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Dopamine D_1 Agonist Facilitates Long-term Depression Induction by Callosal Low-frequency Stimulation in Rat Anterior Cingulate Cortex

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ABSTRACT

Dopamine is known to play roles in the processing of emotion. To test whether dopamine has effects on synaptic plasticity in emotion-processing areas, I examined callosally-evoked field potentials in coronal slices of rat anterior cingulate cortex（ACC）when low-frequency stimulation（LFS）was applied to corpus callosum as a conditioning stimulus. Neither dopamine nor the D_2 agonist, quinpirole, had a significant effect on synaptic plasticity, while the D_1 agonist, SKF-38393, facilitated induction of long-term depression（LTD）. This facilitative effect of SKF-38393 was completely blocked by the D_1 antagonist, SCH-23390. LFS-induced LTD was not blocked by application of the metabotropic glutamatergic receptor antagonist, MCPG or the voltage-gated calcium channel blocker, nifedipine, but was blocked by application of the *N*-methyl-D-aspartate receptor（NMDAR）antagonist, APV. The facilitative effect of SKF-38393 on LTD induction was not mimicked by forskolin, an adenylcyclase activator, but was partially mimicked by phorbol 12, 13-didecanoate, which is known to activate the intracellular PKC pathway. These findings suggest that LTD in ACC is induced via NMDAR and facilitated by D_1 agonists at least in part via the PKC-related intracellular pathway. This type of facilitation of LTD induction may be related to the pathogenesis of schizophrenia or major depression, in which frontal lobe hypofunction has been indicated.

Key words: Dopamine, D_1 agonist, low-frequency stimulation, LTD, anterior cingulate cortex

INTRODUCTION

Emotional behavior is determined genetically, environmentally, or by interactive effects of the two. Most behaviors of animals, including humans, are basically dependent on reward and punishment systems, and behavior patterns are gradually developed through rewarding and/or punishing experiences. These experiences modulate synaptic transmission and thereby remodel neuronal networks. However, the same experience does not necessarily have the same emotional value in all conditions; for example, food will be greatly rewarding when given during starvation but not when given to a well-fed animal. Emotional experience is thus modulated by the emotional state at a given moment.

Anterior cingulate cortex（ACC）participates in higher cognitive functions including working memory, error detection, performance monitoring, and decision-making, and in addition plays roles in emotional information processing[5–9, 13]. The ACC receives several types of emotion-related information through dopaminergic inputs from the ventral tegmental area, noradrenergic inputs from locus ceruleus, and serotonergic inputs from the raphe nuclei. ACC activity thus varies depending on emotional state.

The corpus callosum is composed of commissural fibers, which for the most part connect homotopic regions of the two hemispheres[15, 19], and thereby contributes to interhemispheric communication and synchronization of activity on the two sides of the brain. Callosal connections may contribute to regulation of general ACC activity via plastic changes in synaptic efficacy. Dopamine is known to be released in response to rewarding experiences or the expectancy of reward, and may thus have modulatory effects on synaptic plasticity when conditioning stimuli are applied to the corpus callosum.

Although the effects of dopamine on synaptic plasticity in emotion-processing areas such as the amygdala, hippocampus, prefrontal cortex, and ACC have been vigorously studied with application of several stimuli to various sites, those in ACC with stimulation of the corpus callosum have not been sufficiently examined. I therefore examined the effects of dopamine and dopamine receptor-related agents on synaptic plasticity in the ACC using callosal low-frequency stimulation, and found very frequent induction of long-term depression（LTD）in the presence of D_1 agonist. Which receptors are involved in induction of

LTD was then examined. Furthermore, single bath applications of forskolin and phorbol 12, 13-didecanoate (PDD) were performed to determine which intracellular pathway is involved in facilitatory modulation of induction of LTD.

METHODS

Coronal slices (400μm thick) of ACC were prepared from Sprague-Dawley rats at 20–30 days postnatally under deep anesthesia with isoflurane and maintained in an interface-type chamber perfused with an artificial cerebrospinal fluid (ACSF; in mM: 126 NaCl, 3 KCl, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 1.2 NaH_2PO_4, 26 $NaHCO_3$, and 10 glucose at 33℃). All experimental procedures were approved by the Animal Care Committee, Research Institute of Environmental Medicine, Nagoya University.

