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ORIGINAL ARTICLE

Genes, Brain and Behavior

Biological functions of α2-adrenergic-like octopamine receptor in *Drosophila melanogaster*

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Abstract

Octopamine regulates various physiological phenomena including memory, sleep, grooming and aggression in insects. In *Drosophila*, four types of octopamine receptors have been identified: *Oamb*, *Oct/TyrR*, *Octβ R* and *Octα2R*. Among these receptors, *Octα2R* was recently discovered and pharmacologically characterized. However, the effects of the receptor on biological functions are still unknown. Here, we showed that *Octα2R* regulated several behaviors related to octopamine signaling. *Octα2R* hypomorphic mutant flies showed a significant decrease in locomotor activity. We found that *Octα2R* expressed in the pars intercerebralis, which is a brain region projected by octopaminergic neurons, is involved in control of the locomotor activity. Besides, *Octα2R* hypomorphic mutants increased time and frequency of grooming and inhibited starvation-induced hyperactivity. These results indicated that *Octα2R* expressed in the central nervous system is responsible for the involvement in physiological functions.

KEYWORDS
Drosophila melanogaster, grooming behavior, locomotor activity, octopamine receptor, starvation-induced hyperactivity

1 | INTRODUCTION

In vertebrates, noradrenaline (NA) is a neurotransmitter that plays an important role in controlling behaviors such as sleep–wake and fight-or-flight behaviors in the central nervous system. In *Drosophila*, octopamine (OA) has a structure and function similar to NA. Both OA and NA are biosynthesized in similar pathways from tyrosine. Tyrosine is directly decarboxylated by tyrosine-decarboxylase (TDC) and turns into tyramine (TA), which is then hydroxylated by tyramine beta-hydroxylase (TβH) and yields OA. On the other hand, tyrosine is first hydroxylated at its phenyl residue by tyrosine hydroxylase (TH) and turns into L-3,4-dihydroxyphenylalanine (L-DOPA). Then L-DOPA is decarboxylated by dopa-decarboxylase (DDC) and turns into dopamine (DA), which is then hydroxylated by dopamine beta-hydroxylase (DβH) and yields NA. OA has been reported to be involved in various physiological phenomena like NA. For example, OA is involved in the regulation of fight-or-flight behavior, locomotor activity, sleep, feeding, memory and the proliferation of germline stem cells after mating.[1–4] Elucidation of the details of OA signaling leads to novel regulatory mechanisms of physiological phenomena. The major receptors for monoamines are G protein-coupled receptors. The three types of OA receptors reported are *Oamb*, *Oct/TyrR* and *Octβ R*.[5,6] The

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relationship between each receptor and physiological function was reported. *Oamb* belongs to the α-receptor family and activates cellular calcium and cAMP signaling.[6] *Oamb* is expressed in the mushroom body (MB) and is involved in memory enhancement by providing input of sweet taste information.[7,8] *Oct/TyrR* has a higher affinity for TA than OA and inhibits the activity of adenylate cyclase. *Oct/TyrR* is expressed in astrocytes and regulates DA neuron activity and startle response.[9] *Octβ*R belongs to the β-receptor family and has three subtypes *Octβ1R*, *Octβ2R* and *Octβ3R*.[10] These receptors primarily increase intracellular cAMP levels and are involved in changes of neural plasticity.[11] Recently, a novel OA receptor (*CsOA3*) that decreases cAMP concentration has been identified in the rice stem borer, *Chilo suppressalis*.[12] An orthologous gene for *CsOA3* exists in honeybees, and pharmacological studies have shown that it is an OA receptor.[13]

An orthologous gene *α2-adrenergic-like octopamine receptor* (*Octα2R*) has also been reported in *Drosophila*, and it has been shown that the receptor is responsive to serotonin (5-HT) as well as OA and that there is a difference in expression between males and females, with the expression being particularly strong in males.[14] However, it is unclear whether *Octα2R* regulates the physiological phenomena. In this study, we confirmed that *Octα2R* was a novel OA receptor by the pharmacological assay and explored the physiological phenomena regulated by *Octα2R* signaling.

2 | MATERIALS AND METHODS

2.1 | Functional and pharmacological analysis of *Octα2R* receptors in cultured cells

A nested PCR was performed as a template of cDNA from w^{1118} line to obtain the fragment including *Octα2R* open reading frame (ORF) (also known as *CG18208* or accession No. NM_142497.3). The ORF fragment was ligated into pCR™ 2.1-TOPO® (Thermo Fisher Scientific, Massachusetts). A PCR was performed with a pair of a *Hin*dIII site-attached forward primer and a *Kpn*I site-attached primer and a template of the constructed TOPO vector. The amplified fragment was inserted into the corresponding site of the expression vector pcDNA3 (Invitrogen, Massachusetts).

The constructed pcDNA3-Octα2R vector was transfected into HEK-293 cells or CHO-K1 cells with Lipofectamine® 3000 (Thermo Fisher Scientific, Massachusetts). The transfected cells were cultured and selected in each culture medium (DMEM [Nacalai Tesc., Kyoto, Japan] for HEK-293 and F-12 Nutrient Mixture [Life Technologies, California] for CHO-K1 supplemented with 10% FBS [Life Technologies]) with G418 (1 mg/ml) over a period of about 4 weeks at 37°C and 5% CO_2. The selected polyclonal cells, HEK-Octα2R cells and CHO-Octα2R cells, were stored at −80°C until use.

