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Modulation cholinergique à long terme des potentiels évoqués visuels dans le cortex visuel chez le rat

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Modulation cholinergique à long terme des potentiels évoqués visuels dans le cortex visuel chez le rat

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RÉSUMÉ

Plusieurs études récentes suggèrent un rôle de l'acétylcholine (ACh) dans la plasticité synaptique corticale induite par stimulation sensorielle. En particulier, certaines de nos expériences précédentes ont prouvé que les lésions spécifiques des neurones cholinergiques projetant au cortex visuel primaire (V1) réduisent les capacités d'apprentissage visuel des rats dans un labyrinthe, le visual water maze. Dans l'étude présente, les potentiels évoqués visuels (PEV) ont été analysés en présence de différents composés pharmacologiques. Le but était de déterminer si ce rôle modulateur du système cholinergique était (1) dû à une facilitation à long terme des réponses thalamo-corticales et (2) à l'interaction avec les voies glutamatergiques via les récepteurs NMDA généralement impliqués dans les phénomènes de potentiation à long terme.

44 rats males pigmentés anesthésiés ont été utilisés pour cette étude. Une électrode de tungstène d'enregistrement et un guide de canule «push-pull» pour l'infusion locale de drogues ont été implantés dans V1 deux jours avant l'expérience. Les PEVs ont été évoqués par un réseau sinusoïdal pendant 7 cycles (10 minutes de stimulation et 20 minutes de repos, 3h). Ces injections de drogues ou de véhicule ont été effectuées 1 µl /min pendant la troisième stimulation visuelle, après deux enregistrements du niveau de base du PEV. Sept groupes expérimentaux ont été analysés : groupe de témoin (n=11), injection scopolamine (antagoniste du récepteur muscarinique) i.p. (n=5), injection carbachol (analogue d'ACh) i.c. (n=6), injection aCSF i.c. + carbachol i.c. (n=6), injection CPP (antagoniste du récepteur NMDA) i.c. + carbachol i.c. (n=6), injection mecamylamine (antagoniste du récepteur nicotinique) i.c. + carbachol i.c. (n=5), et injection scopolamine i.p. + carbachol i.c. (n=5).

L'amplitude du PEV enregistré dans le groupe témoin ou scopolamine i.p. n'a pas augmenté à long terme durant les 3 heures d'expérience. Cependant, le carbachol a augmenté de manière significative l'amplitude des PEVs (50%). Cette augmentation était soutenue au moins 2h. Un traitement préliminaire avec la scopolamine, CPP ou mecamylamine, a supprimé cette potentialisation à long terme.

Ces résultats suggèrent que l'administration unitaire de carbachol jumelée avec une stimulation visuelle induit une augmentation à long terme de la réponse corticale aux stimulations visuelles subséquentes est dépendante des récepteurs nicotiniques et muscarinique d'acétylcholine. Ce mécanisme implique la transmission glutamatergique par les récepteurs NMDA.

Cette étude suggère que le système cholinergique peut favoriser le traitement cortical de certains stimuli visuels.

MOTS CLÉS FRANÇAIS

Acétylcholine

Électrophysiologie

Potentiel évoqué visuel

Transmission thalamocorticale

Modulation cholinergique

Récepteur NMDA

Plasticité corticale

Potentialisation à long terme

Cortex visuel

SUMMARY

A growing body of evidence suggests a role of acetylcholine (ACh) in the cortical synaptic plasticity induced by sensory stimulation. Particularly, some of our previous experiments have shown that specific lesions of the cholinergic neurons projecting to the primary visual cortex (V1) reduced the visual learning capacities of the rats in a visual water maze. In the present study, visual evoked potentials (VEP) were analyzed in different experimental procedures to determine whether this cholinergic modulatory role was due (1) to a long-term facilitation of thalamo-cortical responses and (2) to the interaction of cholinergic and long term potentiation pathways, which involves NMDA transmission.

44 anesthetized male rats were used for this study. A recording tungsten electrode and a push-pull canula guide for local drug infusion were implanted in V1 two days before the experiment. The VEP were evoked by sinusoidal gratings during 7 cycles (10 min of stimulation and 20 min of rest, 3h). Drugs or vehicle injections (1 µl /min) were performed during the third visual stimulation, after recording two baseline VEPs. Seven experimental groups were analyzed; control group (n=11), scopolamine (muscarinic receptor antagonist) i.p. injection (n=5), carbachol (an acetylcholine analog) i.c. injection (n=6), aCSF i.c. + carbachol i.c. injection (n=6), CPP (NMDA receptor antagonist) i.c. + carbachol i.c. injection (n=6), mecamylamine (nicotinic receptor antagonist) i.c. + carbachol i.c. injection (n=5), and scopolamine i.p. + carbachol i.c. injection (n=5) were analyzed.

The amplitude of the VEP recorded in control or scopolamine i.p. injection groups did not show any spontaneous long-term enhancement throughout the 3h experiment. However, carbachol significantly increased VEP amplitude (50%), this enhancement being sustained at least 2h. Pre-

treatment with scopolamine, CPP or mecamylamine abolished this long term enhancement effect of carbachol.

These results showed that a single activation of cholinergic system paired with visual stimulation induced long term enhancement of the cortical response. Moreover this enhancement of thalamocortical signals, involves nicotinic and muscarinic acetylcholine receptors as well as glutamatergic NMDA transmission.

This study suggests that cholinergic system could facilitate the visual processing of certain visual stimuli.

KEYWORDS

Acetylcholine

Electrophysiology

Visual evoked potential

Thalamocortical transmission

Cholinergic modulation

NMDA receptor

Cortical plasticity

Long-term potentiation

Visual cortex

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LISTES DES ABRÉVIATIONS

ACh: Acetylcholine

aCSF: Artificial cerebro-spinal fluid

AMPA: α-amino-3-hydroxy-5-methylisoxazole-4- propionic acid)

CaMKII: Calcium/calmodulin kinase II

CNS: Central nervous system

CPP: 3-(2-Carboxypiperazin-4-yl)-propyl-1-phosphonic acid

GABA: Gamma-amino-butyric acid

LGN: Lateral Geniculate Nucleus

LTP: Long-term potentiation

nAChR: Nicotinic cholinergic receptor

NMDA: N-methyl-D-aspartate

NMDAR: NMDA receptor

PKA: Protein kinase A

VEP: Visual evoked potential

V1: Primary visual cortex

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INTRODUCTION

1. Prologue

With a philosophical ideology, Descartes expressed process of vision in two distinguishable states: a mechanical state which reflects light transmitted instantaneously into our senses and a perceptual state which represents signs interpreted by our innate ideas and cognition. Previous work by many neuroscientists had revealed in details visual mechanisms in the mechanical state.

Until few decades, since the study of the brain was technically unavailable, Descartes' perceptual state was considered as mind work and yields its study to psychologists. However, with the development of techniques, electrophysiology being in the forefront, neuroscientists were able to observe the activation of brain during its action. This revolutionary ability allows the study of the perceptual state, i.e. study of the interpretation of visual information in the brain. Descartes' two states are now converging.

2. Visual system in the brain

Vision is provided through highly complex and organized interconnected process among various parties of the brain. And this mechanism, which is still not completely revealed, differs from species to species. Even though in our experiment we only used rat as test subject, to afford a better comprehension primate's visual function is introduced first.

2.1 Primate

Light reflected by objects reaches our retina where two different photoreceptor classes are located: rods which are sensitive to light's intensity and cones sensitive to the wave length of

the afferent light which allow color vision. In order to reach the photoreceptors light must go through other layers in the retina: ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer, and outer nuclear layer (Figure 1). Visual information adapted by photoreceptors is transformed into electrical signals and transferred to the retinal ganglion cells. Axons from retinal ganglion cells project to optic chiasm where they are distributed to three major subcortical targets: the superior colliculus, the pretectum and the Lateral Geniculate Nucleus (LGN). Visual information from retina reaching LGN via optic nerves and optic tracts, are delivered mainly to the cortex in the occipital area which is called primary visual cortex (V1) (Figure 2). Flow of visual information after V1 is illustrated in Figure 3. Although superior colliculus and pretectum have an essential role in visual ability by controlling saccade movement and pupillary reflexes since our studies are performed in visual cortex here we will focus on the flow from LGN.

Visual signals are generally processed in two pathways: the dorsal and the ventral. The dorsal pathway starts in the retina with ganglion cells of M type (M for *Magnus,* meaning large because of the large receptive field of these cells). These cells respond transiently to sustained illumination. M cells projections passing through magnocellular layers (I and II) of LGN (Figure 4) reach the layer IV in the V1. Layer IV is divided into three sublayers designated IV A, IV B, and IV C. Layer IV C is itself subdivided into IV Cα and IV Cβ, and axons in magnocellular layers of LGN project into IV Cα. The majority of neurons in the layer IV are spiny stellate cells whose axons pass information on to the dendrites of the pyramidal cells which are excitatory and use glutamate as their transmitters, in layers IV B and III. From V1, information is conveyed in the dorsal area of brain, the middle temporal area (also referred area MT/V5) extending to the

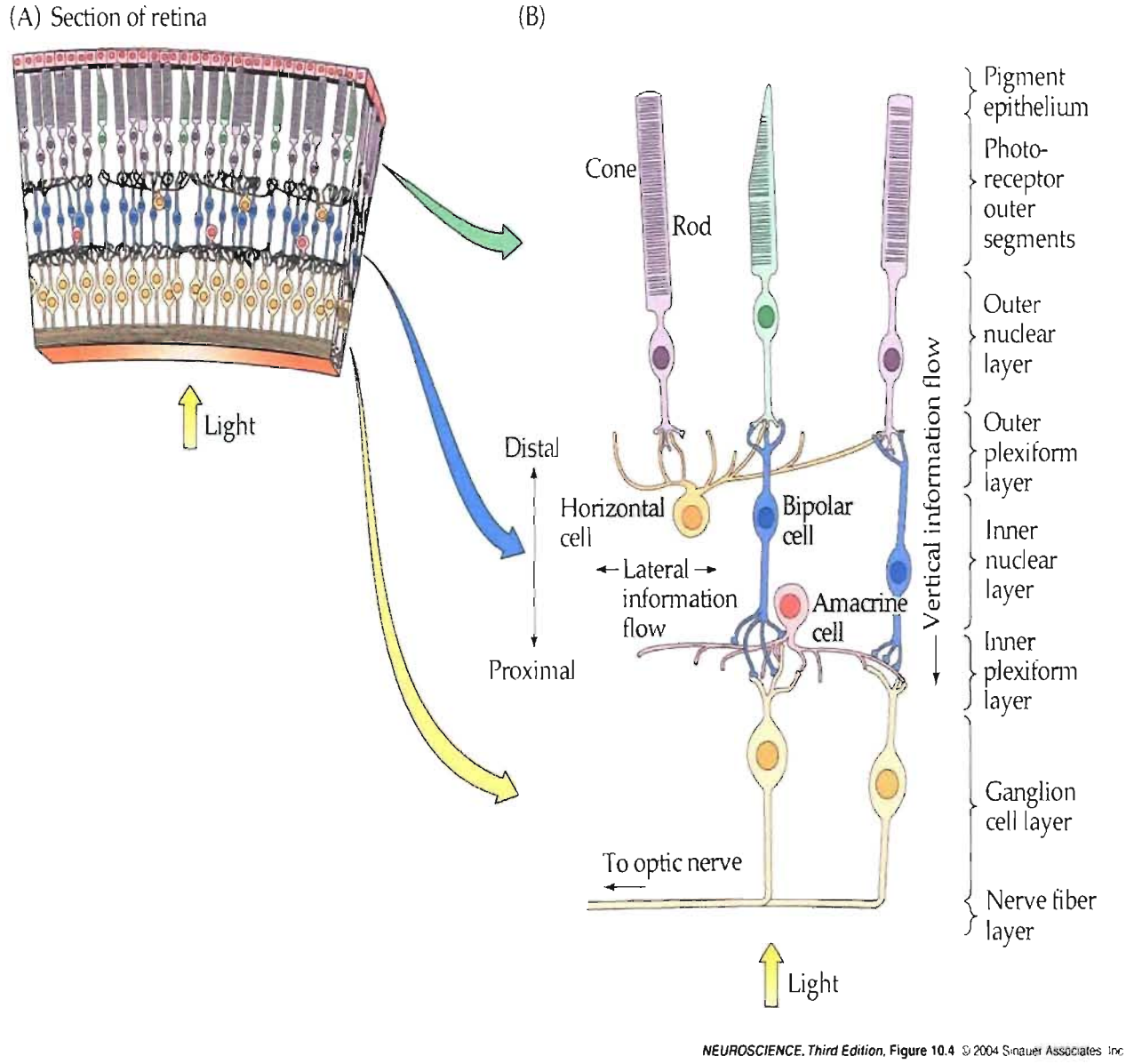

Figure 1: Cross section of the retina. Photoreceptors (rod and cone) are situated in the deep layer. Light must pass through ganglion cell layer, inner plexiform layer, inner nuclear layer, outer plexiform layer and outer nuclear layer to reach photoreceptors.

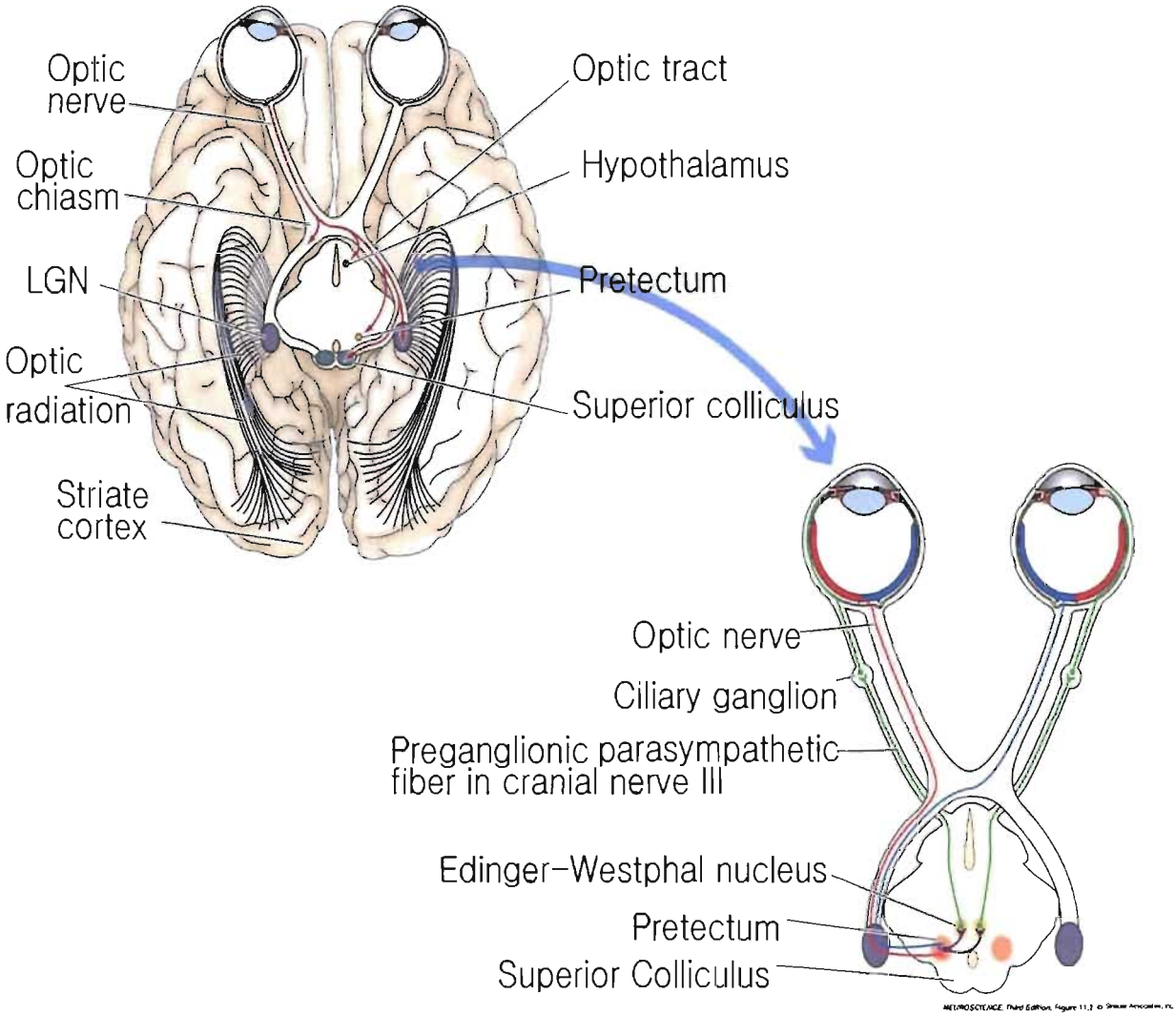

Figure 2: Optic tract. Optic nerves from the gaglion cells in the nasal half of each retina cross each other in optic chiasm and reach the contralateral part of the primary visual cortex. On the contrary axons from temporal visual field do not cross. (LGN: Lateral geniculate nucleus)

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posterior parietal cortex (Figure 3). Relatively, neurons in this area response hardly to colour or to stationary objects analyse (Corbetta *et al.*, 1991).