Extracellular field potentials evoked by callosal stimulation were recorded from layers 5/6 using glass microelectrodes filled with saline containing 2% pontamine sky blue, which was used to mark the recording sites. A pair of bipolar stimulating electrodes separated from each other by ~0.7 mm were placed in the dorsal central region of the corpus callosum. Test stimulation was applied to the electrodes at intervals of 10s. As a conditioning stimulation to induce plastic synaptic changes, low-frequency 2 Hz stimuli were delivered for 15 min (LFS; low-frequency stimulation). The intensity of the test stimulation was adjusted to that eliciting about one-half the maximal response and the same intensity was used for conditioning stimulation. Dopamine receptor-associated agents and other chemical substances were applied by bath perfusion. Dopamine was perfused in the presence of 0.1 mg/ml ascorbic acid (an antioxidant) to avoid deactivation.

Data were normalized to the prestimulus mean value for the 5-minute period just preceding LFS onset and expressed as means ± SE. One-way ANOVA was applied to mean values from 36 to 45 min after LFS. To clarify the effects of dopamine receptor-associated agents, multiple comparison tests (Fisher's PLSD) were performed for post hoc analysis. Statistical analysis was performed on a personal computer using Statview Ver.5.0 (HULINKS), and differences with a probability value of less than 0.05 were considered significant. The compounds used were obtained from the following sources: DL-2-amino-5-phosphonovaleric acid (APV), 6, 7-dinitroquinoxaline-2, 3-dione (DNQX), and (RS)-α-methyl-4-carboxyphenylglycine (MCPG) from Tocris (Bristol, UK); (±)-SKF-38393, (−)-quinpirole hydrochloride, (±)-sulpiride, R-(+)-SCH-23390, and forskolin from Sigma (St. Louis, MO); 3-hydroxytyramine (dopamine) and nifedipine from Research Biochemical International (Natick, MA); phorbol 12, 13-didecanoate (PDD) from Wako (Osaka, Japan); and isoflurane from Abbott Laboratories (North Chicago, IL).

RESULTS

Field potentials recorded from layers 5/6 in response to stimulation of corpus callosum in ACC slices prepared from developing rats at 20–30 days postnatally were examined. These field potentials were considered to be assemblies of monosynaptic EPSPs, since it has been shown that callosal stimulation evokes exclusively monosynaptic EPSPs in ACC[20]. The potentials were evoked by glutamatergic transmission, since they were completely abolished by combined perfusion of 40 μM DNQX (AMPA receptor antagonist) and 100 μM APV (NMDA receptor antagonist).

D_1/D_5 agonist facilitates LTD induction

To determine whether dopamine contributes to modulation of plastic change in synaptic transmission in ACC, single bath applications of dopamine, D_1 family agonists, or D_2 family agonists, and combined bath applications of dopamine with D_1 family antagonists or D_2 family antagonists were performed. The average trends in field potentials when LFS was applied to corpus callosum under the various pharmacological conditions noted above are shown in Fig. 1. Although LFS itself rarely induced LTD (control), 30 μM SKF-38393 (D_1/D_5 agonist) clearly facilitated induction of LTD, while 50 μM quinpirole (D_2/D_3 agonist) and 100 μM dopamine (DA) exhibited no facilitation of induction of LTD. Combined application of 100 μM sulpiride (D_2/D_3 antagonist) or 10 μM SCH-23390 (D_1/D_5 antagonist) with 100 μM DA exhibited no facilitative effects on induction of LTD, even though co-application of D_2/D_3 antagonists with DA would be expected to have effects similar to D_1/D_5 agonists.

