European Journal of Neuroscience, Vol. 20, pp. 1001–1007, 2004

Long-term but not short-term blockade of dopamine release in *Drosophila* impairs orientation during flight in a visual attention paradigm

Yizhou Ye,[1] Wang Xi,[1] Yueqing Peng,[1] Yizheng Wang[1] and Aike Guo[1,2]
[1]Institute of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue-yang Road, Shanghai 200031, China
[2]Institute of Biophysics, Chinese Academy of Sciences, 15 Da-tun Road, Chao-yang Dist., Beijing 100101, China

Keywords: long-term blockade of dopamine release, orientation, transient blockade of dopamine release, visual attention-like behavior

Abstract

Dopamine is a major neuromodulator in both vertebrates and invertebrates and has profound effects on many physiological processes, including the regulation of attention. Most studies of the functions of dopamine use models with long-term blockade of dopamine release and few effects of transient blockade have yet been reported. The goal of the present study was to determine the role of dopamine in attention-like behavior in *Drosophila* by taking advantage of the fly's orientation behavior during flight. The examination of several different transgenic flies in a single-target visual attention paradigm showed that flies lost their orientation ability if dopamine release was blocked from the beginning of the development of dopaminergic neurons. This is similar to the attention loss in mammals. However, if the blockade of dopamine release was induced during the experimental procedure, flies performed normally. Statistical analysis of the behavioral assessment showed a significant difference between long-term and transient blockade. Using the RNA interference approach, we generated flies with down-regulated J-domain protein, which is a potential cochaperone in synaptic vesicle release, to make an alternative form of long-term dopamine-blockade mutant. Behavioral assays revealed that flies with permanent J-domain protein down-regulation specifically in dopaminergic neurons have an attention defect similar to that induced by long-term blockade of dopamine release. Furthermore, dopamine depletion beginning at eclosion also caused an attention deficit. Our results indicate that prolonged but not transient blockade of dopamine release impairs visual attention-like behavior in *Drosophila*.

Introduction

Many lines of evidence in mammals show that dopaminergic neurons are involved in the regulation of a variety of integrative behaviors, including attention. Changes in the activity of dopamine systems in the frontal cortex lead to poor choice accuracy in rats performing an attention task (Puumala & Sirvio, 1998). Numerous studies in insects have shown that dopaminergic signaling modulated many aspects of physiological functions, such as hormone biosynthesis (Neckameyer *et al.*, 2001) and acute response to drugs (Torres & Horowitz, 1998; Bainton *et al.*, 2000). Behavioral assays in flies have elucidated the role of dopamine in experience-dependent courtship (Neckameyer, 1998), aggressive behavior (Baier *et al.*, 2002) and the process of aversive olfactory memory formation (Schwaerzel *et al.*, 2003). Here, we investigate the functional significance of dopaminergic neurons in the regulation of attention-like behavior of flies.

Studies in *Drosophila* have shown that the cysteine string protein associated with the secretory vesicle is required for regulated neurotransmitter release and peptide exocytosis (Umbach *et al.*, 1994; Zinsmaier *et al.*, 1994) via its interaction with Hsc70 through its J-domain as a molecular chaperone (Silver & Way, 1993). Some studies in *Drosophila* have shown that the J-domain protein (JDP), which is conserved from fly to human (Hahn *et al.*, 1999; Lee *et al.*, 2000), has a highly conserved J-domain at its N-terminus. As the orthologue of JDP in mammals plays a role in vesicle release, we propose that JDP participates as a cochaperone in vesicle release in flies. *In vitro* studies have suggested that the J-domain of JDP interacts with a member of the Hsp70 family and stimulates its ATPase activity (Inoue *et al.*, 2000), which is required for regulated neurotransmitter release and peptide exocytosis. We therefore chose this highly conserved JDP, which can be phosphorylated by dopamine application (Inoue *et al.*, 2000), as a target protein to generate an alternative form of long-term blockade of dopamine release.

Selective visual attention-like behavior has already been described in flies by employing a flight simulator. That learning mutants have an attention deficit has been demonstrated in studies of their orientation patterns which are quite different from those of the wild-type flies (Wu *et al.*, 2000; Heisenberg *et al.*, 2001). In the present study, we show that flies with a transient blockade of dopamine release exhibited the normal orientation pattern; flies that were deprived of dopamine from the beginning of the development of dopaminergic neurons or from the beginning of eclosion exhibited a pattern of attention-like orientation different from that of the control flies and flies with JDP down-regulated specifically in dopaminergic neurons performed similarly to flies with long-term blockade of dopamine release, establishing that long-term blockade of dopamine is the cause of defective flight attention-like behavior in *Drosophila*.

Correspondence: Dr Aike Guo, [1]Institute of Neuroscience, as above.
E-mail: akguo@ion.ac.cn

Received 13 November 2003, revised 14 June 2004, accepted 21 June 2004

doi:10.1111/j.1460-9568.2004.03575.x

Materials and methods

Drosophila strains

UAS-TNT-E, UAS-IMPTNT-V1-A and UAS-shi^{ts1} were used to generate dopamine-depletion models by crossing with the p{GAL4} drivers. TH-GAL4, Elav-GAL4 and GH146-GAL4 drivers were used to express certain genes in different tissues. UAS-TNT-E and UAS-IMPTNT-V1-A were from Professor O'Kane (University of Cambridge, Cambridge, UK), UAS- shi^{ts1} from Professor Tully (Cold Spring Harbor Laboratory, USA), TH-GAL4 from Professor Hirsh (University of Virginia, USA), Elav-GAL4 from Dr Deng (Fudan University, China) and GH146-GAL4 from Professor Stocker (University of Fribourg, Switzerland). The wild-type strain, Canton-S, served as the control in behavioral tests. All flies were grown on standard media at 25 °C (Guo & Gotz, 1997). Three-day-old females were randomly selected for all behavioral experiments. To examine the effects of thermal activation on their behavior, flies with a temperature-sensitive mutation (TH-GAL4/UAS-shi^{ts1}) or the control strain (Canton-S) were raised until pupation or eclosion at 25 °C, transferred to a culture room pre-warmed to 30 °C and kept at this temperature until the end of the experiment.