We used aequorin, a bioluminescent Ca^{2+} sensor, to measure Ca^{2+} response of the HEK-Octα2R cells and CHO-Octα2R cells to ligands.[15] Briefly, pcDNA3-mtAEQ (2.5 μg), containing mitochondria-targeted apoaequorin (mtAEQ), with or without 2.5 μg of pCMV-XL4 inserting GNA15 (human Gα15 subunit cDNA) (OriGene, Maryland) was transiently transfected into HEK-Octα2R cells or CHO-Octα2R cells (6×10^5/3 ml, 3.5 mm diameter dish) and then treated with 5 μM coelenterazine h (Wako, Tokyo, Japan) for 4 h. A 180 μl portion of the cell suspension was quickly added to a 20 μl aliquot of OA or other biogenic amines (DA, TA, NA and synephrine [SY]). In an antagonist experiment, phentolamine, epinastine or yohimbine was mixed together with OA and treated with each cell. The luminescence of the sample was recorded for 30 s immediately after drug administration using a GENE LIGHT 55 luminometer (Microtec Co., Ltd., Chiba, Japan).

2.2 | Fly stocks and culture environment

All flies were harvested at 25°C, 12 h/12 h light and dark cycles, in 60% humidity. The conventional food containing 4% cornmeal, 8% glucose, 2.4% dry yeast powder, 1% wheat germ, 0.8% agar and 0.8% propionic acid was used. We used *PBac{WH}Octα2R*^{f03483} (#18659), RNAi control fly (#35786), *UAS-Octα2R* RNAi (#50678), *Octα2R Trojan-Gal4* (#67636), *Octα2R T2A-Gal4* (#84610), *Octα2R T2A-LexA* (#84370), *Tdc2-Gal4* (#9319), *DDC-Gal4* (#7009), *R24B11-Gal4* (#49070), *R69F08-Gal4* (#39499), *R46H04-Gal4* (#50280), *R30G03-Gal4* (#49646) and *UAS-mCD8::RFP*, *LexAop2-mCD8::GFP* (#32229) from the Bloomington *Drosophila* Stock Center. *NP0010* was obtained from the Kyoto Stock Center. *TH-Gal4* was gifted from Dr. Jay Hirsh, *OK371-Gal4* was gifted from Dr. Hermann Aberle, *GAD-Gal4* was gifted from Dr. Takaomi Sakai, *dilp2-Gal4* was gifted from Dr. Linda Partridge and *121Y-Gal4* and *30Y-Gal4* were gifted from Dr. J. Douglas Armstrong.

2.3 | Locomotor activity and sleep analysis

Four to 7 days after eclosion, males were separated under CO_2 anesthesia and placed in glass tubes (length, 65 mm; inside diameter, 3 mm) which contained 1% agar and 5% sucrose. The locomotor activity was monitored as a number of crossing infrared beam (IR) by the *Drosophila* activity monitoring (DAM) System (Trikinetics, Massachusetts). Data were collected every 1 min. Sleep defined as immobile time over 5 min was calculated.[16,17]

2.4 | Grooming analysis using the multibeam monitor

Grooming was defined by the number of hyperactivity times (over 30 counts per 1 min). We used the Trikinetics multibeam activity monitor (MB5) that can detect activity counts and moving positions according to 17 IR beams. Data were collected every 1 min. We analyzed counts of over 30 counts per 1 min at the same position.

2.5 | Grooming analysis of decapitated flies

Male flies of 8–10 h after eclosion were used. We decapitated the flies within 5 min under CO_2 anesthesia. After surgery, flies were

