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Original Article

Reversal of an Unconditioned Behavioral Preference for Specific Food Pellets by Intervention of Whisker Sensory Inputs

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Adenylyl cyclase type-5 (AC5) is preferentially expressed in the dorsal striatum. Recently, we reported that AC5 knockout (KO) mice preferred food pellets carrying an olfactory cue produced by AC5 KO mice during food consumption (AC5 KO pellets) over food pellets that had been taken by wildtype (WT) mice. In the present study, we demonstrated that whisker trimming on the right side of the face but not the left in AC5 KO mice blocked the behavioral preference for AC5 KO pellets. Conversely, whisker trimming on the right but not the left in WT mice induced a behavioral preference for AC5 KO pellets. Mice lacking D2 dopamine receptor (D2 KO mice) also showed a behavioral preference for AC5 KO pellets. In D2 mice, whisker trimming on the right side of the face but not the left blocked a behavioral preference for AC5 KO food pellets. AC5 KO mice had increased level of phospho-CaMKIIα in the dorsal striatum, and WT mice with whiskers cut on either side also showed increased p-CaMKIIα level in the dorsal striatum. The siRNA-mediated inhibition of CaMKIIα in the dorsal striatum in either the right or the left hemisphere in AC5 KO mice and D2 KO mice blocked the behavioral preference for AC5 KO pellets. However, behavioral changes induced by this inhibition on each side showed asymmetrical time courses. These results suggest that an unconditioned behavioral preference for specific food pellets can be switched on or off based on the balance of states of neural activity in the dorsal striatum regulated by a signaling pathway centered on AC5 and D2 and the sensory inputs of whiskers from the right side of the face.

Key words: whisker-trimming, dorsal striatum, unconditioned preference

INTRODUCTION

Animals have an intrinsic tendency to prefer a certain object with a specific cue to other similar objects. Behavioral preference for a specific cue in innate behaviors is automatically produced by

the action of programmed responses in the brain [1]. Behavioral preference for a specific cue in habits is learned through experience, but once it is formed, it is not easily modified by new experiences, thus having a similarity to that of innate behaviors [2]. In certain psychiatric illnesses, such as drug addiction, obsessive-compulsive disorder, and autism, a similar type of behaviors occurred is difficult to control [3, 4]. However, the neural mechanisms regulating this type of behaviors are not clearly understood.

The dorsal striatum is important in decision making that leads to behavioral preference. The dorsal striatum, particularly the

dorsolateral striatum, becomes active as behavioral preference in habits develops [5]. Adenylyl cyclase type 5 (AC5) is expressed preferentially in the dorsal striatum, where it has an essential role in the activity of D2 dopamine receptor [6]. AC5 KO mice exhibit a behavioral preference for food pellets containing an olfactory cue produced by AC5 KO mice (AC5 KO pellets) over food pellets containing an olfactory cue produced by WT mice (WT pellets). Interestingly, bilateral whisker trimming in AC5 KO mice blocks the expression of this behavioral preference for AC5 KO pellets, while such whisker trimming in WT mice induces this behavioral preference [7]. These results suggest that AC5 KO mice can be used as an animal model to study possible modulation of unconditioned behavioral preferences in relation to the neural activity of sensory inputs.

In rodents, whisker sensory inputs project though the thalamus to the somatosensory cortex, called the barrel cortex [8]. The dorsal striatum and its associated neural circuits serve as the neural substrates that integrate glutamatergic afferents coming from the cerebral cortex [9]. Whisker trimming increases glutamatergic synaptic inputs from the barrel cortex to the dorsal striatum, resulting in an increase in CaMKIIα activity [7]. The dorsal striatum is also regulated by dopaminergic inputs [10]. However, the detailed mechanisms for the neural circuit from the whisker sensory input to the dorsal striatum and the neural mechanism through which the dopaminergic system regulates unconditioned behaviors are not clearly understood.

In the present study, we investigated the neural mechanism regulating unconditioned behavior in food-intake using AC5 KO and D2 KO mice. We demonstrated that an unconditioned behavioral preference for specific food pellets was regulated by the AC5 and D2 signaling systems and the sensory inputs from whiskers on the right side of the face.

