Published in final edited form as:

Pharmacol Res. 2006 November ; 54(5): 361–372.

Antipsychotic drugs disrupt normal development in *Caenorhabditis elegans* via additional mechanisms besides dopamine and serotonin receptors

Dallas R. Donohoe¹, Eric J. Aamodt², Elizabeth Osborn³, and Donard S. Dwyer^{1,3,4,*}

¹ Department of Pharmacology, Toxicology and Neuroscience,

² Department of Biochemistry and Molecular Biology, and

³ Department of Psychiatry, Louisiana State University Health Sciences Center, 1501 Kings Highway, Shreveport, LA, 71130, USA

Abstract

Antipsychotic drugs may produce adverse effects during development in humans and rodents. However, the extent of these effects has not been systematically characterized nor have molecular mechanisms been identified. Consequently, we sought to evaluate the effects of an extensive panel of antipsychotic drugs in a model organism, *C. elegans*, whose development is well characterized, and which offers the possibility of identifying novel molecular targets. For these studies, animals were grown from hatching in the presence of vehicle (control) or antipsychotic drugs over a range of concentrations (20–160 μ M) and growth was analyzed by measuring head-to-tail length at various intervals. First-generation antipsychotics (e.g., fluphenazine) generally slowed growth and maturation more than second-generation drugs such as quetiapine, and olanzapine. This is consistent with *in vitro* effects on human neuronal cell lines. Clozapine, a second-generation drug, produced similar growth deficits as haloperidol. Converging lines of evidence, including the failure to rescue growth with high concentrations of agonists, suggested that the drug-induced delay in development was not mediated by the major neurotransmitter receptors recognized by the antipsychotic drugs. Moreover, in serotonin-deficient *tph-1* mutants, the drugs dramatically slowed development and led to larval arrest (including dauer formation), and neuronal abnormalities. Evaluation of alternative targets of the antipsychotics revealed a potential role for calmodulin and underscored the significance of Ca^{2+} -calmodulin signaling in development. These findings suggest that antipsychotic drugs may interfere with normal developmental processes, and provide a tool for investigating the key signaling pathways involved.

Keywords

Antipsychotic drugs; Calmodulin; Developmental toxicity; Dopamine; Serotonin

1. Introduction

To date there has been little systematic study of the effects of psychotropic medications on development. Recently, Ansorge et al. [1] showed that early post-natal exposure to the antidepressant drug fluoxetine produced lasting alterations in the behavior of mice. These studies suggest that a similar evaluation of the effects of antipsychotic drugs on development

*Address correspondence to: Dr. Donard S. Dwyer, Department of Psychiatry, LSU Health Sciences Center, 1501 Kings Highway, Shreveport, LA 71130, USA, PH: 318 675-7612; FAX: 318 675-6148; EMAIL: ddwyer@lsuhsc.edu.

⁴Conflict of interest statement: D.S.D. is funded by grants from NIH (HL84926 and MH68385) and Eli Lilly and Co.

is merited. The FDA considers most of the antipsychotic drugs to be Class C, that is, risk of developmental defects stemming from treatment during pregnancy cannot be ruled out [2]. Phenothiazines, haloperidol, and clozapine have all been reported to produce birth defects [3–6]; however, other studies have not confirmed these findings [7,8]. Acute neonatal effects of *in utero* exposure to clozapine and haloperidol have been described, including convulsions [9], floppy-infant syndrome [10,11], and lower developmental scores [12]. Longer-lasting behavioral deficits in infants may include “jittery” infant syndrome, tremor, and movement disorders, which may persist for up to 6 months [13–15]. Thus, lasting developmental effects of antipsychotic drugs are a possible outcome of early drug exposure.

Antipsychotic drugs produce a variety of adverse effects in patients that include movement disorders and metabolic disturbances such as weight gain and diabetes. The molecular mechanisms responsible for these side effects are not known, however, *in vitro* studies of the drugs have provided some insight into the problem. Antipsychotic drugs adversely affect cell growth [16], energy metabolism [17] and glucose transport [18,19] in cultured cell lines. The major pharmacological actions of the antipsychotic drugs on dopamine and serotonin receptors do not appear to explain the effects of the drugs on cell cultures. On the other hand, there does appear to be a correlation between inhibition of glucose transport and loss of cell viability [16]. It is unclear whether any particular tissues or cells are vulnerable to these same effects *in vivo* or manifest vulnerability in relation to developmental stage, e.g. immature neurons may be more susceptible to the adverse effects of the drugs.

Studies in rats provide some support for the possibility of detrimental effects of antipsychotic drugs on development. Developmental toxicity is listed in The Physician’s Desk Reference as a possible outcome of exposure to newer atypical antipsychotic drugs, quetiapine and ziprasidone, at relevant doses. Treatment of pregnant rats with haloperidol results in low birthweight pups, stunted growth, and reduced brain size [20] that persists through adulthood [21]. Findings of reduced numbers of proliferating neurons in newborn rats exposed *in utero* to antipsychotic drug are consistent with this overall picture [22]. Furthermore, prenatal drug exposure in rats leads to a reduction in nerve growth factor (NGF) protein and mRNA [23], and a significant decrease in expression of genes involved in neuronal plasticity (including calmodulin) in neonatal brain [24]. Although the various findings are not conclusive, further study is clearly needed of the developmental effects of antipsychotic drugs. *Caenorhabditis elegans* may offer a valuable *in vivo* model system for evaluating developmental effects of these drugs and their potential mechanisms of action.

The powerful genetic tools available in *C. elegans* have allowed researchers to gain mechanistic insight into the action of drugs, such as the antidepressants [25–28], cholinergic drugs [29, 30], and CNS stimulants [31,32]. *C. elegans* has also been used to identify new drug targets, as was the case for fluoxetine and the resultant isolation of drug-resistant mutants [33]. Studies of antipsychotics in *C. elegans* are limited [25], although these drugs were used to characterize various dopamine [34,35] and serotonin receptors [36] in this organism. In mammals, antipsychotic drugs mainly antagonize D2 dopaminergic receptors and 5-HT_{2/7} serotonergic receptors [37,38]. As a general rule, dopamine is involved in the control of movement, motivation, and reward, and serotonin regulates feeding, mood, temperature, and sleep [39]. In *C. elegans*, dopamine inhibits egg laying and movement, and regulates the basal slowing response to food, whereas serotonin stimulates egg laying and pharyngeal pumping, and mediates the enhanced slowing response to food [40–44]. Antipsychotic drugs have been noted to stimulate egg laying and defecation in *C. elegans* [25]. We have employed *C. elegans* as an initial platform to gain new insight into the biology of both first-generation (chlorpromazine, fluphenazine, and haloperidol) and second-generation (clozapine, olanzapine, quetiapine, and risperidone) antipsychotic drugs. These two generations of drug have different pharmacological profiles and their diverse biological activities are not fully characterized at

the molecular level. Antipsychotic drugs that adversely affect cell viability, *in vitro* may similarly disrupt cellular processes involved in the normal development of *C. elegans*. The studies described here test this hypothesis and evaluate the role of potential drug targets, including dopamine and serotonin receptors, and calmodulin-mediated pathways. We included analysis of the calmodulin pathway because calmodulin is a known target of many antipsychotic drugs [45,46] and because it is involved in growth regulation [47]. These studies are the first report on the developmental effects of antipsychotic drugs in *C. elegans*. Our data suggest that further studies of the developmental effects of antipsychotic drugs are warranted given the increased use of these medications in young children. A preliminary account of these findings has been presented elsewhere [48].

