**UNIVERSIDADE FEDERAL DO PAMPA**
**PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA**

**DIENIFFER ESPINOSA JANNER**

# AVALIAÇÃO DA EXPOSIÇÃO AO IMIDACLOPRIDA SOBRE O MODELO DE TRANSTORNO NEURODESENVOLVIMENTAL EM *Drosophila melanogaster*

**Uruguaiana, RS, Brasil**
**2020**

**DIENIFFER ESPINOSA JANNER**

# AVALIAÇÃO DA EXPOSIÇÃO AO IMIDACLOPRIDA SOBRE O MODELO DE TRANSTORNO NEURODESENVOLVIMENTAL EM *Drosophila melanogaster*

Dissertação apresentada ao programa de Pós graduação *Stricto Sensu* em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Bioquímica.

Orientador: **Prof. Dr. Gustavo Petri Guerra.**

Coorientadora: **Profª. Drª. Marina Prigol**

**Uruguaiana, RS, Brasil**

**2020**

Ficha catalográfica elaborada automaticamente com os dados fornecidos
pelo(a) autor(a) através do Módulo de Biblioteca do
Sistema GURI (Gestão Unificada de Recursos Institucionais) .

J34a Janner, Dieniffer Espinosa
Avaliação da Exposição ao Imidacloprida Sobre o Modelo de Transtorno Neurodesenvolvimental em Drosophila melanogaster / Dieniffer Espinosa Janner.
89 p.

Dissertação(Mestrado)-- Universidade Federal do Pampa, MESTRADO EM BIOQUÍMICA, 2020.
"Orientação: Gustavo Petri Guerra".

1. Pesticidas. 2. Neonicotinóides. 3. Transtorno do Espectro do Autismo. 4. Transtorno do Déficit de Atenção e Hiperatividade. 5. Dopamina. I. Título.

**DIENIFFER ESPINOSA JANNER**

# AVALIAÇÃO DA EXPOSIÇÃO AO IMIDACLOPRIDA SOBRE O MODELO DE TRANSTORNO NEURODESENVOLVIMENTAL EM *Drosophila melanogaster*

Dissertação apresentada ao programa de Pós-graduação *Stricto sensu* em Bioquímica da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Bioquímica.

Área de concentração: Bioprospecção Molecular.

**Dissertação defendida e aprovada em:** 03/03/2020

**Banca Examinadora**

**Prof. Dr. Gustavo Petri Guerra**
**Orientador**
**UNIPAMPA**

**Prof. Drª. Daiana Silva de Ávila**
**UNIPAMPA**

**Prof. Dr. Rafael Porto Ineu**
**UTFPR**

## RESUMO

Os distúrbios neurodesenvolvimentais possuem como principais constituintes o Transtorno do Espectro do Autismo (TEA) e o Transtorno do Déficit de Atenção Hiperatividade (TDAH). O imidacloprida é um inseticida pertencente à família neonicotinóides, os quais atuam como potentes agonistas dos receptores de acetilcolina, possuindo a capacidade de promover alterações comportamentais em *Drosophila melanogaster*, que apresentam semelhanças aos fenótipos humanos TEA e TDAH. O objetivo do presente estudo foi corroborar a ação do imidacloprida como modelo químico de distúrbios do desenvolvimento neurológico, no nível comportamental, juntamente com a investigação de alterações neuroquímicas induzidas pela exposição ao imidacloprida, as quais estão relacionadas aos sintomas desses transtornos no modelo de *Drosophila melanogaster*. Foram utilizadas moscas virgens e machos de 3 dias de idade, divididos em quatro grupos e expostas a dieta padrão (controle) ou dieta contendo imidacloprida (200, 400 ou 600 pM) durante 7 dias. Após a eclosão da progênie, as moscas foram submetidas à atividades *in vivo* (geotaxia negativa, campo aberto, interação social, agressividade, claro/escuro e grooming) e *ex vivo* (consumo de alimentos, níveis de dopamina, atividade da acetilcolinesterase (AChE), indicadores de estresse oxidativo (superóxido dismutase, SOD; catalase, CAT; glutationa S-transferase, GST; espécies reativas de oxigênio, ROS; substâncias reativas ao ácido tiobarbitúrico, TBARS; e ensaios proteicos e não proteicos, PSH e NPSH) e ensaios de viabilidade celular (resazurina)). A progênie de moscas expostas ao imidacloprida demonstraram aumento da hiperatividade (geotaxia e tarefa de campo aberto), agressividade, ansiedade e movimentos repetitivos, juntamente com uma diminuição da interação social. Além disso, a progênie das moscas expostas ao imidacloprida apresentaram diminuição nos níveis de dopamina e viabilidade celular e aumentaram o estresse oxidativo (ROS e TBARS), no entanto, a atividade de AChE, CAT e GST e PSH e NPSH permaneceram inalterados. Nossos resultados sugerem o envolvimento da dopamina e do estresse oxidativo como um possível mecanismo de ação para danos comportamentais semelhantes ao TEA e TDAH induzido pelo imidacloprida em *Drosophila melanogaster*.