MATERIALS AND METHODS

Animals

AC5 KO mice and D2 KO mice [11, 12] were maintained with genetic backgrounds of C57BL/6 and genotyped as described previously [7, 11]. Mice were housed in a temperature- and humidity-controlled environment with a 12 h light/dark cycle and were provided food and water *ad libitum*. All experiments were conducted in accordance with the Guidelines of Animal Care at Ewha Womans University through permission of the EWU-IACUC (No. 2014-01-003).

Stereotaxic injection of siRNA

Stereotaxic injection of siRNA was performed as described

previously [7]. In brief, mice were anesthetized with a mixture (3.5:1) of ketamine hydrochloride (50 mg/ml) and xylazine hydrochloride (23.3 mg/ml). The FAM-labeled RISK-independent siRNA transfection control siGLO Green (D-001630-01-05), AC5-siRNA (M-059173-00-0005, NM_009792) and CaMKIIα siRNA (M-059173-00-0005, NM_009792) were purchased from Dharmacon Inc. (Chicago, IL, USA). siRNA-control (SN-1012) and D2-siRNA (1351275, NM_010077.1) were purchased from Bioneer Co. (Deajun, Korea).

One volume of the diluted (50 ng/μl) siRNA-control, AC5-siRNA or D2-siRNA, after adding siGLO Green (19:1 ratio), was mixed with 2.5 volumes of neurofect transfection reagent (T800075; Genlantis, San Diego, CA, USA) and 0.5 volumes of 50% sucrose. siRNA-AC5, siRNA-D2 or control siRNA was injected at a volume of 1.5 μl (18 ng siRNA) into each dorsal striatum (AP, +1.0; ML, ±1.5; DV, -3.6 mm). Behavioral tests were performed 48 h after the siRNA injection.

Western blot analysis

Western blot analyses were carried out as described previously [7]. The dorsal striatum on each side was homogenized in lysis buffer (50 mM Tris-HCl at pH 7.5, 0.5% NP-40, 0.5% Triton X-100, 0.25% sodium deoxycholate, 0.25% SDS, 150 mM sodium chloride, 1 mM EDTA) with protease inhibitors (Complete Mini; Roche Applied Science). The protein concentration was determined using a NanoDrop (ND1000, Thermo Scientific), and 20 μg of protein was run in each lane. Blots were blocked with 2% BSA (Armnesco) in 0.1% TBS-T buffer and then probed with anti-p-CaMKIIα (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-CaMKIIα (Santa Cruz Biotechnology). Immunoblots were visualized using a PicoEPD Western Reagent Kit (EBP-1073, ELPis Biotech, Daejeon, Korea). Western blot images were quantified using Image-ProPremier 6.0 (MediaCybernetics, MD, USA).

Statistical analysis

Two-sample comparisons were conducted using the two-tailed t-test, and multiple comparisons were made using two-way repeated-measures ANOVA followed by a *post hoc* test using Graphpad Prism 6 (San Diego, CA, USA). All data are presented as mean±SEM, and a statistical difference was accepted at the 5% level.

RESULTS

AC5 KO mice exhibited an unconditioned behavioral preference for food pellets

Food pellets collected from normal home cages housing WT

mice were termed WT pellets, while those from home cages carrying AC5 KO mice were termed KO pellets. Fresh food pellets weighed approximately 4 g, while the collected AC5 KO pellets and WT pellets were in the range of 2.5~3.5 g. When presented with WT pellets and size-matched AC5 KO pellets in normal home cages, WT mice consumed similar amounts of WT pellets and AC5 KO pellets over 24 h, whereas AC5 KO mice preferably ate AC5 KO pellets over WT pellets (Fig. 1A and B). This result is consistent with our previous report [7].

Lateralization of whisker trimming effects on unconditioned behavioral preferences in AC5 KO mice

