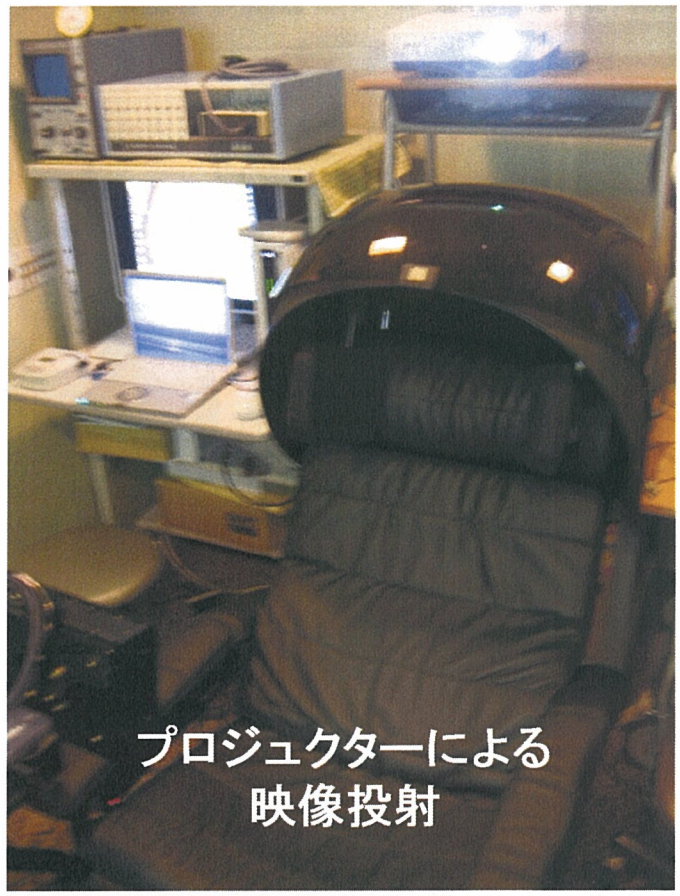
図25 咀嚼運動を利用した脳刺激システムの完成

ボディーソニックチェア、打楽器

ななめ上方からベースペダル、打楽器、
ボディーソニックチェアを見たところ。

図26

画像提示システム

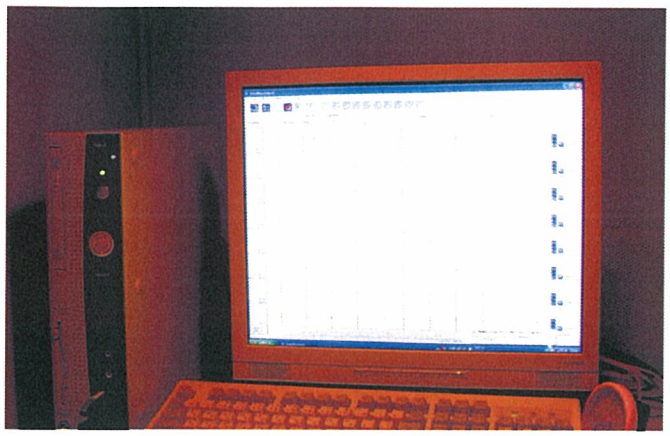


プロジュクターによる
映像投射

スクリーンへの映像提示

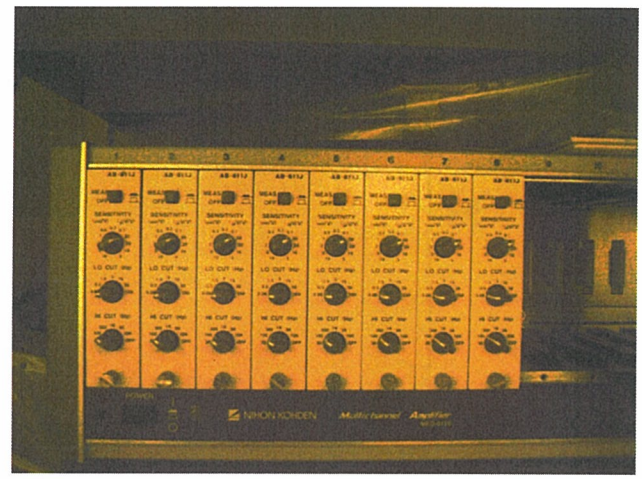
図27

脳波計測解析システム



脳波のパソコンへの取り込み

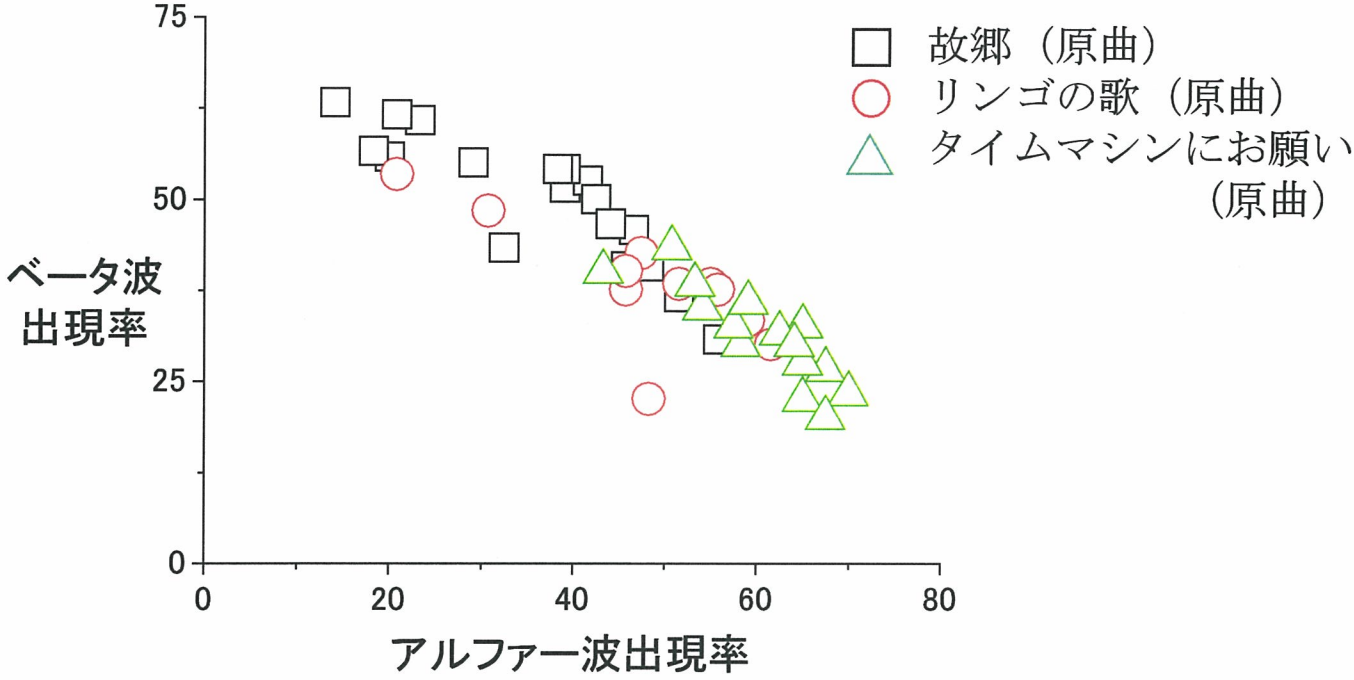
脳波計測アンプ

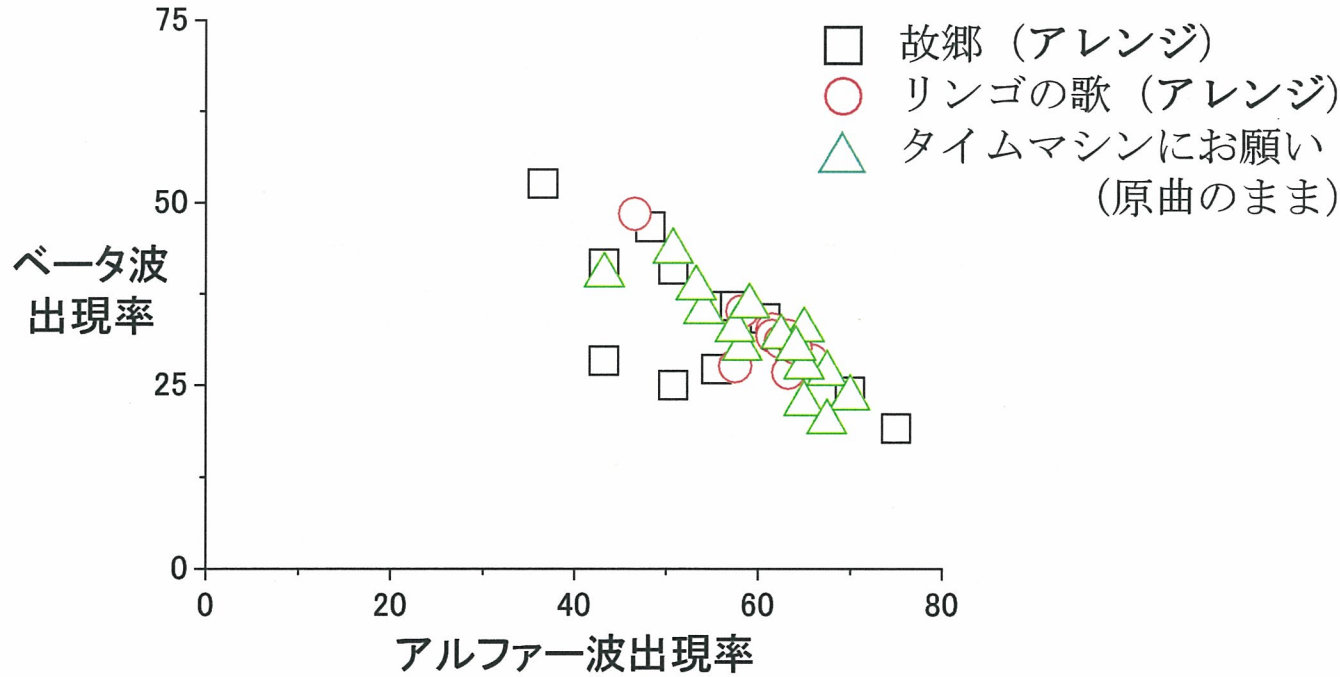


脳波周波数解析

図28

脳波周波数解析による
本研究で作成したアレンジ曲の効果
（原曲との比較）





同一健常者からの記録
提示曲を２分間聴いているのアルファー波・ベータ波の出現率をそれぞれ
X軸、Y軸としてプロットした。
唱歌故郷と歌謡曲リンゴの歌の原曲とアレンジ曲の脳波出現様式を比較した。
タイムマシンにお願いはコントロールとしてプロットした。

図29

受動型治療（積極参加型治療の導入としての役割）

例　原曲提示

とおりゃんせ、七つの子、故郷、
リンゴの歌、青い山脈
＋
それぞれの歌に関連する画像提示