Behavioral paradigm and evaluation

We used a flight simulator to investigate the flies' orientation to a visual object. A test fly was held, by a 0.2-mm wire glued to its head and thorax, in a fixed position and orientation at the center of the arena of the flight simulator. The arena wall consisted of white paper with a vertical black bar (12° width and 40° length) (Fig. 1A). The tested fly was allowed to control, by its intended turns, the rotational speed of the arena (i.e. speed proportional to the fly's yaw torque around its vertical body axis). This enabled the fly to stabilize the rotational movements of the panorama (i.e. to fly straight) and to adjust flight directions with respect to a visual landmark. The fly's flight direction was recorded continuously at a sampling frequency of 20 Hz (Heisenberg & Wolf, 1993). At the same time, the fly's orientation (from -180° to 180°) relative to the landmark was converted into a histogram of the frequency of occurrence of the error angle ψ (Fig. 1B). The error angle (ψ) is defined as $\alpha_p - \alpha_f$ and represents the angular position of the object with respect to the coordinate system of the fly (Fig. 1C). α_f represents the instantaneous direction of flight with respect to an arbitrary zero direction and α_p represents the instantaneous angular position of an object that the fly may or may not track (Reichardt & Poggio, 1976). When $\alpha_p = \alpha_f$, the fly's long axis points directly at the object ($\psi = 0$). The characteristic variable in these experiments is ψ. The average of the absolute value of the error angle ($|\psi|$) during each experiment was used as an error index to describe the orientation performance.

Transient gene silencing

The JDP coding sequence (a fragment of about 600 bp) was amplified by reverse transcription-polymerase chain reaction with primers containing unique restriction sites at their 5′ ends:
5′-CGCCGCTTATCCCAAAAAAAACA-3′ and
5′-CCAACTCCTCCTGCTCGCTCATC-3′.

The polymerase chain reaction product was subcloned into the pGEM-T Easy Vector. The expected fragment, obtained by digesting the vector with EcoR I and Not I, was used in trimolecular ligation with the alkaline phosphatase-treated pUAST vector linearized with EcoR I. The inverted-repeat construct was injected into w^{1118} embryos to generate transgenic flies according to the standard procedure (Spradling & Rubin, 1982).

Antibodies

Polyclonal rabbit antibodies against JDP were generated with the antigenic peptide (CSRKGEWGGENTDV) and conjugated with mcKLH using the maleimide-activated immunogen conjugation kit (Pierce).

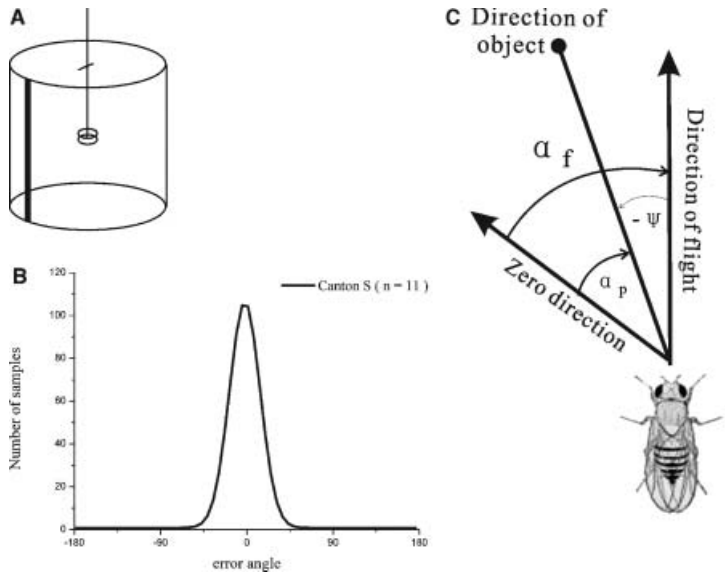

FIG. 1. Angular coordinate system describing the fly's rotational degree of freedom around the vertical axis. (A) A flying *Drosophila* in a fixed position and orientation at the center of a flight simulator. The fly's intended turns control the rotational speed of the arena where the target consists of a vertical black bar (12° width and 40° length). (B) The frequency of occurrence of the error angle ψ of wild-type flies (Canton-S). The flies have a strong tendency to head towards the stripe. (C) Angular position of the fly on the horizontal plane. α_p, angle between an arbitrary zero direction and the direction of an object in the environment of the fly; α_f, angle between the zero direction and the fly's direction of flight; the angle $\psi = \alpha_p - \alpha_f$ is the 'error angle' between the fly's direction of flight and the object.

Western blotting

For western blotting, frozen adult flies' heads were homogenized in ristocetin-induced platelet agglutination buffer [150 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris, 0.2 mM Na vanadate, 10 μM NaF, 0.4 mM EDTA, pH 8.0, 10% glycerol]. The crude extract was cleared by centrifugation at 9300 ***g*** for 1 min. Equal amounts of total proteins were separated on sodium dodecyl sulfate-12% polyacrylamide gels and transferred to nitrocellulose. The following antibody dilutions were used: anti-JDP, 1 : 1000; anti-actin, 1 : 2000 and anti-rabbit antibody conjugated with alkaline phosphatase, 1 : 10 000. Standard immunostaining was carried out using chemiluminescence ECL plus (Amersham Biosciences).