On the other hand, ventral pathway starts with P cells (for *parvi*, or small) and via parvocellular layers in LGN (3 to 6) projects to IV Cβ and IV A layer of V1. V1 projects to V4 linked with inferior temporal cortex where neurons are sensitive to the outline of images or orientation, colour and shape. It is suggested that dorsal pathway is concerned with "where" objects are, and ventral pathway with "what" the objects are (Mishkin *et al.*, 1983). Compelling evidence suggest that different systems are specialized for different visual functions (Zeki, 1978; DeYoe and van Essen, 1988; Livingstone and Hubel, 1988; Zeki and Shipp, 1988). Visual information sent in different area is used differently i.e. visuospatial recognition for dorsal pathway and recognition of complex objects for ventral pathway (Figure 3).

In primate visual cortex specific features of the organization of V1 are orientation columns, blobs and ocular dominance columns. Discovered by Hubel and Wiesel, using 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 receptive field 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 (~75%) reach contralateral visual cortex. In the result according to their input source those two separate tracts compose ocular dominance columns.

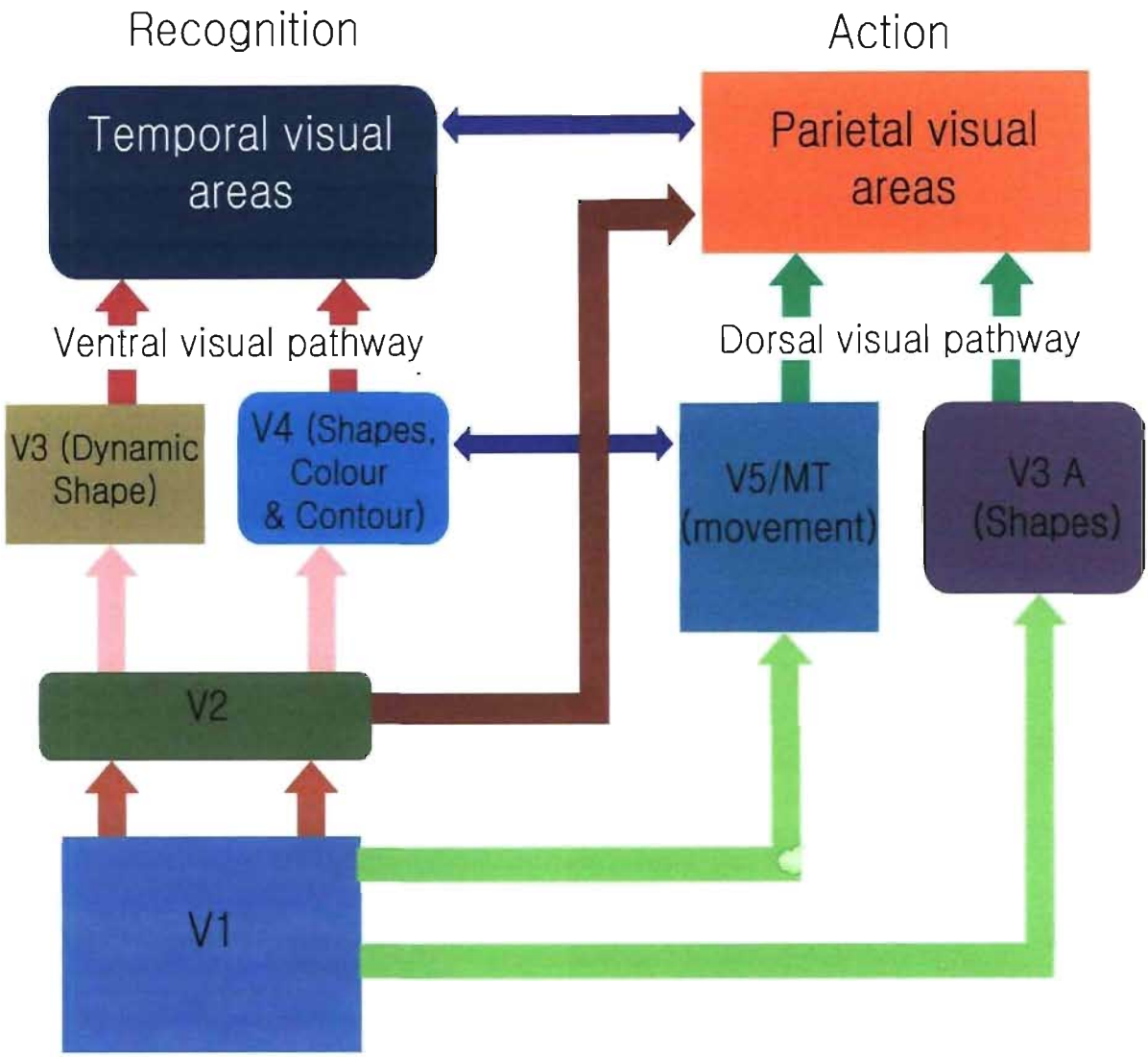


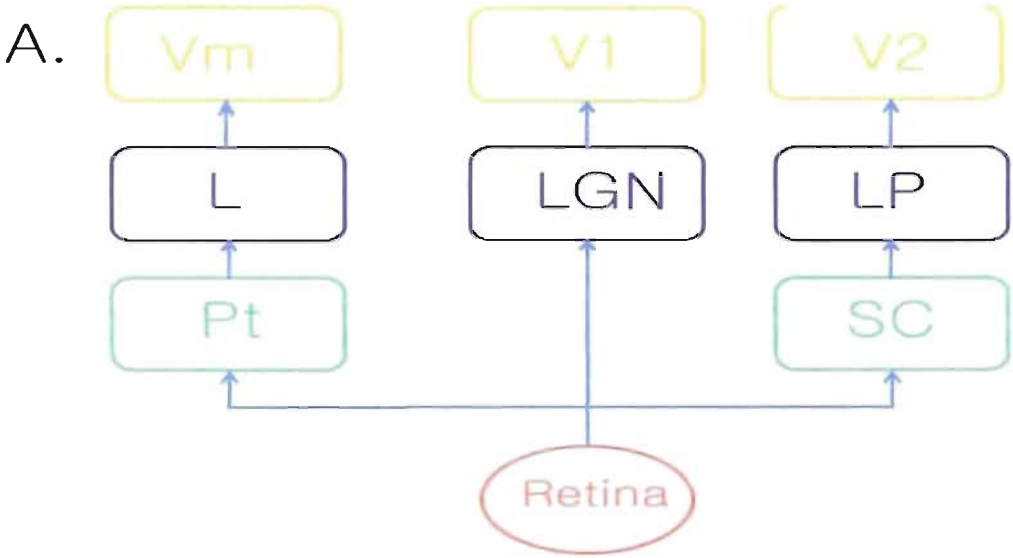
Figure 3: **Visual pathway through visual cortex.** Visual information from LGN reaches V1. V1 distributes information to ventral and dorsal pathway. (For more detail see text)

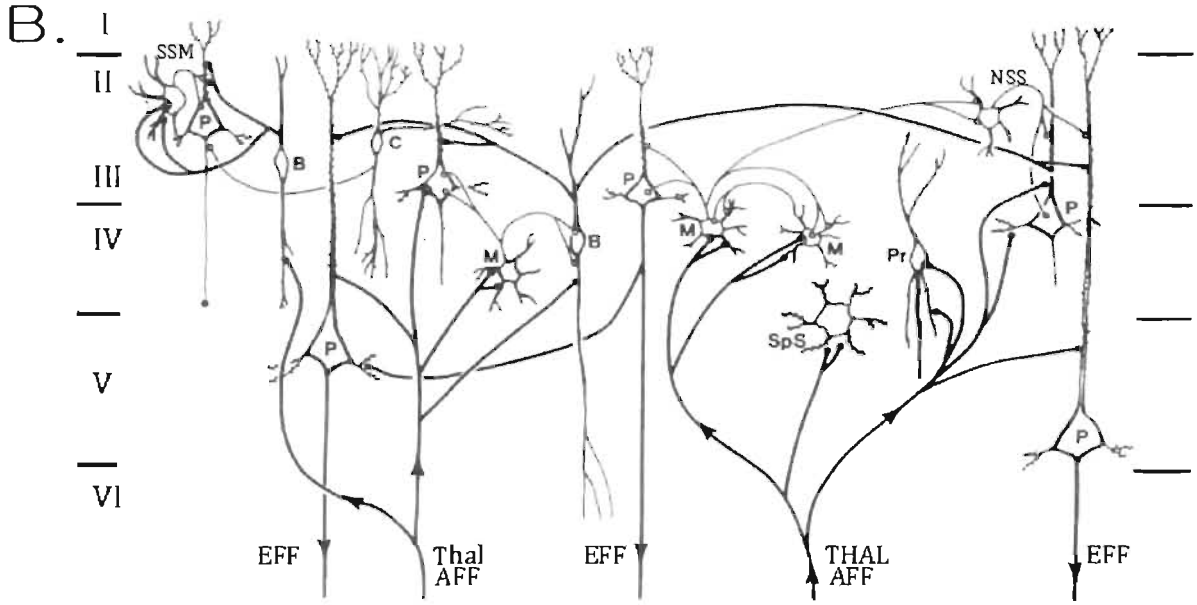
2.2 Rat

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

Although in rat's system visual pathway also includes LGN, superior colliculus and pretectum since in our study the main interest is on LGN afferent pathway physiological description will be focused on the structure of LGN. Precisely, retinal ganglion inputs reach the dorsal area of LGN (dorsal LGN or dLGN). Despite the lack of lamination in dLGN of the rat many studies have discriminated various regions in the distribution of cells of different sizes, in the composition of afferent axons and different patterns of degeneration after lesions (Land, 1987; Martin, 1986; 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, visual information reaches mostly the layer IV of V1 through dLGN. But its projection was 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,





(A) Simplified visual information flow in rodent; V2 is used for Oc2L to compare with primate. IC, inferior colluculus; L, lateral nucleus of the thalamus; PT, pretectum; LP, secondary visual (lateral posterior) thalamic nucleus; Vm, medial visual area (B) Features of thalamocortical and intracortical connections in V1. Cortical layers are indicated by Roman numerals on the left. B, bipolar cell; C, chandelier cell; M, multipolar cell; NSS, nonspiny (smooth) stellate cell; P, pyramidal cell; Pr, bitufted projection cell with a myelinated axon; SpS, spiny geniculocortical. Pyramidal cells are usually glutamatergic. Bipolar, chandelier, multipolar, and smooth stellate cells are mostly GABAergic. Projection between layers is detailed in the text.

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) (Feldman and Peters, 1978; Peters et al., 1976; Peters and Feldman, 1976, 1977). Layer IV cells project prominently in a precise manner to lower layers II/III while weaker projection extends laterally and diffusely in layer II/III. A vertical projection also exists heading to layers V and VI. Vertical intracortical connections conveying information to layers above and below in the visual cortex provide binocular or receptive field properties (Gilbert, 1983). Comparing to cells in layer IV, cells in layer II/III compose a widespread intralaminar connections projecting to lamina V. Pyramidal neurons in layer V receive projections from layer II/III and from layer VI. Whereas lower layer V make clustered projections in a diffuse manner to layer I, the bottom of layers II/III, and the top of layer IV and V. Finally neurons in layer VI make clustered projections to the boarder of layer III and IV. Additional projections were also observed from layer VI to the boarder of layer V and VI and layer I/II (Burkhalter, 1989).

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

Although the functional organization of rodent is similar to that of primate, in the rat's visual cortex there was no evidence of orientation column (Girman *et al.*, 1999). However interestingly, 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.

3. *Plasticity*

Adaptation was the essential ability to survive, expand habitats and propagate during evolution even for bacteria. In MacMillan English dictionary it is described as "abilities that make it possible for organisms to deal with their environment". For mammals, adaptation was readily observable in the nervous system especially in the central nervous system (CNS) including the brain. One of the brain's striking adapting ability is usually referred as 'learning'. Learning is determined as the acquisition and development of memories and behaviours produced by experience. With many studies in CNS, various kinds of adaptation which corresponds in neuroscience to a reorganization of the neuronal circuits, termed "plasticity" had been revealed. Plasticity is also considered as one of the mechanism during learning process in brain. Although plasticity is not an event persisting for millions of years as evolution had occurred, plasticity is not a response against a spontaneous change. Adaptation as a result of long and continuous modification requires long term to launch such function. Plasticity however reflects a long term modification. Two kind of plasticity are studied: synaptic plasticity and cortical plasticity.

