Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 81 (2005) 849 – 853

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

A developmental role for catecholamines in *Drosophila* behavior

Robert G. Pendleton [a,b,*], Aseel Rasheed [a], Prasuna Paluru [a], Jeannettee Joyner [b], Natalie Jerome [a], Robert D. Meyers [c], Ralph Hillman [a]

[a] *Department of Biology Temple University, Philadelphia, Pennsylvania 19122, United States*
[b] *College of Graduate Studies, Thomas Jefferson University, Philadelphia, Pennsylvania 19107, United States*
[c] *Department of Pharmacology, East Carolina University, Greenville, North Carolina 27858, United States*

Received 15 November 2004; received in revised form 1 April 2005; accepted 3 June 2005
Available online 26 July 2005

Abstract

Tyrosine hydroxylase (TH), the enzyme which catalyzes the conversion of tyrosine to L-DOPA and is the rate limiting step in catecholamine biosynthesis, is genetically expressed during development in *Drosophila*. Null mutant alleles of the single copy gene which codes for this enzyme are developmentally lethal as is a conditional TH mutant at its restrictive temperature. In adult flies, inhibition of TH by *alpha*-methyl-*p*-tyrosine (αMT) decreases locomotor activity in a dose-dependent manner. This behavioral effect is accompanied by reductions in brain levels of dopamine, the primary CNS catecholamine in *Drosophila*, and can be prevented by the coadministration of L-DOPA. Similar effects are found with reserpine and at the restrictive temperature in flies with a temperature conditional mutation for TH. In agreement with published studies in mammals, inhibition of TH by αMT during *Drosophila* development results in enhanced expression of this enzyme in the progeny of surviving adults. This biochemical outcome is accompanied behaviorally by increased sensitivity to the locomotor effects of both αMT and reserpine, drugs which act via depletion of brain catecholamines. Since TH is the rate limiting enzyme responsible for the conversion of tyrosine to L-DOPA and L-DOPA is converted to dopamine by aromatic amino acid decarboxylase (AAAD), the results indicate that depletion of catecholamine levels in the fly embryo results in increased dopamine biosynthesis in the next generation accompanied by alterations in behavior.
© 2005 Elsevier Inc. All rights reserved.

Keywords: Tyrosine hydroxylase; Dopamine; Behavioral development

1. Introduction

Tyrosine hydroxylase (TH) is the enzyme that catalyzes the first and rate limiting step in catecholamine biosynthesis, the conversion of tyrosine to L-DOPA (Nagatsu et al., 1964; Axelrod, 1971). In *Drosophila*, the enzyme is coded for by a single copy gene, *pale*, localized on the left arm of the third chromosome. Null alleles of this gene, when homozygous, result in embryonic lethality (Neckameyer and White, 1993). Pharmacological inhibition of TH is also lethal, affecting embryogenesis in *Drosophila* as well as later stages of development (Neckameyer, 1996; Pendleton et al., 1996). *Drosophila melanogaster* has been chosen as a reductionist animal model system for our studies because of its well-defined development; its short life cycle, approximately 10 days at 25 °C; and the availability of simple behavioral test systems (Hillman and Pendleton, 2005). TH inhibitors in mammals, including man, are well known to produce CNS depressant effects (Moore and Dominic, 1971), effects concluded to result mainly from decreased synthesis of dopamine in the brain (Zhou and Palmiter, 1995; Kelly et al., 1998; Meyer et al., 1993). Reserpine also produces CNS depression primarily through inhibition of dopamine reuptake into neuronal storage granules (Kelly et al., 1998). Thus, both pharmacological approaches result in dopamine depletion, albeit by different mechanisms. The

* Corresponding author. Department of Biology, Temple University, Philadelphia, PA 19122, United States. Tel.: +1 215 654 5022; fax: +1 215 654 7766.
E-mail address: robertpendleton@comcast.net (R.G. Pendleton).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.06.008

initial purpose of the present study was to ascertain whether similar effects on CNS locomotor function are found in *Drosophila* when a tyrosine hydroxylase inhibitor (alpha-methyl-p-tyrosine, αMT) or reserpine are administered and in flies containing a homozygous conditional TH mutant allele under restrictive conditions when expression of the allele is suppressed. When positive results were obtained in *all of these models*, we then examined the adult *progeny* of flies treated with either αMT or reserpine to determine if the behavioral sensitivities of the flies to these agents were altered from naive adults. If so, this would suggest that behavioral alterations in progeny may result from altered catecholamine function during development.

