Functional characterization of dopamine transporter in vivo using Drosophila melanogaster behavioral assays

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**INTRODUCTION**

The neurotransmitter dopamine is crucial for arousal, motor function and memory. Further, accurate temporal and spatial regulation of dopamine signaling is required for these activities. The dopamine transporter (DAT) mediates reuptake of dopamine from the synaptic cleft, and has a key role in limiting dopamine signaling. DAT is inhibited by therapeutic agents such as Ritalin (methylphenidate) or psychostimulant drugs of abuse such as amphetamine and cocaine, demonstrating its importance in regulating behaviors. Besides directly regulating dopamine signaling, DAT is thought to participate in gating the spillover of dopamine (Floresco et al., 2003; Cragg and Rice, 2004). This spillover of dopamine from a release site into the extrasynaptic space (i.e., volume transmission) has been reported in mammals, and is proposed to modulate brain function.

The Drosophila dDAT mutant, fumin (fmn), sleeps less than wild-type flies due to an increase in postsynaptic dopamine signaling (Kume et al., 2005). In vivo voltammetric studies have shown that the fumin mutants are defective for dopamine clearance (Makos et al., 2009). Increased dopamine signaling in fumin mutants also alters their activity, metabolic rate, longevity, temperature sensitivity and sensitivity to dietary calories (Ueno et al., 2012a,b; Yamazaki et al., 2012). A similar effect is seen in mammals, where deletion of the dopamine transporter gene results in a decrease in sleep (Wisor et al., 2001). Recent studies have identified a dopamine pathway for controlling fly sleep (Liu et al., 2012b; Ueno et al., 2012c) which is distinct from other dopamine pathways responsible for memory formation (Aso et al., 2010), and the response to ethanol (Kong et al., 2010).

Due to its powerful genetics, Drosophila is an ideal model organism for identifying genes or neural circuits underlying specific behaviors. Currently, little is known about the dynamics and distribution of the released dopamine. For example, it is unknown how far dopamine, released from specific sites, diffuses, and how long the effects of released dopamine last. To investigate the effects of released neurotransmitters, genetic perturbation of plasma membrane transporters can be used to great advantage for controlling the intensity of extracellular signals.

In this report, we describe effects of altered dDAT expression on fly behavior. Using tissue specific knockdown and rescue experiments, we found that dDAT expression in a subset of dopaminergic neurons is sufficient for normal sleep. In addition, we also found that dDAT expression in glial cells could rescue the short sleep phenotype of fumin mutants. dDAT expression in postsynaptic sites for memory formation (the mushroom bodies), abolished olfactory memory formation. These results shed light...
on the physiological function of dDAT in dopamine signaling and in regulating extrasynaptic dopamine spillover in vivo.

**MATERIALS AND METHODS**

**FLY STOCKS**

Flies were reared on a standard corn meal, yeast, glucose agar medium at 24.5°C under a 12 h:12 h light:dark cycle. The fmn (fumin) mutant was described previously (Kume et al., 2005). TH-GAL4 was a gift from J. Hirsh. 104Y was a gift from D. Armstrong. NP lines were obtained from the Kyoto Drosophila Genetic Resource Center. To remove possible modifiers and allow comparisons in a common genetic background, we outcrossed all the alleles into the w1118 or fmn background over at least five consecutive generations. As controls we used w1118 or fmn flies. The transgenic RNA interference (RNAi) line for DAT (Transformant ID: 106961), UAS-Dicer-2 flies (Stock numbers: 60008) and the w1118 (60000) which is the genetic background of the RNAi line were obtained from the Vienna Drosophila RNAi Center (VDRC), Vienna, Austria (Dietzl et al., 2007).

