

Drosophila melanogaster as an indispensable model to decipher the mode of action of neurotoxic compounds

MONALISA MISHRA^{1,2,*}; PUNYATOYA PANDA¹; BEDANTA KUMAR BARIK¹; AMRITA MONDAL¹; MRUTUNJAYA PANDA¹

¹ Neural Developmental Biology Lab, Department of Life Science, NIT Rourkela, Rourkela, 769008, India

² Centre for Nanomaterials, NIT Rourkela, Rourkela, 769008, India

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Abstract: Exposure to some toxic compounds causes structural and behavioral anomalies associated with the neurons in the later stage of life. Those toxic compounds are termed as a neurotoxicant, which can be a physical factor, a toxin, an infection, radiation, or maybe a drug. The incongruities caused due to a neurotoxicant further depend on the toxicity of the compound. More importantly, the neurotoxicity of the compound is associated with the concentration and the time point of exposure. The neurodevelopmental defect appears depending on the toxicity of the compound. A neurodevelopmental defect may be associated with a delay in developmental time, defective growth, structural abnormality of many organs, including sensory organs, behavioral abnormalities, or death in the fetus stage. Numerous model organisms are employed to assess the effect of neurotoxicants. The current review summarizes several methods used to check the effect of neurotoxicant and their effect using the model organism *Drosophila melanogaster*.

Introduction

Neurotoxicity is an anatomical, morphological, physiological, biochemical as well as behavioral abnormality (Coyle *et al.*, 1976) that occurs during the development of the embryo to the fetus and can cause deformities in the adult. The causative agent for neurotoxicity is termed as a neurotoxicant. A neurotoxicant can be physical (thermal), electromagnetic (Wang *et al.*, 2007), magnetic (Ho *et al.*, 1992), ultrasound (Williams and Casanova, 2013), X-ray, or a chemical (Wang *et al.*, 2007) factor. Neurotoxins or neurotoxicants alter the activity of the nervous system in such a way that it causes reversible or irreversible damage to nervous tissue (Cunha-Oliveira *et al.*, 2008). With damaged neurons, the ability of transmission and processing of information in the central and peripheral nervous systems is hampered to a certain extent. A neurotoxicant may cause mutation in the organism by interfering with mitosis and resulting in altered chromosome numbers, nucleic acid synthesis, and function. Often a neurotoxicant causes deficiencies in precursors, substrates, enzymes, and other prerequisites necessary for the normal biochemical and metabolic function of the cell. Thus, it may alter the property of the cell, causing osmotic imbalance and altering

the cell membrane, molecular, and biochemical composition (Wilson, 1968). Different potential neurotoxicants like infection and drugs have dissimilar effects on the developmental pattern. Many model organisms, including *Drosophila melanogaster* are used to identify a potential neurotoxicant (Daston, 2011; Mishra and Barik, 2018). Barik and Mishra reported in their review that nanoparticles act as a behavioral teratogen in the model organism *D. melanogaster* (Barik and Mishra, 2019). At low doses, some of the teratogens affect the behavior of the animal and thus act as a neurotoxicant (Coyle *et al.*, 1976). Since the behavior of an organism originates from the nervous system, a toxicant at a lower dose can cause neurotoxicity, which appears as neurodegeneration in the later part of life. *D. melanogaster* is well studied to identify behavioral abnormalities in several stages of development (Mishra and Barik, 2018).

Drosophila melanogaster as a Model to Study Neurotoxicity

D. melanogaster is widely used to identify innumerable toxicants which can cause teratogenicity and neurotoxicity. The physiology and genetic similarity with higher vertebrates (Abnoos *et al.*, 2013), short life cycle, low rearing cost, completely sequenced genome, and availability of gene-editing tools (Affleck and Walker, 2008) make it an ideal model to analyze the neurotoxicity and teratogenicity of innumerable compounds. Furthermore, the developmental

*Address correspondence to: Monalisa Mishra, mishramo@nitrkl.ac.in
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pathways of *D. melanogaster* and humans have a significant similarity. *D. melanogaster* is also used for the study of glycobiology since the O-linked glycosylation of its cells share similarity with that of mammalian cells (Hagen *et al.*, 2008). Some of the common pathways include the insulin pathway (McClure *et al.*, 2011), the mitogen-activated protein kinase (MAPK) signaling pathway (Abnoos *et al.*, 2013), the Notch signaling pathway (Alattia *et al.*, 2011), folate metabolism (Affleck and Walker, 2008), fatty acid metabolism (Logan-Garbisch *et al.*, 2015), the dopaminergic pathway (Bainton *et al.*, 2000), and Wnt, TGF β , Hedgehog, EGF, and cytokine pathways. Besides the signaling pathways, various channels (Sharma, 2004), which are useful in identifying pathway-specific interactions of different chemicals (including transient receptor channel, TRP), are also conserved between *D. melanogaster* and human being. More importantly, the transport, uptake, and efflux of many metals are conserved (Calap-Quintana *et al.*, 2017). Altogether, *D. melanogaster* makes an ideal model for identifying neurotoxicants.

Factors Affecting Neurotoxicity

Dose of administration

The dose of any compound plays a key role in determining its toxicity (Podratz *et al.*, 2011; Sadler *et al.*, 1988; Sudmeier *et al.*, 2015). A compound at a low dose for a shorter period to an embryo/fetus may cause a neuronal abnormality, while chronic exposure to that drug may cause lethality. Lower doses of a toxic compound affect the nervous system and thus act as a neurotoxicant.

Mode of administration

D. melanogaster development includes stages like an embryo, larva, pupa, and adult (Fig. 1), which help in understanding the influence of myriad compounds on several organs, including the nervous system (Rand, 2010). There are several modes via which a neurotoxicant can be introduced to different developmental stages of *D. melanogaster* (Fig. 2).

The maternal feeding method is widely used as a common mode of exposure (Fig. 2A). During maternal feeding, the exact dose of the compound consumed, metabolized, and absorbed determines the toxicity (Rand *et al.*, 2010). For this method, the neurotoxicant of interest is mixed in food media or with diluted yeast paste for a long and short exposure (Pandey and Nichols, 2011). Often the neurotoxicants are injected into the embryo (Fig. 2B) or adults so that they are circulated to the hemolymph of the whole organism, and their toxicity can be checked (Dzitoyeva *et al.*, 2003). Embryos are also exposed by the permeabilization method for the exposure of the neurotoxicant (Rand *et al.*, 2010). However, due to the presence of a vitelline membrane, sometimes, direct incubation of embryos with the compound of interest fails (Rand, 2010). Thus, the vitelline membrane of the embryo is also taken out (Sabat *et al.*, 2015) before it is exposed to the toxicants (Fig. 2C). The larvae are also injected into the neurotoxicant to study the effect (Fig. 2D). Larvae are preferred for the feeding experiments because they are a rapacious eater. Neurotoxicants like ethanol and cocaine, which are used in the form of vapor (McClung and Hirsh, 1998; Moore *et al.*, 1998), are soaked in filter paper in the saturated form (Nichols *et al.*, 2012) to expose the flies or larvae (Fig. 2E). Often drugs are exposed to the nerve cord of decapitated flies to see the effect (Torres and Horowitz, 1998). A number of times the drugs are introduced via glass microcapillary. This method allows the precise measurement of the drugs consumed by a single or group of flies (Ja *et al.*, 2007). This method is popularly known as the capillary feeder assay.

Time of administration

Besides mode, the developmental stage in which the compound is administered also plays a central role in determining its toxicity. Thus, the same compound administered at different developmental stages shows a different result. Why do different stages respond differently to the same compound? In the embryonic stage, the fate of various cells gets determined and leads to the formation of

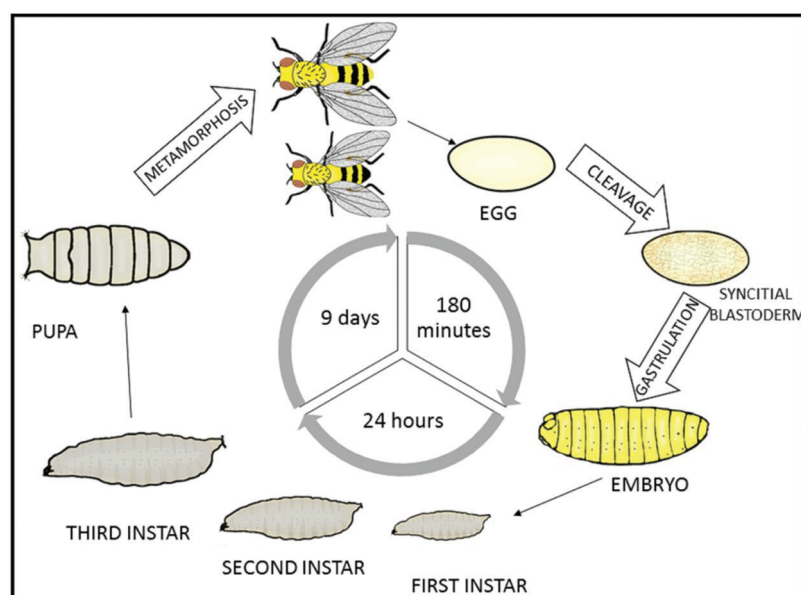


FIGURE 1. Developmental cycle of *Drosophila melanogaster*. The developmental stages include egg, larva, pupae, and adult. Each stage occurs at a particular time point, which does not change unless there is some internal or external stressor.