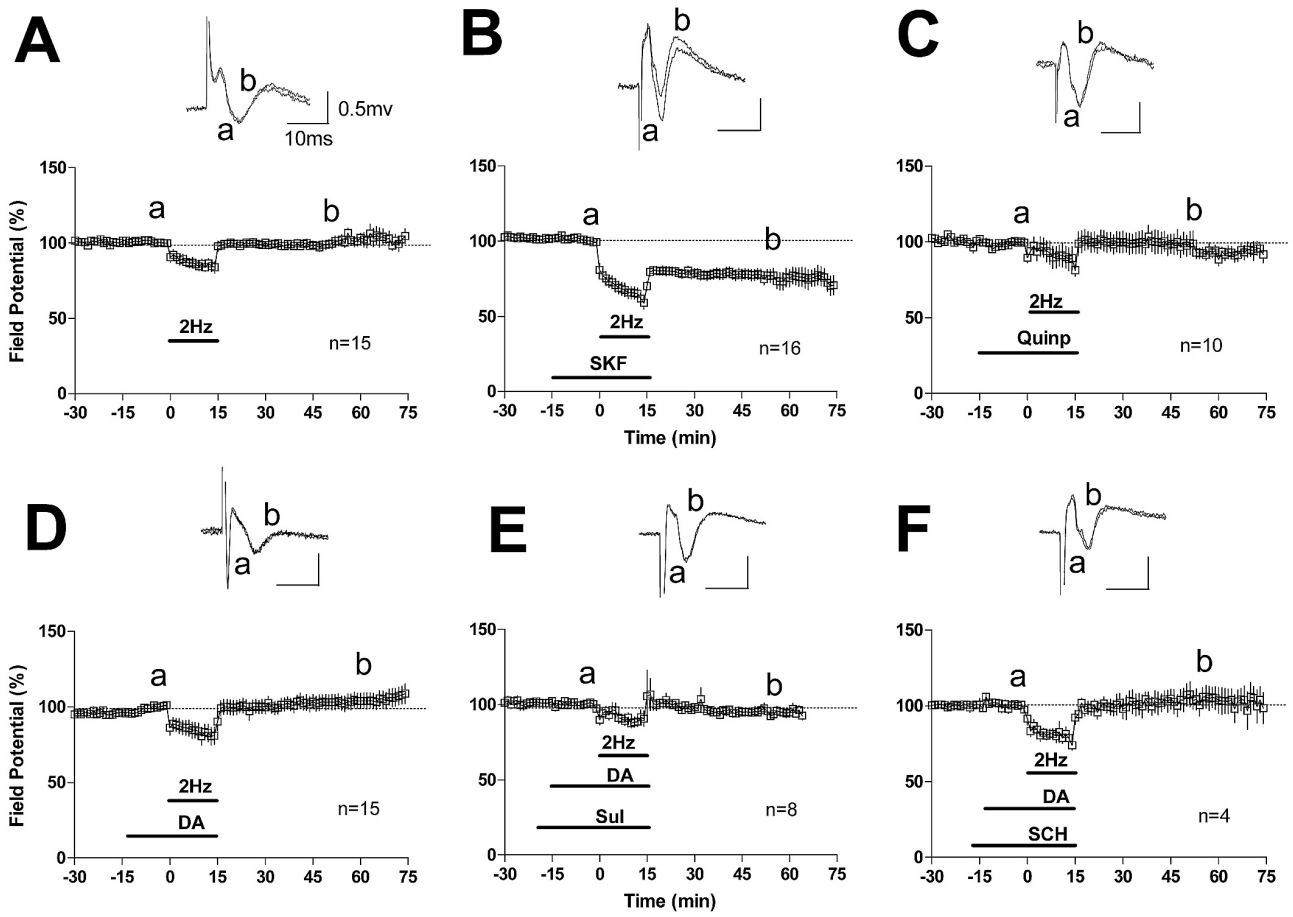

Fig. 1 Effects of dopamine and related substances on field potentials in anterior cingulate cortex during 2 Hz stimulation for 15min (LFS; low-frequency stimulation) of corpus callosum. A: control recording (with no active substance perfusion). B, C, D: D_1/D_5 agonist (SKF 38393), D_2/D_3 agonist (quinpirole), and dopamine (DA) were applied about 15min before onset of LFS, respectively. E, F: D_2/D_3 antagonist (sulpiride) and D_1/D_5 antagonist (SCH-23390) were co-applied about 5 min before DA perfusion. The application period is indicated by the horizontal bar in each figure. Superimposed traces on top of each figure represent sample traces before (a) and 36–45min after (b) LFS. Note that only SKF perfusion facilitated induction of LTD.