2. Methods

2.1. Locomotor assay

Adult wild type Canton S flies were maintained on an agar, sucrose, water, dried yeast media at 25 °C on a 12-h light–dark cycle (Pendleton et al., 1996). Methyl-4-hydroxymethylbenzoate (0.1% w/v) was added to prevent fungal growth. In specified vials containing age-matched flies L-DOPA (L-dihydroxyphenylalanine), reserpine or αMT was added to this media at the concentrations indicated for 7 days. In assays using L-DOPA, vitamin C (0.025% w/v) was added to maintain its stability. Flies were tested at 25 °C using 100×15 mm^2 petri dishes with 13 mm^2 grids etched on the bottom (Ford et al., 1989). Individual organisms were transferred to the petri dish and allowed to acclimate for 30 s. During the following 30 s, the number of grid lines crossed by the fly was recorded as the spontaneous motor activity (SMA) value. Statistical significance between means was assessed using a two-way ANOVA (analysis of variance) with a subsequent Student's *t* test comparison (Snedecor and Cocchran, 1989).

2.2. Biochemical assays

Dopamine levels were measured by HPLC with an electron capture detector (Adell and Myers, 1995) on 0.4 perchloric acid extracts of 6–15 heads removed from age-matched adult flies treated with 1 mM αMT for 7 days. Brain tyrosine hydroxylase studies were performed on the progeny of Canton S flies maintained in sterilized bottles on standard *Drosophila* media. Control groups were fed standard food while the food of the experimental flies contained 0.1 mM, αMT. Both groups were allowed to feed and lay eggs for 10 days. The parents were then discarded and their progeny were then collected for 7 days. Progeny groups were fed on fresh control food for 7 days after which they were decapitated, their heads homogenized in 1 mM PMSF and centrifuged at $5000 \times g$ for I5 min at l–5 °C to removed cell debris and surrounding head structures. The resulting supernatants were stored at −20 °C.

2.3. Tyrosine hydroxylase (TH) quantification

Electrophoretic studies on supernatants obtained from 200 heads were conducted according to the procedure of Laemmli (1970). Samples were prepared by mixing 50 μl of supernatant with 50 μl of SDS-containing buffer followed by heating at 95 °C for 3 min. A control and experimental aliquot and 3 TH standards (Signal Transduction Products) were placed into individual wells of premade 10% Tris gels (Bio-Rad Laboratories).The running buffer contained Tris (1.5% w/v), glycine and 10% SDS and the voltage applied for each gel ranged from 150 to 175 V. The gels were run at room temperature. The proteins were transferred from these gels to nitrocellulose paper using a Bio-Rad Laboratories Semi-Dry transfer cell at 15 V for 40 min. Ponceau-S solution was used to ascertain efficiency of transfer. After protein transfer, the nitrocellulose membranes were incubated for 16 h in a blocking solution (3 g BSA in 100 ml of water) and then incubated with primary rabbit polyclonal anti-TH (Calbiochem). Primary antibody incubation was followed by 4 TTBS washes and incubation with an alkaline phosphate tagged goat anti-rabbit IgG (secondary antibody, American Qualex). Unbound antibodies were removed using 3 TTBS washes and the alkaline phosphatase conjugate was allowed to react for 120 min with BCIT/TNBT to form a purple precipitate. After a 10-min wash in distilled water, the nitrocellulose membranes were air dried