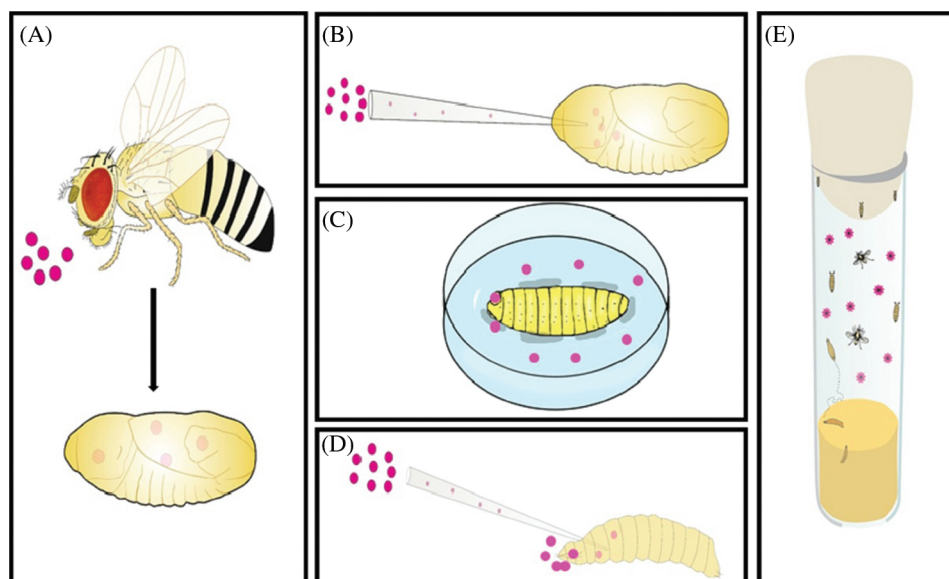


FIGURE 2. Mode of exposure of neurotoxicant to *Drosophila melanogaster*. Any developmental stage of *Drosophila* can be used for exposure to neurotoxicants. (A) Adult flies are exposed to the neurotoxicant via oral mode, (B) The embryos are exposed via injection, (C) Exposure to the neurotoxicant by membrane permeabilization method, (D) Exposure of larvae to the neurotoxicant by microinjection or feeding method, and (E) Various stages exposed to volatile neurotoxicants.

the organ, including neurons and pathfinding for axons (Pandey and Nichols, 2011). The development of central, peripheral, motor, and interneurons occurs in the embryonic stage (Bossing *et al.*, 1996; Brewster and Bodmer, 1996; Schmid *et al.*, 1999). This allows a fly embryo to be used in numerous neurotoxicology studies (Rand *et al.*, 2010). In the larval stage, the nervous system originates from the embryonic stage and differentiates as the ventral neuroectoderm. The larva has fully functional circuits with conventional synapse like a neuromuscular junction, enabling it to perform motor and sensory functions. Using the nervous system, the larvae can detect food, chemical, temperature, light, and sound, as reviewed by Mishra and Barik (Mishra and Barik, 2018). The larva possesses many undifferentiated precursor organs known as imaginal discs, which later transform into adult structures during the pupa stage (Pandey and Nichols, 2011). The pupal stage is important in a developing *D. melanogaster*, as neuronal remodeling and new patterns of synaptogenesis occur in this period (Rand *et al.*, 2010), which subsequently form a healthy adult nervous system capable of performing normal functions. If a chemical interferes with any of the above-mentioned developmental stages, it alters the structure and functioning of several organs, including sensory organs (Bournias-Vardiabasis and Teplitz, 1982; Bournias-Vardiabasis *et al.*, 1983; Mellerick and Liu, 2004).

Absorption, Distribution, and Metabolism of Drugs

The absorption, distribution, and metabolism of a drug depends on the route of administration (Fig. 2). The drug enters into different parts of the body through the route of administration. It is slowly eliminated from the body by absorption, distribution, metabolism, and excretion (Pappus and Mishra, 2018). During the stay within the body, the drug may undergo a structural change through a process known as biotransformation (Baars, 1980). Often the release

of the drug takes place in the air, and this step is known as transportation. The absorption of a drug depends on its physiochemical properties and the route of its entry into the circulatory system. A drug can be transported via transmembrane transport by active or passive or dynamic transport (Stewart, 2002). Passive transportation takes place from higher to lower concentrations by means of diffusion. Active transportation takes place from lower to higher concentration using a carrier protein. Macromolecules get transported via the membrane by pinocytosis or exocytosis.

Drugs are administered into the body by (1) the digestive tract, (2) injection, or (3) transdermal delivery. Drugs that enter through the digestive tract get absorbed via epithelial cells of the gastrointestinal tract via passive transport (Pappus and Mishra, 2018; Sahu *et al.*, 2022). Once the drug is absorbed, it mixes with the hemolymph. The villi present in the small intestine increase the surface area of absorption (Sahu *et al.*, 2022). Drug absorption depends on the pH, absorption, and drug dissolution. High pH facilitates the absorption of alkaline drugs, whereas low pH promotes the absorption of acidic drugs (Pappus and Mishra, 2018). The interaction of drugs with fat soluble compounds affects absorption.

Drugs injected into the body are absorbed faster than those that are orally administered. On being injected, water-soluble drugs diffuse from the site of injection to different parts of the body (Mayer *et al.*, 2009). Drugs administered through injection are fully absorbed. The absorption is directly proportional to the blood flow in the area.

Administration of drugs via the skin, smearing, and spraying is known as transdermal drug delivery (Panonnummal *et al.*, 2021). In this method, drugs enter into the body through the cuticle and enter into the hemolymph via passive diffusion. Fat-soluble drugs are absorbed easily via the epidermis. A drug entering through the olfactory organ reaches the trachea, where it is absorbed via passive diffusion to reach the blood vessel (Scholl *et al.*, 2021). From the blood vessel, it

reaches the central nervous system (CNS). Large molecular weight drugs have a lesser chance of absorption, and peptides and circular proteins are absorbed faster than linear ones.

Once the drug is absorbed, it is transported to different parts of the tissue and organs. The distribution of the drug occurs in an uneven manner. The binding of the drug to the plasma protein is dependent on the pH and physicochemical properties of the drug (Pandey and Nicholas, 2011). The unbound drug binds to the intracellular component of the hemolymph for its distribution. The drug is distributed unevenly in different parts of the body.

The drug metabolism depends on the drug-metabolizing enzyme present in the hemolymph and the tissue (Misra et al., 2011). Drugs are metabolized in the digestive system, nephrocyte, trachea, skin, and CNS. Some of the drugs are metabolized by the bacteria present within the body. Some drugs bind to the enzymes present in the digestive system, thus reducing the bioavailability. Often the drugs undergo reduction, hydrolysis, acetylation, and dealkylation after binding to the microbial flora (Guengerich, 2001). Drug metabolism results in phase I and Phase II reaction. Phase I includes metabolism resulting in carbonyl, carboxyl, sulfhydryl, and amino groups. The oxidation includes oxidation of sulfur, nitrogen, amino, and desulfurization. Cytochrome p450 is the most critical enzyme in the phase I metabolism of drugs (Chung et al., 2009). In phase II reaction, the metabolite covalently binds to glucuronic acid, sulfuric acid, glycine, and glutathione. Genetic factors regulate the drug metabolism, and the non-genetic factors include age, sex, nutrition, and temperature (Rand, 2010). Drug metabolism is affected by different developmental stages. In the early developmental stages, the drug may cause toxicity. With age, metabolic enzymes and endogenous cofactors get reduced. Drugs are excreted from the body by passive or active transport with respect to concentration from the hemolymph.