Palavras-chave: Pesticidas, Neonicotinóides, Transtorno do Espectro do Autismo, Transtorno do Déficit de Atenção e Hiperatividade, Dopamina, Acetilcolinesterase.

## ABSTRACT

Neurodevelopmental disorders have as main constituents Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD). Imidacloprid is an insecticide belonging to the neonicotinoid family, which act as potent acetylcholine receptor agonists, having the ability to promote behavioral changes in *Drosophila melanogaster*, which are similar to the human phenotypes TEA and ADHD. The aim of the present study was to corroborate the action of imidacloprid as a chemical model of neurological development disorders at the behavioral level, together with the investigation of neurochemical changes induced by exposure to imidacloprid, which are related to the symptoms of these disorders in the *Drosophila melanogaster* model. Virgin flies and 3-day-old cheeks were used, divided into four groups and exposed to a standard diet (control) or diet containing imidacloprid (200, 400 or 600 pM) for 7 days. After the progeny hatched, the flies were subjected to in vivo activities (negative geotaxis, open field, social interaction, aggression, light/dark and grooming) and ex vivo [food consumption, dopamine levels, acetylcholinesterase activity (AChE), oxidative stress indicators (superoxide dismutase, SOD; catalase, CAT; glutathione S-transferase, GST; reactive oxygen species, ROS; substances reactive to thiobarbituric acid, TBARS; and protein and non-protein assays, PSH and NPSH) and assays cell viability (resazurin)]. The progeny of flies exposed to imidacloprid showed increased hyperactivity (geotaxis and open field task), aggressiveness, anxiety and repetitive movements, together with a decrease in social interaction. In addition, the progeny of flies exposed to imidacloprid showed decreased levels of dopamine and cell viability and increased oxidative stress (ROS and TBARS), however, the activity of AChE, CAT and GST and PSH and NPSH remained unchanged. Our results suggest the involvement of dopamine and oxidative stress as a possible mechanism of action for behavioral damage similar to TEA and ADHD induced by imidacloprid in *Drosophila melanogaster*.



Keywords: Pesticides, Neonicotinoid, Autism Spectrum Disorder, Attention Deficit Hyperactivity Disorder, Dopamine, Acetylcholinesterase.

# LISTA DE ILUSTRAÇÕES

## LISTA DE ABREVIATURAS

(Referentes a Revisão Bibliográfica)

AChE – Acetilcolinesterase

ATP – Adenosina Trifosfato

Akt – Proteína Quinase B

CAT – Catalase

CNV - Variantes no Número de Cópias

GABA – Ácido Gama-Aminobutírico

GST – Glutationa S-Transferase

LPS – Lipopolissacarídeo

NADPH – Nicotinamida Adenina Dinucleotídeo Fosfato reduzido

ROS – Espécies Reativas de Oxigênio

SOD – Superóxido Dismutase

TBARS – Espécies Reativas ao Ácido Tiobarbitúrico

TEA – Transtorno do Espectro do Autismo

TDAH – Transtorno do Déficit de Atenção com Hiperatividade

TID - Transtorno Invasivo do Desenvolvimento

VPA – Ácido Valpróico

**SUMÁRIO**

# APRESENTAÇÃO

No item **INTRODUÇÃO e REVISÃO BIBLIOGRÁFICA** está descrita uma breve revisão de literatura sobre os temas abordados nesta dissertação seguida pelo item **OBJETIVOS.**

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de artigo científico, os quais são apresentados no item **MANUSCRITO CIENTÍFICO**. As seções: *Introdução, Materiais e Métodos, Resultados, Discussão, Conclusão e Referências Bibliográficas,* encontra-se no próprio artigo e representa a íntegra deste estudo. O manuscrito está estruturado de acordo com as normas da revista científica "*Food and Chemical Toxicology*" para a qual será submetido.