Bilateral whisker trimming in AC5 KO mice blocked the behavioral preference for KO pellets over WT ones. Conversely, bilateral whisker trimming in WT mice led behavioral preference for KO pellets over WT ones (Fig. 1C-E). Interestingly, right-side whisker trimming in AC5 KO mice was sufficient to block the behavioral preference for AC5 KO pellets, thus mimicking bilateral whisker trimming. However, left-side whisker trimming
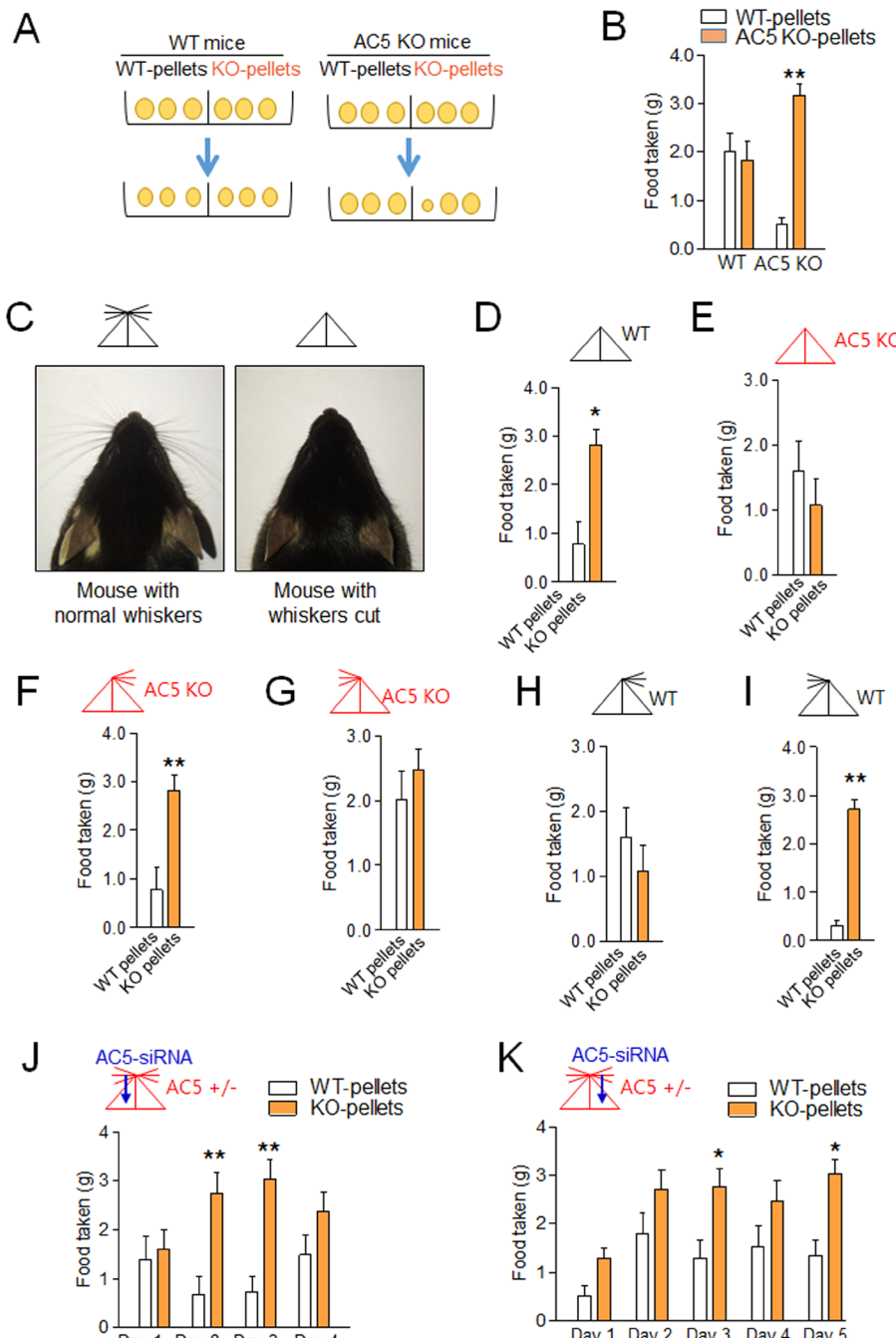