共通点：童謡、唱歌、昭和20年代流行歌の歌詞をかなり正確に覚えており、提示曲に合わせて歌う。童謡唱歌に関連する画像、流行歌原曲とその当時の画像の同時提示は記憶の呼び起こしに非常に効果的であった。

世代による違い：

大正末期生まれ世代---昭和20年代流行歌提示曲を聴くと、戦争体験にかかわる思い出が鮮明によみがえる傾向にあった。

昭和初期生まれ世代---昭和20年代流行歌提示曲を聴くと、学生時代の楽しい出来事を思い出す傾向にあった。

認知症進行度による違い：

重度進行症例---童謡・唱歌をとくに好む傾向

軽度進行症例---流行歌を好む傾向にあった。

図30

積極参加型治療

アレンジを加えた本研究課題のための新規作成楽曲
（とおりゃんせ、七つの子、故郷、リンゴの歌、青い山脈）の提示
＋
リズムに合わせた咀嚼運動、打楽器、ベース演奏
⇒音声、振動、画像のフィードバック刺激

提示楽曲について

明確なリズムセクション、シンコペーション、魅力的コード進行、補続音、多種のメロディーパートなどを織り込んで、楽しく積極参加できるような楽曲を作製した。その際、積極参加を促すには、ボーカルパート（歌詞）の存在は非常に重要であった。

顎運動について

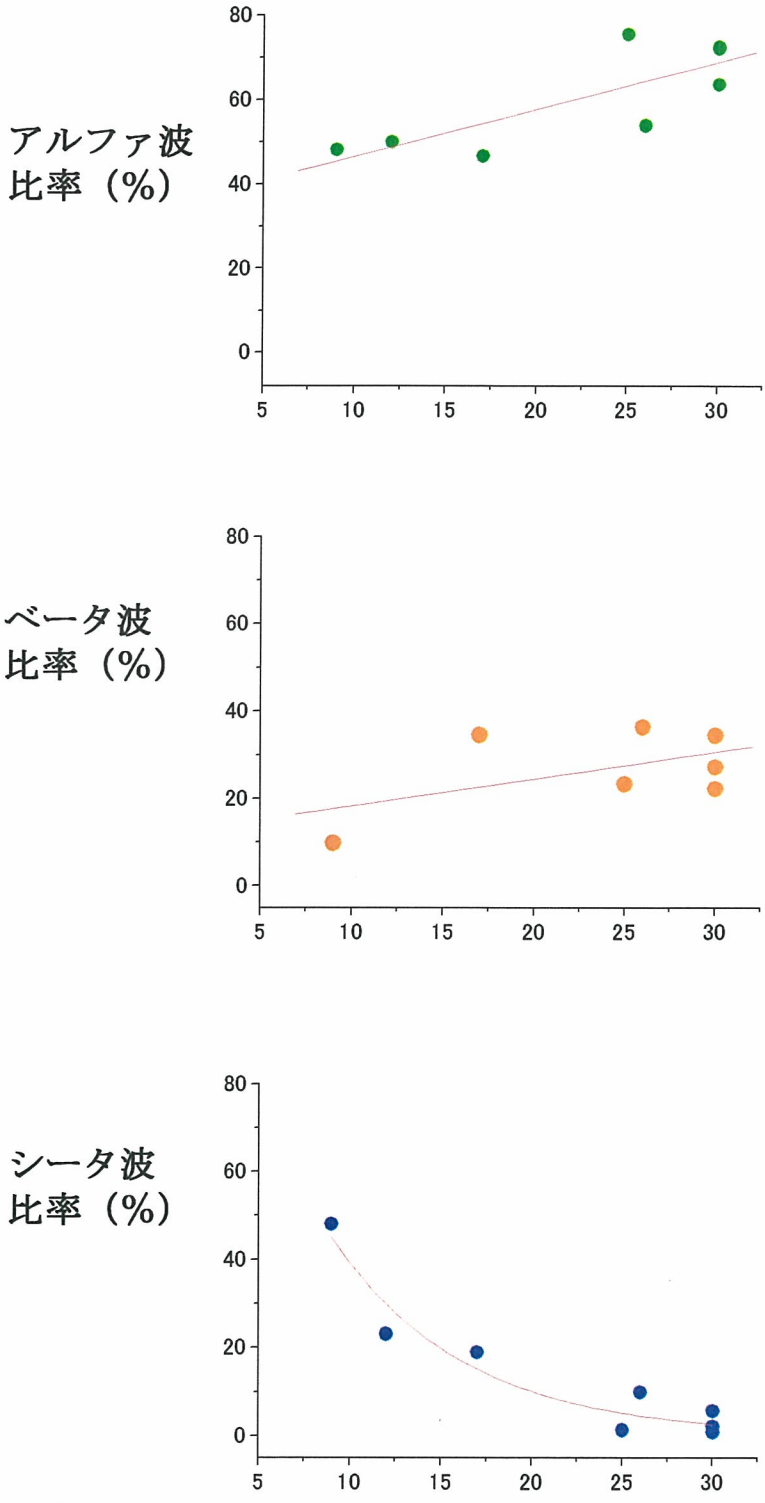
顎を動かして音が鳴ったり椅子が振動したり映像が動くこと自体が大きな驚きとなっていた。　安定して筋電位が発生し、かつ積極的に楽しく咀嚼してもらうには、チューインガムが効果的であった。

