

# Chapter 8

## Circuit-Dependent Striatal PKA and ERK Signaling Underlying Action Selection



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Cell-specific, time-lapsed changes in activities of dMSNs and iMSNs were examined by biosensors that allowed monitoring the active and inactive forms of PKA and ERK (Kamioka et al., *Cell Struct. Funct.* 2012). Four lines of transgenic mice were generated by crossing two lines of biosensor-expressing transgenic mice (floxed AKAR3EV for PKA and floxed EKAREV for ERK) with D1-Cre and D2-Cre BAC transgenic mice (Goto et al., *PNAS*, 2015, Fig. 8.1a). The D1-PKA and D1-ERK mice and the D2-PKA and D2-ERK mice exclusively expressed the respective FRET biosensors in the neural pathways of dMSNs and iMSNs, respectively. FRET imaging of the striatum of freely moving mice was achieved by developing fiber bundle-based micro-endoscope techniques (Fig. 8.1b). The pencil like-shaped tip of the optical fiber bundle was implanted into the mouse brain, and the flat end of the bundle was scanned with a confocal laser scanning microscope equipped with a 445-nm laser for excitation and a pair of band-pass filters,  $483 \pm 16$  nm for CFP and  $542 \pm 13$  nm for FRET. Fluorescence excitation of the FRET biosensors was exclusively detected in the striatum of the transgenic mice. Upon quantitative analysis, *in vivo* administration of SP-8-Br-cAMPs (cAMP analog) through the cannula attached close to the endoscope caused a progressive increase in FRET responses (changes in FRET/CFP ratio) in both D1-PKA and D2-PKA mice (Fig. 8.1c), but not in PKA-neg mice. Conversely, the MEK inhibitor PD184352 gradually decreased FRET responses in both D1-ERK and D2-ERK mice (Fig. 8.1c). The FRET micro-endoscopy thus allowed us to monitor dynamic changes in activities of PKA and ERK specific for dMSNs and iMSNs.

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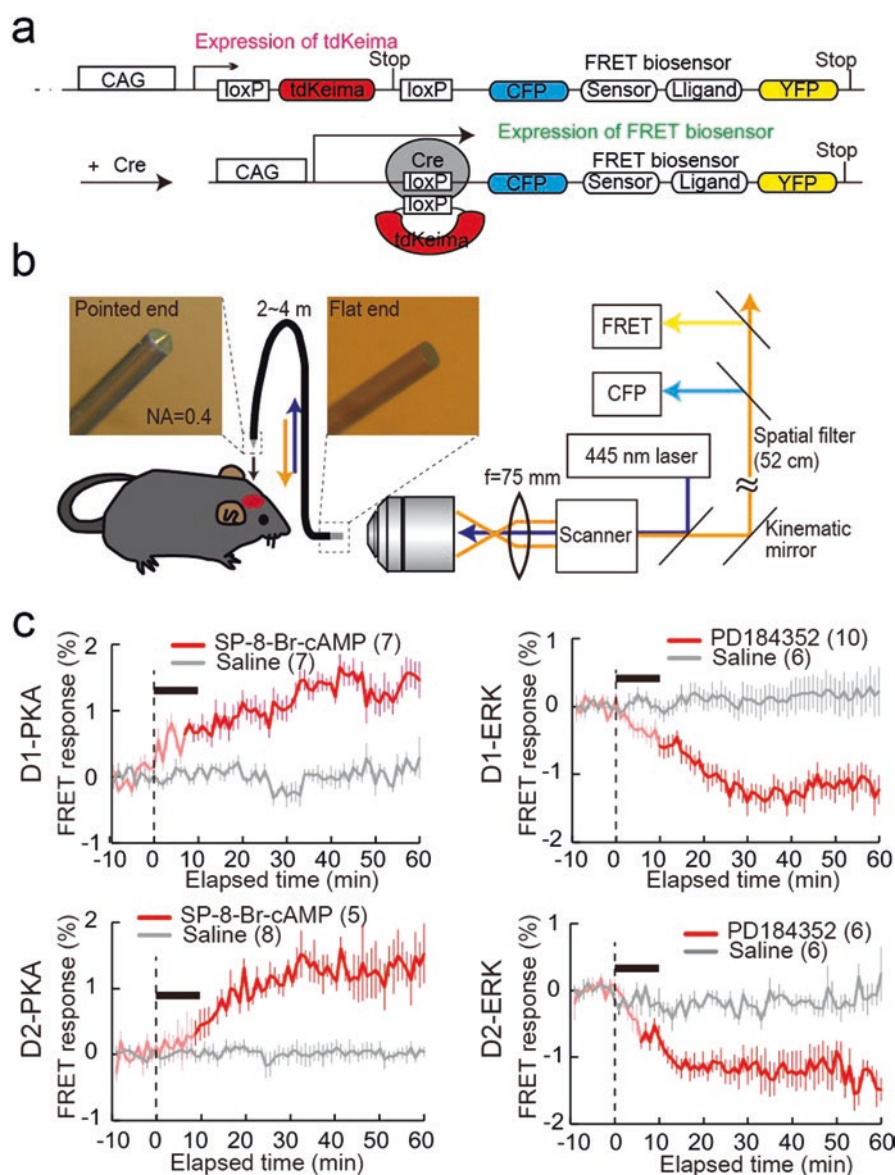
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**Fig. 8.1** Live monitoring of PKA and ERK activities in dMSNs and iMSNs of transgenic mice expressing FRET biosensors