Quantitative determination of dopamine

Adult flies were quick-frozen in liquid nitrogen. The heads were removed and homogenized in 0.1 M perchloric acid (10 μL/head) and then the homogenate was centrifuged at 12 000 *g* at 4 °C for 30 min. Dopamine in the supernatant fluid was measured with an ^{125}I-radioimmunoassay dopamine kit (LDN, Germany).

Locomotor assay

Newborn females were placed individually into 60 × 3-mm glass tubes supplied with sufficient food. Each tube was placed in a slot of the *Drosophila* Activity Monitor System (TriKinetics Inc., Waltham, MA, USA) with a centrally located infrared beam. Beam breaks were automatically collected at a 5-min interval by the TriKinetics software. All locomotor studies were conducted in an alternating light and dark condition (12 : 12 h) at 25 °C. The total daily activity (counts/24 h) of the third day was used as a criterion.

Statistical analysis

The ANOVAs to determine the significance of differences between strains were performed with the SAS general linear model procedure (SAS Institute Inc., Cary, NC, USA). To investigate differences between independent variables after rejecting the null hypothesis, Duncan's test procedure was conducted as a *post-hoc* investigation. *T*-tests (Cochran procedure) were used to determine differences between shi^{ts1}-expressing flies at different temperatures, toxin-expressing flies and the control flies.

Results

Long-term blockade of dopamine release affected attention-like behavior

As tethered flies have a tendency to head toward the stripe in the center of the visual field, which serves as a reference for choosing a particular orientation (Heisenberg & Wolf, 1993), we took advantage of this behavioral phenomenon to explore the functions of dopamine in visual attention.

To obtain mutants with long-term blockade of dopamine release, we used a transgenic *Drosophila* strain in which synaptic transmission is constitutively blocked by expression of the catalytic subunit of bacterial tetanus toxin (TNT-E) in dopaminergic neurons (Friggi-Grelin *et al.*, 2003). Expression of another transgene, an inactive form of the tetanus toxin light chain (IMPTNT-V1-A), controlled for possible deleterious effects of protein over-expression (Sweeney *et al.*, 1995). The mutated flies could not continuously keep the black

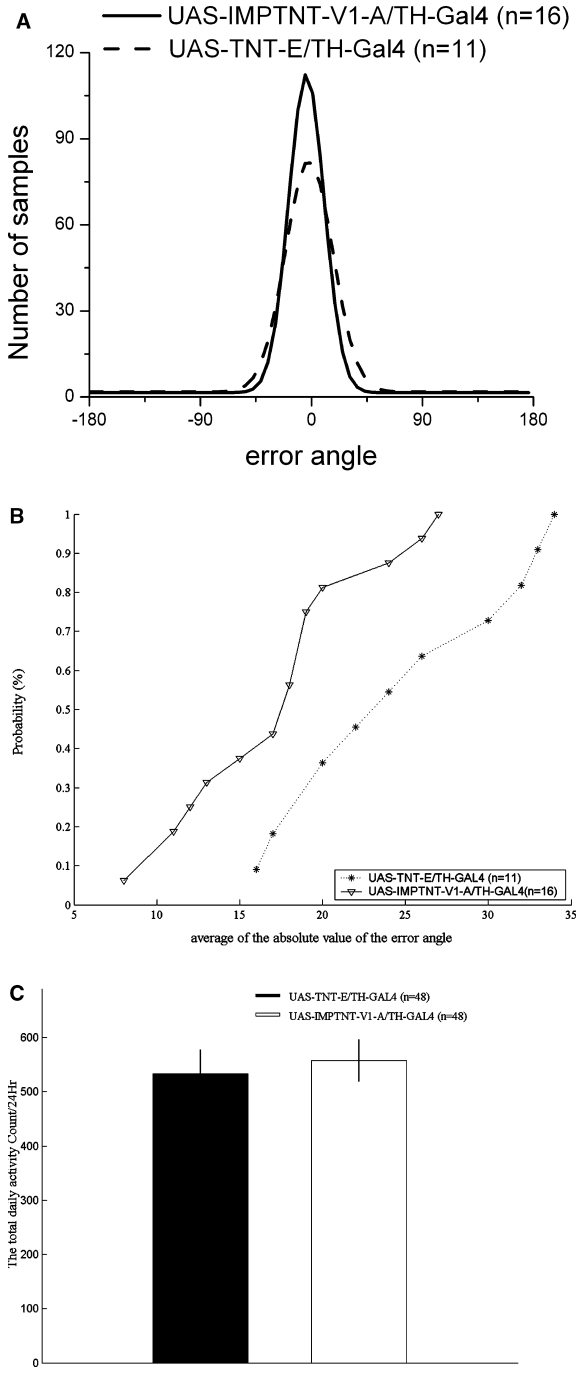

FIG. 2. Flies with long-term blockade of dopamine release showed a significant defect in attention-like behavior and unchanged locomotor activity. (A) Histograms of the frequency of occurrence of error angle. The height of the distribution of the error angle decreased markedly when tetanus toxin light-chain was expressed in dopaminergic neurons (UAS-TNT-E/TH-GAL4) relative to UAS-IMPTNT-V1-A/TH-GAL4 controls. (B) Statistical results of the attention-like orientation shown as cumulative error distribution curves. The curve for UAS-TNT-E flies was significantly different from that for UAS-IMPTNT-V1-A/TH-GAL4 flies (*t*-test, $P = 0.016$, i.e. < 0.05). The probability at any average error angle for the control flies was higher than that of flies with impaired dopamine release, i.e. more flies performed worse without dopamine. (C) Blockade of dopamine release did not cause the change in activity. The total daily activity (counts/24 h) of flies with TNT-E expression in dopaminergic neurons ($n = 48$; mean ± SEM, 533.7 ± 43.9) was not different from that of the control flies ($n = 48$; mean ± SEM, 557.8 ± 38.2) (*t*-test, $P = 0.683$).