咀嚼運動だけでは、やがて単調になる傾向にあった。しかし、打楽器、ベースを加えると、難易度が急激に上昇し、咀嚼のリズムパターンを複雑にすることが可能となった。難易度が上昇したにもかかわらず、患者は積極的に手や足、顎を動かして参加し、一緒に歌うこともあった。これにより、咀嚼運動による脳刺激効果をより上昇させることが出来たと考えられる。

治療効果について

音楽療法訓練を重ねると、複雑なリズムパターンの組み合わせが可能になり、ほとんどの患者で、記憶がよみがえり、意欲性が上昇した。

図31 認知症進行度の脳波周波数分析による新たな指標



受動型治療遂行時の知見

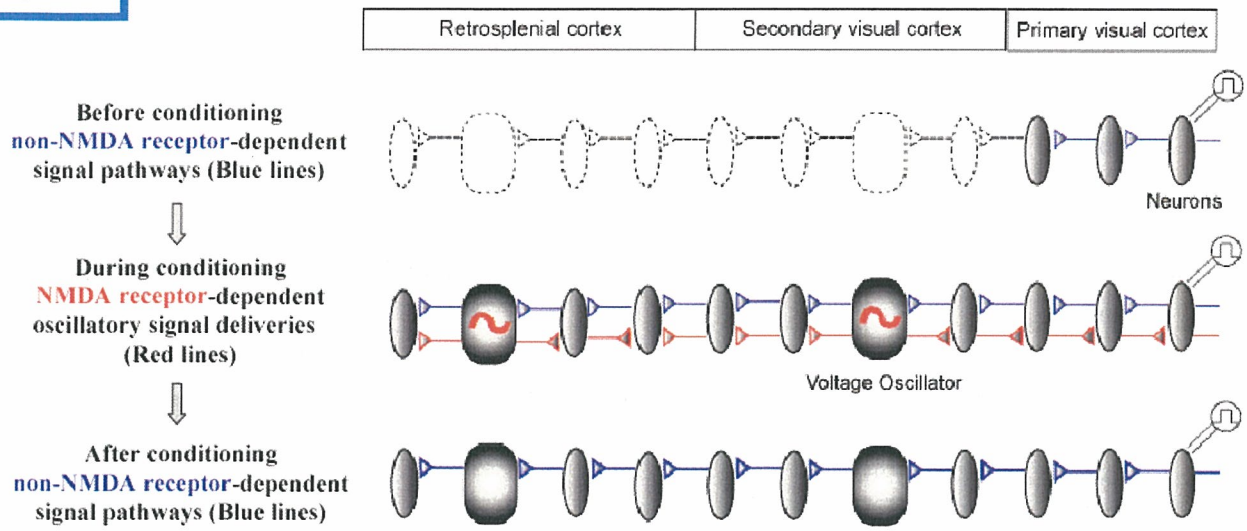
・映像+音楽同時刺激を行なった後，音楽刺激のみ行い、アルファ波・ベータ波・シータ波出現率の長谷川式スケール値に対する依存性を調べた。

・健常者では音楽刺激のみでアルファ波が増加しシータ波が減少する傾向にあるが、認知症患者ではシータ波が減少しない傾向にあった.

・映像や音楽刺激に対する脳波周波数分析のパラメーターを長谷川式簡易知能スケールにあてはめることにより、『脳波分析による認知症・アルツハイマー病の進行度の新たな指標』の可能性が示された。

図32

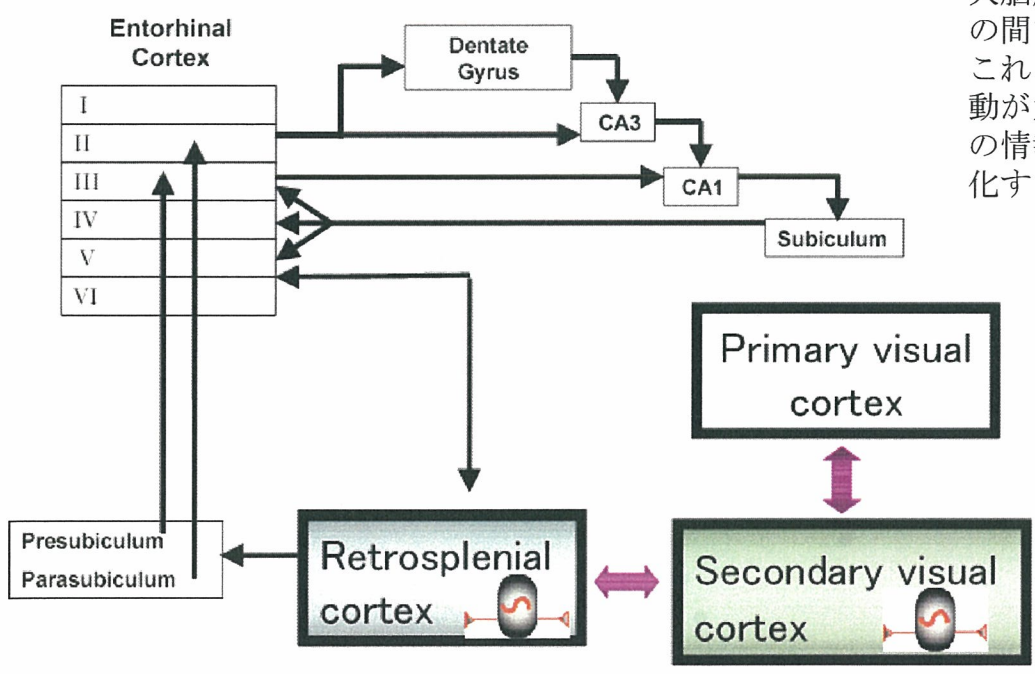
振動装置依存性可塑性仮説



膜電位振動を引き起こす振動源がある間隔で存在し、NMDA受容体依存性の信号を周囲へと配信し、皮質間結合が強化される.

振動装置の配置

Signal flows between visual cortex and hippocampal formation and voltage oscillators



大脳皮質視覚野と海馬の間で振動源が存在し、これらによる振動性活動が大脳皮質-海馬間の情報のやりとりを強化するという仮説.

研究成果の刊行に関する一覧表

雑 誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yoshimura, H.	The potential of caffeine for functional modification from cortical synapses to neuron networks in the brain.	Current Neuropharmacology	3	309-316	2005
Yoshimura, H., Honjo, M., Segami, N., Kaneyama, K., Sugai, T., Mashiyama, Y., Onoda, N.	Cyclic AMP-dependent attenuation of oscillatory-activity-induced intercortical strengthening of horizontal pathways between insular and parietal cortices.	Brain Research	1069	86-95	2006

医学系新聞発表

1．Medical Tribune Vol.39. No.17、2006年11月23日、P43
「脳機能維持には口腔機能を低下させないことが必要」

2．Dental Tribune Vol.3. No.1、2007年1月号、P11
「脳機能維持には口腔機能を低下させないことが必要」

The Potential of Caffeine for Functional Modification from Cortical Synapses to Neuron Networks in the Brain

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Abstract: Structure and function of the brain are use-dependent variables based on "synapse plasticity". Since synapses are driven by chemical transmitters, synaptic functions are liable to be modified by extrinsic chemicals displaying affinities for synaptic receptors or modulators. Caffeine is a widely used chemical substance that can invade synapses, and has several biochemical and metabolic actions on synaptic activities. This review focuses on the actions of caffeine on changes in structure and function in the region of the hippocampal formation and neocortex, which exhibit high synapse plasticity. At the synapse level, various synaptic receptors and channel activities are modulated by caffeine *via* mobilization of intracellular calcium, inhibition of phosphodiesterase, antagonism of adenosine receptors and GABA receptors. These actions of caffeine enable neurons to induce plastic changes in the properties of synaptic activities, such as synaptic transmission efficiency and morphology. At the network level, caffeine has the ability to activate cortical neural oscillators that deliver repetitive *N*-methyl-D-aspartate receptor-dependent signals to surrounding areas, causing strengthening of long-range inter-cortical communications. Caffeine might thus allow reorganization of cortical network functions *via* synaptic mobilizations.