2. Materials and methods

2.1. Experimental growth conditions and mutant strains

Wild-type (N2) and mutant strains of *C. elegans* were grown and maintained at 20 °C on NGM plates (60 mm) seeded with *Escherichia coli* strain OP50 according to previously described culture conditions [49]. The mutant strain *tph-1(mg280)* was provided by the Caenorhabditis Genetics Center (Minneapolis, MN).

2.2. Neurotransmitters and drugs

Serotonin, dopamine, quinpirole, clozapine, risperidone, haloperidol, fluphenazine, chlorpromazine, butaclamol, metergoline, and trifluoperazine were purchased from Sigma-Aldrich (St. Louis, MO). Calmidazolium chloride and W-13 were purchased from Calbiochem (San Diego, CA). Quetiapine was obtained as a gift from Astra-Zeneca and olanzapine was provided by Eli Lilly and Company.

2.3. Preparation of serotonin and drug-plates

All drugs were dissolved in DMSO, with the exceptions of risperidone (dissolved in ethanol), serotonin, and quinpirole (both dissolved in M9 buffer). Final concentrations of the antipsychotic drugs were prepared in dilute acetic acid (1:10,000) to maximize solubility on the plates. The final concentrations were calculated based on the total volume of each plate, which included the NGM/agar (6.4 ml) plus the amount of drug-solution added to the top of each plate (700 µl for serotonin and 200 µl for all other drugs used). Solutions containing serotonin or the various compounds were added directly to the top of seeded NGM plates. Plates were then gently swirled to allow drug to spread over the entire dish and were equilibrated overnight. Drugs and compounds are stable on the plates, which can be used up to one week after their initial preparation with no loss of activity. Each experimental condition was run in duplicate and experiments were repeated at least twice.

2.4. Analysis of developmental maturation and growth

Twenty gravid adult N2 or mutant animals were transferred to serotonin, quinpirole, control (vehicle), or drug-plates and allowed to lay eggs for ~ 6 hr and then were removed from the plates leaving only unhatched eggs. Juvenile animals that hatched into drug were cultured for 24, 48, or 72 hr before transfer to microscope slides (containing 50 mM sodium azide in M9 buffer) for observation. At each time point, animals were photographed with a 10X objective on a Nikon Eclipse TE300 inverted microscope with an epi-fluorescence attachment, Nomarski optics, and a CoolSNAP monochrome CCD camera. The body length (head to tail) of 25 animals per time point and condition was measured with Image J software (NIH, Bethesda, MD). Experimental conditions were evaluated in duplicate and ~ 12–13 worms (chosen at random) were analyzed from each plate at every time point.

2.5. Rescue experiments with serotonin and dopamine agonist, and inhibition of dopamine-induced immobilization with antipsychotics

Plates containing serotonin or the dopamine agonist quinpirole were made in accordance with previous studies [41,43]. Serotonin creatine sulfate (50 mM) or quinpirole (60 mM) were dissolved in M9 buffer and added together with vehicle (control group) or drug at the desired concentrations. The biological effects of serotonin and quinpirole were confirmed in egg-laying assays. The growth of animals on the serotonin and quinpirole rescue plates was measured as described in the above section.

Dopamine paralysis experiments were performed as described previously [42]. Briefly, dopamine was added to 1.7 % Difco bacto agar (~ 50 °C) and 2 mM acetic acid to obtain a final concentration of 15 mM. Control plates were prepared identically except without dopamine. The final volume of each plate was 6.4 ml. The plates were allowed to dry overnight. The next day, drug or vehicle (DMSO or ethanol) was added to the top of the plates (200 µl) to obtain the final desired concentration. Well-fed adult N2 animals (25–35) were placed on unseeded plates (no dopamine) containing drug or vehicle for 20 minutes. These animals were then transferred to experimental plates containing dopamine plus drug or vehicle. Animals were scored as immobile based on failure to show spontaneous movement for 10 seconds at 15, 20, 25, 30, and 35 minutes. A similar criterion for dopamine-induced immobilization has been used [50].

2.6. Pharyngeal pumping, egg-laying, and developmental arrest

Pharyngeal pumping was assayed in duplicate for each experimental condition by counting the number of pharyngeal pumps in thirty-second intervals for 20 animals per plate (N=40). Egg-laying in response to serotonergic or dopaminergic agonists was measured by transferring 10 synchronized adults to control, serotonin, or quinpirole plates and counting the number of eggs laid after 1, 3, and 5 hr. Developmental arrest assays were performed by placing 20 gravid *tph-1* animals on control (vehicle) or drug (clozapine or fluphenazine) plates (made as described above) and allowing them to lay eggs for ~ 6 hr before the mothers were removed from the plates. Eggs were allowed to hatch and the number of adults, dauer larvae, and developmentally-arrested animals was counted at 72 hr and 96 hr after hatching. Dauer or partial dauer were identified by a thin, rigid, and granular appearance, lack of pharyngeal pumping, and absence of spontaneous movement for 30 sec, while retaining touch sensitivity. We considered animals to be arrested that moved and maintained pharyngeal pumping, but failed to develop past the fourth larval stage after 4–5 days.

2.7. Data analysis and statistics

A one-way ANOVA followed by a post-hoc Tukey test was used to test for differences in development and behavior between drug-treated groups and controls. Each experiment was performed twice and data were collected from duplicate plates that were averaged for each time point. Differences between drug-treated and control groups were considered significant with a p-value < 0.05.

3. Results

3.1. First-generation antipsychotic drugs slow growth and development

We previously showed that the first-generation antipsychotics, including chlorpromazine, fluphenazine, and haloperidol, reduced cell viability and slowed growth in cell cultures. In contrast, the second-generation antipsychotics produced a modest reduction in cell viability over the same concentration range or even promoted cell growth in some cases [16]. To further characterize the adverse effects of antipsychotic drugs, we initiated studies in *C. elegans*

because this system offers the potential to identify the molecular mechanism(s) involved. We reasoned that the antipsychotic drugs that produced the greatest effects on cell growth and viability *in vitro* would adversely affect growth and development in *C. elegans*. To test this possibility, animals were incubated from hatching in the presence of various antipsychotic drugs, and growth was monitored over time. We chose the concentration range (20–320 μM) to be tested based on concentrations of drug previously shown to affect cell growth *in vitro* by our group and others [16,51]. For comparison, clozapine reaches concentrations of 1 μM in blood [52] and 20–30 μM in brain tissue [53].