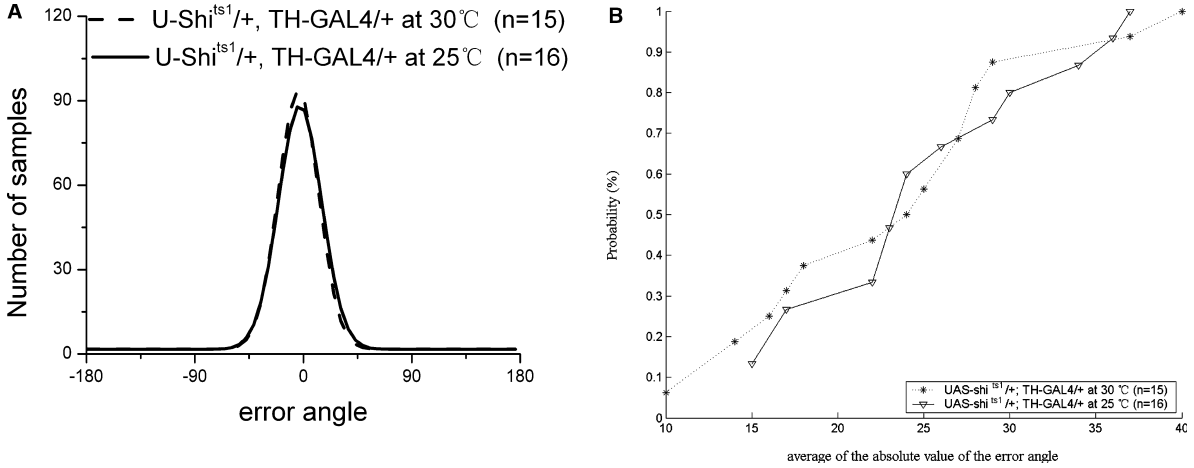

FIG. 3. Flies with short-term blockade of dopamine release showed similar performance in attention-like behavior. (A) Histograms of the frequency of occurrence of error angle. No difference was found between the error angle distributions at the permissive (25 °C) and restrictive temperatures (30 °C) in UAS-shi^{ts1}/+ TH-GAL4/+flies. (B) Statistical results of the attention-like orientation shown as cumulative error distribution curves. The cumulative distribution curves for UAS-shi^{ts1}/+ TH-GAL4/+ flies at the permissive and restrictive temperatures were not significantly different.

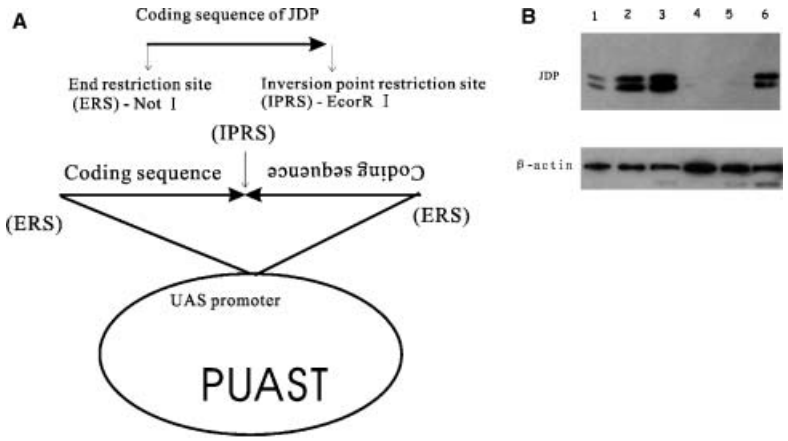

FIG. 4. Analysis of RNA interference (RNAi) effects in different strains. (A) Construction of inducible inverted repeated genes. cDNA of J-domain protein (JDP) was amplified using two primers and inserted into the vectors by trimolecular ligation. (B) Insertion dependence of RNAi efficiency. Western blots from the brains of five randomly chosen strains of UAS-dsRNA JDP × Elav-GAL4 flies showed loss of JDP expression in transgenic flies expressing RNAi under the control of the UAS promoter. Lanes: 1–5, different strains of UAS-dsRNA JDP/Elav-GAL4; 6, UAS-dsRNA JDP alone. Expression of a control protein (β-actin) served as the quantitative marker.

bar in the center of their visual field so the error angles in these dopamine-depleted flies were larger than those in the controls (Fig. 2A). From the cumulative curves of error indices, it was clear that flies with dopaminergic neurons expressing tetanus toxin always had significantly larger errors (at a level of $P < 0.05$, *t*-test) (Fig. 2B). In addition, blockade of dopamine release did not cause major changes in activity as shown in the locomotor assay ($P = 0.683$, *t*-test, Fig. 2C). These results suggest that long-term dopamine depletion was related to the reduced accuracy of attention-like behavior.

In contrast, we over-expressed shi^{ts1}, which encodes a temperature-sensitive form of the protein dynamin (Chen *et al.*, 1991), in dopaminergic neurons to generate a model of short-term blockade of dopamine release. Synaptic transmission in these flies is rapidly and reversibly inhibited at the restrictive temperature (30 °C; Kitamoto, 2001; Koenig *et al.*, 1983). Unexpectedly, when the temperature was raised to 30 °C from 2 min before until the end of the experiment, the flies showed visual attention ability as good as that at the permissive temperature (25 °C) (Fig. 3A and B). These results exclude the involvement of transient dopamine release in the visual attention behavior.