1. INTRODUCTION

The brain is a complex system for information processing. The intellective device requires harmonic and coherent action of the component neuron network units, resulting in consistent and intensive operation of the network systems [23,76]. One prominent property of the brain is that structure and function, such as neural wiring and signal communicating efficiency, remain use-dependent and developmentally variable, allowing the brain to acquire the ability to process various modes of information in accordance with changing circumstances [3,9,13,78]. Mechanisms at the synapse level in local dimensions provide this brain variability. Use-dependent induction of synaptic changes is called "synapse plasticity" [11,19,43,52,53,54,59,75]. In general, induction of the synapse plasticity requires repetitive synaptic experiences. Ionotropic or metabotropic receptor activities elicited by synaptic transmission play important roles in the generation of use-dependent synapse plasticity. Production of the electro-motive forces that drive the network systems is triggered at the synapse level. Synapto-motive forces are generated by presynaptic chemical-transmitter release and postsynaptic receptor activities. Interestingly, various natural and synthetic chemicals in the external environment display affinities for synapse receptors and modulators. When these chemicals invade the synaptic cleft and chemical actions are exerted, synaptic functions are liable to be modified.

Among the natural chemicals in the external environment, caffeine is one of the most well-known chemicals able to invade the synaptic cleft. Caffeine displays affinities for several kinds of receptors embedded in the synaptic membranes and internal calcium store, and also has an affinity for cytoplasmic phosphodiesterases (PDEs), enabling caffeine to modify synaptic activities [31,32,66]. Caffeine thus displays various biochemical and metabolic actions at the synapse level. In general, plastic changes in synaptic transmission efficiency and synaptic architecture are induced according to synaptic activities *via* various kinds of modulation system [6,16,48]. If local synaptic changes are induced systematically and extensively, local changes may develop into network changes. The chemical activity of caffeine might therefore provide the potential for reorganization of brain function from synapse to wide-ranging networks.

Among the various areas of the brain, the hippocampal formation and neocortex exhibit a high susceptibility to the induction of synapse plasticity [11,13,53,75]. The present review focuses attention on these cortical regions, and explores the action of caffeine on plastic changes in structure and function from synapse to cortical network levels.

2. BASIC NEUROPHARMACOLOGICAL EFFECTS OF CAFFEINE

2.1. Effects of Caffeine on Adenosine A1 and A2A Receptors

Purines such as adenosine triphosphate (ATP) and adenosine play central roles in energy metabolism for all cells, and purinergic receptors are located on the cell surface and hence bind purines in the extracellular space [14,31,34]. Interestingly, xanthines such as caffeine block adenosine receptors, but not ATP receptors [20,31]. Adenosine receptors are coupled with G-protein, and can be divided into subtypes A1, A2A, A2B and A3 [20,22,24,31,32,34,69]. Among these subtypes, caffeine blocks A1 receptors that inhibit adenylyl cyclase (AC), in addition to A2A receptors that activate AC [22,24,31,32,34]. In neurons, A1 and A2A receptors are expressed at presynaptic terminals. A1 receptors negatively influence transmitter release from presynaptic terminals, whereas A2A receptors positively influence transmitter release [32]. While A1 receptors are widely distributed

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1570-159X/05 $50.00+.00

throughout the brain, A1 receptors are expressed at the highest level in the hippocampus and neocortex, where glutamate is used as an excitatory transmitter. Conversely, A2A receptors are not distributed widely, but are distributed locally at the highest level in the striatum and nucleus accumbens [22,24,32]. A2A receptors are expressed in dopamine-rich regions, and are co-expressed with dopamine D2 receptors [28,29,35,49,80]. A2A receptors are thus dominantly linked with the dopaminergic system, whereas A1 receptors in the hippocampus and neocortex are dominantly linked with the glutamatergic system. In addition, in hippocampal CA3 neurons, A1 receptor-selective blockade induces bursting activities, but not A2A receptor-selective blockade [82]. Caffeine is therefore considered to act predominantly on A1 receptors in the cortical regions, and positively influence presynaptic transmitter release *via* blockade of A1 receptors.

2.2. Effects of Caffeine on PDEs

The cyclic AMP (cAMP) cascade is one of the most important intracellular signaling pathways, playing a key role in the expression and modulation of neural function in the central nervous system (CNS) [8]. Activation of membrane receptors coupled to a specific G protein, Gs, such as β-adrenergic receptors or specific metabotropic glutamate (mGlu) receptors, initiates the operation of membrane-bound AC and production of cAMP as a second messenger. Protein phosphorylation or gene expression is finally induced by way of cAMP-dependent protein kinase (PKA) or cAMP response element-binding (CREB) proteins [18,55,85]. These cAMP cascades are negatively controlled by PDEs that breakdown cAMP and turn off the cAMP signaling pathways [4,30,79]. Caffeine depresses PDE activity, and intracellular cAMP is accumulated, resulting in the enhancement of cAMP signaling pathways [5,15,32].

2.3. Effects of Caffeine on Ryanodine Receptors

Calcium signaling pathways play an important role in regulating various brain functions [7]. In particular, increases in cytoplasmic calcium triggers down-stream of the intracellular calcium-dependent cascades. There are extra- and intracellular sources of calcium. Neurons include endoplasmic reticulum (ER) to store high concentrations of calcium. Calcium-release channels called ryanodine receptors are expressed in the membrane of the ER. When extracellular calcium enters the endoplasm through voltage- or receptor-operated calcium channels, ryanodine receptor channels are opened by the binding of calcium with the ryanodine receptors, and calcium is then released from the calcium store into the cytoplasm as a calcium-induced calcium release (CICR) [7,26]. Concentrations of endoplasmic calcium are thus amplified, and intracellular calcium signaling pathways are activated in a feed-forward manner. Caffeine permeating into the cell through the cell membrane combines with ryanodine receptors. This results in activation of the ryanodine receptors, reducing the threshold of the CICR and resulting in intense facilitation of CICR [25,32,36,61]. In rat hippocampal CA3 neurons, caffeine promotes epileptic discharges *via* enhancement of the CICR [56], and caffeine enhances action potential-triggered CICR in rat hippocampal CA1 neurons [71]. Amplification of intracellular calcium is thus positively controlled by caffeine through ryanodine receptors.

2.4. Effects of Caffeine on GABA Receptors

Neuron network activities are based on excitatory and inhibitory synaptic activities. The GABAergic network plays important roles in the stabilization of overall network activities. A recent study revealed that caffeine can modulate the GABAergic system. In ganglion cells of the turtle retina, caffeine depresses the activities of GABA-A receptors. This depression is mediated by caffeine facilitating CICR [1]. Similarly, in dentate gyrus cells of the hippocampus, CICR elicited by caffeine depresses the activities of GABA-A receptors [21]. In neonatal hippocampal neurons, mobilization of Ca^{2+} from caffeine-ryanodine-sensitive stores facilitates GABA release, while caffeine simultaneously depresses the activities of postsynaptic GABA-A receptors [72]. Caffeine thus affects GABA-A receptor activities by way of facilitating CICR. Conversely, although the mechanisms remain unclear, Ca^{2+}-independent inhibition of GABA-A receptor activities by caffeine occurs in hippocampal neurons [81].

3. NEUROPHARMACOLOGICAL EFFECTS OF CAFFEINE AT CORTICAL SYNAPSES

At the synapses, local synaptic potentials are generated by synaptic inputs. As local synaptic potentials are summated spatio-temporally, neurons have the ability to integrate various input signals. In addition, synapses can display changes in the efficiency of synaptic transmissions and induce morphological changes according to activity. The basic targets of caffeine mentioned above are concentrated at the synapses (Fig. **1**). Synapses are therefore considered to represent the dominant targets of caffeine.