For these experiments, control animals were grown on plates containing identical amounts of solvent (DMSO or ethanol), and at the maximum concentrations used (1%) there were negligible effects of solvent on development. Previously, we showed that most animals on control plates reached the L3–L4 stage by day 2 as judged by characteristic anatomical features. By comparison, the majority of animals grown on plates with fluphenazine, chlorpromazine and clozapine (80 μM and above) failed to develop past the L2 stage at this time point and the effect was dose-dependent [54]. Thus, the drugs slowed the larval stage maturation of *C. elegans*. For the present studies, we chose to examine a more quantitative measure of growth (body length) rather than developmental staging. The first-generation antipsychotics, chlorpromazine, fluphenazine, and haloperidol, slowed the growth of *C. elegans* in a dose-dependent manner (Fig. 1). These effects on growth were found to be significant at 40 μM and above for chlorpromazine (Fig. 1B) and fluphenazine (Fig. 1D) and 80–160 μM for haloperidol (Fig. 1F). The drugs slowed maturation, but had little or no effect on the size of the animals at a particular developmental stage. All of the animals treated with drug at the concentrations shown here eventually became viable adults of normal size and laid eggs, although the brood sizes were smaller than for vehicle-treated animals (data not shown). The time to reach adulthood depended upon the concentration of drug on the plates, but typically took about 5 d for animals treated with 160 μM fluphenazine. We conclude that the first-generation drugs produced a dose-dependent slowing in the growth and development of *C. elegans*.

3.2. Second-generation antipsychotic drugs produce minimal effects on development

The first-generation antipsychotic drugs are primarily considered dopamine antagonists, although to varying degrees they also antagonize serotonin receptors. Therefore, we also sought to test the second-generation antipsychotics, which are more potent antagonists of serotonin receptors than dopamine receptors in mammals, for their effects on growth and development (Fig. 2). Clozapine, a second-generation drug that binds to *C. elegans* serotonin and dopamine receptors, caused a dose-dependent slowing in growth and maturation (Fig 2B). In contrast, risperidone produced modest effects on growth at 160 μM , and quetiapine and olanzapine were essentially inactive (Fig. 2D, 2F, and 2H). Thus, the first-generation drugs showed far greater effects on gross development in *C. elegans* than the second-generation drugs, which is consistent with findings on cultured cells.

3.3. Pharmacological analysis of drug-induced developmental delay

To address possible mechanism(s) of action, we evaluated the drugs that slowed development in a head-to-head comparison with established ligands for *C. elegans* dopamine and serotonin receptors, butaclamol and metergoline, respectively. Butaclamol potently antagonizes dopamine D2 receptors in *C. elegans* [44], but acts as an inverse agonist at D1 receptors [34]. Neither the selective receptor ligands nor the antipsychotic drugs produced a significant developmental delay at the 24 hr time point (Fig. 3A). This is likely due to the fact that the animals are approximately the same size across the experimental groups at hatching and maintain this relative relationship for the first 24 hr. At 48 and 72 hr, the interference by drugs with normal growth is clearly evident (Fig. 3B and C). The rank order, greatest to least, of the drugs tested in Fig. 3 is chlorpromazine > fluphenazine > haloperidol ~ clozapine = metergoline

> butaclamol. Butaclamol, which binds to the *C. elegans* D2-like receptor, *dop-2*, with a higher affinity than does haloperidol or clozapine [35], did not slow development to the same extent as the antipsychotic drugs (Fig. 3A, B, and C). Butaclamol is an inverse agonist at D1 dopamine receptors, therefore, the possible involvement of other dopamine receptors in the response is not ruled out by this experiment.

Differences in the ability of the drugs to inhibit development of *C. elegans* could be related to their differential effects on dopamine receptors or failure of a drug to be taken up by the worms. To address these issues, drugs were tested for their ability to antagonize dopamine-induced immobilization, which has been shown to be mediated through dopamine receptors [50]. Animals were incubated on plates containing solvent (control) or antipsychotic drug for 20 min prior to transfer to plates containing dopamine at a concentration (15 mM), which produces paralysis in 80–90% of the animals. The 20 min preincubation with antipsychotic drug was necessary to observe protection against paralysis and indicates rapid uptake of the antipsychotics. The results of these experiments (Fig. 4A & B) illustrate two important points. First, the antipsychotic drugs used in these studies antagonized dopamine's effects in the locomotion assay and reduced the number of paralyzed animals. By contrast, quinpirole, a dopaminergic agonist, enhanced the paralytic effect of dopamine as expected. Second, olanzapine and quetiapine, which did not affect development of *C. elegans*, were the most potent dopamine antagonists in the locomotion assay. This indicates that all of the drugs are indeed being taken up by the animals and produce significant biological effects over the concentration range tested. There was no correlation between dopamine antagonism in this assay and inhibition of development, which argues against dopamine receptor blockade as the main explanation for the growth delay induced by antipsychotic drugs.

To evaluate the role of serotonin in the response, we examined the effects of metergoline, a ligand that binds to *C. elegans* serotonin receptors with a similar affinity as clozapine. Metergoline and its close analogs have been reported to block the SER-1, SER-4 and SER-7 receptors in *C. elegans* [36,55,56]. In addition, growth of the *tph-1* strain was analyzed as a positive control for developmental impairment and to provide a baseline for the effects of serotonin antagonism because these animals completely lack serotonin [57]. Metergoline slowed development of the animals to the same extent as clozapine, which suggested that antipsychotic drugs may produce their effects through actions on serotonin receptors (Fig. 3). The fact that, as shown here, serotonin deficiency in *tph-1* mutants is accompanied by slower development in comparison to wild-type N2 animals is consistent with this interpretation (Fig. 3B and C). Decreased serotonergic tone is also associated with a reduction in pharyngeal pumping and this could conceivably affect the rate of growth. However, the drugs did not appreciably slow pharyngeal pumping (except chlorpromazine at 80 μ M) as shown in Fig. 4C, nor did they adversely affect neuromuscular function as judged by normal movement on drug plates.

