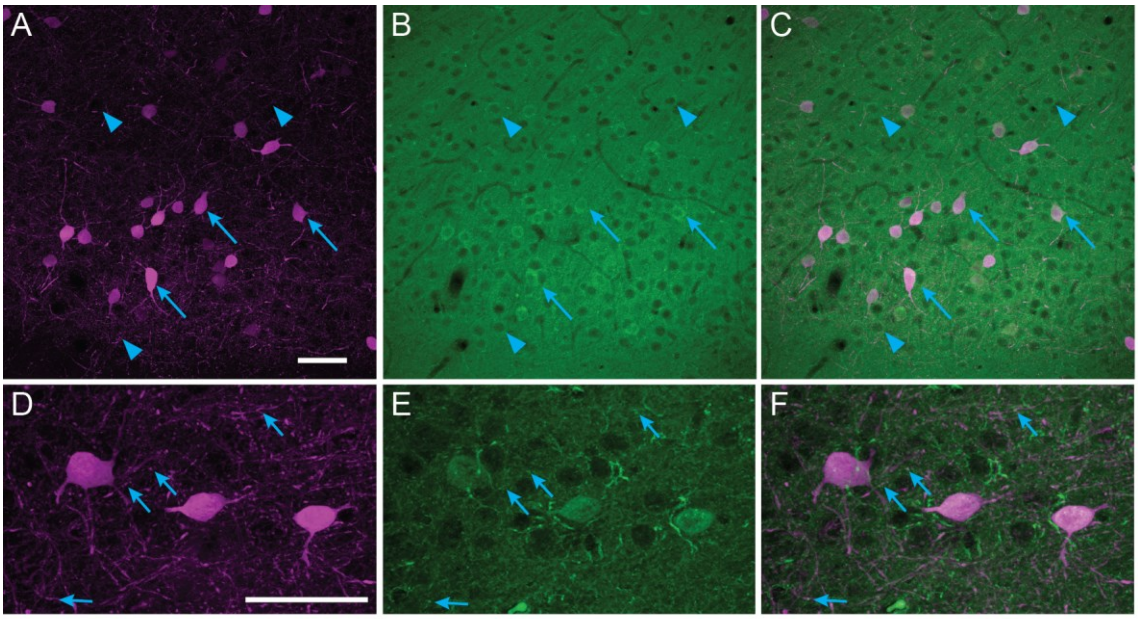

Figure 4. Fluorescent images were observed under a 40x (A-C) and 100x (D-F) magnification. Most of parvalbumin positive neurons or varicosities (magenta; A and D) were double-immunostained (arrows) with M2 mAChR (green; B and E), whereas some M2 mAChR neurons did not co-express parvalbumin (arrowheads). The scale bar is 50 μm.

The presence of M2 mAChRs on GABAergic neurons

Double-label immunofluorescence of PV and M2 mAChR revealed that M2 mAChRs were expressed on the surface of most of the PV-positive neurons and some PV varicosities in V1 (Fig. 4), as already shown in the primate (Disney et al., 2006, Disney and Aoki, 2008). M2 mAChRs positive neurons were occasionally expressed by some PV-negative cells. M2 mAChRs have been shown to reduce GABA release, which suggests the possibility that the AF-DX116 effect might occur via disinhibition of GABAergic interneurons.

Gamma band cortical oscillations increase following repetitive VS/HDB stimulation

To evaluate the frequency changes in the cortical response to the visual stimulation, we performed a time-frequency analysis utilizing a short-time Fourier transformation of the VEP results (50 ms time window) and compared the power spectral densities (PSDs). Because significant differences in the PSDs were not observed across the spatial frequencies (data not shown), the data were pooled together, and only the orientations were analyzed in relation to time. The spectral analyses of the VEP results revealed that, 2 days after the repetitive VS/HDB stimulation, the neuronal activation in the gamma band frequency began to increase at 100 ms after the stimulus onset (one-way ANOVA, $F [6, 53] = 2.052$, post-hoc Dunnett's test, $p= 0.032$, compared to CTL) and remained significantly different until 400 ms after the stimulus presentation (Fig. 5B, C). This effect was abolished by each of the drugs, which is suggestive of a combined action of ACh and GABA. These results suggest that the enhancement of the cortical response after VS/HDB pairing was correlated with an increase of neuronal synchronization in the gamma band frequency.

Figure IV.5 Time-frequency VEP analyses.

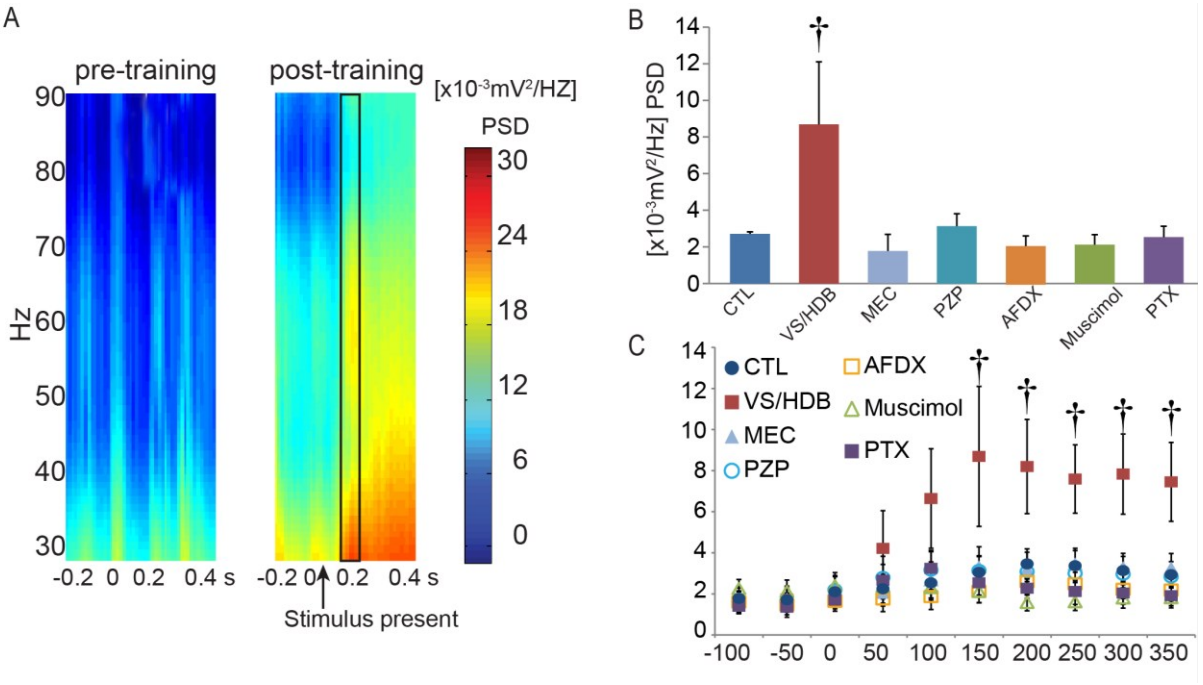

Figure 5. A) Representative comparison of the power spectral densities (PSDs) of the pre- and post-training VEPs from the VS/HDB group as analyzed with the short-time Fourier transformation. The gamma band oscillation (30-90 Hz) gradually increased and reached a plateau 150-200 ms after stimulus onset (square on the figure). B) Comparison of the PSDs in the 150-200 ms time window after the stimulus presentation. Only the VS/HDB group exhibited an increase in gamma band oscillations (†, one-way ANOVA, $p < 0.05$).

Discussion

The purposes of the present study were to (1) examine the long-term effects of pairing visual stimulation with electrical HDB stimulation (VS/HDB) on visual efficacy as measured with VEP responses and (2) to determine the neurotransmitters that are involved in this potentiation effect. Our results revealed that repetitive VS/HDB stimulation induced an increase in VEP amplitude that was sustained across subsequent visual stimulations. This increase was disrupted when nAChR, M1 mAChR or $GABA_AR$ antagonists were simultaneously applied in the VS/HDB stimulation regimen. Moreover, M2 mAChR antagonists and $GABA_AR$ agonists not only prevented VEP potentiation but also decreased VEP amplitudes compared to the control levels. Together, these results suggest that pairing visual stimulation with HDB stimulation can boost the V1 response to subsequent visual stimuli. This effect was mediated by M2 mAChRs via the disinhibition of excitatory cells and by nAChRs and M1 mAChRs via the facilitation of excitatory inputs.

Repetitive VS/HDB stimulation increases sensitivity to visual stimuli

We analyzed whether the visual training might enhance visual acuity as indicated by larger responses to high spatial frequencies as measured by VEP recording. The increased amplitudes of the VEPs in response to 0.12-0.7 CPD stimuli observed in the present study are indicative of enhanced visual acuity, although visual acuity was not directly behaviorally measured. These results agree with those of one of our previous studies that showed that repetitive VS/HDB stimulation induces an increase in visual discrimination capacity that is not restricted to the trained stimulus but extends to higher spatial frequency stimuli (Kang et al., 2013). Furthermore, the improvement in visual discrimination shown in this previous study was correlated with an increase in VEP amplitudes (Kang et al., 2013; Pearson's correlation,

R=0.725, p=0.001). It is probable that cholinergic stimulation during visual training improves stimulus sensitivity (Wesnes et al., 1983, Froemke et al., 2013) or cortical plasticity (Morishita et al., 2010). For example, the deletion of the lynx1 gene, which produces Lynx1 that prevents cortical plasticity via nAChR inhibition, reverses the loss of visual acuity in mice that are raised in monocular deprivation. Although direct evidence relating VEPs to perceptibility is needed, the increase in the electrophysiological response shown here might reflect an increased number of synchronized neurons during visual processing (Niedermeyer and Lopes da Silva, 2005, Nunez and Srinivasan, 2006). Overall, the cortical modifications that result from visual training might increase stimulus sensitivity, facilitate discrimination and increase the perceptibility of visual stimuli.

The second main result of the present study revealed that repetitive VS/HDB stimulation induces a long-term increase in the V1 neuronal response to subsequent visual stimuli because the VEP recordings were performed 2 days after the repetitive stimulation paradigm was stopped. Several recent studies have demonstrated that pairing cholinergic and sensory stimulation can induce perceptual learning (Rokem and Silver, 2010, Bhattacharyya et al., 2013, Froemke et al., 2013, Kang et al., 2013), and these results are comparable to the results presented here. Other studies have also showed that repetitive visual stimulation can increase long-term electrophysiological responses after perceptual learning induction in other modalities (Frenkel et al., 2006, Cooke and Bear, 2010). Together, these studies and the current results suggest that repetitive VS/HDB stimulation can improve perceptual abilities by eliciting long-term increases in the cortical responses to subsequent visual stimuli.

The increases in gamma band oscillations shown in the present study suggest that the VEP enhancement effect is correlated with synchronized neuronal activity. It has been suggested

that oscillation in the gamma frequency reflects cognitive activities such as the processing of sensory input (Cardin et al., 2009) or attention (Fries, 2009). Previous studies have also demonstrated that cholinergic stimulation can increase gamma band activity (Rodriguez et al., 2004, Pafundo et al., 2013) in a manner that is correlated with the enhancement of visual encoding (Goard and Dan, 2009) or contrast sensitivity (Bhattacharyya et al., 2013). In the present study, we observed that only the pairing of visual and cholinergic stimulation increased gamma band activity and the VEPs. This finding suggests that VS/HDB pairing promotes long-term synchronization of neuronal activities and amplifies visual information for transfer to higher cortical areas.

Although structural perceptual learning tasks should *stricto sensu* be selective to the trained attributes, we did measure a transfer of the VS/HDB stimulation effect to the untrained orientation (i.e., X+90°). Some studies have reported that ACh release increase neurons' orientation selectivities (Sillito and Kemp, 1983, Murphy and Sillito, 1991), and other studies have shown that the cholinergic effect results in a decrease in orientation selectivity (Muller and Singer, 1989, Zinke et al., 2006, Bhattacharyya et al., 2013). It can be deciphered that ACh release-dependent amplification of VEPs might be transferred to other neurons with different orientation preferences through attentional priming (Anton-Erxleben and Carrasco, 2013). The cholinergic action on GABAergic neurons (Disney et al., 2012, Nunez et al., 2012, Kang et al., 2013, Yang et al., 2014), which is essential for orientation tuning (Atallah et al., 2012, Lee et al., 2014), might also be the reason for transfer. It is possible that visual training induces synaptic changes that enhance sensitivity to visual information without being orientation-specific.

Pharmacological mechanisms involved in repetitive VS/HDB stimulation

The similar reductions of the VEPs by $GABA_AR$ activation and M2 mAChR inhibition observed in the present study suggest that M2 mAChR activation might reduce GABAergic drive. This putative role is supported by the anatomical co-localization of M2 mAChRs with GABA neurons found here and in previous studies (Disney et al., 2006). VS/HDB stimulation thus disinhibits excitatory neurons via the M2 mAChR-mediated inhibition of GABAergic neurons as already suggested (Salgado et al., 2007, Nunez et al., 2012). Alternatively, excitatory AChRs are expressed at the cell surface of GABAergic neurons (e.g., M1 mAChR: Salgado et al. (2007) or nAChR: Ji et al. (2001), Alitto and Dan (2012)), which could explain the reductions in VEP amplitudes to below control levels following the blockade of M2 mAChRs. This process might have resulted to changes to the weights of cholinergic inputs to the GABAergic neurons in favor of an excitatory effect. Surprisingly, $GABA_AR$ inhibition did not induce a significant increase in VEP amplitudes. This lack of effect could be related to the involvement of a reduction of GABAergic function in plasticity in the adult V1 (Harauzov et al., 2010). Given that neuronal activities are increase during VS/HDB pairing (Kang and Vaucher, 2009), it is possible that $GABA_AR$ inhibition elevates the lateral competition between excitatory neurons or up-regulates the baseline activity, which masks VEP enhancement.

The injection of a nAChR antagonist abolished the VS/HDB training effect, which is consistent with the findings of a previous study (Kang and Vaucher, 2009). It has been shown that nAChRs regulate glutamate release in the sensory cortex (Lambe et al., 2003, Metherate and Hsieh, 2003, Konradsson-Geuken et al., 2009). Because nAChRs are primarily found in the presynaptic thalamocortical afferents in layer 4 (Gil et al., 1997, Disney et al., 2007), this

regulation could be a priming event that enhances the effect of repetitive VS/HDB stimulation. Because the activation of nAChRs has been proposed to be crucial for the induction of the recovery of visual acuity (Morishita et al., 2010), the increase in thalamocortical responses mediated by nAChRs might also be essential for the perceptual learning that is induced by VS/HDB stimulation.

Similar to nAChRs, the antagonism of M1 mAChRs blocked the effects of VS/HDB training. Compared to nAChRs, M1 mAChRs are widely distributed in V1 (Lucas-Meunier et al., 2009), and their functions vary depending on location (Gil et al., 1997). Although we cannot determine the exact actions of M1 mAChRs, they might act through a post-synaptic activation that would emphasize feedforward transmission (Gulledge et al., 2009) or through a pre-synaptic reduction of inhibitory lateral connections that would decrease the lateral spread of thalamocortical inputs (Kimura et al., 1999) and prevent competition between feedforward excitations. Moreover, it has been demonstrated that the deletion of the M1 mAChR gene increases the receptive fields of V1 neurons (Groleau et al., 2014); i.e., the overlapping areas between neurons thus decrease the efficacy of feedforward connections due to competition. Despite this technical limitation, it is likely that pre-synaptic M1 mAChRs focus thalamocortical inputs and promote the transmission of visual training effects by increasing the feedforward response at the post-synaptic level.

In summary, ACh and glutamate release during repetitive VS/HDB stimulation enhance feedforward processing through (i) nAChRs, increasing thalamocortical transmission; (ii) M1 mAChRs, resulting in a restriction of lateral spread); and (iii) M2 mAChRs, which may disinhibit neighboring neurons and enhance cortical activity during visual processing via the

inhibition of GABAergic drive. Visual function is amplified by the synchronized neuronal activity in the gamma band that is induced by VS/HDB pairing. Repetitive VS/HDB stimulation is a novel method that can improve visual capacity by facilitating perceptual learning.

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CHAPTER V: Article 4

Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception

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Review article

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Number of words: 11,876
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Abstract

The cholinergic system is a potent neuromodulatory system that plays critical roles in cortical plasticity, attention and learning. In this review, we propose that the cellular effects of acetylcholine in the primary visual cortex during the processing of visual inputs might induce perceptual learning; i.e., long-term changes in visual perception. Specifically, the pairing of cholinergic activation with visual stimulation increases the signal-to-noise ratio, cue detection ability and long-term facilitation in the primary visual cortex. This cholinergic enhancement would increase the strength of thalamocortical afferents to facilitate the treatment of a novel stimulus while decreasing the cortico-cortical signaling to reduce recurrent or top-down modulation. This balance would be mediated by different cholinergic receptor subtypes that are located on both glutamatergic and GABAergic neurons of the different cortical layers. The mechanisms of cholinergic enhancement are closely linked to attentional processes, long-term potentiation and modulation of the excitatory/inhibitory balance. Recently, it was found that boosting the cholinergic system during visual training robustly enhances sensory perception in a long-term manner. Our hypothesis is that repetitive pairing of cholinergic and sensory stimulation over a long period of time induces long-term changes in the processing of trained stimuli that might improve perceptual ability. Various non-invasive approaches to the activation of the cholinergic neurons have strong potential to improve visual perception.

Keywords: attention, cholinergic system, cognitive enhancement, cortical plasticity, nicotinic receptors, muscarinic receptors, perceptual learning, visual cortex.

INTRODUCTION

Boosting the brain's functioning during rehabilitation paradigms might help individuals with cognitive or sensory deficits to better recover their abilities. In this review, we will examine how the cholinergic system might help in this regard by specifically focusing on visual function. Recent knowledge about the cellular and functional organization of the primary visual cortex (V1) is particularly interesting for the deciphering of the neurobiological mechanisms of perceptual learning and its modulation by the cholinergic system. V1 is the first cortical step of the integration of complex visual stimuli and is decisive in the selection of specific stimuli from the visual field. This process further orients processing in higher cognitive cortical areas involved in elaboration of fine visual conscious perception. Thus, cholinergic modulation of visual processing in V1 would have strong effects on the fine-tuning of perception and the acquisition of memory traces.

Perceptual learning is the long-term improvement of the ability to detect or discriminate specific sensory stimuli without interfering with or diminishing other skills that results from training over a sustained period of time (Fahle and Poggio, 2002, Fahle, 2009, Roelfsema et al., 2010). In vision, improvements in the discrimination of specific attributes of a stimulus, such as its orientation (Ramachandran and Braddick, 1973, Fiorentini and Berardi, 1980, Mayer, 1983), contrast (Hua et al., 2010) or vernier acuity (McKee and Westheimer, 1978), have been demonstrated using such paradigms. Increases in visual capacity should go together with increases in the numbers of neurons that encode the trained stimulus in the V1 and the expansions of the cortical maps that represent the stimulus (Kilgard and Merzenich, 1998). The signal-to-noise ratio is usually increased. The connectivity between neurons and

efficiency of the neuronal transmission, i.e. the strength of the input they transmit as well as the short processing time, should also be increased. Changes in dendritic spines number, morphology and synaptic plasticity (i.e., long-lasting modifications of the strength of the post-synaptic electrical signal) have also been demonstrated during perceptual learning (Gilbert and Li, 2012). However, it should be assumed that the neurons involved in perceptual learning increase the amount of information that they carry while preserving their primary selective response properties (Gilbert et al., 2001). Perceptual learning is also facilitated either by attention (Ahissar and Hochstein, 1993) or reinforcement by reward expectation (Seitz et al., 2009); both of these process enhance neuronal transmission efficiency.

Perceptual learning or increased cortical processing of specific stimuli is generally achieved with repetitive training. It has been recently suggested that it can also be boosted by neuromodulation and extrinsic control of the cerebral neuromodulatory systems by electrical or pharmacological means. The cholinergic system, which uses acetylcholine (ACh) as a neurotransmitter, is particularly relevant because it widely innervates V1 and alters the efficiency of neurons. The injection of ACh or its analogs into V1 has been shown to increase neuronal responses and trigger synaptic plasticity (Gu, 2003) and cortical plasticity (Bear and Singer, 1986). More specifically, the administration of ACh during visual processing increases thalamocortical input while reducing intracortical recurrence (Gil et al., 1997, Disney et al., 2007, Soma et al., 2013a) and thus enhances specific stimulus processing and output. This diversity of the actions of ACh is due to the ubiquitous localization of both ionotropic nicotinic receptors (nAChRs) and metabotropic muscarinic receptors (mAChRs) in V1 (Levey et al., 1991, Disney et al., 2006, Amar et al., 2010), which are involved in the facilitation of

cortical activity and synchronized cortical activity. In addition to the direct and acute effects of ACh, an increasing number of studies have recently shown that repetitive cholinergic activation of the visual cortex has also the ability to enhance visual perception. The repetitive pairing of ACh release with exposure to a visual stimulus improves several visual capacities, such as contrast sensitivity (Mayer, 1983, Hua et al., 2010), motion detection (Rokem and Silver, 2010), working memory (Furey et al., 2000, Bentley et al., 2004), texture discrimination (Beer et al., 2013) and visual acuity (Kang et al., 2013) in both humans and animals. Many animal studies have also demonstrated the involvement of the cholinergic system in perceptual learning in different sensory modalities, including olfaction (Wilson et al., 2004) and audition (Bakin and Weinberger, 1996). These improvements suggest that paired visual and cholinergic stimulation induces perceptual learning possibly via synaptic and cortical modifications linked to attention mechanisms (Herrero et al., 2008) or reward expectation (Chubykin et al., 2013) and cortical plasticity. The repetition of such pairings would result in a more efficient processing and increased automaticity of visual stimuli. This could be related to reduced strength of connectivity between attention regions and V1 (Ricciardi et al., 2013) and a role of ACh in perceptual inference and repetition suppression (Moran et al., 2013).

Our research hypothesis proposes that cholinergic effects in V1 contribute to perceptual learning and can thus be used to voluntarily develop one's brain capacity and aid the restoration of visual function. In the present review, we will discuss how ACh might improve perceptual capacities, particularly during repetitive stimulation paired with visual stimulation, which are related to its roles in the long-term enhancement of cortical responsiveness and

Figure V.1 Hypothesis of the effect of the cholinergic system on visual perception.