3.1. Effects of Caffeine on Presynaptic Sites

Release of excitatory transmitter is more strongly inhibited by adenosine than release of inhibitory neurotransmitters [33]. Blockade of adenosine receptors by caffeine can thus occasionally generate overactivity at excitatory synapses [2,47]. In hippocampal CA3 in guinea pigs, blockade of A1 receptors by caffeine generates paroxysmal depolarizing shifts, and the underlying mechanisms may be increased by intracellular cAMP and Ca^{2+} influxes [62,63]. In hippocampal CA1 neurons, caffeine enhances excitatory postsynaptic potentials (EPSPs), which are mediated by antagonism of presynaptic adenosine receptors [37].

In both glutamatergic and cholinergic neurons, caffeine affects presynaptic sites. In rat hippocampal neurons, caffeine enhances acetylcholine (Ach) release from presynaptic terminals *via* blockade of A1 receptors [17].

Changes in the probability of transmitter release induced by caffeine have been investigated by focusing miniature excitatory postsynaptic currents (EPSCs) [74]. The study proposed that in rat barrel cortex, caffeine enhances glutamate release from presynaptic terminals *via* calcium release from ryanodine-sensitive internal Ca^{2+} stores.

3.2. Effects of Caffeine on Postsynaptic Sites

Postsynaptic activities can be divided into two categories: direct synaptic transmission, and indirect synaptic trans-

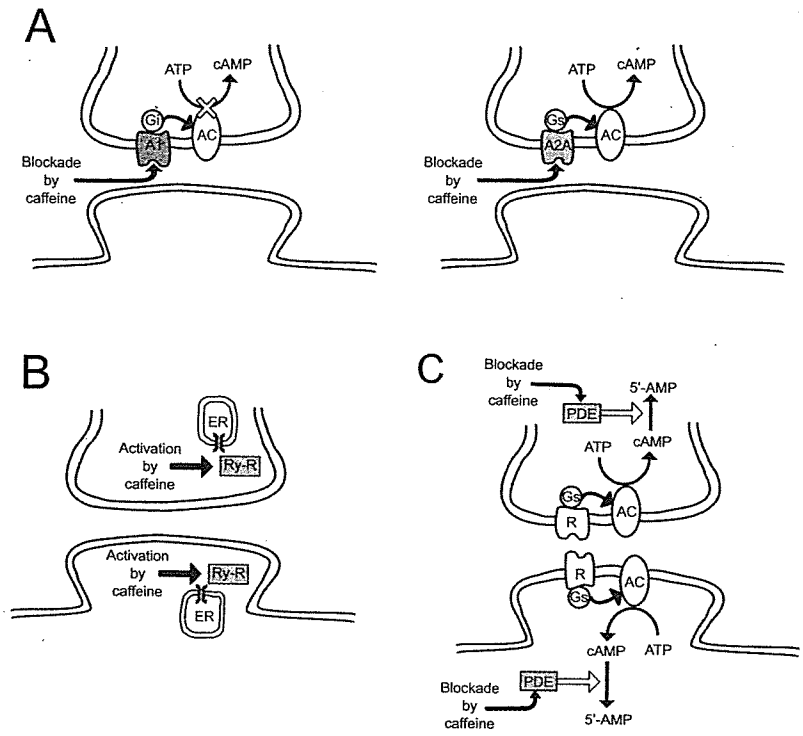

Fig. (1). Basic actions of caffeine at synapses.

(A) Presynaptic A1 receptors inhibit adenylyl cyclase (AC), resulting in decreased levels of intracellular cAMP, whereas presynaptic A2A receptors stimulate AC, increasing intracellular cAMP levels. Caffeine blocks both A1 and A2 receptors, resulting in increased and decreased intracellular cAMP levels, respectively. (B) Intracellular calcium stores are present for both pre- and postsynaptic sites. Ryanodine-sensitive calcium release channels are expressed on the calcium stores. Caffeine activates ryanodine receptor channels, reducing the threshold for calcium-induced calcium-release (CICR). (C) Intracellular phosphodiesterases (PDEs) breakdown intracellular cAMP at both pre- and postsynaptic sites. Caffeine blocks PDEs, increasing intracellular cAMP levels.

mission. Direct synaptic transmission is mediated by ligand-gated ionic channel-coupled receptors. Electro-motive force at the synapse is produced by ligand-gated ionic channel-coupled receptors. In general, two types of ionotropic glutamate receptors produce EPSPs: *N*-methyl-D-aspartate (NMDA) receptors, and non-NMDA receptors. NMDA receptors are postsynaptic activity-dependent calcium permeable channels, and play a central role in the induction of synapse plasticity, as mentioned below.

In rat hippocampal CA3 neurons, caffeine enhances both NMDA and non-NMDA receptor activities, inducing high-frequency oscillations [39]. In rat visual and parietal cortices, caffeine also enhances both NMDA and non-NMDA receptor activities, inducing α-range oscillations [89,90,92,93]. Caffeine-enhanced synaptic activities are triggered by activation of both receptors, in turn causing enhancement of the receptor activities themselves. Since adequate repetitive synaptic inputs are required for caffeine-dependent enhancement of synaptic activities, this enhancement is considered use-dependent.

Indirect synaptic transmission is mediated by G-protein-coupled metabotropic receptors. Expressed at both pre- and postsynaptic sites, mGlu receptors are involved in modulation of synaptic activities by activation of a second messenger system. Generation of a transient increase in intracellular Ca^{2+} to switch on the Ca^{2+}-signaling second messenger pathway is one of the principal roles of mGlu receptors [68]. In hippocampal CA1 neurons, the Ca^{2+} transient induced by postsynaptic mGlu receptor activation is blocked by caffeine [10]. Caffeine thus acts on mGlu receptor activities at postsynaptic sites.

GABA receptors are concerned with generation of inhibitory postsynaptic potentials, so GABA receptors negatively influence synaptic activities. In hippocampal dentate gyrus neurons, elevation of intracellular calcium levels by caffeine depresses postsynaptic ionotropic GABA-A receptor activities, showing that depression of GABA-A receptors by caffeine is dependent on intracellular calcium elevation [21, 72]. In contrast, caffeine depresses GABA-A receptor activities in hippocampal CA3 neurons, which are independent of intracellular calcium elevations [81].

3.3. Effects of Caffeine on Long-Term Potentiation and Long-Term Depression

At many cortical synapses, repetitive synaptic activities can produce long-term changes in synaptic efficiency [11,12, 59]. According to the patterns of temporal coincidence, location and intensity of pre- and potsynaptic activities, synaptic efficiency is potentiated or depressed over the long term, and termed long-term potentiation (LTP) or long-

term depression (LTD) respectively. Various kinds of LTP, LTD and associated mechanisms have been investigated and summarized in many reviews [43,48,52,53,54,59, 75]. Considering the mechanisms of LTP and LTD, whether LTP and LTD are NMDA receptor-dependent represents a central issue, since induction of activity-dependent synapse plasticity is deeply affected by NMDA receptor activities [16,43,50,73,75]. Activities of postsynaptic NMDA receptors are blocked by extracellular Mg^{2+}, and reduction of this Mg^{2+} block requires postsynaptic depolarization, allowing NMDA receptors to function as important detectors of coincident pre- and postsynaptic activities [60,67]. The coincidence between pre- and postsynaptic activities is deeply involved in the induction of synapse plasticity [40].