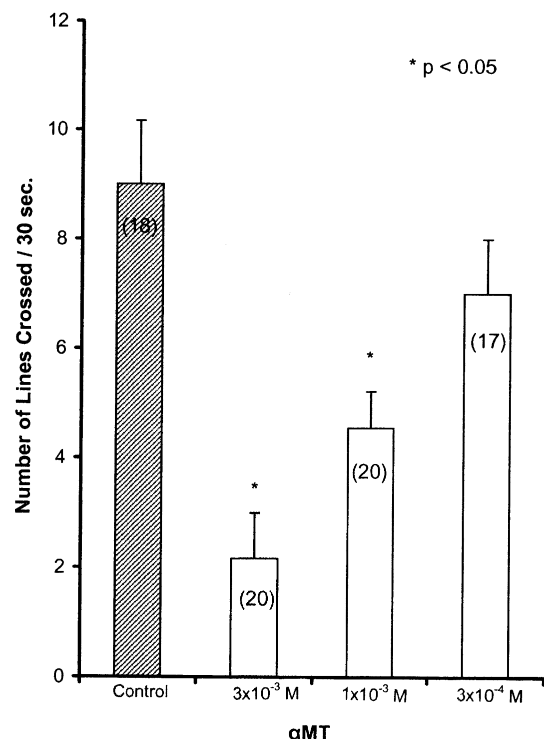

Fig. 1. Spontaneous locomotor activity of αMT treated flies. Adult organisms were treated for 7 days and then assayed for locomotor activity. Bars indicate the means and standard errors for the number of flies indicated in parenthesis. Asterisks show significant differences ($p < 0.05$) from control using ANOVA and group Student's "*t*" test.

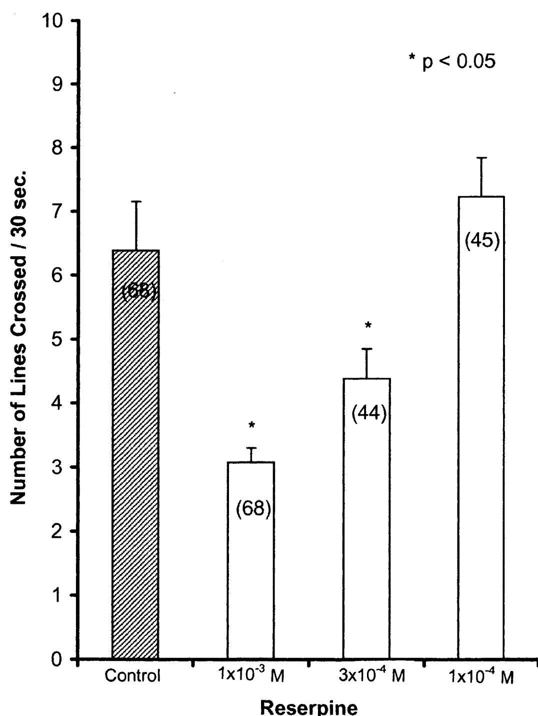

Fig. 2. Spontaneous locomotor activity of reserpine treated flies. Adult organisms were treated for 7 days and then assayed for locomotor activity. Bars indicate the means and standard errors for the number of flies indicated in parenthesis. Asterisks show significant differences from control using ANOVA and group Student's "t" test.

overnight and laminated. Quantitative density analysis of TH on the blots was done using a Molecular Dynamics Densitometer with Image Quant software. The protein content of the samples was determined by the Bradford (1976) method. Three TH standards, a control and an αMT sample were run on each gel and the combined results of 5 such assays (Table 3) were statistically analyzed using paired Student's "t" test (Snedecor and Cocchran, 1989).

3. Results

Our initial behavioral studies showed that αMT (Fig. 1) and reserpine (Fig. 2) significantly reduced locomotor activity in normal flies in a dose-dependent manner. The motor depressant effects of the highest doses of both drugs were abolished by coadministration of L-DOPA at a dose which did not itself affect this behavior (Table 1). The behavior effect of αMT was also associated with a decreased brain dopamine concentration (Table 2). Other catecholamines were not identifiable in confirmation of Neckameyer (1996). The above results were reinforced by the finding that a pale conditional mutant showed reduced locomotor activity at its restrictive temperature, an effect which also could be prevented by coadministration of L-DOPA (Fig. 3). Taken together, these results indicate that

Table 1
Effects of L-DOPA on spontaneous locomotor activity

Treatment	αMT (mM)	Reserpine (mM)	L-DOPA (mM)	SMA
Control 1	–	–	–	8.1±1.8
Control 2	–	–	3	8.8±0.3
Control 3	3	–	–	2.5±0.6
Control 4	–	1	–	3.1±0.2
αMT	3	–	3	9.1±0.2*
Reserpine	–	1	3	8.5±0.2*

Groups of ten flies were incubated on the media indicated for 7 days. They were they assayed to determine the number of grid lines crossed per 30-s period (SMA Value) as outlined in Methods. Asterisks indicate significant difference ($p<0.05$) from control 3 or 4 using Student's "t" test.

Table 2
Dopamine levels in *Drosophila* heads

Sample	N	Pg/Head
Control	6	239±21
αMT (1 mM)	6	101±24*

Groups of 6–15 flies were incubated on the media indicated for 7 days. They were then decapitated, the heads homogenized and assayed for dopamine (see Methods). Values indicate group means and standard errors. Asterisk indicates significant difference ($p<05$) from control using Student's "t" test.