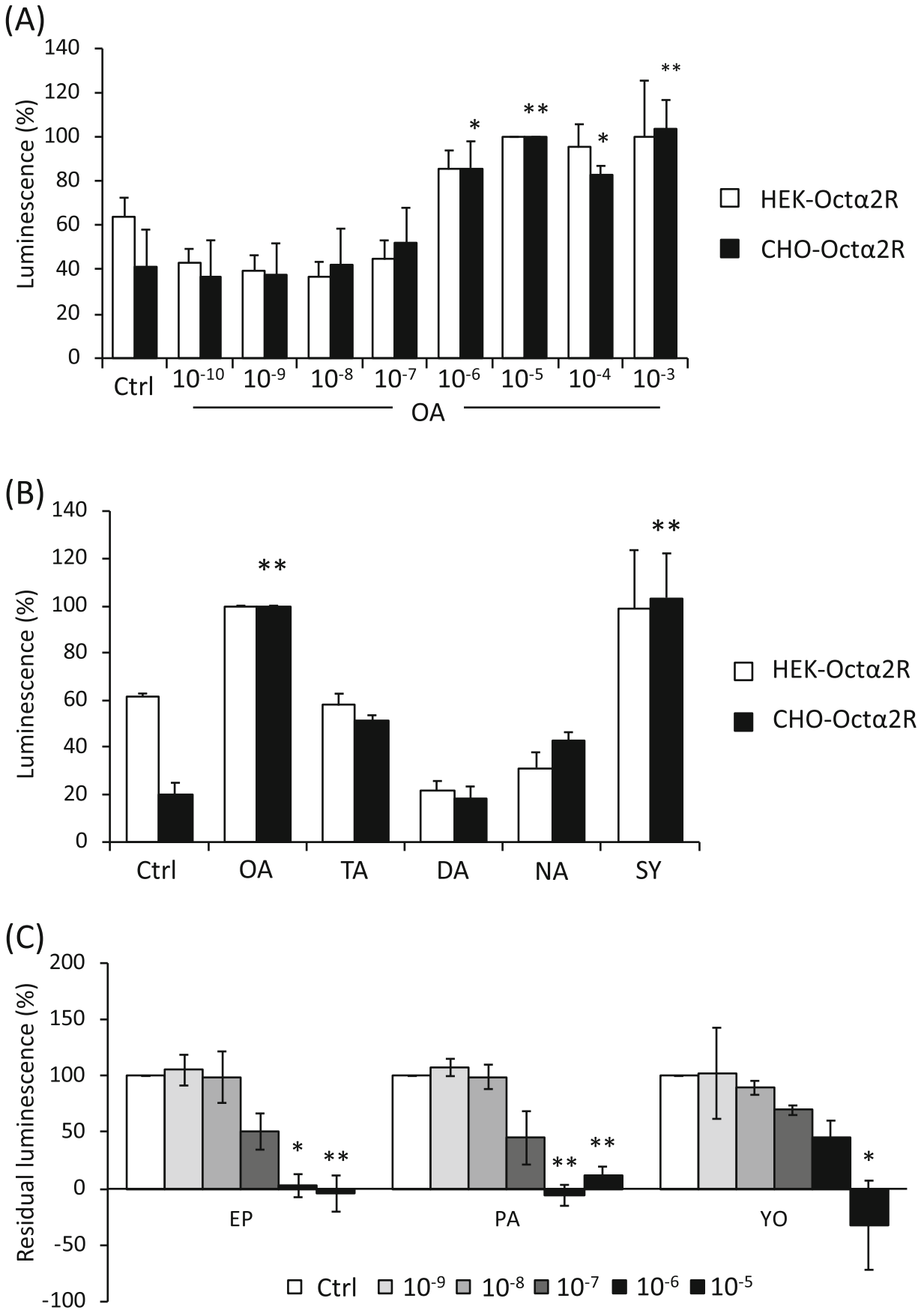

FIGURE 1 Legend on next page.

recovered for 0.5 to 2 h in a humid petri plate. Only decapitated flies that showed an upright posture and a response of grooming after mechanical stimulation by birdwing were used in this study. 10 mM (+)−octopamine hydrochloride (Sigma-Aldrich) were dissolved in PBS. After applying the drug to the exposed nerve cord using a micropipette, we observed grooming behavior for 2 min. We measured the time of foreleg grooming.

2.6 | Measurement of mRNA level by qPCR

All of the male flies were frozen at ZT 6 using liquid nitrogen. Total RNA was collected from 10 frozen heads of flies using RNAiso (Takara). Total RNA was reverse transcripted to cDNA using ReverTra Ace® qPCR RT Master Mix (TOYOBO). cDNA was used for qPCR using THUNDERBIRD® SYBR qPCR Mix (TOYOBO) and Thermal Cycler Dice® Real-Time System II (Takara). The primers were 5′-TGGTACGACA ACGAGTTTGG-3′ and 5′-TTTCAGGCCG TTTCTGAAGT-3′ for *GAPDH2* and 5′-CAGTTGCATC ATGGTCTTTG TGT-3′ and 5′-GCATCGGTAT TGTTCCTTTC TTG-3′ for *Octα2R*. Each mRNA expression level was normalized to housekeeping gene *GAPDH2*. Data were shown as normalized values to the control sample.

2.7 | Statistical analysis

We analyzed data using by Microsoft Excel and the statistical software package RStudio Ver1.4.1717.

3 | RESULTS

3.1 | Octα2R responds to OA and representative OA antagonists specifically

Firstly, HEK-Octα2R or CHO-Octα2R cells were examined for response to OA using AEQ assays without co-transfection with Gα15. When tested with OA at 10^{-9}–10^{-5} M, both cells showed no OA-dependent luminescence signal (data not shown). In contrast, co-transfection with Gα15 induced a dose-dependent luminescence at a range of 10^{-10}–10^{-3} M OA in both cells (Figure 1A). In HEK-293 cells without *Octα2R* expression, OA administration did not increase luminescence (Figure S1).

Next, representative six biogenic amines including OA were examined for agonist activity on *Octα2R* expressed in each cell.[10] In both HEK-Octα2R cells and CHO-Octα2R cells, 10^{-5} M SY showed agonist activity nearly equal to 10^{-5} M OA. TA, DA and NA were equivalent or inferior to control (Figure 1B). Although Qi et al. reported that Octα2R responds not only to OA but also 5-HT using a cAMP assay,[14] we found that HEK-Octα2R cells displayed no Ca^{2+} response to 5-HT at concentrations from 10^{-8} to 10^{-4} M in our assay (Figure S2). Considering the general OA receptor pharmacology,[18] Octα2R is a subtype of *Drosophila* OA receptor.

We also evaluated whether Ca^{2+}-dependent luminescence stimulated by OA is blocked by representative OA receptor antagonists (phentolamine [PA], epinastine [EP] and yohimbine [YO]) in HEK-Octα2R cells.[18] The 10^{-5} M OA-induced luminescence was reduced by more than 90% by 10^{-6} M or higher concentrations of EP and PA and by 10^{-5} M YO (Figure 1C). IC_{50} values of EP, PA and YO were 1.0×10^{-7} M, 9.3×10^{-8} M and 1.8×10^{-5} M, respectively. These results showed that Octα2R has a further pharmacological feature as OA receptors.

3.2 | Octα2R hypomorph and knockdown mutants decreased locomotor activity

Figure 1 showed that *Octα2R* responded to OA. These results were consistent with the previous study.[14] However, the biological function of *Octα2R* is unknown. OA signaling is related to various biological functions such as sleep, locomotor activity, feeding, grooming, and aggression in *Drosophila*. To examine whether that OA signaling through Octα2R regulates the biological functions in adult *Drosophila*, we used Piggy Bac transposon insertion mutants (*Octα2R* hypo) in which *Octα2R* mRNA level decreased by 90% (Figure 2A). We analyzed locomotor activity using the DAM under the 12 h:12 h light and dark conditions. *Octα2R* hypo decreased evening peak and total locomotor activity (Figure 2B,C). *Octα2R* hypo did not show a significant change in the total amount of sleep (Figure S3). The *Octα2R* mRNA levels tended to be decreased in adult heads of *Octα2R* RNAi-expressing flies using *elav-Gal4*, but did not reach statistical