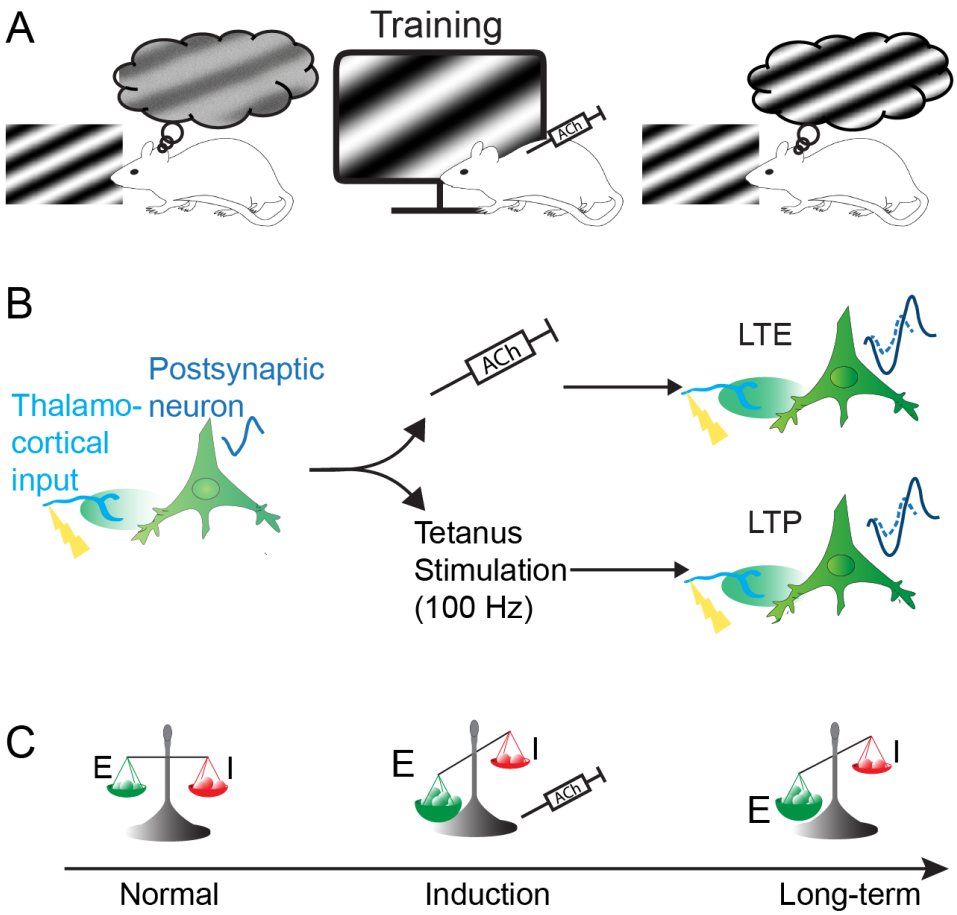

Figure 1. Increase of perceptual capacity (perceptual learning) can be obtained by naturally or artificially activating the cholinergic system during sensory training. This perceptual learning might be achieved by long-term facilitation of cortical responses and/or change of the excitatory/inhibitory balance. (**A**) Representation of the improvement of visual perception in the rat by pairing the presentation of a specific sinusoidal grating coupled to cholinergic system activation (represented by injection of acetylcholine, ACh) (**B**) Long-term enhancement (LTE) of the cortical responses by acetylcholine (upper path) share common features with classical long-term potentiation (LTP, lower path): visual stimulation of presynaptic input evokes small responses (represented by a resulting small VEP signal waveform) in post-synaptic neurons. If paired to cholinergic activation, the presynaptic stimulation induces a long-term enhancement of neuronal responses (upper path, represented by an increased VEP signal waveform). This mechanism is similar to LTP where theta-burst stimulation (100 Hz) in lateral geniculate nucleus induces an increase of postsynaptic potentiation in the cortex (lower path). VEP signals are imaginary waveform to compare neuronal response magnitude, as recorded in our previous experiments. (**C**) Cortical plasticity induced by ACh could also result from a change in excitatory and inhibitory balance by changing the strength of the excitatory synapse over inhibitory synapses, resulting in long-term modification of cortical responses.

cortical plasticity (Fig. 1). Specifically, we will first discuss the diverse effects of ACh on V1 neuron function and connectivity and relate these effects to the background theory of the cholinergic modulation of neural mechanisms and brain function. To assess these neuronal mechanisms, we will primarily discuss studies that have been performed in rodents and non-human primates (for more information about cholinergic effects on human cognition, see Drevets et al. (2008), Bentley et al. (2011)).

ORGANIZATION OF THE CHOLINERGIC SYSTEM IN V1

Cholinergic fibers are distributed throughout the cortical layers of V1 (Lysakowski et al., 1989, Avendano et al., 1996, Mechawar et al., 2000), which suggests that ACh might affect every step of visual processing (Fig. 2A).

Local effect of the cholinergic fibers

The cholinergic system influences the local network by diffuse transmission rather than by synaptic transmission (Descarries et al., 1997, Yamasaki et al., 2010). This property is related to the fact that ACh is released from the varicosities that are distributed along the cholinergic axons and that these varicosities show only rare synaptic organizations at the ultrastructural level (Umbriaco et al., 1994, Vaucher and Hamel, 1995, Mechawar et al., 2000). However, the modulation of the cortex by ACh is not widespread and is primarily selective and adapted to the local microfunction due to the differential distribution of varicosities along the cholinergic axons (Zhang et al., 2011) and the differential distribution of the cholinergic receptor subtypes on different neuronal targets. Moreover, ACh release might be triggered by local neuronal activity to induce locally restricted rather than generalized action of the cholinergic system (Laplante et al., 2005).

Figure V.2 Schematic representation of the primary visual cortex (V1) and its cholinergic modulation on cortical processing.

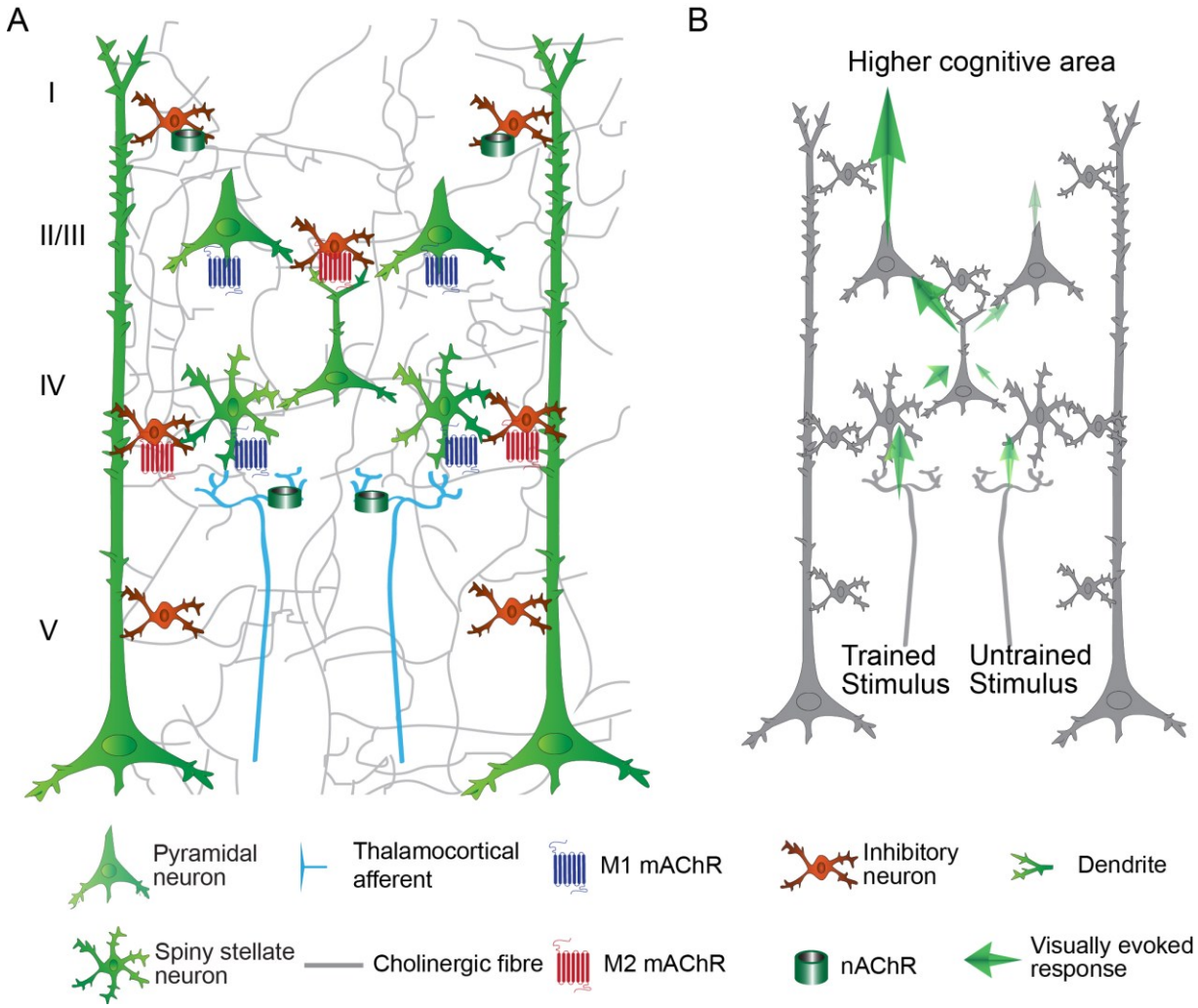

Figure 2. (**A**) Thalamocortical afferent (light blue fibers) from lateral geniculate nucleus conveying stimulus information reach spiny stellate neuron in the layer IV. The input is transferred to the layer II/III, then layer V and to higher visual area. The cholinergic activation modulates the visual processing in virtually all the levels of V1 connectivity by nicotinic (green cylinder) and muscarinic (7 transmembrane domains molecules) receptors. B) Cortical processing after VS/HDB training. The cortical processing for the trained stimulus is significantly enhanced after VS/HDB training but un-trained stimulus processing is not affected. Note that the input from the thalamus is similar but the feedforward propagation is increased. Excitatory influences are shown in blue arrows. The strength of the response enhancement is represented by the contrast of the arrow. Layer VI and horizontal connections are omitted for clarity.

The variety of the cholinergic receptors and their distributions convey subtype-specific functions (Thiele, 2013, Groleau et al., 2014). In V1, AChRs exhibit differential subtype densities across the cortical layers (I-VI) on both excitatory (Gulledge et al., 2009, Thiele, 2013) and inhibitory neurons (Hashimoto et al., 1994). The distinct actions of cholinergic receptors can be related to differences in the conductances of the ionotropic receptor nAChRs for Na^+, K^+ ($\alpha_4\beta_2$) and Ca^{2+} (α_7) (Rang, 2003) and in the intracellular pathways of the different subtypes of the G-protein coupled mAChRs. Amongst the five mAChR subtypes identified, the M1, M3 and M5 mAChRs are coupled with Gq/11 proteins, which activate phospholipase C and lead to increases in intracellular Ca^{2+} and the M2 and M4 mAChRs are bound with Gi protein that inhibits adenylyl cyclase, which leads to a decrease in cAMP, the inhibition of voltage-gated Ca^{2+} channels and an increased K^+ efflux (Caulfield and Birdsall, 1998, Wess, 2003). In addition, M1 promotes the opening of NMDARs and induces LTP in the hippocampus (Buchanan et al., 2010, Giessel and Sabatini, 2010) .

Cholinergic fibers activation in V1

Stimulation of the cholinergic system in V1 can be achieved via the administration of ACh analogs (e.g., carbachol), cholinergic receptor agonists (e.g., nicotine and selective mAChR drugs) or cholinesterase inhibitors or through electrical or optogenetic stimulation of the cholinergic neurons that project to V1. The cholinergic neurons that project to V1 are located in the basal forebrain (BF), particularly the ventral pallidum, substantia innominata and the horizontal limb of the diagonal band of Broca (HDB) (Gaykema et al., 1990, Laplante et al., 2005). Although the nucleus basalis magnocellularis is the main cholinergic nucleus of the BF which innervates the cortical mantle, it projects only weakly to V1 (Luiten et al., 1987,

Vaucher and Hamel, 1995); nevertheless, some studies report that the stimulation of this nucleus might induce functional changes in the visual cortex (Goard and Dan, 2009, Pinto et al., 2013). Moreover, although there are GABAergic neurons in the BF, many studies have confirmed that the effects of BF stimulation are identical to those of intracerebral injections of ACh agonists and are primarily mediated by the cholinergic fibers (Dauphin et al., 1991, Ma and Suga, 2005, Dringenberg et al., 2007, Kocharyan et al., 2008, Kang and Vaucher, 2009). There are also intrinsic cholinergic neurons that represent only 10-15% of the total cortical innervation (Eckenstein et al., 1988, Chedotal et al., 1994), and the involvement of these neurons in cortical processing remains unclear.

ACETYLCHOLINE MODULATES THE FLOW OF VISUAL INFORMATION IN V1

The efficiencies of the cortical inputs and outputs are altered by the different cholinergic receptors in both the glutamatergic and GABAergic systems according to the cortical layer, neuron and receptor subtype reached by ACh (Fig. 2). V1 integrates visual information via different pathways that include the following: the feedforward thalamocortical pathways, V1 intracortical connectivities, and the feedback influence from higher cortical areas (Fig. 3). The visual information arriving to layer IV of V1 from the lateral geniculate nucleus (LGN) is considered to be the dominant thalamocortical visual pathway. In contrast, the intracortical pathway might arise from neighboring neurons, local recurrent axons or more broadly from horizontal networks. The cholinergic system induces facilitation, suppression or does not affect the visual cells. Direct local effects of ACh might be opposed to the indirect effects of ACh due to neuronal interactions across layers. The general picture of the cholinergic influence on V1 is that the response to a stimulus is increased by cholinergic modulation in the

Figure V.3 Neuronal connectivity within the primary visual cortex (V1).

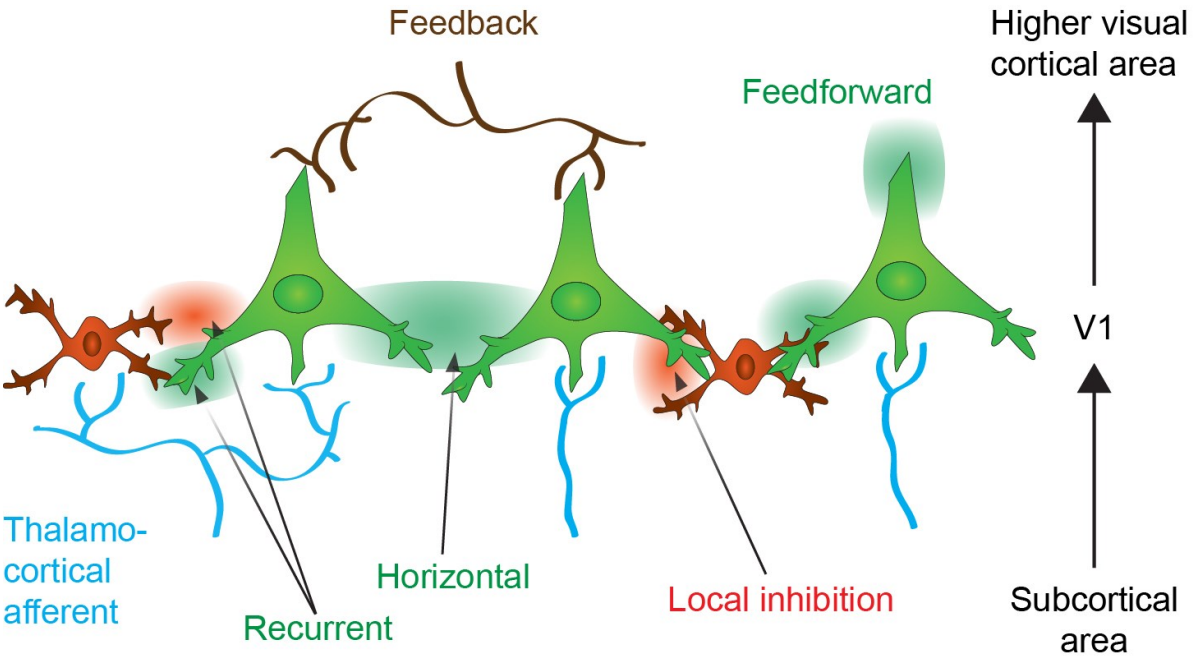

Figure 3. Neurons from V1 receive thalamocortical (in blue) and corticocortical inputs originating from upper cortical areas (feedback control, in brown). The thalamocortical information is integrated within V1 and further transmitted to upper cortical areas (feedforward transmission). The activation of neurons might enhance activation or inhibition of neighboring neuron by the horizontal connections or through the local inhibitory interneurons. Recurrent connections auto-regulates neuronal activity (see text for more details). Excitatory effect is expressed as green color and inhibitory effect as red.

thalamocortical pathway while the intracortical influence is suppressed. The cholinergic influence described in the following paragraph represents the acute effects in V1 that can participate in attention and trigger perceptual learning. The effects of the cholinergic system on long-range corticocortical relationships are also of interest but are beyond the scope of this review.

Cholinergic modulation of thalamocortical inputs

Cortical responses to sensory stimuli transmitted by the LGN are amplified during learning and experience-dependent plasticity to emphasize relevant information (Sarter et al., 2005, Wang et al., 2013). These thalamic afferents are of prime relevance because they define the receptive fields and other properties of V1 neurons. Complex information is extracted according to its properties (e.g., orientation) via projections to different columns (in primates) or specific cells (in rodents). Cholinergic activation in this layer induces a general increase in responsiveness regardless of the features of the visual stimuli (e.g., orientation; Disney et al., 2012), which allows the cortex to respond reliably to weak stimulation (Disney et al., 2007). ACh increases the thalamocortical input through presynaptic nAChRs on the thalamocortical fibers (Gil et al., 1997, Disney et al., 2007) (Fig. 2A, 4). The M1 mAChR also amplifies the spiny stellate cell/pyramidal cell response through a postsynaptic intracellular pathway (Gu, 2003), but inhibition through the M4 mAChR has also been observed on spiny neurons in the somatosensory cortex (Eggermann and Feldmeyer, 2009). Interestingly, the cholinergic facilitation of thalamocortical inputs in sensory cortex slices is ACh-concentration dependent. High doses of ACh enhance the thalamocortical afferents both *in vitro* and in computational models (Hasselmo, 2006, Deco and Thiele, 2011). Together, these results indicate that, under

conditions of high levels of ACh release, the enhancement of the thalamocortical inputs in layer IV facilitates the transmission of sensory information and induces experience-dependent plasticity (e.g., learning).

Cholinergic modulation of intracortical interactions

In addition to the enhancement of thalamocortical inputs, ACh might modulate intracortical connectivity either by suppressing lateral inhibition (Kimura and Baughman, 1997, Metherate et al., 2005, Metherate, 2011) or suppressing the spread of the excitation of thalamic inputs (Kimura et al., 1999, Silver et al., 2008). The presynaptic mAChRs that are located on the glutamatergic fibers induce a suppression of the intracortical neurons (Gil et al., 1997), although the inhibition of GABAergic terminals induces a disinhibition of the pyramidal cells (Ji and Dani, 2000, Christophe et al., 2002, Seeger et al., 2004, Salgado et al., 2007). Intracortical connectivity modulates the response intensity and the output of V1 neurons (Fig. 3). The lateral connections also synchronize the firing of similar neuronal populations (Gilbert and Wiesel, 1989, Lien and Scanziani, 2013), which allows for lateral correlation between neurons with similar orientation preferences during typical perceptual learning tasks (e.g., the Vernier acuity test) (Ramalingam et al., 2013). The differential action of ACh on lateral connections might simultaneously enhance specific modules of the same orientation (lateral correlation) while depressing adjacent irrelevant modules (McGuire et al., 1991, Stettler et al., 2002). A recent study using optogenetics showed that inhibition of the intracortical excitatory neurons leads to a receptive field reduction (Li et al., 2013), and this finding is consistent with the effect of ACh release in V1 (Roberts et al., 2005, Zinke et al., 2006) and the increases in the population receptive fields of M1/M3 mAChR knock-out mice (Groleau et al., 2014).

Furthermore, an ACh esterase inhibitor reduces surround suppression in a perceptual study in humans (Kosovicheva et al., 2012), which could be indicative of a weakening of lateral connections. Hasselmo (2006) proposed that high ACh levels suppress the magnitude of feedback excitation, whereas low ACh levels result in weaker afferent input to the cortex. Similarly, Deco and Thiele (2011) also proposed that high ACh levels decrease the intracortical interactions and that low ACh increase these interactions. The hypothesis of these authors was confirmed in an *in vitro* study that showed that the enhancement of the recurrent cortical activity in low-dose ACh conditions was independent of the thalamocortical input (Wester and Contreras, 2013). Together, these results suggest that during intense ACh release, the intracortical connections are inhibited, which relieves the sensory cortices from recurrent connections. However, in low concentration ACh situations, the lateral connections might amplify the thalamocortical activity amongst similarly tuned neurons.

These effects have primarily been recorded within layer II/III; however, in layers I, V and VI, which are primarily involved in feedback mechanisms, ACh might also influence feedforward processing by interacting with neurons in layers IV and II/III (De Pasquale and Sherman, 2012). Layer I neurons are densely innervated by the cholinergic projections (Vaucher and Hamel, 1995, Mechawar et al., 2000). It has been shown that inhibitory actions mediated by AChRs can suppress layer II/III (Zinke et al., 2006, Alitto and Dan, 2012, Soma et al., 2013b) and layer V pyramidal neuron activity (Lucas-Meunier et al., 2009, Amar et al., 2010) and can also inhibit the cortical GABAergic network and thus result in the disinhibition of the majority of the cortical layers (Christophe et al., 2002). It has been observed that local ACh application primarily suppresses the activity of layer VI neurons (Disney et al., 2012), which can alter the

activation of all of the layers of V1 in a linear manner via the intracortical pathway (Olsen et al., 2012) and alter the activation of the thalamocortical fibers (Cudeiro and Sillito, 2006, Sillito et al., 2006). Cholinergic action might thus disinhibit the activities of other layers by suppressing layer VI. Topical injections of ACh into layer V produce the predominant effect of facilitation of the regular and fast-spiking cells (Soma et al., 2013b), although local ACh activation seems to decrease excitatory drive through presynaptic M1 mAChRs (Kimura and Baughman, 1997) and to increase inhibitory drive through M3 mAChRs (Amar et al., 2010). Similarly, an increase in the activation of GABAergic neurons activation in layer V has been observed following repetitive BF/visual pairing (Kang et al., 2013). Layer V pyramidal neurons send dense projections to the superior colliculus and diverse thalamic nuclei that are involved in focused attention.

Finally, ACh can promote the co-activation of different cortical areas and layers which might be an efficient method for the selection of visual information via a summation of the temporally coincident presynaptic spikes (Fries et al., 2007). It has been shown that visually driven gamma power is differentially distributed across the layers of V1 (Xing et al., 2012) and that gamma oscillations can be induced by cholinergic stimulation (Rodriguez et al., 2004, Bhattacharyya et al., 2013).

In conclusion, basal forebrain stimulation that facilitates the release of ACh in multiple layers of V1 might act in diverse manners and results in the enhancement of visual stimulus-driven responses. The pre-amplified responses of layer IV are filtered out by GABAergic neurons of layer II/III to transfer task-relevant information to higher visual cortical areas. The activated

synaptic connections can be modulated by layers V and VI or by the feedback mechanism of layer I. Differential responses across layers might be integrated by the synchronization of their activities in the gamma-band to facilitate visual processes.

CELLULAR EFFECTS OF ACETYLCHOLINE IN V1-RELATED ATTENTION

Most of these cellular mechanisms contribute to attentional mechanisms in V1. Attention increases the cortical response to stimuli (i.e., the signal) while lowering interference from the background (i.e., the noise). Several animal studies have described deficits of attention following cholinergic lesions or injections of cholinergic antagonists (Voytko et al., 1994, McGaughy and Sarter, 1998, 1999) and ACh has been shown to be involved in attention in V1 (Herrero et al., 2008). However, ACh release promotes rather than initiates attention (Reed et al., 2011). Because ACh-mediated attention and perceptual learning have crucial effects on each other, the role of ACh during visual attention is delineated in the following section to better understand how ACh enhances cortical functioning.