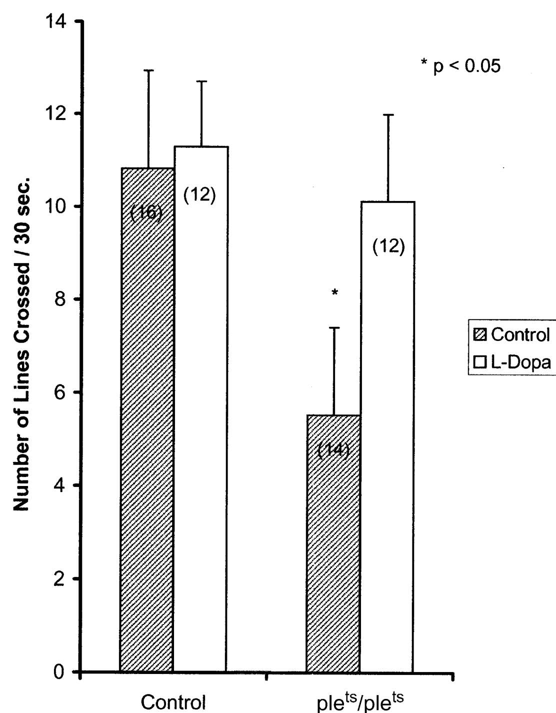

Fig. 3. Effects of L-DOPA on spontaneous locomotor activity in homozygous ple^{ts} flies. Homozygous ple^{ts} mutants maintained at their permissive temperature (18 °C) and control flies of similar age were placed on media containing 3 mM L-DOPA at 25 °C for 72 h. They were then transferred to 29 °C (restrictive temperature) for 72 h and then assayed for spontaneous locomotor activity after 24 h at 25 °C. Bars indicate the means and standard errors for the number of flies indicate in parenthesis. Asterisk indicates a significant difference ($p<0.05$) from corresponding control flies using group Student's "t" test.

αMT and reserpine produce depressant effects on locomotor activity in *Drosophila* by decreasing dopamine release in the brain.

However, the lowest doses of both αMT and reserpine in the above dose response studies, which did not alter locomotor activity, were effective in reducing locomotor activity in adults treated with them throughout development, but then fed drug-free food for 7 days prior to testing (Fig. 4). Thus, treatment of parents with doses of reserpine and αMT which did not alter their behavior did alter the behavior of their progeny.

In a subsequent study, adult flies were treated with 0.1 mM αMT for 10 days and discarded. Their progeny, all of which survived (Pendleton et al., 1996), were placed on untreated media for 7 days to allow the flies to clear the drug from their system and were then assayed for brain tyrosine hydroxylase by Western blotting. The results (Fig. 5, Table 3) indicated that these progeny expressed significantly higher ($p<0.05$) levels of brain TH than did the progeny of untreated parents. Moreover, the total brain protein content did not differ significantly between the two groups (data not shown) suggesting that this effect on TH was specific. The Western blots also showed through the use of protein ladders that the molecular weight in all tissue samples was approximately 60 kDa, indicative of the neural form of the enzyme. The isoform of TH in the hypoderm (epidermis) which forms the cuticle is 80 kDa (Friggi-Grelin et al., 2003).