**SLEEP ANALYSIS**

Flies were placed individually in glass tubes (length, 65 mm; inside diameter, 3 mm) containing 1% agar and 5% sucrose at 24.5°C. Male flies were used for sleep analysis. They were entrained for at least 3 d to LD conditions before being transferred to constant dark (DD) conditions. Locomotor activity was monitored by recording infrared beam crossings by individual flies in 1 min bins using the Drosophila activity monitoring system (Trikinetics, Waltham, MA, USA). Based on previous reports (Hendricks et al., 2000; Shaw et al., 2000), sleep was defined as periods of inactivity lasting 5 min or longer. For the pharmacological manipulations, 3-iodo-d-tyrosine and RU486 were obtained from Sigma-Aldrich. Flies were transferred to vehicle (1% agar + 5% sucrose) with 3-iodo-d-tyrosine and RU486 were obtained from Sigma-Aldrich. Daily sleep time was defined as periods of inactivity separated by electrical tape of ~200 μm thickness, so that the brain sample was not flattened. Immunolabeled adult brains were imaged under a Leica TCS SP2 AOB5 confocal microscope using 20× magnification. Z-stack images were scanned at 2 μm section intervals with a resolution of 1024 × 1024 pixels.

For quantification of dDA1 signal, a confocal slice image containing the holizontal lobes of the mushroom body was selected from a stack. The average pixel intensity values in the horizontal lobes and the adjacent control region (aimpr: anterior inferior medial protocerebrum) was measured by NIH ImageJ. The signal of the dDA1 in horizontal lobes was normalized by the control region of an identical confocal slice.

**AVERSIVE ODOR MEMORY**

Olfactory conditioning with two odors (4-methylcyclohexanol and 3-octanol) was performed as described previously (Tully and Quinn, 1985). Briefly, approximately 100 flies were placed in a training chamber where they were exposed to odors and electric shocks. One of the aversive odors, OCT (3-octanol, Sigma) or MCH (4-methylcyclohexanol, Sigma), was paired with 12 pulses of electrical shocks (60 V DC) for 1 min, whereas the other was not. Testing was performed for 2 min after the training by placing flies at a choice point in a T-maze between the two odors for 2 min. If no flies moved to either arm of the T-maze or if a small number of flies were trapped in the middle compartment, it indicated that the flies did not choose either odor. A performance index (PI) was calculated so that a 50:50 distribution (no memory) yielded a PI of 0 and a 0:100 distribution away from the shock-paired odor yielded a PI of 100. Individual PIs were always the average of two experiments where the shock-paired odor was alternated.