FIGURE 1 Response of HEK-Octα2R and CHO-Octα2R cells to OA with Gα15. (A) Graph values are shown as relative luminescence (%) of the maximum AEQ response at 10^{-5} M OA. Data represent the mean ± SEM of three independent experiments each performed in duplicate. Asterisks mean a significant difference from the control (Ctrl) using the Dunnett test (*$p < 0.05$ and **$p < 0.01$) in multiple comparisons. Response of HEK-Octα2R and CHO-Octα2R cells to other biogenic amines. (B) Each biogenic amine was administrated at 10^{-5} M. Values are shown as relative luminescence (%) of the AEQ response at OA application. Data represent the mean ± SEM of three independent experiments each performed in duplicate. Asterisks mean a significant difference from the control (Ctrl) using the Dunnett test (**$p < 0.01$) in multiple comparisons. Ctrl; control, OA; octopamine, TA; tyramine, DA; dopamine, NA; noradrenaline, SY; synephrine. Antagonist evaluation in HEK-Octα2R. (C) Each antagonist was administrated at 10^{-9}–10^{-5} M along with 10^{-5} M OA. The inhibitory activity is shown as residual luminescence (%) to OA-induced luminescence (100%). Data represent the mean ± SEM of three independent experiments each performed in duplicate. Asterisks mean a significant difference from each OA-induced luminescence using the Dunnett test (*$p < 0.05$ and **$p < 0.01$). PA; phentolamine, EP; epinastine, YO; yohimbine

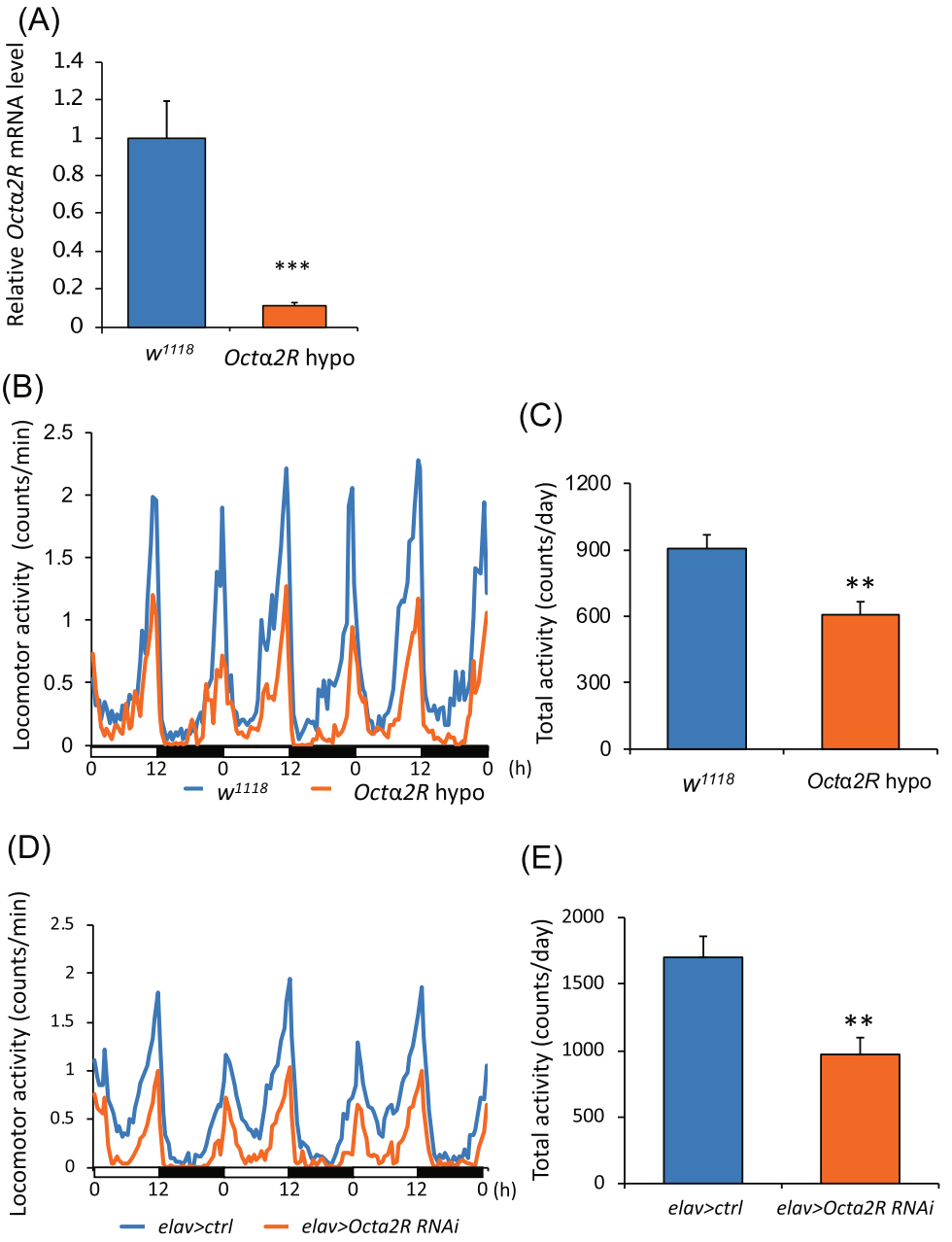

FIGURE 2 *Octα2R* hypo and pan-neuronal knockdown mutants showed decreased activity counts. (A) Relative mRNA level of *Octα2R* in the heads of w^{1118} (blue) and *Octα2R* hypo (BDSC 18659) (orange). Data represent average ± SEM which normalized to *GAPDH2* (n = 3). Student's *t*-test detected a significant difference. (***$p < 0.001$). (B) Locomotor activity profiles in 1 min interval for w^{1118} (blue) or *Octα2R* hypo (orange). Locomotor activity was monitored over 3 days in the 12 h:12 h/light: dark (LD) conditions. (C) Total daily locomotor counts for w^{1118} (blue) and *Octα2R* hypo (orange). Data represent average ± SEM (n = 32, 22). Student's *t*-test detected a significant difference (**$p < 0.01$). (D) Locomotor activity profiles in 1 min interval for *elav* > ctrl (blue) or *elav* > *UAS-Octα2R RNAi* (orange). Locomotor activity was monitored over 3 days in LD conditions. (E) Total daily locomotor counts for *elav* > ctrl (blue) or *elav* > *UAS-Octα2R RNAi* (orange). Data represent average ± SEM (n = 16). Student's *t*-test detected a significant difference (**$p < 0.01$)

significance (Figure S4A). On the other hand, The *Octα2R* mRNA levels were significantly decreased in *Octα2R* knockdown flies using *Actin-Gal4*, which is a *Gal4* driver of all cells (Figure S4B). Pan-neuronal knockdown of *Octα2R* decreased locomotor activity similarly to *Octα2R* hypo (Figure 2D,E). These data suggested that *Octα2R* expressed in neurons regulated locomotor activity.

