主論文の要旨

Cell type-specific activation of mitogen-activated protein kinase in D1 receptor-expressing neurons of the nucleus accumbens potentiates stimulus-reward learning in mice

（側坐核Ｄ１受容体発現神経細胞における分裂促進因子活性化
タンパク質キナーゼの細胞種特異的活性化は
マウスの刺激–報酬学習を促進する）

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【Introduction】

Actions and responses to reward-associated stimuli are essential components in daily life. It is well known that dopamine (DA) signaling plays a crucial role in this type of learning, and dysfunctions in dopaminergic neurons can lead to psychiatric disorders including depression, schizophrenia, parkinsonism and other disorders. Among the brain regions involved in the reward circuit, the nucleus accumbens (NAc) is of particular interest because of its involvement in the affective and motivational aspects of behavior. Most neurons in the NAc are inhibitory GABAergic medium spiny neurons (MSNs), which have been separated into two populations, DA D1 receptor (D1R)- and DA D2 receptor (D2R)-expressing MSNs. One of the common signaling pathways that are modulated by D1Rs and D2Rs is mitogen-activated protein kinase (MAPK) signaling. Although the role of dopaminergic receptors and MAPK signaling in food reward-associated instrumental tasks have been studied, the precise effects of the cell type-specific activation of MAPK signaling in the NAc on Pavlovian and instrumental conditioning have not yet been clarified. In the present study, I investigated the cell type-specific role of MAPK signaling in accumbal D1R-MSNs and D2R-MSNs in natural reward-associated learning and memory as well as the motivation for rewards. I also investigated their roles in methamphetamine (METH)-associated conditioned place preference (CPP).

【Materials and methods】

Animals

Drd1a-Cre, Drd2-Cre, Drd1a-YFP, Drd2-YFP and C57BL/6 male mice were used in this study. In food reward-associated behavioral experiments, mice were restricted food to achieve 85% of their ad libitum bodyweight.

【Methods】

Stereotaxic injection of AAVs

AAV viral vector containing wild-type, constitutively active mutant MAP2K1 or control vectors were injected into the NAc of both *Drd1a-Cre* and *Drd2-Cre* mice. Three weeks after the surgery all the behavioral analysis was performed.

Immunohistochemistry

Brain sections (25 μm) were collected at different stages of experimental schedule and were immunostained with appropriate antibodies to detect pMAPK1/3, total MAPK1/3, GFP and RFP. Confocal microscopic images were analyzed using Metamorph software.

Pavlovian conditioning

Mice were trained in Pavlovian conditioning for 7 days. 20 mg food pallet was given after 10 s conditioned stimulus (CS) presentation followed by 50 s intertrial interval (ITI) period. Each day mice received 25 trials. Head entry for food during both the periods was recorded.

Instrumental conditioning

Pavlovian conditioning was followed by the fixed ratio 1 (FR1) and FR2 fixed side nose-poking task and then by the FR2 random nose-poking task to receive a single food pellet. Criteria to pass each step was 75% correct response for three consecutive sessions. Subsequently, mice were subjected to a single progressive ratio nose poking session to determine motivation.

CPP

Two chambered CPP box was used. Mice were given 2 mg/kg METH (i.p.) in one of the boxes and saline in the other one for 3 days. In the pre-test and test session, mice were allowed to explore both the boxes without any drug treatment and CPP score was calculated from these two sessions.

【Results】

In order to analyze the involvement of MAPK signaling within the NAc in natural reward-associated learning, we trained wild-type C57BL/6 mice in food reward-associated Pavlovian conditioning (Fig. 1a). Head entry rate during conditioned stimulus (CS) presentation increased significantly after 7-days of training only in the paired CS-unconditioned stimulus (US) group (Fig. 1b). Head entry rate during inter-trial interval (ITI) period was not significantly different between groups (Fig. 1c). On day 7, the number of pMAPK1/3-positive cells in the paired CS-US group was markedly increased as compared to the respective cell number in the same group on day 1 and in the unpaired CS-US group on day 7 (Fig. 1d, e). The number of total MAPK1/3-positive cells was not different between two groups on either day 1 or day 7 (Fig. 1f, g).