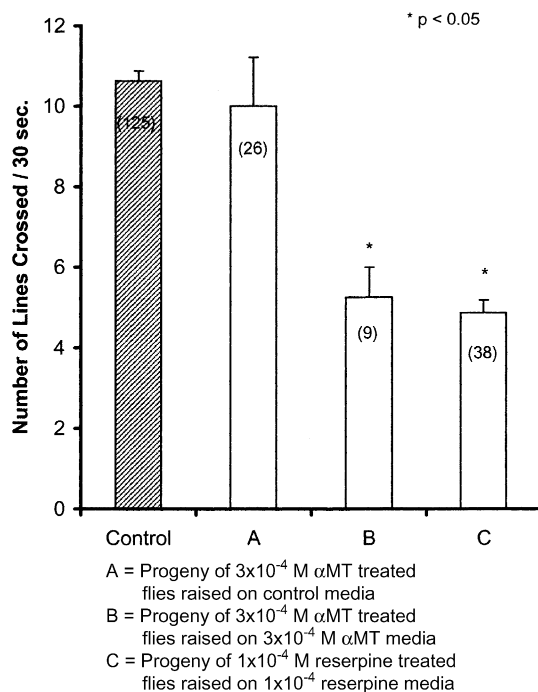

Fig. 4. Spontaneous locomotor activity of drug treated progeny. Adult flies were treated for 7 days at 25 °C and discarded. Progeny were assayed for locomotor activity after 5 days. Bars indicate the means and standard errors for the number of flies indicated in parenthesis. Asterisks show significant differences from the control using ANOVA and group Student's "t" test. A reserpine progeny group was also raised on control media and assayed. There was no difference from control.

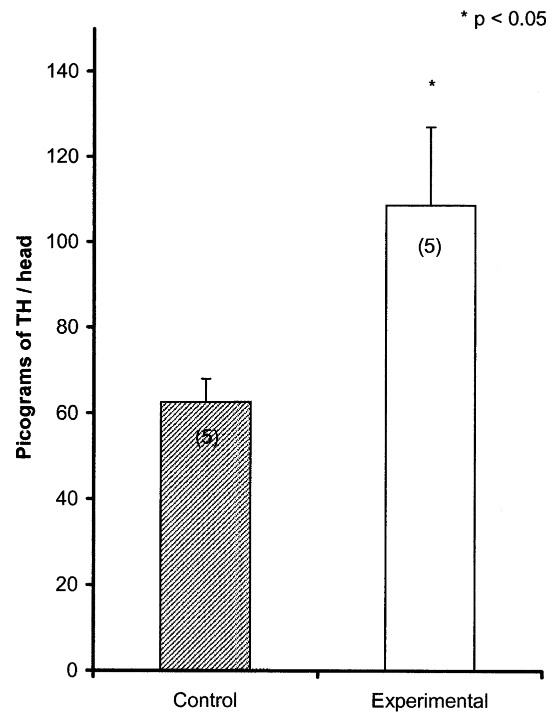

Fig. 5. Brain TH levels in progeny of flies treated with αMT. Parents in the experimental group were placed on food containing 0.1 mM αMT at 25 °C for 10 days. The control group received no drug. The parents were then discarded and the progeny transferred to normal food for 7 days, decapitated and the heads analyzed for TH. Each of 5 samples contained 200 heads. Bars indicate the mean and standard error for each group. The asterisk indicates that the TH level in the progeny of the αMT treated parental group was significantly ($p<0.05$) higher than in the non-treated controls using paired Student's "t" test.

4. Discussion

It is well established that tyrosine hydroxylase is required for development in *Drosophila.* This enzyme is coded for by *pale*, a single copy gene located on the third chromosome. Null mutant alleles of this gene are lethal to embryos when homozygous or when hemizygous over a small deficiency (Jurgens et al., 1984; Neckameyer and White,

Table 3
Tyrosine hydroxylase levels in *Drosophila* heads

Experiment	(Pg/Head)	
	Control	Experimental
1	43	47
2	73	176
3	75	110
4	68	108
5	54	102

Paired analysis: Student's "t" value is 2.86; $p<0.05$. Experimental details and averages are described in Methods and Fig. 5.

1993). A temperature sensitive conditional mutant of pale (*ple*^{ts1}) recently reported by us is also lethal at its restrictive temperature (Pendleton et al., 2002). These genetic findings are reinforced by pharmacological studies indicating that inhibition of TH by αMT (Pendleton et al., 1996) or iodotyrosine (Neckameyer, 1996) also inhibits embryogenesis as well as larval development. These effects are preventable by L-DOPA coadministration.

Our data also show that treatment of flies during development with low dosages of αMT result in increased levels of brain TH when they emerge as adults. Such flies are also behaviorally more sensitive to drugs which deplete catecholamines either by affecting their biosynthesis (αMT) or storage in nerve terminals (reserpine). Similar results have been reported in chicken eggs (Lydiard et al., 1975; Lydiard and Sparber, 1974) where the administration of αMT or reserpine is followed by postnatal increases in the TH levels in 28-day-old progeny and is associated with behavioral alterations in response to certain drugs (Lydiard and Sparber, 1977).