3.1 *Synaptic plasticity*

Nervous system is composed of two types of different cells: neurons and glia. Although recently their functioning importance has been revised, glia's main role is contributing to brain function by supporting and nourishing neurons. On the other hand, neurons are responsible of sensing the changes, deliver it to other neurons and command the body's responses to these senses. Neuron receives outer signal through dendrite and transfer it to neighbouring neuron by axon (Figure 5). Junction between neurons are called synapses, generally synapses are established between axons and dendrites, axons and axons or cell bodies. Nervous sense is transported under form of electrical signal and at the end of the axon it is transferred by releasing chemical molecules (neurotransmitters) delivered by synaptic vesicles. At the dendritic membrane of neuron receiving neurotransmitters there are proteins (receptor) which bind specifically to the released neurotransmitter. Outer-membrane of neuron is positively charged and near synapse inner-membrane is negatively charged. Binding of neurotransmitter induces conformational change of ionic channel and allows positive ions enter in the neuron. Entrance of positive ions changes membrane polarity and this potential change is propagated to the next neuron (Figure 6). Neurotransmitter releasing neuron is called presynaptic neuron and receiving neuron is called postsynaptic neuron. Neurotransmitter receptor permitting positive ion (Na^+, Ca^{2+}) entrance is called excitatory (e.g. glutamate) and negative ion (Cl^-) admitting receptor is called inhibitory e.g. GABA: γ-amino-butyric-acid. Synaptic plasticity is the ability of a synapse between presynaptic and postsynaptic neurons to change its strength by changing the efficacy of receptor response and/or changing postsynaptic transduction. Earlier works in laboratories such

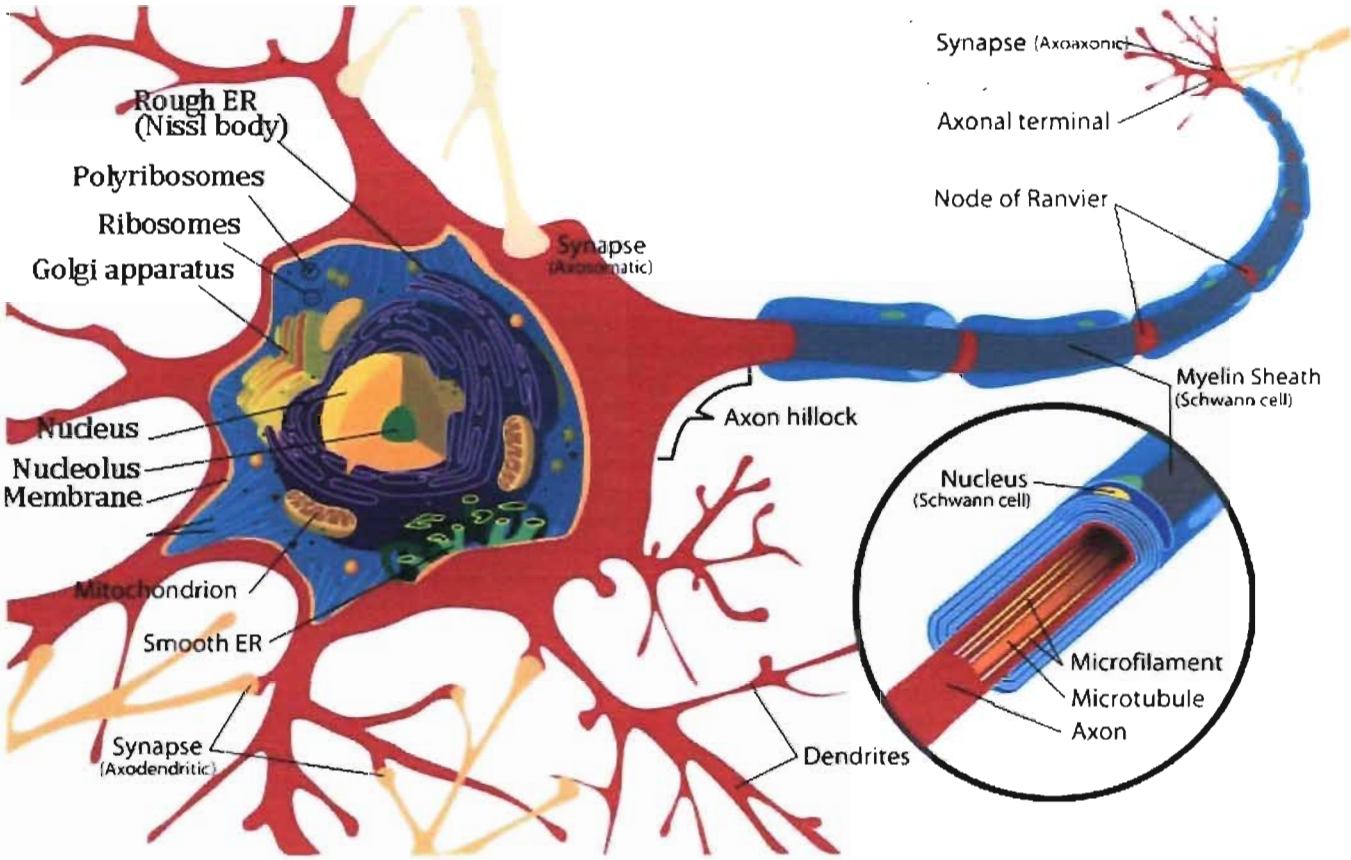


Figure 5: Structure of a neuron. Neuron is consisted of axon, dendrite and soma (cell body). Myelin sheath is a phospholipid layer surrounding axon. It is an outgrowth of glial cells.

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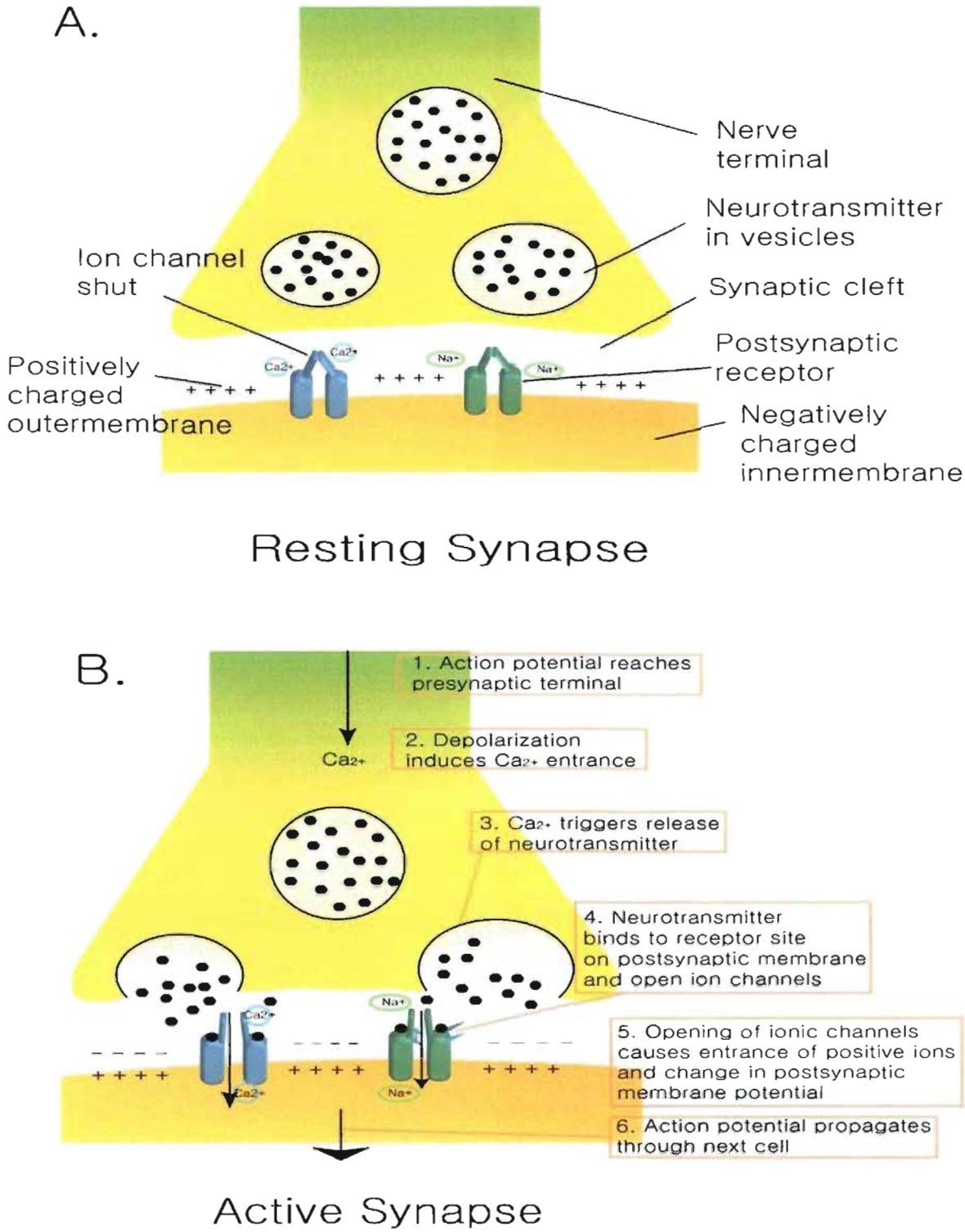

Figure 6: **Synapse and action potential**. (A) Action potential is propagated through synapse and delivered to next cell. Outer membrane of neuron is positively charged due to the sodium and calcium ion. (B) When ionic channel is opened those ions enter in the neuron and convert the polarity. This signal is transmitted through the cell.

as the one of Eric Kandel in aplysia had revealed part of the molecular mechanisms for synaptic plasticity (Castelluci *et al.*, 1978).

Presynaptic facilitation is likely to be a result of Ca^{2+} influx that activate calcium/calmodulin-dependent protein kinases II (CaMKII). These kinases prominently phosphorylate synaptic vesicle associated proteins, synapsin and detach them from cytoskeleton. Increase of Ca^{2+} level can be induced directly from voltage-gated Ca^{2+} channels or indirectly from the modulation of presynaptic K+ channels. This facilitation can occur autonomously by homosynaptical transmitter release from the terminal itself or heterosynaptically by a modulatory neuron at axo-axonic synapses.

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.

3.1.1 Long-term potentiation

A representative effect of changes in the efficacy of synaptic connection is observed during the phenomenon called long-term potentiation (LTP) (Figure 7). Discovered in the rabbit hippocampus (Andersen *et al.*, 1966), LTP is the long-lasting enhancement of communication and results when postsynaptic neuron shows a persistent increase in synaptic strength following

high frequency stimulation of a chemical synapse. Possessing common features with long-term memory LTP has been the most attractive candidate for cellular mechanism of learning.

LTP is usually induced with a short tetanic stimulation (100 Hz in 1 sec) in presynaptic area and observed as an increase of excitatory postsynaptic potential (EPSP) in postsynaptic area lasting more than an hour (Huang and Kandel, 1994). Non-tetanic stimulus induced in presynaptic area causes release of neurotransmitter glutamate which binds to AMPA (α-amino-3-hydroxy-5-methylisoxazole-4- propionic acid) receptors embedded in the postsynaptic membrane. This binding opens the sodium channels and ion influx cause a short EPSP. This depolarization however, when repeated stimuli at high frequency are given to the presynaptic fibre, causes the postsynaptic neuron to depolarize progressively. When such a train of stimuli was applied it expresses stronger and prolonged EPSP which will remove magnesium ion blocking the NMDA (N-methyl-D-aspartate) receptors. This opening allows calcium influx when glutamate is bound. The rise in intracellular Ca^{2+} concentration triggers the activation of several protein kinase enzymes, enzyme serving to transfer phosphate to donor molecule. For example, calcium/calmodulin-dependent protein kinase II (CaMKII), protein kinase C (PKC) and cAMP-dependent protein kinase A (PKA) (Sweatt, 1999) are activated by calcium entrance (Figure 7A).

During LTP, CaMKII and PKC become independent on calcium and autonomously active. Consequently CaMKII and PKC will activate AMPA receptors (AMPAR) by phosphorylation to increase their activity and induce the insertion of additional AMPAR into the postsynaptic membrane (Malenka and Bear, 2004). It is suggested that this AMPAR insertion does not involve protein synthesis. During non-stimulated state AMPAR are generally internalized inside the synapse and with LTP inducting stimulation under the influence of protein kinases they are trafficked into the membrane (Malinow, 2003).

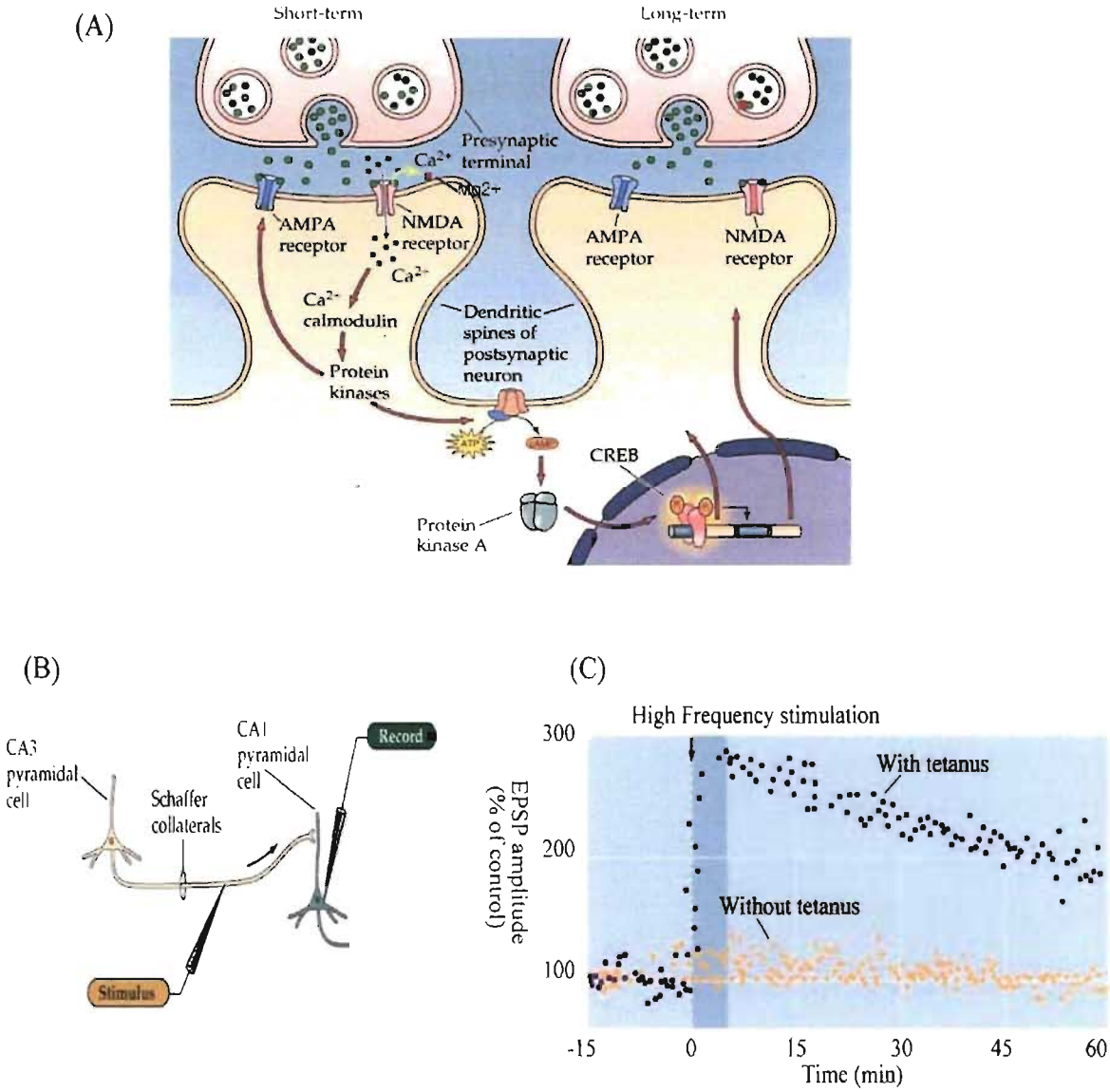

Figure 7: Long-term potentiation. A) LTP mechanism in hippocampus. Sustained stimulation of postsynaptic cell with sufficient strength removes Mg2+ ion and allows calcium entrance. B) Recording procedure of LTP in hippocampus. C) After tetanic stimulation synaptic response increases 200% comparing to baseline and last for an hour.

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Protein synthesis appears during the late phase of LTP, called L-LTP. Persistent activation of protein kinases such as MAPK, especially extracellular signal-regulated kinase (ERK), can induce L-LTP (Sweatt, 1999; Lynch, 2004; Kelleher *et al.*, 2004) followed by activating transcription factors like protein kinase Mζ.

3.1.2 Long-term depression

Another form of synaptic plasticity is long-term depression (LTD). As the name imply unlike LTP, LTD shows a depression of EPSP amplitude after delivering a prolonged trains of presynaptic stimulation at low (0.5-10 Hz) frequency (Dudek and Bear, 1992). Although low frequency stimulation (LFS) is a standard method to induce a homosynaptic LTD in hippocampal neuron, NMDAR antagonists were able to block this process. Application of NMDA showed that just like the LTP, an appropriate activation of postsynaptic NMDARs is sufficient to induce LTD (Lee *et al.*, 1998; Kamal *et al.*, 1999). And another result demonstrate that rise in postsynaptic calcium ion concentration through NMDAR was the critical variables not the stimulation frequency itself (Neveu and Zucker, 1996) (Figure 8).