Figure 4 shows average field potential magnitudes assessed at 36–45 min after LFS under various pharmacological conditions. One-way analysis of variance (one-way ANOVA) revealed significant effects of pharmacological condition ($p = 0.0002$). Post hoc comparison with Fisher's PLSD revealed a significant difference between control and SKF condition, clearly indicating a facilitative effect of SKF-38393 on induction of LTD.

LTD induction requires NMDA receptor activation

To clarify which receptors contribute to the induction of LTD in ACC, bath applications of 100 μM APV (NMDA receptor antagonist), 500μM MCPG (metabotropic glutamatergic receptor antagonist: mGluR antagonist), and 20μM nifedipine (voltage-gated Ca^{2+} channel blocker: VGCC blocker) were combined with application of 30 μM SKF-38393. In addition, 10 μM SCH23390 (D_1/D_5 antagonist) was applied with 30μM SKF-38393 to confirm the involvement of D_1 family receptors in facilitation of LTD induction. Figure 2 shows the average trends in field potentials when LFS was applied to corpus callosum under the several pharmacological conditions noted above.

One-way ANOVA (Fig. 4) revealed significant effects of pharmacological condition ($p = 0.0001$). Post hoc comparison with Fisher's PLSD revealed a significant difference between SKF and SCH + SKF conditions, indicating that the effect of SKF was completely blocked by prior application of SCH − 23390. Post hoc comparison revealed a significant difference between SKF and APV + SKF conditions, but no significant difference between SKF and MCPG + SKF or nifedipine + SKF conditions, indicating that this type of LTD was not induced while APV was coapplied with SKF, but was induced with co-application of MCPG or nifedipine.