In view of the effects of metergoline on development of *C. elegans*, we undertook further characterization of the possible role of serotonin in the response to antipsychotic drugs. For these studies, *tph-1* mutants were grown in the absence (control) or presence of clozapine (second-generation drug) or fluphenazine (first-generation drug) and development was monitored as before. If the drugs work primarily via serotonergic mechanisms, there should be little difference between control and drug-treated *tph-1* animals. Alternatively, drug-induced exacerbation of growth defects in the *tph-1* strain would suggest involvement of additional pathways. As seen in Fig. 5A and B, clozapine and fluphenazine at 200 μ M reduced the rate of growth of *tph-1* mutants even further. Moreover, many drug-treated animals failed to develop to the adult stage and arrested as dauer/partial dauer or as immature larvae. Dauer/partial dauer animals were distinguished by morphological features and behavior as described in the Materials and methods section. There were no observable adults even after 8 days on

the drug plates. In a separate experiment, we confirmed that the antipsychotic drugs induced *bona fide* dauer larvae in the *tph-1* strain. *tph-1* animals were hatched onto plates containing vehicle, clozapine, or fluphenazine, 4 days later they were exposed to 1 % SDS for 30 min, and then transferred to fresh seeded plates. The number of surviving animals was counted 24 hr later. Both clozapine- and fluphenazine-treated plates had a larger number of animals that survived SDS treatment (dauer larvae) than the vehicle control groups (data not shown). Therefore, the antipsychotic drugs worsen the developmental deficits in *tph-1* mutants as indicated by the larger number of animals that failed to mature to the adult stage. Thus, it appears that serotonergic mechanisms do not fully explain the adverse effects of the antipsychotic drugs on development. Nevertheless, the slower growth of the *tph-1* strain and animals treated with butaclamol suggest that serotonin and dopamine may play some role in controlling the rate of development in *C. elegans*.

3.4. Failure to rescue developmental effects with serotonin and quinpirole

To further evaluate possible contributions of the serotonergic and dopaminergic systems to the drug-induced developmental delay, we attempted to rescue animals from the adverse effects of the antipsychotic drugs by the addition of serotonin or quinpirole. As a first step, we confirmed that the agonists produced their normal biological effects on *C. elegans*. Serotonin has been reported to induce egg-laying in wild-type N2 animals. On the other hand, dopamine exerts the opposite effect and inhibits egg-laying in wild-type animals. We confirmed that in *C. elegans* serotonin promoted egg-laying at 2.5 and 5 mM (358 and 416 eggs, respectively vs. 220 eggs for the controls at 5 hr; N = 10 animals per group), whereas quinpirole at 750 μ M and 1 mM reduced egg-laying as judged by the number of eggs present on plates 3 and 5 hr after transfer of N2 animals. The values at the 5 hr time point were 187 and 179 eggs for 750 μ M and 1 mM quinpirole, respectively, compared to 217 eggs in the control condition. A similar result was reported by Segalat et al. [41]. Therefore, serotonin and quinpirole were biologically active as applied to the plates. Data in Fig. 5C clearly show that addition of serotonin to the clozapine-treated plates did not restore normal growth to the animals. Similarly, quinpirole (750 μ M and 1 mM) failed to rescue animals from the developmental delay induced by clozapine and fluphenazine (Fig. 5D). In fact, serotonin and quinpirole by themselves reduced the rate of development in *C. elegans*. Quinpirole acts as a dopaminergic agonist in *C. elegans* ([41] and see Fig. 4B), however, its modest effect to slow growth may be due to additional actions. Taken together with the findings presented above, these results suggest that the drugs may produce their adverse effects on development independent of their actions on dopamine and serotonin receptors. Consequently, it appears that the drugs target additional pathways to inhibit growth in *C. elegans*.

3.5. Role of calmodulin in growth and development

Calmodulin is Ca^{2+} binding protein that exerts a broad spectrum of biological effects and is highly conserved from *C. elegans* to mammals. Many of the antipsychotic drugs are potent inhibitors of calmodulin [45,46]. Importantly, chlorpromazine and fluphenazine had the greatest effect on development (Fig. 3), and are also considered to be strong calmodulin inhibitors. By comparison, clozapine and haloperidol produced smaller effects on development and are weaker calmodulin antagonists. Therefore, we sought to test whether other known calmodulin inhibitors (calmidazolium, trifluoperazine, and W-13) slowed development in comparison to chlorpromazine and fluphenazine in wild-type *C. elegans*. Calmidazolium, trifluoperazine, and W-13 all produced significant inhibition of growth at 80 μ M and 160 μ M (Fig 6A). Furthermore, trifluoperazine had the greatest effect, and was comparable to chlorpromazine. These data suggested a link between inhibition of calmodulin and inhibition of growth in *C. elegans*. When this relationship was examined quantitatively through linear regression analysis, a striking correlation ($R^2 = 0.93$) was found between the IC_{50} values for inhibition of calmodulin activity and a drug's ability to slow development (Fig. 6B). These

data are consistent with phenotypes produced in *C. elegans* by reduction of calmodulin levels by RNAi [58,59]. Thus, calmodulin may represent a common mechanism through which the antipsychotic drugs adversely affect growth and development in *C. elegans*.

4. Discussion

The data presented here showed that many antipsychotic medications, especially the first-generation drugs, interfered with the normal rate of development of *C. elegans*. Thus, chlorpromazine, fluphenazine, and haloperidol produced greater effects than the second-generation drugs, quetiapine and olanzapine. The drugs slowed reproductive maturation through the four larval stages and even arrested development at high concentrations. The adverse effects on development were not the result of interference with pharyngeal pumping or feeding. The animals fed, remained viable, and reproduced (albeit with smaller brood sizes), but matured much more slowly in the presence of drug. The fact that the first-generation drugs were more potent than the second-generation antipsychotics initially suggested that dopaminergic mechanisms might be involved in the developmental delay because first-generation drugs show a preferential inhibition of dopamine vs. serotonin receptors in comparison to second-generation drugs where the reverse is true. However, normal growth rates were not restored by adding dopamine or serotonin together with the antipsychotic drugs. Moreover, the development of serotonin-deficient *tph-1* mutants was slowed even further by the antipsychotic drugs. Interestingly, *cat-4* and *bas-1* mutants (that are deficient in both serotonin and dopamine) and *dop-1* mutants (which lack functional dopamine receptors) develop at a normal rate [34,60]. Therefore, additional receptor proteins appear to be involved in the developmental delay produced by the antipsychotic drugs, which are known to target other proteins besides neurotransmitter receptors.

The results presented here support our initial line of reasoning that there would be a close correspondence between the adverse effects of antipsychotic drugs on cultured cells and their effects in *C. elegans*. In fact, there was no exception to this relationship among the drugs tested. This included the previously-noted distinction between clozapine (toxic, slows development) and olanzapine (neutral to slightly positive effects), despite their close structural resemblance [16]. It is noteworthy that the drug concentrations that produced significant delays in development are among the lowest effective concentrations reported for *C. elegans*. In many cases, drug concentrations as high as 1 mM have been used to produce the desired biological effect in this organism [25,27]. It has been suggested that higher concentrations are necessary due to the limited uptake of drugs via ingestion and the barrier properties of the cuticle [61]. We found that the antipsychotic drugs affected development in *C. elegans* at concentrations that were very similar (about 2-fold higher) to those required to produce significant effects on cultured cell lines [16]. Thus, *C. elegans* may be an appropriate model system for investigating potential adverse effects of antipsychotic drugs on growth and development.