Os itens **CONSIDERAÇÕES FINAIS E PERSPECTIVAS** encontram-se no final desta dissertação e apresentam interpretações e comentários gerais sobre o artigo contido neste trabalho.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações que aparecem nos itens introdução e revisão bibliográfica.

# 1. INTRODUÇÃO

Os distúrbios do neurodesenvolvimento são condições neurológicas que ocorrem precocemente na infância, frequentemente antes da idade escolar, afetando o desenvolvimento do funcionamento pessoal, social e profissional. Sendo o Transtorno do Espectro do Autismo e o Transtorno do Déficit de Atenção com Hiperatividade os principais constituintes desses distúrbios (JONES; KLIN, 2013; LUKE et al., 2012).

Estima-se que a prevalência para esses distúrbios é de aproximadamente 16,8 em 1.000, e que essas taxas de prevalência do TEA e TDAH parecem estar crescendo em todo o mundo (BAIO et al., 2018; LANDRIGAN; LAMBERTINI; BIRNBAUM, 2012; CHRISTENSEN et al., 2019). Esses transtornos normalmente envolvem distúrbios de hiperatividade, atenção, memória, linguagem, solução de problemas ou interação social, no qual essas características acompanham o indivíduo ao longo da vida (KOLOZSI et al., 2009).

A etiologia do TEA e do TDAH permanecem desconhecidas, todavia estudos apontam apara o envolvimento de variados genes e de fatores ambientais, o que contribui para que sua etiologia não seja completamente compreendida (HALLMAYER, 2011; SANDIN et al., 2014). Supõe-se que a exposição a produtos químicos como pesticidas pode contribuir significativamente para a prevalência desses transtornos neurodesenvolvimentais (QUIRÓS-ALCALÁ; MEHTA; ESKENAZI, 2015; ROBERTS; KARR, 2012).

O imidacloprida é um inseticida neonicotinóide o qual provoca a morte de insetos via ingestão ou contato, atuando no sistema nervoso central como potente agonista dos receptores nicotínicos de acetilcolina (TOMIZAWA; CASIDA, 2005). É largamente empregado em lavouras para o controle de pragas no solo, em sementes revertidas, controle de pulgas, baratas e insetos (KARAHAN et al., 2015; MENGONI; FARINA, 2015).

A *Drosophila melanogaster* vem sendo amplamente utilizada como modelo alternativo para diversos estudos, devido ao fato de apresentar vantagens importantes, tais como estrutura genômica simples, ciclo de vida curto, baixo custo de manutenção, apresentar comportamentos semelhantes aos humanos, e principalmente homologia de aproximadamente 75% de genes relevantes com organismos superiores, além da facilidade de obtenção de fenótipos mutantes (ROBERTS; DAWLEY; REIGART, 2019; TAUBER; VANLANDINGHAM; ZHANG, 2011).

Têm sido proposto um modelo de transtorno neurodesenvolvimental por KIM et al., (2017), avaliando os efeitos do imidacloprida, entretanto faz-se necessário uma maior compreensão de sua ação como modelo químico para distúrbios do desenvolvimento neurológico em *Drosophila melanogaster*. Desta forma no presente estudo avaliou-se o efeito

da exposição ao imidacloprida em diferentes concentrações sobre o comportamento e principalmente marcadores bioquímicos, sugerindo um possível mecanismo de ação, a fim de gerar conhecimento sobre o imidacloprida como um modelo experimental de transtorno neurodesenvolvimental com sintomas semelhantes ao TEA e TDAH em *Drosophila melanogaster*. Assim a realização desse estudo possui como hipótese o fato de que o imidacloprida pode vir a ser uma ferramenta útil para o desenvolvimento de um modelo químico, o qual permitirá avaliar fatores ambientais relacionados ao TEA e ao TDAH em *Drosophila melanogaster*, e principalmente investigar as alterações neuroquímicas induzidas pela exposição ao imidacloprida, as quais são relacionadas aos sintomas de TEA e TDAH.