These results suggest that inhibiting catecholamine synthesis or their neuronal retention in embryos may alter adult behavior by enhancing the levels and activity of TH leading to increased catecholamine turnover. This effect on neuronal plasticity may have implications in the pathogenesis and/or treatment of CNS disorders such as schizophrenia, which is considered to be the result of hyperactivity of dopaminergic neurons in mesolimbic areas of the human brain (Carlsson, 2001; Woods, 1998; Thome et al., 1998).

References

Adell A, Myers RD. 5-HT,dopamine,norepinephrine, and related metabolites in brain of low alcohol drinking (LAD) rats shift after chronic intrahippocampal administration. Neurochem Res 1995;20(2):209–15.

Axelrod J. Noradrenaline: fate and control of its biosynthesis. Science 1971;179:598–606.

Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.

Carlsson A. A half-century of neurotransmitter research: impact on neurology and psychiatry Nobel lecture. Bio Rep 2001;21:691–710.

Ford SC, Napolitano LM, McRobert SP, Tompkins L. Development of behavioral competence in young *Drosophila melanogaster* adults. J Insect Behav 1989;2:575–87.

Friggi-Grelin F, Iche M, Birman S. Tissue-specific developmental requirements of *Drosophila* tyrosine hydroxylase isoforms. Genesis 2003;35(3):175–84.

Hillman R, Pendleton RG. Behavior in *Drosophila*: analysis and control. In: LeDoux M, editor. Animal models of movement disorders; 2005. p. 101–10.

Jurgens G, Weischaus E, Nuslein-Volhard C, Kluding H. Mutations affecting the pattern of larval cuticle in *Drosophila melanogaster*. Wilhelm Rouxs Arch Dev Biol 1984;193:283–95.

Kelly MA, Rubinsterin M, Phillips TJ, Lessov CN, Burkhart-Kasch S, Bunrow JR, et al. Locomotor activity in D2 dopamine receptor deficient mice is determined by gene dosage, genetic background, and developmental adaptations. J Neurosci 1998;18:3470–9.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.

Lydiard RB, Sparber SB. Evidence for a critical period for postnatal elevation of brain tyrosine hydroxylase activity resulting from reserpine administration during embryonic development. J Pharmacol Exp Ther 1974;189:370–9.

Lydiard RB, Sparber SB. Postnatal behavioral alterations resulting from prenatal administration of DL-alpha-methyl-para-tyrosine. Dev Psychobiol 1977;10:305–14.

Lydiard RB, Fossom LH, Sparber SB. Postnatal elevation of brain tyrosine hydroxylase activity, without concurrent increases in steady-state catecholamine levels, resulting from DL-a-methylparatyrosine administration during embryonic development. J Pharmacol Exp Ther 1975;194:27–36.

Meyer ME, Cottrell GA, VanHartesveldt C, Potter TJ. Effects of dopamine D_1 antagonists SCH 23390 and SK&F 83566 on locomotor activities in rats. Pharmacol Biochem Behav 1993;744:429–32.

Moore KE, Dominic JA. Tyrosine hydroxylase inhibitors. Fed Proc 1971;30:859–70.

Nagatsu T, Levitt M, Undenfriend S. Tyrosine hydroxylase The initial step in norepinephrine biosynthesis. JBC 1964;239:2910–7.

Neckameyer WS. Multiple roles for dopamine in *Drosophila* development. Dev Biol 1996;176:206–19.

Neckameyer WS, White K. *Drosophila* tyrosine hydroxylase is encoded by the pale locus. J Neurogenet 1993;8:189–99.

Pendleton RG, Robinson N, Roychowdhury R, Rasheed A, Hillman R. Reproduction and development in *Drosophila* are dependent upon catecholamines. Life Sci 1996;59:2083–91.

Pendleton RG, Rasheed A, Sardina T, Tully T, Hillman R. Effects of tyrosine hydroxylase mutants on locomotor activity in *Drosophila*: a study in functional genomics. Behav Genet 2002;32:89–94.

Snedecor GW, Cocchran WG. Statistical methods8th ed. Ames: Iowa State Univ. Press; 1989.

Thome J, Foley P, Riederer P. Neurotrophic factors and the maldevelopmental hypothesis of schizophrenic psychoses. J Neural Transm 1998;105:85–100.

Woods BT. Is schizophrenia a progressive neurodevelopmental disorder? Toward a unitary pathogenic mechanism. Psychiatry 1998;155:1661–70.

Zhou QY, Palmiter RD. Dopamine deficient mice are severely hypoactive, adipsic, and aphagic. Cell 1995;83:1197–209.