In general, LTP in hippocampal CA1 neurons requires both postsynaptic NMDA receptors and increased levels of intracellular Ca^{2+} by way of NMDA receptors. In contrast, caffeine induces another form of LTP in hippocampal CA1 neurons. Caffeine-dependent LTP requires neither postsynaptic NMDA receptors nor increased intracellular Ca^{2+} by way of NMDA receptors, but does require the interaction of caffeine with P1 adenosine receptors, P2 prinoreceptors and ryanodine receptors, indicating that caffeine-dependent CA1 LTP is caused by increases in presynaptic transmitter release [57, 58]. Another presynaptically induced caffeine-dependent LTP in hippocampal CA1 has been reported. In rat hippocampal CA1 neurons, caffeine promotes forskolin-induced LTP, where adenosine A1 receptor antagonism underlies the effects of caffeine [51]. Caffeine thus increases susceptibility to the induction of cAMP-dependent LTP, *via* enhancement of presynaptic cAMP accumulation. Actions of caffeine at presynaptic sites may be sufficient to induce such cAMP-dependent NMDA receptor-independent LTP.

As for LTD, postsynaptically induced caffeine-dependent LTD has been reported. Caffeine-dependent LTD in hippocampal CA neurons is postsynaptically induced in a stimulation frequency-dependent manner. LTD requires both NMDA receptor activities and calcium release from internal calcium store [65].

3.4. Effects of Caffeine on Morphological Changes in Synapses

The morphology of dendritic spines exerts a substantial effect on important aspects of synaptic activities, such as synaptic transmission and integration of synaptic information [41,73,77,87,95,96]. In dendritic spines, calcium dynamics play an important role in the expression of those synaptic functions, by way of various calcium-dependent biochemical processes. Particularly in hippocampal CA1 neurons, individual spines play an important role in detecting temporal coincidence between pre- and postsynaptic activities *via* NMDA receptors [96].

In cultured hippocampal neurons, application of caffeine causes a transient rise in intracellular calcium levels *via* ryanodine receptors in dendrites and spines, resulting in increased size of excitatory dendritic spines and changes in spine shapes [44,45]. Calcium from internal stores elicited by caffeine can thus modify dendritic spine shape [38]. Dynamics of intracellular calcium increases differently between short- and long-neck dendritic spines, suggesting that control of spatio-temporal calcium increases is provided by the shape of dendritic spines [84]. Since changes in spine structure contribute to changes in brain function [64,95], caffeine might modulate brain function *via* increases in intracellular Ca^{2+} level [38].

4. NEUROPHARMACOLOGICAL EFFECTS OF CAFFEINE ON CORTICO-CORTICAL SIGNAL INTERACTIONS

Local synaptic changes may in turn induce reorganization of cortical network function. However, this might require strong and long-range synchronization of synaptic activities or firing between neuron clusters [27,46,70,76]. In this respect, a strong relationship may exist between neural oscillations and synapse plasticity [27,83]. To understand the mechanisms of neural oscillation, several network oscillation models have been proposed [27]. Theoretically, synchronization as a non-local event is convenient for induction of synapse plasticity between long-range discrete cortical areas. Even if synchronization is a local event, however, synapse plasticity is inducible between long-range discrete areas, on the condition that the propagating system is strong and stable.

Recently, a protocol for inducing synchronized membrane potential oscillation at a frequency of 8-10 Hz in the visual cortex has been developed, by applying caffeine to rat brain slices [90,92,93]. The start of oscillation requires a trigger input, and oscillation comprises several propagating wavelets. Oscillation induction requires low-frequency activation of input fibers in conjunction with caffeine application, suggesting that use-dependent mechanisms underlie oscillation induction. Notably, induction of oscillation requires both NMDA receptor activation and the release of intracellular calcium from the internal calcium store, suggesting that functional coupling between NMDA and ryanodine receptors underlies caffeine-dependent oscillation [92,93]. In the absence of caffeine, the strength of functional coupling between NMDA and ryanodine receptors in hippocampal neurons depends on the magnitude of NMDA receptor activation [42]. In the presence of caffeine, caffeine activates ryanodine receptors and potentiates presynaptic glutamate release, resulting in an increased likelihood of functional coupling between NMDA and ryanodine receptors.

Strictly speaking, caffeine-dependent oscillation comprises initial propagating components and subsequent oscillatory components. These subsequent oscillatory components emerge from the local area in the visual cortex, showing that the oscillator is localized. Although synchrony is a local event, the neural oscillator delivers NMDA receptor-dependent signals to the surrounding areas [90,94]. These signal deliveries finally cause strengthening of non-NMDA receptor-dependent inter-cortical functional connections between long-range discrete areas [94]. The oscillators are separately located in the medial and lateral secondary visual cortices. Horizontal connections in layer II/III between the primary and secondary visual cortices are strengthened after repetitive NMDA receptor-dependent signal delivery originating from the oscillators.

Another study revealed that the oscillator is also located outside the visual cortex. The retrosplenial cortex is located

at a critical position between the visual cortex and hippocampal formation. In the area of the retrosplenial cortex, the oscillator is present in the retrosplenial granular a cortex (RSGa). Activation of oscillators in both the secondary visual cortex and RSGa under application of caffeine finally opens functional connections from primary visual cortex to the postsubiculum [91]. Hence, in the presence of caffeine, an oscillator with local synchronization can induce spatially wide-ranging synapse plasticity from the visual cortex to the hippocampal formation.

These studies resulted in the "oscillator-dependent plasticity hypothesis" [90,91,94]. This hypothesis is illustrated in Fig. 2, showing the induction of plastic changes in the visual cortex in the presence of caffeine. Caffeine, in combination with low-frequency electrical stimulation, promotes the voltage oscillator delivering NMDA receptor-dependent signals at a frequency of 8-10 Hz from the secondary visual cortex to surrounding cortical areas. This induces opening and strengthening of non-NMDA receptor-dependent signal pathways. Repetitive activities of an NMDA receptor-dependent voltage oscillator thus induce use-dependent network plasticity in the cortical regions.

The same mechanism is present between the gustatory insular cortex and somatosensory parietal cortex. In these areas, the oscillator that delivers NMDA receptor-dependent signals is located in the parietal cortex, and is driven under application of caffeine by repetitive low-frequency stimulation. Oscillatory NMDA receptor-dependent signal delivery causes strengthening of functional connections between the insular and parietal cortices [88,89].

Theoretical investigation has demonstrated that oscillation-dependent mechanisms underlie the establishment of working memory. The study showed that NMDA receptor-mediated synaptic transmission at a frequency of 8 Hz is required to sustain persistent network activities of the prefrontal cortex [86]. Results collected by experimental and theoretical studies thus suggest that NMDA receptor-mediated α-range signal delivery plays a critical role in the generation and stabilization of functional networks *via* plastic changes from synapses to networks.

5. CONCLUSION

Caffeine displays various general pharmacological actions: 1) blockade of presynaptic A1 and A2A receptors, resulting in modulation of transmitter release; 2) activation of internal ryanodine receptors, resulting in reduction of CICR threshold; 3) blockade of PDEs, resulting in intracellular cAMP accumulation; and 4) blockade of GABA-A receptors, resulting in depression of inhibitory synaptic activities. In the brain, these general actions of caffeine are liable to take place at the synapses, as the targets of caffeine and its effects are concentrated at the synapses. Particularly in the regions of the hippocampal formation and neocortex, where use-dependent synapse plasticity is liable to be established, caffeine is able to enhance synaptic NMDA receptor activities and intracellular calcium signaling pathways, through which plastic changes in synaptic morphology and transmission efficiency are induced. In cortical neuron networks, the actions of caffeine in combination with adequate input fiber activation produce opening and strengthening of long-rage inter-cortical signal

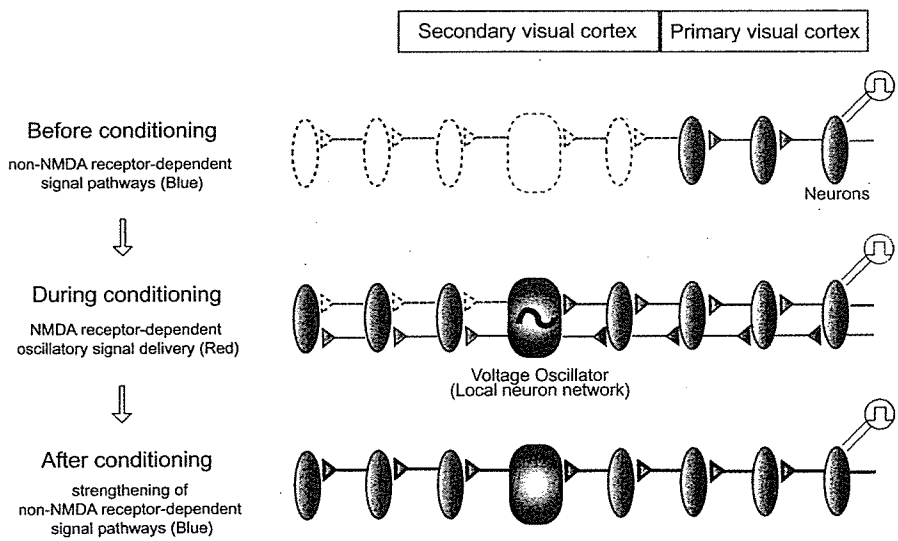

Fig. (2). Oscillator-dependent plasticity hypothesis.