3.3 | Octα2R is expressed in the central complex, PI and MB

OA neurons project to various region including the optic lobe, the mushroom body (MB), the central complex.[19] The expression pattern of each OA receptor is showed. For example, *Oamb* is expressed mainly in MB and the broad brain region.[6] However, the expression region of *Octα2R* is unknown in the central nervous system (CNS) of *Drosophila*. To examine expression patterns in adult CNS, we used the *T2A-Gal4* line in which *T2A-Gal4* sequence is inserted to the 3′ terminus of *Octα2R* coding sequence and the *Trojan-Gal4* line, in which MiMIC cassette is exchanged by *T2A-Gal4*-containing cassette.[20,21] These lines were crossed with *UAS-mCD8::GFP*. We confirmed that both lines labeled the pars intercerebralis (PI), the ellipsoid body (EB) of the central complex, the MB and the ventral nerve code (VNC) (Figure 3). To confirm whether these drivers were expressed in the same cells, we used *Octα2R Trojan-Gal4* and *Octα2R T2A-LexA*. We found that *Octα2R T2A-LexA* and *Octα2R Trojan-Gal4* labeled the

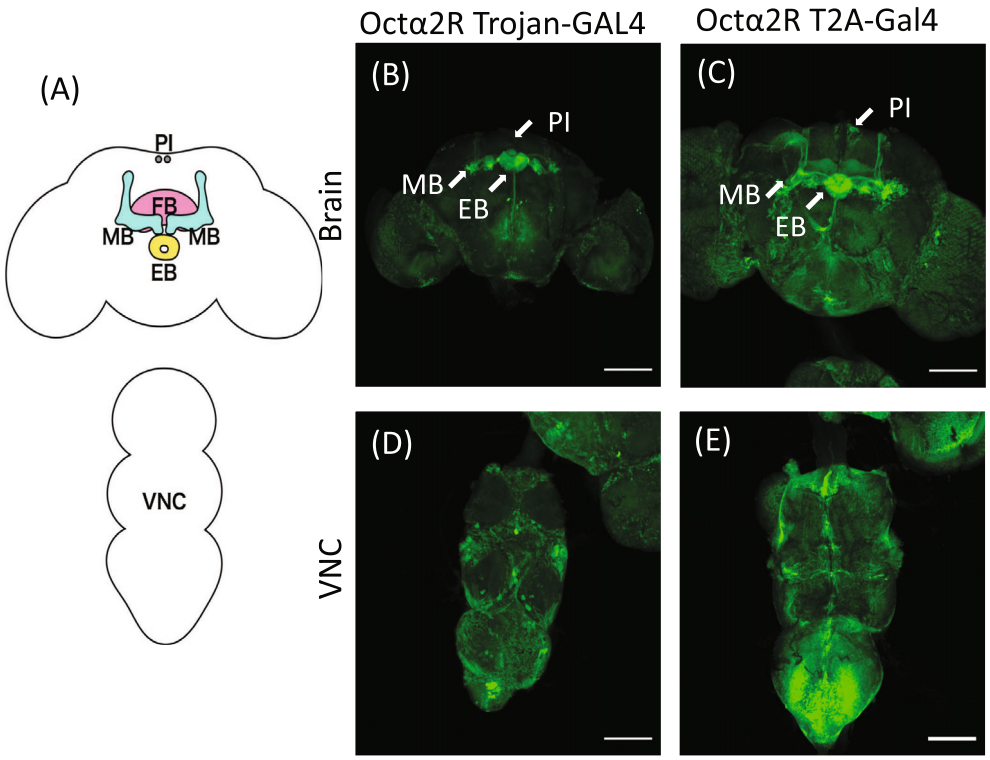

FIGURE 3 Expression patterns of *Octα2R-Gal4* drivers in CNS. (A) A schema of the anatomy of the brain and VNC in *Drosophila*. (B–E) The expression patterns of the *Octα2R-Gal4* lines were visualized with a GFP in the brain (B, C) or VNC (D, E). Expression was characterized by crossing *Octα2R Trojan-Gal4* (B, D) or *Octα2R T2A-Gal4* (C, E) with *UAS-mCD8::GFP*. Scale bar = 100 μm