Fig. 1. Right-side whisker trimming in WT and AC5 KO mice changed the behavioral preference for AC5 KO pellets in opposite directions. (A, B) Diagram showing three WT-food *vs.* three KO-food pellets presented to each mouse (A; top panels) and food pellets remaining on the next day (A; bottom panels). The reduced sizes of food pellets on the bottom panels indicate that WT randomly ingested all six pellets, whereas AC5 KO mice preferentially consumed one AC5 KO-food pellet among 6 pellets (B). (C-E) Photographs showing a mouse with normal whiskers (left) and whiskers cut to the fur level (right). Whisker trimming in WT (D) and AC5 KO mice (E) reversed the behavioral preference for AC5 KO pellets over WT pellets. (F-I) AC5 KO mice with whiskers cut on the right side lost their behavioral preference for AC5 KO pellets (F, G), while WT mice with whiskers cut on the right side gained a behavioral preference for AC5 KO pellets (H and I). (J, K) siRNA-mediated inhibition of AC5 on the left side (J) or the right side (K) in AC5 heterozygous mice (AC5$^{+/-}$) transiently induced a behavioral preference for AC5 KO pellets. Naive AC5$^{+/-}$ mice showed WT-like behaviors in this test. Data are presented as the mean±SEM (n=7-11). * and **denote the difference between the indicated groups at p<0.05 and p<0.01, respectively (Student's *t*-test, two-way ANOVA or two-way repeated-measures ANOVA, and *post-hoc* test).

in AC5 KO mice did not change the behavioral preference for AC5 KO pellets (Fig. 1F and G). Conversely, right-side but not left-side whisker trimming in WT mice induced a behavioral preference for AC5 KO pellets (Fig. 1H and I). Thus, right-side whisker trimming in WT and AC5 KO mice produced similar effects to those induced by bilateral whisker trimming.

Unilateral suppression of AC5 in the dorsal striatum on the right or the left produced a behavioral preference for AC5 KO food pellets with different time courses

We examined whether unilateral inhibition of AC5 in the dorsal

striatum mimicked the lateralization effects of whisker trimming on behavioral preference. Unilateral siRNA-mediated inhibition of AC5 in the dorsal striatum of AC5 heterozygote mice (AC5$^{+/-}$) induced a behavioral preference for AC5 KO pellets, but inhibition on each side produced different time courses of behavioral changes (Fig. 1J and K).

D2 KO mice exhibited an unconditioned behavioral preference for AC5 KO pellets

AC5 is essential for normal function of D2 dopamine receptor in the dorsal striatum [6]. Indeed, D2 knockout (KO) mice also
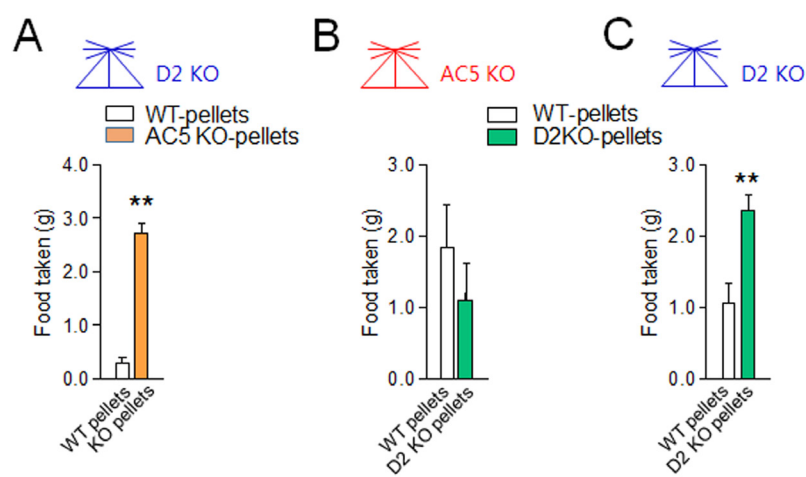

