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**MEETING ABSTRACT**

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Phospho-proteomic analysis of the dopamine pathway enables discovery of a novel reward signal in vivo
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Dopamine type 1 receptor (D1R) signaling in the striatum presumably regulates neuronal excitability and reward-related behaviors through PKA. However, whether and how D1R and PKA regulate neuronal excitability and behavior remains largely unknown. To identify PKA substrates that regulate the excitability changes that are associated with rewarding experiences and to more broadly examine PKA substrates, we developed a phospho-proteomic analysis method that uses affinity beads coated with 14-3-3 proteins to enrich phosphorylated proteins. Using this approach, we comprehensively identified PKA substrates downstream of D1Rs in the striatum of mice, and found more than one hundred candidate substrates of PKA, including Rap1 GEF (Rasgrp2). PKA directly phosphorylated Rasgrp2, and PKA-mediated Rasgrp2 phosphorylation enhanced its guanine nucleotide exchange activity on Rap1. Treatment with a single dose of cocaine significantly and dose-dependently increased the level of Rasgrp2 phosphorylation and activated Rap1 in the nucleus accumbens (NAc) of mice. Cocaine-induced phosphorylation of Rasgrp2 was detected in accumbal D1R-expressing medium spiny neurons (D1R-MSNs). The AAV-mediated expression of constitutively active PKA or Rap1 in accumbal D1R-MSNs enhanced neuronal firing rates and behavioral responses to cocaine exposure through MAPK. Knockout of Rap1 in the accumbal D1R-MSNs was sufficient to decrease these phenotypes. The deficit in cocaine-induced behavioral response in Rap1 conditional knockout mice was restored by co-transfection with constitutively active MAP2K1 (MEK1). These findings demonstrate a novel DA–PKA–Rap1–MAPK intracellular signaling mechanism in D1R-MSNs that increases neuronal excitability to enhance reward-related behaviors [1]. We believe that our phospho-proteomic screening is a powerful and useful tool to increase molecular-level understanding of multifarious brain functions by elucidating the function of the dopamine.

**Reference**


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