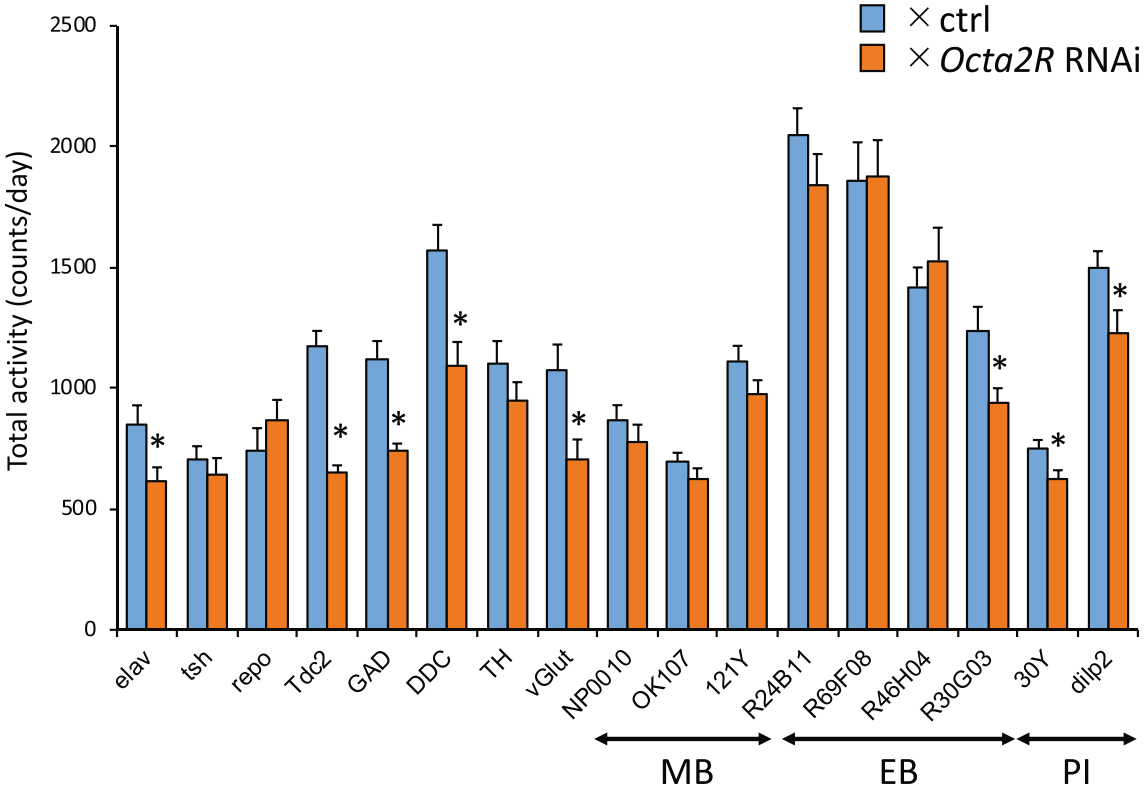

FIGURE 4 Knockdown of *Octα2R* in PI region decreased locomotor activity. Total daily locomotor counts for progeny collected from between 17 Gal4 drivers and *UAS-Octα2R RNAi* (orange). For controls, each Gal4 driver was crossed to ctrl flies (blue). Data represent average ± *SEM* (n = 10–16). Student's *t*-test detected a significant difference (*$p < 0.05$)

same cell types of EB neurons, but did not find any PI cells in which these two drivers were co-expressed (Figure S5). These results suggested that *Octα2R* is expressed in PI region, the EB and the MB.

3.4 | Octα2R knockdown in PI decreased locomotor activity

We confirmed that *Octα2R* expressed in the MB, the EB and PI. For other OA receptors, the relationship between the receptor-expressing regions and physiological functions is reported. For example, *Oamb* expressed in PI is related to sleep regulation and *Octα2R* expressed in MB α'β' Kenyon cells is related to the formation of the anesthesia-resistant memory.[11,22] To detect the region that was related to the regulation of locomotor activity, we screened Gal4 drivers. Knockdown of *Octα2R* in *dilp2-Gal4* and *30Y-Gal4*, which are PI labeling drivers (Figure S6), decreased the locomotor activity (Figure 4). To identify the neuronal types in which *Octα2R* is expressed, we performed knockdown *Octα2R* by using neurotransmitter drivers and found a decrease in locomotor activity in various types of neurons (Figure 4). These results suggest that the *Octα2R* expressed in PI regulates the locomotor activity.

3.5 | Octα2R is involved in hyperactivity during starvation

OA signaling has a relationship with feeding behavior. OA signaling has a key role in transmitting information about food, such as sweetness and nutrition and promotes hyperactivity during starvation.[23] To investigate whether *Octα2R* is involved in starvation-induced hyperactivity, we measured the locomotor activity under starvation condition, in which the food contains only 1% agarose. Starvation induced hyperactivity in wild type (w^{1118}) flies, but not in *Octα2R* hypo (Figure 5A,B). These results suggested that *Octα2R* is involved in starvation-induced hyperactivity.

3.6 | Octα2R is involved in the regulation of grooming

In the locomotor activity data recorded by the DAM, *Octα2R* hypo showed a characteristic activity peak (Figure 6A). A frequency of locomotor activity peak which is over 30 counts per 1 min was increased in *Octα2R* hypo, despite reduced total daily activity (Figure 6B). The over 30 counts per 1 min in DAM data were reported to indicate grooming on the IR beam.[24] Flies spend their time near food in glass tubes, therefore detection sensitivity of grooming in the central part of the tube, where infrared beam detector locates, is low in this condition.[16] For more detailed analysis, we used a multibeam monitor with 17 IR beams across the tube (Figure 6C).[25] We found that over 30 counts per 1 min was significantly decreased in *Octα2R* hypo (Figure 6D,E).