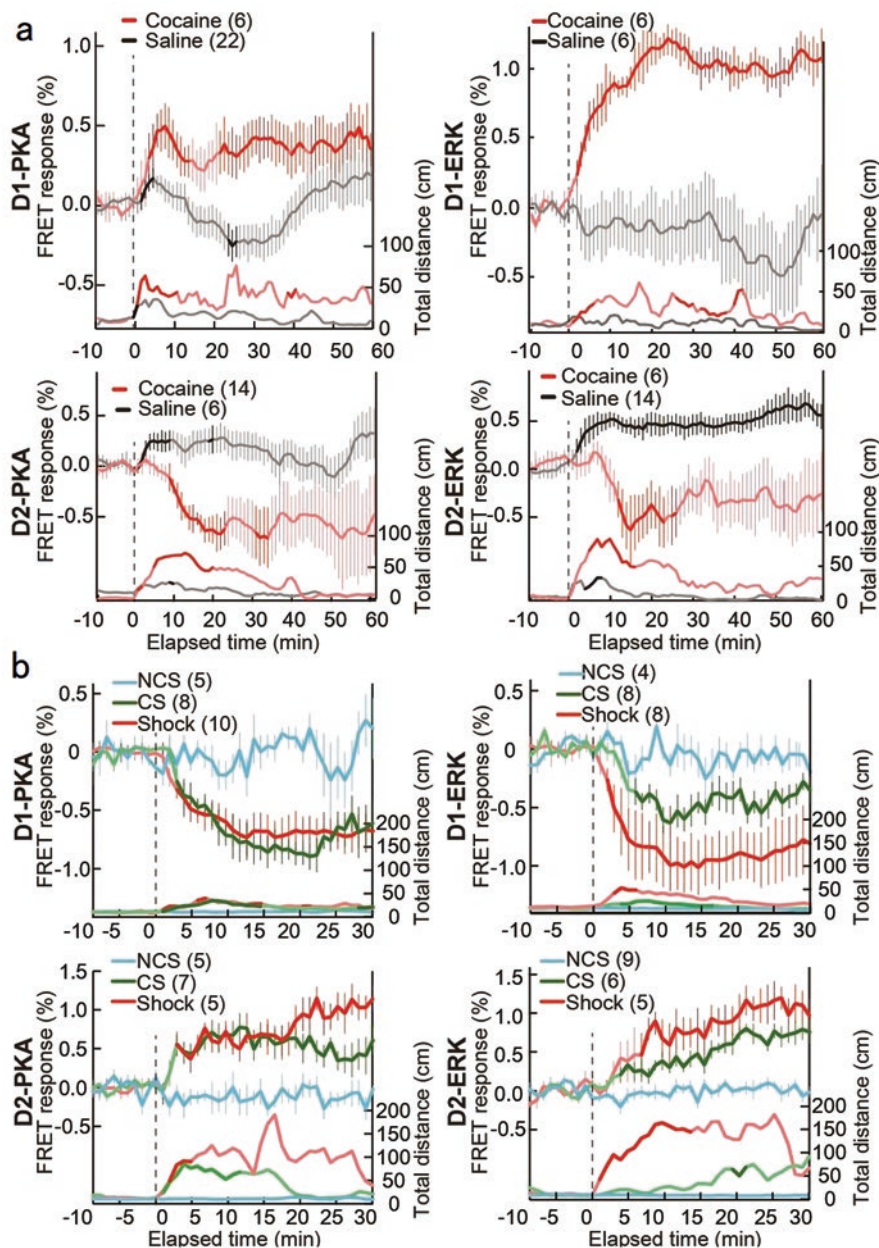
(a) Construct for Cre-dependent expression of FRET biosensors