The antipsychotic drugs are mainly noted for their ability to inhibit dopamine and serotonin receptors. While these actions may contribute to some of the effects on growth in *C. elegans*, e.g., serotonin deficiency is associated with slower development (as shown here), they do not fully account for the effects of the drugs. Previously, we have shown that these drugs block glucose transport into neuronal, muscle, and other cell types *in vitro* [18,19]. Glucose is essential for growth in mammals [62]. If the antipsychotic drugs produce a similar reduction in glucose uptake in *C. elegans*, this may explain how they could interfere with normal development. The rank ordering of the drugs in terms of their effects on glucose transport in cell lines is similar to the rank ordering of their adverse effects on development. However, the exceptions to this trend cast doubt on this simple interpretation. Thus, olanzapine is a modest inhibitor of glucose transport, whereas haloperidol is completely ineffective, yet haloperidol significantly retards development in *C. elegans* and olanzapine does not.

Based on these observations, we evaluated the possible involvement of another established target of many antipsychotic drugs, namely calmodulin, in the disruption of development. Unfortunately, knockout of calmodulin in *C. elegans* is embryonic lethal. Therefore, we relied on pharmacological methods to determine whether calmodulin might be a relevant target of the antipsychotic drugs. These studies revealed that two additional antipsychotic drugs, trifluoperazine and pimozide (data not shown), which are well-established, high affinity antagonists of calmodulin [45,46], produced significant retardation of growth in *C. elegans*. Moreover, two selective calmodulin antagonists (calmidazolium and W-13) with very different chemical structures also inhibited normal development. These compounds are quite distinct from the antipsychotic drugs in terms of their pharmacological profiles, e.g., they do not block dopamine or serotonin receptors. Calmidazolium has previously been reported to inhibit the development of rat embryos *in vitro* [63]. The data in Fig. 6 show the strong correlation between antagonism of calmodulin and interference with development in *C. elegans*. Our data are consistent with the results of studies using RNAi to reduce the level of calmodulin in *C. elegans*. Kamath et al. [58] reported slowed growth in animals fed RNAi against calmodulin. Karabinos et al. [59] confirmed this observation and showed that injection of RNAi produced a more severe phenotype of embryonic lethality. The latter group demonstrated that injection of RNAi was superior to feeding in the reduction of calmodulin. In our system, there is significant lethality for the worms when the concentrations of drug are increased beyond the levels reported here, which is consistent with the data of Karabinos et al. Although the correlation shown in Fig. 6 does not prove a direct mechanistic connection, it suggests that calmodulin is a prime candidate whose involvement merits further study.

Calmodulin is critical for the regulation of various aspects of growth and development [47]. Moreover, recent studies from our group revealed that antagonism of calmodulin with antipsychotic drugs or selective inhibitors abrogates the activation of Akt by nerve growth factor [64]. Akt regulates cell size, cell proliferation, and glucose (and nutrient) transport and metabolism [65,66]. Thus, drug-induced interference with calmodulin-dependent activation of Akt and other important signaling molecules is an attractive mechanism for the disruption of normal development in *C. elegans*. Mutant strains with altered expression of certain upstream or downstream components of calmodulin-signaling pathways, calcium channels (*unc-2*) and calmodulin kinase II (*unc-43*), respectively, produce small brood sizes and are slower to develop [42,67,68], which is consistent with the scheme proposed here. However, further studies will be necessary to establish the mechanisms involved in the response to the drugs. In an initial attempt to identify the mechanism of action, we have devised a selection method based on complete developmental arrest of *tph-1* animals exposed to 300 μ M clozapine. Animals were mutagenized with ethylmethane sulfonate and F₁ mutants that developed to the adult stage in 300 μ M clozapine were selected for further analysis. The drug-resistant animals were propagated for several generations and three mutant strains show clear resistance to clozapine, fluphenazine, and risperidone (data not shown). Detailed characterization of these mutants is underway.

Regardless of the precise mechanism of action, the striking effects of the antipsychotic drugs in *C. elegans* may provide more general insights into the regulation of developmental processes. The ultimate target(s) of the drugs clearly play an important role in normal development. Genetic approaches in *C. elegans* may allow identification of these targets and characterization of the pathways involved. In addition, to developmental delays, we have observed drug-induced disturbances in neuronal migration and axonal pathfinding in *C. elegans* (ref. 48; unpublished observations). The fact that the drugs also interfere with neuronal development is not surprising in view of the significant effects on reproductive maturation.

There are limited data concerning the effects of antipsychotic drugs on development in higher organisms. Studies of the drugs in pregnant rats have revealed that mothers treated with high

doses of drug give birth to smaller pups with stunted growth and reduced brain size [20]. In humans, there have been reports of transient behavioral and neurological symptoms (tremors and “floppy infant” syndrome) [10,11,13,15] and sporadic accounts of birth defects in babies born to mothers who were treated during pregnancy with antipsychotic drugs, including the phenothiazines, and haloperidol and clozapine [3–6]. Other studies did not find an increase in birth defects [7,8]. The overall conclusion based on an extensive survey of the literature is that the safety of the antipsychotic drugs during early developmental periods has not been established. Increasingly, antipsychotic drugs are being prescribed for various disorders in children as young as 2 years old [69,70]. This trend has emerged in the absence of data to support the advisability of this therapeutic approach. The results presented here suggest that great caution should be used in prescribing antipsychotic drugs for children, especially at early stages of development. Furthermore, our data suggest that some antipsychotic drugs, such as olanzapine and quetiapine, might be safer than others for use during vulnerable developmental periods. Additional studies of these important issues are clearly needed and *C. elegans* may represent an attractive system for pursuing this research in order to characterize relevant pathways and molecular mechanisms.

Acknowledgements

The authors wish to thank Ray Jarvis for expert technical assistance and NIH for funding this research.