With an elevation of Ca^{2+} concentration CaMKII is able to activate Calcineurin, which in turn is able to activate a postsynaptic substrate PP1. The activity of PP1 is persistently increased while LTD inducing stimulation (Thiels *et al.*, 1998). LTD is associated with dephosphorylation of the GluR1 subunit of the AMPAR. GluR1 contains serine 831 that can be phosphorylated by CaMKII and PKC while serine-845 is phosphorylated by PKA (Roche *et al.*, 1996; Barria *et al.*, 1997). The PKA site shows higher basal phosphorylation than the CaMKII-PKC. LFS causes dephosphorylation of the PKA site and LTD. On the contrary, TBS causes phosphorylation of the CaMKII-PKC site and LTP. Complementary with these, activation of postsynaptic PKA

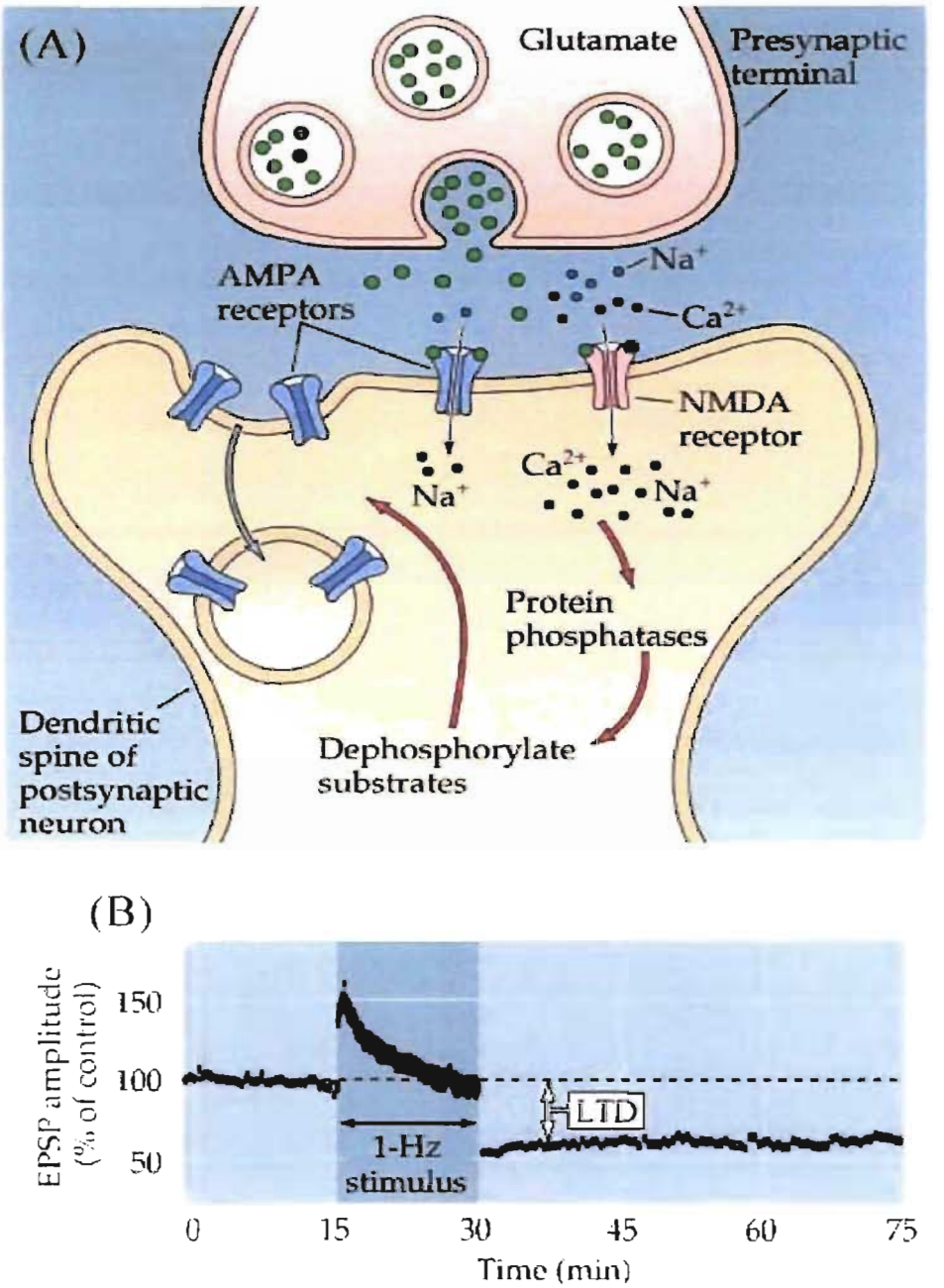

Figure 8: **Long-term depression.** (A) Dephosphorylation mechanism induces internalization of AMPAR. (B) Low frequency stimulation generates long-term depression.

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reversing previously induced LTD showed that dephosphorylation of AMPARs is a mechanism 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 presynaptic stimulation at 5 Hz 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 expression mechanism for LTD. It was proposed that 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 interaction 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).

3.1.3 Plasticity of plasticity: Metaplasticity

Continuous reinforcement or weakening of synapse will cause destabilization of neuronal networks over time by driving neurons towards maximal and/or minimal action potential firing frequency ranges. However, other forms of plasticity than LTP or LTD provide the negative feedback; scaling and metaplasticity. To maintain synaptic strength and plasticity within a functional dynamic range synaptic scaling lowers amplitudes of small EPSP in response to continual excitation and raise them after prolonged blockage (Turrigiano *et al.*, 1998; Watt *et al.*, 2000; Leslie *et al.*, 2001). This takes effect gradually by changing the numbers of NMDAR at

the synapse (Watt *et al.*, 2000). Metaplasticity, introduced by W.C. Abraham and M.F. Bear (Abraham and Bear, 1996), refers to the plasticity of synaptic plasticity. Principle meaning is that previous activation history of synapse determines the present plasticity. Depending on current synaptic state the level of synaptic inhibition or the activity of modulatory afferents (or neurotransmitters, e.g. Acetylcholine) can influence the effect of plasticity overtime (Abraham and Tate, 1997). For example, if a neuron was already exposed to many plasticity, metaplasticity drops the future plasticity efficacy by changing NMDAR subunits and lower the concentration of Ca^{2+} influx (Flint *et al.*, 1997; Carmignoto and Vicini, 1992; Philpot *et al.*, 2001).

3.2 *Cortical plasticity*

While synaptic plasticity occurs between two neurons, cortical plasticity refers to the changes occurring in the organization of the cortex according to the experience. Brain activity transferring from the given function to a different location results of normal experience or brain damage is the remarkable consequence of cortical plasticity.

Few decades ago when neocortex was considered unalterable, Hubel and Wiesel had demonstrated that during development before a specific period, called the critical period, ocular dominance column in V1 was highly plastic (Wiesel and Hubel, 1963). Depriving monocular vision during development results in an expansion of the columns serving the open eye and those which were responding to the deprived eye become reduced in size and afferent complexity. Following the result of Hubel and Wiesel, cortical plasticity had been shown to somatosensory cortex (Van der Loos and Woolsey, 1973), auditory cortex (Moore, 1985) and in diverse experimental and natural conditions.

Cortical synaptic plasticity has many features that are similar to those of hippocampal synaptic plasticity. For example when a rat's whisker of postnatal day 12-14 was stimulated it drove a recombinant AMPAR subunits into synapses in the somatosensory cortex (Takahashi *et al.*, 2003). Also in the visual cortex, similarly with LTD, 24h monocular deprived rat showed various changes in the GluR1 phosphorylation level (Heynen *et al.*, 2003). Another important common mechanism is NMDAR dependency. Blocking NMDARs in developing visual cortex blocks the effects of monocular deprivation suggests the involvement of NMDAR (Bear *et al.*, 1990) for a long term effect. Confirming previous experiment and without affecting visual responses, suppression of NMDAR subunit (NR1) expression shows that NMDAR is involved in visual cortex plasticity (Roberts *et al.*, 1998). Involvement of NMDAR signifies Ca^{2+} entrance after its opening. Similarly with synaptic plasticity numerous results indicate three essential kinases during monocular deprivation; PKA (Beaver *et al.*, 2001), extracellular-signal-regulated kinase (ERK; Di Cristo *et al.*, 2001) and αCaMKII (Taha *et al.*, 2002). In the cytoplasm those kinases are responsible to phosphorylate substrates like synapsin (Hosaka *et al.*, 1999), AMPAR (Barria *et al.*, 1997; Benke *et al.*, 1998), GABAR (Brandon *et al.*, 2003), or actin (Matus, 2000). Those are molecules that have crucial role in synaptic transmission, neuronal excitability and morphological stabilization. Additionally, kinase activity during ocular-dominance plasticity drives to activation of CREB (cAMP response element binding) (Mower *et al.*, 2002; Liao *et al.*, 2002). CREB proteins, being transcription factors, bind to certain DNA sequences called cAMP response elements (CRE) has been in concern since its implication during long-term synaptic facilitation (Martin and Kandel, 1996). Starting by cell surface receptor activation, production of a second messenger such as cAMP or Ca2+ activates in turn protein kinase which induces CREB protein to bind to a CRE region. With successive binding of CREB-binding protein, CREB

regulates certain 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 essential for both ocular dominance plasticity (Taha *et al.*, 2002) and long-term changes in synaptic strength (Silva *et al.*, 1998).

Although cortical plasticity shares numerous consequences with synaptic plasticity there is no consistent correlation founded between the monocular deprivation effect in vivo and the ability to induce homosynaptic plasticity in vitro (Renger *et al.*, 2002; Bartoletti *et al.*, 2002). Continual induction of LTP in synapse does not induce facilitation of cortical plasticity (Hensch, 2003). This disassociation suggests some more conditions have impact on cortical plasticity. Neurotrophin, depending of visual experience, modulates electrical activity and synaptic transmission by increasing transmitter release or depolarisation of neuron (Sala *et al.*, 1998; Kafitz *et al.*, 1999). Development of GABA-mediated inhibition, known as triggering the critical period (Fagiolini and Hensch, 2000), was accelerated in BDNF (brain derived neurotrophic factor)-overexpressing mice (Huang *et al.*, 1999). Neurotransmitters, other than GABA, also have modulator effect during cortical plasticity. Lesion in the basal forebrain, the main source of ACh in the neocortex, accompanied with destruction of cortical adrenergic innervations retarded ocular dominance plasticity (Bear and Singer, 1986). In vitro study in prefrontal cortex have demonstrated that dopamine facilitates LTD of glutamatergic transmission (Otani *et al.*, 1998), and serotonergic axons destructed kitten showed no ocular dominance shift after monocular deprivation (Gu and Singer, 1995). Those supplementary experiments imply that cortical plasticity can be induced through numerous variables e.g. injection of neuromodulator.

Cortical plasticity was not only found in juvenile cortex but also in adult cortex after critical period. Although composition of NMDAR subunit (Yoshimura *et al.*, 2003) or spine

motility (Holtmaat *et al.*, 2005) status are unfavourable comparing those of young cortex, this phenomenon was confirmed through behavioural observation (Karni and Sagi, 1991), electrophysiology (Heynen *et al.*, 2001) and fMRI (Furmanski *et al.*, 2004).

In the visual cortex, cortical plasticity was mainly observed by ocular dominance shift. However, recently, more experiments demonstrate that modulatory ability in cortex level can be reflected through various experiments. Teyler et al showed that with visual tetanic stimulation it is possible to induce an LTP-like increased cortical response in human (Teyler *et al.*, 2005). Supplementing experiences showing about cortical modification, for example orientation tuning (Fregnac *et al.*, 1988) or contextual modulation (Crist *et al.*, 2001) indicate that ocular dominance plasticity is not the only representative model of cortical plasticity.

4. *Acetylcholine*

As stated above the neurotransmitter ACh has a functional effect during cortical modulation and consequently on cortical plasticity. Influence of ACh in various regions of the brain is well demonstrated in cognitive functions like attention, consciousness, learning, memory and sleep (Nobili and Sannita, 1997; Baxter and Chiba, 1999; Hasselmo, 1999; Sarter and Bruno, 2000; Hobson and Pace-Schott, 2002).

Many animal experiments have consistently shown that by blocking muscarinic receptors, subjects had deficiency of learning acquisition ability (Torres *et al.*, 1994; Baxter and Gallagher, 1996; Dornan *et al.*, 1997; Davidson and Marrocco, 2000). Moreover, excitotoxic lesions of neurons in nucleus basalis induced a severe impairment in memory (Dekker *et al.*, 1991). It was suggested that cholinergic activity promote the cortical processing of thalamic inputs and at the same time inhibit the intracortical associations (McCormick *et al.*, 1993; Tang

et al., 1997). Especially during attentional function some special cortical cholinergic inputs are found to be enhanced (Conner *et al.*, 2003).

4.1 *Cholinergic pathways in the brain*

Cholinergic forebrain projections are generally divided into six distribution pathways; Ch1-Ch6 (Mesulam *et al.*, 1983) (Figure 9). 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.

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).

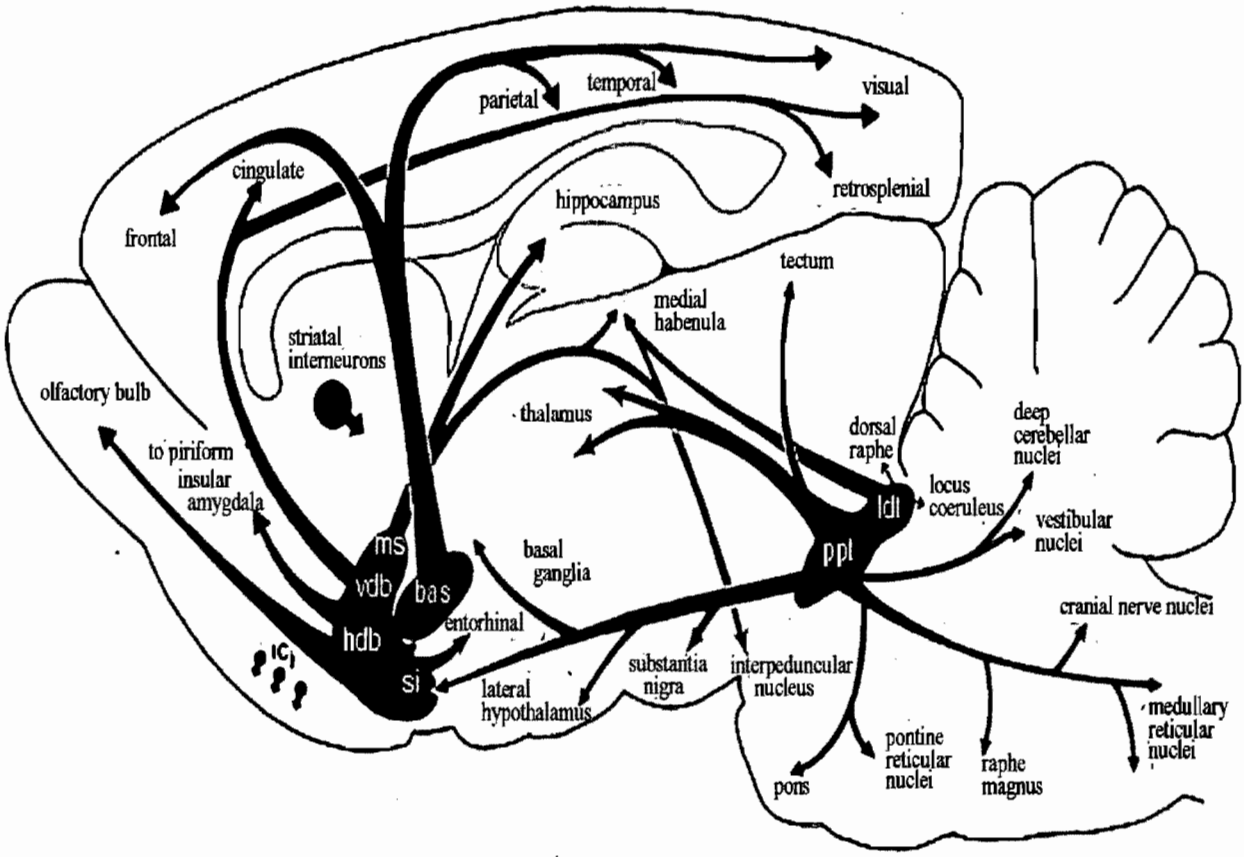

Figure 9: Rat cholinergic central pathway. Diagonal band of Broca (vdb & hdb) is the main ACh distributor in the occipital cortex (Gaykema et al., 1990; Zaborsky et al., 1997). Abbreviation; ms: medial septum, vdb: vertical diagonal band of broca, hdb: horizontal diagonal band of Broca, bas: nucleus basalis, si: substantia innominata, ppt: pedunculopontine tegmentum, ldt: laterodorsal tegmentum

Activation of nicotinic receptors opens Na+, K+ and Ca^{2+} channels. Comparing to muscle nAChRs which are more permeable to Na+ ion, neuronal nAChR are highly permeable to Ca^{2+}. 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 Ca^{2+} permeability ratio of nAChR demonstrated that increase of Ca^{2+} concentration induced by nAChR activation was high enough to influence intracellular Ca^{2+} dependent mechanisms and may act in synaptic plasticity (McGehee, 2002). In 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 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 supplemental injection of nicotine enhance the attention in human and rodents (Levin et al., 1998; Mirza and Stolerman, 1998). Deletion of α7 nicotinic receptor showed impairing of attention in a 5-choice serial reaction-time task.