Cholinergic involvement in bottom-up and top-down attention

ACh has been suggested to control the balance between bottom-up and top-down processing through attentional mechanisms (Yu and Dayan, 2002, Sarter et al., 2005, Yu and Dayan, 2005). This influence is mediated by pre-synaptic thalamocortical nAChRs (Gil et al., 1997, Disney et al., 2007). Attention that is prompted by the properties of a stimulus, i.e., the saliency of the stimulus relative to the background, is said to be bottom-up attention, whereas attention that is prompted by the voluntary direction of focus toward a specific stimulus is defined as top-down attention. Although it can be difficult to separate bottom-up and top-

down attentional control (Ansorge et al., 2010, Egeth et al., 2010, Eimer and Kiss, 2010, Theeuwes, 2010), some studies have shown that cholinergic activity influences bottom-up attention. The effect of ACh on bottom-up attention might occur not only in V1 but also in early processing areas such as the thalamus. For example, the direct injection of 192-IgG saporin into the BF causes a complete loss of cholinergic projections to the neocortex but causes restricted fiber lesions when injected into V1. The injection of 192-IgG saporin into the BF but not V1 affects performance in the sustained attention task (McGaughy and Sarter, 1998, 1999). In addition, compared to controls and ex-smokers, human smokers have been shown to exhibit increased subcortical activity during an attentional task (Nestor et al., 2011). These data indicate that attentional dysfunction following cholinergic lesions might be due to the disruption of detection processes that are independent of V1. However, there is no direct evidence of cholinergic enhancement effect in bottom-up attention in human studies (Rokem et al., 2010). In contrast, there is a growing body of evidence showing that ACh is involved in top-down attention. Direct effects of ACh on attention in the visual cortex have been measured (Herrero et al., 2008, Bauer et al., 2012). Specifically, Herrero and colleagues provided direct evidence that ACh in V1 enhances the cortical response to an attentional demand (Herrero et al., 2008). It has also been shown that lesions to the cholinergic system impair attention performance and increase neuronal activity in the PFC upon the presentation of distractors (which trigger top-down attention) (Gill et al., 2000). Taken together, these results indicate that ACh can facilitate task-relevant learning in V1 by promoting attentional states in both top-down and bottom-up manners.

Cholinergic modulation of response gain

Response gain modulation by ACh has frequently been observed (Disney et al., 2007, Aggelopoulos et al., 2011, Bhattacharyya et al., 2013, Soma et al., 2013a) and follows the gain control model at least in terms of the contrast-response function. Increasing thalamocortical pathway input in a context-independent manner while context-dependent intracortical suppression occurs might facilitate the transmission of information related to novel stimuli. In V1, context-dependent (i.e., increases in the maximal response) and independent (i.e., increases in the baseline response) gain control due to cholinergic effects have both been observed (80% and 20%, respectively) without any laminar bias (Soma et al., 2013b). These findings could be related to the optimization of the gain of supragranular pyramidal cells controlled by ACh which could result in the detection of novel stimuli and hence perceptual learning (Moran et al., 2013). Interestingly, gain modulation was proposed as function that underlies of attentional control (Keitel et al., 2013) and network connectivity (Haider and McCormick, 2009). The high gain that results from the amplification of the responses of excited neurons is similar to attention processes (Servan-Schreiber et al., 1990, Eldar et al., 2013) and hence facilitates learning. Taken together, these results suggest that ACh might assist in visual perceptual learning via modulation of cortical responses through gain control in both stimulus-dependent and -independent manners.

CELLULAR EFFECTS OF ACETYLCHOLINE IN V1 IN RELATION TO CORTICAL PLASTICITY

Learning and perceptual learning are sustained by cortical plasticity which triggers anatomical reorganization of the cortical connectivity. The cholinergic system plays also a key role in cortical plasticity. For example, the blockade of cholinergic activation via cholinergic antagonists or cholinergic fiber lesions results in robust impairment of learning in rats (Conner

et al., 2003, Dotigny et al., 2008) and ocular dominance plasticity in kittens (Bear and Singer, 1986). In acute preparations, cholinergic pairing is also involved in plasticity as observed in the cat auditory cortex; the application of ACh during acoustic processing alters the receptive fields of single neurons in a tone-specific manner (Metherate and Weinberger, 1990). The pairing of cholinergic and auditory stimulation also leads to the reorganization of the cortical map (Kilgard and Merzenich, 1998); i.e., an enlargement of the representation of the specifically trained frequency. Cholinergic pairing with sensory stimulation also induces long-lasting effects on cortical responsiveness observed in both the visual cortex (Dringenberg et al., 2007, Kang et al., 2013) and the somatosensory cortex (Verdier and Dykes, 2001). Cortical plasticity is essential for the occurrence of perceptual learning (for review see Fahle (2009), although not systematic, cholinergic-sensory paired activation would thus facilitate the induction of perceptual learning in the sensory cortices (Reed et al., 2011).

Cholinergic modulation of long-term cortical responsiveness

At the neuronal level, ACh has been shown to contribute to cortical plasticity through both the acute and long-term modulation of synaptic responses (Sato et al., 1987, Soma et al., 2012). The impairment of learning by cholinergic antagonists is similar to the effect of blocking cortical plasticity mechanisms and long-term potentiation (LTP) with NMDA receptor (NMDAR) antagonists (Morris et al., 1986, Artola and Singer, 1987, Cooke and Bear, 2010). In most situations, LTP in the visual cortex induced by high theta-burst stimulation (100 Hz) (Heynen and Bear, 2001, Dringenberg et al., 2007) has been found to be NMDAR-dependent. Interestingly, cholinergic system-induced cortical plasticity has also been found to be NMDAR-dependent (Verdier and Dykes, 2001, Dringenberg et al., 2007, Kang and

Vaucher, 2009) but independent of theta-burst stimulation (Kirkwood et al., 1999)(Fig. 1B). Previous studies in hippocampal slices have shown that NMDAR opening during LTP induction is facilitated by mAChR activation (Buchanan et al., 2010) and administration of ACh to pyramidal neurons (Shinoe et al., 2005). Additionally, NMDAR-dependent long-term facilitation of synaptic responses is associated with ACh release in V1, and LTP is impaired in the visual cortices of mAChR knock-out mice (Origlia et al., 2006).

Cholinergic modulation of the excitation-inhibition balance

Another contribution of the cholinergic system to cortical plasticity mechanisms in V1 is the alteration of the excitatory and inhibitory (E-I) balance (Fig. 1C). The excitatory and inhibitory synaptic inputs tend to equilibrate during maturation to optimally tune the neurons according to sensory experiences (Hensch et al., 1998, Sun et al., 2010) during the critical period; i.e., the post-natal time window during which mammals visual cortices are highly plastic that terminates with the maturation of the neurons. It has been proposed that disrupting the E-I balance can re-open the critical period after maturation (Hensch, 2004). Neuromodulation can also disrupt the E-I balance and contribute to cortical plasticity. Recent studies have also demonstrated numerous examples of cortical plasticity that are modified by the inhibitory system (Hensch, 2005). The onset of the critical period is accelerated by GABAA inhibitory receptor activation (Fagiolini and Hensch, 2000, Iwai et al., 2003). Conversely, it is also possible to re-induce plasticity after the critical period by reducing the inhibitory drive via the injection of GABAA receptor antagonists (Harauzov et al., 2010). As the inhibitory system is strongly modulated by the cholinergic system through the protein Lynx1 (Takesian and Hensch, 2013), which acts as a brake on nAChR-dependent plasticity

(Morishita et al., 2010), by nAChRs (Christophe et al., 2002, Arroyo et al., 2012), or by mAChRs (Salgado et al., 2007), cholinergic activation might modulate the E-I balance and facilitate cortical plasticity in adults that would promote perceptual learning. An interaction between the cholinergic and GABAergic systems has been shown to occur following BF stimulation that increases the activation of PV+ (Christophe et al., 2002) neurons through mAChRs (Dotigny et al., 2008, Alitto and Dan, 2012). Interestingly, Alitto and Dan used an optogenetic method to show that the nAChRs on vasoactive intestinal peptide-positive (VIP+) neurons and layer I neurons can inhibit excitatory and PV+ neurons (Christophe et al., 2002).

The cholinergic modulation of V1 thus promotes cortical plasticity through LTP-like long-term enhancement of synaptic responses to subsequent presentations of a visual stimulus and through control of the excitatory-inhibitory balance that regulate the strength of cortical output and internal connectivity. The cortical plasticity induced by cholinergic stimulation could transfer the acute cholinergic effect into long-term scale to produce visual precision.

REPETITIVE CHOLINERGIC STIMULATION TRIGGERS PERCEPTUAL LEARNING

In summary, acute effects of cholinergic activation might amplify the thalamocortical response that promotes the transmission of sensory inputs. Intensive release of ACh might also inhibit intracortical interactions and relieve the internal brake on processing in the sensory cortices. Simultaneously, neurons with similar tuning characteristics (e.g., orientation) are co-activated via lateral connections to enhance the transfer of visual information. This cholinergic alternation might contribute to gain control modulation in both stimulus-dependent or and -independent manners and prioritize the processing of selected visual stimuli; this process

might be linked to attention and is the first step of perceptual learning. The cholinergic activation also induces the NMDAR-dependent LTP-like long-term enhancement (i.e., cortical plasticity) and relief of the brakes on plasticity by altering the E-I balance. The repetitive coupling of visual and cholinergic stimulation results in reinforcement of all of these acute mechanisms and generates gamma-band synchronization. This would result in the consolidation of the synaptic strengths of new and existing neuronal connections, facilitation of the processing of certain thalamocortical inputs while suppressing others. It has been shown that increases in the cortical responses by expanding the number of neurons to a stimulation (via increases in the strength of the connections) would improve perceptual capacity (Anton-Erxleben and Carrasco, 2013). The repetitive cholinergic-visual stimulation would also increase the efficiency and automaticity of these selected pathways. These processes contribute to perceptual learning.

Repetitive cholinergic stimulation promotes long-term potentiation

As mentioned above, ACh can induce NMDAR-dependent long-term modifications of postsynaptic glutamatergic neurons which are related to memory formation. The opening of the NMDAR launches a second messenger cascade and guides the expression of synaptic glutamate receptors (Regehr and Tank, 1990, Zhong et al., 2006) but also activates autoregulated kinases that confer a persistent improved response of the neuron to the stimulus. Immunohistochemistry for the c-Fos, which is an immediate early gene and also a transcription factor for synaptogenesis genes, has revealed that c-Fos is increased in layer II/III pyramidal neurons following a repetitive BF/visual stimulation (Kang et al., 2013), which may be indicative of the formation of new synapses and LTP mechanisms. Repetitive pairing of the

cholinergic and visual stimulation also induces morphological reorganization, i.e. increase in the numbers of cholinergic varicosities in the proximity of the neurons that are sensitive to the orientation of the stimulus (Zhang et al., 2011). This increased number of cholinergic inputs, along with postsynaptic mechanisms, would increase and consolidate the response of the activated neurons to ameliorate its long-term efficiency. Thus repetitive cholinergic stimulation might enhance the encoding of the memory and morphological modifications.

Repetitive cholinergic stimulation promotes stimulus selection and amplification

We suggest that selection of decisive inputs is controlled by the cholinergic system and contributes to the specific enhancement of a particular stimulus in perceptual learning. Modulation of the orientation selectivity of the neurons provides a great example of the possible improvement of perceptual sensitivity. Training of the rat to a preferred or a non-preferred orientation might increase the cortical response for this orientation (Cooke and Bear, 2010) (Fig. 4). These mechanisms are facilitated by repetitive cholinergic activation, which improve orientation discrimination of human or rats (Rokem and Silver, 2010, Kang et al., 2013). Repetitive cholinergic stimulation coupled with a certain orientation stimulus might favor the discrimination of this stimulus by two different cellular mechanisms (Fig. 4). First, ACh can harmonize the activation of the whole dendritic tree of layer II/III neurons to preserve their orientation selectivity and confer responsiveness to new orientation - the dendrites of the layer II/III neurons receive inputs randomly over all of their branches, some of which are selective for the neurons' un-preferred orientations (Jia et al., 2010). Second, the cholinergic system can enhance orientation discrimination through its interaction with the GABAergic system which assists in the sharpening (Isaacson and Scanziani, 2011) of the

convergent input in the layer II/III neurons (Nassi and Callaway, 2009) but also filters out task-relevant information during perceptual learning (Roberts and Thiele, 2008). Parvalbumin-positive (PV+) and somatostatin-positive (SOM+) GABAergic neurons are particularly involved in orientation tuning in V1 (Atallah et al., 2012, Wilson et al., 2012). It has been shown that the specific activation of PV+ neurons in V1 improves orientation discrimination abilities in awake rats during perceptual learning (Lee et al., 2012) and repetitive coupling of ACh to visual stimulation activates the V1 GABAergic neurons (Dotigny et al., 2008, Kang et al., 2013).

Thus repetitive cholinergic pairing to sensory training enhances the cortical response to trained feature of the sensory stimulus that increases the influence of the feedforward afferent.

Repetitive cholinergic stimulation promotes perceptual learning related to attention, reward expectation and connectivity

Repetitive cholinergic stimulation first promotes attentional mechanisms that are necessary to perceptual learning (Ahissar and Hochstein, 1993, Schoups et al., 2001, Li et al., 2004, Mukai et al., 2007). These attentional processes might be also related to synchronization in the gamma band (30-90 Hz) (Fries et al., 2008) induced by repetitive cholinergic stimulation which has been proposed to facilitate the transfer of the visual information to higher visual

Figure V.4 Summary of the effect of acetylcholine on neuronal transmission of the visual inputs.

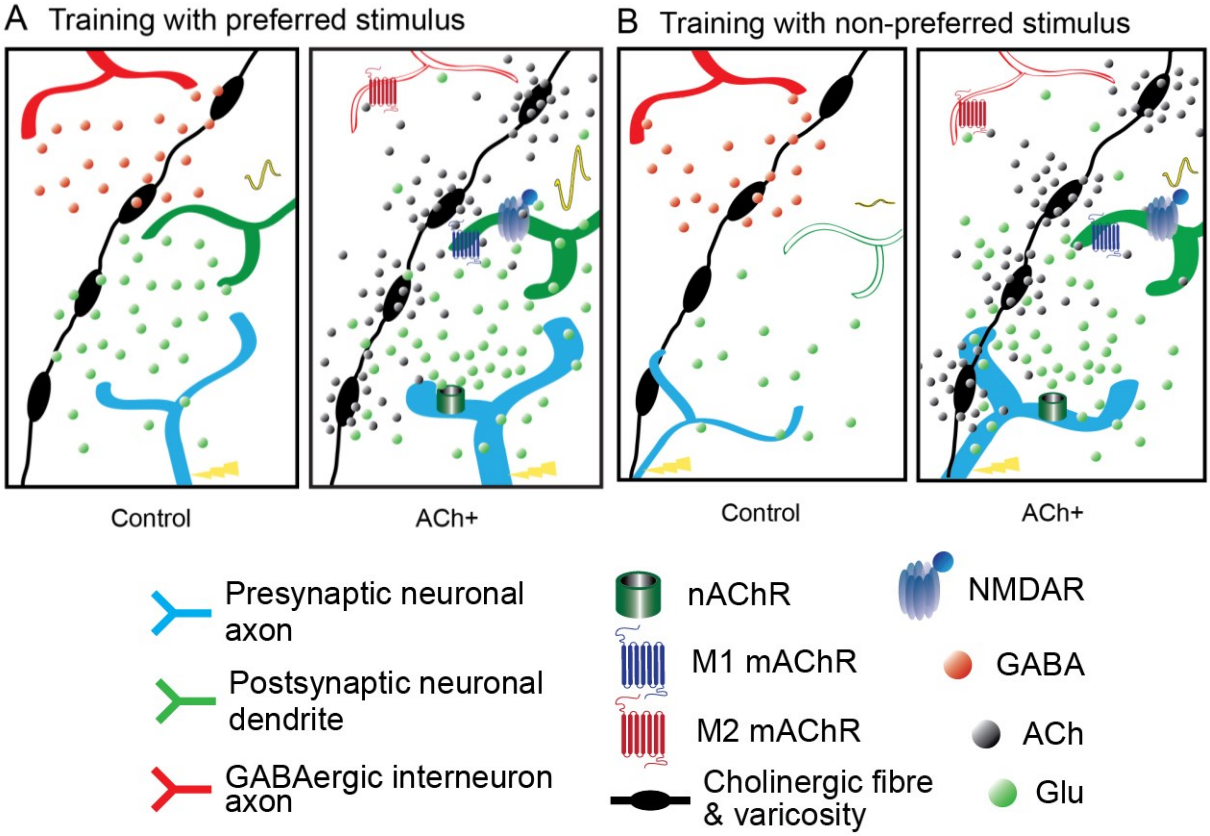

Figure 4. The varicose cholinergic fiber (black fiber with swellings) can act on excitatory input (blue axon), neighboring GABAergic inhibitory input (red axon) and on V1 neurons (green dendrite). Excitatory/inhibitory influences are represented by red and green dots, respectively. Cholinergic activation (ACh+, right panel) is represented by black dots. The cortical response to the stimulus is represented by a VEP signal waveform which changes are elicited by increased numbers of neurons responding to the trained stimulus or increased neurons efficiency (**A**) Response of the V1 neuron after a training with preferred stimulus coupled to cholinergic activation (right panel, ACh+) or without (left panel, control). The cortical response to this stimulus is increased (high VEP signal waveform in right panel compared to small VEP signal waveform in left panel). In presence of cholinergic activation the inhibitory influence is reduced by M2 muscarinic receptors, the postsynaptic excitatory influence is increased by M1 muscarinic receptors located on the postsynaptic neuron and nicotinic receptors located on the thalamocortical fiber and a long-term effect is triggered by NMDA receptor activation, compared to normal condition (control, left panel). In a normal visual process (control) local or recurrent inhibition via GABAergic interneuron (in red) blocks the development to a long-term modification. (**B**) Response of the V1 neuron after a training with non-preferred stimulus coupled to cholinergic activation (right panel, ACh+) or without (left panel, control). The neuronal response to this stimulus is increased (small VEP signal waveform in right panel compared to flat VEP signal waveform in left panel). In normal condition (control, left panel), non-preferred orientation stimulus does not evoke activation in postsynaptic neurons in V1. Weak thalamocortical innervation is suppressed by GABAergic inhibition and hence fails to transmit to postsynaptic neuron. Acetylcholine can amplify the weak presynaptic input (ACh+) by nicotinic receptors and activates postsynaptic neuron through M1 muscarinic receptor. GABAergic inhibition is suppressed by M2 muscarinic receptor and NMDA receptor opening occurs leading to long-term modification.

areas. ACh can also promote task-irrelevant perceptual learning that occurs in the absence of conscious effort (Skrandies and Fahle, 1994, Watanabe et al., 2002, Gutnisky et al., 2009). Compared to task-relevant learning, which utilizes focused attention as reinforcement, studies of task-irrelevant learning have suggested that reward serves as the reinforcement signal (Seitz et al., 2009, Chubykin et al., 2013). During task-irrelevant learning, the response to a feature on which attention was not directed can also be enhanced (Watanabe et al., 2001, Giordano et al., 2009, Gutnisky et al., 2009). Interestingly, rewards can affect the visual response in V1 (Shuler and Bear, 2006), and the cholinergic system can influence reward timing expectancy (Chubykin et al., 2013). To reconcile studies showing a role of attention in perceptual learning or not, Roelfsema proposed that the attentional feedback signal related to the cholinergic system that enhances the plasticity of task-relevant features in the visual cortex also causes the inhibition of task-irrelevant features so that their plasticity is switched off (Roelfsema et al., 2010).

To a cognitive point of view, by modulating synaptic transmission in V1 and modifying the cortical dynamics, ACh can also participates in the perceptual inference to increase the strength of the representation of trained stimuli and reduce the sensory noise (Yu and Dayan, 2002) and induce sensory precision (Moran et al., 2013). It might suppress the top-down sources in the balance between top-down and bottom-up information integration in V1 (Yu and Dayan, 2005). This is in agreement with a recent study demonstrating that the cholinergic enhancement reduces the connectivity strength between cortical regions involved in attention and V1 (Ricciardi et al., 2013) and reduce the activity in frontoparietal regions (Furey et al., 2008). This suggests an increased neural efficiency in the processing of the trained stimulus

that leads to an improved perceptual task performance (Ricciardi et al., 2013) linked to an automation of the cortical processing and a reduction of the attentional load required to process the trained stimulus (Furey, 2011) .

Together, the findings from recent work using different techniques suggests that cholinergic pairing induces perceptual learning via different mechanisms that include the following: 1) the use of the layer II/III GABAergic system to filter the pre-amplified response from layer IV; 2) NMDAR-dependent modification at the postsynaptic level to induce long-term augmentations of individual neurons, and an increase in the numbers of cholinergic varicosities to facilitate ACh release and 3) changes in the efficiency of the connectivity between cortical areas and bottom-up and top-down control.

CLINICAL PERSPECTIVES OF CHOLINERGIC MODULATION OF BRAIN`S FUNCTION

Similar with experimental data, some clinical studies have demonstrated that enhancing cholinergic system improves perception (Furey et al., 2000, Bentley et al., 2004, Wilson et al., 2004, Rokem and Silver, 2010, Beer et al., 2013, Ricciardi et al., 2013). Clinically, a method to enhance cholinergic function might involve the use of acetylcholine esterase inhibitors, such as physostigmine, galantamine, rivastigmine or donepezil. Nicotine is also a well-known molecule that enhances cognitive function. These drugs are currently used to the treatment of Alzheimer's disease or diverse dementia. Orally administered nicotine or smoking improve attentional performance (Nestor et al., 2011, Newhouse et al., 2011), learning (Riekkinen and Riekkinen, 1997, Olausson et al., 2004), attention (Thiel et al., 2005, Nestor et al., 2011) and

memory consolidation (Beer et al., 2013) through the activation of nAChRs. Increases in ACh action due to the administration of acetylcholinesterase inhibitors or direct mAChRs agonists alleviate cognitive deficits in Alzheimer's disease (Cummings, 2003), Parkinson's disease (Fagerstrom et al., 1994, Holmes et al., 2011) and schizophrenia patients (Shekhar et al., 2008). An α7 nAChR agonist is also used as a cognitive enhancer in patients with schizophrenia (Freedman, 2013) and Alzheimer's disease (Hilt et al., 2009). As shown in an fMRI study, cholinergic action potentiates communication efficiency between cortical areas (Wylie et al., 2012). The use of these drugs in cholinergically healthy subjects might also be beneficial for enhancing cognitive function (Buchanan et al., 2008, Demeter and Sarter, 2013).

Some pharmacological approaches have been developed to increase the perceptual learning in healthy humans. Performance improvements following the use of donepezil during a motion direction discrimination task have confirmed that systemic blockade of acetylcholine esterase can induce perceptual learning (Rokem and Silver, 2010, 2013). Cholinergic amplifications paired with sensory stimulations might also be a promising approach to accelerating visual recovery following lesions to the retina or the optical nerve. If the neuronal mechanisms that occur during perceptual learning and after retinal lesions are similar (Gilbert and Li, 2012) (i.e., they both involve changes in the responsiveness of cortical neurons to inputs from outside the neurons' preferred receptive fields (Darian-Smith and Gilbert, 1994)), then ACh might also aid to boost structural and functional plasticity of the visual cortex to recover from losses of retinal input.