References

1. Ansorge MS, Zhou M, Lira A, Hen R, Gingrich JA. Early-life blockade of the 5-HT transporter alters emotional behavior in adult mice. *Science* 2004;306:879–881. [PubMed: 15514160]
2. Committee on Drugs. Use of psychoactive medication during pregnancy and possible effects on the fetus and newborn. *Pediatrics* 2000;105:880–887. [PubMed: 10742343]
3. Kopelman AE, McCullar FW, Heggeness L. Limb malformations following maternal use of haloperidol. *JAMA* 1975;231:62–63. [PubMed: 1243570]
4. Elia J, Katz IR, Simpson GM. Teratogenicity of psychotherapeutic medications. *Psychopharmacol Bull* 1987;23:531–586. [PubMed: 2893424]
5. Godet PF, Marie-Cardine M. Neuroleptics, schizophrenia and pregnancy. Epidemiological and teratologic study. *Encephale* 1991;17:543–547. [PubMed: 1687216]
6. Altshuler LL, Cohen L, Szuba MP, Burt VK, Gitlin M, Mintz J. Pharmacologic management of psychiatric illness during pregnancy: dilemmas and guidelines. *Am J Psychiatry* 1996;153:592–606. [PubMed: 8615404]
7. Ernst CL, Goldberg JF. The reproductive safety profile of mood stabilizers, atypical antipsychotics, and broad-spectrum psychotropics. *J Clin Psychiatry* 2002;63(Suppl 4):42–55. [PubMed: 11913676]
8. Nguyen HN, Lalonde P. Clozapine and pregnancy. *Encephale* 2003;29:119–124. [PubMed: 14567163]
9. Stoner SC, Sommi RW Jr, Marken PA, Anya L, Vaughn J. Clozapine use in two full-term pregnancies. *J Clin Psychiatry* 1997;58:364–365. [PubMed: 9515978]
10. DiMichele V, Ramenghi LA, Sabatino G. Clozapine and lorazepam administration in pregnancy. *Eur Psychiatry* 1996;11:214.
11. Lanczik M, Knoche M, Fritze J. Psychopharmacotherapy during pregnancy and lactation. 1. *Pregnancy Nervenarzt* 1998;69:1–9.
12. Yoshida K, Smith B, Craggs M, Kumar R. Neuroleptic drugs in breast-milk: a study of pharmacokinetics and of possible adverse effects in breast-fed infants. *Psychol Med* 1998;28:81–91. [PubMed: 9483685]
13. Sexson WR, Barak Y. Withdrawal emergent syndrome in an infant associated with maternal haloperidol therapy. *J Perinatol* 1989;9:170–172. [PubMed: 2738729]
14. Shimohira M, Iwakawa Y, Kohyama J. Rapid-eye movement in jittery infants. *Early Hum Dev* 2002;66:25–31. [PubMed: 11834345]
15. Collins KO, Comer JB. Maternal haloperidol therapy associated with dyskinesia in a newborn. *Am J Health-Sys Pharm* 2003;60:2253–2255.

16. Dwyer DS, Lu XH, Bradley RJ. Cytotoxicity of conventional and atypical antipsychotic drugs in relation to glucose metabolism. *Brain Res* 2003;971:31–39. [PubMed: 12691834]
17. Dwyer DS, Donohoe D, Lu XH, Aamodt EJ. Mechanistic connections between glucose/lipid disturbances and weight gain induced by antipsychotic drugs. *Int Rev Neurobiol* 2005;65:211–247. [PubMed: 16140058]
18. Dwyer DS, Pinkofsky HB, Liu Y, Bradley RJ. Antipsychotic drugs affect glucose uptake and the expression of glucose transporters in PC12 cells. *Prog Neuropsychopharmacol Biol Psychiatry* 1999;23:69–80. [PubMed: 10368857]
19. Ardizzone TD, Bradley RJ, Freeman AM 3rd, Dwyer DS. Inhibition of glucose transport in PC12 cells by the atypical antipsychotic drugs risperidone and clozapine, and structural analogs of clozapine. *Brain Res* 2001;923:82–90. [PubMed: 11743975]
20. Holson RR, Webb PJ, Grafton TF, Hansen DK. Prenatal neuroleptic exposure and growth stunting in the rat: an *in vivo* and *in vitro* examination of sensitive periods and possible mechanisms. *Teratology* 1994;50:125–136. [PubMed: 7801300]
21. Williams R, Ali SF, Scalzo FM, Soliman K, Holson RR. Prenatal haloperidol exposure: effects on brain weights and caudate neurotransmitter levels in rats. *Brain Res Bull* 1992;29:449–458. [PubMed: 1356601]
22. Patel AJ, Barochovsky O, Lewis PD. Psychotropic drugs and brain development: effects on cell replication *in vivo* and *in vitro*. *Neuropharmacology* 1981;12:1243–1249. [PubMed: 7033814]
23. Alberch J, Brito B, Notario V, Castro R. Prenatal haloperidol treatment decreases nerve growth factor receptor and mRNA in neonate rat forebrain. *Neurosci Lett* 1991;131:228–232. [PubMed: 1662344]
24. Castro R, Brito B, Segovia J, Martin-Trujillo JM, Notario V. Prenatal haloperidol induces a selective reduction in the expression of plasticity-related genes in neonate rat forebrain. *Brain Res Mol Brain Res* 1994;26:74–80. [PubMed: 7854069]
25. Weinshenker D, Garriga G, Thomas JH. Genetic and pharmacological analysis of neurotransmitters controlling egg laying in *C. elegans*. *J Neurosci* 1995;15:6975–6985.
26. Schafer WR. How do antidepressants work? Prospects for genetic analysis of drug mechanisms. *Cell* 1999;98:551–554. [PubMed: 10490094]
27. Ranganathan R, Sawin ER, Trent C, Horvitz HR. Mutations in the *Caenorhabditis elegans* serotonin reuptake transporter MOD-5 reveal serotonin-dependent and -independent activities of fluoxetine. *J Neurosci* 2001;21:5871–5884. [PubMed: 11487610]
28. Dempsey CM, Mackenzie SM, Gargus A, Blanco G, Sze JY. Serotonin (5HT), fluoxetine, imipramine, and dopamine target distinct 5HT receptor signaling to modulate *Caenorhabditis elegans* egg-laying behavior. *Genetics* 2005;169:1425–1436. [PubMed: 15654117]
29. Rand JB, Russell RL. Choline acetyltransferase-deficient mutants of the nematode *Caenorhabditis elegans*. *Genetics* 1984;106:227–248. [PubMed: 6698395]
30. Alfonso A, Grundahl K, Duerr JS, Han HP, Rand JB. The *Caenorhabditis elegans unc-17* gene: a putative vesicular acetylcholine transporter. *Science* 1993;261:617–619. [PubMed: 8342028]
31. Hartman PS. Caffeine-resistant mutants of *Caenorhabditis elegans*. *Genet Res* 1987;49:105–110. [PubMed: 3596232]
32. Adachi R, Kagawa H. Genetic analysis of ryanodine receptor function in *Caenorhabditis elegans* based on *unc-68* revertants. *Mol Genet Genomics* 2003;269:797–806. [PubMed: 12898220]
33. Choy RK, Thomas JH. Fluoxetine-resistant mutants in *C. elegans* define a novel family of transmembrane proteins. *Mol Cell* 1999;4:143–152. [PubMed: 10488330]
34. Sanyal S, Wintle RF, Kindt KS, Nuttley WM, Arvan R, Fitzmaurice P, et al. Dopamine modulates the plasticity of mechanosensory responses in *Caenorhabditis elegans*. *EMBO J* 2004;23:473–482. [PubMed: 14739932]
35. Suo S, Sasagawa N, Ishiura S. Cloning and characterization of a *Caenorhabditis elegans* D2-like dopamine receptor. *J Neurochem* 2003;86:869–878. [PubMed: 12887685]
36. Hamdan FF, Ungrin MD, Abramovitz M, Ribeiro P. Characterization of a novel serotonin receptor from *Caenorhabditis elegans*: cloning and expression of two splice variants. *J Neurochem* 1999;72:1372–1383. [PubMed: 10098838]
37. Strange PG. Antipsychotic drugs: importance of dopamine receptors for mechanisms of therapeutic actions and side effects. *Pharmacol Rev* 2001;53:119–133. [PubMed: 11171942]