**IMMUNOHISTOCHEMISTRY**

Brains of adult flies were dissected in cold PBS and fixed in 4% paraformaldehyde in PBS for 20 min at room temperature. After three 20 min washes in 0.3% Triton X-100 in PBS, samples were blocked and penetrated in 5% normal sheep serum (NSS) for 30 min at room temperature. Samples were then incubated in a primary antibody solution in 5% NSS at 4°C overnight. The following primary antibodies were used in this study: chicken anti-GFP (1:1000; Abcam), rabbit anti-TH (1:25 Pel-Freez), rat anti-DAT (1:1000; Millipore) and rabbit anti-dDA1 (1:1250; gift from Dr. Fred W. Wolf). After three 20 min washes in 0.3% Triton X-100 in PBS, brains were incubated at 4°C overnight in a secondary-antibody solution in 5% NSS. The following secondary antibodies were used: Alexa Fluor 568 goat anti-rat IgG (1:200; Invitrogen), Alexa Fluor 488 goat anti-chicken IgG (1:200; Invitrogen) and Alexa Fluor 568 goat anti-rabbit IgG (1:200; Invitrogen). After three 20 min washes in PBS, brains were mounted using PermaFluor (Thermo Scientific) between two coverslips separated by electrical tape of ~200 μm thickness, so that the brain sample was not flattened. Immunolabeled adult brains were imaged under a Leica TCS SP2 AOB5 confocal microscope using 20× magnification. Z-stack images were scanned at 2 μm section intervals with a resolution of 1024 × 1024 pixels.
investigate behavioral effects of dDAT expression, we examined the sleep phenotype of dDAT knockdown flies. Using GAL4/UAS system (Brand and Perrimon, 1993), we expressed dsRNA to induce RNAi in a cell specific manner. As shown in Figures 1A, B, flies with pan-neuronal knockdown of dDAT using Elav-GAL4 displayed a significant sleep reduction compared with control flies. The total daily sleep of dDAT knockdown using various GAL4 drivers (n = 16–32 flies). Data are represented as the mean ± s.e.m. * P < 0.05; one-way ANOVA with Tukey-Kramer HSD post hoc test. (D) Efficiency of dDAT knockdown. The expression levels of dDAT gene in the head of flies expressing dDAT RNAi transgene by GAL4 drivers are expressed as the relative values to the control flies. n = 3 for each group. Data are represented as the mean ± s.e.m. * P < 0.05; one-way ANOVA with Tukey-Kramer HSD post hoc test. (E) Expression patterns of GAL4 drivers for dopaminergic neurons in the central brain. The GAL4 expression for each driver was visualized using UAS-mCD8::GFP (green). The brain was stained with antibody to nc82 to stain the neuropil (magenta). PAM, protocerebral anterior medial; PAL, protocerebral anterior lateral. Scale bars = 50 µm.
TH-GAL4 or Ddc-GAL4 both showed decreased dDAT mRNA level to one-fourth of control, which is consistent with the previous report that showed dDAT expression is restricted to dopaminergic neurons. These results suggest that GAL4 expression pattern rather than GAL4 expression level contribute to the sleep phenotype. To further investigate the effect of dDAT knockdown in a subset of dopaminergic neurons, we crossed with HL5-, HL7-, HL9-GAL4 drivers. These drivers have GAL4 expression in PAM clusters whereas other clusters such as PPL1 neurons are sparcely labeled (Claridge-Chang et al., 2009; Figure 1E). Despite dense expression in PAM clusters, dDAT knockdown using these drivers showed no significant sleep reduction. We further knocked down dDAT in mushroom body and fan-shaped body. These neuropiles receive projection of dopaminergic neurons and contribute to arousal effect of dopamine (Andretic et al., 2008; Liu et al., 2012b; Ueno et al., 2012c). Consistent with Portzgen et al. that showed restricted dDAT expression in dopamine neurons, dDAT knockdown in these postsynaptic neurons has no effect on sleep phenotype. Taken these results together, we hypothesize that dDAT expression in a subset of dopamine neurons is sufficient for the reuptake of dopamine from synaptic cleft then shut down postsynaptic dopamine signaling for normal sleep.

**dDAT EXPRESSION IN A SUBSET OF DOPAMINE NEURONS CAN RESCUE THE SHORT SLEEP PHENOTYPE IN fumin MUTANTS**

To further investigate the effect of dDAT expression on fly sleep, we rescued dDAT in fumin mutant. Using GAL4 drivers which labels a subset of dopamine neurons, we expressed dDAT in dopaminergic neurons in fumin mutant. Although we could not observe significant sleep reduction with dDAT knockdown using TH-GAL4 (Figure 1C), fumin with dDAT expression in TH-GAL4 labeled neurons showed significant increase of sleep (Figure 2A). As described above, dopaminergic neurons except for PAM cluster neurons are labeled by TH-GAL4 line. Other GAL4 drivers which labels a subset of dopaminergic neurons, HL5- and HL7-GAL4, could also rescue the short sleep phenotype of fumin by cell specific dDAT expression. HL5-GAL4 has dense expression in PAM cluster neurons whereas PPL1 and PPM3 cluster neurons which are one of the wake promoting dopaminergic neurons are not labeled by this GAL4 driver. HL7-GAL4 also labels PAM cluster neurons but PPL1 and PPM3 clusters are sparcely labeled (Kong et al., 2010). Based on the discrepancy between dDAT knockdown and dDAT rescue experiments, we consider that dDAT expression in a subset of dopamine neurons is sufficient to rescue the short sleep phenotype of fumin mutant. On the other hand, fumin with dDAT expression by HL9-GAL4 showed no significant increase of sleep. It might be due to its low expression level since we observed lower expression in HL9-GAL4 compared with HL5- and HL7-GAL4 drivers.