CONCLUSION

In this review, we proposed that the neuromodulator ACh, which is known for its involvement in attention and learning, might participate in and promote perceptual learning. We proposed that, via the inhibition of intracortical feedback, ACh can render V1 more sensitive to incoming thalamocortical information and enhance sensory performance. During visual processing, ACh acts on different layers to amplify the encoding of weak stimuli by strengthening synaptic connectivity, which leads to behavioral improvements. Furthermore, ACh might not only facilitate task-relevant perceptual learning via attention but also facilitate task-irrelevant learning via reward reinforcement. However, much remains to be uncovered regarding whether the cholinergic system has the potential to be used as a key mechanism for improving the function of the brain and speeding rehabilitation. Specifically, because perceptual learning occurs easily under conditions of attentional control, the development of a method to improve one's brain capacity through improved attention and cholinergic stimulation is very attractive.

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DISCLOSURE STATEMENT

The authors declare that they have no conflict of interest.

CHAPTER VI: General Discussion

In the present thesis, we studied the role of the cholinergic system in rat's V1 during cognitive function by behavioral, electrophysiological and neuro-pharmacological methods. Through electrophysiological study we observed that 10 minutes of cholinergic system activation paired with visual stimulation induces a long-term increase of cortical response. Similar with LTP this enhancement is NMDAR dependent. When the pairing continued for 14 days, we observed that it can induce an improvement of behavioral task (i.e. visual discrimination capacity in the water maze) by facilitating perceptual learning. Intracortical injection of different pharmacological agents during pairing demonstrated that nAChR and M1 mAChR are able to amplify cortical response while M2 mAChR suppresses GABAergic neurons to disinhibit excitatory neurons. Infusion of GABAergic antagonist supported that inhibitory system is crucial to induce cortical plasticity. Altogether these results open the possibility to induce perceptual learning in V1 to enhance cortical activity.

Technical aspect: Cortical response measurement by electrophysiological method

In order to analyze the modification of the cortical response, we measured the extracellular field potential by the electrophysiological method (Chapter 2, 3, and 4). Since most of the study was to observe a long-term effect, we applied field potential (FP) recording. FP recording is useful to observe a large area up to 0.5 mm ((Mitzdorf, 1985, Katzner et al., 2009, Xing et al., 2012) but see also Kajikawa and Schroeder (2011) asserting 3 mm). During a chronic experiment it is unlikely to record the same neuron every time. And compared to intracellular recording or single unit activity depending mainly on single neuron FP rely less

on one cell. Although the source of FP is not perfectly clear it is suggested that FPs are generated by cortical neurons synchronizing their synaptic currents (Niedermeyer and Lopes da Silva, 2005, Nunez and Srinivasan, 2006).

Although FP recording is a powerful method to observe the change of cortical response in a large scale it has some limitations. First, as mentioned above the generator of FPs is not yet clarified. Many models were suggested but not proven (Nunez and Srinivasan, 2006, Pettersen and Einevoll, 2008, Bedard and Destexhe, 2009). Second, the exact area recorded by FPs also varies depending on the model from few hundreds of microns up to 3 mm (Mitzdorf, 1985, Katzner et al., 2009, Xing et al., 2009, Kajikawa and Schroeder, 2011). Lastly, recording during an anesthetized state may affect certain visual properties of neurons in V1. Especially, since the FP recording in this study was performed under isoflurane anesthetized rat, the inhibitory system was more activated than awake state (Larsen et al., 1998). Single neuron recording revealed that optimal responses to orientation are affected by isoflurane (Villeneuve and Casanova, 2003). It is possible that isoflurane effect on individual neuron also influenced FP results.

Despite those weaknesses, abundant studies use FPs to measure cortical response change in sensory cortex (Verdier and Dykes, 2001, Frenkel et al., 2006, Dringenberg et al., 2007, Katzner et al., 2009, Cooke and Bear, 2010, Morishita et al., 2010, Bhattacharyya et al., 2013). It is probably because FP is more stable (less affected by slight difference of electrode position), and can quickly measure a large number of neurons. During FP recording, high frequency responses are filtered out. Such filtering reduces the influence of nearest neuron activating at high frequency rate. Since FP is little affected by the property of adjacent neuron,

consequently slight change of electrode has little effect on electrophysiological values. I also examined the relation between visual discrimination capacity and electrophysiological response and observed that they were highly correlated (Kang et al., 2013, Kang et al., 2014b). The technical properties of FP are supplemented by another study measuring visual acuity of the mouse by FP result (Morishita et al., 2010). These studies validate that electrophysiological FP recording can be used to measure cortical response change and visual acuity.

For the FP analysis, I applied the method and the visual stimulation paradigm (during electrophysiology recording) that was previously used in the literature for other sensory modalities (Verdier and Dykes, 2001, Morishita et al., 2010). I calculated the amplitude of electrophysiological response to visual stimulation by subtracting the negative peak potential from the positive peak potential (Kang and Vaucher, 2009, Kang et al., 2013, Kang et al., 2014b). The visual evoked potential (VEP) was obtained by measuring 20 amplitudes and averaging those values (except for Chapter 4 where 40 amplitudes were measured for each VEP) as used by Verdier and Dykes (2001). In contrast to the noise signal which occurred randomly, since the event-related potential is evoked almost at the same time after stimulus onset, this method could reduce the noise effect. Contrast reversion of visual stimulus was also used as visual stimulus to measure visual acuity (Morishita et al., 2010). Averaging and contrast reversion methods allowed us to measure cortical response and evaluate visual capacity by FP recording.

In sum, despite the not perfectly clear source of FP, due to its stability and large range recording FP was used as a method to measure cortical plasticity. I used contrast reversion of

sinusoidal grating pattern as visual stimulus and analyzed FP results with peak-to-peak difference averaging.

VI.1 Discussion of the objective 1: Does the visual stimulation paired with HDB stimulation (VS/HDB) induce cortical plasticity?

Stimulation in the HDB during visual stimulus presentation increased VEP amplitude. The injection of ACh has been shown to modulate pyramidal neuron's response (Shinoe et al., 2005, Zinke et al., 2006), facilitate synaptic plasticity (Kirkwood et al., 1999) or affect ocular dominance plasticity (Gu and Singer, 1989, Morishita et al., 2010). In contrast, cholinergic fiber depletion suppresses cortical map reorganization (Conner et al., 2003, Ramanathan et al., 2009) and cholinergic antagonist blocks ocular dominance plasticity (Bear and Singer, 1986, Gu and Singer, 1993). Since an increase of cortical response after LTP induction in V1 is observed (Heynen and Bear, 2001, Clapp et al., 2006) it is possible that cholinergic activation facilitate the cortical plasticity in V1. Altogether electrical or pharmacological stimulation of the cholinergic system changes neuronal connectivity probably by inducing cortical plasticity represented by an increase of VEP.

The cholinergic stimulation-induced VEP increase is NMDAR dependent (Kang and Vaucher, 2009) which is consistent with other studies (Verdier and Dykes, 2001, Dringenberg et al., 2007). An increase of cortical response was also observed after theta burst stimulation (100 Hz) to the thalamocortical input (Heynen and Bear, 2001). Theta burst stimulation evoked an LTP like augmentation which was also NMDAR dependent. The opening of NMDAR will launch Ca2+ influx and an up-regulation of glutamatergic receptors (Yoshimura

et al., 2003). Activation of NMDAR inducing the expression of glutamatergic receptor on synapse or facilitating synaptogenesis can strengthen the neuronal connectivity, hence increasing cortical response. The implication of NMDAR suggests that VS/HDB pairing follow similar mechanism with LTP and thus cortical plasticity is induced.

The visual training also increases neuronal synchronization in gamma band (Kang et al., 2014b). The gamma oscillation can represent synaptic and cortical plasticity (Paik and Glaser, 2010, Headley and Weinberger, 2011, Shao et al., 2013) Oscillation in gamma frequency is suggested to reflect cognitive activity such as sensory perception (Cardin et al., 2009) and attention (Fries, 2009). Previous studies have demonstrated that cholinergic stimulation could increase gamma band activity (Rodriguez et al., 2004, Pafundo et al., 2013) and this can enhance visual encoding (Goard and Dan, 2009) or contrast sensitivity (Bhattacharyya et al., 2013). Gamma-band oscillation is probably reflecting coherent firing of large numbers of cells. Neuron, by synchronizing its activity timing with other neurons can affect spike-time dependent plasticity and thus, cortical response. Synchronized activity of large numbers of neurons can give rise to macroscopic oscillations and can be another cortical mechanism to increase its cortical response after VS/HDB pairing.

Overall, cholinergic activation can induce cortical plasticity (Gu, 2003). Infusion of cholinergic agonist and stimulation in the cholinergic system increase VEP via NMDAR dependent mechanism. Synchronized activity between large numbers of neurons after cholinergic stimulation can also enhance cortical plasticity. All these results suggest that cholinergic activation through agonist administration or electrical stimulation in the basal forebrain during visual stimulation induces an NMDAR dependent cortical plasticity.

VI.2 Discussion of the objective 2: Does HDB stimulation have a similar effect to ACh in V1?

In this research, I focused on the role of cholinergic system during cognitive function. Since the basal forebrain is the major cortical region that distributes cholinergic fibers in the sensory cortex, I examined whether electric stimulation in basal forebrain results in similar effect with cholinergic agonist injection. HDB stimulation with visual stimulus projection during 10 minutes (visual training) increased VEP in V1 lasting for several hours suggesting that cortical plasticity occurred. Similar result with ACh agonist carbachol injection supports that electric stimulation of basal forebrain resembles with ACh release effect in V1.

VI.2.1 Pairing visual and basal forebrain stimulation

Since ACh modulates the neuronal response (Ji and Dani, 2000, Metherate, 2004, Thiel et al., 2005, Zinke et al., 2006, Soma et al., 2012) I induced cholinergic system activation in V1 and observed its cortical effect. In order to analyze the cholinergic effect during visual process, I developed a novel method of visual training. This visual training consists of simultaneous stimulation in the cholinergic system and the visual system in an awake or anesthetized rat. This was performed by stimulating the basal forebrain or intracortical infusion of ACh agonist carbachol. During the training the rat was restrained in front of 3 monitors showing visual stimulus (sinusoidal grating, 0.12 CPD, contrast reversion, 1 Hz). The experimental paradigm was originally created by Kilgard and Merzenich to observe the auditory cortical map reorganization after training (Kilgard and Merzenich, 1998). As mentioned above (Chapter I) since ACh is involved in multiple cortical functions this setup

was designed to affect cognitive abilities (e.g. attention or learning) during process of visual information.

VI.2.2 The basal forebrain stimulation has similar effect to ACh release

I found that electrical stimulation in the basal forebrain (HDB) has similar effect to carbachol injection. A previous study in the laboratory showed that V1 stimulation by showing sinusoidal grating pattern on an anesthetized rat promotes ACh release in V1 (Laplante et al., 2005). Since this study also demonstrated that the cholinergic fibers releasing ACh originate from the HDB, I applied an electrical stimulation paradigm (Vaucher et al., 1997) in HDB to enhance ACh release in V1 during visual training. The HDB stimulation or carbachol infusion induced an increase of VEP which last for several hours (Kang and Vaucher, 2009). ACh release in the visual cortex facilitates cortical plasticity (Gu, 2003) and thus, easily modified by novel information upcoming from thalamocortical afferent (Aggelopoulos et al., 2011). This method is used in other cortical area such as auditory (Kilgard and Merzenich, 1998, Froemke et al., 2007) and sensory cortex (Verdier and Dykes, 2001). I estimated that basal forebrain stimulation or ACh release could both induce cortical reorganization and/or long-term enhancement of cortical response.

Moreover, injection of cholinergic antagonist or cholinergic fiber lesion by 192-IgG saporin blocked the VEP enhancement effect (Kang and Vaucher, 2009, Kang et al., 2013). This implies that the stimulation in the HDB boost the cortical response via cholinergic system in the sensory cortex. This confirms that the increase in cortical response by electrical stimulation in the basal forebrain follows the similar mechanism as cholinergic agonist application.

Although many evidences support that ACh can induce cortical plasticity, there are many other factors that can also induce cortical plasticity. Mostly neuromodulators system, such as serotonergic (Ji and Suga, 2007), adrenergic (Bear and Singer, 1986, Salgado et al., 2011), GABAergic (Hensch et al., 1998, Harauzov et al., 2010) or dopaminergic system (Goto and Grace, 2005) has the potential to promote cortical plasticity. Indeed, it was found GABAergic and dopaminergic neurons fibres are closely located in the basal forebrain or in the prefrontal cortex (Zhang et al., 2010, Yang et al., 2014). Adrenergic system also have an effect on cholinergic afferent (Berntson et al., 2003). Interaction between these systems needs to be determined to clearly understand the cortical plasticity induction mechanism.

Altogether, electrical stimulation of the HDB seems to have a similar effect with cholinergic agent injection. This proposes that cortical plasticity mechanism HDB stimulation is mediated by cholinergic system activation. However, interaction with different neuromodulators needs to be carefully verified to delineate the exact function.

VI.3 Discussion of the objective 3: Does a repetitive VS/HDB pairing improve behavioral performance similar to perceptual learning?

The present results show orientation specific perceptual improvement when VS was paired with HDB stimulation (Kang et al., 2013). Continuous pairing during 14 days improves rats' visual discrimination capacity in the water maze. These findings demonstrate that when coupled with visual training, the cholinergic system improves visual performance for the

trained orientation probably through cortical plasticity in V1 related to the ratio of excitatory/inhibitory inputs.

VI.3.1 Measuring visual discrimination capacity in the water maze

I applied the Prusky's water maze (Prusky et al., 2000) to evaluate the behavioral change after visual training (Kang et al., 2013). The task of the rat was to discriminate between a grey screen and sinusoidal grating pattern. The spatial frequency of grating pattern is increased gradually after each session until rat fails to discriminate. During one session, the rat has to make seven successful discriminations out of ten trials and the last succeeded spatial frequency was considered as its visual acuity. Originally, Prusky et al., used optimal stimulus (Girman et al., 1999) such as vertical or horizontal grating pattern. Since we observed that these stimulus has a ceiling effect after visual training we used an oblique (30°) stimulus (Kang et al., 2013).

During the training on an awake rat, head movement of the rat could disrupt the enhancement effect of visual training. Rat body was restricted to a frame surrounded by three computer monitors but because of ethical reason its head was not completely restricted. It is possible that the visual system was not properly stimulated compared to the rat with head fixed. Indeed, in the water maze task (Kang et al., 2013), I observed some odd results e.g. 3 rats (among 13 rats) that received visual training failed to improve their visual capacity. Despite these problems, most of the rats remained calm during visual training and received visual training head fixed toward the screen. We overcame these weaknesses by increasing the number of subjects.

Although it has limitations, Prusky's water maze is an excellent tool to evaluate the rat visual capacity at the level of the visual cortex. Recognizing the visual stimulus (perception and attention), associating with escape platform (memory) and swims toward it (decision making) demands cognitive process. Task success rate of the rat can be easily measured. Rat's success rate in the water maze is high with easy task (low spatial frequency) but decreased markedly at the visual acuity threshold. Furthermore, compared to other behavioral method, the water maze uses a negative reward (i.e. water) which segregates the dopamine and cholinergic effect. It was shown that reward triggers the dopaminergic system. Both dopamine and cholinergic systems are closely related in the prefrontal cortex (Zhang et al., 2010, Zhang et al., 2011). Since the purpose of this study was to examine the cholinergic function the water maze was an optimal method.

VI.3.2 VS/HDB stimulation induced perceptual learning related to cortical plasticity

I have shown that increase of perceptual ability occurred after repetitive visual training (Kang et al., 2013). Improved visual ability for the trained orientation (i.e. 30°) was long-lasting, orientation selective, and preserved the initial visual acuity for the optimal stimulus (0° orientation). Since recognition of stimulus demands perception, the amelioration of visual capacity can be considered as perceptual learning. Its effect was abolished when cholinergic fibres were lesioned with 192-IgG saporin. Numerous studies confirm that repetitive training induces perceptual learning (Crist et al., 1997, Crist et al., 2001, Cooke and Bear, 2010, Hua et al., 2010) and visual/cholinergic stimulation can facilitate it (Rokem and Silver, 2010,

Froemke et al., 2013). As discussed above, pairing cholinergic and visual stimulation induces cortical plasticity represented by increase of VEP. Therefore, I suggest that VS/HDB stimulation facilitates perceptual learning by cortical plasticity mechanism.

Increase of visual acuity signifies that there was a transfer to untrained stimuli (i.e. higher spatial frequencies than trained stimulus). Rats that were trained with 0.12 CPD stimulus showed better discrimination capacity in 0.7, 0.75, 0.8, and 0.88 CPD. Such behavioral amelioration is followed by an increase of VEP in both trained (0.12 CPD) and high spatial frequency stimulus (i.e. 0.7 CPD; Kang et al. (2013)). This indicates that there was a transfer of perceptual learning to untrained stimulus. Although specificity is a hallmark of perceptual learning transfer can occur when there is identical element between tasks (Golcu and Gilbert, 2009). It is estimated that the transfer of perceptual learning to higher spatial frequency stimulus was due to its common orientation (i.e. 30°) between stimuli.

I found that cholinergic stimulation facilitates perceptual learning; however, ACh is also implicated in different brain states, such as wakefulness, arousal or attention (Sarter and Bruno, 2000, Harris and Thiele, 2011). It was shown that ACh has an important role in controlling cortical state (Buzsaki et al., 1988, Harris and Thiele, 2011). Generally, cortical state can affect sensory responses due to synchronized neuronal activity in a specific frequency range (e.g. gamma band during attention, (Fries et al., 2007)). Gamma oscillation power increases after repetitive VS/HDB pairing (Kang et al., 2014b) or after cholinergic activation (Rodriguez et al., 2004, Bhattacharyya et al., 2012). It is possible that cholinergic stimulation during visual training shifts the cortex into an attentive state and increased visual acuity. Since mAChR has been shown to be essential for top-down modulation during spatial

attention, I cannot exclude the possibility that increased visual acuity is due to increased attention after visual training.

VI.4 Discussion of the objective 4: Does repetitive VS/HDB pairing increase neuronal activity?

The increase of perception may be due to increased neuronal activity and this can be analyzed by immunohistochemistry method. I observed that VS/HDB pairing induced an increase of c-fos. I also observed that the immunoreactivity of both pyramidal and GABAergic interneuron were increased for long-term in V1.

VI.4.1 Increase of neuronal activity shown by c-fos immunoreactivity

To observe the change of neuronal activity, I analyzed c-fos immunoreactivity. There was an increase in the number of activated neurons in all cortical layers of V1 (Kang et al., 2013). The c-fos, is an immediate early gene, its expression is indicative of neuronal activity (Kaczmarek and Chaudhuri, 1997, Laplante et al., 2005, Dotigny et al., 2008). Such augmentation is consistent with the increase of zif-268, another immediate early gene, after induction of LTP in V1 by stimulating in the thalamocortical input (Heynen and Bear, 2001). There was also an increase of c-fos activity in prefrontal cortex as well as the number of varicosities after VS/HDB pairing (Zhang et al., 2011). Increase number of c-fos immunoreactive neurons implies that the cortical plasticity after VS/HDB pairing increase the total of activated neurons (Kaczmarek and Chaudhuri, 1997). Such results also suggest that enhancement of VEP and perception is correlated with the number of neurons implicated. It is possible that the cortical plasticity represented by an increase of VEP and perception may be

due to change of neuronal network (Gilbert et al., 2001). The strengthening of connectivity between neurons can promote neuronal activation with a weak pre-synaptic stimulation. Therefore, the increased reactivity of c-fos probably indicates an augmentation of activated neurons during visual training that results in increase of VEP and perception. Such enhancement can be caused by strengthening of connection within neuronal network.

VI.4.2 Excitatory/inhibitory ratio is modulated differently between layers by visual training

To compare the rate of excitatory and inhibitory neurons activated we performed double immunostaining with either c-fos/pyramidal (rat brain pyramidal cell antigen) or c-fos/GABAergic (parvalbumin and calretinin) cells. There was an increase of excitatory neurons double stained in the layers II/III and IV. Comparatively, inhibitory neurons show increased activities in the layers V/VI and IV. Increase of excitation in the layers II/III is probably due to the augmentation of the layer IV spiny stellate neurons activity which receive thalamocortical inputs (Nassi and Callaway, 2009). This result is in agreement with previous studies showing facilitation of thalamocortical afferents and increase of firing capacity in the layers II/III (Soma et al., 2012, Thiele et al., 2012). On the other hand, the influence of cholinergic system on the inhibitory system of layer V might be due to enhanced GABAergic activity (Lucas-Meunier et al., 2009). As the ratio between excitatory and inhibitory drive has been suggested to participate in cortical plasticity (Gandhi et al., 2008, Yazaki-Sugiyama et al., 2009, Morishita et al., 2010), the alternation of excitatory or inhibitory expression between layers might be the influence of cholinergic modulation after repetitive visual training.

VI.5 Discussion of the objective 5: What is the pharmacological mechanism of visual training?

To determine the mechanism of visual training, I injected different pharmacological agents during repetitive VS/HDB pairing and compared its electrophysiological response. The VEP show a long-term increase in V1 but not when nAChR, M1 subtype mAChR or GABAAR was blocked. Comparatively, M2 subtype mAChR antagonist or GABAAR agonist injection decreased the VEP. These findings demonstrate that visual training coupled with the cholinergic stimulation enhances cortical plasticity in V1. This enhancement is mediated by nAChR, M1 mAChR and M2 mAChR, which the latter may induce a disinhibition by inhibiting GABAergic neuron.

VI.5.1 VS/HDB stimulation induced cortical response enhancement transfers to untrained stimulus

Enhancement of cortical response after visual training was also observed in untrained orientation and spatial frequency (Kang et al., 2014b). Rats that were trained in X° (chosen between 30°, 45° or 60°), 0.12 CPD stimulus shows increase of VEP at not only X° and high spatial frequency stimulus but also at X+90° stimulus. This is in contrast with studies showing perceptual learning is not transferable to different orientation (Sagi, 2011). Such transfer can be explained by 1) cholinergic effect: In contrast of some studies ACh release increases neuron's orientation selectivity (Sillito and Kemp, 1983, Murphy and Sillito, 1991) there are other studies reporting decrease of selectivity (Muller and Singer, 1989, Zinke et al., 2006, Bhattacharyya et al., 2013). It is possible that visual training induces cortical plasticity and

augments the response of visual system. Orientation selectivity may be acquired later during water maze by a top-down modulation (Schummers et al., 2005) or by locomotive state (Polack et al., 2013). Since the possibility of mAChR involvement was excluded by scopolamine injection, nAChR can induce an orientation specific effect during water maze task. Another possible answer is that 2) water maze learning before visual training causes stimulus selectivity. Taking into account that rodents are not visual animals, the learning process in the water maze can increase visual sensitivity before the actual training. Interestingly, it was suggested that during object recognition process the expectation of the object can create a set of filter (Ullman, 2007). Such filter can decrease the interference by suppressing activation of unexpected stimuli while increasing selectivity for expected one. For example in this research, rat that has learned to detect a specific orientation stimulus (Kang et al., 2013) its V1 may possess a selectivity and thus filtering out other orientation. Comparatively, rat which did not learn the water maze task may lack of orientation specificity and thus cortical response enhancement was transferred. Notably, it could be unreasonable to argue that visual training alone can induce perceptual learning.