Methods to Check Neurotoxicity

A neurotoxicant can alter the morphology of the neurons as well as functions or behaviors associated with it. The behavior of an animal is regulated by many sensory organs (Hirsch et al., 2012) and the neurons associated with it (Markow and Gottesman, 1993). During development, a network of genes cross-talk with each other and forms the nervous system, which regulates the physiology and behavioral pattern of an animal. If the stressor becomes uncontrollable and reoccurs for a longer period, then it causes chronic stress. Chronic stress results in allostatic load and initiates molecular changes within the key regulator of the nervous system, i.e., the brain (Min and Condron, 2005). Thus a neurotoxicant may cause developmental instability in the CNS, alter the symmetry of neuronal numbers, chemistry, or connections (Markow and Gottesman, 1993), and can cause behavioral changes in any species in later developmental stages (Alves-Pimenta et al., 2018). Also, any contact with toxins during development affects the development of the nervous and endocrine systems resulting in behavioral defects in adults (Hirsch et al., 2012). Parameters like developmental cycle, morphological parameters of different developmental stages, immunohistochemistry and histology, biochemical assay, and several behavioral assays are used to assess neurotoxicity.

Developmental cycle

The developmental cycle of *D. melanogaster* is used widely to check the toxic effect of a neurotoxicant or teratogen (Bianchini et al., 2018; Li and Bi, 2018). Many developmental time points like the hatching of eggs to larva, a transition of larvae to pupae, pupation time, hatching of pupae to eclosion of adult flies, and their survivability or life span are already known (Fig. 1). Developmental time points are noted after the exposure of neurotoxicants and compared with the control flies. Alteration in the time point of any of these stages is considered a defect caused due to neurotoxicants (Bianchini et al., 2018). Besides time, the number of animals affected at the developmental stages due to the effect of a neurotoxicant is also calculated (Figs. 3A–3E). The hatching of fewer larvae suggests the death of the embryos (Fig. 3C). The dead embryos can be imaged under light and scanning electron microscope to identify the phenotypic defect (Bianchini et al., 2018; Rand et al., 2014). Similarly, if the death occurs during the larval stage, then the dead larvae (Fig. 3D) can be checked under light and scanning electron microscope to identify the phenotypic defect (Affleck et al., 2006b; Rand et al., 2014). Death may also occur in the pupa stage (Fig. 3E). The dead pupa appears black, indicating incomplete pupation (Fig. 3E), and in such cases, fewer flies are hatched (Rand et al., 2014). The dead pupa is dissected and compared with the control pupa to check the developmental defect due to the neurotoxicant. Thus, the analysis of the number of animals in all the stages gives a clear picture of the effect of the neurotoxicant in the development of *D. melanogaster* (Fig. 3).

Morphometric validation of various stages

A neurotoxicant can alter the size of the various developmental stages (described in the above section). The teratogen-induced morphometric developmental defect is reported for many chemicals (Lynch et al., 1991; Schuler et al., 1982). Those strategies can be adopted to check the toxic effect of neurotoxicants. Some of the neurotoxicants even produce more than one phenotypic defect. To check the abnormality of the embryo, it is collected and imaged under both light and scanning electron microscopes. Defective regions can be assessed using Image J for any kind of morphological defect. Similarly, the larvae are collected after the treatment of neurotoxicants and imaged under a stereomicroscope. From the image, the length, breadth, diameter, and area of the larvae are measured using Image J software. Similarly, in case of any phenotypic abnormality in the adult fly, an image of that region is taken and subjected to quantification to deduce a value. An increase or decrease in the size of the larva or adult flies can be measured through its weight for quantification purposes.

Immunohistochemistry and histology

A teratogen can alter the cell cycle and cell division. To check the effect of a teratogen on the cell division and cell cycle, apoptosis is checked in various tissues. If the teratogen is administered orally, the midgut of the larvae is analyzed for apoptosis (Priyadarsini et al., 2020). Double staining of Caspase and DAPI/Hoescht can provide information about apoptosis in various tissues. Hoescht/DAPI staining detects

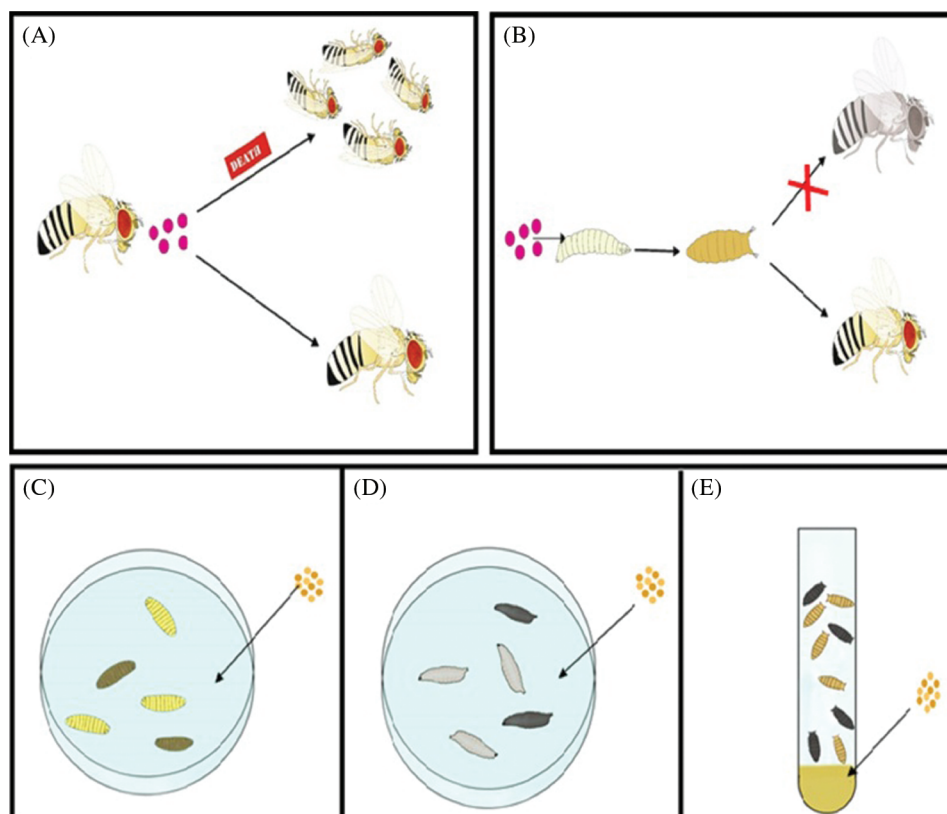


FIGURE 3. Toxicity evaluation of neurotoxicants using developmental cycle. (A) Quantification of death of adult flies after feeding the neurotoxicants, (B) Reduction of the adult flies number after feeding the neurotoxicant from first instar larval stage onwards, (C) Quantification of dead embryos after exposure to the neurotoxicant, (D) Quantification of dead larvae after exposure to the neurotoxicant, and (E) Quantification of dead pupa formed from larvae exposed to the neurotoxicant.

the nuclei shape within the tissue (Fig. 4A). Apoptotic nuclei are smaller in size in comparison to the normal nuclei. From the shape and size of the nuclei, the apoptotic condition of the cell can be determined (Fig. 4A). DAPI binds to DNA, and Caspase binds to apoptotic cells. The TUNEL (TdT-mediated dUTP-biotin nick end labeling) assay is also used to check the apoptosis in the cell (Vasudevan and Ryoo, 2016). Several studies also use acridine orange and propidium iodide to detect cell death (Sahu and Mishra, 2020b; Vasudevan and Ryoo, 2016). Imaginal discs (eye and wing) of the larvae are stained with acridine orange to

detect the cell death caused by neurotoxicants (Figs. 4B and 4C). Newly eclosed flies were examined under the stereomicroscope. After detecting the defect, they are analyzed under the scanning electron microscope for visualization at a higher resolution. The defective organ of the adult stage is analyzed in a developmental time window to detect the role of cell death and cell cycle in the formation of a phenotypic defect (Mishra *et al.*, 2010; Sahu and Mishra, 2020a). To check the internal defect, the defective tissue of organs is fixed for histological analyses. The sections are cut, stained, and imaged under the microscope for phenotypic