To further clarify which subtype of MSN is involved in MAPK activation in the NAc core after the training session, both *Drd1a-YFP* and *Drd2-YFP* mice expressing a variant of YFP under the control of D1R promoter or D2R promoter were used for Pavlovian conditioning. After the 7^{th} day training session, no significant differences were observed in the total number of YFP- and pMAPK1/3-positive cells in the NAc core between *Drd1a-YFP* and *Drd2-YFP* mice (2a-c). However, the number and percentage of cells co-expressing pMAPK1/3 and YFP in the NAc core was significantly higher in *Drd1a-YFP* mice than in *Drd2-YFP* mice (Fig. 2d, e).

Activity of MAPK signaling was assessed after genetic manipulation in the NAc of *Drd1a-Cre* mice (Fig. 3a-d). Constitutively active MAP2K1 (caMAP2K1) gene significantly potentiated pMAPK1/3 expression both in the presence and absence of acute dose of METH injection (Fig. 3c, d). Similar effect on MAPK signaling was observed after genetic manipulation in the NAc of *Drd2-Cre* mice (Fig. 3e-h).

I then investigated natural reward-associated learning in Pavlovian conditioning. The learning curve only in caMAP2K1-transfected *Drd1a-Cre* mice significantly shifted to the left (Fig. 4a, b). Analysis of the behavioral subtype of conditional responses in the last conditioning

session reveled that only caMAP2K1-transfected *Drd1a-Cre* mice had a significant increase in sign-tracking, but not goal-tracking, behaviors (Fig. 4c, d). However, overexpression of mutant MAP2K1 gene in the NAc of *Drd1a-Cre* and *Drd2-Cre* mice failed to influence instrumental learning and performance although motivation for food reward was increased in both the groups (Fig. 5a, b). Finally, effects of MAPK signaling on drug reward-associated behavioral changes were analyzed using METH-induced CPP. Activation of MAPK signaling in accumbal D1R-MSNs facilitated METH-induced CPP while the activation in D2R-MSNs has little effects.

【Discussion】

In the present study, I investigated the role of MAPK in accumbal MSNs in reward-associated learning and memory. AAV mediated gene delivery enabled to manipulate MAPK signaling pathway exclusively in either D1R or D2R-MSNs of the NAc. D1R-MSNs showed to be more involved in reward-associated activation of MAPK signaling. Overexpression of MAPK signaling in the D1R-MSNs facilitated reward-associated learning and memory. MAPK signaling in neither D1R nor D2R-MSNs of the NAc was found to be involved in instrumental learning and performance while both cell types were involved in motivation. A previous study reported that the repeated presentation of a reward-associated cue increased the phasic DA release in the NAc during Pavlovian conditioning. It has been shown that repeated training for natural reward increases intracellular and surface expression of glutamatergic receptors in the NAc. Concomitant activation of D1Rs and NMDARs causes DARPP-32 and MAPK phosphorylation and expression of long-term potentiation. In our previous study it has been proposed that phosphorylation of MAPK1/3 after phasic dopamine release increases the excitability of D1R-MSNs and subsequently increases their response to excitatory glutamatergic signals which in turn facilitates further phosphorylation of MAPK1/3. In the present study it has been demonstrated that increased phosphorylation of MAPK1/3 in the NAc potentiates reward-associated learning and memory.

【Conclusion】

In conclusion, our results indicate that MAPK signaling in the D1R-MSNs of NAc core contributes to reward-associated learning, while this intracellular signaling in D1R- and D2R-MSNs modulates motivation. Taken together with previous studies, our findings imply that therapies that enhance MAPK signaling in the NAc may be successfully applied to psychiatric disorders that involve learning deficits and motivational dysfunction.