Each ellipse indicates a postsynaptic neuron or neuron cluster, and small triangles indicate presynaptic terminals. In the presence of caffeine, low-frequency electrical stimulation is applied to the primary visual cortex as "conditioning". Horizontal pathways in blue represent non-NMDA receptor-dependent pathways. A voltage oscillator comprising local neuron networks is equipped in the secondary visual cortex, and horizontal pathways in red represent *N*-methyl-D-aspartate (NMDA) receptor-dependent pathways. (Upper) Before conditioning, signals elicited by the primary visual cortex stimulation propagate within a short distance. (Middle) During conditioning, non-NMDA receptor-dependent signals switch on the oscillator that delivers NMDA receptor-dependent oscillatory signals back and forth. (Lower) After conditioning, non-NMDA receptor-dependent pathways are strengthened, and signals propagate a long distance. Note that when NMDA receptor activities are blocked from the beginning, strengthening of non-NMDA receptor-dependent pathways is not induced [90,91,94].

communications *via* activation of cortical neural oscillators that deliver NMDA receptor-dependent signals to surrounding areas. The actions of caffeine at a synapse level thus cause plastic changes at the cortical network level.

Most of the experimental evidence has been collected from basic studies using peculiar conditions *in vitro*. However, these basic studies have elicited the potential of caffeine, and the evidence indicates that caffeine exerts profound actions from synapse to neuron networks in the cortical regions. Caffeine might thus provide the potential for use-dependent reorganization of brain function.

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Research Report

Cyclic AMP-dependent attenuation of oscillatory-activity-induced intercortical strengthening of horizontal pathways between insular and parietal cortices

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ABSTRACT

Cyclic AMP (cAMP) is a key intracellular second messenger, and the intracellular cAMP signaling pathway acts to modulate various brain functions. We have previously reported that low-frequency insular cortex stimulation in rat brain slices switches on a voltage oscillator in the parietal cortex that delivers signals horizontally back and forth under caffeine application. The oscillatory activities are N-methyl-D-aspartate (NMDA) receptor-dependent, and the role of oscillation is to strengthen functional intercortical connections. The present study investigated actions of the cAMP signaling pathway on caffeine-induced strengthening of intercortical connections and tried to confirm the role of oscillation on intercortical strengthening by focusing on the cAMP pathway. After induction of parietal oscillation by insular cortex stimulation in caffeine-containing medium, application of membrane-permeable cAMP analog, bromo-cAMP, diminished oscillatory signal delivery from the parietal cortex, but initial insulo-parietal signal propagation remained strong. When oscillatory activities were reduced with co-application of caffeine and bromo-cAMP from the beginning, initial insulo-parietal propagation was established, but amplitudes of propagating wavelets and propagating velocity were reduced. Thus, cAMP-dependent diminution of caffeine-induced NMDA-receptor-dependent oscillatory signal delivery causes attenuation of intercortical strengthening of horizontal pathways between insular and parietal cortices. This finding suggests that the intracellular cAMP signaling pathway has the ability to regulate extracellular communications at the network level, and also that full expression of strengthened intercortical signal communication requires sufficient NMDA-receptor-dependent oscillatory neural activities.

1. Introduction

In general, one of the roles of intracellular signaling pathways is to regulate extracellular signal communication (Bhella and Iyengar, 1999; Butcher and Sutherland, 1962). Such signal regulation enables cells to make feedback or feedforward loops for cellular interaction and communication. Cyclic AMP (cAMP) is a critical intracellular second messenger, and the

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cAMP signaling pathway modulates various biological functions (Butcher and Sutherland, 1962; Cho et al., 2002; Crump et al., 2001; Frey et al., 1993; Mao and Wang, 2002; Renden and Broadie, 2003; Wang et al., 1999). In the brain, neurotransmitters and receptors dominantly intermediate extracellular signal communication at the synapses, and the cAMP signaling pathway largely operates at the synapses. The cAMP signaling pathway may thus have the ability to regulate extracellular signal communication in the brain via modulation of synaptic activities (Evans and Morgan, 2003; Ma et al., 1999; Thakur et al., 2004; Yoshimura and Kato, 2000).

Caffeine displays several pharmacological actions and can invade the synapses in the brain (Fredholm et al., 1999; Nehlig and Boyet, 2000). We recently demonstrated that caffeine enhances intercortical neural activities between primary and secondary visual cortices (Yoshimura et al., 2003a, 2005b). In those studies, we reported that intercortical horizontal connections are functionally opened and strengthened by caffeine-induced α-range voltage oscillations. The oscillatory signal deliveries that activate N-methyl-D-aspartate (NMDA) receptor components to surrounding areas cause strengthening of functional neural connections (Yoshimura et al., 2003a, 2005a,b). One important action of caffeine is thus the opening and strengthening of intercortical signal communications by way of driving NMDA-receptor-dependent neural oscillators.

Since synapses are major sites where both the cAMP signaling pathway and caffeine operate and since caffeine has the ability to affect the cAMP signaling pathway (Beavo and Reifsnyder, 1990; Butcher and Sutherland, 1962; Fredholm et al., 1999), increased intracellular cAMP level by pharmacological manipulation in addition to application of caffeine might induce up- or down-regulation of synaptic activities. We have focused attention on areas between the gustatory insular cortex where chemosensory information is processed and the parietal oral somatosensory cortex where somatosensory information is processed (Hanamori et al., 1999; Katz et al., 2001; Kosar et al., 1986; Ogawa and Wang, 2002; Paxinos