(b) Schema of the micro-endoscope system. Changes in FRET responses were monitored to measure the activity of PKA or ERK

(c) Local application of SP-8-Br-cAMP (2 mM) and PD184352 (10  $\mu$ M) into the dorsal striatum of anesthetized transgenic mice (indicated by black bars) respectively increased FRET responses of D1-PKA and D2-PKA and decreased those of D1-ERK and D2-ERK

Exposure to cocaine causes a massive increase in the dopamine (DA) level in the striatum and results in activation of PKA and ERK in this brain region (Valjent et al., *J. Neurosci.*, 2000; Bertran-Gonzales et al., *J. Neurosci.*, 2008). Upon FRET micro-endoscopic analysis, administration of cocaine (25 mg/kg, i.p.), but not that of saline, rapidly and continuously increased the activities of both D1-PKA and D1-ERK with a concomitant increase in locomotor activity (Fig. 8.2a). This increase was in marked contrast to the response of iMSNs, in which the activities of both D2-PKA and D2-ERK were gradually decreased by the cocaine administration (Fig. 8.2a). Importantly, saline injection appreciably activated both D2-PKA and D2-ERK (Fig. 8.2a). Aversive stimuli transiently suppress tonic firings of most of DA neurons in the ventral tegmental area and disinhibit iMSNs via inactivation of Gi-coupled inhibitory D2 receptors (Gerfen and Surmeier, *Annu. Rev. Neurosci.*, 2011; Ungless, *Science*, 2004). Because saline injection supposedly serves as an aversive stimulus, we more directly addressed how a strong aversive stimulus, i.e., an electric foot shock, would affect PKA and ERK in dMSNs and iMSNs. Both D2-PKA and D2-ERK were markedly activated in response to an electric foot shock (2 mA, 40 Hz for 2 sec) (Fig. 8.2b). Conversely, the activities of D1-PKA and D1-ERK were notably decreased in response to the electric shock (Fig. 8.2b). Thus, the activities of PKA and ERK are reciprocally controlled not only by rewarding and aversive stimuli but also between dMSNs and iMSNs. We further investigated involvement of D2-PKA and D2-ERK in the induction of aversive learning by pairing electric shocks with the sound of a bell (Fig. 8.2b). After habituation with repeated exposure to a bell sound, an additional bell sound never influenced the activities of PKA and ERK in either dMSNs or iMSNs and thus served as neutral information. Notably, both PKA and ERK were activated and inactivated in iMSNs and dMSNs, respectively, by the conditioned bell sound without electric shocks. Thus, the cell-specific regulation of PKA and ERK underlies the induction of both the acute aversive reaction and aversive learning behavior.