Overall, the paired stimulation in the HDB and visual system seems to increase cortical response in long-term rendering the cortex more vulnerable to novel stimuli, hence facilitates learning process. It is likely that 1) learning prior to visual training influences the visual sensitivity of the rat, or it is also possible that 2) behavioral performance after visual training affects the modulation properties through nAChR or through top-down control.

VI.5.2 Presynaptic enhancement and disinhibition by nAChR

Injection of nAChR antagonist blocking VEP enhancement effect (Kang and Vaucher, 2009, Kang et al., 2014b) demonstrates that it has an essential role for visual training. Since nAChR inhibition can affect behavioral performance its effect was not tested in the water maze task (Kang et al., 2013). However, our electrophysiological results and numerous studies show that nAChR is crucial to increase cortical response (Verdier and Dykes, 2001, Disney et al., 2007, Lucas-Meunier et al., 2009, Morishita et al., 2010). The action of nAChR to enhance visual cortical response probably occurs in layer IV of V1 through presynaptic thalamocortical afferent. Considering that layer IV is the first cortical region receiving thalamocortical feedforward input, it is possible that nAChR has a crucial role to initiate the VEP enhancement.

Unlike mecamylamine which blocks both α4β2 and α7 subtype of nAChR (Christophe et al., 2002, Disney et al., 2007, Albuquerque et al., 2009) methyllycaconitine blocks only α7 and fails to completely abolish VEP enhancement (Kang and Vaucher, 2009). Activation of α7 of layer 1 GABAergic interneurons has been shown to mediate disinhibition of cortical networks (Christophe et al., 2002, Alitto and Dan, 2012), which can increase VEP response. Consequently, inactivation of GABAR could decreases VEP amplitude while an increase of VEP amplitude could be induced by inhibition of GABAergic cells from layer 4. Such blockade of α7 has been shown to induce LTP in the hippocampus (Ge and Dani, 2005, Wang et al., 2006) due to their location on inhibitory interneurons (Yamazaki et al., 2005). Although the α7 subtype of nAChRs is considered a key participant in cortical plasticity (Albuquerque et al., 2009), its role in the visual cortex has not been clearly elucidated (Chapter 2). The α4β2 subtype is also found on GABAergic neuron (Alkondon et al., 2000) but it was suggested that

its main role in sensory cortex is to alter thalamocortical transmission (Lambe et al., 2003). In sum, nAChR can act both on excitatory and inhibitory neurons. Disinhibition by activating layer I interneurons or amplifying by layer IV thalamocortical input can be both the underlying mechanism of VEP enhancement.

VI.5.3 Postsynaptic amplification by M1 and presynaptic disinhibition by M2 mAChR

Given that mAChRs are widely found in V1 – the predominant postsynaptic mAChR being M1 subtype and the presynaptic mAChR being M2 (Levey et al., 1991) – it was expected that inhibition of these receptors express different results. Blocking mAChRs during visual/cholinergic stimulation or M1 mAChR during repetitive visual training both abolished the VEP enhancement (Chapter 2, 4). On the other hand, M2 mAChR inhibition reduces the VEP amplitude suggesting that it has a disinhibition effect. These results indicate a postsynaptic amplification by M1 mAChR and presynaptic disinhibition by M2 mAChR.

The M1 mAChR increases the postsynaptic signal on excitatory neuron to enhance VEP during visual/cholinergic stimulation. Although the function of M1 mAChR varies according to its location (Gil et al., 1997), under our stimulation paradigm the role of M1 seems to be amplifying visual response (Gulledge et al., 2009, Kang et al., 2014b). Double-immunostaining revealed that more excitatory neurons are activated in the layers II/III after repetitive VS/HDB pairing indicating that synaptic amplification occurred (Kang et al., 2013). Since M1 is involved during LTP induction in hippocampus (Colgin et al., 2003) it can have similar function in layers II/III of V1. The activation of M1 mAChR most likely elevates intracellular Ca^{2+} level followed by synaptic plasticity mechanism. The M1 mAChR is mostly

found in the layers II/III and VI while M2 is densely labeled in layer IV or at the border of layers V/VI (Levey et al., 1991, Mrzljak et al., 1993, Hohmann et al., 1995, Tigges et al., 1997)(but see also Amar et al., 2010 which shows high elevation of M2 in layer II). It is likely that the enhancement effect of M1 mAChR inducing LTP occurs in the layer II/III of V1.

The M2 mAChR decreases inhibitory drive and hence, increases pyramidal activation during visual training. Present study demonstrates that inhibition of M2 mAChR resembles with GABAergic activation effect and M2 mAChR exists on GABAergic neuron (Kang et al., 2014b). This result is in agreement with other study showing by confocal microscopy and electrophysiological methods that M2 mAChR can inhibit GABAergic neuron (Disney et al., 2006, Salgado et al., 2007). Suppression of GABAergic neuron decreases stimulus selectivity (Wilson et al., 2012) and disinhibited neurons may respond to an un-preferred stimulus increasing cortical response (Frenkel et al., 2006, Zinke et al., 2006). Activation of adjacent neuron may increase horizontal inhibition between cells. However, since GABAergic neuron has an essential role for cortical oscillation (Muthukumaraswamy et al., 2009), controlling the spike timing can synchronize neuronal activity (Kang et al., 2014b) and prevent the competition between pyramidal neuron. Interestingly, it was proposed that mAChR suppress the lateral spread of feedforward activation (Kimura 1999, Silver 2008). It is possible that under high concentration of ACh lateral spread is blocked by M2 but low ACh enable the propagation through M1 mAChR (Wester and Contreras, 2013). Altogether, M2 mAChR disinhibits neighboring neuron to respond to an un-preferred stimulus and enhances cortical response at a low ACh dose. The competition between neurons can be avoided by synchronizing the neuronal activity.

Overall, it is possible that thalamocortical input increases its glutamate release by nAChR activation to spiny stellate neuron in layer IV, which in turn, is disinhibited by M2 mAChR activity on GABAergic neuron. This enhanced response can be amplified again during transferring to the layer II/III pyramidal neuron. Increase of cortical response is also due to the activation of adjacent neurons which its stimulus selectivity is lowered by disinhibition. Competition between neurons can be prevented by forming neuronal ensemble and synchronizing their activities. Repetitive augmentation of cortical response can induce change in synaptic connection and network, thus cortical plasticity.

VI.5.4 Long-term effect of cholinergic stimulation

In this Ph.D. study, since I evaluated the long-term response of cholinergic stimulation, it is necessary to distinguish the mechanism between during and after VS/HDB pairing. For example, compared to acute application which by blocking M2 mAChR or GABAR increases neuron's response (Egan and North, 1986, Salgado et al., 2007, Katzner et al., 2011), long-term application decreases or disrupts VEP enhancement (Kang et al., 2014b). During visual training (i.e. acute effect), it is probable that cholinergic stimulation follows the mechanism mentioned above: post-synaptic M1 mAChR increases thalamocortical afferent which is amplified by presynaptic nAChR, while M2 mAChR inhibits GABAergic drive. However, it is important to note that visual acuity or VEP were measured without cholinergic stimulation but still show enhancement (Kang et al., 2013, Kang et al., 2014b). It is reasonable to estimate that continuous acute cholinergic stimulation induces a distinct long-term modification mechanism. Although I cannot exclude the possibility that persistent cholinergic activation increase the

affinity of cholinergic receptors, it is more likely that the cortical response enhancement effect is due to modulation of neuronal connections (Ahissar et al., 1998).

Continuous cholinergic activity can strengthen neuronal connectivity. Since synaptic plasticity mechanism includes both short term effect (e.g. expression of AMPAR; (Takahashi et al., 2003, Anggono and Huganir, 2012)) and long term effect (e.g. synaptogenesis; (Kleim et al., 2004)), it is probable that short-term effect precedes long-term effect and modify synaptic strength. It was proposed that after perceptual learning occurs with specific orientation, it increases the overall number of responding neurons without changing neurons' preferred orientation (Frenkel et al., 2006). According to Hebbian rule, neurons that fire together their neuronal connections can be strengthened (Ahissar et al., 1998). Enhancement of neuronal response by cholinergic stimulation can increase layer IV neuron's connectivity with layer II/III neuron which has different orientation preference (Jia et al., 2010). Such strengthening can evoke the neuron to respond to an unpreferred stimulus. However, this modification does not affect the genuine property of layer II/III neuron (e.g. receptive field size, orientation preference) since the optimal connection from feedforward neuron is unchanged. This speculation is supported by studies showing that perceptual learning does not affect pre-learned capacity (Ahissar and Hochstein, 1997, Crist et al., 1997). I also observed that the visual acuity of an optimal orientation (i.e. vertical) was not changed even after an augmentation of unoptimal orientation (30°) visual acuity (Kang et al., 2013). Altogether, I can estimate that neuronal connections are strengthened and thus preserve their amplified activities after visual training.

Persistent activation during perceptual learning can also prompt synchronized neuronal activities (Beierlein et al., 2000) by enhancing lateral connections with similar property neuron (McGuire et al., 1991, Ramalingam et al., 2013). Cortical oscillation, controlled by GABAergic network (Galarreta and Hestrin, 2002), can affect cortical response without cholinergic stimulation after repetitive VS/HDB pairing. Overall, neuronal connection strengthening or synchronized neurons' activities can be the underlying mechanism to increase visual capacity in long-term.

VI.6 Future perspective

VI.6.1 Cortical function enhancement

The present thesis studied the effect of cholinergic system during process of visual information. It was demonstrated that cholinergic activation during visual stimulation induces cortical plasticity. Continuous pairing of the stimulation improves perception by increasing visual sensitivity. It was estimated that it is mediated by thalamocortical afferent amplification through presynaptic nAChR and postsynaptic M1 mAChR, while M2 mAChR disinhibit GABAergic neuron. Although some properties of cholinergic stimulation induced cortical plasticity are revealed, it remains to clarify its cognitive effect. For instance, since the electrophysiology recording was performed in anesthetized animal the cholinergic influence on attention could not be tested. Moreover, we were not able to distinguish the cognitive effect between perception and attention in behavioral test. Examination on the effect of attention can be the next step. Sustained attention task in a sound-attenuated chamber is normally used to examine the effect of attention (St Peters et al., 2011). Measuring VEP in an awake or behaving animal can also fulfill the purpose.

Understanding the exact laminar mechanism can be another future perspective. I speculate some arguments based on immunoreactivity results but it should be noted that immunohistochemistry analysis is not an online method. The exact neuronal activation time in different layers during VS/HDB pairing or the modulation effect after VS/HDB can be assessed by current source density analysis or polytrode recording (Goard and Dan, 2009). Moreover, these techniques combined with injection of pharmacological agents, the exact laminar mechanisms of VS/HDB pairing can be explained.

Isolating cholinergic function from GABAergic interference can be also expected. Cholinergic and GABAergic neurons are closely located in the HDB (Yang et al., 2014). Although I attempted to delineate the function of cholinergic system during VS/HDB pairing by cholinergic fiber lesions or antagonist injection, it is still debatable about the implication of GABAergic neuron. Recently, with the development of optogenetics activating specific types of neuron in the implanted area is possible (Deisseroth, 2010). Cholinergic fiber stimulation by optogenetic method instead of electrode implantation can help to better delineate the function of cholinergic system. Altogether, examining more cognitive effect (e.g. attention), determining laminar mechanism, or isolating cholinergic system activation can be proposed as an extension of the present research.

VI.6.2 Visual restoration by perceptual learning mechanism

Beyond the scientific meaning of understanding the brain function, a cholinergic amplification paired with a sensory stimulation could also be a promising method to accelerate the visual recovery after lesion in the retina or in optical nerve. In adult, lesions of the retina or the optic nerve create a silencing zone in the corresponding retinotopic region in V1 called the lesion projection zone. After lesions, recovery occurs and neurons within lesion projection zone restore responsiveness to visual information from unharmed retinal areas surrounding the lesioned region (Sabel et al., 2011). Such restoration is mediated by cortical reorganization. Following lesions, long-range horizontal connections are strengthened and drive surrounding neuronal activity within the lesion projection zone to spiking levels (Yamahachi et al., 2009). Therefore, shifting RFs outside of the retinal lesion locations is induced. Similar with restoration long-range horizontal connections are used during perception. For example, in

contour recognition, neurons extend their RFs via these connections and integrate inputs from larger areas (Gilbert and Wiesel, 1989, Darian-Smith and Gilbert, 1994). Long-range horizontal connection, which normally connects between neurons with similar stimulus preference, can be strengthened by perceptual learning (Gilbert and Wiesel, 1989, Lien and Scanziani, 2013). These connections augment the sensitivity of neurons by supporting its activation with weak stimulus (FIGURE VI.1). Since VS/HDB pairing facilitates perceptual learning the similarities between perceptual learning and restoration imply that VS/HDB training can accelerate visual rehabilitation.

It is to note that more experimental researches are needed to exactly understand the process of pairing cholinergic and visual system activation. Cognitive effect, laminar mechanism or isolating cholinergic activation is one of the assignments left to be fulfilled. By clarifying its function, it is possible that the visual training is used to enhance cortical function or to accelerate visual recovery.

VI.7 Conclusion

This research shows the neuromodulatory role of cholinergic system inducing cortical plasticity. Cholinergic system activation paired with visual stimulus can lead to perceptual learning and may facilitate visual recovery in V1. Brain is the organ responsible for cognition and mind. Compared to the extreme complexity of the brain, understanding the functional mechanism of cholinergic system in V1 might be equal to finding a piece of puzzle. However, by matching the pieces one by one we can eventually assemble and see the grand picture. Further knowledge will help to access the remedy of various cognitive diseases such as

Alzheimer, dementia or Parkinson's. I hope that my study can be used as a cornerstone of a brighter future.

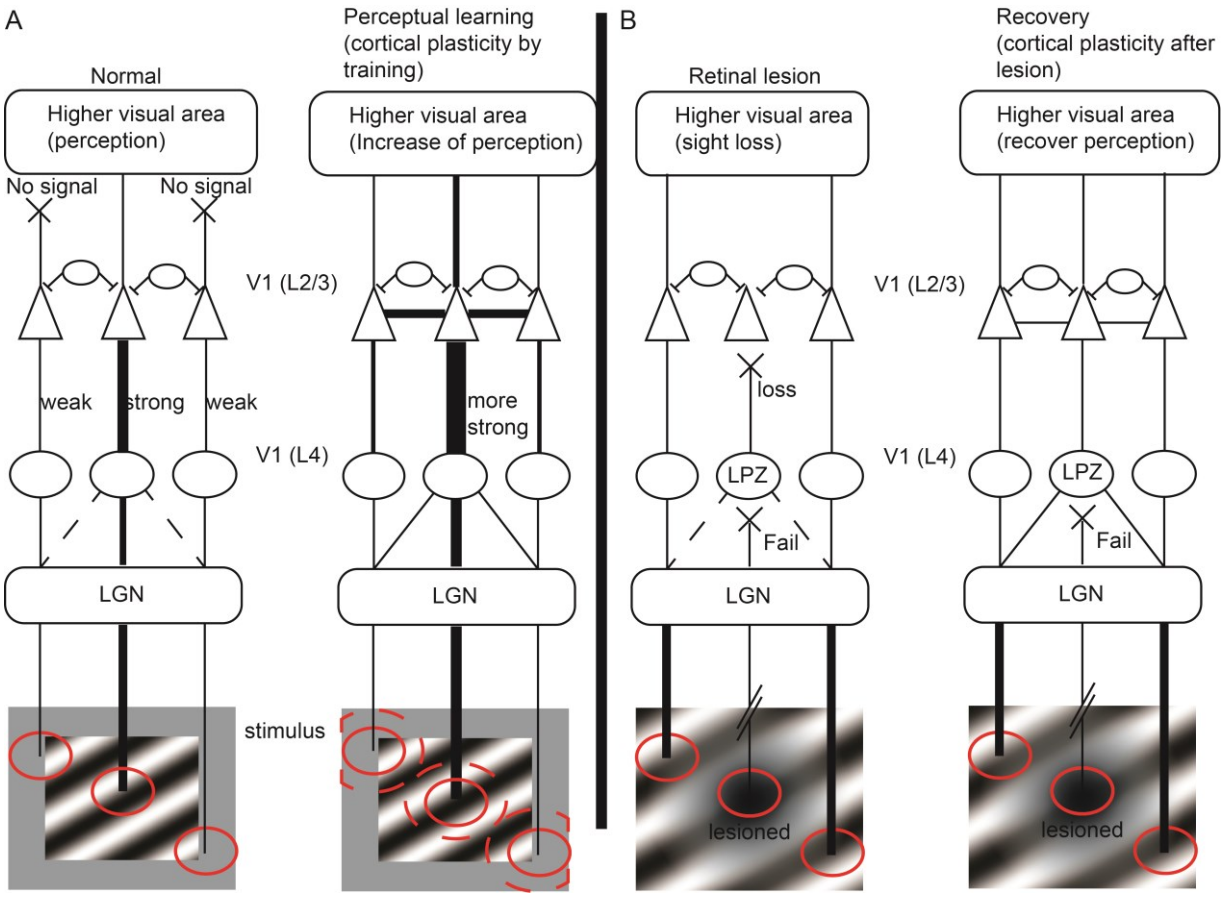

Figure VI.1. Hypothetical mechanism of perceptual learning and visual recovery.

A) During normal visual process, neurons in V1 have weak activation to the stimulus outside of its receptive field (RF: red circle) (left panel). After perceptual learning neurons can extend the RF (dashed red circle) and integrate information (right panel). Such change can increase the information carried to higher visual area or activate horizontally connected neuron to send higher visual area, consequently increasing perception. B) After a retinal lesion, neurons in lesion projecting zone (LPZ) fail to transmit feedforward signals (left panel). During visual recovery neurons in LPZ regain signals by surrounding neurons and transmit to higher visual area (right panel).

REFERENCES

Aggelopoulos NC, Liebe S, Logothetis NK, Rainer G (2011) Cholinergic control of visual categorization in macaques. Frontiers in behavioral neuroscience 5:73.

Ahissar E, Abeles M, Ahissar M, Haidarliu S, Vaadia E (1998) Hebbian-like functional plasticity in the auditory cortex of the behaving monkey. Neuropharmacology 37:633-655.

Ahissar M, Hochstein S (1993) Attentional control of early perceptual learning. Proceedings of the National Academy of Sciences of the United States of America 90:5718-5722.

Ahissar M, Hochstein S (1997) Task difficulty and the specificity of perceptual learning. Nature 387:401-406.

Albright TD, Stoner GR (2002) Contextual influences on visual processing. Annual review of neuroscience 25:339-379.

Albuquerque EX, Pereira EF, Alkondon M, Rogers SW (2009) Mammalian nicotinic acetylcholine receptors: from structure to function. Physiol Rev 89:73-120.

Alenda A, Nunez A (2007) Cholinergic modulation of sensory interference in rat primary somatosensory cortical neurons. Brain Res 1133:158-167.

Alitto HJ, Dan Y (2012) Cell-type-specific modulation of neocortical activity by basal forebrain input. Frontiers in systems neuroscience 6:79.

Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX (2000) Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:66-75.

Amar M, Lucas-Meunier E, Baux G, Fossier P (2010) Blockade of different muscarinic receptor subtypes changes the equilibrium between excitation and inhibition in rat visual cortex. Neuroscience 169:1610-1620.

Andersen GJ, Ni R, Bower JD, Watanabe T (2010) Perceptual learning, aging, and improved visual performance in early stages of visual processing. Journal of vision 10:4.

Andersen P, Andersson SA, Lomo T (1966) Patterns of spontaneous rhythmic activity within various thalamic nuclei. Nature 211:888-889.

Anggono V, Huganir RL (2012) Regulation of AMPA receptor trafficking and synaptic plasticity. Current opinion in neurobiology 22:461-469.

Ansorge U, Horstmann G, Scharlau I (2010) Top-down contingent attentional capture during feed-forward visual processing. Acta psychologica 135:123-126; discussion 133-129.

Anton-Erxleben K, Carrasco M (2013) Attentional enhancement of spatial resolution: linking behavioural and neurophysiological evidence. Nature reviews Neuroscience 14:188-200.

Aramakis VB, Bandrowski AE, Ashe JH (1999) Role of muscarinic receptors, G-proteins, and intracellular messengers in muscarinic modulation of NMDA receptor-mediated synaptic transmission. Synapse 32:262-275.

Arroyo S, Bennett C, Aziz D, Brown SP, Hestrin S (2012) Prolonged disynaptic inhibition in the cortex mediated by slow, non-alpha7 nicotinic excitation of a specific subset of cortical interneurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:3859-3864.

Artola A, Singer W (1987) Long-term potentiation and NMDA receptors in rat visual cortex. Nature 330:649-652.
Atallah BV, Bruns W, Carandini M, Scanziani M (2012) Parvalbumin-expressing interneurons linearly transform cortical responses to visual stimuli. Neuron 73:159-170.
Avendano C, Umbriaco D, Dykes RW, Descarries L (1996) Acetylcholine innervation of sensory and motor neocortical areas in adult cat: a choline acetyltransferase immunohistochemical study. Journal of chemical neuroanatomy 11:113-130.
Avillac M, Deneve S, Olivier E, Pouget A, Duhamel JR (2005) Reference frames for representing visual and tactile locations in parietal cortex. Nature neuroscience 8:941-949.
Baker CI, Peli E, Knouf N, Kanwisher NG (2005) Reorganization of visual processing in macular degeneration. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:614-618.
Bakin JS, Weinberger NM (1996) Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. Proceedings of the National Academy of Sciences of the United States of America 93:11219-11224.
Bao S, Chan VT, Merzenich MM (2001) Cortical remodelling induced by activity of ventral tegmental dopamine neurons. Nature 412:79-83.
Baroncelli L, Sale A, Viegi A, Maya Vetencourt JF, De Pasquale R, Baldini S, Maffei L (2010) Experience-dependent reactivation of ocular dominance plasticity in the adult visual cortex. Experimental neurology 226:100-109.
Barria A, Derkach V, Soderling T (1997a) Identification of the Ca2+/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. The Journal of biological chemistry 272:32727-32730.
Barria A, Muller D, Derkach V, Griffith LC, Soderling TR (1997b) Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. Science 276:2042-2045.
Bartoletti A, Cancedda L, Reid SW, Tessarollo L, Porciatti V, Pizzorusso T, Maffei L (2002) Heterozygous knock-out mice for brain-derived neurotrophic factor show a pathway-specific impairment of long-term potentiation but normal critical period for monocular deprivation. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:10072-10077.
Bauer M, Kluge C, Bach D, Bradbury D, Heinze HJ, Dolan RJ, Driver J (2012) Cholinergic enhancement of visual attention and neural oscillations in the human brain. Current biology : CB 22:397-402.
Bear MF, Kleinschmidt A, Gu QA, Singer W (1990) Disruption of experience-dependent synaptic modifications in striate cortex by infusion of an NMDA receptor antagonist. The Journal of neuroscience : the official journal of the Society for Neuroscience 10:909-925.
Bear MF, Singer W (1986) Modulation of visual cortical plasticity by acetylcholine and noradrenaline. Nature 320:172-176.
Beaver CJ, Ji Q, Fischer QS, Daw NW (2001) Cyclic AMP-dependent protein kinase mediates ocular dominance shifts in cat visual cortex. Nature neuroscience 4:159-163.
Bedard C, Destexhe A (2009) Macroscopic models of local field potentials and the apparent 1/f noise in brain activity. Biophysical journal 96:2589-2603.