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 ($G_{q/11}$) 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 Ca^{2+} channel by deactivating adenylate cyclase via G-protein (G_i) (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). Stimulation of postsynaptic muscarinic receptors induces neuron depolarization by inhibiting K+ efflux, usually Ca^{2+} independent (McCormick and Prince, 1986). M2/M4 receptors are traditionally considered to be situated at presynaptic area for an autoreceptor as a negative feedback implication (Douglas *et al.*, 2001).

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).

Relation between vision and ACh has yet many undiscovered mysteries. Direct application of ACh to visual cortex modifies neuronal responses showing increase of spontaneous activity, facilitation of evoked responses, or suppression of evoked responses. Assumed to be action of arousal state or attention it has been demonstrated that the visual response can be enhanced by stimulating the mesencephalic reticular nuclei (Singer 1977). 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 from cholinergic basal forebrain and dorsal noradrenaline bundle, its ocular dominance shift was

blocked despite the monocular eyelid suture (Bear and Singer, 1986). Following experiments by blocking muscarinic but not 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 with selective cholinergic immunolesion with cholinergic fibre toxin 192 IgG saporin confirmed that M1 and M2 lesioned juvenile mouse the synaptic plasticity was gravely affected (Kuczewski *et al.*, 2005). Those indicate that muscarinic M1 receptors may have a critical role during cortical plasticity.

Cholinergic modulation effect is also shown in orientation dominance column shift. Normally neurons do not alternate its preferred orientation simply by continual exposure to another. However, when this repeated visual stimulus of sub-optimal orientation is paired with application of ACh, responses of neurons become stronger at the expense of diminishing response against 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 delete of a digit (e.g. a whisker) followed by neighbouring 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). Also stimulation of basal forebrain paired with whisker showed a long-term enhanced somatosensory response (Verdier and Dykes, 2001). Additionally in the auditory cortex, combining nucleus basalis stimulation and tone emission it induced changes of receptive field (Ma and Suga, 2003). On the contrary this effect was not shown when muscarinic receptors were blocked with antagonist (Miasnikov *et al.*, 2001).

Although the exact mechanism of how ACh application can induce increase of 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 Ca^{2+} level (Yamamoto *et al.*, 2000) which will promote the plasticity in the visual cortex (Kato *et al.*, 2000). This pre-increased Ca^{2+} level can activate intracellular protein kinases (Hamilton and Nathanson, 2001) which may facilitate responses induced by NMDA receptor (Aramakis *et al.*, 1999). Another possibility of cholinergic contribution is to reduce membrane K+ conductance. While activation of muscarinic receptor increasing the depolarization of cortical pyramidal cells associated with NMDA receptor-gated conductance is supporting result (Kirkwood *et al.*, 1999), this effect will facilitate depolarization in response to visual input which is transmitted through glutamatergic neurons. These cholinergic actions enhance the opening of NMDAR dependent synaptic transmission. With a direct contact in visual cortex ACh can regulate GABAergic neuronal inhibition (Xiang *et al.*, 1998; Erisir *et al.*, 2001) and since GABAergic interneurons have crucial role in cortical plasticity (Fagiolini and Hensch, 2000) ACh can influence modification threshold of cortical plasticity.

PURPOSE OF THIS STUDY

Even though plasticity in adult cortex has now received much recognition, since detailed mechanisms are different from those of critical period there still remain much to understand. Although other neurotransmitters (e.g. norepinephrine or serotonin: reviewed by Gu, 2002) also participated in synaptic plasticity, ACh seems to have a crucial role in modulatory effect by facilitating and/or by accelerating the plasticity mechanism.

In a previous study, we have demonstrated that in the V1 of an anaesthetized rat, ACh was released via a repetitive stimulation of sinusoidal grating (Laplante *et al.*, 2005). Here, during this experiment we tried to observe if this endogenous distribution of ACh could affect the cortical response and be examined by change of visual evoked potential (VEP). Also by decomposing the activating pathway of cholinergic modification we tried to clarify the underlying mechanism of cortical plasticity in adult visual cortex and observe whether this effect was valuable for long term. Since several studies suggest the role of nicotinic (Rosato-Siri *et al.*, 2006; Kawai *et al.*, 2007) and muscarinic receptor (Origlia *et al.*, 2006; McCoy and McMahon, 2007) during synaptic plasticity we blocked both receptors alternatively and observe the change of cortical response through VEP. Also based on studies showing in vitro that ACh or muscarinic receptor activation can induce LTP in slices of V1 mediated by an enhancement effect of NMDA receptor conductance (Brocher *et al.*, 1992; Kirkwood *et al.*, 1999; Kojic *et al.*, 2001) we tested whether blocking NMDA receptor affected ACh induced facilitating effect.

In the result we demonstrate that during patterned visual stimulation paired with cholinergic activation could enhance thalamocortical LTP which is mediated through both muscarinic and nicotinic receptors in vivo increasing the amplitude of VEP. Thus synaptic

mechanisms involving ACh promote plasticity by increasing the input thalamic signals that will launch translational cascades indicating long term enhancing effect on synaptic strength.

METHODS

1. Animal preparation

Guidelines set out by the Canadian Council for the Protection of Animals were followed for all procedures. 44 Long-Evans rats (250-300g) were obtained from Charles River Canada (St-Constant, Quebec, Canada) and maintained in a 12h light/dark cycle with free access of food during both pre- and post-implantation period. During all experiments every effort to reduce both the suffering and number of animals used was made. Experiments were performed during 210 minutes (from t0 to t7). Experimental groups were control (n=11), carbachol(n=6) injected (injection time=90 min: t2) group, scopolamine (n=5) injected group (t2), CPP (n=6) (t2) + carbachol (t=120 min: t4) injected group, aCSF (n=6) (t2) + carbachol (t4) injected group, scopolamine (n=5) (t=60 min: t1) + carbachol (t2) injected group, and mecamylamine (n=5) (t2) +carbachol (t4) injected group.

2. Implantation

Two days before recording, animals were placed in a Plexiglas box and anaesthetized with a gaseous mixture of isoflurane (5%) along with oxygen and air. After transferring the animal to the stereotaxic apparatus, anaesthesia (isoflurane 1.5%) was administered through a mask. The rectal temperature was monitored and maintained at 37°C with a thermostatically controlled heating pad (FHC, Bowdoinham, ME, USA). A dental drill was used to make an hole (~3.0 x 3.5 mm) in the skull above the left visual cortex and a push-pull cannula guide (plastics1, Roanoke, VA) and the electrode guide (polyurethane tubing) were implanted. The dura mater was left intact except the cannula guide inserted location where a small incision was made with

	T1 (0 min)	T2 (30 min)	T3 (60 min)	T4 (90 min)	T5 (120 min)	T6 (150 min)	T7 (180 min)
Control (n=11)							
Carbachol (n=6)			Injection				
Scopolamine (n=5)			Injection				
aCSF+CCh (n=6)			aCSF injection		CCh injection		
CPP+CCh (n=6)			CPP injection		CCh injection		
Sco+CCh (n=5)		Sco injection	CCh injection				
Mec+CCh (n=5)			Mec injection		CCh injection		

Table 1: Injection procedure. Recording result from T1 was used as baseline for each group. Carbachol, aCSF, CPP and mecamylamine was injected intracortical. Only scopolamine was injected intraperitoneal.

30 gauge needle. The cannula guide was inserted at the V1 (mm from Bregma: AP -7.5, L +3.6, V -0.7 mm) with an angle of 30° and the tubing was placed perpendicularly on the dura mater 0.4 mm left lateral (Figure 10A). Final recording location was decided by the optimum response generating area after multiple recording tests. Two nylon screws (Small parts, Miami Lakes, FL, USA) were also screwed in the skull (3.0 mm anterior and 4.0 mm right lateral of the hole) and the guides were secured with dental cement after covering the exposed region with agarose gel to prevent dryness and direct contact with dental cement solvent. After suturing incised skins and applying a local anaesthetic (Xylocaine) to sutured point, animals were returned to the cage.

3. Visual stimulation

The visual stimulation during 10 minutes was provided with a patterned sinusoidal grating displayed on a computer screen every 30 minutes. The computer monitor (30x25 cm, Titanium; Apple Computer Inc., Cupertino, CA, USA) was placed 30 cm unilaterally parallel to the midline of the rat. A horizontal sinusoidal grating (contrast 100%, 0.12 cyc/deg, 0.033 Hz) was produced by Vpixx software (v 8.5; Sentinel Medical Research Corp., Quebec, Canada) and displayed on the computer monitor (Figure 10A). Each visual stimulus (100 ms) was displayed 20 times and evoked potential changes in the visual cortex were recorded during that period. Selected orientation and spatial frequency of the grating were based on published values that have shown to induce an optimal response in V1 of the rat, Girman (Girman *et al.*, 1997) and from previous studies (Laplante *et al.*, 2005).

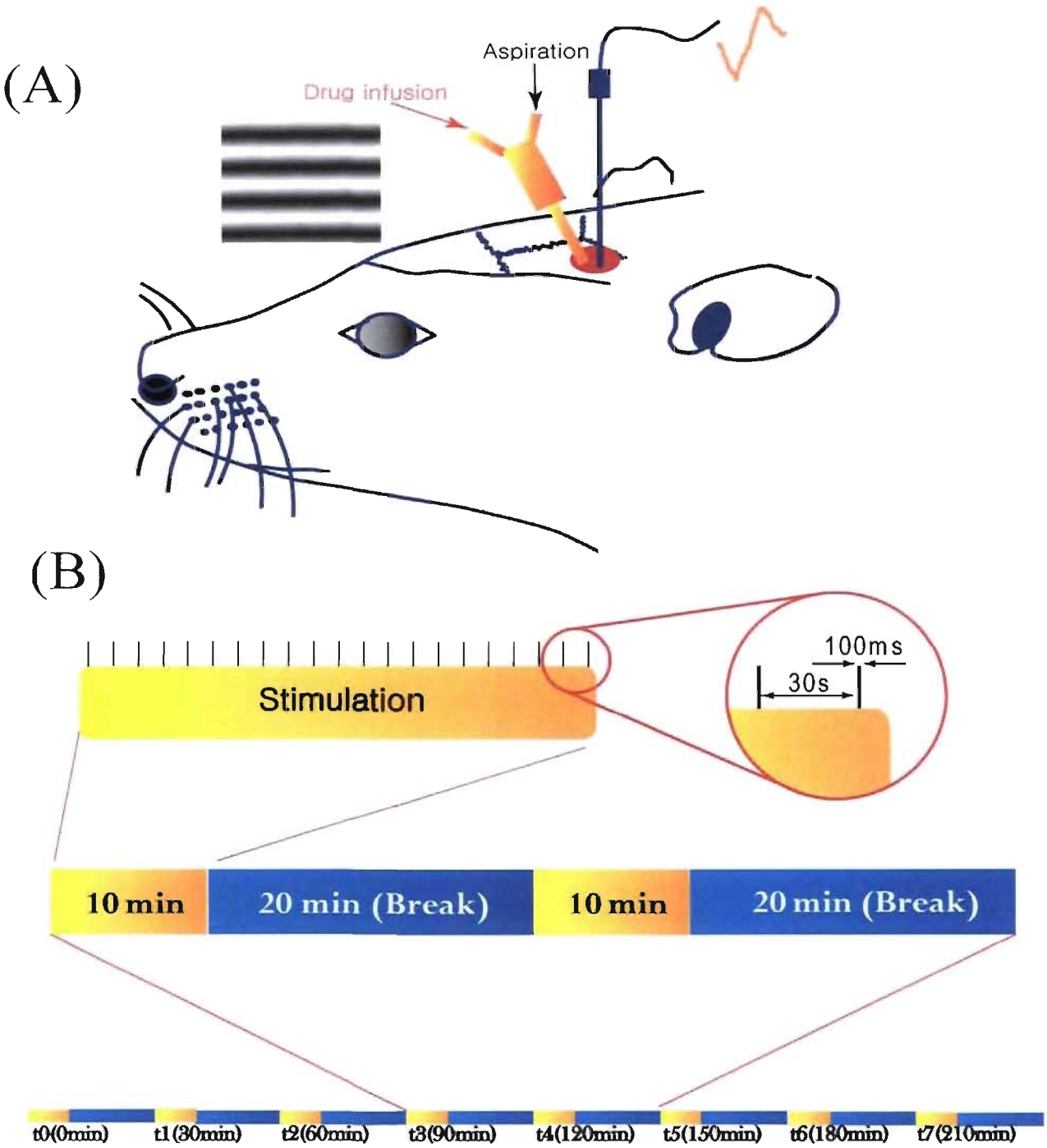

Figure 10: **Experimental procedure.** A) Chronic implantation. Push-pull cannula was implanted in striate cortex 2 days before recording. B) Visual stimulation. Rat was stimulated for 8 cycles. Each cycle was consisted of 10 min stimulation and 20 min break. Sinusoidal grating was projected for 100 ms with 30 sec of interval.

4. ***Recording procedure***

Two days after implantation, rat was replaced on the stereotaxic frame under anaesthesia for recording. Polyurethane tubing was removed, leaving a hole through dental cement over V1 (AP -7.5, L +4.0). Electrode (tungsten microelectrode < 0.8 MΩ; FHC, Bowdoinham, ME) insertion was performed through this hole (Figure 10A). The final vertical position of the recording electrode was determined by eventually descending electrode through the electrode guide. When electrode reached the dura mater the noise ratio reduced instantly both on the screen and at the speaker. Histological analysis demonstrated that the tip of the electrode was positioned 400~500 μm below the dura mater. (Figure 11)

The recording procedure consisted in 10 min of continuous assessment of the changes of the field potentials (extracellular recording) elicited with 20 visual stimuli of 100 msec at 30 sec of interval followed by 20 min of rest in darkness. 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 (biopac) and Acqknowledge software (v 3.8; Biopac system Inc, Goleta, CA, USA). This was repeated during 4 hours (Figure 10B). Visual evoked potential (VEP) was obtained by averaging the electrical responses elicited by the visual stimuli. Each visual response was evoked by sinusoidal grating 20 exposure over 10 min. Before the beginning of each experiment baseline responses were collected in the same experimental condition except the monitor covered with black curtain.

The design of the experiment (see below) was adapted from a study performed in the somato-sensory cortex stimulation paradigm (Verdier and Dykes, 2001).