**dDAT EXPRESSION IN NON-DOPAMINERGIC CELLS CAN RESCUE THE SHORT SLEEP PHENOTYPE IN fumin MUTANTS**

Since we could rescue the short sleep phenotype of fumin by dDAT expression in different subset of dopaminergic neurons which were insensitive to dDAT knockdown, we further investigated the effect of ectopic dDAT expression in non-dopaminergic cells. We presumed if dDAT expression in a subset of dopamine neurons is sufficient for the shut down of postsynaptic dopamine signaling, it is possible to control dopamine signaling by dDAT expression in postsynaptic neurons or extrasynaptic cells such as glia.

In order to express dDAT in various regions of the brain in a fumin background, we outcrossed the GAL4 drivers to the fumin mutant. Next, we examined the effect of dDAT expression on the short sleep phenotype of the fmn mutant. We found that fmn flies with dDAT expression in non-dopaminergic neurons showed a significant sleep increase (Figure 2A). Mushroom body (Joiner et al., 2006; Pitman et al., 2006) and fan-shaped body (Donlea et al., 2011) receive dopaminergic projection and regulates sleep and arousal. Although we observed no significant changes in sleep by dDAT knockdown in these postsynaptic structure (Figure 1C), dDAT expression in these neuropile rescued short sleep of fumin mutant. In addition, dDAT expression in glial cells also rescued short sleep in fumin. To confirm that the drivers have no GAL4 expression in dopamine neurons, we expressed GFP using the GALA/UAS system and then counter stained with anti-TH antibody. As the merged signal patterns shown in Figure 2B, TH-GAL4 drives GFP expression in most, but not all, dopamine neurons. Conversely, we could not detect any merged signals when expressing GFP using the other drivers, which suggests that these drivers only expressed dDAT in non-dopaminergic neurons. Next, we analyzed the localization of ectopically expressed dDAT using immunohistochemistry. Although we could not detect endogeneous dDAT expression due to high background with the antibody, ectopic expression of dDAT in the fmn mutant using the 30Y-GAL4 driver, which has strong expression in the mushroom body, showed dDAT immunostaining in all lobes and calyx of the mushroom body (Figure 2C). These lobes and calyx are composed of axon bundles and dendrites of Kenyon cells, thus ectopically expressed dDAT appears to be transported and localized to the synaptic area.

To further investigate if the effect of dDAT expression in non-dopaminergic neurons is due to developmental changes or not, we expressed dDAT in postsynaptic neurons or glial cells using spatiotemporal expression system, TARGET system (McGuire et al., 2003). In this system, dDAT expression is suppressed at 19°C by the temperature sensitive GAL4 inhibitor GAL80° which is under the control of tubulin promoter. After shifting to 31°C, GAL80° was inactivated and dDAT expression will be induced. All the flies were raised at 19°C to adulthood. At the permissive temperature, progeny from the experimental crosses showed similar sleep patterns to control flies. The temperature shift to 31°C significantly increased sleep time of flies which has temporal dDAT expression in mushroom body and glial cells (Figure 3). These results suggest that dDAT expression in non-dopaminergic neurons, such as the mushroom body or glial cells, can rescue the short sleep phenotype in fmn without developmental changes.