38. Meltzer HY, Li Z, Kaneda Y, Ichikawa J. Serotonin receptors: their key role in drugs to treat schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;27:1159–1172.
39. Siegel, G.; Albers, RW.; Brady, S.; Price, D., editors. *Basic Neurochemistry*. Academic Press, Inc.; London: 2005. p. 1-1016.
40. Horvitz HR, Chalfie M, Trent C, Sulston JE, Evans PD. Serotonin and octopamine in the nematode *Caenorhabditis elegans*. *Science* 1982;216:1012–1014. [PubMed: 6805073]
41. Segalat L, Elkes DA, Kaplan JM. Modulation of serotonin-controlled behaviors by Go in *Caenorhabditis elegans*. *Science* 1995;267:1648–1651. [PubMed: 7886454]
42. Schafer WR, Kenyon CJ. A calcium-channel homologue required for adaptation to dopamine and serotonin in *Caenorhabditis elegans*. *Nature* 1995;375:73–78. [PubMed: 7723846]
43. Sawin ER, Ranganathan R, Horvitz HR. *C.elegans* locomotory rate is modulated by the environment through a dopaminergic pathway and by experience through a serotonergic pathway. *Neuron* 2000;26:619–631. [PubMed: 10896158]
44. Suo S, Ishiura S, Van Tol HHM. Dopamine receptors in *C. elegans*. *Eur J Pharmacol* 2004;500:159–166. [PubMed: 15464029]
45. Roufogalis BD, Minocherhomjee AM, Al-Jobore A. Pharmacological antagonism of calmodulin. *Can J Biochem Cell Biol* 1983;61:927–933. [PubMed: 6138140]
46. Weiss B, Prozialeck W, Cimino M, Barnette MS, Wallace TL. Pharmacological regulation of calmodulin. *Ann NY Acad Sci* 1980;356:319–344. [PubMed: 6112947]
47. Means AR, VanBerkum MFA, Bagchi I, Lu KP, Rasmussen CD. Regulatory functions of calmodulin. *Pharmacol Ther* 1991;50:255–270. [PubMed: 1763137]
48. Donohoe, DR.; Aamodt, EJ.; Dwyer, DS. Neurodevelopmental effects of antipsychotic drugs in *C. elegans*. 15th International *C. elegans* Conference; 2005.
49. Brenner S. The genetics of *Caenorhabditis elegans*. *Genetics* 1974;1:71–94. [PubMed: 4366476]
50. Chase DL, Pepper JS, Koelle MR. Mechanism of extrasynaptic dopamine signaling in *Caenorhabditis elegans*. *Nat Neurosci* 2004;7:1096–1103. [PubMed: 15378064]
51. Bai O, Wei Z, Lu W, Bowen R, Keegan D, Li XM. Protective effects of atypical antipsychotic drugs on PC12 cells after serum withdrawal. *J Neurosci* 2002;22:278–283.
52. Perry PJ, Miller DD, Arndt SV, Cadoret RJ. Clozapine and nortriptyline plasma concentrations and clinical response of treatment-refractory schizophrenic patients. *Am J Psychiatry* 1991;148:231–235. [PubMed: 1670979]
53. Baldessarini RJ, Centorrino F, Flood JG, Volpicelli SA, Huston-Lyons D, Cohen BM. Tissue concentrations of clozapine and its metabolites in the rat. *Neuropsychopharmacology* 1993;9:117–124. [PubMed: 8216694]
54. Cusick R, Dwyer DS, Bradley RJ, Aamodt EA. Antipsychotic drugs affect development in *Caenorhabditis elegans*. *Soc Neurosci Abstr* 2003;29:479.9. Online
55. Olde B, McCombie WR. Molecular cloning and functional expression of a serotonin receptor from *Caenorhabditis elegans*. *J Mol Neurosci* 1997;8:53–62. [PubMed: 9061615]
56. Hobson RJ, Hapiak VM, Xiao H, Buehrer KL, Komuniecki PR, Komuniecki RW. SER-7, a *Caenorhabditis elegans* 5-HT₇-like receptor, is essential for the 5-HT stimulation of pharyngeal pumping and egg laying. *Genetics* 2006;172:159–169. [PubMed: 16204223]
57. Sze JY, Victor M, Loer C, Shi Y, Ruvkun G. Food and metabolic signaling defects in a *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature* 2000;403:560–564. [PubMed: 10676966]
58. Kamath RS, Fraser AG, Dong Y, Poulin G, Durbin R, Gotta M, Kanapin A, Le Bot N, Moreno S, Sohrmann M, Welchman DP, Zipperlen P, Ahringer J. Systemic functional analysis of the *Caenorhabditis elegans* genome using RNAi. *Nature* 2003;421:231–237. [PubMed: 12529635]
59. Karabinos A, Bussing I, Schulze E, Wang J, Weber K, Schnabel R. Functional analysis of the single calmodulin gene in the nematode *Caenorhabditis elegans* by RNA interference and 4-D microscopy. *Eur J Cell Biol* 2003;82:557–563. [PubMed: 14703012]
60. Loer CM, Kenyon CJ. Serotonin-deficient mutants and male mating behavior in the nematode *Caenorhabditis elegans*. *J Neurosci* 1993;13:5407–5417. [PubMed: 8254383]
61. Rand JB, Johnson CD. Genetic pharmacology: interactions between drugs and gene products in *Caenorhabditis elegans*. *Methods Cell Biol* 1995;48:187–204. [PubMed: 8531725]