Naturally occurring rewarding stimuli such as food, drinking, and mating enhance DA release in the striatum and induce motivational reward-seeking behavior (Damsma et al., *Behav. Neurosci.*, 1992; Salamone and Correa, *Neuron*, 2012). The role of striatal PKA and ERK in naturally occurring rewarding behavior was addressed by measuring the activities of PKA and ERK during the mating behavior of a male mouse after inclusion of a female mouse in the same chamber. The male mice showed a wide variety of mating reactions, including frequent sniffing, grooming, and mounting (Park 2011). We counted the percentages of time exhibiting mating behaviors (sniffing, grooming, mounting) every minutes during a 30-min period after inclusion of the female mouse (%PMR), and used it as an index to estimate the motivational strength for the male mice to the presented female mouse. When overall 30 min period (interaction with female) was averaged, a significant positive correlation was noted between the extent of the D1-PKA and D1-ERK activation and the percentage of positive mating reactions, and this correlation was inverted in the responses of D2-PKA and D2-ERK (Fig. 8.3a). When mating behaviors were arbitrarily divided into two groups exhibiting either frequent or infrequent mating reactions by values of 20% PMR (Fig. 8.3b), the D1-PKA and D1-ERK activities



**Fig. 8.2** Reciprocal regulation of PKA and ERK activities between dMSNs and iMSNs in response to cocaine administration and electric foot shocks

(a) PKA and ERK activities (upper traces) with SEM (vertical lines) and locomotor activities (lower traces) in response to cocaine or saline injection

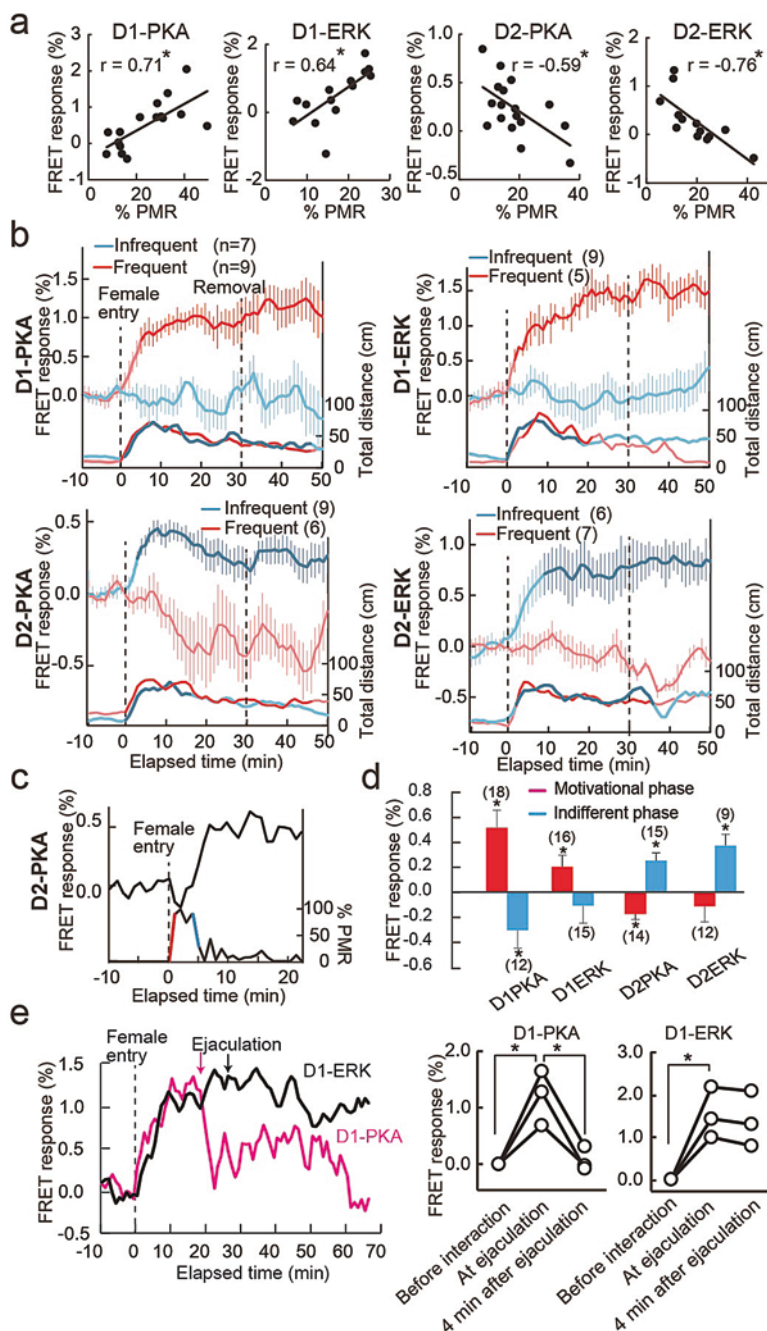
(b) PKA and ERK activities in response to electric foot shocks (Shock), bell sound without unconditioned stimulus (NCS), and bell sound after conditioning with electric shocks (CS)