**ENHANCEMENT OF EXTRACELLULAR Dopamine CAUSES DOWNREGULATION OF THE D1-LIKE RECEPTOR dDA1**

The D1-like receptor dDA1 is a dopamine receptor expressed in the mushroom body and in central complex structures such as the ellipsoid body and fan-shaped body. dDA1 expression in the mushroom body mediates olfactory memory (Kim et al., 2007). As shown in Figure 4A, fmn mutants demonstrated decreased...
FIGURE 2 | Effect of dDAT expression on sleep. (A) The short sleep phenotype of fmn was rescued by expression of dDAT in various brain regions. Black bar indicates control fly without dDAT mutation. Blue bars indicate fmn GAL4 drivers without UAS-dDAT. Red bars indicate fmn GAL4 drivers with UAS-dDAT (n = 16–32 flies). Error bars represent s.e.m. * P < 0.05; two-sided Student’s t-test. (B) Expression patterns of GAL4 drivers in the central brain. The GAL4 expression for each driver was visualized using UAS-mCD8::GFP (green). Dopamine neurons were visualized using anti-TH antibody counterstaining (magenta). PPM, protocerebral posterior medial; PPL, protocerebral posterior lateral. Scale bars = 50 µm. (C) dDAT expression in the fly brain detected with immunohistochemistry. dDAT expression in fmn flies and fmn 30Y>dDAT flies are shown. Left panel shows anterior view and right panel shows posterior view. Scale bars = 50 µm.

**dDA1** expression in the mushroom body. Quantification of dDA1 expression based on image analysis of immunohistochemistry results (Knapek et al., 2011) also confirmed reduced dDA1 expression in the mushroom body (Figure 4B). Administration of a dopamine synthesis inhibitor (tyrosine hydroxylase inhibitor) rescued the short sleep phenotype of fmn mutants (Figure 4C) and increased dDA1 expression in the mushroom body (Figures 4D,E). Conditional rescue of dDAT in all neurons using the GeneSwitch system (Osterwalder et al., 2001) increased dDA1 expression in the mushroom body (Figure 4F). Without administration of RU486, there was also significant leakage of dDAT expression in the mushroom body as previously pointed out (Poirier et al., 2008). To investigate whether the decreased dDA1 expression was the result of short sleep or increased dopamine signaling, we checked the dDA1 expression in another sleep mutant. Both calcineurin knockout and knockdown result in the short sleep phenotype (Nakai et al., 2011; Tomita et al., 2011), but dDA1 expression in the calcineurin mutants was not reduced (Figure 4G). Taken these results together, increased dopamine signaling, rather than short sleep in fmn
FIGURE 3 | Effect of spatiotemporal dDAT expression on sleep.
(A) Sleep profiles and daily sleep amounts for flies expressing dDAT in the mushroom body using the TARGET system. (B) Sleep profiles and daily sleep amounts for flies expressing dDAT in glial cells using the TARGET system. (C) Total daily sleep amounts for flies expressing dDAT in the mushroom body using the TARGET system. Daily sleep at 31°C is shown. (D) Total daily sleep amounts for flies expressing dDAT in glial cells using the TARGET system. Daily sleep at 31°C is shown. Data are represented as the mean ± s.e.m. * P < 0.05; one-way ANOVA with Tukey-Kramer HSD post hoc test (n = 32 flies).

mutants, downregulates dDA1 expression in the mushroom body. Thus dDA1 expression level is applicable as an indicator of the dopamine signaling intensity.

dDAT EXPRESSION RESCUES DOWNREGULATION OF dDA1
To examine the effect of dDAT expression on dopamine signaling, we verified the dDA1 level in fmn with dDAT expression. As shown in Figure 5, dDAT expression in a subset of dopaminergic neurons with TH-GAL4 rescued dDA1 expression in fmn. In addition, dDAT expression in the mushroom body (121Y-GAL4, 11Y-GAL4), glial cells (dEAAT1-GAL4) and pars intercerebralis (c767-GAL4) increased dDA1 expression in the mushroom body, indicating decrease of dopamine signaling. These results are consistent with the rescued sleep phenotype in fmn by ectopic
dDAT expression (Figure 2A). On the other hand, npf-GAL4 driven dDAT expression in fmn flies could not rescue the short sleep phenotype (Figure 2A) and showed no significant changes in dDA1 expression in the mushroom body (Figure 5). These results indicate that the ectopic expression of dDAT suppresses the increased dopamine signaling in the fmn mutant.