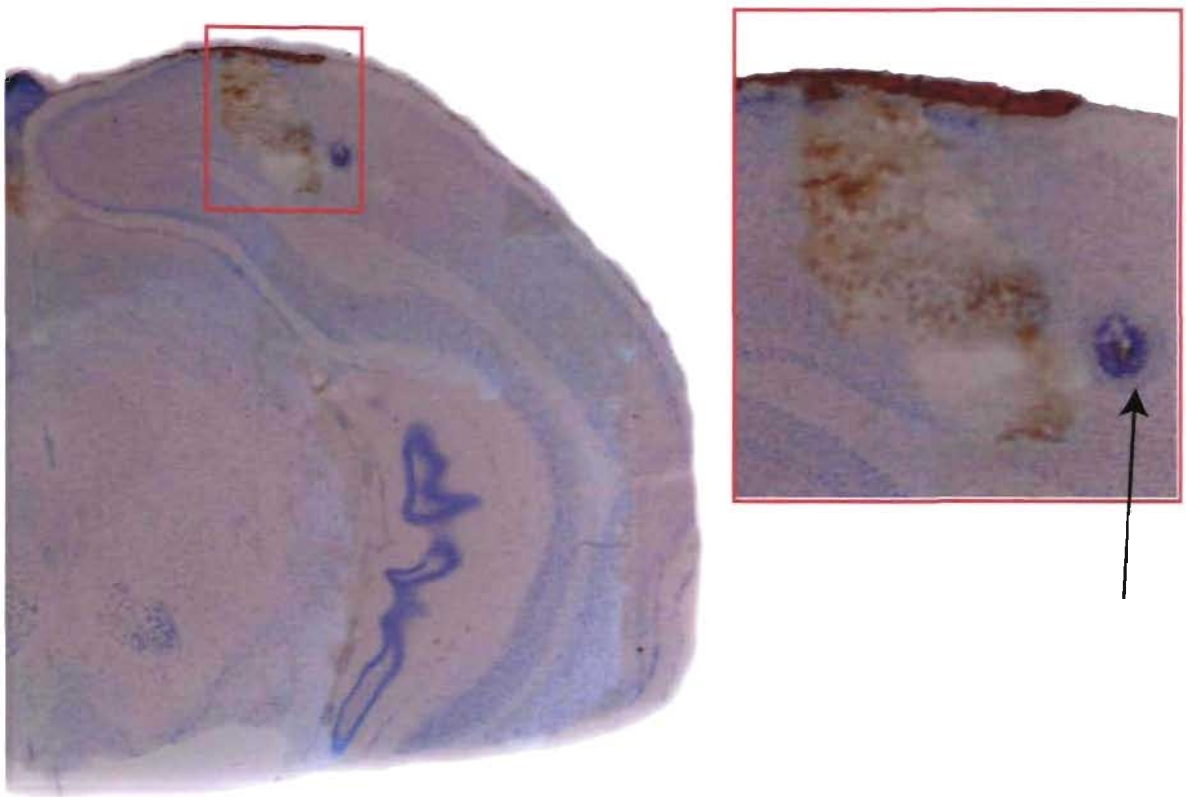

Figure 11: **Cresyl violet staining**. Final position of electrode was traced by cresyl violet staining. Lesioned area was marked by arrow.

5. ***Drug infusion***

All drugs were obtained from Sigma Chemical Co and dissolved in artificial cerebro spinal fluid (aCSF: NaCl, 1.0M; $NaHCO_3$, 0.5M; KCl, 1.47M; $MgSO_4$, 1.25M; KH_2PO_4, 0.25M; $C_6H_{12}O_6$, 0.01M; $CaCl_2$, 1.73M). During experiment 1 the control group, carbachol (10mM), cholinergic receptor agonist, injected i.c. (intracortical) group and scopolamine (10 mg/kg), muscarinic receptors antagonist, injected i.p. group were tested. Two different drugs were delivered separately during experiment 2. ACSF i.c. paired with carbachol i.c., (±)-3-(2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid (CPP: NMDAR antagonist; 50ng) i.c. with carbachol i.c., mecamylamine (10 μM), nicotinic receptor antagonist, i.c. with carbachol i.c., and scopolamine i.p. with carbachol i.c. All intracortical injected drugs were delivered (1 μl/min) via push-pull cannula connected with a 1.0 ml syringe (Becton Drive, Franklin Lakes, NJ, USA) controlled by an injection pump (Harvard Apparatus, Holliston, MA, USA) and at the same time aspiration was performed at the other side with another pump.

6. Histology

Toward the end of experiment, to mark electrode recording point and drug infused area, we injected Bromophenol blue (5%) and made an electrolytic lesion. The animal was then sacrificed by intraperitoneal administration of pentobarbital and brain was removed to freeze at -50°C isopentane. Frozen brain was cut at a thickness of 20μm in the visual cortex region and the slices were stained with cresyl violet permitting to track the electrode position. Histology confirmed that the electrode inserted area was the primary visual cortex and the tip was within 200 μm of the cannula (Figure 11).

7. Data analysis

Amplitude difference between negative peak and positive peak was compared between each session. Latency was measured as time difference between those two peaks. First VEP was considered as 100%. A one-way analysis of variance (ANOVA) was used to compare VEP at different time. Turkey tests, with $P<0.05$ being considered significant were conducted.

RESULTS

Repetition during 10 minutes of 0.03Hz pattern visual stimulation does not affect the amplitude of the VEPs for at least 4 hours after the first stimulation

Before testing any drugs, it was investigated whether the amplitude of evoked potentials was changed in function of time or was dependant on cholinergic mechanisms.

A 100ms horizontal sinusoidal grating evoked a wave composed of a negative peak followed by a positive deviation. The VEP registered in the first session for each rat was considered as baseline (100%) and compared with VEPs collected the subsequent session.

The results showed that no significant increase or decrease of VEP amplitude or latency was induced by patterned visual stimulation after seven repetitive stimulations compared to the baseline (Figure 12 and table 1; 12% decrease of amplitude and 3 ms in latency comparing to baseline, p=0.264). Blockade of muscarinic receptors by scopolamine injection prior to the experiment also didn't show any difference in amplitude comparing to the control group (Figure 12 and table 1; 16 % decrease in amplitude and 2.6 ms in latency).

Carbachol induces a long term augmentation of VEP amplitude

A single injection of the cholinergic agent carbachol (10mM, 1µl/min) paired with visual stimulation (at t3 = 60min), induced an increase in VEP amplitude that lasted for 3h and 1/2. (ANOVA, p=0.0001, VEP amplitude increase was 73% at maximal potentiation, Figure 13). This enhancement was not seen in control animals, i.e. when saline was injected by i.p. or i.c. injections (n=5, data not shown), showing that the augmentation of amplitude was specifically

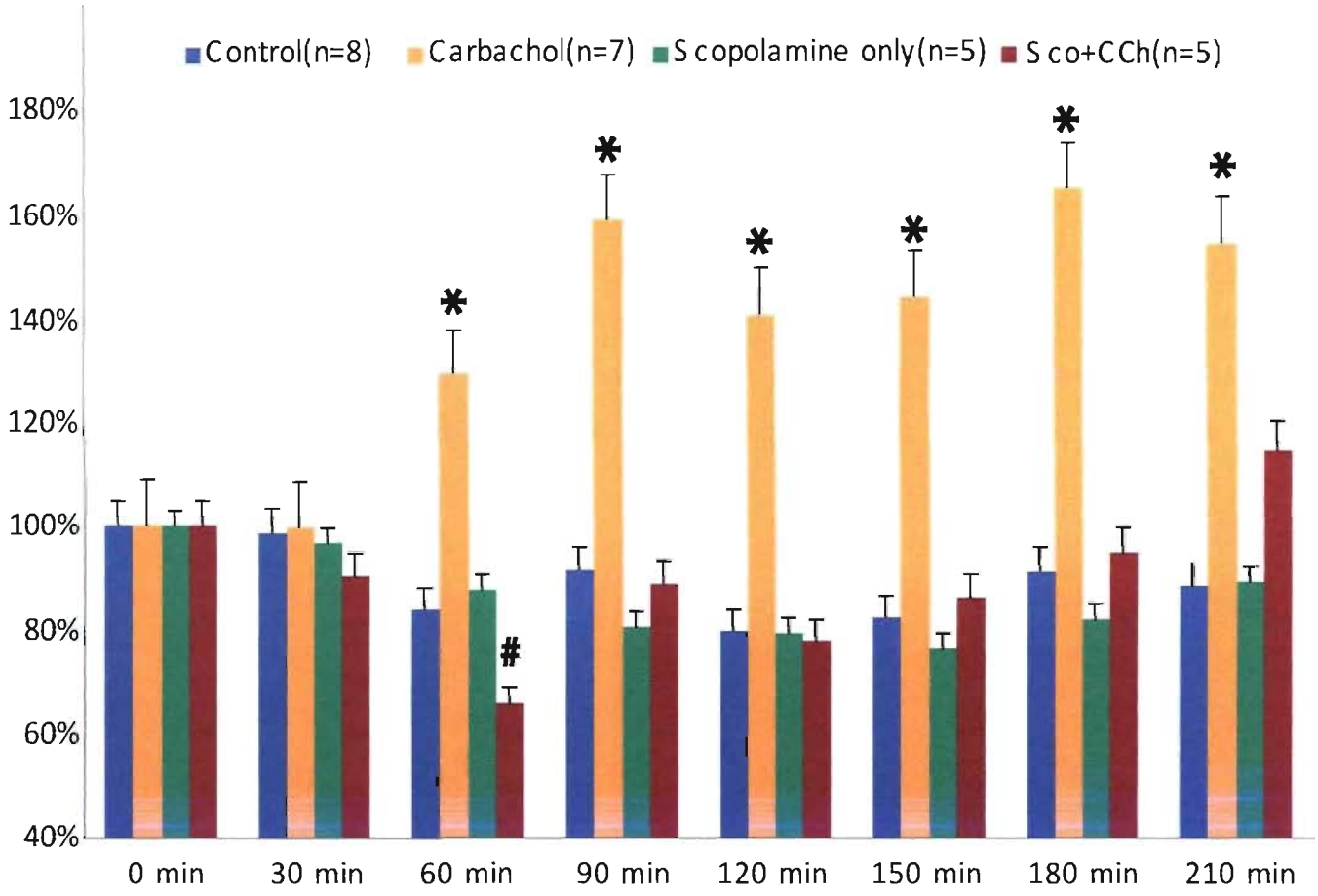

Figure 12: Graphical representation of the changes in the amplitude of the VEP of control, carbachol injected, scopolamine injected and sco+ CCh group as a function of time. Note that when carbachol was injected VEP amplitude shows a crucial increase (*). On the other hand, scopolamine pre-treated groups showed a decreased during CCh infusion period (#). (see DISCUSSION for more details)

(A)

(%)	0 min (t0)	30 min (t1)	60 min (t2)	90 min (t3)	120 min (t4)	150 min (t5)	180 min (t6)	210 min (t7)
Control (n=11)	100.0	98.6	83.8	91.6	80.0	82.4	91.2	88.4
Carbachol (n=6)	100.0	99.8	129.2	159.0	141.0	144.5	164.9	154.7
Scopolamine only (n=5)	100.0	96.6	87.8	80.6	79.4	76.5	82.1	89.2
Sco + CCh (n=5)	100.0	100.0	65.8	88.9	78.1	86.3	94.9	114.6

(B)

(ms)	0min (t0)	30min (t1)	60min (t2)	90min (t3)	120min (t4)	150min (t5)	180min (t6)	210min (t7)
Control (n=11)	35.8±3.8	38.4±6.4	37.5±4.8	35.9±3.8	35.7±4.3	34.7±4.7	38.3±6.5	36.5±2.8
Carbachol (n=6)	35±3.3	35.2±3.4	34.8±3.9	33.8±4.2	33.7±2.5	31.8±3.9	35.3±3.44	36.5±3.2
Scopolamine only (n=5)	32.2±2.6	33.8±5.1	35.0±3.2	33.4±2.9	34.6±2.2	34.4±3.9	34.8±3.8	35.2±4.3
Sco + CCh (n=5)	33±4.6	33.2±4.0	35.8±4.4	37±1.6	33.4±3.9	35±2.2	38.2±2.6	34.2±5.8

Table 2: **Amplitude and latency.** (A) Amplitude of control, carbachol, scopolamine, scopolamine and carbachol; Amplitude changes are normalized according to the first (0 min) VEP (100%). Carbachol injection induced an increase of amplitude (60min) lasting several hours but blocked with scopolamine injection. (B) Latency; Values of the latency of the VEPs for the different time points analysed. No significant change was observed.

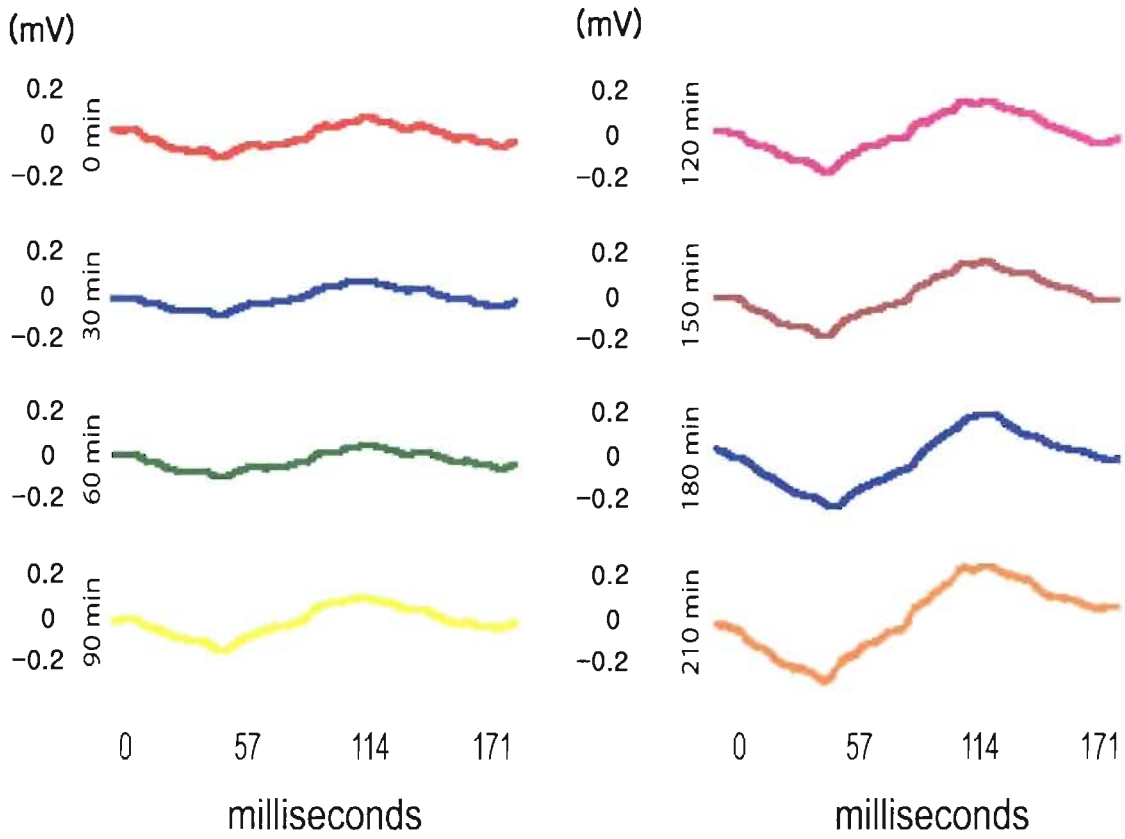

Figure 13: **Representative example of carbachol injected rat VEP** at different time points. Increase in amplitude is notable after carbachol injection (t=60min). Although it is not apparent on the graph the averaged increase rate is significant (see table 2).

due to the carbachol injection. Carbachol infusion did not induce any significant changes in latency of the VEP across the groups (Table 1; 4 ms maximal difference).