significantly increased in frequently interacting mice, whereas the D2-PKA and D2-ERK activities were increased in the infrequently interacting ones (Fig. 8.3b). We noticed that the observed activity changes in frequently and infrequently interacting animals depended on whether these mice became successively motivated or indifferent to a female mouse during mating behaviors. Thus, the PKA and ERK activities of individual mice were pursued during the shift between the motivational phase and indifferent phase of mating behavior (defined as more than 50% PMR increase and decrease within 1–4 min, respectively) (Fig. 8.3c). This analysis revealed that the activities of D1-PKA and D1-ERK were elevated during the motivational phase of the mating reaction. In contrast, the activities of D2-PKA and D2-ERK were increased when a male mouse became indifferent to or escaped from a female mouse. Thus, the activities of both PKA and ERK (but more PKA) rapidly changed during mating behavior, and this rapid change reflected the shift between motivational and indifferent phases of the mating reaction. The above results suggest that activation of PKA and ERK in dMSNs and iMSNs underlies the induction and suppression of mating reactions, respectively. To substantiate this possibility, the ejaculation of a male mouse was facilitated by pairing the male mouse with a hormonally-primed female mouse (Ogawa et al., *Endocrinology*, 1998) and the activities of D1-PKA and D1-ERK were measured before and after ejaculation (Fig. 8.3e, f). The D1-PKA activity elevated during the mating reaction was rapidly reduced to lower levels within 4 min after ejaculation, whereas the D1-ERK activity continued to be elevated even after ejaculation, suggesting that PKA in the dMSNs was more relevantly associated with the rapid shift in the mating reaction.

To further address the causality of PKA activity in mating behavior, we artificially activated Gi or Gs by DREADDs (Designer Receptors exclusively Activated by a Designer Drug, Rogan & Roth, *Pharmacological reviews*, 2011) which were induced by adeno-associate virus in a Cre-dependent manner. CNO was injected 10 or 20 min after the female entry. Around 10 min after CNO injection, PKA activity was either decreased in AAV-hM4Di-mCherry injected mice, or increased in AAV-hDs-mCherry injected ones (Goto et al., *PNAS*, 2015). Concomitant to the increase or decrease in D1-PKA, male mouse exhibited increase or decrease in %PMR, respectively. Conversely, increase or decrease in D2-PKA either induced either decrease or increase in %PMR, respectively. These results indicate that the causal relationship between PKA activity of dMSNs and iMSNs in the dorsal striatum and the mating reactions in male mice.

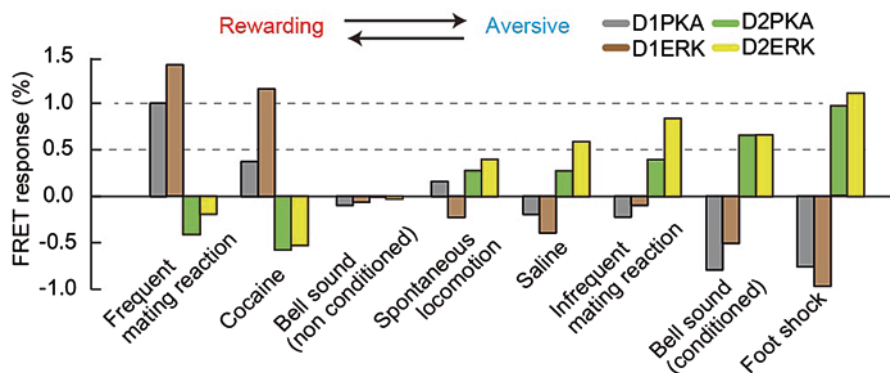
Figure 8.4 summarizes differences in the amplitudes of the PKA and ERK responses in MSNs along different behavioral conditions. This summary explicitly demonstrates that PKA and ERK are coordinately stimulated or inhibited in both dMSNs and iMSN but oppositely regulated between these two cell types under different conditions. Animal behaviors can thus be orderly aligned from rewarding to aversive behaviors by taking into account the extents of activation and inactivation of PKA and ERK in individual behaviors. Intriguingly, highly interacting male mice with female mice exhibited higher PKA and ERK activities in dMSNs than those of cocaine-treated mice, suggesting that sexual behavior is highly emotional and motivational for male mice. Importantly, the motivational and indifferent phases of mat-