THE EFFECT OF dDAT EXPRESSION ON LEARNING AND MEMORY

Next, we investigated the effect of dDAT expression on aversive olfactory memory formation. Dopamine signaling mediates associative olfactory memory via dDA1 receptor in mushroom body (Kim et al., 2007). As previously reported (Zhang et al., 2008), fmn mutants have an impaired aversive olfactory memory due to
excessive dopaminergic signaling (Figure 6A). dDAT expression in glial cells (using Repo-GAL4) as well as dopamine neurons (using TH-GAL4) promoted memory formation in fmn mutants although we could not find statistical significance. Surprisingly, however, when some GAL4 drivers which are expressed in the mushroom body (such as 30Y-GAL4 and OK107-GAL4) were used to drive dDAT, these flies did not demonstrate aversive olfactory memory (Figure 6A). 30Y-GAL4 and OK107-GAL4 have broad and strong GAL4 expression in the mushroom body (Aso et al., 2009), which suggest that post-synaptic strong dDAT expression abolished dopamine signaling after activation of dopamine neurons with electric punishment. Another possibility is that strong expression of dDAT in the mushroom body caused a developmental defect in the Kenyon cells since flies with chemical ablation of mushroom body are unable to perform in a classical conditioning paradigm (de Belle and Heisenberg, 1994). To exclude this possibility, we checked the memory performance of flies with temporal dDAT expression using Elav-GeneSwitch. Without administration of RU486, there was significant leakage of dDAT expression which may have affected memory performance (Figures 6B,C), since there was a slight improvement in the PI even in flies fed with vehicle (compare control of Figure 6A and vehicle of Figure 6B). However, administration of RU486 results in the impairment of memory performance in a dose dependent manner. Furthermore, the flies fed with 500 µM of RU486 did not demonstrate associative memory (Figure 6B). These results suggest that strong expression of dDAT at the post-synaptic site results in abolition of dopamine signaling after activation of dopamine neurons.

**DISCUSSION**

In this study, we investigated the effect of dDAT expression on sleep and memory. We showed that dDAT expression in a subset of dopaminergic neurons is sufficient for normal sleep. We also demonstrated that dDAT expression in non-dopaminergic neurons rescue the short sleep phenotype of fmn mutant through the reduction of dopaminergic signaling. Finally, we also demonstrated that postsynaptic strong dDAT expression results in the abolishment of memory formation.

To investigate the role of dDAT on sleep, we set out to map the neural circuit in which the dDAT expression mediates sleep. Mushroom body receives projection of dopaminergic neurons and controls fly sleep (Joiner et al., 2006; Pitman et al., 2006). Previous study demonstrated that dDA1 expression in mushroom body mediates arousal effect of caffeine and methamphetamine (Andretic et al., 2008). Dopaminergic neurons also have projection to sleep promoting neuropeptide fan-shaped body and regulates sleep (Donlea et al., 2011; Liu et al., 2012b; Ueno et al., 2012c). Pan-neuronal knockdown of dDAT resulted in decrease of sleep similar to fmn mutant. On the other hand, flies with dDAT RNAi in glial cells or postsynaptic neurons showed no sleep changes which is consistent with the previous report that demonstrated restricted dDAT expression in dopaminergic neurons (Porzgen et al., 2001). We also demonstrated that dDAT knockdown in a subset of dopaminergic neurons using TH-, HL5-, HL7- and HL9-GAL4 is not sufficient to cause a short sleep phenotype although Ddc-GAL4 driven RNAi resulted in a sleep decrease. TH-GAL4 labels most dopaminergic neurons including fan-shaped body projecting PPL1 and PPM3 clusters but lacks GAL4 expression in PAM clusters. On the other hand, HL5-, HL7- and HL9-GAL4 drivers heavily labels PAM clusters. It is possible that GAL4 expression pattern in dopaminergic neurons is responsible for sleep regulation by dDAT since Ddc-GAL4 driven RNAi resulted in a sleep decrease. Another explanation is that dDAT expression in serotonin positive neurons contribute to sleep regulation since Ddc-GAL4 labels some serotonin-releasing neurons (Pech et al., 2013). In contrast to the results of knockdown experiments, we demonstrated that dDAT expression in a subset of dopaminergic neurons is sufficient to rescue the short sleep phenotype in fmn mutant. Taken these results together, we concluded that dDAT expression in a subset of dopaminergic neurons is sufficient for normal sleep. It is possible that diverse cells can redundantly fulfill the task of clearing the extracellular space from dopamine since dDAT expression in PAM-cluster neurons is not required but sufficient for proper sleep. We further investigated the effect of dDAT expression in non-dopaminergic neurons. dDAT expression rescued the short sleep phenotype of the fmn mutant not only in dopamine neurons but also in other cells such as Kenyon cells and glial cells. The effect of ectopic dDAT expression on dopamine signaling was confirmed by the rescue of dDA1 expression in the mushroom body which is down-regulated in the fmn mutant. The ectopic expression of dDAT in mushroom body resulted in a poor memory performance. These results may suggest the phenotype of dDAT mutant is not only the result of a simple enhancement of physiological dopamine pathway, but also the result of an extrasynaptic volume transmission, i.e., spillover. This should be taken into account when we interpret the phenotype of a neurotransmitter transporter mutant.