# 2. REVISÃO BIBLIOGRÁFICA

## 2.1.1 Transtornos Neurodesenvolvimentais

Os distúrbios mentais assim como do desenvolvimento infantil envolvem distúrbios do desenvolvimento neurológico, emocionais e comportamentais que têm amplos e graves impactos adversos no bem-estar, tanto psicológico quanto social (AMERICAN PSYCHIATRIC ASSOCIATION, 2013).

O Transtorno do Espectro do Autismo juntamente com o Transtorno do Déficit de Atenção com Hiperatividade são denominados como os transtornos mais representativos dos distúrbios neurodesenvolvimentais, possuindo como principais características padrões de interesse restritos, falha nas interações sociais, assim como falta de atenção e hiperatividade (LUKE et al., 2012).

O TEA sendo um transtorno neurodesenvolvimental é reconhecido por seu início precoce, antes dos três anos de idade, e é definido através de uma avaliação clínica apresentando como principais características comportamentais o comprometimento sócio-comunicativo e de interação social, exibindo sinais como agressividade, hiperatividade, comportamentos repetitivos e estereotipados entre outros conforme ilustrados na Figura 1, onde o nível de alteração nessas características é distinto entre os indivíduos autistas (GESCHWIND; LEVITT, 2007; ROULLET; CRAWLEY, 2011).


**Figura 1:** Sintomas característicos do TEA**.**

Fonte: adaptado de autismoinfantil.com.br.

Já o Transtorno do Déficit de Atenção com Hiperatividade é caracterizado por um padrão persistente de desatenção e/ou hiperatividade mais frequente do que o observado em crianças da mesma faixa etária (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Assim como para o TEA o diagnóstico para o TDAH é clinico, não havendo exames laboratoriais para o seu diagnóstico, sendo levados em consideração as seguintes características comportamentais descritos na Figura 2 abaixo.

| Desatenção | Hiperatividade/impulsividade |
|---|---|
| Prestar pouca atenção a detalhes e cometer erros por falta de atenção | Mover de modo incessantes pés e mãos quando sentado |
| Dificuldade em se concentrar (em deveres ou brincadeiras) | Dificuldade de permanecer sentado em situações em que isto é esperado (sala de aula, mesa de jantar, etc.) |
| Parecer estar prestando atenção em outras coisas numa conversa | Correr ou trepar em objetos freqüentemente, em situações nas quais isto é inapropriado |
| Dificuldade em seguir as instruções até o fim ou deixar atividades sem terminá-las | Dificuldades para se manter em atividades de lazer em silêncio |
| Dificuldade de se organizar ou planejar com antecedência | Parecer ser movido por um "motor" sempre "ligado" |
| Relutância ou antipatia para fazer deveres de casa ou iniciar tarefas que exijam esforço mental por muito tempo | Falar demais |
| Perder objetos ou esquecer compromissos | Responder as perguntas antes das mesmas serem concluídas |
| Distrair-se com muita facilidade com coisas a sua volta ou com seus pensamentos | Não conseguir aguardar a vez |
| Esquecer coisas do dia-a-dia | Interromper freqüentemente os outros em suas atividades ou conversas |

**Figura 2:** Sintomas para o diagnóstico do TDAH.

Fonte: Araújo, A.P.Q.C (2002).

### 2.1.2 Epidemiologia e Etiologia

O primeiro estudo epidemiológico sobre o TEA realizou-se por Victor Lotter, em 1966, sendo relatado um índice de prevalência de 4,5 em 10.000 crianças, em toda a população de crianças de 8 a 10 anos de Middlesex, um condado ao noroeste de Londres (KLIN, 2006). Desde a década de 1960 inúmeras pesquisas sobre a prevalência do TEA baseadas na população foram conduzidas, juntamente com uma série de revisões recentes resumindo essas pesquisas e avaliando mudanças nas estimativas mencionadas ao longo do tempo (NEWSCHAFFER, 2005; WING; POTTER, 2002).

O aumento alarmante no número de indivíduos que recebem auxílio de agências de serviços relacionados a deficiência educacional e de desenvolvimento sob classificação do TEA, juntamente com os dados epidemiológicos nos últimos 15 anos chamaram a atenção para a propensão secular na prevalência do TEA e suas subjacentes (NEWSCHAFFER, 2005; SHATTUCK, 2006).