**Fig. 8.3** PKA and ERK activities of dMSNs and iMSNs during sexual interaction

(a) A male mouse was exposed to an unfamiliar female mouse for 30 min and analyzed by the FRET micro-endoscopy. Percentages of PMR were measured, and average changes in activities of PKA and ERK of dMSNs and iMSNs during the 30-min period were plotted against % PMR



**Fig. 8.4** Summary of PKA and ERK activities under rewarding and aversive/impulsive conditions

Values of the PKA and ERK activities were calculated by averaging FRET responses during 5 min at the peak response under the different conditions.  $***p < 0.001$ ,  $**p < 0.01$ ,  $*p < 0.05$ , as compared with the non-conditioned bell sound (Mann-Whitney's U test). Reward-seeking and aversive/impulsive animal behaviors can be orderly aligned according to the extents of activation and inactivation of PKA and ERK

ing reactions are tightly associated with the rapid shift in the activities of PKA and ERK in both dMSNs and iMSNs. Phosphorylation/dephosphorylation of PKA and ERK in these two types of MSNs could thus be a potential mechanism that controls this rapid shift between rewarding and aversive behaviors. Notably, the bell sound after conditioning with electric shocks induced profound effects on both dMSNs and iMSNs, indicating that robust and strong adaptive alterations in the striatal circuit are involved in the aversive learning behavior. Furthermore, saline injection, spontaneous locomotion, and indifference of male mice toward female mice caused up-regulation of the activities of PKA and ERK in iMSNs. This finding is consistent with the predominant expression of high-affinity (nM order) D2 receptors in iMSNs, which are capable of sensing subtle changes in synaptic DA concentrations in the

**Fig. 8.3** (continued) (b) Changes in activities of PKA and ERK (upper traces) and locomotor activities (lower traces) of frequently or infrequently interacting male mice (c, d) Up- and down-regulation of PKA and ERK activities at the active and indifferent phases of the mating reaction ( $*p < 0.05$ , Mann-Whitney's U test). The numbers in parentheses indicate the sample numbers from 6 to 9 animals (e), (left) Examples of changes in the PKA and ERK activities in dMSNs after ejaculation. Ejaculation is marked with the arrow in the D1-PKA (red) and D1-ERK (black) mouse (right) Rapid inactivation of PKA in the D1-PKA mice after ejaculation ( $*p < 0.05$ ,  $n = 5$ , Wilcoxon signed-rank test)

striatum (Gerfen & Surmeier, *Annu. Rev. Neurosci.*, 2011). The iMSN transmission would thus greatly contribute to the innate tendency of animals to be more concerned about and to rapidly avoid uncomfortable environments and predators. Conversely, when animals encounter rewarding stimuli such as sexual interaction, these stimuli increase DA levels in the striatum and stimulate low-affinity ( $\mu\text{M}$  order) D1 receptors in dMSNs. Thus, the D1 and D2 receptors serve as key determinants to distinctly sense changes in synaptic DA transmission in a pathway-specific manner and to induce reward-directed and aversive behaviors via common PKA and ERK signaling cascades (Nakanishi et al., *Neuroscience*, 2014).

We also applied these techniques to measure the temporal dynamics of PKA response in the formation of aversive memory in the core part of nucleus accumbens (NAc). We found that PKA activities of iMSNs at NAc occurred not instantaneously after footshock but in a delayed and progressive manner (Yamaguchi et al., *PNAS*, 2015). We believe that the above methodologies allow us to study regulatory mechanisms of neural circuits involved in a wide range of animal behaviors.

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