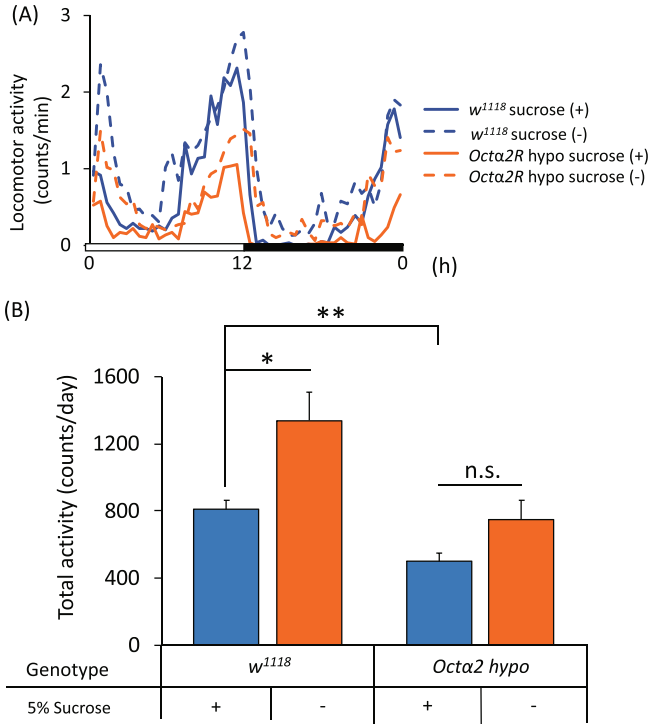

FIGURE 5 *Octα2R* hypo suppressed starvation-induced hyperactivity. (A) Activity profiles in 1 min intervals for w^{1118} (blue) and *Octα2R* hypo (orange) in sucrose- (dot) or sucrose+ (line) conditions (n = 10–13). Locomotor activity was monitored 1 day after the change of food in the 12-h light: dark cycle (LD) conditions. (B) Total daily locomotor counts for w^{1118} or *Octα2R* hypo under sucrose- (orange) or sucrose+ (blue) conditions. Data represent average ± SEM. One-way ANOVA with Bonferroni's multiple comparison test detected a significant difference (**$p < 0.01$, *$p < 0.05$ n.s.: $p \geq 0.05$)

Previous reports showed that grooming was increased in decapitated flies treated with OA.[26] To confirm that whether *Octα2R* hypo response to OA, we performed grooming assay using decapitated flies (Figure 7A). *Octα2R* hypo showed increased grooming time in conditions without OA administrations (Figure 7B). In wild type (w^{1118}) treated with OA, the time of foreleg grooming was increased (Figure 7B). In contrast, *Octα2R* hypo treated with OA did not show significant increased time of grooming (Figure 7B). These results suggested that OA signaling via Octα2R inhibits grooming.

4 | DISCUSSION

Qi and colleagues have reported that activation of Octα2R reduced intracellular cAMP levels using a cAMP assay, while it does not affect Ca^{2+} levels.[14] Consistent with these results, we found that *Octα2R*-expressing cells showed no response to OA at concentrations from 10^{-10} to 10^{-3} M in AEQ assays without Gα15 subunit. Although Octα2R is a Gi protein-coupled receptor, in this study, use of AEQ assays with the Gα15 subunit enables the response of Octα2R to