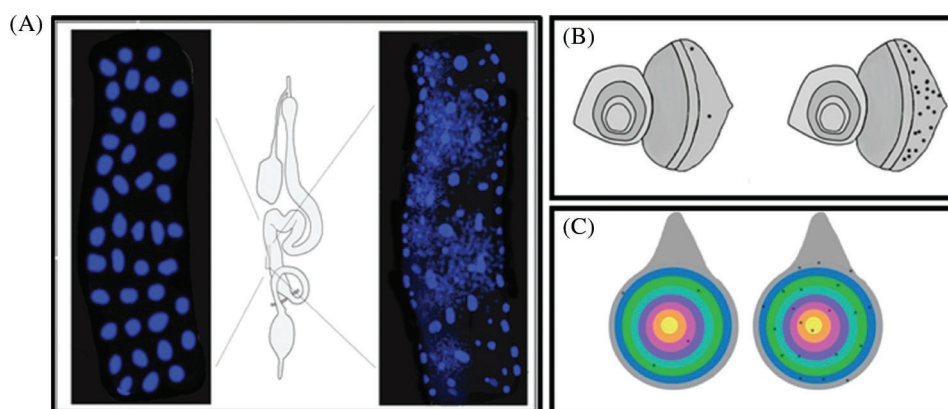


FIGURE 4. Apoptosis detection in *Drosophila melanogaster* tissues using histological staining. (A) Drawing of control gut (Left one) and a gut exposed towards a neurotoxicant orally (Right; note the difference between the size and number of nuclei present in the left and right side), (B) Eye imaginal disc drawing of a control (left) and after treatment with the neurotoxicant (right), and (C) Wing imaginal disc drawing of a control (left) and neurotoxicant treated one (right).

analysis. For the analysis of fine structural defects, the tissues are fixed and analyzed under a transmission electron microscope. Any abnormality at the organelle level can be detected after the analysis of the sample under a transmission electron microscope (Moreira et al., 2010).

Biochemical assays

Biochemical assays are used to detect the neurotoxicity of an unknown compound. Some of the common endpoints of measurements are (1) glutathione content, (2) glutathione-S-transferase, (3) lipid peroxidation, (4) protein carbonylation, (5) acetylcholinesterase (AChE) activity, (6) monoamine oxidase, and (7) caspase-9 and caspase-3 activity. Glutathione content after the treatment of neurotoxicants can be studied using Ellman's Reagent (Jollow et al., 1974). Depletion of glutathione results in the death of the neurons (White and Cappai, 2003) because it is an essential neuronal antioxidant necessary to detoxify free radicals and prevent oxidative stress (Bains and Shaw, 1997). Glutathione-S-transferase (GST) is a major detoxifying defense enzyme of the antioxidant enzyme system (Dasari et al., 2018). GSTs activity is measured at 340 nm from the end product of the reaction. The lipid peroxidation assay measures the level of stress within the body. Stress can be estimated following the protocol of Mishra and Acharya (Mishra and Acharya, 2004) by quantifying the end product of the reaction, i.e., thiobarbituric acid (TBARs) at 535 nm. More TBARs content leads to apoptosis-induced neuronal cell death. The protein carbonyl content can be estimated following Hawkins et al. (2009). Carbonylation alters the protein functions and leads to several intermolecular aggregates and crosslinks that degrade the intracellular proteases. Thus, the accumulation of carbonylated protein content leads to several CNS disorders. Acetylcholinesterase is an essential neurotransmitter. The activity of AChE directly defines the effects of neurotoxicants on the nervous system. AChE activity can be estimated at 412 nm following Ellman and Courtney (1961). Monoamine oxidase (MAO) plays a significant role in the bioactivation of neurotoxic analogs (Heikkila et al., 1988). Although the MAO homolog is not present in the fly (Roelofs and van Haastert, 2001), its activity has been reported by several authors (Chaudhuri et al., 2007; Wang et al., 2011). Interestingly, drugs which can inhibit the activity of MAO can also inhibit the activity of flies (Yellman et al., 1997). Recently, the activity of MAO was measured from the fly head (Oyeniran et al., 2021). Lemons can protect from Alzheimer's disease by decreasing the MAO activity (Oyeleye et al., 2021). Capsaicin can also reduce the MAO activity in the Parkinson's model of *D. melanogaster* (Siddique et al., 2018). MAO can be quantified following McEwen (McEwen, 1965). Caspase-9 (Dronc) and Caspase-3 (Drice) reveal the neurotoxicity leading to neuronal cell death and are quantified by detecting the chromophore p-nitroanilide at 405 nm (Shakya and Siddique, 2018).

Behavioral assays associated with developmental stages

Behavioral assays are well studied to check the effect of a neurotoxicant or teratogen. A neurotoxicant/teratogen can modify the behavior of an organism by altering the

expression of genes and neurotransmitters associated with it (Barik and Mishra, 2019; Moore et al., 1998; Nichols et al., 2012). The alteration of the nervous system in developmental time offers *D. melanogaster* as a model to study behaviors associated with the nervous system (Dhar et al., 2020b; Rand, 2010). The exposure of the embryonic stage to a neurotoxicant can interrupt the development of the nervous system and the glia associated with it (Rand et al., 2010). Those embryos later have a defective nervous system. (Bianchini et al., 2018; Rand et al., 2010). A functional nervous system makes the fly respond toward light (Hardie, 2012), odors (Montell, 2009), sound, tastants (Montell, 2009; Weiss et al., 2011), humidity (Liu et al., 2007), temperature (McKemy, 2007), and gravity (Inagaki et al., 2010; Kamikouchi et al., 2009). These responses are used to check the functionality of the nervous system by doing numerous behavioral assays in adult flies (Fig. 5). The assay is choice to detect the response towards the light (Lilly and Carlson, 1990), vision (Gerber et al., 2004), smell (Shaver et al., 1998), heat (Liu et al., 2003), and taste (Heimbeck et al., 1999). Third instar larvae are used to check for foraging behavior (Pereira et al., 1995; Sokolowski et al., 1997), light sensing ability (Busto et al., 1999), and the coordination of neuromuscular junction (Fig. 6A). The larval response towards light and determines the functionality of circadian rhythm (Luna et al., 2013). Larvae can detect the right type of food using olfaction (Kim et al., 2015), this behaviour is called feeding behavior (Fig. 7B). For this assay, yeast paste is kept at the center of the Petri plate, and the time taken by the larvae to reach the area is calculated (Min and Condron, 2005). The time taken by the control and the treatment larva is calculated and compared for the abnormality. Self-righting behavior is associated with the functioning of mechanosensory organs. The self-right test is used to keep the animal maintained at its right position. For this, the first instar larvae are placed towards the ventral side up (Fig. 6C). The time taken by the larva to turn to its original position is monitored. This behavior was recently reviewed by Dhar et al. (2020b), who used third instar larva to detect their ability to differentiate between heat and cold (Fig. 6D). To respond to the cold temperature, the larval body contracts (Turner et al., 2016) by activating class III (CIII) multicentric neurons (Turner et al., 2016). The touch-sensitive assay is used to detect the larval response towards touch by gently touching the thoracic segment (Fig. 6E) (Caldwell et al., 2003). Similarly, the larval body has numerous channels to detect the sound. To detect the sound sensing ability of the larva, sound avoidance behavioral assay is used in the third instar larvae (Dhar et al., 2020a, 2020b). If there is any defect in the channel, the larvae do not respond to the sound properly. Late third instar larvae can climb the wall of the vial to form pupae (Fig. 6F). The height climbed by the untreated and treated larvae is calculated. Larvae with defective motor neurons are unable to climb high either due to defects in the morphology or in the signaling molecule (Bianchini et al., 2018; Lozinsky et al., 2012; Lozinsky et al., 2013). Thus a defect in the sensory organ can be screened. Larvae also choose the right place for pupation for their survival. This performance is called pupation site preference (PSP)