Nos Estados Unidos um estudo realizado em 2016 relatou que a prevalência do TEA é de aproximadamente 1 em cada 40 crianças (SCHMIDT et al., 2017). Com relação ao sexo o TEA apresenta prevalência desigual, sendo cerca de quatro vezes mais frequente em meninos do que em meninas (CALABRESEC et al., 2016), no entanto, quando afetadas, as meninas

tendem a desenvolver a forma mais severa de TEA, associada à grave comprometimento cognitivo (SADOCK; SADOCK, 2017).

Estima-se que a prevalência mundial para o TDAH é em torno de 5% a 15%, e os sintomas iniciam-se na infância e persistem na adolescência e na idade adulta na grande maioria dos casos sendo a ocorrência desse transtorno três vezes maior em indivíduos do sexo masculino (BIEDERMAN, 2005; POLANCZYK et al., 2007).

Com relação à etiologia, no geral o TEA é pouco compreendido, sendo que, a hipótese mais aceita no presente, é a de que o transtorno resulte de uma interação entre características genéticas e fatores ambientais, com envolvimento de múltiplos fatores de risco e proteção nos níveis biológico, psicológico e social (MANDY; LAI, 2016; SANDIN et al., 2014).

Nenhuma variante genética específica é identificada como necessária ou suficiente para causar o TEA, sugerindo que exista uma heterogeneidade genética, em que essas alterações genéticas incluem mutações novas e herdadas, variantes no número de cópias (CNV) e polimorfismos (BOURGERON, 2015).

Tais alterações epigenéticas podem resultar em um excesso de espécies reativas de oxigênio (ROS), os quais podem levar ao comprometimento da metilação do DNA, levando a um mecanismo de *feedback* positivo, logo indivíduos com TEA possuem uma maior vulnerabilidade ao estresse oxidativo e a neurotoxicidade conforme demonstrado na Figura 3 (ESSA, 2020).