Effects of muscarinic, nicotinic and NMDA receptor antagonist on the augmentation of the amplitude of the VEPs

Representative example of the effect of mecamylamine (14% increase at maximal potentiation comparing to control group, one-way ANOVA p=0.7), scopolamine (3.8% increase at maximal potentiation comparing to control group, p=1.0) and CPP (25% increase at maximal potentiation comparing to control, p=0.089) on the VEP is illustrated on Figure 14. ACSF injection paired with carbachol was served to demonstrate that the double injection itself does not affect the electric signals. Comparing to other groups the difference of amplitude before (0 min) and after (180 min) carbachol injection in aCSF paired group was significant (Figure 14; 41% increase at maximal potentiation, p=0.001).

1. Muscarinic receptor antagonist

In order to analyze the mechanisms of carbachol long-term enhancement, various drugs were injected prior to carbachol in different groups. To examine whether the carbachol actions was mediated through nicotinic or muscarinic receptors we injected scopolamine intraperitoneally 30 minutes before carbachol infusion -latency to obtain the maximal inhibition effect of the drug. Figure 11 demonstrates a graphical representation of the changes in the amplitude of the VEPs as a function of time with scopolamine injection. A prior blockade of muscarinic receptors with scopolamine significantly suppressed the enhancing effect of the carbachol at t5, t6 and t7 which was not seen in control groups; aCSF injected before carbachol

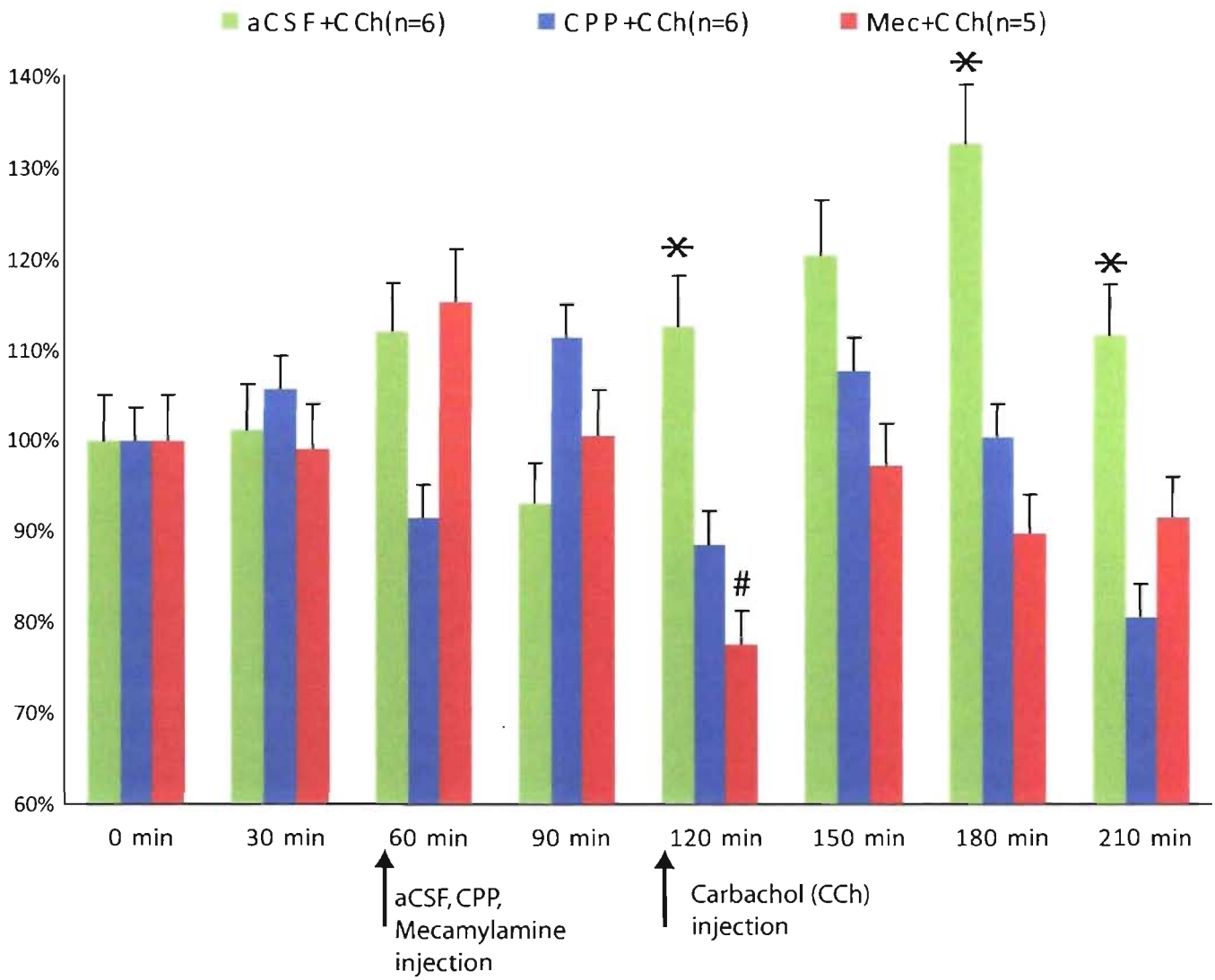

Figure 14: Graphical representation of the changes in the amplitude of the VEPs as a function of time during inhibition of muscarinic, nicotinic and NMDA receptors. Comparing to CPP and mecamylamine pre-injected group, aCSF injected group still induced an increase of amplitude (*). Even though mecamylamine injected group showed a similarity with scopolamine pre-injected group (figure 12) during carbachol injection (#), its effect is not statistically significant.

(35% of amplitude difference comparing to aCSF injected group, p=0.02). When carbachol was applied right after scopolamine i.p. injection during t3, meaning before activation of scopolamine, we observed that carbachol still induced an augmentation of VEP (n=2, data not shown). An interesting result is that there was a significant decrease of VEP amplitude evoked during carbachol injection (46% decrease comparing to control, p=0.034) when muscarinic receptors were blocked compared to control animals. However, this effect was shown only during the infusion.

2. Nicotinic receptor antagonist

Several studies also demonstrated the role of nicotinic receptor during synaptic plasticity (Hsieh *et al.*, 2000). To test whether augmentation of cortical responses also involves nicotinic receptors we injected mecamylamine, a nicotinic receptors antagonist. In order to observe the specific cortical effects of the drug we performed a local injection directly into the brain via a push-pull cannula 1h prior to the carbachol injection. Similarly with scopolamine, inhibition of nicotinic receptors also abolished carbachol induced long term enhancement of the VEP amplitude (42% of amplitude difference comparing to aCSF injected group, p<0.001). However, decrease of VEP was not observed during carbachol injection (one-way ANOVA, p<0.05, Figure 14).

3. NMDA receptor antagonist

Since the cholinergic system enhances the response of NMDAR mediated potentiation in the hippocampus (Kirkwood *et al.*, 1999), NMDAR inhibitor CPP was injected in the visual cortex to observe the involvement of NMDAR in the long term component of the response. The CPP infusion in V1 abolished the long-term effect of carbachol (Figure 13; 32% of amplitude difference comparing to aCSF group, p=0.007). This result indicates that long term change of

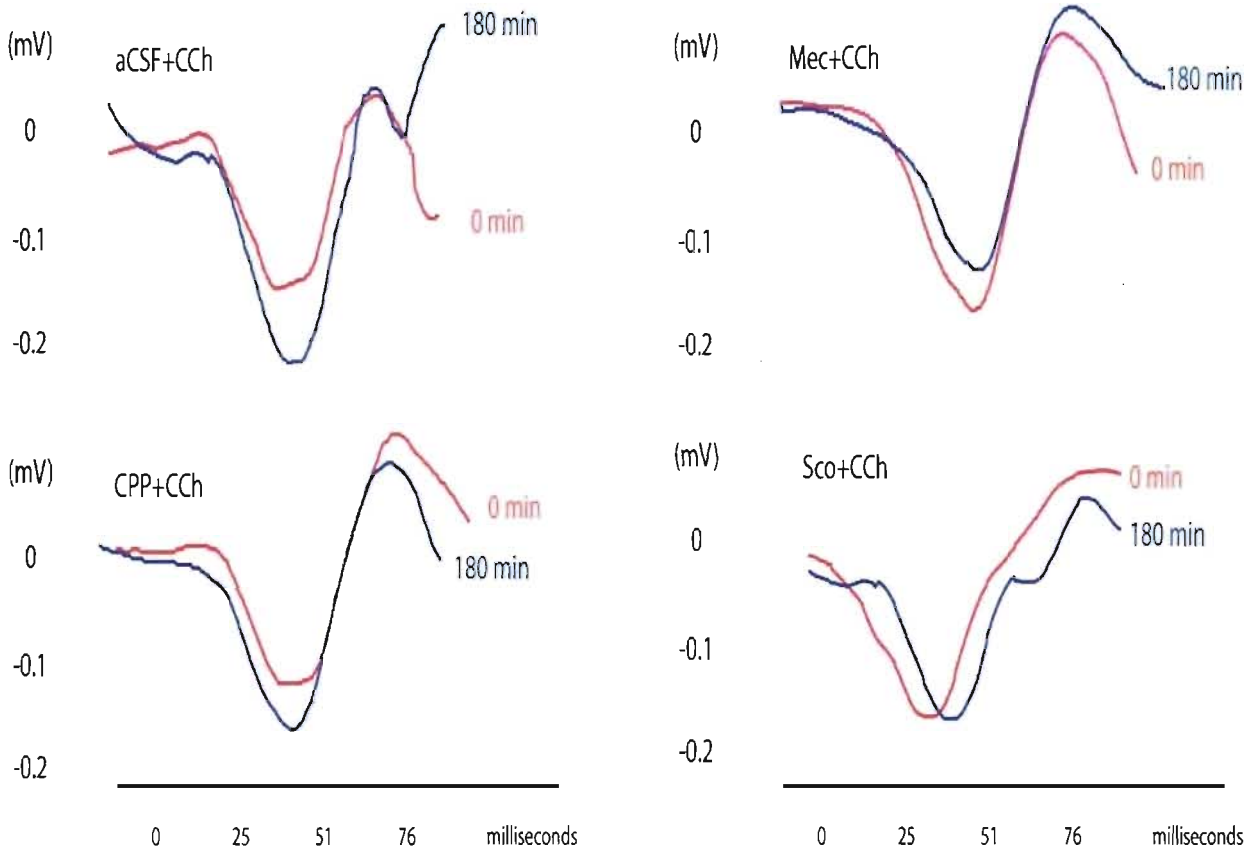

Figure 15: Representative example of the effect of mecamylamine, CPP and scopolamine on the VEP at t=0 and t=180.

(A)

(%)	0min (t0)	30min (t1)	60min (t2)	90min (t3)	120min (t4)	150min (t5)	180min (t6)	210min (t7)
aCSF+CCh (n=6)	100.0	101.1	111.8	93.0	112.5	120.4	132.5	111.5
CPP+CCh (n=6)	100.0	105.6	91.4	111.3	88.5	107.6	100.2	80.5
Mec+CCh (n=5)	100.0	99.0	115.3	100.6	77.4	97.1	89.6	91.4

(B)

(ms)	0min (t0)	30min (t1)	60min (t2)	90min (t3)	120min (t4)	150min (t5)	180min (t6)	210min (t7)
aCSF+CCh (n=6)	36.3±3.7	40.3±5.4	37.7±4.1	35.5±2.7	38.2±1.5	31.2±2.1	37.3±3.6	38.7±2.1
CPP+CCh (n=6)	36.3±3.2	34.5±3.4	41.2±5.0	34.0±2.5	33.5±3.5	36.8±3.1	33.7±3.0	37.2±2.7
Mec+CCh (n=5)	37.4±3.1	35.6±4.0	36.8±1.6	36.2±4.3	38.4±4.1	37.2±1.6	35.6±4.2	37±1.9

Table 3: **Amplitude and latency values of aCSF and carbachol, scopolamine and carbachol, mecamylamine and carbachol.** (A) Amplitude: aCSF pre-injected group showed similar increase of VEP after carbachol injection (t5). Pre-treatment of CPP or mecamylamine abolished this effect. (B) Latency; no significant difference in latency was observed.

VEP amplitude induced by carbachol is partly mediated by NMDAR. Table 2 shows the values of the amplitude and latency of the VEPs for different time points analyzed. Note that once again there is no significant increase or decrease amongst groups (ANOVA tukey HSD, $p>0.05$).

DISCUSSION

The present study demonstrates that coupling cholinergic activation to visual activation induces a long term enhancement in the amplitude of the subsequent VEPs. It appeared that endogenous spontaneous release of ACh during a patterned stimulation is not sufficient to activate this long term effect. Moreover, cholinergic transmission through muscarinic and/or nicotinic receptors seems to facilitate the opening of the NMDA receptors via second messenger activation or decreasing membrane threshold by lowering potassium conductance, most likely through post-synaptic and presynaptic mechanisms (Figure 16).

Technical aspects

VEP is most likely generated by calcium-activated potassium currents (Harada and Takahashi, 1983; Walton and Fulton, 1986; Higashi *et al.*, 1993; Kobayashi *et al.*, 1997) reflecting synaptic potentials and includes the activity of excitatory and inhibitory interneurons. Origin of the potentiation was tracked by current source density (Mitzdorf, 1985) and comparison with rhythmic EEG. With a substantially low impedance microelectrode (<1 MΩ) positioned a little apart from spike-generating source it is able to record an averaged potentiation of that area (Mitzdorf, 1985). Activity of a large number of neurons contributes to the signal by the low impedance of the electrode. Depending on the impedance of the electrode, the unfiltered signal indicates the sum of action potentials from cells grouped approximately 50-350 μm from its tip (Legatt *et al.*, 1980; Gray *et al.*, 1995). Henze *et al.* discriminated signals from ~140 μm away from the tip to calculate the numbers of neuron contributing to the response (Henze *et al.*, 2000). Considering the number of neurons in the hippocampus CA1 region (~400 000: Boss *et*

al., 1987) it is estimated that signal collected in a cylindrical region of 140 μm was generated from about 1000 neurons (Henze *et al.*, 2000). Since V1 possesses ~200 000 neurons in 1 mm^2 of cortical surface (O'Kusky and Colonnier, 1982), number of neurons is estimated higher than hippocampus.

Theoretically, during single unit recording, tip of the electrode placed close to the soma or axon of a neuron will measure spike traffic of that neuron and of its direct neighbours as well. It is estimated to generate accurate information of neurons placed within 50 μm radius sphere (Harris *et al.*, 2000; Henze *et al.*, 2000). Depolarization of a large neuron generates a physically bigger flow of membrane current than a small cell. Supported by experimental works single unit recording is estimated to reflect activity of very small neural populations of large principal cells (Towe and Harding, 1970; Humphrey and Corrie, 1978) which in cortex correspond to pyramidal cells. Interneurons (e.g. GABAergic) are hardly detected by this technique. Experimental evidence was provided in human study (Kreiman *et al.*, 2000). Since the purpose of this study is to determine systemic interference during cholinergic activation including excitatory and inhibitory neurons, single unit area recording was not selected.

Unlike single unit recording VEP has no bias toward cell-size or cell-type. Distance from the tip of the electrode generates a bigger effect. Since our purpose is to analyze the cortical local network among various neurotransmitter systems we chose VEP as analyzing tool. Moreover placing electrode close to cannula permits to observe the influence of injected pharmaceutics. And also to examine the effect of ACh on thalamocortical response, we recorded VEP in layer IV of V1 where it receives synaptic input from LGN.

Acetylcholine modulates cortical responses in adult visual cortex

The principal objective of this study was to analyze the cholinergic effect on cortical plasticity during visual stimulation and determine its molecular pathway. As cortical plasticity changes and memory trace are achieved through long term modification of the neuron functioning, we tested long term changes in evoked potentials elicited by the cholinergic system.