Beer AL, Vartak D, Greenlee MW (2013) Nicotine facilitates memory consolidation in perceptual learning. Neuropharmacology 64:443-451.
Beierlein M, Gibson JR, Connors BW (2000) A network of electrically coupled interneurons drives synchronized inhibition in neocortex. Nature neuroscience 3:904-910.
Benke TA, Luthi A, Isaac JT, Collingridge GL (1998) Modulation of AMPA receptor unitary conductance by synaptic activity. Nature 393:793-797.
Bentley P, Driver J, Dolan RJ (2011) Cholinergic modulation of cognition: insights from human pharmacological functional neuroimaging. Progress in neurobiology 94:360-388.
Bentley P, Husain M, Dolan RJ (2004) Effects of cholinergic enhancement on visual stimulation, spatial attention, and spatial working memory. Neuron 41:969-982.
Berntson GG, Shafi R, Knox D, Sarter M (2003) Blockade of epinephrine priming of the cerebral auditory evoked response by cortical cholinergic deafferentation. Neuroscience 116:179-186.
Bhattacharyya A, Biessmann F, Veit J, Kretz R, Rainer G (2012) Functional and laminar dissociations between muscarinic and nicotinic cholinergic neuromodulation in the tree shrew primary visual cortex. The European journal of neuroscience 35:1270-1280.
Bhattacharyya A, Veit J, Kretz R, Bondar I, Rainer G (2013) Basal forebrain activation controls contrast sensitivity in primary visual cortex. BMC neuroscience 14:55.
Boulton AA, Baker GB, Vanderwolf CH (1990) Neurophysiological techniques. Clifton, N.J.: Humana Press.
Boutillier AL, Barthel F, Roberts JL, Loeffler JP (1992) Beta-adrenergic stimulation of cFOS via protein kinase A is mediated by cAMP regulatory element binding protein (CREB)-dependent and tissue-specific CREB-independent mechanisms in corticotrope cells. The Journal of biological chemistry 267:23520-23526.
Brandon NJ, Jovanovic JN, Colledge M, Kittler JT, Brandon JM, Scott JD, Moss SJ (2003) A-kinase anchoring protein 79/150 facilitates the phosphorylation of GABA(A) receptors by cAMP-dependent protein kinase via selective interaction with receptor beta subunits. Molecular and cellular neurosciences 22:87-97.
Briand LA, Gritton H, Howe WM, Young DA, Sarter M (2007) Modulators in concert for cognition: modulator interactions in the prefrontal cortex. Progress in neurobiology 83:69-91.
Brocher S, Artola A, Singer W (1992) Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. Brain Res 573:27-36.
Buchanan KA, Petrovic MM, Chamberlain SE, Marrion NV, Mellor JR (2010) Facilitation of long-term potentiation by muscarinic M(1) receptors is mediated by inhibition of SK channels. Neuron 68:948-963.
Buchanan RW, Conley RR, Dickinson D, Ball MP, Feldman S, Gold JM, McMahon RP (2008) Galantamine for the treatment of cognitive impairments in people with schizophrenia. The American journal of psychiatry 165:82-89.
Burkhalter A (1989) Intrinsic connections of rat primary visual cortex: laminar organization of axonal projections. The Journal of comparative neurology 279:171-186.
Burne RA, Parnavelas JG, Lin CS (1984) Response properties of neurons in the visual cortex of the rat. Experimental brain research 53:374-383.

Buzsaki G, Bickford RG, Ponomareff G, Thal LJ, Mandel R, Gage FH (1988) Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. The Journal of neuroscience : the official journal of the Society for Neuroscience 8:4007-4026.

Calabresi P, Centonze D, Gubellini P, Pisani A, Bernardi G (1998) Endogenous ACh enhances striatal NMDA-responses via M1-like muscarinic receptors and PKC activation. The European journal of neuroscience 10:2887-2895.

Callaway E (2004) Feedforward, feedback and inhibitory connections in primate visual cortex. Neural Networks 17:625-632.

Callaway EM (1998) Local circuits in primary visual cortex of the macaque monkey. Annual review of neuroscience 21:47-74.

Caporale N, Dan Y (2008) Spike timing-dependent plasticity: a Hebbian learning rule. Annual review of neuroscience 31:25-46.

Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI (2009) Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 459:663-667.

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA, Malenka RC (1999) Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. Nature neuroscience 2:454-460.

Carulli D, Pizzorusso T, Kwok JC, Putignano E, Poli A, Forostyak S, Andrews MR, Deepa SS, Glant TT, Fawcett JW (2010) Animals lacking link protein have attenuated perineuronal nets and persistent plasticity. Brain : a journal of neurology 133:2331-2347.

Carulli D, Rhodes KE, Brown DJ, Bonnert TP, Pollack SJ, Oliver K, Strata P, Fawcett JW (2006) Composition of perineuronal nets in the adult rat cerebellum and the cellular origin of their components. The Journal of comparative neurology 494:559-577.

Castellucci VF, Carew TJ, Kandel ER (1978) Cellular analysis of long-term habituation of the gill-withdrawal reflex of Aplysia californica. Science 202:1306-1308.

Caulfield MP, Birdsall NJ (1998) International Union of Pharmacology. XVII. Classification of muscarinic acetylcholine receptors. Pharmacological reviews 50:279-290.

Changeux JP, Bertrand D, Corringer PJ, Dehaene S, Edelstein S, Lena C, Le Novere N, Marubio L, Picciotto M, Zoli M (1998) Brain nicotinic receptors: structure and regulation, role in learning and reinforcement. Brain research Brain research reviews 26:198-216.

Chattopadhyaya B, Cristo GD (2012) GABAergic circuit dysfunctions in neurodevelopmental disorders. Frontiers in psychiatry 3:51.

Chedotal A, Cozzari C, Faure MP, Hartman BK, Hamel E (1994) Distinct choline acetyltransferase (ChAT) and vasoactive intestinal polypeptide (VIP) bipolar neurons project to local blood vessels in the rat cerebral cortex. Brain research 646:181-193.

Christophe E, Roebuck A, Staiger JF, Lavery DJ, Charpak S, Audinat E (2002) Two types of nicotinic receptors mediate an excitation of neocortical layer I interneurons. Journal of neurophysiology 88:1318-1327.

Chubykin AA, Roach EB, Bear MF, Shuler MG (2013) A cholinergic mechanism for reward timing within primary visual cortex. Neuron 77:723-735.

Clapp WC, Eckert MJ, Teyler TJ, Abraham WC (2006) Rapid visual stimulation induces N-methyl-D-aspartate receptor-dependent sensory long-term potentiation in the rat cortex. Neuroreport 17:511-515.

Colgin LL, Kubota D, Lynch G (2003) Cholinergic plasticity in the hippocampus. Proc Natl Acad Sci 100:2872-2877.
Conner JM, Culberson A, Packowski C, Chiba AA, Tuszynski MH (2003) Lesions of the Basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning. Neuron 38:819-829.
Cooke SF, Bear MF (2010) Visual experience induces long-term potentiation in the primary visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:16304-16313.
Cooke SF, Bear MF (2012) Stimulus-selective response plasticity in the visual cortex: an assay for the assessment of pathophysiology and treatment of cognitive impairment associated with psychiatric disorders. Biological psychiatry 71:487-495.
Corbetta M, Miezin FM, Dobmeyer S, Shulman GL, Petersen SE (1991) Selective and divided attention during visual discriminations of shape, color, and speed: functional anatomy by positron emission tomography. The Journal of neuroscience : the official journal of the Society for Neuroscience 11:2383-2402.
Creutzfeldt OD, Heggelund P (1975) Neural plasticity in visual cortex of adult cats after exposure to visual patterns. Science 188:1025-1027.
Crist RE, Kapadia MK, Westheimer G, Gilbert CD (1997) Perceptual learning of spatial localization: specificity for orientation, position, and context. Journal of neurophysiology 78:2889-2894.
Crist RE, Li W, Gilbert CD (2001) Learning to see: experience and attention in primary visual cortex. Nature neuroscience 4:519-525.
Cudeiro J, Sillito AM (2006) Looking back: corticothalamic feedback and early visual processing. Trends in neurosciences 29:298-306.
Cummings JL (2003) Use of cholinesterase inhibitors in clinical practice: evidence-based recommendations. The American journal of geriatric psychiatry : official journal of the American Association for Geriatric Psychiatry 11:131-145.
Dan Y, Poo MM (2004) Spike timing-dependent plasticity of neural circuits. Neuron 44:23-30.
Dani JA (2001) Overview of nicotinic receptors and their roles in the central nervous system. Biological psychiatry 49:166-174.
Darian-Smith C, Gilbert CD (1994) Axonal sprouting accompanies functional reorganization in adult cat striate cortex. Nature 368:737-740.
Darian-Smith C, Gilbert CD (1995) Topographic reorganization in the striate cortex of the adult cat and monkey is cortically mediated. The Journal of neuroscience : the official journal of the Society for Neuroscience 15:1631-1647.
Dauphin F, Lacombe P, Sercombe R, Hamel E, Seylaz J (1991) Hypercapnia and stimulation of the substantia innominata increase rat frontal cortical blood flow by different cholinergic mechanisms. Brain research 553:75-83.
De Pasquale R, Sherman SM (2012) Modulatory effects of metabotropic glutamate receptors on local cortical circuits. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:7364-7372.
Deco G, Thiele A (2011) Cholinergic control of cortical network interactions enables feedback-mediated attentional modulation. The European journal of neuroscience 34:146-157.

DeFelipe J, Alonso-Nanclares L, Arellano JI (2002) Microstructure of the neocortex: comparative aspects. J Neurocytol 31:299-316.
Deisseroth K (2010) Controlling the brain with light. Scientific American 303:48-55.
Demeter E, Sarter M (2013) Leveraging the cortical cholinergic system to enhance attention. Neuropharmacology 64:294-304.
Descarries L, Gisiger V, Steriade M (1997) Diffuse transmission by acetylcholine in the CNS. Progress in neurobiology 53:603-625.
DeYoe EA, Van Essen DC (1988) Concurrent processing streams in monkey visual cortex. Trends in neurosciences 11:219-226.
Di Cristo G, Berardi N, Cancedda L, Pizzorusso T, Putignano E, Ratto GM, Maffei L (2001) Requirement of ERK activation for visual cortical plasticity. Science 292:2337-2340.
Dilks DD, Serences JT, Rosenau BJ, Yantis S, McCloskey M (2007) Human adult cortical reorganization and consequent visual distortion. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:9585-9594.
Disney AA, Aoki C (2008) Muscarinic acetylcholine receptors in macaque V1 are most frequently expressed by parvalbumin-immunoreactive neurons. J Comp Neurol 507:1748-1762.
Disney AA, Aoki C, Hawken MJ (2007) Gain modulation by nicotine in macaque v1. Neuron 56:701-713.
Disney AA, Aoki C, Hawken MJ (2012) Cholinergic suppression of visual responses in primate V1 is mediated by GABAergic inhibition. Journal of neurophysiology 108:1907-1923.
Disney AA, Domakonda KV, Aoki C (2006) Differential expression of muscarinic acetylcholine receptors across excitatory and inhibitory cells in visual cortical areas V1 and V2 of the macaque monkey. The Journal of comparative neurology 499:49-63.
Donato F, Rompani SB, Caroni P (2013) Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. Nature 504:272-276.
Dotigny F, Ben Amor AY, Burke M, Vaucher E (2008) Neuromodulatory role of acetylcholine in visually-induced cortical activation: behavioral and neuroanatomical correlates. Neuroscience 154:1607-1618.
Douglas CL, Baghdoyan HA, Lydic R (2001) M2 muscarinic autoreceptors modulate acetylcholine release in prefrontal cortex of C57BL/6J mouse. J Pharmacol Exp Ther 299:960-966.
Douglas CL, Baghdoyan HA, Lydic R (2002) Postsynaptic muscarinic M1 receptors activate prefrontal cortical EEG of C57BL/6J mouse. Journal of neurophysiology 88:3003-3009.
Drevets WC, Price JL, Furey ML (2008) Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain structure & function 213:93-118.
Dringenberg HC, Hamze B, Wilson A, Speechley W, Kuo MC (2007) Heterosynaptic facilitation of in vivo thalamocortical long-term potentiation in the adult rat visual cortex by acetylcholine. Cereb Cortex 17:839-848.
Dudek SM, Bear MF (1992) Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proceedings of the National Academy of Sciences of the United States of America 89:4363-4367.

Eckenstein FP, Baughman RW, Quinn J (1988) An anatomical study of cholinergic innervation in rat cerebral cortex. Neuroscience 25:457-474.
Egan TM, North RA (1986) Acetylcholine hyperpolarizes central neurones by acting on an M2 muscarinic receptor. Nature 319:405-407.
Egeth HE, Leonard CJ, Leber AB (2010) Why salience is not enough: reflections on top-down selection in vision. Acta psychologica 135:130-132; discussion 133-139.
Eggermann E, Feldmeyer D (2009) Cholinergic filtering in the recurrent excitatory microcircuit of cortical layer 4. Proceedings of the National Academy of Sciences of the United States of America 106:11753-11758.
Eimer M, Kiss M (2010) The top-down control of visual selection and how it is linked to the N2pc component. Acta psychologica 135:100-102; discussion 133-109.
Eldar E, Cohen JD, Niv Y (2013) The effects of neural gain on attention and learning. Nature neuroscience 16:1146-1153.
Erisir A, Levey AI, Aoki C (2001) Muscarinic receptor M(2) in cat visual cortex: laminar distribution, relationship to gamma-aminobutyric acidergic neurons, and effect of cingulate lesions. J Comp Neurol 441:168-185.
Erreger K, Dravid SM, Banke TG, Wyllie DJ, Traynelis SF (2005) Subunit-specific gating controls rat NR1/NR2A and NR1/NR2B NMDA channel kinetics and synaptic signalling profiles. The Journal of physiology 563:345-358.
Eslinger PJ, Damasio AR (1985) Severe disturbance of higher cognition after bilateral frontal lobe ablation: patient EVR. Neurology 35:1731-1741.
Eysel UT, Schweigart G, Mittmann T, Eyding D, Qu Y, Vandesande F, Orban G, Arckens L (1999) Reorganization in the visual cortex after retinal and cortical damage. Restorative neurology and neuroscience 15:153-164.
Fagerstrom KO, Pomerleau O, Giordani B, Stelson F (1994) Nicotine may relieve symptoms of Parkinson's disease. Psychopharmacology 116:117-119.
Fagiolini M, Hensch TK (2000) Inhibitory threshold for critical-period activation in primary visual cortex. Nature 404:183-186.
Fahle M (2004) Perceptual learning: a case for early selection. Journal of vision 4:879-890.
Fahle M (2009) Perceptual learning and sensomotor flexibility: cortical plasticity under attentional control? Philosophical transactions of the Royal Society of London Series B, Biological sciences 364:313-319.
Fahle M, Poggio T (2002) Perceptual learning. Cambridge, Mass.: MIT Press.
Feldman ML, Peters A (1978) The forms of non-pyramidal neurons in the visual cortex of the rat. The Journal of comparative neurology 179:761-793.
Fiorentini A, Berardi N (1980) Perceptual learning specific for orientation and spatial frequency. Nature 287:43-44.
Freedman R (2013) alpha7-Nicotinic Acetylcholine Receptor Agonists for Cognitive Enhancement in Schizophrenia. Annual review of medicine.
Frenkel MY, Sawtell NB, Diogo AC, Yoon B, Neve RL, Bear MF (2006) Instructive effect of visual experience in mouse visual cortex. Neuron 51:339-349.
Fries P (2009) Neuronal gamma-band synchronization as a fundamental process in cortical computation. Annual review of neuroscience 32:209-224.
Fries P, Nikolic D, Singer W (2007) The gamma cycle. Trends in neurosciences 30:309-316.
Fries P, Womelsdorf T, Oostenveld R, Desimone R (2008) The effects of visual stimulation and selective visual attention on rhythmic neuronal synchronization in macaque area

V4. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:4823-4835.
Froemke RC, Carcea I, Barker AJ, Yuan K, Seybold BA, Martins AR, Zaika N, Bernstein H, Wachs M, Levis PA, Polley DB, Merzenich MM, Schreiner CE (2013) Long-term modification of cortical synapses improves sensory perception. Nature neuroscience 16:79-88.
Froemke RC, Merzenich MM, Schreiner CE (2007) A synaptic memory trace for cortical receptive field plasticity. Nature 450:425-429.
Furey ML (2011) The prominent role of stimulus processing: cholinergic function and dysfunction in cognition. Current opinion in neurology 24:364-370.
Furey ML, Pietrini P, Haxby JV (2000) Cholinergic enhancement and increased selectivity of perceptual processing during working memory. Science 290:2315-2319.
Furey ML, Ricciardi E, Schapiro MB, Rapoport SI, Pietrini P (2008) Cholinergic enhancement eliminates modulation of neural activity by task difficulty in the prefrontal cortex during working memory. J Cogn Neurosci 20:1342-1353.
Galarreta M, Hestrin S (1999) A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402:72-75.
Galarreta M, Hestrin S (2002) Electrical and chemical synapses among parvalbumin fast-spiking GABAergic interneurons in adult mouse neocortex. Proceedings of the National Academy of Sciences of the United States of America 99:12438-12443.
Gandhi SP, Yanagawa Y, Stryker MP (2008) Delayed plasticity of inhibitory neurons in developing visual cortex. Proceedings of the National Academy of Sciences 105:16797-16802.
Gaykema RP, Luiten PG, Nyakas C, Traber J (1990) Cortical projection patterns of the medial septum-diagonal band complex. The Journal of comparative neurology 293:103-124.
Ge S, Dani JA (2005) Nicotinic acetylcholine receptors at glutamate synapses facilitate long-term depression or potentiation. J Neurosci 25:6084-6091.
Gibson JR, Bartley AF, Huber KM (2006) Role for the subthreshold currents ILeak and IH in the homeostatic control of excitability in neocortical somatostatin-positive inhibitory neurons. Journal of neurophysiology 96:420-432.
Giessel AJ, Sabatini BL (2010) M1 muscarinic receptors boost synaptic potentials and calcium influx in dendritic spines by inhibiting postsynaptic SK channels. Neuron 68:936-947.
Gil Z, Connors BW, Amitai Y (1997) Differential regulation of neocortical synapses by neuromodulators and activity. Neuron 19:679-686.
Gilbert CD (1983) Microcircuitry of the visual cortex. Annual review of neuroscience 6:217-247.
Gilbert CD, Li W (2012) Adult visual cortical plasticity. Neuron 75:250-264.
Gilbert CD, Sigman M, Crist RE (2001) The neural basis of perceptual learning. Neuron 31:681-697.
Gilbert CD, Wiesel TN (1989) Columnar specificity of intrinsic horizontal and corticocortical connections in cat visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 9:2432-2442.
Gill TM, Sarter M, Givens B (2000) Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. The Journal of neuroscience : the official journal of the Society for Neuroscience 20:4745-4757.

Giordano AM, McElree B, Carrasco M (2009) On the automaticity and flexibility of covert attention: a speed-accuracy trade-off analysis. Journal of vision 9:30 31-10.
Girman SV, Sauve Y, Lund RD (1999) Receptive field properties of single neurons in rat primary visual cortex. Journal of neurophysiology 82:301-311.
Goard M, Dan Y (2009) Basal forebrain activation enhances cortical coding of natural scenes. Nature neuroscience 12:1444-1449.
Golcu D, Gilbert CD (2009) Perceptual learning of object shape. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:13621-13629.
Golmayo L, Nunez A, Zaborszky L (2003) Electrophysiological evidence for the existence of a posterior cortical-prefrontal-basal forebrain circuitry in modulating sensory responses in visual and somatosensory rat cortical areas. Neuroscience 119:597-609.
Gonchar Y, Burkhalter A (1997) Three distinct families of GABAergic neurons in rat visual cortex. Cereb Cortex 7:347-358.
Gonchar Y, Burkhalter A (2003) Distinct GABAergic targets of feedforward and feedback connections between lower and higher areas of rat visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:10904-10912.
Goto Y, Grace AA (2005) Dopamine-dependent interactions between limbic and prefrontal cortical plasticity in the nucleus accumbens: disruption by cocaine sensitization. Neuron 47:255-266.
Gray CM, Maldonado PE, Wilson M, McNaughton B (1995) Tetrodes markedly improve the reliability and yield of multiple single-unit isolation from multi-unit recordings in cat striate cortex. Journal of neuroscience methods 63:43-54.
Greuel JM, Luhmann HJ, Singer W (1988) Pharmacological induction of use-dependent receptive field modifications in the visual cortex. Science 242:74-77.
Groleau M, Nguyen HN, Vanni M, Huppe-Gourgues F, Casanova C, Vaucher E (2014) Impaired functional organization in the visual cortex of muscarinic receptors knock-out mice. NeuroImage 98:233-242.
Grutzendler J, Kasthuri N, Gan WB (2002) Long-term dendritic spine stability in the adult cortex. Nature 420:812-816.
Gu Q (2003) Contribution of acetylcholine to visual cortex plasticity. Neurobiology of learning and memory 80:291-301.
Gu Q, Singer W (1989) The role of muscarinic acetylcholine receptors in ocular dominance plasticity. Exs 57:305-314.
Gu Q, Singer W (1993) Effects of intracortical infusion of anticholinergic drugs on neuronal plasticity in kitten striate cortex. The European journal of neuroscience 5:475-485.
Gulledge AT, Bucci DJ, Zhang SS, Matsui M, Yeh HH (2009) M1 receptors mediate cholinergic modulation of excitability in neocortical pyramidal neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:9888-9902.
Gutnisky DA, Hansen BJ, Iliescu BF, Dragoi V (2009) Attention alters visual plasticity during exposure-based learning. Current biology : CB 19:555-560.
Haider B, McCormick DA (2009) Rapid neocortical dynamics: cellular and network mechanisms. Neuron 62:171-189.
Hamilton SE, Nathanson NM (2001) The M1 receptor is required for muscarinic activation of mitogen-activated protein (MAP) kinase in murine cerebral cortical neurons. The Journal of biological chemistry 276:15850-15853.