Down-regulation of J-domain protein by transgenic RNA interference

To further confirm the effect of long-term blockade of dopamine release on orientation behavior, we generated another transgenic fly in which JDP was permanently down-regulated. Taking the transgenic RNA interference approach (Tavernarakis *et al.*, 2000) (Fig. 4A), we used the GAL4-UAS binary expression system (Brand & Perrimon, 1993) to test the efficiency of RNA interference. Having generated UAS-JDP inverted repeat transgenic flies capable of expressing double-stranded RNA (UAS-dsRNA JDP), we crossed different UAS-dsRNA JDP flies with Elav-GAL4 flies in which GAL4 was highly expressed throughout the central nervous system. Notably, the

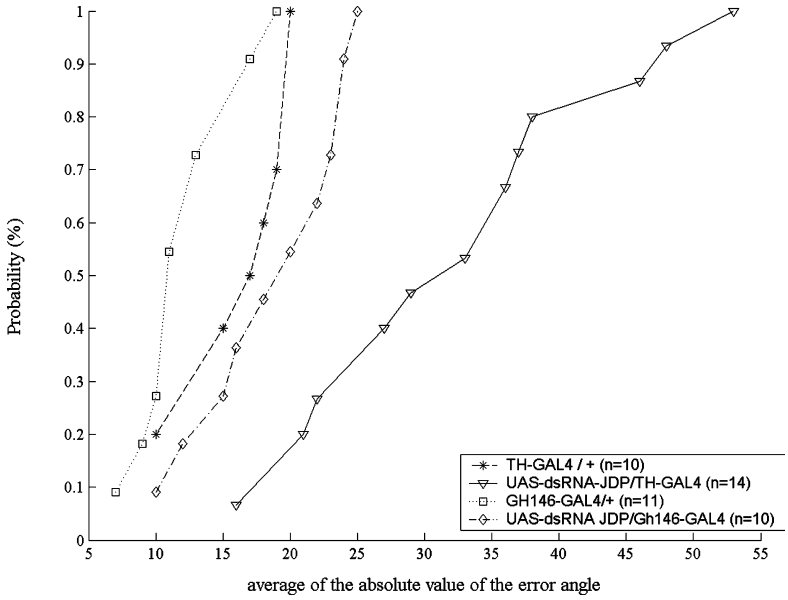

FIG. 5. Deficit of attention-like behavior in flies with J-domain protein (JDP) specifically down-regulated in dopaminergic neurons. Flies with a high efficiency of interference in dopaminergic neurons had significantly greater error angles than any of the control groups (TH-GAL4/+, GH146-GAL4/UAS-dsRNA JDP, GH146-GAL4/+) (ANOVA, $P < 0.001$). The results of Duncan multiple range tests showed no significant difference among the control groups.

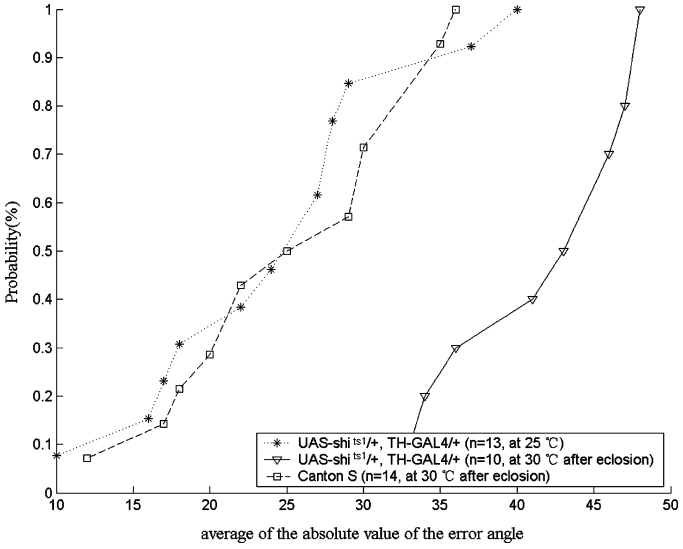

FIG. 6. Chronic dopamine depletion in impaired attention-like behavior of shi^{ts1}-expressing flies. Flies expressing shi^{ts1} in dopaminergic neurons, raised at 30 °C after eclosion, showed significantly greater error angles than those of both control groups (flies of the same genotype raised at 25 °C and wild-type flies raised at 30 °C after eclosion) (ANOVA, $P < 0.0001$). The results of Duncan multiple range tests showed no significant differences between the control groups.

expression of JDP in most strains of RNA interference flies was down-regulated and RNA interference constructs in the UAS-dsRNA JDP strain inhibited the expression of JDP with the highest efficiency (Fig. 4B, lane 5). Therefore, we chose this strain for the subsequent assays of movements and attention-like behavior. A ^{125}I-radioimmunoassay was used to quantitatively determine the levels of dopamine in adult brain. A fivefold decrease in dopamine (about 0.075 μg/mL) was observed in the flies with dsRNA JDP expressed in dopaminergic neurons (UAS-dsRNA JDP/+, TH-GAL4/+) compared with the control strain (about 0.375 μg/mL).