62. Dwyer DS, Vannucci SJ, Simpson IA. Expression, regulation, and functional role of glucose transporters (GLUTs) in brain. *Int Rev Neurobiol* 2002;51:159–188. [PubMed: 12420359]
63. Smedley M, Stanisstreet M. Effect of the calmodulin inhibitor R24571 (calmidazolium) on rat embryos cultured *in vitro*. *Experientia* 1984;40:992–994. [PubMed: 6468629]
64. Lu XH, Dwyer DS. Second generation antipsychotic drugs, olanzapine, quetiapine, and clozapine enhance neurite outgrowth in PC12 cells via PI3K/Akt, ERK, and pertussis toxin-sensitive pathways. *J Mol Neurosci* 2005;27:43–64. [PubMed: 16055946]
65. Hajdуч E, Litherland GJ, Hundal HS. Protein kinase B (PKB/Akt) – a key regulator of glucose transport? *FEBS Lett* 2001;492:199–203. [PubMed: 11257494]
66. Lawlor MA, Alessi DR. PKB/Akt: a key mediator of cell proliferation, survival and insulin responses? *J Cell Sci* 2001;114:2903–2910. [PubMed: 11686294]
67. Reiner DJ, Newton EM, Tian H, Thomas JH. Diverse behavioural defects caused by mutations in *Caenorhabditis elegans unc-43* CaM kinase II. *Nature* 1999;402:199–202. [PubMed: 10647014]
68. Tam T, Mathews E, Snutch TP, Schafer WR. Voltage-gated calcium channels direct neuronal migration in *Caenorhabditis elegans*. *Dev Biol* 2000;226:104–117. [PubMed: 10993677]
69. Findling RL, Schulz SC, Reed MD, Blumer JL. The antipsychotics. A pediatric perspective. *Pediatr Clin North Am* 1998;45:1205–1232. [PubMed: 9884683]
70. Zito JM, Safer DJ, dosReis S, Gardner JF, Boles M, Lynch F. Trends in the prescribing of psychotropic medications to preschoolers. *JAMA* 2000;283:1025–1030. [PubMed: 10697062]

Fig. 1.

First-generation antipsychotic drugs affect developmental growth in *C. elegans*. Differential interference contrast (DIC) images (100X) of animals grown for 72 hr in the presence of vehicle (control, left panels), or drugs (right panels): (A) chlorpromazine (160 μ M), (C) fluphenazine (160 μ M), or (E) haloperidol (160 μ M). In addition, head-to-tail body length was analyzed in N2 animals at 24, 48, and 72 hr (N=25 at each time point/condition) treated with vehicle (DMSO) or (B) chlorpromazine, (D) fluphenazine, or (F) haloperidol at 20, 40, 80, and 160 μ M as indicated in the legends. The data represent the means and standard deviations (error bars) at each time interval.

Fig. 2. Second-generation antipsychotic drugs produce modest effects on development in *C. elegans*. The data are presented according to the same scheme as Fig. 1. Drugs and the concentrations tested are indicated in the legends.

Fig. 3.

Comparison of antipsychotic drugs that slow growth in *C. elegans*. Growth was measured as a function of body length in *tph-1(mg280)* and N2 animals treated with vehicle (control), butaclamol, mergoline, clozapine, haloperidol, fluphenazine, or chlorpromazine at (A) 24, (B) 48, and (C) 72 hr after hatching onto drug plates. All drugs were tested at the same concentration (160 μM). The data represent the means and standard deviations calculated from 25 animals per group. The conditions that are listed in the legend from top to bottom correspond to the bars presented from left to right for this and subsequent figures.

Fig. 4.

Control experiments for biological effects of antipsychotic drugs. (A, B) First-generation and second-generation antipsychotic drugs were evaluated at the concentrations indicated for their ability to inhibit paralysis in *C. elegans* induced by 15 mM dopamine. In addition, the dopamine receptor agonist, quinpirole, was tested as a positive control for the paralytic effects of dopamine. For these experiments, adult N2 animals were preincubated with drugs or solvent (control) for 20 min prior to their introduction to test plates containing dopamine and drug or solvent. Animals (25–35 per plate) were examined for paralysis over time and the experiment was performed in triplicate. The data represent the averages from the 3 plates and are expressed as the fraction of immobile animals. All of the antipsychotic drugs produced significant inhibition of dopamine effects at all time points. (C) Pharyngeal pumping was measured in control (DMSO) or drug-treated animals (clozapine, olanzapine, haloperidol, or chlorpromazine at the concentrations indicated) at the 4th larval stage (L4) by counting the number of pharyngeal pumps over 30 sec. Duplicate plates were tested for each condition and twenty animals were analyzed from each plate (N=40). Significant differences from the control group are indicated by asterisks: **p < 0.01.

Fig. 5.

Effects of antipsychotic drugs on development in relation to serotonergic and dopaminergic systems. (A) *tph-1* mutants were grown in the presence of vehicle (DMSO), clozapine (200 μ M), or fluphenazine (200 μ M) at 20 °C and photographed (60X magnification) on the fourth day after hatching. (B) Quantitative analysis of the developmental staging (Stage %) of animals classified as adults, arrested, or dauer/partial dauer larvae (refer to Methods for criteria) on the third and fourth day after hatching. (C) Body length was measured for N2 animals treated with vehicle (DMSO), serotonin (2.5 or 5 mM), clozapine (160 μ M), and clozapine (160 μ M) with serotonin (2.5 or 5 mM) at 24, 48, and 72 hr after hatching onto plates. (D) In a similar manner, N2 animals were grown in the presence of vehicle (DMSO), quinpirole (750 μ M or 1 mM), clozapine (160 μ M), fluphenazine (160 μ M), clozapine with quinpirole (750 μ M or 1 mM), and fluphenazine (160 μ M) with quinpirole (750 μ M or 1 mM) and body length was measured 72 hr after hatching. Each data point represents the average of 25 animals per condition. Error bars represent standard deviations from the mean. Statistical significance is indicated as before.

Fig. 6.

Effect of calmodulin inhibitors on development in *C. elegans*. (a) Body length (μm) was measured and averaged from 25 animals that were grown in the presence of vehicle (control), selective calmodulin inhibitors (W-13 and calmidazolium), or antipsychotic drugs (chlorpromazine, fluphenazine, and trifluoperazine) for 72 hr after hatching. All compounds were tested at 80 and 160 μM. Error bars represent the standard deviations from the mean. Statistical differences from the control group are indicated as before. (B) Linear regression analysis of the potency of antipsychotic drugs as calmodulin antagonists (x-axis) vs. their effects on growth (body length) in *C. elegans*. This analysis was limited to drugs that were pharmacologically characterized as calmodulin antagonists and included, trifluoperazine, fluphenazine, chlorpromazine, haloperidol, and clozapine. The body length data were obtained from head-to-head comparisons of the drugs at the 72 hr time point. The potency (IC₅₀) data were compiled from different sources and refer to inhibition of functional activity of calmodulin [45,46].