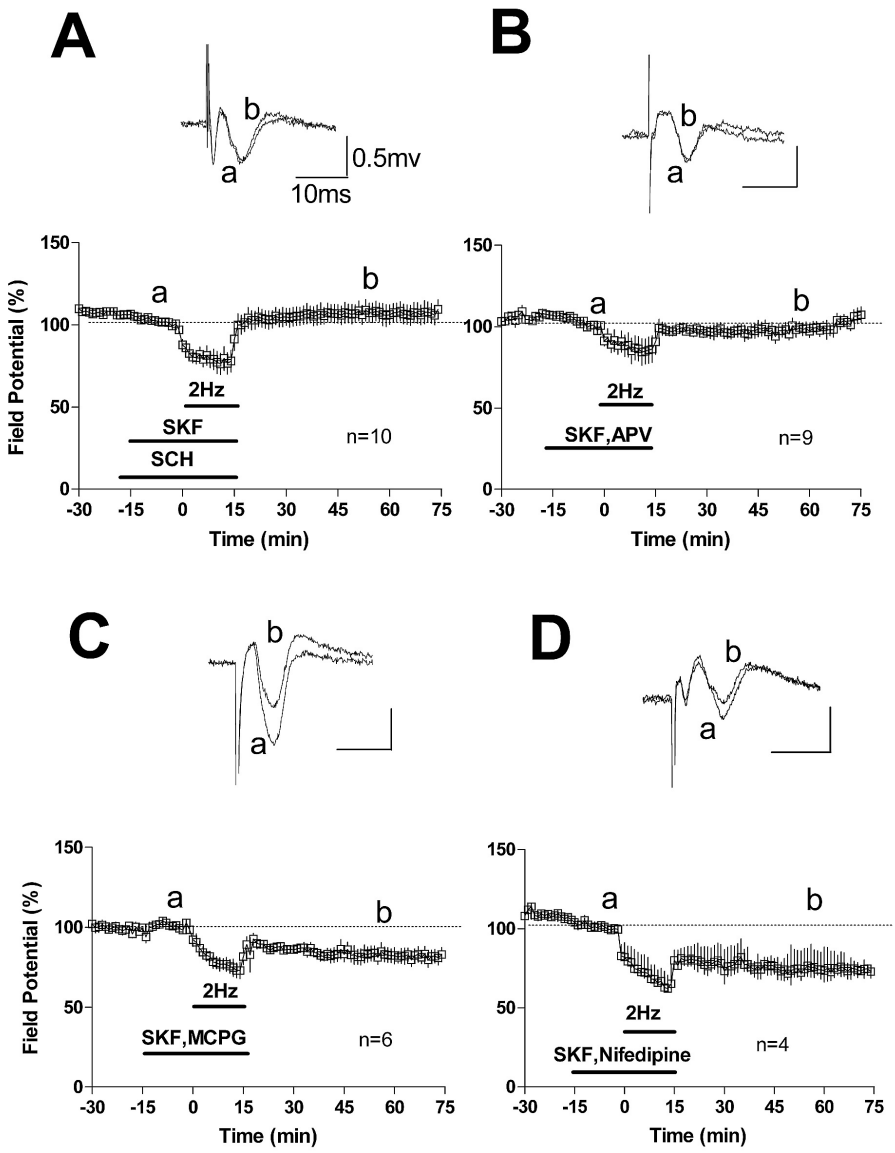

Fig. 2 Effects of co-application of D_1 antagonist, NMDA, MGluR, and VGCC blockers with SKF on field potentials in anterior cingulate cortex during LFS of corpus callosum. A: D_1 / D_5 antagonist (SCH-23390) was applied about 5 min before SKF perfusion. B, C and D: NMDA receptor blocker (APV), MGluR blocker (MCPG), and VGCC blocker (nifedipine) were co-applied with SKF, respectively. The application period is indicated by the horizontal bar in each figure. Superimposed traces on top of each figure represent sample traces before (a) and 36–45min after (b) LFS. Note that the facilitative effect of SKF perfusion on LTD induction was blocked by SCH and APV, but not by MCPG or nifedipine.

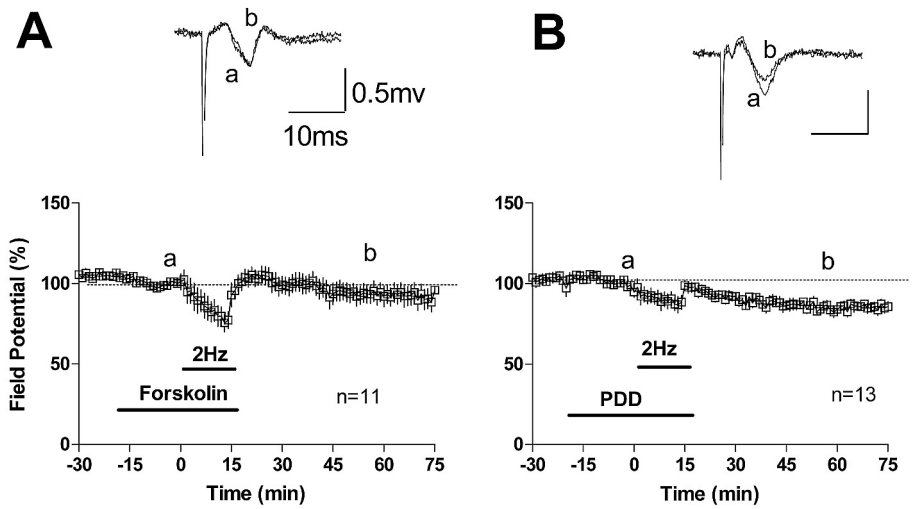

Fig. 3 Effects of PKA and PKC activators on field potentials in anterior cingulate cortex during 2 Hz stimulation for 15 min (LFS; low-frequency stimulation) of corpus callosum. A and B: PKA activator (Forskolin) and PKC activator (PDD) were applied about 15min before onset of LFS, respectively. Superimposed traces on top of each figure represent sample traces before (a) and 36–45min after (b) LFS. Note that PDD perfusion facilitated induction of LTD.