Fig. 2. D2 KO mice exhibited an unconditioned behavioral preference for AC5 KO pellets. (A) D2 KO mice preferred AC5 KO-food to WT-food. (B,C) AC5 KO mice did not prefer D2 KO-food to WT-food (B), whereas D2 KO mice preferred D2 KO-food to WT-food (C). Data are presented as the mean±SEM (n=7-8). **denote the difference between the indicated groups at p<0.01 (Student *t*-test).
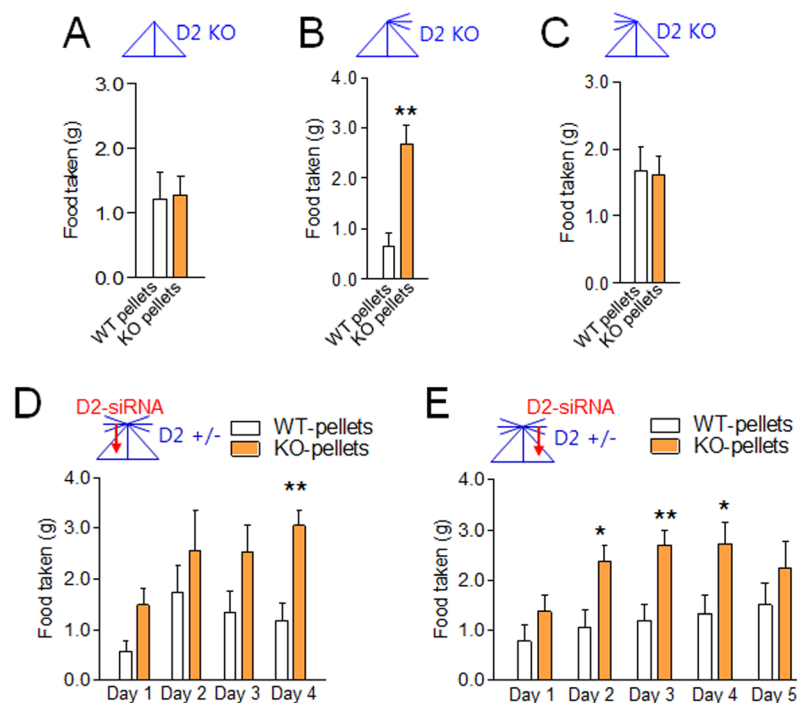

Fig. 3. Right-side whisker trimming in D2 KO mice changed their behavioral preference for AC5 KO pellets. (A-C) Bilateral whisker trimming in D2 KO mice reversed the behavioral preference for AC5 KO pellets over WT pellets. D2 KO mice with whiskers cut on the right side of the face (C), but not the left (B), lost their behavioral preference for AC5 KO pellets. (D, E) siRNA-mediated inhibition of D2 unilaterally on the left side (D) or the right side (E) of the face in D2 heterozygous mice (D2$^{+/-}$) transiently induced a behavioral preference for AC5 KO pellets. Naive D2$^{+/-}$ mice showed WT-like behaviors in this test. Data are presented as the mean SEM (n=6-9). * and **denote the difference between the indicated groups at p<0.05 and p<0.01, respectively (Student's *t*-test, or two-way repeated-measures ANOVA, and *post-hoc* test).

exhibited a behavioral preference for AC5 KO pellets over WT pellets (Fig. 2A). Cross-check analysis of behavior driving-cue generator and cue responder relationships in AC5 KO mice and D2 KO mice indicated that AC5 KO mice did not prefer D2 KO pellets to WT pellets. This analysis also found that D2 KO mice preferred D2 KO pellets to WT pellets, suggesting that D2 KO mice produce an olfactory cue that directs behavioral preference by D2 KO mice (Fig. 2B and C).

Lateralization of whisker trimming effects on unconditioned behavioral preferences in D2 KO mice

Bilateral whisker trimming in D2 KO mice blocked their behavioral preference for AC5 KO pellets over WT ones (Fig. 3A). Right-side whisker trimming in D2 KO mice also blocked

the behavioral preference for AC5 KO pellets. However, left-side whisker trimming in D2 KO mice did not produce such effects (Fig. 3B and C).

Unilateral suppression of D2 in the dorsal striatum on the right or the left produced a behavioral preference for AC5 KO food pellets with different time courses