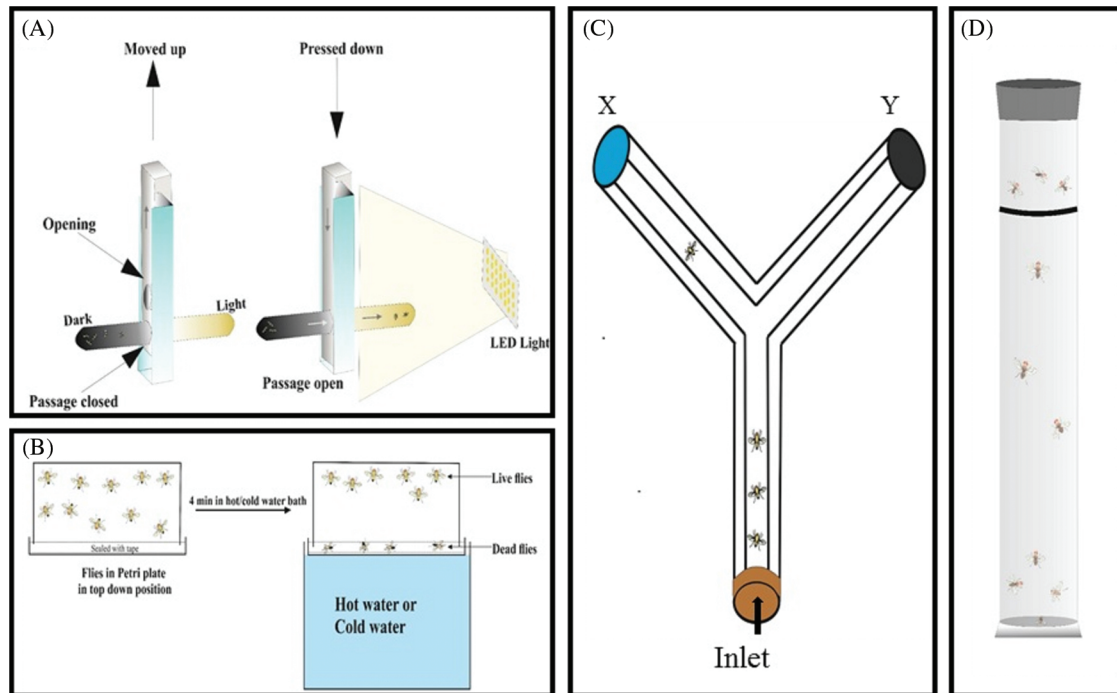


FIGURE 5. Behavioral assays in adult flies. (A) Light-dark choice assay, (B) Heat or cold sensitivity assay, (C) Y-maze device for odor test, and (D) Climbing assay.

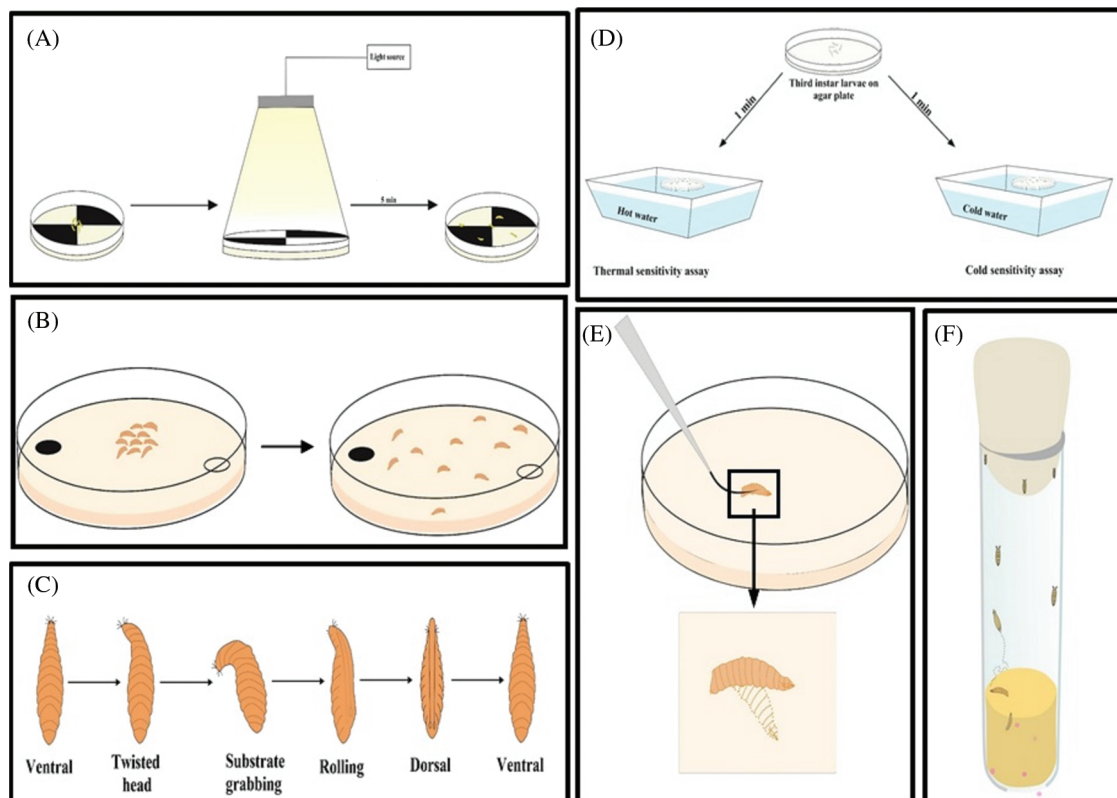


FIGURE 6. Behavioral assays in larvae. (A) Light-dark choice assay, (B) Choice assay for odor test, (C) Self-righting behavior, (D) Heat or cold sensitivity assay, (E) Touch response in larvae, and (F) Pupation site preference behavior of the late third instar larvae.

behavior (Sameoto and Miller, 1968). A larva having a defective nervous system shows altered PSP behavior in response to epileptic drugs (Beltrami *et al.*, 2012). Temporal evaluation is also considered one of the parameters to measure behavioral teratogenesis. For this evaluation, flies

are released into food-containing vials and allowed to lay eggs. After 24 h, the number of larvae are counted. Next, the formation of second and third instar larvae was checked with time. The time taken to form the first pupa and the number of pupae are counted. Similarly, the hatching time

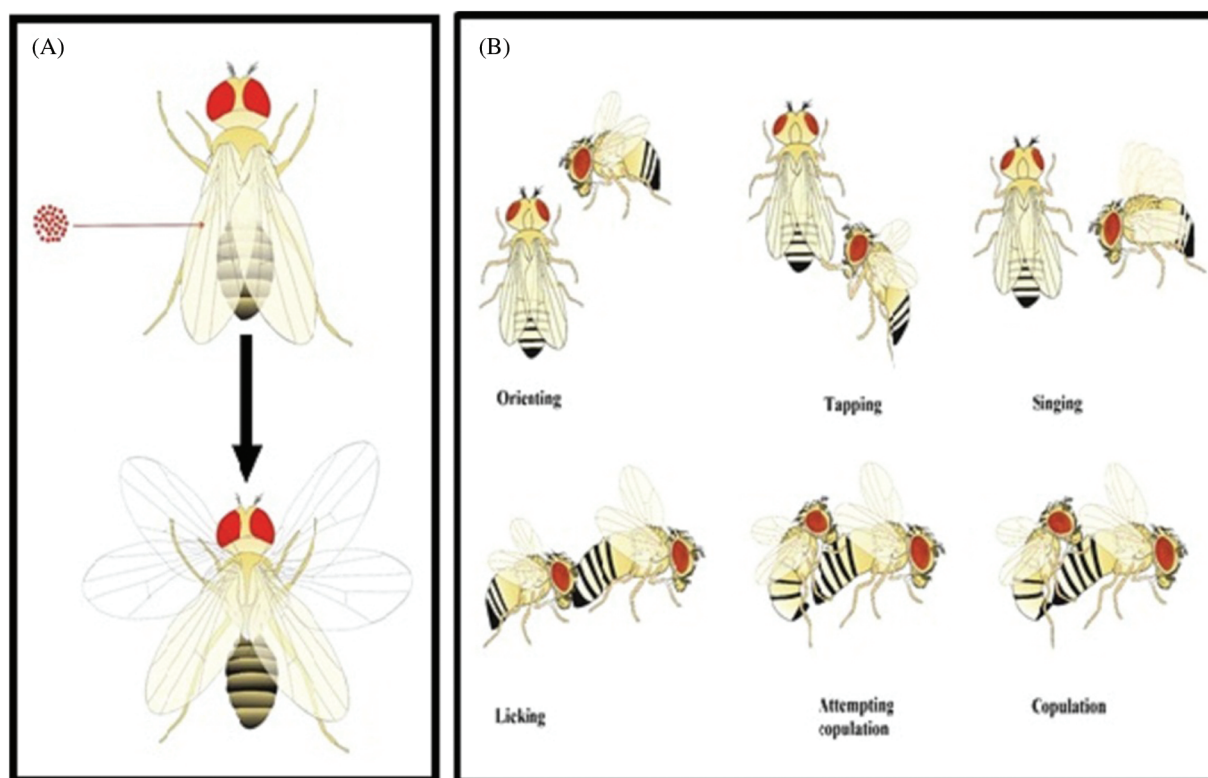


FIGURE 7. Complex behavior in adult flies after treatment of neurotoxicant. (A) Grooming behavior (B) Steps involved in mating behavior.

