論文の内容の要旨

論文題目　Cell-type specific detection of transient dopamine signal for structural plasticity in the nucleus accumbens（側坐核形態可塑性のための細胞種別の一過性ドーパミン変化の検出機構）

氏名　飯野　祐介

Introduction

Midbrain dopamine (DA) neurons act as teaching signal in the brain to drive associative learning. DA neurons have a baseline activity (tonic DA) with a firing rate around 5 Hz in the absence of any salient events. If the actual reward is better than expected, midbrain DA neurons show bursting activity (15 ~ 30 Hz) which leads to appetitive associative learning. In contrast, if the actual reward is worse than expected (e.g. omission of expected reward), the firing rate of DA neurons temporarily decrease from the baseline (DA dips), which results in avoidance learning or inhibiting behaviors. DA neurons mainly project to the striatum, including the nucleus accumbens (NAc), where changes in firing rate induce bidirectional transient changes in DA concentration (DA transients).

In the NAc, principal neurons are spiny projection neurons (SPNs), which are classified as either D1R- or D2R-SPNs according to almost dichotomous expression of dopamine D1 and D2 receptors. D2R-SPNs co-express adenosine A2A receptors (A2AR). Bidirectional DA transients are thought to be separately detected by D1R and D2R. D1R is a low affinity receptor, which is activated by high DA concentration evoked by DA burst. On the contrary, D2R is a high affinity receptor which is activated by even low tonic DA concentration, allowing DA dips to deactivate D2R. Gs-coupled D1R and A2AR activates adenylyl cyclase (AC) to increase 3', 5'-cyclic monophosphate (cAMP) and Protein kinase A (PKA), whereas Gi-coupled D2R inhibits AC. A previous study shows transient increase in DA activated somatic PKA in D1R-SPNs, whereas *in silico* studies suggested DA dips activated somatic PKA in D2R-SPNs, although direct experimental evidences are lacking. However, these somatic PKA signal, which facilitate long-term potentiation (LTP), cannot explain specificity of associative learning, because DA signaling in the NAc has low spatial specificity. Then, how DA transients can modulate specific somatosensory signals conveyed by glutamatergic inputs from prefrontal cortex, thalamus, amygdala, and hippocampus?

For DA burst, a previous study shows that in D1R-SPNs, a DA burst alone is insufficient to activate PKA in dendrites due to high phosphodiesterase (PDE) activity. Instead, PKA is activated when action potentials precede a DA burst in dendrites. If glutamatergic input is accompanied, N-methyl-D-aspartate glutamate receptors (NMDAR) allow [Ca2+]i influx. Then Ca2+/calmodulin-dependent protein kinase II (CaMKII) were activated, leading to input-specific structural LTP (sLTP) of dendritic spines. These results suggest D1R-SPNs detect DA burst for sLTP. On the other hand,

cellular and synaptic mechanisms by which DA dips are detected and induce plasticity are unknown. Because D2R in the NAc was necessary for DA-dip dependent avoidance learning, I hypothesized that D2R-SPNs detect DA dips for sLTP.

In this study, I mimicked tonic DA and DA transients in mouse NAc slices using optogenetics, and found D2R-SPNs and D1R-SPNs specifically detected DA dip or DA burst for sLTP. Tonic DA had no effect on sLTP in either SPN. FRET imaging revealed PKA was activated when DA dip or DA burst were coincided with action potentials in the dendrites. Even with tonic DA, phosphodiesterase (PDE)-inhibition resulted in sLTP in both SPNs, suggesting that SPNs were insensitive to tonic DA because of PDE activity. Thus, the regulation of PKA allows the cell-type specific detection of DA bursts and DA dips in the NAc for sLTP, which might be the neural basis for learning by bidirectional DA signals.

Results

First, I explored condition where sLTP in D2R-SPNs occured. D2-SPNs were labeled by AAV vectors which express mCherry specifically in D2R-SPNs. Identified D2R-SPNs were patch-clamped and dendritic spines were imaged by two-photon microscopy. Spike-timing-dependent plasticity (STDP) protocols, which consisted of glutamate uncaging followed by action potentials induced by current injection, was used to induce sLTP. Not STDP alone but STDP with A2AR agonist (CGS21680, 0.3 μM) resulted in sLTP, which was specific to stimulated spines. This sLTP was dependent on NMDAR, CaMKII, and PKA, consistent with D1R-SPNs.

Next, I mimic DA transient in brain slice. To do so, light-activated cation channel, ChR2 was specifically expressed in DA axon terminal. Blue-light induced DA release was measured by amperometry. Using 5 Hz blue light stimulation, stable tonic DA concentration was maintained in slice. Inserting transient pause or burst during 5 Hz continuous stim resulted in transient decrease or increase in DA concentration, respectively, suggesting that optogenetic stimulation of DA terminal can mimic tonic DA and bidirectional DA transients. Optogenetically-mimicked tonic DA inhibited sLTP in D2R-SPNs in D2R dependent manner, suggesting that D2R could counteract with A2AR activation. This sLTP was restored when DA dips (duration of 0.4 ~ 2 s), but not DA burst, was imposed simultaneously with synaptic activity, indicating that D2R-SPNs specifically detect DA dips. FRET imaging revealed protein kinase A (PKA) was activated when DA dips coincided with action potentials in the dendrites.

In D1R-SPNs, DA bursts, but not tonic DA, induced PKA activation and sLTP when the bursts coincided with action potentials. Even with tonic DA, phosphodiesterase (PDE) inhibition provoked sLTP in both SPNs, suggesting that SPNs were insensitive to tonic DA because of PDE.

Conclusion

The regulation of PKA allows the cell-type specific detection of DA bursts and dips in the NAc for sLTP, which might be the neural basis for learning by bidirectional DA signals.