Down-regulation of J-domain protein in dopaminergic neurons disrupts attention-like behavior

After obtaining the UAS-dsRNA JDP strain with high RNA interference efficiency, we subjected it to several behavioral assays. As flies with JDP down-regulation throughout the central nervous system (UAS-dsRNA JDP/Elav-GAL4) displayed a normal walking posture and jumped well, we considered whether other aspects of behavior were related to the level of dopamine in the central nervous system. We therefore applied our flight visual attention paradigm to this model. Behavioral assays of flies with dsRNA-JDP expression directed to dopaminergic neurons gave results similar to those of flies that were dopamine depleted by tetanus toxin expression. The down-regulation of JDP in dopaminergic neurons is functional. When the expression of JDP was only down-regulated in non-dopaminergic olfactory projection neurons from the antennal lobe to the mushroom bodies and the lateral horns (Stocker *et al.*, 1997), the flies (UAS-dsRNA JDP/GH146-GAL4) exhibited normal orientation behavior. Attention-like orientation assays were then carried out on flies with one wild-type chromosome to further test whether the mutant gene caused the defect. A significant defect of attention-like orientation ability in flies expressing dsRNA-JDP in dopaminergic neurons was evident (Fig. 5). This indicates that dopamine depletion caused by down-regulation of JDP is important for attention in the flies. The results of Duncan multiple range tests showed no significant difference among the control groups UAS-dsRNA JDP/GH146-GAL4, TH-GAL4/+ and GH146-GAL4/+. Neither the introduction of UAS-dsRNA JDP nor TH-GAL4 contributed to the reduction in UAS-dsRNA JDP/+, TH-GAL4/+ flies. Thus, we can relate these findings to evidence that attention-like behavior is not directly mediated by transient dopamine release but chronic loss of dopamine influences orientation behavior in flies.

Chronic dopamine depletion in shi ^{ts1}-expressing flies impaired attention-like behavior

If the difference in behavior between the two temperatures in shi^{ts1}-expressing flies was only due to chronic dopamine depletion, then flies raised at the restrictive temperature (30 °C) should show a different orientation behavior. We raised flies at the restrictive temperature after egg hatching but most larvae died. In addition, adults that were raised at the restrictive temperature from pupation appeared abnormal with smaller bodies and coiled wings. To avoid larval lethality and abnormal appearance, the flies were raised to eclosion at the permissive temperature (25 °C) and then transferred to a culture room pre-warmed to 30 °C. Three-day-old females showed impaired attention-like behavior which was significantly different from both control groups, flies of the same genotype raised at 25 °C and wild-type flies raised at 30 °C after eclosion (Fig. 6).

Discussion

Dopamine is widely known as a key neurotransmitter in brain and is involved in regulating attention in mammals. Previous studies have demonstrated dopamine's regulatory role in motor and limbic functions in mammals (Kamei *et al.*, 1994; Baker *et al.*, 1995). Our results indicate that dopamine may function to subtly modulate attention-like behavior in insects.

We took both TNT-E- and shi^{ts1}-expressing flies as models of dopamine depletion. The result from TNT-E-expressing flies (UAS-TNT-E/TH-GAL4), representing a long-term dopamine depletion, supports the conclusion that the altered attention-like behavior might be caused by the impairment of dopaminergic neurons, while the result from transgenic flies (UAS-shi^{ts1}/+, TH-GAL4/+) with transient dopamine depletion excluded the involvement of transient dopamine release.

We therefore sought another constitutively expressed factor that functions in dopaminergic neurons in order to model the long-term effects of dopamine dysfunction and its role in the attention deficit. The flies with JDP specifically down-regulated in dopaminergic neurons showed impaired performance similar to that of flies with constitutive blockade of dopamine by tetanus toxin expression. The quantitative determination of dopamine showed that JDP down-regulation decreased the level of dopamine. Therefore, we suggest that JDP may act as a cochaperone via its J-domain and affect the intracellular concentration of dopamine, resulting in the fly's behavioral change. Furthermore, we used the GH146-GAL4 strain, which specifically expressed dsRNA in the olfactory projection neurons from the antennal lobe to the mushroom bodies and lateral horns, to drive JDP down-regulation. The behavior of the fly was unaffected, assuring us that the behavioral change is due to dopamine depletion caused by JDP down-regulation.

It has been pointed out that defective synaptic vesicle recycling occurred temporarily in shi^{ts1}-expressing flies (Kitamoto, 2001), while synaptic transmission was constitutively blocked (Sweeney *et al.*, 1995) in both the TNT-E- and dsRNA JDP-expressing lines. Our result suggested that the defect in attention-like behavior was due to the chronic dopamine depletion during development. As dopamine plays a growth factor-like role in the permanent neurogenesis (Feron *et al.*, 1999) and an essential role in the correct terminal differentiation of specific tissues in flies (Neckameyer, 1996), we assume that functions of dopamine in development may explain the difference between long- and short-term blockade of dopamine release.

We cultured the shi^{ts1}-expressing flies at the restrictive temperature in an attempt to induce shi^{ts1} expression in dopaminergic neurons throughout development, mimicking long-term blockade of dopamine release. Only the flies raised at 30 °C after eclosion were capable of the flight procedure and showed impaired attention-like behavior. It has been reported that flies with a temperature-sensitive paralytic mutation in shibire exposed to heat induction during the late pupal stage remain similar to the control flies in the anatomy of the giant fiber pathway (Hummon & Costello, 1987). Dopamine receptors are important for synaptic plasticity in mammals (Huang *et al.*, 2004). It is thus possible that the long-term inhibition of dopamine release alters synaptic strength or neuronal excitability.