of the first fly and the number of flies hatched are counted after metamorphosis (Bianchini *et al.*, 2018). Adult flies hatched after neurotoxic compound treatment are checked for their climbing ability against gravity (Fig. 6D). Dopamine, octopamine, tyramine, and serotonin are associated with locomotory behavior (Pendleton *et al.*, 2000; Sombati and Hoyle, 1984; Saraswati *et al.*, 2004; Silva *et al.*, 2014). Several functions of the antennae, including balancing, have been reviewed by Bokolia and Mishra (Bokolia and Mishra, 2015). A defective antennae make the fly positively geotaxis. Thus, the climbing assay quantifies the neuronal defects and aging in *D. melanogaster* (symptoms of Parkinson's disease) (Feany and Bender, 2000; Shaltiel-Karyo *et al.*, 2010). Similarly, the aversive phototaxis suppression (APS) assay (Le Bourg and Buecher, 2002; Seugnet *et al.*, 2009) is used to study Alzheimer's, Parkinson's, and Huntington's diseases in the *D. melanogaster* model to enumerate the deviations in locomotion, learning, and memory (Ali *et al.*, 2011). Dopamine is involved in memory formation (Berry *et al.*, 2012) and octopamine is involved in both learning and memory. Serotonin is responsible for long-term memory (Sitaraman *et al.*, 2015; Scheunemann *et al.*, 2018), and gamma-aminobutyric acid (GABA) convert sleep into memory consolidation (Haynes *et al.*, 2015). Adult flies also respond towards starvation and desiccation (Hoffmann and Harshman, 1999). Any anomaly after neurotoxicant treatment can amend this behavior, pointing to a defect in the nervous system. The larval photosensitive pattern is analyzed under a regulated light-dark cycle, and the locomotion is monitored. A neurotoxicant may alter the 24-h light-dark cycle by altering the sleep cycle (Hirsch *et al.*, 2012). *D. melanogaster* Activity Monitors (DAMs) is widely used to track the locomotor activity of adult flies. Dopamine

is involved in sleep and arousal (van Swinderen and Andretic, 2011), acetylcholine regulate sleep promotion (Aso *et al.*, 2014), and glutamate (Guo *et al.*, 2016) and serotonin (Liu *et al.*, 2019) regulate the sleep.

Complex behaviors

Some of the *D. melanogaster* behaviors need the signal from many sensory organs, as recently reviewed by (Sahu *et al.*, 2020). They are courtship, grooming, aggression, social avoidance, and predator fear behavior (Fig. 7). These behaviors depend on the signal from the eye, hearing and the chemosensory organ (Nichols *et al.*, 2012). Many neurotransmitters are also involved in this process. Male courtship behavior is associated with dopamine and tyramine (Huang *et al.*, 2016). Octopamine regulates both male and female courtship behavior (Zhou *et al.*, 2012; Rezával *et al.*, 2014). Courtship behavior follows particular steps before mating (Spieth, 1974); this behavior is used to check the functionality of neurons (Nichols *et al.*, 2012). Any anomaly in the mating step indicates defective neurons. Defective courtship behavior is seen as a fly model of Parkinson's disease (Shaltiel-Karyo *et al.*, 2012). Similarly, grooming behavior helps the fly to keep itself clean. A defective mechanosensory organ resulted in faulty grooming behavior keeping the fly dirty (Sahu and Mishra, 2020a). Aggressive behavior helps the animal to find its food and partner, and protect the territory (Dhar *et al.*, 2020a; Sahu *et al.*, 2020). Aggressive behavior is associated with the environment, and (Dhar *et al.*, 2020a) the level of serotonin and dopamine also regulates aggression (Aleksyenko *et al.*, 2013). A defective aggressive behavior is associated with serotonin and the dysfunction of the antennae (Dhar *et al.*, 2020a). When exhibiting social avoidance behavior, flies

move away from a stress signal released by the stressed fly. The stress signal released by the flies is known as dS (Suh *et al.*, 2004). For this assay, flies are placed in a T-maze device. Flies have a chance to avoid dSO present in the vial and a fresh vial. This experiment is carried out at 25°C–30°C, with even light and more than 30% humidity. After one minute of introduction to the T-maze device, flies present in both the chambers are counted. This experiment is repeated for three independent data sets and a T-test is carried out to compare the result (Fernandez *et al.*, 2014). *D. melanogaster* perform different behavior in presence of a predator (Parigi *et al.*, 2019). The behaviors include (1) lifting of the abdomen, (2) flying (moving in space using wings), (3) jumping (instantaneous movement between points without the use of the wing), (4) pausing (inactive period, similar to stopping; the duration lasts for less than one second), (5) turn orient 180° without changing the position, (6) wing display (lifting of wing without singing or vibration), (7) grooming (rolling of legs over different parts of the body while in a stationary stage), (8) walking (movement through space), (9) running (rapid movements of the body), (10) stopping (immobile for more than one second), and (11) retreating (walking in reverse direction while encountered with a predator). The predatory response behavior varies with species and different stages of development.

Neurotoxicants Tested Using *Drosophila melanogaster*

D. melanogaster is an effective model to investigate the neurotoxicity of many chemicals and physical parameters. Several factors like UV, gamma radiation, numerous drugs, pesticide, fungicide, herbicides, water pollutants, and air pollutants also show neurotoxicity and teratogenicity (Abnoos *et al.*, 2013; Bednářová *et al.*, 2020; Cui *et al.*, 2020; de Moraes *et al.*, 2017; Dokucu *et al.*, 2005; Ferdenache *et al.*, 2019; Finetti *et al.*, 2020; Hoang and Rand, 2015; Inamdar and Bennett, 2014; Kaur *et al.*, 2015; Kaya *et al.*, 2006; Kissoum *et al.*, 2020; Piccoli *et al.*, 2019; Rajak *et al.*, 2018; Rand *et al.*, 2009; Senthilkumar *et al.*, 2020; Sharma *et al.*, 2012; Verghese and Su, 2018; Zhang *et al.*, 2020; Zhang *et al.*, 2017). The neurotoxic or teratogenic effects of those compounds are summarized in Table 1. Many neurotoxicants alter the redox activity of essential metals necessary to maintain brain functions and homeostasis. The redox activity of the metals induces oxidative stress and impairs the nervous system (Sharma *et al.*, 2014). Heavy metal toxicity can also cause deafness and loss of vision. We next discuss the compounds which are exclusively tested for their neurotoxic or teratogenic effect in the fly model.

Ethanol

D. melanogaster model is used to study ethanol-induced developmental and behavioral defects. Prenatal exposure to ethanol causes reduced viability, delay in developmental time, and smaller size of the hatched flies due to less cell division (McClure *et al.*, 2011). Ethanol vapor affects the behavior of *D. melanogaster* and makes the fly hyperactive, disoriented, and uncoordinated (Cohan and Hoffmann, 1986). Ethanol can target receptors and channels like

voltage-gated channels, NMDA, serotonin, and GABA (Diamond and Gordon, 1997). Some of the behavioral defects in *D. melanogaster* are dependent on dopamine (Bainton *et al.*, 2000). Among signaling pathways, the insulin pathway is largely affected (reduced insulin receptor and *D. melanogaster* insulin-like peptide (DILP)) due to the action of mediating developmental and behavioral defects (McClure *et al.*, 2011). DILP regulates the growth, reproduction, longevity, metabolism of carbohydrates and fat (Géminard *et al.*, 2009).

Methotrexate (MTX)

MTX, a commonly used drug in chemotherapy, is also checked for its teratogenic effect using *D. melanogaster* (Affleck and Walker, 2008). MTX inhibits the dihydrofolate reductase in *D. melanogaster* (Affleck *et al.*, 2006a) and in humans, affects the folate metabolism, which is mediated by dihydrofolate reductase (Affleck and Walker, 2008).