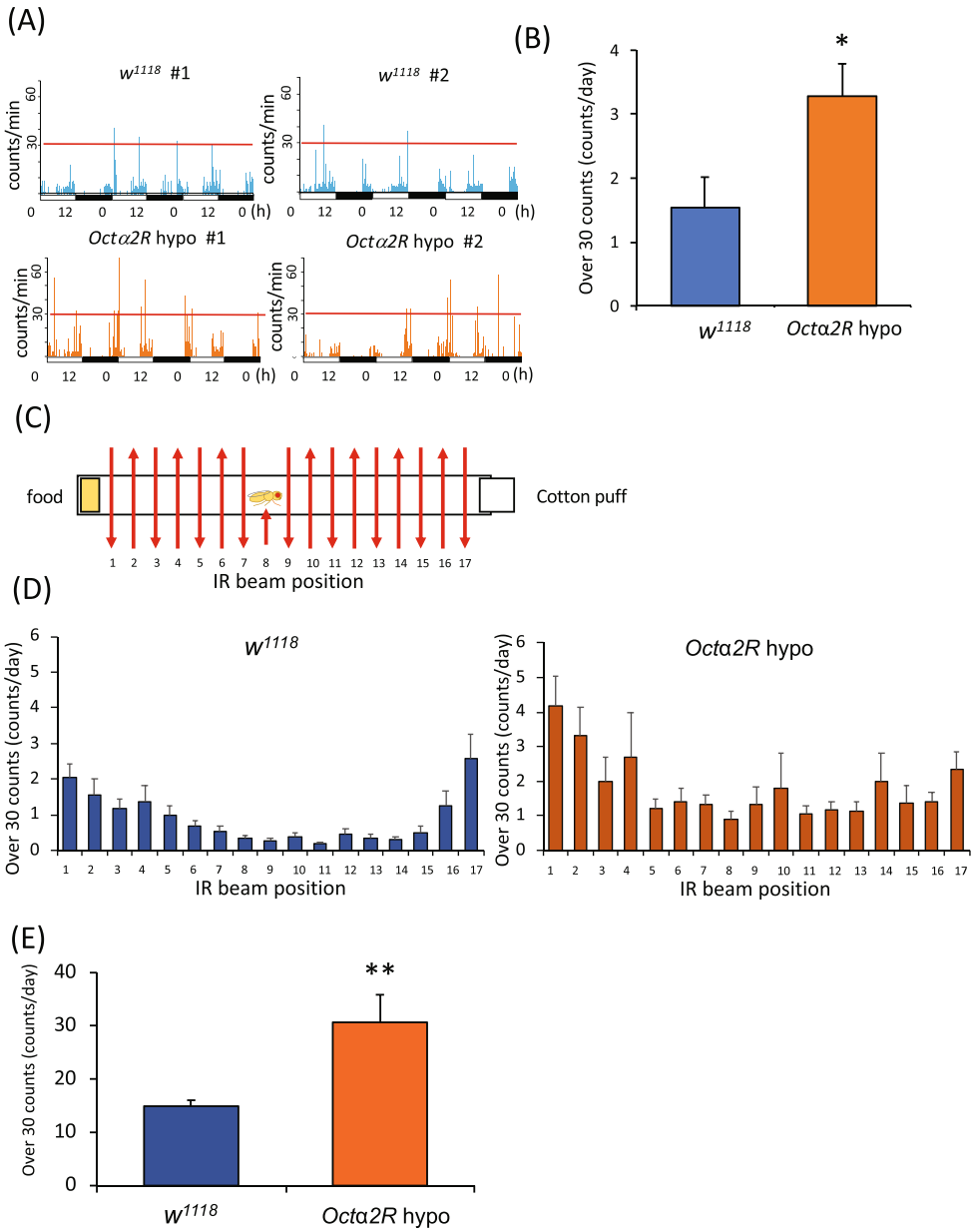

FIGURE 6 *Octα2R* hypo increased grooming frequency. (A) Representative locomotor records of individual w^{1118} and *Octα2R* hypo by DAM system. The red line indicates 30 counts/min. (B) Average of frequency over 30 counts for w^{1118} (blue) or *Octα2R* hypo (orange). Data represent average ± SEM (n = 32). Student's *t* test detected a significant difference ($^{*}p < 0.05$). (C) Schematic of Multibeam Monitor. 17 IR beams can detect the activity and position of fly in tubes. (D, E) Average of frequency over 30 counts of each beam position and total of all for w^{1118} (blue) or *Octα2R* hypo (orange). Student's *t*-test detected a significant difference ($^{**}p < 0.01$)

octopamine to be detected as intracellular Ca^{2+}-dependent luminescence.

This study showed that Octα2R signaling regulates the locomotor activity, grooming and starvation-induced hyperactivity. Results in Figure 2 suggested that *Octα2R* in neurons regulated the locomotor activity. Most of *Octα2R* knockdown flies using neurotransmitter Gal4 drivers decreased the locomotor activity, suggesting that the function of *Octα2R* is not limited to specific neurons (Figure 4). Two drivers, *dilp2-Gal4* and *30Y-Gal4*, showed a significant decrease in locomotor activity (Figure 4). The brain region where the expression of these two drivers overlapped was the PI (Figure S6). In addition, we found that two different *Octα2R-Gal4* drivers labeled PI region (Figure 3). These results suggested that *Octα2R* expressed in the PI is involved in the regulation of locomotor activity. On the other hand, *30Y-Gal4* and *dilp2-Gal4* were not specific for the PI. These results did not exclude the possibility that *30Y-Gal4* and *dilp2-Gal4*-expressing cells other than PI neurons may also affect locomotor activity. Knockdown of *Octα2R* in *R30G03-Gal4* expressing cells decreased the locomotor activity. *R30G03-GAL4* is expressed in not only the EB but also PI region. The PI is projected by octopaminergic neurons, and it has been reported that activation and inhibition of PI cells decreases and increases the amount of sleep, respectively.[22] To elucidate the details of the effect of *Octα2R* on PI region, the recording of neuronal activity in the PI region using Ca^{2+} imaging and electrophysiology with the *Octα2R* mutants is required.

Octα2R hypo did not show hyperactivity during starvation (Figure 5). In humans and many other organisms, activity is increased and sleep is reduced during starvation. In *Drosophila*, hyperactivity during starvation is regulated by OA signaling.[23] Adipokinetic hormone (AKH), an analog of glucagon secreted during starvation,

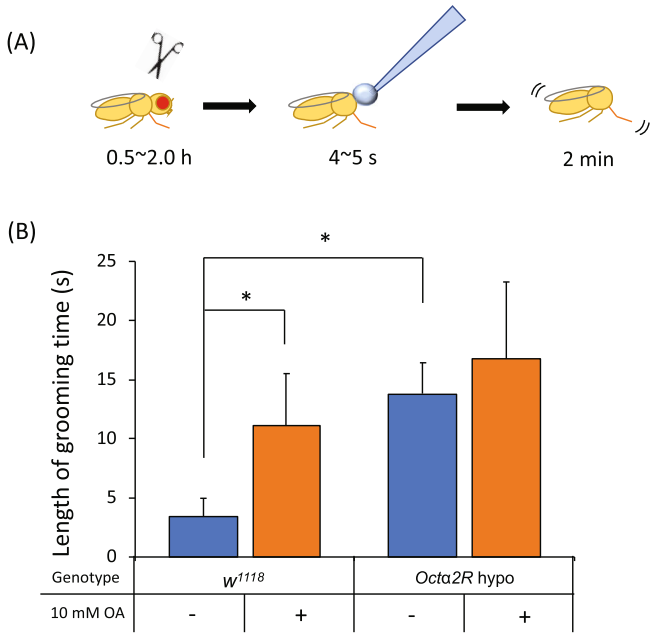

FIGURE 7 Decapitated *Octα2R* hypo increased grooming length of time. (A) A schematic of grooming assay. Decapitated flies were treated with PBS (blue) or 10 mM OA (orange) in 4 ~ 5 s using P20 micropipette. (B) The average length of grooming time during 2 min for w^{1118} or *Octα2R* hypo. Data represent average ± SEM (n = 7–11). One-way ANOVA ($F = 3.2439$, $p = 0.0356$) with Bonferroni's multiple comparison test (*$p < 0.05$)

activates octopaminergic neurons and causes hyperactivity.[4] It is possible that Octα2R received OA signal activated by AKH during starvation.

The time and frequency of grooming were increased in *Octα2R* hypo, suggested that the Octα2R signaling in the VNC involved in grooming behavior (Figures 6 and 7). Furthermore, *Octα2R* hypo treated with OA did not increase time of grooming. These results suggest that Octα2R inhibits OA signaling.

In addition, the octopamine synthase *Tβh* knockout mutants increased the number of grooming despite the increased amount of sleep.[27] In this study, the amount of the locomotor activity measured by DAM system was reduced, but over 30 counts of activity was increased (Figures 2 and 6). The grooming behavior may inhibit locomotor activity. We measured foreleg grooming of decapitated flies by video analysis (Figure 7). Elucidation of detail of regulation mechanism of grooming by Octα2R signaling is possible to provide a novel link between grooming behavior and locomotor activity.

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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