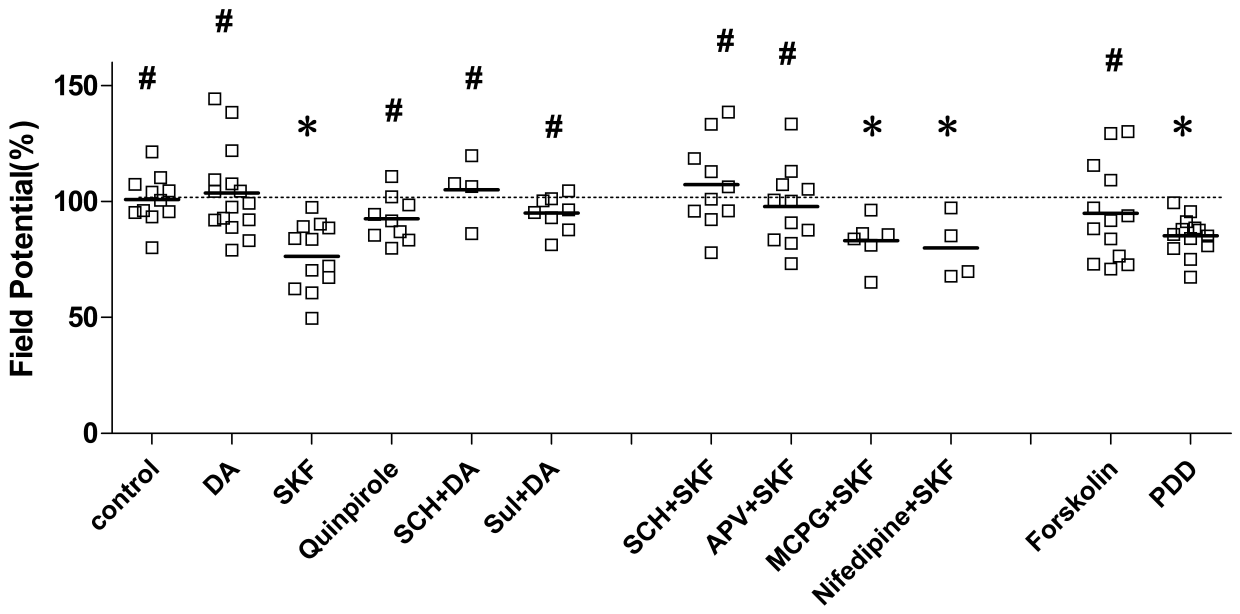

Fig. 4 Field potential magnitude assessed at 36–45min after LFS for control, dopamine (DA), SKF, Quinpirole, SCH + DA, sulpiride (Sul) + DA, SCH + SKF, APV + SKF, MCPG + SKF, Nifedipine + SKF, Forskolin, and PDD conditions. The asterisk (*) and hash marks (#) indicate that values were significantly different from those for control and SKF, respectively.

PKC pathway contributes to facilitation of LTD induction

D_1/D_5 receptors are coupled to G-protein and are believed to activate adenylcyclase, and thus increase generation of cAMP. In addition, D_1/D_5 receptors are believed to activate the PKC pathway[18]. Therefore, to clarify which intracellular signaling pathways contribute to the facilitation of induction of LTD in ACC, I applied 100 μM forskolin (adenylcyclase activator), which increases cytoplasmic cAMP concentration, and 20 μM PDD (intracellular PKC pathway activator) with LFS. Figure 3 shows the average trends in field potentials when LFS was applied to corpus callosum under the two conditions noted above. Although forskolin exhibited no clear facilitative effect on induction of LTD, PDD did facilitate LTD induction. Post hoc comparison revealed no significant difference between the control and forskolin conditions, but did reveal a significant difference between control and PDD conditions, indicating that this type of induction of LTD was not facilitated by forskolin, but was facilitated by PDD.