Dopamine needs to be cleared from synaptic cleft to maintain the proper signaling levels. dDAT reuptake dopamine from synaptic cleft into presynaptic dopamine neurons. In Drosophila, recycling of dopamine through glial cells also regulates dopamine.
signaling (Suh and Jackson, 2007). Ebony converts dopamine to N-β-alanyl dopamine which is passed from glial cells to presynaptic neurons and then converted back to dopamine. Fly homolog of Dysbindin, a human schizophrenia susceptibility gene, regulates dopamine signaling through upregulation of Ebony (Shao et al., 2011). Although we could not find any significant sleep changes with dDAT knockdown in glial cell, glial ebony might have contribution to sleep phenotype in fmn with dDAT expression in glial cells. Another mechanism to clear dopamine from synaptic cleft is degradation of dopamine by metabolic enzyme. In Drosophila, dopamine is metabolized primarily by arylalkylamine N-acetyltransferase (aaNAT). Flies with aaNAT mutation show defects in sleep homeostasis (Shaw et al., 2000).

Since synaptic and extrasynaptic glutamate uptake transporters are enriched on multiple presynaptic and postsynaptic cells (including glial cells), neurotransmitters such as glutamate are restricted to the synaptic cleft of the release site (Seal and Amara, 1999; Danbolt, 2001). In contrast, neurotransmitters such as gamma-aminobutyric acid (GABA) and monoamines show extrasynaptic (volume) transmission which is mediated by the diffusion of transmitters through the extracellular space (Cragg and Rice, 2004; Syková, 2004; Farrant and Nusser, 2005).
of experimental methods such as microdialysis or voltammetry with DAT-knockout mice, or psychostimulants such as cocaine or methylphenidate, revealed that DAT inhibition increases the extracellular dopamine concentration (Benoit-Marand et al., 2000; Bradberry et al., 2000), although these invasive methods may affect the extracellular environment. On the other hand, using the fruit fly and its highly sophisticated genetic toolbox we can spatially and temporally control dDAT expression and analyze physiological function using behavioral assays. Park et al., studied the effect of ectopic Drosophila serotonin transporter (dSERT) expression in the fly brain and suggested additional factors may be needed for dSERT function (Park et al., 2006). By combining ectopic expression, a knockout mutant, a behavioral assay and immunohistochemistry, we revealed that ectopic dDAT expression affects the kinetics of dopamine elimination from the synaptic cleft under physiological conditions (i.e., without invasive experimental methods). Cell-type-specific ectopic expression of transporters for neurotransmitters in Drosophila will be a favorable tool by which the mechanisms underlying precise inter-cellular signaling in space and time will be elucidated.

A previous study explored the relative roles of the DAT on striatal extracellular dopamine during tonic vs. phasic activity of dopamine neurons in the substantia nigra (Floresco et al., 2003). They suggested that dopamine released during burst firing is normally rapidly removed by uptake before it can escape the synaptic cleft. Although the fly brain is tiny for electrophysiology, ectopic expression of dDAT in the fly brain might shed light on the relationship between physiological function and neural activity of dopamine neurons.

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