Hammer R, Berrie CP, Birdsall NJ, Burgen AS, Hulme EC (1980) Pirenzepine distinguishes between different subclasses of muscarinic receptors. Nature 283:90-92.
Hanover JL, Huang ZJ, Tonegawa S, Stryker MP (1999) Brain-derived neurotrophic factor overexpression induces precocious critical period in mouse visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:RC40.
Harauzov A, Spolidoro M, DiCristo G, De Pasquale R, Cancedda L, Pizzorusso T, Viegi A, Berardi N, Maffei L (2010) Reducing intracortical inhibition in the adult visual cortex promotes ocular dominance plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 30:361-371.
Harris KD, Thiele A (2011) Cortical state and attention. Nature reviews Neuroscience 12:509-523.
Hashimoto T, Shu H, Kuriyama K (1994) Muscarinic M1 receptor mediated inhibition of GABA release from rat cerebral cortex. Neurochemistry international 24:389-394.
Hasselmo ME (2006) The role of acetylcholine in learning and memory. Current opinion in neurobiology 16:710-715.
Headley DB, Weinberger NM (2011) Gamma-band activation predicts both associative memory and cortical plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:12748-12758.
Hendry SH, Yoshioka T (1994) A neurochemically distinct third channel in the macaque dorsal lateral geniculate nucleus. Science 264:575-577.
Hensch TK (2003) Controlling the critical period. Neuroscience research 47:17-22.
Hensch TK (2004) Critical period regulation. Annual review of neuroscience 27:549-579.
Hensch TK (2005) Critical period plasticity in local cortical circuits. Nature reviews Neuroscience 6:877-888.
Hensch TK, Fagiolini M, Mataga N, Stryker MP, Baekkeskov S, Kash SF (1998) Local GABA circuit control of experience-dependent plasticity in developing visual cortex. Science 282:1504-1508.
Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A (2008) Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. Nature 454:1110-1114.
Heynen AJ, Bear MF (2001) Long-term potentiation of thalamocortical transmission in the adult visual cortex in vivo. The Journal of neuroscience : the official journal of the Society for Neuroscience 21:9801-9813.
Hille B (2001) Ion channels of excitable membranes /Bertil Hille. Sunderland, Mass.: Sinauer Associates, Inc.
Hilt D, Gawryl M, Koenig G (2009) Safety, tolerability and cognitive effects of a novel a7 nicotinic receptor agonist in Alzheimer's disease patients on stable donepezil or rivastigmine therapy. Alzheimer's & Dementia: The Journal of the Alzheimer's Association 5:1.
Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. The Journal of physiology 117:500-544.
Hohmann CF, Potter ED, Levey AI (1995) Development of muscarinic receptor subtypes in the forebrain of the mouse. The Journal of comparative neurology 358:88-101.
Holmes AD, Copland DA, Silburn PA, Chenery HJ (2011) Acute nicotine enhances strategy-based semantic processing in Parkinson's disease. The international journal of

neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum 14:877-885.
Hosaka M, Hammer RE, Sudhof TC (1999) A phospho-switch controls the dynamic association of synapsins with synaptic vesicles. Neuron 24:377-387.
Hoyle E, Genn RF, Fernandes C, Stolerman IP (2006) Impaired performance of alpha7 nicotinic receptor knockout mice in the five-choice serial reaction time task. Psychopharmacology 189:211-223.
Hua T, Bao P, Huang CB, Wang Z, Xu J, Zhou Y, Lu ZL (2010) Perceptual learning improves contrast sensitivity of V1 neurons in cats. Current biology : CB 20:887-894.
Huang YY, Kandel ER (1994) Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. Learning & memory 1:74-82.
Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L, Tonegawa S (1999) BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. Cell 98:739-755.
Hubel DH, Wiesel TN (1965) Receptive Fields and Functional Architecture in Two Nonstriate Visual Areas (18 and 19) of the Cat. Journal of neurophysiology 28:229-289.
Hubel DH, Wiesel TN (1968) Receptive fields and functional architecture of monkey striate cortex. The Journal of physiology 195:215-243.
Inglis FM, Fibiger HC (1995) Increases in hippocampal and frontal cortical acetylcholine release associated with presentation of sensory stimuli. Neuroscience 66:81-86.
Isaacson JS, Scanziani M (2011) How inhibition shapes cortical activity. Neuron 72:231-243.
Iwai Y, Fagiolini M, Obata K, Hensch TK (2003) Rapid critical period induction by tonic inhibition in visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 23:6695-6702.
Jacobs GH, Neitz J, Deegan JF, 2nd (1991) Retinal receptors in rodents maximally sensitive to ultraviolet light. Nature 353:655-656.
Ji D, Dani JA (2000) Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. Journal of neurophysiology 83:2682-2690.
Ji D, Lape R, Dani JA (2001) Timing and location of nicotinic activity enhances or depresses hippocampal synaptic plasticity. Neuron 31:131-141.
Ji W, Suga N (2007) Serotonergic modulation of plasticity of the auditory cortex elicited by fear conditioning. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:4910-4918.
Jia H, Rochefort NL, Chen X, Konnerth A (2010) Dendritic organization of sensory input to cortical neurons in vivo. Nature 464:1307-1312.
Juergens E, Guettler A, Eckhorn R (1999) Visual stimulation elicits locked and induced gamma oscillations in monkey intracortical- and EEG-potentials, but not in human EEG. Experimental brain research 129:247-259.
Juliano SL, Ma W, Eslin D (1991) Cholinergic depletion prevents expansion of topographic maps in somatosensory cortex. Proceedings of the National Academy of Sciences of the United States of America 88:780-784.
Kaang BK, Kandel ER, Grant SG (1993) Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in Aplysia sensory neurons. Neuron 10:427-435.

Kaczmarek L, Chaudhuri A (1997) Sensory regulation of immediate-early gene expression in mammalian visual cortex: implications for functional mapping and neural plasticity. Brain research Brain research reviews 23:237-256.
Kajikawa Y, Schroeder CE (2011) How local is the local field potential? Neuron 72:847-858.
Kamal A, Ramakers GM, Urban IJ, De Graan PN, Gispen WH (1999) Chemical LTD in the CA1 field of the hippocampus from young and mature rats. The European journal of neuroscience 11:3512-3516.
Kandel ER, Schwartz JH, Jessell TM (2013) Principles of neural science. New York: McGraw-Hill, Health Professions Division.
Kang JI, Groleau M, Dotigny F, Giguere H, Vaucher E (2013) Visual training paired with electrical stimulation of the basal forebrain improves orientation-selective visual acuity in the rat. Brain structure & function.
Kang JI, Huppe-Gourgues F, Vaucher E (2014a) Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception. Frontiers in systems neuroscience 8.
Kang JI, Huppe-Gourgues F, Vaucher E (2014b) Pharmacological mechanisms of cortical enhancement induced by repetitive pairing of visual/cholinergic stimulation. In: PLoS one (submitted).
Kang JI, Vaucher E (2009) Cholinergic pairing with visual activation results in long-term enhancement of visual evoked potentials. PloS one 4:e5995.
Kato N, Isomura Y, Tanaka T (2000) Intracellular calcium releases facilitate induction of long-term depression. Neuropharmacology 39:1107-1110.
Katzner S, Busse L, Carandini M (2011) GABAA inhibition controls response gain in visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:5931-5941.
Katzner S, Nauhaus I, Benucci A, Bonin V, Ringach DL, Carandini M (2009) Local origin of field potentials in visual cortex. Neuron 61:35-41.
Kaur S, Lazar R, Metherate R (2004) Intracortical pathways determine breadth of subthreshold frequency receptive fields in primary auditory cortex. Journal of neurophysiology 91:2551-2567.
Kawai H, Lazar R, Metherate R (2007) Nicotinic control of axon excitability regulates thalamocortical transmission. Nat Neurosci 10:1168-1175.
Keck T, Scheuss V, Jacobsen RI, Wierenga CJ, Eysel UT, Bonhoeffer T, Hubener M (2011) Loss of sensory input causes rapid structural changes of inhibitory neurons in adult mouse visual cortex. Neuron 71:869-882.
Keitel C, Andersen SK, Quigley C, Muller MM (2013) Independent effects of attentional gain control and competitive interactions on visual stimulus processing. Cereb Cortex 23:940-946.
Kilgard MP, Merzenich MM (1998) Cortical map reorganization enabled by nucleus basalis activity. Science 279:1714-1718.
Kimura F, Baughman RW (1997) Distinct muscarinic receptor subtypes suppress excitatory and inhibitory synaptic responses in cortical neurons. Journal of neurophysiology 77:709-716.
Kimura F, Fukuda M, Tsumoto T (1999) Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. The European journal of neuroscience 11:3597-3609.

Kirkwood A, Rozas C, Kirkwood J, Perez F, Bear MF (1999) Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. The Journal of neuroscience : the official journal of the Society for Neuroscience 19:1599-1609.

Kleim JA, Hogg TM, VandenBerg PM, Cooper NR, Bruneau R, Remple M (2004) Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:628-633.

Kocharyan A, Fernandes P, Tong XK, Vaucher E, Hamel E (2008) Specific subtypes of cortical GABA interneurons contribute to the neurovascular coupling response to basal forebrain stimulation. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 28:221-231.

Konradsson-Geuken A, Gash CR, Alexander K, Pomerleau F, Huettl P, Gerhardt GA, Bruno JP (2009) Second-by-second analysis of alpha 7 nicotine receptor regulation of glutamate release in the prefrontal cortex of awake rats. Synapse 63:1069-1082.

Kosovicheva AA, Sheremata SL, Rokem A, Landau AN, Silver MA (2012) Cholinergic enhancement reduces orientation-specific surround suppression but not visual crowding. Frontiers in behavioral neuroscience 6:61.

Kruglikov I, Rudy B (2008) Perisomatic GABA release and thalamocortical integration onto neocortical excitatory cells are regulated by neuromodulators. Neuron 58:911-924.

Kuczewski N, Aztiria E, Leanza G, Domenici L (2005) Selective cholinergic immunolesioning affects synaptic plasticity in developing visual cortex. Eur J Neurosci 21:1807-1814.

Kuhlman SJ, Olivas ND, Tring E, Ikrar T, Xu X, Trachtenberg JT (2013) A disinhibitory microcircuit initiates critical-period plasticity in the visual cortex. Nature 501:543-546.

Lambe EK, Picciotto MR, Aghajanian GK (2003) Nicotine induces glutamate release from thalamocortical terminals in prefrontal cortex. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 28:216-225.

Land PW (1987) Dependence of cytochrome oxidase activity in the rat lateral geniculate nucleus on retinal innervation. The Journal of comparative neurology 262:78-89.

Laplante F, Morin Y, Quirion R, Vaucher E (2005) Acetylcholine release is elicited in the visual cortex, but not in the prefrontal cortex, by patterned visual stimulation: a dual in vivo microdialysis study with functional correlates in the rat brain. Neuroscience 132:501-510.

Larsen M, Haugstad TS, Berg-Johnsen J, Langmoen IA (1998) Effect of isoflurane on release and uptake of gamma-aminobutyric acid from rat cortical synaptosomes. British journal of anaesthesia 80:634-638.

Lee HK, Barbarosie M, Kameyama K, Bear MF, Huganir RL (2000) Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. Nature 405:955-959.

Lee HK, Kameyama K, Huganir RL, Bear MF (1998) NMDA induces long-term synaptic depression and dephosphorylation of the GluR1 subunit of AMPA receptors in hippocampus. Neuron 21:1151-1162.

Lee SH, Kwan AC, Dan Y (2014) Interneuron subtypes and orientation tuning. Nature 508:E1-2.

Lee SH, Kwan AC, Zhang S, Phoumthipphavong V, Flannery JG, Masmanidis SC, Taniguchi H, Huang ZJ, Zhang F, Boyden ES, Deisseroth K, Dan Y (2012) Activation of specific

interneurons improves V1 feature selectivity and visual perception. Nature 488:379-383.
Legatt AD, Arezzo J, Vaughan HG, Jr. (1980) Averaged multiple unit activity as an estimate of phasic changes in local neuronal activity: effects of volume-conducted potentials. Journal of neuroscience methods 2:203-217.
Levey AI, Kitt CA, Simonds WF, Price DL, Brann MR (1991) Identification and localization of muscarinic acetylcholine receptor proteins in brain with subtype-specific antibodies. J Neurosci 11:3218-3226.
Levin ED, Conners CK, Silva D, Hinton SC, Meck WH, March J, Rose JE (1998) Transdermal nicotine effects on attention. Psychopharmacology 140:135-141.
Levy RB, Reyes AD, Aoki C (2006) Nicotinic and muscarinic reduction of unitary excitatory postsynaptic potentials in sensory cortex; dual intracellular recording in vitro. J Neurophysiol 95:2155-2166.
Li S, Cullen WK, Anwyl R, Rowan MJ (2007) Muscarinic acetylcholine receptor-dependent induction of persistent synaptic enhancement in rat hippocampus in vivo. Neuroscience 144:754-761.
Li W, Piech V, Gilbert CD (2004) Perceptual learning and top-down influences in primary visual cortex. Nature neuroscience 7:651-657.
Li YT, Ibrahim LA, Liu BH, Zhang LI, Tao HW (2013) Linear transformation of thalamocortical input by intracortical excitation. Nature neuroscience 16:1324-1330.
Liao DS, Mower AF, Neve RL, Sato-Bigbee C, Ramoa AS (2002) Different mechanisms for loss and recovery of binocularity in the visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 22:9015-9023.
Lien AD, Scanziani M (2013) Tuned thalamic excitation is amplified by visual cortical circuits. Nature neuroscience 16:1315-1323.
Livingstone M, Hubel D (1988) Segregation of form, color, movement, and depth: anatomy, physiology, and perception. Science 240:740-749.
Lozada AF, Wang X, Gounko NV, Massey KA, Duan J, Liu Z, Berg DK (2012) Glutamatergic synapse formation is promoted by alpha7-containing nicotinic acetylcholine receptors. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:7651-7661.
Lucas-Meunier E, Monier C, Amar M, Baux G, Fregnac Y, Fossier P (2009) Involvement of nicotinic and muscarinic receptors in the endogenous cholinergic modulation of the balance between excitation and inhibition in the young rat visual cortex. Cereb Cortex 19:2411-2427.
Luiten PG, Gaykema RP, Traber J, Spencer DG, Jr. (1987) Cortical projection patterns of magnocellular basal nucleus subdivisions as revealed by anterogradely transported Phaseolus vulgaris leucoagglutinin. Brain research 413:229-250.
Luscher C, Xia H, Beattie EC, Carroll RC, von Zastrow M, Malenka RC, Nicoll RA (1999) Role of AMPA receptor cycling in synaptic transmission and plasticity. Neuron 24:649-658.
Luthi A, Chittajallu R, Duprat F, Palmer MJ, Benke TA, Kidd FL, Henley JM, Isaac JT, Collingridge GL (1999) Hippocampal LTD expression involves a pool of AMPARs regulated by the NSF-GluR2 interaction. Neuron 24:389-399.

Lysakowski A, Wainer BH, Bruce G, Hersh LB (1989) An atlas of the regional and laminar distribution of choline acetyltransferase immunoreactivity in rat cerebral cortex. Neuroscience 28:291-336.
Ma X, Suga N (2005) Long-term cortical plasticity evoked by electric stimulation and acetylcholine applied to the auditory cortex. Proceedings of the National Academy of Sciences of the United States of America 102:9335-9340.
Maffei A, Turrigiano GG (2008) Multiple modes of network homeostasis in visual cortical layer 2/3. The Journal of neuroscience : the official journal of the Society for Neuroscience 28:4377-4384.
Marik SA, Yamahachi H, Meyer zum Alten Borgloh S, Gilbert CD (2014) Large-scale axonal reorganization of inhibitory neurons following retinal lesions. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:1625-1632.
Markram H, Segal M (1990) Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine. J Physiol 427:381-393.
Martin KC, Kandel ER (1996) Cell adhesion molecules, CREB, and the formation of new synaptic connections. Neuron 17:567-570.
Martin PR (1986) The projection of different retinal ganglion cell classes to the dorsal lateral geniculate nucleus in the hooded rat. Experimental brain research 62:77-88.
Masquilier D, Sassone-Corsi P (1992) Transcriptional cross-talk: nuclear factors CREM and CREB bind to AP-1 sites and inhibit activation by Jun. The Journal of biological chemistry 267:22460-22466.
Matus A (2000) Actin-based plasticity in dendritic spines. Science 290:754-758.
Mayer MJ (1983) Practice improves adults' sensitivity to diagonals. Vision research 23:547-550.
McCormick DA (1992) Neurotransmitter actions in the thalamus and cerebral cortex. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 9:212-223.
McCormick DA, Prince DA (1985) Two types of muscarinic response to acetylcholine in mammalian cortical neurons. Proc Natl Acad Sci 82:6344-6348.
McCoy PA, McMahon LL (2007) Muscarinic receptor dependent long-term depression in rat visual cortex is PKC independent but requires ERK1/2 activation and protein synthesis. J Neurophysiol 98:1862-1870.
McGaughy J, Sarter M (1998) Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of physostigmine and FG 7142. Behavioral neuroscience 112:1519-1525.
McGaughy J, Sarter M (1999) Effects of ovariectomy, 192 IgG-saporin-induced cortical cholinergic deafferentation, and administration of estradiol on sustained attention performance in rats. Behavioral neuroscience 113:1216-1232.
McGehee DS (2002) Nicotinic receptors and hippocampal synaptic plasticity ... it's all in the timing. Trends in neurosciences 25:171-172.
McGuire BA, Gilbert CD, Rivlin PK, Wiesel TN (1991) Targets of horizontal connections in macaque primary visual cortex. The Journal of comparative neurology 305:370-392.
McKee SP, Westheimer G (1978) Improvement in vernier acuity with practice. Perception & psychophysics 24:258-262.

Mechawar N, Cozzari C, Descarries L (2000) Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. The Journal of comparative neurology 428:305-318.
Mesulam MM, Mufson EJ, Wainer BH, Levey AI (1983) Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience 10:1185-1201.
Metherate R (2004) Nicotinic acetylcholine receptors in sensory cortex. Learn Mem 11:50-59.
Metherate R (2011) Functional connectivity and cholinergic modulation in auditory cortex. Neuroscience and biobehavioral reviews 35:2058-2063.
Metherate R, Hsieh CY (2003) Regulation of glutamate synapses by nicotinic acetylcholine receptors in auditory cortex. Neurobiology of learning and memory 80:285-290.
Metherate R, Kaur S, Kawai H, Lazar R, Liang K, Rose HJ (2005) Spectral integration in auditory cortex: mechanisms and modulation. Hearing research 206:146-158.
Metherate R, Weinberger NM (1990) Cholinergic modulation of responses to single tones produces tone-specific receptive field alterations in cat auditory cortex. Synapse 6:133-145.
Miasnikov AA, Chen JC, Weinberger NM (2008) Specific auditory memory induced by nucleus basalis stimulation depends on intrinsic acetylcholine. Neurobiology of learning and memory 90:443-454.
Miasnikov AA, McLin D, 3rd, Weinberger NM (2001) Muscarinic dependence of nucleus basalis induced conditioned receptive field plasticity. Neuroreport 12:1537-1542.
Mirza NR, Stolerman IP (1998) Nicotine enhances sustained attention in the rat under specific task conditions. Psychopharmacology 138:266-274.
Mishkin M, Ungerleider LG, Macki KA (1983) Object vision and spatial vision: two cortical pathways. Trends in neurosciences 6:414-417.
Mitzdorf U (1985) Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. Physiological reviews 65:37-100.
Moore DR (1985) Postnatal development of the mammalian central auditory system and the neural consequences of auditory deprivation. Acta oto-laryngologica Supplementum 421:19-30.
Moran RJ, Campo P, Symmonds M, Stephan KE, Dolan RJ, Friston KJ (2013) Free energy, precision and learning: the role of cholinergic neuromodulation. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:8227-8236.
Morishita H, Miwa JM, Heintz N, Hensch TK (2010) Lynx1, a cholinergic brake, limits plasticity in adult visual cortex. Science 330:1238-1240.
Morris RG, Anderson E, Lynch GS, Baudry M (1986) Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319:774-776.
Mrzljak L, Levey AI, Belcher S, Goldman-Rakic PS (1998) Localization of the m2 muscarinic acetylcholine receptor protein and mRNA in cortical neurons of the normal and cholinergically deafferented rhesus monkey. JComp Neurol 390:112-132.
Mrzljak L, Levey AI, Goldman-Rakic PS (1993) Association of m1 and m2 muscarinic receptor proteins with asymmetric synapses in the primate cerebral cortex: morphological evidence for cholinergic modulation of excitatory neurotransmission.

Proceedings of the National Academy of Sciences of the United States of America 90:5194-5198.
Mukai I, Kim D, Fukunaga M, Japee S, Marrett S, Ungerleider LG (2007) Activations in visual and attention-related areas predict and correlate with the degree of perceptual learning. The Journal of neuroscience : the official journal of the Society for Neuroscience 27:11401-11411.
Muller CM, Singer W (1989) Acetylcholine-induced inhibition in the cat visual cortex is mediated by a GABAergic mechanism. Brain research 487:335-342.
Murphy PC, Sillito AM (1991) Cholinergic enhancement of direction selectivity in the visual cortex of the cat. Neuroscience 40:13-20.
Murthy VN, Schikorski T, Stevens CF, Zhu Y (2001) Inactivity produces increases in neurotransmitter release and synapse size. Neuron 32:673-682.
Muthukumaraswamy SD, Edden RA, Jones DK, Swettenham JB, Singh KD (2009) Resting GABA concentration predicts peak gamma frequency and fMRI amplitude in response to visual stimulation in humans. Proceedings of the National Academy of Sciences of the United States of America 106:8356-8361.
Nassi JJ, Callaway EM (2009) Parallel processing strategies of the primate visual system. Nature reviews Neuroscience 10:360-372.
Nathanson NM (2000) A multiplicity of muscarinic mechanisms: enough signaling pathways to take your breath away. Proceedings of the National Academy of Sciences of the United States of America 97:6245-6247.
Nestor L, McCabe E, Jones J, Clancy L, Garavan H (2011) Differences in "bottom-up" and "top-down" neural activity in current and former cigarette smokers: Evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. NeuroImage 56:2258-2275.
Neves G, Cooke SF, Bliss TV (2008) Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nature reviews Neuroscience 9:65-75.
Newhouse PA, Potter AS, Dumas JA, Thiel CM (2011) Functional brain imaging of nicotinic effects on higher cognitive processes. Biochemical pharmacology 82:943-951.
Newman EL, Gupta K, Climer JR, Monaghan CK, Hasselmo ME (2012) Cholinergic modulation of cognitive processing: insights drawn from computational models. Frontiers in behavioral neuroscience 6:24.
Niedermeyer E, Lopes da Silva FH (2005) Electroencephalography : basic principles, clinical applications, and related fields. Philadelphia: Lippincott Williams & Wilkins.
Nishimune A, Isaac JT, Molnar E, Noel J, Nash SR, Tagaya M, Collingridge GL, Nakanishi S, Henley JM (1998) NSF binding to GluR2 regulates synaptic transmission. Neuron 21:87-97.
Nunez A, Dominguez S, Buno W, Fernandez de Sevilla D (2012) Cholinergic-mediated response enhancement in barrel cortex layer V pyramidal neurons. Journal of neurophysiology 108:1656-1668.
Nunez PL, Srinivasan R (2006) Electric fields of the brain : the neurophysics of EEG. Oxford ; New York: Oxford University Press.
Olausson P, Jentsch JD, Taylor JR (2004) Nicotine enhances responding with conditioned reinforcement. Psychopharmacology 171:173-178.