DISCUSSION

In the present study, it was found that SKF-38393 (D_1/D_5 agonist) clearly facilitates induction of LTD in rat ACC, and this facilitation was eliminated by SCH-23390 (D_1/D_5 antagonist). In contrast, neither dopamine nor quinpirole, a selective D_2-like dopamine receptor agonist with some selectivity for D_3 sites, had significant modulatory effects on the plastic changes in synaptic efficacy induced by application of LFS to corpus callosum. It is unclear why combined application of sulpiride (D_2/D_3 antagonist) and DA did not facilitate induction of LTD, even though SKF-38393 (D_1/D_5 agonist) clearly facilitated it. However, it is likely that sulpiride hardly binds to dopamine D_4 receptors, which results in little suppression of the D_4 receptors, and activation of the D_4 receptors by DA may have played some role in the process of modulation of plastic changes in synaptic efficacy. Intense immunostaining of D_4 receptors was found in pyramidal neurons of the frontal cortex in rodents[3]. D_4 receptors might thus have opposed the effects of D_1/D_5 receptor stimulation and have confounded the findings obtained.

D_1 receptor family members are believed to activate adenylcyclase, which in turn increases cytoplasmic cAMP, and to exhibit effects on subsequent intracellular signal transmission. Facilitation of induction of LTD was not mimicked by forskolin, which also increases cytoplasmic cAMP, while facilitation was at least partially mimicked by PDD, which activates the intracellular PKC pathway. Recently, a D_1 receptor-interacting protein, Calcyon, has been discovered, which can couple with the D_1 receptor to the phospholipase C pathway to activate PKC[4, 14]. The D_1-Calcyon-phospholipase C pathway may thus contribute to facilitation of induction of LTD. D_1 receptors are known to be located in close proximity to dendritic Ca^{2+} channels and to modulate Ca^{2+} potential[17, 21, 22]. Ca^{2+} channels might thus contribute to facilitation of induction of LTD, though in the present study antagonists of Ca^{2+} channels could not be used since these channels should affect transmitter release.

Although the biological significance of callosally-induced LTD is unclear, it may dampen noisy information irrelevant to ongoing tasks or contribute to generalized cortical hypofunction.

This type of facilitation of induction of LTD would rarely occur in a physiological condition, since D_1/D_5 agonists do not naturally exist in vivo, however, similar conditions may occur when extreme predominance of D_1 receptor distribution in ACC is combined with high DA release. The D_1/D_2 receptor distribution ratio in this region may be determined genetically or by interaction between genetic factors and emotional experiences. Persistent low-intensity activation, similar to the LFS administered in the present study, may facilitate induction of LTD when activation occurs during periods of high DA concentration in ACC, if D_1 receptor distribution is extremely predominant in this region. Yang et al. hypothesized that dysfunction of the mesocortical dopamine system may lead to abnormal modulation of prefrontal cortical neurons, which may account for some symptoms in schizophrenic or other types of psychotic patients. Rostral ACC controls stimuli relevant to unexpected fear, and low rostral ACC activity consequently enhances anxiety[5]. Facilitation of LTD induction might thus contribute to the pathogenesis of schizophrenia, major depression, or other types of psychosis involving frontal lobe hypo-or dysfunction[10, 11, 16]. D_1 receptor density in PFC has been reported to be directly correlated to the severity of negative symptoms in schizophrenia[1, 2, 12]. Therefore, extreme predominance of D_1 receptors in this region might be associated with vulnerability to the psychotic diseases, persistent low-frequency stimulation during high DA release might correspond to the effects of certain environmental factors, and the facilitation of induction of LTD observed in this study might thus be responsible for the pathogenesis of schizophrenia or other types of psychosis. The right ACC has been implicated in processing of happiness and anger[8]. Dopamine is known to be released in response to reward or expectation of reward. Then, it is possible to speculate that when strong reward (which would correspond to high DA release) is followed by unpleasant stimuli, such as bullying or ill treatment (although it is uncertain which type of stimuli actually corresponds to LFS, unpleasant stimuli might correspond to the LFS in the present study), or high expectation of reward (which would also correspond to high DA release) is not met or results in disappointment (which might correspond to the LFS in the present study) in a vulnerable individual (whose D_1 distribution in ACC is extremely predominant), these situations might cause the facilitation of LTD induction, which might in turn play some role in the induction of psychoses.

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