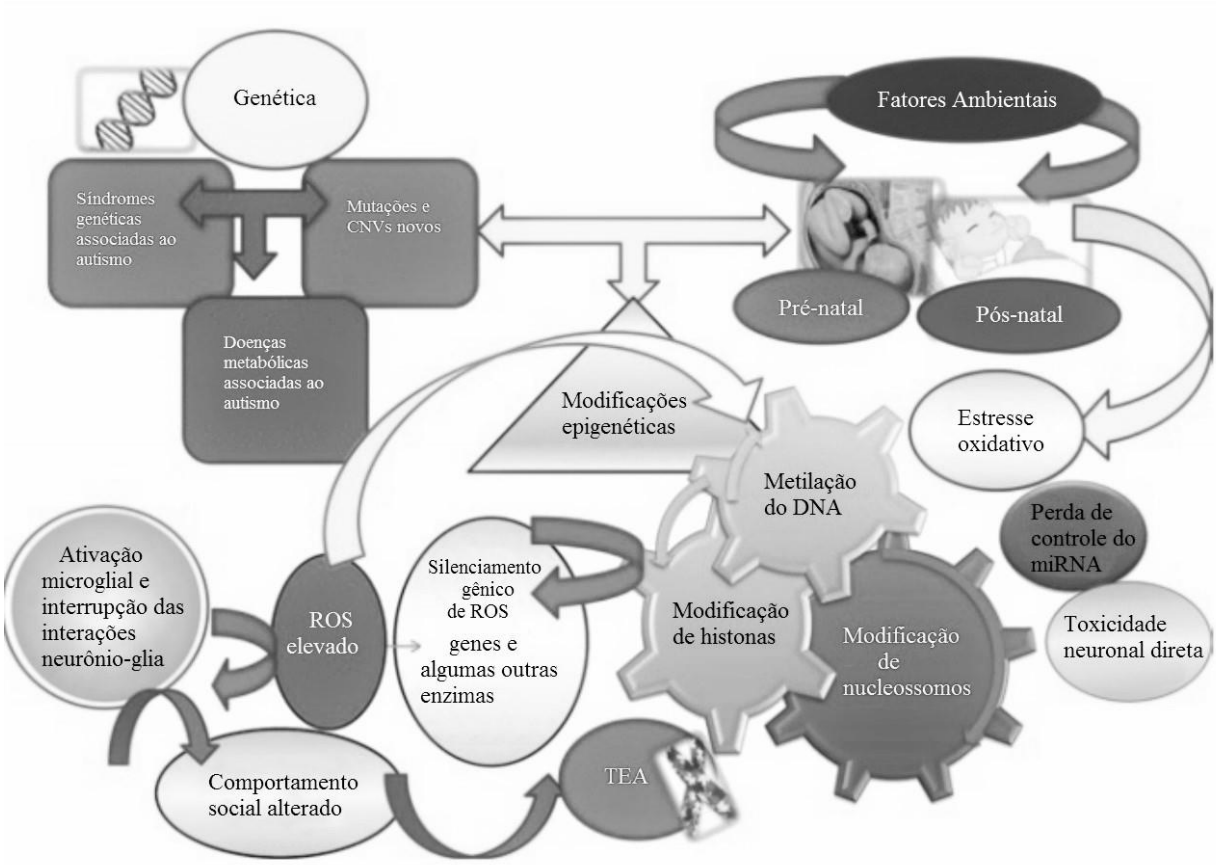



**Figura 3.** Diagrama de ilustração complexa de ambiente, genética e epigenética no desenvolvimento do TEA.

Fonte: Adaptado de ESSA & QORONFLEH, (2020).

Foram identificados vários genes como fortes candidatos, envolvendo genes de andaimes pós-sinápticos como por exemplo SHANK3, genes de contato como CNTN4, genes de remodelação da cromatina tendo como exemplo CHD2. Estas evidências servem para sugerir que variantes genéticas de risco identificadas entre pessoas com TEA convergem em vias genéticas comuns, onde resultado cumulativo de múltiplas variantes genéticas, ou seja um risco poligênico apresenta-se como um importante indicador de risco para outros transtornos psiquiátricos bem como para o TEA (DE LA TORREUBIETA et al., 2016).

O primeiro trabalho sobre o TDAH foi realizado pelo médico escocês Alexander Crichton em 1798, onde o mesmo descreveu aspectos de desatenção encontrados em jovens, os quais são muito semelhantes aos critérios propostos pelo DSM-IV para o tipo desatento do TDAH (PALMER; FINGER, 2001).

Com relação a etiologia das particularidades neurobiológicas do TDAH também não estão completamente esclarecidas, em que estudos demonstram que é um transtorno complexo

e multifatorial, causado pela influência de inúmeros fatores de risco, onde cada um possui um pequeno efeito auxiliando para o aumento da vulnerabilidade através de seus efeitos aditivos e interativos (BIEDERMAN; FARAONE, 2005; GENRO et al., 2010; NEALE et al., 2008).

A partir de um estudo realizado com gêmeos, estima-se uma herdabilidade de aproximadamente 76%, sugerindo assim um forte componente genético no TDAH (FARAONE; MICK, 2010). Genes de distintos sistemas de neurotransmissores que codificam transportadores, receptores ou enzimas são extremamente investigados no TDAH, tendo-se como exemplo, o DAT1 (gene do transportador de dopamina), genes dos receptores adrenérgicos α2A (ADRA2A) e α2C (ADRA2C), a COMT (gene que codifica a enzima catecol-O-metiltransferase), entre outros (GENRO et al., 2012; GIZER; FICKS; WALDMAN, 2009; NEALE et al., 2010).

### 2.1.3 Tratamentos

Em relação ao tratamento, não há cura, havendo somente intervenções psicológicas e educacionais precoces as quais constituem a base do tratamento, que tem por objetivo intensificar comportamentos socialmente apropriados, reduzir comportamentos indesejáveis e facilitar o desenvolvimento cognitivo, social e afetivo. O tratamento farmacológico é utilizado em alguns casos como adjuvante das estratégias psicopedagógicas. Medicação antipsicótica têm se mostrado eficaz em casos de agressividade severa e automutilação, inibidores seletivos e não seletivos de serotonina são utilizados para auxiliar a redução de comportamentos perturbadores e o ácido valpróico vem sendo utilizado para redução da agressividade (MACHADO-VIEIRA et al., 2003).