Oldford E, Castro-Alamancos MA (2003) Input-specific effects of acetylcholine on sensory and intracortical evoked responses in the "barrel cortex" in vivo. Neuroscience 117:769-778.
Olsen SR, Bortone DS, Adesnik H, Scanziani M (2012) Gain control by layer six in cortical circuits of vision. Nature 483:47-52.
Origlia N, Kuczewski N, Aztiria E, Gautam D, Wess J, Domenici L (2006) Muscarinic acetylcholine receptor knockout mice show distinct synaptic plasticity impairments in the visual cortex. The Journal of physiology 577:829-840.
Origlia N, Kuczewski N, Pesavento E, Aztiria E, Domenici L (2008) The role of cholinergic system in neuronal plasticity: focus on visual cortex and muscarinic receptors. Arch Ital Biol 146:165-188.
Pafundo DE, Miyamae T, Lewis DA, Gonzalez-Burgos G (2013) Cholinergic modulation of neuronal excitability and recurrent excitation-inhibition in prefrontal cortex circuits: implications for gamma oscillations. The Journal of physiology 591:4725-4748.
Paik SB, Glaser DA (2010) Synaptic plasticity controls sensory responses through frequency-dependent gamma oscillation resonance. PLoS computational biology 6.
Papke RL, Sanberg PR, Shytle RD (2001) Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. J Pharmacol Exp Ther 297:646-656.
Paxinos G, Watson CR (1995) The rat brain in stereotaxic coordinates. Sydney: Academic Press.
Penschuck S, Chen-Bee CH, Prakash N, Frostig RD (2002) In vivo modulation of a cortical functional sensory representation shortly after topical cholinergic agent application. J Comp Neurol 452:38-50.
Peters A, Feldman M, Saldanha J (1976) The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. II. Terminations upon neuronal perikarya and dendritic shafts. J Neurocytol 5:85-107.
Peters A, Feldman ML (1976) The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. I. General description. J Neurocytol 5:63-84.
Peters A, Feldman ML (1977) The projection of the lateral geniculate nucleus to area 17 of the rat cerebral cortex. IV. Terminations upon spiny dendrites. J Neurocytol 6:669-689.
Pettersen KH, Einevoll GT (2008) Amplitude variability and extracellular low-pass filtering of neuronal spikes. Biophysical journal 94:784-802.
Pfister M, Boix F, Huston JP, Schwarting RK (1994) Different effects of scopolamine on extracellular acetylcholine levels in neostriatum and nucleus accumbens measured in vivo: possible interaction with aversive stimulation. J Neural Transm Gen Sect 97:13-25.
Pinto L, Goard MJ, Estandian D, Xu M, Kwan AC, Lee SH, Harrison TC, Feng G, Dan Y (2013) Fast modulation of visual perception by basal forebrain cholinergic neurons. Nature neuroscience 16:1857-1863.
Polack PO, Friedman J, Golshani P (2013) Cellular mechanisms of brain state-dependent gain modulation in visual cortex. Nature neuroscience 16:1331-1339.
Porciatti V, Pizzorusso T, Maffei L (1999) The visual physiology of the wild type mouse determined with pattern VEPs. Vision Res 39:3071-3081.
Pouille F, Scanziani M (2001) Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. Science 293:1159-1163.

Priebe NJ, Ferster D (2012) Mechanisms of neuronal computation in mammalian visual cortex. Neuron 75:194-208.
Prusky GT, West PW, Douglas RM (2000) Behavioral assessment of visual acuity in mice and rats. Vision research 40:2201-2209.
Quinlan EM, Philpot BD, Huganir RL, Bear MF (1999) Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex in vivo. Nature neuroscience 2:352-357.
Radcliffe KA, Dani JA (1998) Nicotinic stimulation produces multiple forms of increased glutamatergic synaptic transmission. The Journal of neuroscience : the official journal of the Society for Neuroscience 18:7075-7083.
Ramachandran VS, Braddick O (1973) Orientation-specific learning in stereopsis. Perception 2:371-376.
Ramalingam N, McManus JN, Li W, Gilbert CD (2013) Top-down modulation of lateral interactions in visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:1773-1789.
Ramanathan D, Tuszynski MH, Conner JM (2009) The basal forebrain cholinergic system is required specifically for behaviorally mediated cortical map plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:5992-6000.
Rang HP (2003) Pharmacology. Edinburgh ; Toronto: Churchill Livingstone.
Recanzone GH, Merzenich MM, Jenkins WM, Grajski KA, Dinse HR (1992) Topographic reorganization of the hand representation in cortical area 3b owl monkeys trained in a frequency-discrimination task. Journal of neurophysiology 67:1031-1056.
Recanzone GH, Schreiner CE, Merzenich MM (1993) Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. The Journal of neuroscience : the official journal of the Society for Neuroscience 13:87-103.
Reed A, Riley J, Carraway R, Carrasco A, Perez C, Jakkamsetti V, Kilgard MP (2011) Cortical map plasticity improves learning but is not necessary for improved performance. Neuron 70:121-131.
Reese BE (1988) 'Hidden lamination' in the dorsal lateral geniculate nucleus: the functional organization of this thalamic region in the rat. Brain research 472:119-137.
Regehr WG, Tank DW (1990) Postsynaptic NMDA receptor-mediated calcium accumulation in hippocampal CA1 pyramidal cell dendrites. Nature 345:807-810.
Renger JJ, Hartman KN, Tsuchimoto Y, Yokoi M, Nakanishi S, Hensch TK (2002) Experience-dependent plasticity without long-term depression by type 2 metabotropic glutamate receptors in developing visual cortex. Proceedings of the National Academy of Sciences of the United States of America 99:1041-1046.
Ricciardi E, Handjaras G, Bernardi G, Pietrini P, Furey ML (2013) Cholinergic enhancement reduces functional connectivity and BOLD variability in visual extrastriate cortex during selective attention. Neuropharmacology 64:305-313.
Riekkinen M, Riekkinen P, Jr. (1997) Nicotine and D-cycloserine enhance acquisition of water maze spatial navigation in aged rats. Neuroreport 8:699-703.
Roberts MJ, Thiele A (2008) Spatial integration and its moderation by attention and acetylcholine. Frontiers in bioscience : a journal and virtual library 13:3742-3759.

Roberts MJ, Zinke W, Guo K, Robertson R, McDonald JS, Thiele A (2005) Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex. Journal of neurophysiology 93:2062-2072.

Rockland KS, Lund JS (1982) Widespread periodic intrinsic connections in the tree shrew visual cortex. Science 215:1532-1534.

Rodriguez R, Kallenbach U, Singer W, Munk MH (2004) Short- and long-term effects of cholinergic modulation on gamma oscillations and response synchronization in the visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:10369-10378.

Roelfsema PR, van Ooyen A (2005) Attention-gated reinforcement learning of internal representations for classification. Neural Comput 17:2176-2214.

Roelfsema PR, van Ooyen A, Watanabe T (2010) Perceptual learning rules based on reinforcers and attention. Trends Cogn Sci 14:64-71.

Rokem A, Landau AN, Garg D, Prinzmetal W, Silver MA (2010) Cholinergic enhancement increases the effects of voluntary attention but does not affect involuntary attention. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 35:2538-2544.

Rokem A, Silver MA (2010) Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans. Current biology : CB 20:1723-1728.

Rokem A, Silver MA (2013) The benefits of cholinergic enhancement during perceptual learning are long-lasting. Frontiers in computational neuroscience 7:66.

Runyan CA, Schummers J, Van Wart A, Kuhlman SJ, Wilson NR, Huang ZJ, Sur M (2010) Response features of parvalbumin-expressing interneurons suggest precise roles for subtypes of inhibition in visual cortex. Neuron 67:847-857.

Rusakov DA (2006) Ca2+-dependent mechanisms of presynaptic control at central synapses. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 12:317-326.

Sabel BA, Henrich-Noack P, Fedorov A, Gall C (2011) Vision restoration after brain and retina damage: The "residual vision activation theory". Progress in brain research 192:199-262.

Sagi D (2011) Perceptual learning in Vision Research. Vision research 51:1552-1566.

Sale A, De Pasquale R, Bonaccorsi J, Pietra G, Olivieri D, Berardi N, Maffei L (2011) Visual perceptual learning induces long-term potentiation in the visual cortex. Neuroscience 172:219-225.

Salgado H, Bellay T, Nichols JA, Bose M, Martinolich L, Perrotti L, Atzori M (2007) Muscarinic M2 and M1 receptors reduce GABA release by Ca2+ channel modulation through activation of PI3K/Ca2+ -independent and PLC/Ca2+ -dependent PKC. Journal of neurophysiology 98:952-965.

Salgado H, Garcia-Oscos F, Patel A, Martinolich L, Nichols JA, Dinh L, Roychowdhury S, Tseng KY, Atzori M (2011) Layer-specific noradrenergic modulation of inhibition in cortical layer II/III. Cereb Cortex 21:212-221.

Sanz B, Exposito I, Mora F (1997) M1 acetylcholine receptor stimulation increases the extracellular concentrations of glutamate and GABA in the medial prefrontal cortex of the rat. Neurochemical research 22:281-286.

Sarter M, Bruno JP (2000) Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. Neuroscience 95:933-952.
Sarter M, Hasselmo ME, Bruno JP, Givens B (2005) Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain research Brain research reviews 48:98-111.
Sasaki Y, Nanez JE, Watanabe T (2010) Advances in visual perceptual learning and plasticity. Nature reviews Neuroscience 11:53-60.
Sato H, Hata Y, Masui H, Tsumoto T (1987) A functional role of cholinergic innervation to neurons in the cat visual cortex. Journal of neurophysiology 58:765-780.
Sawtell NB, Frenkel MY, Philpot BD, Nakazawa K, Tonegawa S, Bear MF (2003) NMDA receptor-dependent ocular dominance plasticity in adult visual cortex. Neuron 38:977-985.
Schoups A, Vogels R, Qian N, Orban G (2001) Practising orientation identification improves orientation coding in V1 neurons. Nature 412:549-553.
Schummers J, Sharma J, Sur M (2005) Bottom-up and top-down dynamics in visual cortex. Progress in brain research 149:65-81.
Seeger T, Fedorova I, Zheng F, Miyakawa T, Koustova E, Gomeza J, Basile AS, Alzheimer C, Wess J (2004) M2 muscarinic acetylcholine receptor knock-out mice show deficits in behavioral flexibility, working memory, and hippocampal plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience 24:10117-10127.
Seitz A, Watanabe T (2005) A unified model for perceptual learning. Trends Cogn Sci 9:329-334.
Seitz AR, Kim D, Watanabe T (2009) Rewards evoke learning of unconsciously processed visual stimuli in adult humans. Neuron 61:700-707.
Servan-Schreiber D, Printz H, Cohen JD (1990) A network model of catecholamine effects: gain, signal-to-noise ratio, and behavior. Science 249:892-895.
Shallice T, Burgess PW (1991) Deficits in strategy application following frontal lobe damage in man. Brain : a journal of neurology 114 (Pt 2):727-741.
Shao YR, Isett BR, Miyashita T, Chung J, Pourzia O, Gasperini RJ, Feldman DE (2013) Plasticity of recurrent l2/3 inhibition and gamma oscillations by whisker experience. Neuron 80:210-222.
Shekhar A, Potter WZ, Lightfoot J, Lienemann J, Dube S, Mallinckrodt C, Bymaster FP, McKinzie DL, Felder CC (2008) Selective muscarinic receptor agonist xanomeline as a novel treatment approach for schizophrenia. The American journal of psychiatry 165:1033-1039.
Shinoe T, Matsui M, Taketo MM, Manabe T (2005) Modulation of synaptic plasticity by physiological activation of M1 muscarinic acetylcholine receptors in the mouse hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 25:11194-11200.
Shuler MG, Bear MF (2006) Reward timing in the primary visual cortex. Science 311:1606-1609.
Sillito AM, Cudeiro J, Jones HE (2006) Always returning: feedback and sensory processing in visual cortex and thalamus. Trends in neurosciences 29:307-316.
Sillito AM, Kemp JA (1983) Cholinergic modulation of the functional organization of the cat visual cortex. Brain research 289:143-155.

Silva AJ, Kogan JH, Frankland PW, Kida S (1998) CREB and memory. Annual review of neuroscience 21:127-148.
Silver MA, Shenhav A, D'Esposito M (2008) Cholinergic enhancement reduces spatial spread of visual responses in human early visual cortex. Neuron 60:904-914.
Skrandies W, Fahle M (1994) Neurophysiological correlates of perceptual learning in the human brain. Brain topography 7:163-168.
Soma S, Shimegi S, Osaki H, Sato H (2012) Cholinergic modulation of response gain in the primary visual cortex of the macaque. Journal of neurophysiology 107:283-291.
Soma S, Shimegi S, Suematsu N, Sato H (2013a) Cholinergic modulation of response gain in the rat primary visual cortex. Scientific reports 3:1138.
Soma S, Shimegi S, Suematsu N, Tamura H, Sato H (2013b) Modulation-specific and laminar-dependent effects of acetylcholine on visual responses in the rat primary visual cortex. PloS one 8:e68430.
St Peters M, Demeter E, Lustig C, Bruno JP, Sarter M (2011) Enhanced control of attention by stimulating mesolimbic-corticopetal cholinergic circuitry. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:9760-9771.
Steinlein O (1998) New functions for nicotinic acetylcholine receptors? Behavioural brain research 95:31-35.
Stettler DD, Das A, Bennett J, Gilbert CD (2002) Lateral connectivity and contextual interactions in macaque primary visual cortex. Neuron 36:739-750.
Sun YJ, Wu GK, Liu BH, Li P, Zhou M, Xiao Z, Tao HW, Zhang LI (2010) Fine-tuning of pre-balanced excitation and inhibition during auditory cortical development. Nature 465:927-931.
Sur C, Mallorga PJ, Wittmann M, Jacobson MA, Pascarella D, Williams JB, Brandish PE, Pettibone DJ, Scolnick EM, Conn PJ (2003) N-desmethylclozapine, an allosteric agonist at muscarinic 1 receptor, potentiates N-methyl-D-aspartate receptor activity. Proc Natl Acad Sci 100:13674-13679.
Suudhof TC (2008) Neurotransmitter release. Handbook of experimental pharmacology 1-21.
Sweatt JD (1999) Toward a molecular explanation for long-term potentiation. Learning & memory 6:399-416.
Taha S, Hanover JL, Silva AJ, Stryker MP (2002) Autophosphorylation of alphaCaMKII is required for ocular dominance plasticity. Neuron 36:483-491.
Takahashi T, Svoboda K, Malinow R (2003) Experience strengthening transmission by driving AMPA receptors into synapses. Science 299:1585-1588.
Takesian AE, Hensch TK (2013) Balancing plasticity/stability across brain development. Progress in brain research 207:3-34.
Tamas G, Buhl EH, Lorincz A, Somogyi P (2000) Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. Nature neuroscience 3:366-371.
Theeuwes J (2010) Top-down and bottom-up control of visual selection. Acta psychologica 135:77-99.
Thiel CM, Zilles K, Fink GR (2005) Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology 30:810-820.
Thiele A (2013) Muscarinic signaling in the brain. Annual review of neuroscience 36:271-294.
Thiele A, Herrero JL, Distler C, Hoffmann KP (2012) Contribution of cholinergic and GABAergic mechanisms to direction tuning, discriminability, response reliability, and

neuronal rate correlations in macaque middle temporal area. The Journal of neuroscience : the official journal of the Society for Neuroscience 32:16602-16615.
Tigges M, Tigges J, Rees H, Rye D, Levey AI (1997) Distribution of muscarinic cholinergic receptor proteins m1 to m4 in area 17 of normal and monocularly deprived rhesus monkeys. The Journal of comparative neurology 388:130-145.
Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature 420:788-794.
Tsubokawa H, Ross WN (1996) IPSPs modulate spike backpropagation and associated [Ca2+]i changes in the dendrites of hippocampal CA1 pyramidal neurons. Journal of neurophysiology 76:2896-2906.
Turrigiano GG, Leslie KR, Desai NS, Rutherford LC, Nelson SB (1998) Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391:892-896.
Ullman S (2007) Object recognition and segmentation by a fragment-based hierarchy. Trends Cogn Sci 11:58-64.
Umbriaco D, Watkins KC, Descarries L, Cozzari C, Hartman BK (1994) Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. The Journal of comparative neurology 348:351-373.
Van der Loos H, Woolsey TA (1973) Somatosensory cortex: structural alterations following early injury to sense organs. Science 179:395-398.
Vaucher E, Borredon J, Bonvento G, Seylaz J, Lacombe P (1997) Autoradiographic evidence for flow-metabolism uncoupling during stimulation of the nucleus basalis of Meynert in the conscious rat. JCerebBlood Flow Metab 17:686-694.
Vaucher E, Hamel E (1995) Cholinergic basal forebrain neurons project to cortical microvessels in the rat: electron microscopic study with anterogradely transported Phaseolus vulgaris leucoagglutinin and choline acetyltransferase immunocytochemistry. The Journal of neuroscience : the official journal of the Society for Neuroscience 15:7427-7441.
Verdier D, Dykes RW (2001) Long-term cholinergic enhancement of evoked potentials in rat hindlimb somatosensory cortex displays characteristics of long-term potentiation. Experimental brain research 137:71-82.
Vidnyanszky Z, Sohn W (2005) Learning to suppress task-irrelevant visual stimuli with attention. Vision research 45:677-685.
Villeneuve MY, Casanova C (2003) On the use of isoflurane versus halothane in the study of visual response properties of single cells in the primary visual cortex. Journal of neuroscience methods 129:19-31.
Voytko ML, Olton DS, Richardson RT, Gorman LK, Tobin JR, Price DL (1994) Basal forebrain lesions in monkeys disrupt attention but not learning and memory. The Journal of neuroscience : the official journal of the Society for Neuroscience 14:167-186.
Wang L, Kloc M, Gu Y, Ge S, Maffei A (2013) Layer-specific experience-dependent rewiring of thalamocortical circuits. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:4181-4191.

Wang Y, Sherwood JL, Lodge D (2006) The alpha4beta2 nicotinic acetylcholine receptor agonist TC-2559 impairs long-term potentiation in the dentate gyrus in vivo. Neuroscience letters 406:183-188.
Watanabe T, Nanez JE, Sasaki Y (2001) Perceptual learning without perception. Nature 413:844-848.
Watanabe T, Nanez JE, Sr., Koyama S, Mukai I, Liederman J, Sasaki Y (2002) Greater plasticity in lower-level than higher-level visual motion processing in a passive perceptual learning task. Nature neuroscience 5:1003-1009.
Weinberger NM (2003) The nucleus basalis and memory codes: auditory cortical plasticity and the induction of specific, associative behavioral memory. Neurobiology of learning and memory 80:268-284.
Wesnes K, Warburton DM, Matz B (1983) Effects of nicotine on stimulus sensitivity and response bias in a visual vigilance task. Neuropsychobiology 9:41-44.
Wess J (2003) Novel insights into muscarinic acetylcholine receptor function using gene targeting technology. Trends in pharmacological sciences 24:414-420.
Wester JC, Contreras D (2013) Differential modulation of spontaneous and evoked thalamocortical network activity by acetylcholine level in vitro. The Journal of neuroscience : the official journal of the Society for Neuroscience 33:17951-17966.
Wiesel TN, Hubel DH (1963) Single-Cell Responses in Striate Cortex of Kittens Deprived of Vision in One Eye. Journal of neurophysiology 26:1003-1017.
Wilson DA, Fletcher ML, Sullivan RM (2004) Acetylcholine and olfactory perceptual learning. Learning & memory 11:28-34.
Wilson NR, Runyan CA, Wang FL, Sur M (2012) Division and subtraction by distinct cortical inhibitory networks in vivo. Nature 488:343-348.
Wonnacott S (1997) Presynaptic nicotinic ACh receptors. Trends in neurosciences 20:92-98.
Woolf NJ (1991) Cholinergic systems in mammalian brain and spinal cord. Progress in neurobiology 37:475-524.
Wylie KP, Rojas DC, Tanabe J, Martin LF, Tregellas JR (2012) Nicotine increases brain functional network efficiency. NeuroImage 63:73-80.
Xiang Z, Huguenard JR, Prince DA (1998) Cholinergic switching within neocortical inhibitory networks. Science 281:985-988.
Xing D, Yeh CI, Burns S, Shapley RM (2012) Laminar analysis of visually evoked activity in the primary visual cortex. Proceedings of the National Academy of Sciences of the United States of America 109:13871-13876.
Xing D, Yeh CI, Shapley RM (2009) Spatial spread of the local field potential and its laminar variation in visual cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience 29:11540-11549.
Yamahachi H, Marik SA, McManus JN, Denk W, Gilbert CD (2009) Rapid axonal sprouting and pruning accompany functional reorganization in primary visual cortex. Neuron 64:719-729.
Yamamoto K, Hashimoto K, Isomura Y, Shimohama S, Kato N (2000) An IP3-assisted form of Ca2+-induced Ca2+ release in neocortical neurons. Neuroreport 11:535-539.
Yamasaki M, Matsui M, Watanabe M (2010) Preferential Localization of Muscarinic M1 Receptor on Dendritic Shaft and Spine of Cortical Pyramidal Cells and Its Anatomical Evidence for Volume Transmission. Journal of Neuroscience 30:4408-4418.

Yamazaki Y, Jia Y, Hamaue N, Sumikawa K (2005) Nicotine-induced switch in the nicotinic cholinergic mechanisms of facilitation of long-term potentiation induction. Eur J Neurosci 22:845-860.

Yang C, McKenna JT, Zant JC, Winston S, Basheer R, Brown RE (2014) Cholinergic neurons excite cortically projecting basal forebrain GABAergic neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 34:2832-2844.

Yazaki-Sugiyama Y, Kang S, Cateau H, Fukai T, Hensch TK (2009) Bidirectional plasticity in fast-spiking GABA circuits by visual experience. Nature 462:218-221.

Yoshimura Y, Ohmura T, Komatsu Y (2003) Two forms of synaptic plasticity with distinct dependence on age, experience, and NMDA receptor subtype in rat visual cortex. J Neurosci 23:6557-6566.

Yu AJ, Dayan P (2002) Acetylcholine in cortical inference. Neural Netw 15:719-730.

Yu AJ, Dayan P (2005) Uncertainty, Neuromodulation, and Attention. Neuron 46:681-692.

Zaborszky L, Pang K, Somogyi J, Nadasdy Z, Kallo I (1999) The basal forebrain corticopetal system revisited. Annals of the New York Academy of Sciences 877:339-367.

Zeki S, Shipp S (1988) The functional logic of cortical connections. Nature 335:311-317.

Zeki SM (1978) Functional specialisation in the visual cortex of the rhesus monkey. Nature 274:423-428.

Zhang ZW, Burke MW, Calakos N, Beaulieu JM, Vaucher E (2010) Confocal Analysis of Cholinergic and Dopaminergic Inputs onto Pyramidal Cells in the Prefrontal Cortex of Rodents. Frontiers in neuroanatomy 4:21.

Zhang ZW, Kang JI, Vaucher E (2011) Axonal varicosity density as an index of local neuronal interactions. PloS one 6:e22543.

Zhao C, Dreosti E, Lagnado L (2011) Homeostatic synaptic plasticity through changes in presynaptic calcium influx. The Journal of neuroscience : the official journal of the Society for Neuroscience 31:7492-7496.

Zhong WX, Dong ZF, Tian M, Cao J, Xu L, Luo JH (2006) N-methyl-D-aspartate receptor-dependent long-term potentiation in CA1 region affects synaptic expression of glutamate receptor subunits and associated proteins in the whole hippocampus. Neuroscience 141:1399-1413.

Zinke W, Roberts MJ, Guo K, McDonald JS, Robertson R, Thiele A (2006) Cholinergic modulation of response properties and orientation tuning of neurons in primary visual cortex of anaesthetized Marmoset monkeys. The European journal of neuroscience 24:314-328.