主論文の要旨

Phosphorylation of Npas4 by MAPK Regulates Reward-Related Gene Expression and Behaviors

（MAPK による Npas4 のリン酸化は報酬関連の遺伝子発現と行動を制御する）

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【Introduction】

Dopamine (DA) is important for motor function, motivation, working memory, and the reward system (Girault and Greengard, 2004; Phillips et al., 2008). Functional deficits in DA signaling have been implicated in various neuropsychological diseases, including Parkinson's disease, drug addiction, compulsive behavior, autism spectrum disorders, and schizophrenia (Carlsson Carlsson, 2001; Hyman et al., 2006; Iversen and Iversen, 2007; Koob and Volkow, 2010; Swanson et al., 2007). Experiencing reward and using various drugs of abuse, such as cocaine or methamphetamine, activate intracellular pathways in the brain reward system, including the ventral tegmental area (VTA), the nucleus accumbens (NAc), and the prefrontal cortex (Rogge and Wood, 2013). These pathways regulate the expression of genes that are essential for long-lasting forms of synaptic plasticity, memory processes, and drug-induced neuronal and behavioral changes (McClung and Nestler, 2008; Renthal and Nestler, 2008). Despite this knowledge, our current understanding of the mechanisms by which some transcriptional factors (TFs) are stimulated by DA and how they regulate several genes that are important for synaptic plasticity remains incomplete. It is even less well-understood how the changes that occur at the intracellular level manifest in behavioral changes.

The principal target of DA is medium spiny neurons (MSNs), which are a special type of GABAergic inhibitory cell that comprises 90% of the neurons within the striatum, including the NAc. The MSNs in the NAc receive inputs from the dopaminergic neurons of the VTA and the glutamatergic neurons of the hippocampus, amygdala, and medial prefrontal cortex. There are two distinct classes of spatially intermixed MSNs that express dopamine type 1 or 2 receptors (D1R MSNs or D2R-MSNs, respectively; Smith et al., 2013). D1R-MSN and D2R-MSN subpopulations were also originally characterized as direct and indirect pathways in the dorsal striatum. D1R is coupled to adenylate cyclase through Golf (Gs) and activates protein kinase A (PKA), whereas D2R inhibits adenylate cyclase through Gi (Herve´ et al., 1993; Stoof and Kebabian, 1984). We previously found that DA stimulates Rap1 GEF (Rasgrp2) phosphorylation via PKA in D1R-MSNs, thereby activating Rap1, and then Rap1 regulates neuronal excitability and behavioral responses to cocaine reward through mitogen-activated protein kinase 1/3 (MAPK1/3), also known as extracellular-signal-regulated kinase 1/2 (ERK1/2) (Nagai et al., 2016). MAPK1/3 also may phosphorylate several nuclear TFs that are involved in gene expression, long-term synaptic plasticity, and memory formation. However, it remains unclear how MAPK1/3 regulates gene expression and memory formation downstream of the D1R/PKA/Rap1 pathway.

TFs such as c-Fos, FosB, and cyclic AMP response element (CRE) binding protein

(CREB) are activated by DA and mediate the expression of genes that are involved in synaptic plasticity (Hyman and Malenka, 2001; Nestler, 2001; Robinson and Kolb, 1999). A variety of protein kinases, including PKA, RSK, and CaMKII, activate CREB via serine-133 (S133) phosphorylation (Choe and McGinty, 2000, 2001; Dash et al., 1991; Montminy et al., 1990; Sheng et al., 1991; Xing et al., 1996, 1998). The phosphorylation of CREB at S133 recruits CREB-binding protein (CBP) to promote transcription. CBP and its homolog p300 are essential transcriptional coactivators for many TFs (Karamouzis et al., 2007; Vo and Goodman, 2001). CBP/p300 can also act as a scaffold protein, stabilizing the transcription complex by simultaneously binding to other proteins. CBP also regulates transcription during memory and synaptic plasticity (Barrett and Wood, 2008). The deletion of CBP in the NAc correlates with impairments in cocaine sensitivity and reward-related learning and memory (Malvaez et al., 2011; Rogge and Wood, 2013). However, how CBP cooperates with many TFs during reward-related memory processing is not fully understood.

【Methods and Results】

We isolated and concentrated TFs from the mouse striatum using affinity beads coated with CBP, which acts as a transcriptional coactivator. We used a fragment of CBP that we called CBP-N-TAD (Figure 1A) to pull down nuclear proteins. This method combined with a LC-MS/MS analysis identified more than 400 CBP-interacting proteins, including Neuronal Per Arnt Sim domain protein 4, Npas4 (Figure 1B). Using an in vitro kinase assay we found that MAPK phosphorylates Npas4 downstream of PKA, increasing the Npas4-CBP interaction and the transcriptional activity of Npas4 at the brain-derived neurotrophic factor (BDNF) promoter. Because Npas4 and its phosphorylation were required for cocaine-induced CPP, our findings raise the possibility that Npas4 and its phosphorylation regulate BDNF expression to form long-term reward-related memory. It has been reported that the expression of Bdnf is upregulated in the NAc of animals conditioned in a CPP task (Tian et al., 2016) and that the expression of lentivirus-BDNF and TrkB enhanced cocaine-induced CPP (Bahi et al., 2008). In addition to BDNF, Npas4 can regulate the expression of several genes that are important for neuronal survival and synaptic plasticity, such as c-Fos, Zif268, Arc/Arg3.1, and Homer1a (Lin et al., 2008; Pruunsild et al., 2011; Shan et al., 2018; Spiegel et al., 2014; Yoshihara et al., 2014).

We also found that Npas4 was mainly expressed in D1R-MSNs after CPP training. To analyze D1R-specific Npas4 function, Npas4-DN was expressed in the NAc under the control of the D1R or Adora2a promoter using AAV-mediated conditional transgenic techniques (Figure 2 A-B). The rewarding effects of cocaine were markedly potentiated in mice that expressed Npas4-DN in D1R-MSNs, but not in D2R-MSNs, of the NAc (Figure

2C). We also administered AAV-SP-Cre into the NAc of homozygous floxed Npas4 mice (Npas4 fl/fl). The D1R-specific knockout of Npas4 notably diminished cocaine-induced CPP. This deficit in mice with local knockout of Npas4 was restored by the expression of Npas4-WT, but not Npas4-6A, indicating that Npas4 phosphorylation in D1R-MSNs is important for reward-related learning and memory (Figure 2 D-E).

【Conclusions】

In this study we reported that MAPK phosphorylates Npas4 downstream of PKA, increasing the Npas4-CBP interaction and the transcriptional activity of Npas4 at the BDNF promoter. Additionally, the deletion of Npas4 in D1R-expressing MSNs impairs cocaine-induced place preference, which is rescued by Npas4-wild-type, but not by a phospho-deficient Npas4 mutant. These observations suggest that MAPK phosphorylates Npas4 in D1R-MSNs and increases transcriptional activity to enhance reward-related learning and memory.