Unilateral siRNA-mediated inhibition of D2 receptor in the dorsal striatum in D2 heterozygote mice (D2$^{+/-}$) induced a behavioral preference for AC5 KO pellets over WT pellets. However, the inhibition of D2 on each side produced different time courses of behavioral responses (Fig. 3D and E).
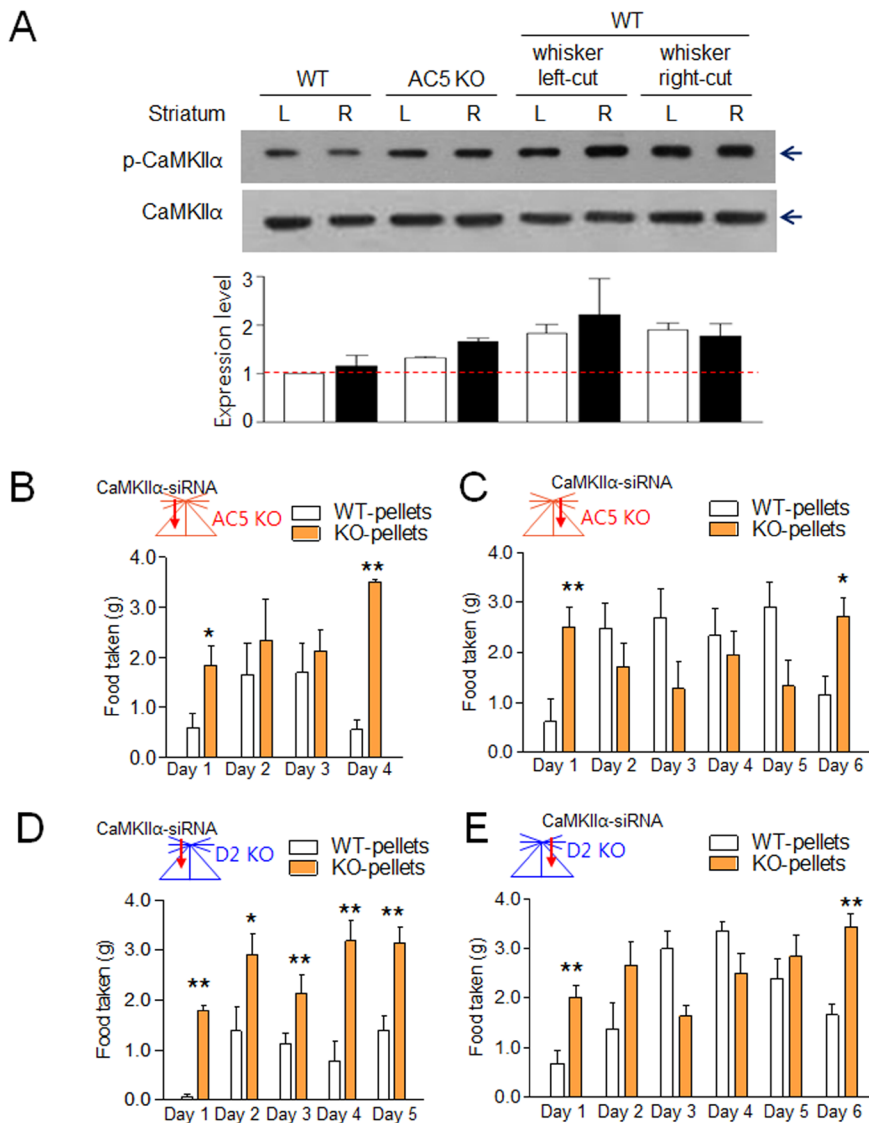

Fig. 4. Selective suppression of CaMKIIα in the dorsal striatum in AC5 KO mice and D2 KO mice blocked the behavioral preference for AC5 KO food pellets. (A) Western blots showing p-CaMKIIα levels in the dorsal striatum in the right (R) and left (L) hemispheres in WT mice, AC5 KO mice, and WT mice with whiskers trimmed unilaterally on the right side (whisker, right-cut) or the left side (whisker, left-cut) of the face. Quantification of p-CaMKIIα level in the dorsal striatum (bottom). (B, C) The siRNA-mediated inhibition of p-CaMKIIα in AC5 KO mice in either the left (B) or the right (C) dorsal striatum suppressed a behavioral preference for AC5 KO pellets. (D, E) The siRNA-mediated inhibition of p-CaMKIIα in D2 KO mice in either the left (D) or the right (E) dorsal striatum suppressed a behavioral preference for AC5 KO pellets. Data are presented as the mean±SEM (n=7-8). * and **denote the difference between the indicated groups at p<0.05 and p<0.01, respectively (Student's *t*-test, or two-way repeated-measures ANOVA, and *post-hoc* test).

Selective suppression of CaMKIIα in the dorsal striatum in AC5 KO mice and D2 KO mice blocked the behavioral preference for AC5 KO food pellets

AC5 KO mice had increased level of phospho-CaMKIIα in the dorsal striatum compared to WT mice. Unilateral whisker trimming in WT mice also increased the level of phospho-CaMKIIα in the dorsal striatum but not in a lateralized manner (Fig. 4A).