MTX can cause irreversible defects in gestation and embryogenesis (Affleck *et al.*, 2006a) in *D. melanogaster*, and malformed eye (photoreceptor organ), wing, bristle (mechanoreceptor organ), and the curvature of appendage are some of the developmental defects caused due to MTX (Affleck *et al.*, 2006a).

Volatile organic compounds (Fungal VOCs)

Exposure of VOCs to flies causes defective coordination during rapid mobility, restlessness, and frequent fall during jumping near the vial. Later, the flies become sluggish in movement or suffer from bradykinesia after the exposure to the toxicant for 12–18 h. This behavior shows similarity with the fly model for Parkinson's disease when dopamine gets affected (Inamdar *et al.*, 2010). Low concentrations of fungal VOCs in adult *D. melanogaster* results in the locomotory defect by altering the activity of dopaminergic neurons (Inamdar *et al.*, 2010). Industrial VOC exposure increases the production of reactive oxygen species (Bayil *et al.*, 2008; Singh *et al.*, 2009) which can induce lipid peroxidation with the production of toxic products (Inamdar *et al.*, 2010).

Antiepileptic drugs (AEDs)

Exposure to AED during the first trimester causes anatomical abnormalities, whereas exposure during the third trimester causes behavioral anomalies in *D. melanogaster*. AEDs such as phenytoin, valproic acid (VPA), and carbamazepine alter the genotoxicity of *D. melanogaster* in a dose-dependent manner (Yüksel *et al.*, 2010). Mating propensity, larval PSP, and climbing ability are largely affected after treatment with AEDs (Harini, 2016). AEDs are reported to act via the GABA channel, which along with dopamine, regulates the female receptivity during courtship and mating (Gayathri and Harini, 2012). VPA reduces the copulation duration, whereas pentyleneetetrazole amends the climbing speed of adult flies. The larva changes the PSP concerning different AEDs. PSP has an important role in pre-adult development as per the survivability of pupae (hence adult *D. melanogaster*) is concerned (Harini, 2016). A high dose of phenytoin resulted in death in all the developmental stages of *D. melanogaster* (Gayathri and Harini, 2013).

TABLE 1

List of day to day exposed chemicals or radiation which causes neurotoxicity in *Drosophila*

Name of chemicals/radiations	Mode of exposure	Developmental defect	Defect in larvae/pupae/adults	Phenotypic/behavioural defect in adults	Reference
Ionising radiation	γ radiation	Induced stem cell-like property	Imaginal disc of larvae		Verghese and Su, 2018
UV A, B, C	UV radiation	Larval and pupal death	Death during larvae and pupae	Wing spot in adults	Kaya et al., 2006
Bisphenol A	via food	Delay larval development	Larvae growth	Neurodevelopmental disorder	Kaur et al., 2015
Mercury chloride	via food	Larvae and pupae development	Defective hatching rate	Non-functioning of glia, Abnormal neural development	Rand et al., 2009; Engel et al., 2012
Acrylamide	via food	embryo		Behavioural defect in adults	Senthilkumar et al., 2020
Volatile organic compounds	via food	yes	Adults	Locomotory defect along with dopaminergic neurons	Inamdar et al., 2010
Microplastic	Food	Larval gut damage	Larval behaviour	Silence epigenetic genes	Zhang et al., 2020
Methylmercury	Food	Embryo	Embryo	Adult behaviour	Abnoos et al., 2013
Acephate	Food	Larvae gut damage	Adults	Reduced adult longevity	Rajak et al., 2018
Azadirachtin	Food	life span, yes		Less survival and alter reproduction	Ferdenache et al., 2019
Natural harmine	Food	eclosion rate	Pupation site, pupal length, weight, height	Larvae and pupae	Cui et al., 2020
Monoterpene	Fumigation			Survivability	Finetti et al., 2020
POEA	Food	Life span		Fecundity	Bednářová et al., 2020
Endosulphan	Food	Delay hatching		Locomotory behaviour	Sharma et al., 2012
Thiamethoxam	Food	Developmental time, life span	Pupation, growth	Fecundity	de Moraes et al., 2017
Dichlorov	Food			Nervous system defect	Hoang and Rand, 2015; Zhang et al., 2017
Vinylcyclohexene	Food			Locomotory behaviour	Piccoli et al., 2019
Spiromesifen	Food			Less body weight and fewer oocytes, courtship behaviour affected	Kissoum et al., 2020
Lithium	Food			Sleep associated behaviour	Dokucu et al., 2005

Cocaine

It is a potent psychostimulant in *D. melanogaster*. A low dose of cocaine can make flies hyperactive, uncontrollable, and continuous grooming behavior as a defective behavioral endpoint. A locomotory defect such as circling behavior is seen among flies (Schafer, 2002). A high dose of cocaine was shown to induce irregular activity, shock, and complete immobility (akinesia) in *D. melanogaster* (Bainton et al., 2000). Repeated exposure of flies to cocaine increases the behavioral response (sensitization) (Schafer, 2002) and male flies show more sensitivity towards cocaine than females. Alteration of dopamine levels can change the grooming and locomotive behaviors of *D. melanogaster* (Torres and Horowitz, 1998).

Nicotine

The effect of nicotine is confined to the CNS in *D. melanogaster* since acetylcholine receptors for nicotine in insects are found

only in the nervous system (Gundelfinger, 1992; Restifo and White, 1990). Nicotine elicits similar behavioral defects as cocaine. Volatilized nicotine exposure can make flies hyperactive and induce occasional movements, resulting in grooming, hypokinesia, and akinesia in the maximum treated individuals, along with impaired negative geotaxis movement (Bainton et al., 2000; Heberlein et al., 2009). Interestingly, flies treated with both cocaine and nicotine simultaneously show impaired climbing ability in a dose-dependent manner (Bainton et al., 2000).

Hydrogen peroxide

Oral ingestion of hydrogen peroxide (0.1%–2%) as well as the injection of hydrogen peroxide (1%) can increase the locomotory activity of adult flies. Injected hydrogen peroxide mixed with the hemolymph thus causes an alteration of the activity of the fly. Superoxide dismutase

(SOD), a hydrogen peroxide-producing enzyme, also increases fly activity. Injection of hydrogen peroxide and expression of SOD resulted in abnormal walking movement in flies (Grover *et al.*, 2009).

Physical stress (thermal and magnetic field)

Environmental stress, such as thermal stress, can disrupt the CNS (Wang *et al.*, 2007). The mushroom body (MB) is associated with the integration of sensory and associated center of the insect brain, which is further responsible for memory and conditioned behaviors (Davis, 2005). Thermal exposure of larva and pupa during development can change the MB by reducing intrinsic Kenyon cells and the number of neurons by affecting the associative odor learning in adults (Bahrdorff *et al.*, 2016; Wang *et al.*, 2007). Similarly, brief exposure to a weak static magnetic field during the early period of development greatly affects the cuticular pattern of *D. melanogaster* larva (Ho *et al.*, 1992) and the negative geotaxis behavior of adults (Fedele *et al.*, 2014). The hatching rate of the larva decreases due to weak static magnetic fields (Ramirez *et al.*, 1983).

Elements depicting neurotoxicity

Elements are essential for several biological activities in an organism, and their roles are conserved between *D. melanogaster* and higher vertebrates (Calap-Quintana *et al.*, 2017). The human body utilizes metals from food. The body adjusts to the deficiency or excess of metals at the cellular level by altering the metabolic pathways. In the fly, the metals are metabolised using the secretory pathways. If the concentration of the metal exceeds the tolerance level within the body for any reason, it causes neurotoxicity or teratogenicity in flies. We have listed a few elements which are validated as teratogen using *D. melanogaster*.

Mercury (Hg)

Hg is an environmental pollutant and teratogen in *D. melanogaster*. Hg increases the larva to pupa transition time, and reduces the size of the larva, pupae, and hatching rate of pupae. It affects the phosphorylation of various kinases such as MAPK, extracellular signal-regulated kinase (ERK), and c-JUN N-terminal kinase (JNK). In *D. melanogaster*, ERK regulates cell growth and differentiation (Posser *et al.*, 2009), whereas JNK is responsible for cell cytoskeleton and cell formation (Pereira *et al.*, 2011). The toxic effects of Hg include non-functioning of the nervous system and glia (Rand *et al.*, 2009), polarity loss in follicular cells (Baffet *et al.*, 2009), and differentiation defect of nerve and muscle cell (Bournias-Vardiabasis *et al.*, 1990). Abnormal cell differentiation during metamorphosis was also observed in the latter study. More importantly, Hg inhibits the notch pathway and thus disrupts normal neural development (Engel *et al.*, 2012). The inhibition of the notch pathway reduces the hatching rate of the larva (Abnoos *et al.*, 2013).