Although dopamine modulates some behaviors related to locomotion and grooming in flies (Yellman *et al.*, 1997), its effects on movement in these paradigms may not play an important role. Most dopamine-deficient flies displayed normal posture, walking and jumping (they maintained normal flight ability, showing changes of the error angle as smooth as those of the wild-type fly throughout the entire flight) and their yaw torque modulations were similar to those in the wild-type fly. Furthermore, locomotor assay of flies with long-term dopamine depletion displayed unchanged activity. Therefore, this behavioral defect is probably a result of the attention-like deficit caused by dopamine depletion in the central nervous system.

The physiological roles of dopamine in the insect nervous system appear to be diverse. Attention is currently focused on two main structures, the central complex and the mushroom bodies (Liu *et al.*, 1999; Tang & Guo, 2001; Heisenberg, 2003). In the adult fly brain, six clusters of dopaminergic neurons have been shown to project to specific regions of the mushroom bodies and to the central complex (Budnik & White, 1988; Nassel & Elekes, 1992). Both structures are related to learning and memory formation (Sitnik *et al.*, 2003). In several behavioral paradigms, mutant flies with a structural alteration in the central complex walk more slowly than wild-type flies and show altered orientation behavior toward landmarks (Strauss & Heisenberg, 1993). They are either less active or quickly lose activity or fail to start walking or flying under circumstances in which wild-type flies would readily do so (Strauss & Heisenberg, 1993; Ilius *et al.*, 1994). Dopaminergic terminals are abundant in the central complex so dopamine obviously plays an important role as a messenger there (Nassel & Elekes, 1992). Therefore, further work on the local circuits participated in by dopamine-containing cells in insects should be very interesting.

Acknowledgements

This work was supported by the NSFC (grants 39770187, 69835020 and 30270341), Multidisciplinary Research Program 'Brain and Mind' of the CAS, Major State Basic Research Program (G2000077800) and Science & Technology Commission of Shanghai Municipality. We thank Professor Bruce (University of Hong Kong), Professor Hennig (Shanghai Institute for Biological Sciences, Shanghai, China) and Professor Buchner (University of Wurzburg) for critically reading the manuscript. We also thank K.J. Deng and Y. Wu (Fudan University, China) for their help with microinjection.

Abbreviations

JDP, J-domain protein; TNT-E, catalytic subunit of bacterial tetanus toxin; UAS-dsRNA JDP, UAS-JDP inverted repeat transgenic flies capable of expressing double-stranded RNA.

References

Baier, A., Wittek, B. & Brembs, B. (2002) Drosophila as a new model organism for the neurobiology of aggression? *J. Exp. Biol.*, **205** (9), 1233–1240.

Bainton, R.J., Tsai, L.T., Singh, C.M., Moore, M.S., Neckameyer, W.S. & Heberlein, U. (2000) Dopamine modulates acute responses to cocaine, nicotine and ethanol in Drosophila. *Curr. Biol.*, **10**, 187–194.

Baker, M.W., Croll, R.P., Dyakonova, V., Khabarova, M., Sakharov, D.A. & Voronezhskaya, E. (1995) Mode of action of antipsychotic drugs: lessons from simpler models. *Acta Biol. Hung.*, **46**, 221–227.

Brand, A.H. & Perrimon, N. (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development*, **118**, 401–415.

Budnik, V. & White, K. (1988) Catecholamine-containing neurons in Drosophila melanogaster: distribution and development. *J. Comp. Neurol.*, **268**, 400–413.

Chen, M.S., Obar, R.A., Schroeder, C.C., Austin, T.W., Poodry, C.A., Wadsworth, S.C. & Vallee, R.B. (1991) Multiple forms of dynamin are encoded by shibire, a Drosophila gene involved in endocytosis. *Nature*, **351**, 583–586.

Feron, F., Vincent, A. & Mackay-Sim, A. (1999) Dopamine promotes differentiation of olfactory neuron in vitro. *Brain Res.*, **845**, 252–259.

Friggi-Grelin, F., Coulom, H., Meller, M., Gomez, D., Hirsh, J. & Birman, S. (2003) Targeted gene expression in Drosophila dopaminergic cells using regulatory sequences from tyrosine hydroxylase. *J. Neurobiol.*, **54**, 618–627.

Guo, A. & Gotz, K. (1997) Association of visual objects and olfactory cues in Drosophila. *Learn. Mem.*, **4**, 192–204.

Hahn, Y., Lee, J., Seong, C., Yoon, J. & Chung, J. (1999) Structural analysis of phylogenetically conserved J domain protein gene. *Biochim. Biophys. Acta*, **1447**, 325–333.

Heisenberg, M. (2003) Mushroom body memoir: from maps to models. *Nat. Rev. Neurosci.*, **4**, 266–275.

Heisenberg, M. & Wolf, R. (1993) The sensory-motor link in motion-dependent flight control of flies. *Rev. Oculomot. Res.*, **5**, 265–283.

Heisenberg, M., Wolf, R. & Brembs, B. (2001) Flexibility in a single behavioral variable of Drosophila. *Learn. Mem.*, **8**, 1–10.

Huang, Y.Y., Simpson, E., Kellendonk, C. & Kandel, E.R. (2004) Genetic evidence for the bidirectional modulation of synaptic plasticity in the prefrontal cortex by D1 receptors. *Proc. Natl Acad. Sci. U.S.A.*, **101**, 3236–3241.

Hummon, M.R. & Costello, W.J. (1987) Induced disruption in the connectivity of an identified neuron in the Drosophila ts mutant shibire. *J. Neurosci.*, **7**, 3633–3638.

Ilius, M., Wolf, R. & Heisenberg, M. (1994) The central complex of Drosophila melanogaster is involved in flight control: studies on mutants and mosaics of the gene ellipsoid body open. *J. Neurogenet.*, **9**, 189–206.