Unilateral siRNA-mediated inhibition of CaMKIIα in the right or left dorsal striatum in AC5 KO mice blocked the behavioral preference for AC5 KO pellets, although inhibition of CaMKIIα in the right hemisphere produced protracted effects compared to inhibition in the left hemisphere (Fig. 4B and C).

The siRNA-mediated inhibition of CaMKIIα in the right dorsal striatum in D2 KO mice blocked their behavioral preference for AC5 KO pellets, and inhibition of CaMKIIα in the dorsal striatum on the right side produced protracted effects on behavior. On the contrary, siRNA-mediated inhibition of CaMKIIα in the dorsal striatum on the left side in D2 KO mice produced insignificant or subtle effects on behavior (Fig. 4D and E).

DISCUSSION

In the present study, we demonstrated that right-side whisker trimming in AC5 KO mice blocked their behavioral preference for AC5 KO pellets. Conversely, such whisker trimming in WT mice induced a behavioral preference for AC5 KO pellets (Fig. 1). D2 KO mice also showed a behavioral preference for AC5 KO pellets with right-side whisker trimming also blocking the behavioral preference for AC5 KO pellets (Fig. 2 and 3). These results suggest the following points: First, behavioral preference for AC5 KO pellets is not learned behavior, but occurs unconditionally. Even in AC5 KO or D2 KO mice, it is unlikely related to developmental defects. Second, the neural system centered on AC5 and D2, preferentially expressed in the dorsal striatum [13, 14, 15], is critical for this unconditioned behavioral preference. Moreover, AC5 is essential for normal function of D2 dopamine receptors in the dorsal striatum [6]. siRNA-mediated inhibition of AC5 in the dorsal striatum is sufficient to replicate the AC5 KO effects on the whole brain [7; Fig. 1J and K]. Collectively, these findings support the notion that the dorsal striatum is the core neural substrate acting in the process of this behavior. Third, the effects of whisker trimming on behavioral preference for AC5 KO pellets are lateralized to the right side. The lateralization of whisker trimming effects presumably represents a change in the ipsilateral neural input from the trimmed whisker side, rather than the contralateral neural input from the untrimmed intact whisker side.

The unilateral siRNA-mediated inhibition of AC5 or D2 in the dorsal striatum induced a behavioral preference for AC5 KO pellets. However, behavioral responses induced by this inhibition on each side showed different time courses of behavioral changes in both AC5 and D2 inhibition cases. Inhibition of AC5 in the dorsal striatum on the left side produced relatively faster responses than inhibition on the right side, whereas inhibition of D2 dopamine receptors in the dorsal striatum on the right side induced more rapid changes (Fig. 1 and 3). The level of CaMKIIα in the dorsal striatum increased after whisker trimming, while unilateral siRNA-mediated inhibition of CaMKIIα in the dorsal striatum of AC5 KO mice or D2 KO mice produced partially asymmetrical effects (Fig. 4). Although the detailed mechanism needs to be studied further, we speculate that the lateralization of whisker trimming effects is produced by a neural activity change in specific microcircuits within the dorsal striatum, rather than by a neural activity change of striatal neurons in mass that could be induced by siRNA-mediated inhibition. Alternatively, we can not rule out the possibility that whisker trimming-induced activation of CaMKIIα in the dorsal striatum is not the primary player mediating the lateralized effects of whisker trimming on food-intake behaviors.

The activity of the barrel cortex is profoundly influenced by ipsilateral and contralateral whisker stimuli [16]. Functional MRI (fMRI) study in rats shows that the barrel cortex in the right hemisphere is activated more strongly than that in the left during bilateral whisker stimulation [17]. Individual rats are polarized either to the left side or the right side in the use of the right vs. left paw in a tactile discrimination task, and unilateral whisker trimming differentially affects these behaviors [18]. Rats learn a novel foraging pattern using right-side whiskers more effectively than left-side whiskers and this lateralization to the right is extended to the left cerebral hemisphere [19]. Thus, the whisker sensory system is functionally more lateralized to the right side than the left with regard to whisker-dependent detection of physical environment. In the present study, we provide novel evidence that whisker trimming can change unconditioned behavioral preference for specific cue-directed food pellets and whisker trimming effects are lateralized to the right side.

ACKNOWLEDGEMENTS

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