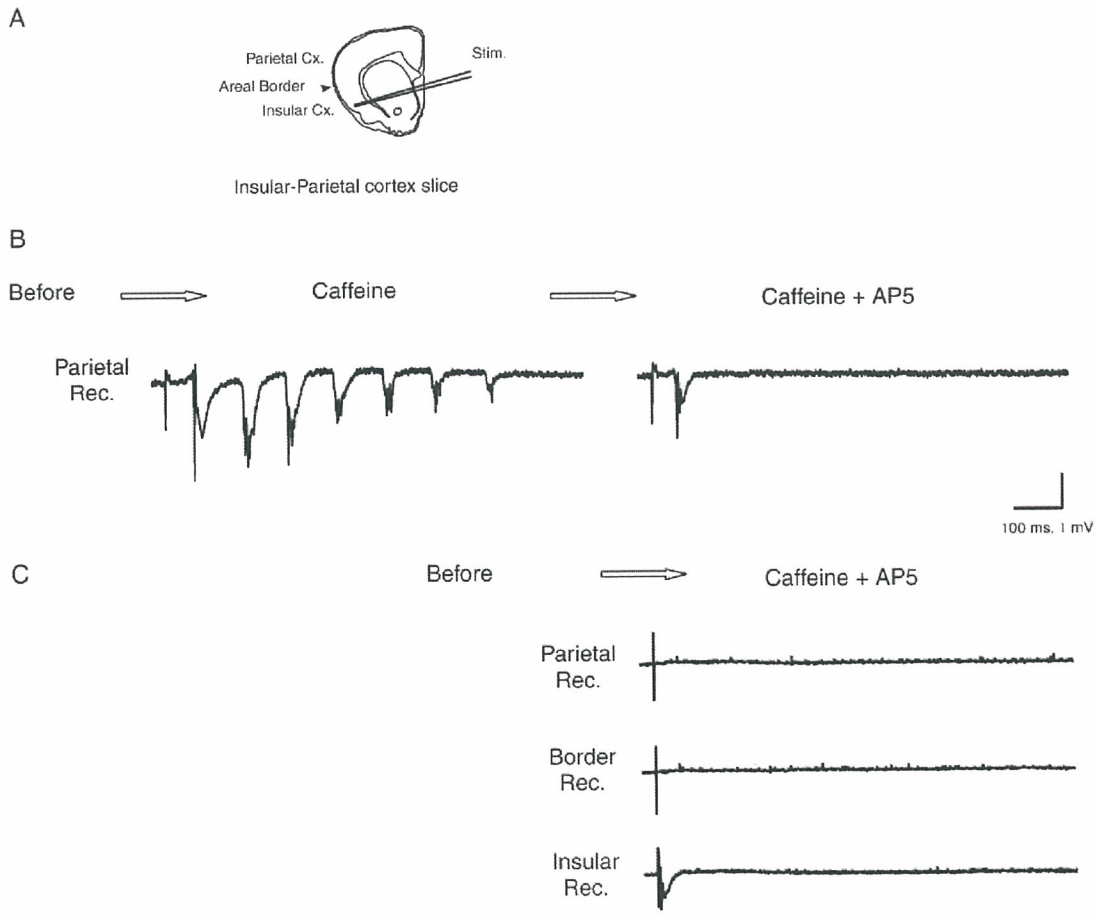

Fig. 1 – Comparing insulo-parietal signal propagation under caffeine application and NMDA receptor blockade after establishing voltage oscillation in the parietal cortex with propagation under application of caffeine and NMDA receptor blockade from the beginning. (A) Brain slices including insular and parietal cortices and electrical stimulation site. Low-frequency stimulation was delivered to the insular cortex, and field potentials were recorded from layer II/III in the insular cortex, parietal cortex and areal border. (B) After induction of parietal oscillation in caffeine-containing medium, D-AP5, an NMDA receptor blocker, was added. Note that initial insulo-parietal component remained. (C) Caffeine and D-AP5 co-applied to medium from the beginning. Note that initial component did not penetrate the parietal cortex.

et al., 1997; Remple et al., 2003; Shi and Cassell, 1998; Swanson et al., 1992; Yamamoto et al., 1981; Yoshimura et al., 2004b; Zilles and Wree, 1995) We recently reported that insulo-parietal neural connections are functionally strengthened after oscillatory activities in the parietal cortex (Yoshimura et al., 2003b, 2004a). The present study investigated how intracellular cAMP elevation affects opening and strengthening of insulo-parietal connections to examine whether cAMP pathways have the ability to regulate extracellular signal communications at the intercortical network level. In addition, we tried to verify that NMDA-receptor-dependent oscillatory activities cause strengthening of intercortical signal communications by focusing on the intracellular cAMP signaling pathway.

2. Results

Low-frequency intracortical electrical stimulation was delivered to layer IV in the gustatory insular cortex, and field potentials were recorded from layer II/III in the parietal cortex (Fig. 1A). In normal medium, a small solitary potential was observed only in the gustatory insular cortex and did not propagate horizontally toward the parietal cortex (24/24 slices, not shown). After application of caffeine into the medium, the evoked potential was enlarged, and the potential propagated from the insular cortex to the parietal cortex. However, marked oscillation was only generated in the parietal cortex (22/24 slices). These propagation and oscillation have been precisely described in our previous report, and histological identification of the stimulation and recording sites has also been demonstrated (Yoshimura et al., 2003b, 2004a). The same kinds of propagation and oscillation are induced in both the insulo-parietal cortex and primary–secondary visual cortex (Yoshimura et al., 2003a, 2005b). We have repetitively reported that later oscillatory components are entirely NMDA-receptor-dependent in both the parietal and visual cortices (Yoshimura et al., 2003a,b, 2004a, 2005b). The present findings again confirmed this. Oscillation in the parietal cortex was induced in caffeine-containing medium after low-frequency stimulation to the insular cortex. After oscillation in the parietal cortex stabilized, D-2-amino-5-phosphonovaleric acid (D-AP5), a blocker of NMDA receptor, was applied to the medium. Approximately 30 min later, the insulo-parietal signal propagation remained, but oscillatory activities in the parietal cortex were depressed (Fig. 1B; "Caffeine + AP5"; 12/12 slices). The remaining initial response was non-NMDA-receptor-dependent since additional application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a blocker of non-NMDA receptor, completely depressed the initial response (15/15 slices, not shown).

In the visual cortex, co-application of caffeine and D-AP5 from the beginning abolishes not only induction of oscillatory signal deliveries from the secondary visual cortex, but also

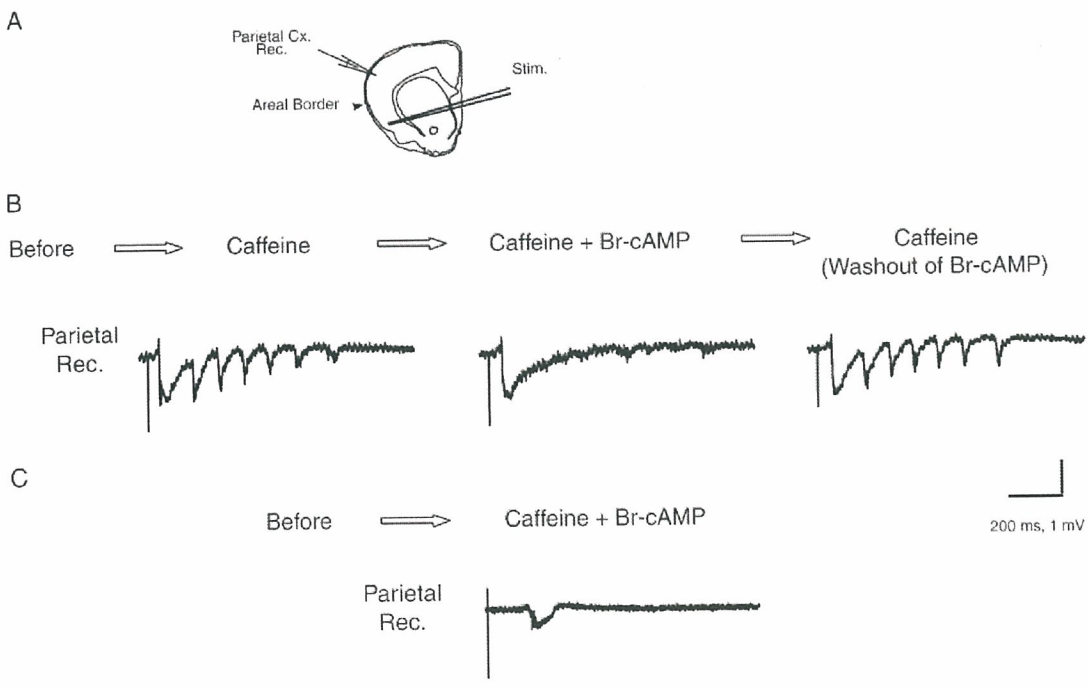

Fig. 2 – Comparing effects of bath application of bromo-cAMP on responses in parietal cortex evoked by insular cortex stimulation under co-application of caffeine and bromo-cAMP after caffeine conditioning with effects under co-application of caffeine and bromo-cAMP from the beginning. (A) Location of stimulation and recording electrodes. (B) After inducing marked parietal oscillations in caffeine-containing medium, bromo-cAMP was added to the medium. When oscillatory phases were depressed, bromo-cAMP was washed out from medium. (C) Bromo-cAMP co-applied with caffeine from the beginning. Note that induction of oscillatory activities was depressed, but depression of oscillatory activities was incomplete, and initial response with smaller amplitude and longer latency was observed.