When carbachol was administrated (mimicking an increase in ACh availability) its effect was sufficient to induce an augmentation of VEP amplitude. This effect lasted for several hours. Similar result was also demonstrated by combining direct TBS (theta burst stimulation) LGN stimulation and carbachol injection (Dringenberg et al., 2006).

It was shown with checkerboard stimulation (Clapp et al., 2006) that when rat was stimulated at a low frequency (0.067Hz) no subsequent change was observed after 1h. But a notable augmentation of VEP was induced by high frequency (9Hz) stimulation. Since it was induced by a natural stimulation and resembled with LTP in the hippocampus Clapp et al. named this effect "sensory long term potentiation (sLTP)". Their results complement our hypothesis showing continual exposure of visual stimulation at a low frequency does not influence the VEP level or induce sLTP. Long lasting augmentation of VEP was comparable with sLTP in the similarity of their increase level. Moreover the facts that tetanic stimulation and carbachol injection were both performed unitarily during both experiments and their effects lasted for few hours were also identical.

Secondly, without any further treatment after 3 hours, no change in VEP amplitude was shown in the present study, although we have shown previously that sinusoidal horizontal grating induces

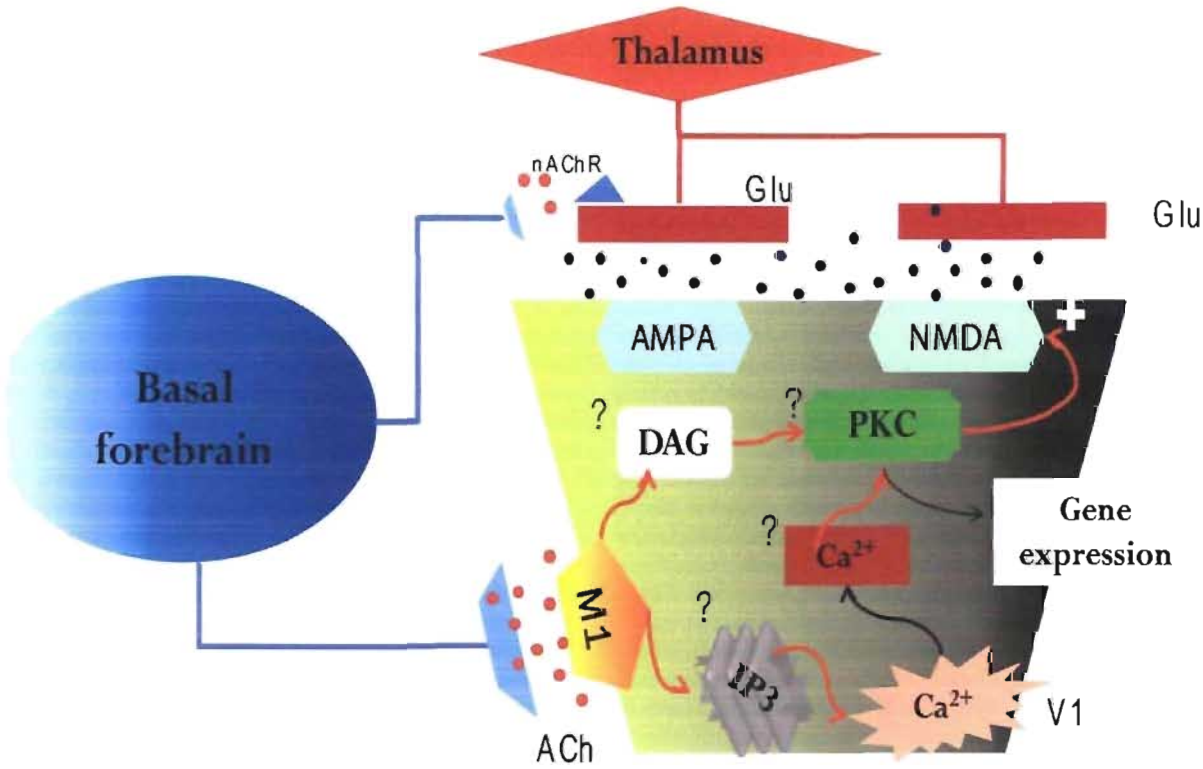

Figure 16: **Hypothetical model of cholinergic function pathway.** Nicotinic receptor functions in presynaptic area amplifying thalamocortical transmission and muscarinic receptor facilitates the opening of NMDA receptor via 2nd messenger on postsynaptic area.

ACh release in the visual cortex (Laplante et al., 2005). A possible reason can be, since our recording area is not a single neuron it is possible that potential change induced by ACh release during visual stimulation was simply neutralized and/or reduced by simultaneously activated influx and efflux of ion at different neurons. Depending on the stimuli, it has been proposed that ACh release level can vary (Inglis and Fibiger, 1995), especially ACh increase is prominent when the subject was introduced to a novel stimulation (Inglis et al., 1994). In that case, CCh levels would be too low to elicit a change in VEP. We propose that to induce an observable cholinergic long term modification of the VEPs a stronger stimulation is required.

Moreover, note that our experiences were performed on rats which were approximately 9 weeks old, obviously beyond critical period which is 6 weeks. Plasticity in adult was well demonstrated in different species like cat (Creutzfeldt and Heggelund, 1975), mouse (Sawtell et al., 2003) and rat (He et al., 2006). Our study thus confirms that the adult brain is consistently modulated by new stimulation.

Carbachol modification through muscarinic and nicotinic receptors

In order to decompose the molecular pathway activated by the cholinergic modulation is activating, different antagonists were injected prior to carbachol. A general consent, supported by abundant results, was established that activating muscarinic receptors (Shinoe *et al.*, 2005; Colgin *et al.*, 2003; Burgard and Sarvey, 1990) and nicotinic receptors (Wang *et al.*, 2006; Ge and Dani, 2005) enhances synaptic plasticity in hippocampus. Our results show that this mechanism can also be applied in the visual cortex as long-term modulation e.g. sLTP. Consistent with many other results our experiment also shows that muscarinic receptor

antagonist and nicotinic antagonist inhibit VEP potentiation which could be apparent to sLTP. This indicates that those receptors are essentials to induce an increase of cortical response.

Injection of carbachol before muscarinic receptor blockade still induced an increase in VEP. These results indicate that long term increase effect of VEP through cholinergic system is more prominent when activated during stimulation i.e. paired with thalamocortical stimulation. And after its induction, the triggered mechanism is able to maintain the elevated response state subsequently via transmission of other neurotransmitter (e.g. glutamate).

Unexpectedly, we observed that when muscarinic receptors were inhibited, VEPs show a significant decrease during carbachol injection. This reduction of VEP was not observed when nicotinic or NMDA receptors were blocked. We can interpret that this effect was due to the different activity pattern of nicotinic and muscarinic receptors (Levy *et al.*, 2006; Oldford and Castro-Alamancos, 2003). It was demonstrated in peduncular nucleus (Lena *et al.*, 1993) and in prefrontal cortex, that an application of nicotine can enhance GABAergic inhibition (Couey *et al.*, 2007). In our experiment, decrease of VEP with muscarinic receptor inhibition may result from the effect of nicotinic receptors. However, in this case, VEP amplitude should increase during carbachol injection when nicotinic receptors are blocked, which is not observed. On the contrary, blocking nicotinic receptors on the thalamocortical pathway decreases the sound-evoked cortical response (Kawai *et al.*, 2007) in the auditory cortex. Another explanation is the inhibition of the receptor M2 located on the dendritic area of GABAergic interneuron (Erisir *et al.*, 2001; Salgado *et al.*, 2007). Blocking M2 receptors could facilitate the release of GABA which will decrease the entrance of ions reaching reduction of VEP amplitude. Different position of cholinergic receptors allows regulating their own electrophysiological activity, synthesis of neurotransmitter, and transmitter release (i.e. autoreceptor) (reviewed by Schlicker and Gother, 1998). Through

this way it permits to selectively suppress intracortical transmission, while sparing or boosting thalamocortical pathway by itself (Gil *et al.*, 1997; Hsieh *et al.*, 2000; Kobayashi, 2000). As our experiment shows, ACh with other neurotransmitter (reviewed in Gu, 2002) play a permissive role in experience dependent plasticity in the neocortex allowing cortical modification based on experiences.

NMDA receptor dependent modulation mechanism

Another important feature in cortical plasticity is the involvement of NMDAR. In synaptic plasticity, since opening NMDAR launches a gene cascade translation and insertion of new receptors on synaptic area it is assumed that NMDAR is the trigger of long lasting effect. Since increase of VEP amplitude induced by carbachol was absent in CPP injected animals, we concluded that cholinergic enhancement of VEP was mediated in interaction with NMDAR. Our results are compatible with the experiment of Frenkel et al, who showed that consistent with the features of LTP, continual exposure to oriented stimuli induced potentiation of mouse cortical responses dependent of both NMDAR and AMPAR (Frenkel *et al.*, 2006). Numerous results indicate NMDAR dependent plasticity in the juvenile (Allen *et al.*, 2003; Quinlan *et al.*, 1999; Bear *et al.*, 1990;) and in the adult visual cortex (He *et al.*, 2006; Yashiro *et al.*, 2005) induced by visual deprivation. Opening of NMDAR through LTP signifies excessive entrance of Ca^{2+} (Malenka and Bear, 2004) and consecutively it activates calcium-dependent enzyme (i.e. CaMKII: Gordon *et al.*, 1996; PKA: Fisher *et al.*, 2004; calcineurin: Yang *et al.*, 2005) followed by a gene cascade. While it is tempting to connect cortical plasticity during critical period with adult visual cortex and interpret the increase of cortical response due to AMPARs induction to

synaptic surface, applying the same NMDAR-dependent mechanism can be impetuous. Inducting LTP and LTD depends on Ca^{2+} influx through the NMDAR and its molecular composition change during development (Yoshimura *et al.*, 2003; Sheng *et al.*, 1994). The ratio shift from NR2B to NR2A leads to kinetic changes resulting weaker Ca^{2+} response (Flint et al, 1997). Moreover, studies in the adult hippocampus showed that after LTP induction surface expression of AMPAR was not increased. Instead on an SRC- and PKC-family-dependent manner NMDAR was delivered on the synapse (Grosshans *et al.*, 2002). It is also reported that the dendrites of GABAergic interneurons are less static compared to the dendrites of glutamatergic pyramidal neurons (Lee *et al.*, 2006). Although to delineate sLTP pathway detailed examination on other neurotransmitter receptors and second messengers should be followed it is possible to infer from the mechanism in other brain area. As our figure 15 demonstrates, in the hippocampus slices, activation of cholinergic system could function on M1 receptor which will again activate PKC and launch a gene transcription mechanism. Nicotinic receptor on the other hand, it is possible that nicotinic receptors are served to not only increase incoming signal from thalamus but also to lower the threshold for thalamocortical innervations.

Attentional process through cholinergic system in V1

According to recent literature a mechanisms explaining the effects of pairing Ach release and visual stimulation facilitates opening of NMDAR through M1 receptor by increasing NMDAR-gated conductance (Kirkwood *et al.*, 1999) and through M2 receptor by inhibiting GABAergic neurons (Fukudome *et al.*, 2004; Salgado *et al.*, 2007). As mentioned above it is observed that when muscarinic receptors were blocked by inhibitors, not only carbachol

enhancing effect was blocked but also induced a decrease of VEP amplitude during carbachol. Decrease of GABA release by activating M2 receptor is reported at the layer 2/3 of auditory cortex (Salgado *et al.*, 2007). Even though the exact mediatory mechanism is not clarified, intuitively we can deduce that cholinergic system can influence both glutamatergic transmission and GABAergic transmission. This suggests the dual-control effect of ACh in V1. This manipulative feature is mainly required during attentional process to amplify selectively a response among many and suppress others. Unlike reminiscent view considering V1 as a static bank of spatio-temporal filters or pre-passing area before reaching the higher visual areas, V1 is expected to have the altering ability over the information (e.g. orientation shift: Ringach *et al.*, 1997) receiving from LGN. Since several studies demonstrate involvement of cholinergic system during attentional process in V1 (Bentley *et al.*, 2004; Thiel *et al.*, 2005; Roberts *et al.*, 2005) this modulatory function may be the essence for V1 to participate in attention mechanisms. It is proposed that cholinergic system modulated by prefrontal activation induces attentional process by suppressing visual information generated from distractors and increases feedforward response from information of the "meaningful" object (Sarter *et al.*, 2001). Presence of excitatory and inhibitory cholinergic neurons allows higher cortical areas to regulate thalamocortical input responses in V1 level by feedback regulation. Although direct projection of cholinergic fibres to visual cortex generates from basal forebrain, showing that prefrontal cortex (PFC) activation is necessary for ACh release level in sensory cortex (Rasmusson *et al.*, 2007) corroborates this hypothesis. Signals in V1 seem to be influenced by PFC mediated via cholinergic system during attention.

Clinical significance and perspective application

Clinical significance of this experiment can be stated as two forms: attention and visual rehabilitation.

Attention is the cognitive process of concentrating on a selected aspect among environment while ignoring surrounding cues. It is well demonstrated that Alzheimer disease patients suffer from degeneration of cholinergic fibres (Thal *et al.*, 1983; Candy *et al.*, 1983; Musial *et al.*, 2007) and consequently general symptom is lack of attention (Gordon and Carson, 1990; Lawrence and Sahakian, 1995) followed by deficit of new short-term memory production (Spaan *et al.*, 2003). As during anaesthesia neurotransmitter activity is disturbed (Kubota *et al.*, 1999; Jansson *et al.*, 2004) we were not able to observe attentional process. Nevertheless, when cholinergic system was activated, the effect was demonstrated by amplifying the thalamocortical response of a specific image which is identical as the feature of attention, amplifying response of selected item and suppressing others. Although to delineate more precisely and to examine whether during anaesthetized state rat can have attention, more studies about molecules involved during stimulation (e.g. norepinephrine) should be measured and compared.

Another important significance of this study is the link with visual rehabilitation. Visual field deficit (e.g. anopia, scotoma) resulted from stroke, trauma, ocular diseases or tumours is extremely debilitating for many people these days. Moreover as intricate the brain is, a small injury can lead to severe visual loss and may remain perpetually especially when it occurred after the loss of plasticity (i.e. critical period). The enhancing effect of cholinergic system on synaptic and cortical plasticity is well illustrated through various experiments. Contribution on cortical

plasticity gives the possibility that ACh can trigger or at least influence on the visual rehabilitation mechanism. As illustrated in our study, a single injection of cholinergic agent increases the thalamocortical response and its effect persist for a few hours. Since loss of neuron can result weakening of certain visual field, ACh can reinforce the response itself or supplement the missing region by boosting information from neighbouring cell. Another possible reaction is regeneration of synapse. Although we did not show here when early LTP state is maintained it is known that neuron convert to protein synthesis dependent state; late LTP. As introduced above, MAPK, especially the extracellular signal-regulated kinase (ERK) subfamily of MAPK is the candidate of mediatory protein kinase between early LTP and late LTP (Lynch, 2004; Kelleher *et al.*, 2004). Deducing from experiments showing MAPK activation is muscarinic (Roberson *et al.*, 1999; Rosenblum *et al.*, 2000) and nicotinic alpha-7 receptor (Dineley *et al.*, 2001) dependent, persistent application of ACh can induce protein synthesis and lead to a growth of new synapse by launching late LTP state. We have also shown indirectly the possibility of GABAergic neurons regulation mediated by cholinergic receptor stimulation and since unlike other neurotransmitters dendritic arbour of GABAergic interneurons possess its plasticity after critical period (Lee *et al.*, 2006) we interpreted that ACh can possibly trigger the induction of plasticity in adult visual cortex. With proper guidance this mechanism can serve to rehabilitate the visual loss.

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