Inoue, H., Chikaoka, Y., Takahashi, M. & Yoshioka, T. (2000) Identification of a protein phosphorylated by cAMP-dependent protein kinase in Drosophila brain. *Brain Res.*, **875**, 160–163.

Kamei, J., Saitoh, A., Iwamoto, Y., Funada, M., Suzuki, T., Misawa, M., Nagase, H. & Kasuya, Y. (1994) Effects of diabetes on spontaneous locomotor activity in mice. *Neurosci. Lett.*, **178**, 69–72.

Kitamoto, T. (2001) Conditional modification of behavior in Drosophila by targeted expression of a temperature-sensitive shibire allele in defined neurons. *J. Neurobiol.*, **47**, 81–92.

Koenig, J.H., Saito, K. & Ikeda, K. (1983) Reversible control of synaptic transmission in a single gene mutant of Drosophila melanogaster. *J. Cell Biol.*, **96**, 1517–1522.

Lee, J., Hahn, Y., Yun, J., Mita, K. & Chung, J. (2000) Characterization of JDP genes, an evolutionarily conserved J domain-only protein family, from human and moths. *Biochim. Biophys. Acta*, **1491**, 355–363.

Liu, L., Wolf, R., Ernst, R. & Heisenberg, M. (1999) Context generalization in Drosophila visual learning requires the mushroom bodies. *Nature*, **400**, 753–756.

Nassel, D.R. & Elekes, K. (1992) Aminergic neurons in the brain of blowflies and Drosophila: dopamine- and tyrosine hydroxylase-immunoreactive neurons and their relationship with putative histaminergic neurons. *Cell Tissue Res.*, **267**, 147–167.

Neckameyer, W.S. (1996) Multiple roles for dopamine in Drosophila development. *Dev. Biol.*, **176**, 209–219.

Neckameyer, W.S. (1998) Dopamine and mushroom bodies in Drosophila: experience-dependent and -independent aspects of sexual behavior. *Learn. Mem.*, **5**, 157–165.

Neckameyer, W.S., O'Donnell, J., Huang, Z. & Stark, W. (2001) Dopamine and sensory tissue development in Drosophila melanogaster. *J. Neurobiol.*, **47**, 280–294.

Puumala, T. & Sirvio, J. (1998) Changes in activities of dopamine and serotonin systems in the frontal cortex underlie poor choice accuracy and impulsivity of rats in an attention task. *Neuroscience*, **83**, 489–499.

Reichardt, W. & Poggio, T. (1976) Visual control of orientation behavior in the fly. *Q. Rev. Biophys.*, **9**, 311–375.

Schwaerzel, M., Monastirioti, M., Scholz, H., Friggi-Grelin, F., Birman, S. & Heisenberg, M. (2003) Dopamine and octopamine differentiate between aversive and appetitive olfactory memories in Drosophila. *J. Neurosci.*, **23**, 10 495–10 502.

Silver, P.A. & Way, J.C. (1993) Eukaryotic DnaJ homologs and the specificity of Hsp70 activity. *Cell*, **74**, 5–6.

Sitnik, N.A., Tokmacheva, E.V. & Savvateeva-Popova, E.V. (2003) The ability of Drosophila mutants with defects in the central complex and mushroom bodies to learn and form memories. *Neurosci. Behav. Physiol.*, **33**, 67–71.

Spradling, A.C. & Rubin, G.M. (1982) Transposition of cloned P elements into Drosophila germ line chromosomes. *Science*, **218**, 341–347.

Stocker, R.F., Heimbeck, G., Gendre, N. & de Belle, J.S. (1997) Neuroblast ablation in Drosophila P[GAL4] lines reveals origins of olfactory interneurons. *J. Neurobiol.*, **32**, 443–456.

Strauss, R. & Heisenberg, M. (1993) A higher control center of locomotor behavior in the Drosophila brain. *J. Neurosci.*, **13**, 1852–1861.

Sweeney, S.T., Broadie, K., Keane, J., Niemann, H. & O'Kane, C.J. (1995) Targeted expression of tetanus toxin light chain in Drosophila specifically eliminates synaptic transmission and causes behavioral defects. *Neuron*, **14**, 341–351.

Tang, S. & Guo, A. (2001) Choice behavior of Drosophila facing contradictory visual cues. *Science*, **294**, 1543–1547.

Tavernarakis, N., Wang, S.L., Dorovkov, M., Ryazanov, A. & Driscoll, M. (2000) Heritable and inducible genetic interference by double-stranded RNA encoded by transgenes. *Nat. Genet.*, **24**, 180–183.

Torres, G. & Horowitz, J.M. (1998) Activating properties of cocaine and cocaethylene in a behavioral preparation of Drosophila melanogaster. *Synapse*, **29**, 148–161.

Umbach, J.A., Zinsmaier, K.E., Eberle, K.K., Buchner, E., Benzer, S. & Gundersen, C.B. (1994) Presynaptic dysfunction in Drosophila csp mutants. *Neuron*, **13**, 899–907.

Wu, Z., Gong, Z., Feng, C. & Guo, A. (2000) An emergent mechanism of selective visual attention in Drosophila. *Biol. Cybern.*, **82**, 61–68.

Yellman, C., Tao, H., He, B. & Hirsh, J. (1997) Conserved and sexually dimorphic behavioral responses to biogenic amines in decapitated Drosophila. *Proc. Natl Acad. Sci. U.S.A.*, **94**, 4131–4136.

Zinsmaier, K.E., Eberle, K.K., Buchner, E., Walter, N. & Benzer, S. (1994) Paralysis and early death in cysteine string protein mutants of Drosophila. *Science*, **263**, 977–980.