Lead (Pb)

Pb²⁺ can change the developmental neuronal plasticity in *D. melanogaster* (Jin *et al.*, 2005). Ca²⁺ contributes to synaptic development at different steps, such as guiding the growth cone (Jin *et al.*, 2005), the formation of the synapse

(Xu *et al.*, 2009), elimination, and stabilization (Lohmann and Bonhoeffer, 2008; Pratt *et al.*, 2003). Pb²⁺ affects Ca²⁺ binding proteins (Hirsch *et al.*, 2012) and causes abnormal larval neuromuscular junction and mitochondria with defective ATP synthesis (Flora *et al.*, 2008). Less ATP delays pupal development (Hirsch *et al.*, 2012). Recently it was found that behavioral abnormality induced by Pb²⁺ in flies is by altering the microbiota (Sun *et al.*, 2020).

Chromium (Cr)

Chromium exists in various forms like Cr(III)Cl₃, K₂Cr(VI)O₄, and K₂Cr(VI)₂O₇, and its toxicity was checked using *D. melanogaster*. Wing spots are seen in the adults when the larvae were fed with Cr (VI) salt (Katz *et al.*, 2001; Yeşilada, 2001). Feeding CrCl₃ does not cause any wing spots in adults. [Cr(pic)₃], another chromium (III) compound was fed at a concentration of 200–600 µg per day (Hepburn *et al.*, 2003). In the offspring, it causes a delay in development and death during the process of development (Hepburn *et al.*, 2003). [Cr(pic)₃] does not cause any phenotypic or behavioral, or survival defect when fed to the adult flies. [Cr(pic)₃] does not cause defects genetically but interferes with the metabolic pathways (Hepburn *et al.*, 2003).

Nickel (Ni)

Ni exposure is known to exert many epidemiological and mutagenic effects on human beings. The Ni effect was investigated using *D. melanogaster*. Two different Ni compounds (NiCl₂ and NiSO₄) were employed to check the toxic effect of Ni (Carmona *et al.*, 2011). The genotoxic potential of two Ni-compounds was assessed using *D. melanogaster* using the wing-spot assay and comet assay in the hemolymph. Single- and double-strand DNA breaks were detected after Ni exposure. The frequencies of the wing spot do not increase significantly. However, NiSO₄ can significantly induce DNA damage, as evidenced by comet assay (Carmona *et al.*, 2011).

Aluminium (Al)

Wild type (Canton S) flies were grown on Al mixed food media (20–240 mg/kg). Larvae grown in Al-supplemented food have defective walking behavior. The locomotory activity decreases with the increasing concentration of alumina. The number of pupae and imago count decreases after the treatment of alumina (Kijak *et al.*, 2014). The adult flies hatched after alumina treatment have defective sleep cycle and arrhythmic behavior. The toxicity of alumina depends on concentration and time of exposure. Alumina further decreases the life span of the flies. At 120 mg/kg, the male life span, as well as locomotor activity, were found to be increased (Kijak *et al.*, 2014).

Zinc (Zn)

Dietary intake of several concentrations of Zn (0.1 to 1000 ppm) was checked by mixing it with the fly food (Al-Momani and Massadeh, 2005). Third instar larvae grown on Zn-added food did not exhibit altered growth and development in the first generation up to 500 ppm. At 500 ppm, 75% of pupa and adult death occurred. At 1000 ppm, survival percentages were further significantly

reduced (35%) for pupa and adult stages. For the second generation, survival, growth, and development remain unaltered up to 100 ppm; however, at 500 ppm, a significant reduction in these activities was seen (Al-Momani and Massadeh, 2005). Zn upregulates the expression of metallothionein, and thus serves as an antioxidant. Zn can prevent apoptosis and thus prevent oxidative damage in the brain (Kocatürk et al., 1996). Oral supplementation of ZnCl₂ at a concentration of 4 mM to *parkin* mutant flies (*park*^{25/25}) increased the eclosion frequency and life span of the adult (Saini and Schaffner, 2010). The same concentration of ZnCl₂ decreases the life span of the adults and alters the feeding behavior.

Copper (Cu)

Cu has a role in antioxidant mechanisms, the formation of pigments, and neurotransmitters. It is used in many fungicides. The teratogenic effect of Cu was checked using *D. melanogaster*. Several concentrations (40 to 320 mgL⁻¹) of CuSO₄ were mixed with the fly food (Ding and Wang, 2006) and fed to the first instar larvae. At low concentration, the larval growth rate was slow; with the increasing concentration of Cu, the larval body length decreased (Ding and Wang, 2006). At 320 mgL⁻¹ concentration, the larval body length decreased, and they took 11 days to reach the pupae stage compared to 5 days for control flies (Ding and Wang, 2006).

Cobalt (Co)

CoCl₂ was administered orally and the adults hatched from the pupae were analyzed for morphological defects. Trans-heterozygous larvae for the *multiple wing hairs* (*mwh*) and *flare* (*flr*) were used in this study. The wing spots were observed in the wing after treatment with CoCl₂. CoCl₂ can induce both small and large spots in the wings (Ogawa et al., 1994). In *mwh*/TM3 flies, CoCl₂ could not induce large spots in the wings due to suppressed mitotic crossing-over. To induce large spots, *mwh*/*flr* system clones were generated.

Manganese (Mn)

Mn is an essential element but can induce toxicity if it exceeds the limit. Several concentrations of MnCl₂ were supplemented to the food medium (at 0.1, 0.5, or 1 mM) (Ternes et al., 2014). MnCl₂ treatment was given throughout the development from the egg to the adult stage. Significantly enhanced locomotor activity was observed at 0.5 and 1 mM of Mn. These concentrations can induce reactive oxygen species within the body. At 1 mM, Mn can increase the mRNA expression level of catalase, superoxide dismutase, and Hsp83 without altering the activity. Mn can increase the activity of thioredoxin reductase and GST (Ternes et al., 2014).

Lithium (Li)

Li affects the phosphoinositide signaling pathway and thus distresses neuronal function (Schafer, 2002). Alteration of the inositol polyphosphate pool can cause defects in synaptic function and plasticity (Acharya et al., 1998; de Camilli et al., 1996). Li₂CO₃ is a well-known sedative in psychiatric literature. Li has a role in aging, and at a moderate dose, it has a beneficial effect on longevity. Li treatment affects male mating success and female fecundity

(Matsagas et al., 2009). However, chronic treatment with a low to moderate dose of lithium chloride does not alter the lifespan of *D. melanogaster* (Zhu et al., 2015). Affymetrix Genome Arrays from the Li-treated head of mRNA of *D. melanogaster* suggest an alteration of 12 genes associated with amino acid metabolism and functioning of the nervous system (Kasuya et al., 2009).

Conclusion

Diverse biological, chemical or physical agents that we come across in our day-to-day life may be toxic to our neurons and are referred to as neurotoxicants. The toxin released by these neurotoxicants is known as a neurotoxin. These neurotoxins, upon accumulation, can alter the functioning of the nervous system by altering the electrical and chemical transmission. The toxic effect of these neurotoxicants can be assayed using *D. melanogaster* as the model organism. *D. melanogaster* and vertebrate central and peripheral nervous systems share similarities in their functioning. Thus, the toxicity of the neurotoxicants observed using *D. melanogaster* cannot be neglected. The neurotoxicant effect varies with respect to age, time, and mode of administration. Different modes of administration and their effect on the metabolism of neurotoxicants are described in this paper. The deleterious effect of the neurotoxicant with respect to age and dose can be checked using different developmental stages of *D. melanogaster*. Innumerable well-studied behavioral assays help us to check the effect of neurotoxicant in a dose and time-dependent manner. Stress-related enzymes, which change with respect to the neurotoxicant, can be estimated by numerous biochemical assays reviewed in this paper. The effect of the neurotoxicant on the developmental pattern or timing can also be checked by comparing the developmental cycle. The neurotoxin may cause neuronal degeneration by inducing apoptosis. The apoptotic cell death can be checked easily in many tissues using easy histochemical staining methods described in this review. Change in morphology is a robust assay to detect the effect of the neurotoxicant. The morphometric analysis explained in this paper will help to check the effect of neurotoxicants on developmental stages. Thus this paper summarizes the multidimensional approaches to screening the neurotoxicity of any unknown compounds.

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