### 2.1.4 Modelos experimentais

Devido à complexibilidade do TEA e do TDAH juntamente com a frequente associação a comorbidades, surgem barreiras que dificultam extremamente a pesquisa desses distúrbios, tornando-se necessário o desenvolvimento de modelos animais (SCHLICKMANN; FORTUNATO, 2013).

O surgimento dos modelos experimentais permitem a investigação de inúmeros fatores de risco, tanto ambientais quanto genéticos, assim como a pesquisa de vias moleculares e mecanismos neurofisiológicos envolvidos no transtorno, uma vez que a compreensão desses

aspectos é essencial para o desenvolvimento de métodos de prevenção bem como terapias eficazes (KIM et al., 2016).

Entretanto, os modelos experimentais utilizando animais apresentam suas limitações, não podendo simular integralmente uma doença humana (BERNARDI; KIRSTEN; TRINDADE, 2012; MARKRAM, 2007). Assim a validação de um modelo experimental de TEA e TDAH se dá por meio da observação de características também vistas em pacientes com esses transtornos, ou seja, prejuízos comportamentais na comunicação (verbal e não verbal), interação social e comportamentos estereotipados (WÖHR; SCATTONI, 2013).

Em roedores as substâncias mais utilizadas para mimetizar o modelo de TEA se baseiam através da administração de ácido valproico (VPA), ou lipopolissacarídeo (LPS) podendo ser administrados por via intraperitoneal (i.p) e subcutânea (KIM et al., 2011, 2016).

A *Drosophila melanogaster* (mosca da fruta) tem se tornado um modelo alternativo para utilização em diversos métodos, devido a sua versatilidade. Dessa forma inúmeras pesquisas sobre as funções dos diferentes genes e comportamentos associados ao TEA e TDAH tem sido realizados nesse organismo modelo, entretanto a realização de modelos com exposições a produtos químicos, como por exemplo o imidacloprida não estão totalmente consolidados, havendo assim uma ampla utilização de modelos genéticos para esses transtornos (DOLL; BROADIE, 2014; KIM; LEE; PARK, 2017; LEE et al., 2014; YAMAMOTO et al., 2014).

Em modelos para o FMR1 é um gene humano cuja função é codificar uma proteína chamada proteína de retardo mental X frágil, observou-se que a desregulação do ortólogo de *Drosophila melanogaster*, o dFMR1, é capaz de provocar defeitos da extensão/projeção de neuritos (MORALES et al., 2002), alterações do ciclo celular e apoptose (WAN et al., 2000), anormalidades no ciclo circadiano e déficit de interação social, tais como ausência de interesse em manter o comportamento de corte e dificuldades em desempenhar gestos motores que levam a interação social (BOLDUC et al., 2010; DOCKENDORFF et al., 2002). Modelos de TEA e TDAH knockdown em *Drosophila melanogaster* vem sendo amplamente utilizados, observando alterações nos níveis dopaminérgicos além de um aumento da atividade locomotora e redução do sono nesses modelos, corroborando estudos experimentais relacionadas a esses transtornos (HARICH et al.,2019; KLEIN et al., 2020).

### 2.1.5 Estresse oxidativo, Neurotransmissores e transtornos neurodesenvolvimentais (TEA e TDAH).

O desequilíbrio entre pró-oxidantes e antioxidantes é denominado como estresse oxidativo, no qual as espécies reativas de oxigênio (ROS) ou espécies reativas de nitrogênio (RNS) causam danos prejudiciais à célula (CHIRUMBOLO; BJØRKLUND, 2017).

O desenvolvimento do estresse oxidativo no TEA atribui-se a geração de radicais livres, os quais são responsáveis pela disfunção mitocondrial (ESSA, 2020). As principais consequências da disfunção mitocondrial são: a) redução da produção de ATP, b) produção elevada de espécies reativas de oxigênio (ROS) e consequentemente danos oxidativos e c) indução da apoptose (ROSSIGNOL; FRYE, 2012), sendo tais alterações comumente implicadas no TEA e TDAH, podendo ser induzidas principalmente pela exposição a pesticidas (FRANCO et al., 2009b; ROHLMAN; ANGER; LEIN, 2011). O mecanismo do estresse oxidativo no TEA está ilustrado na Figura 4 abaixo.

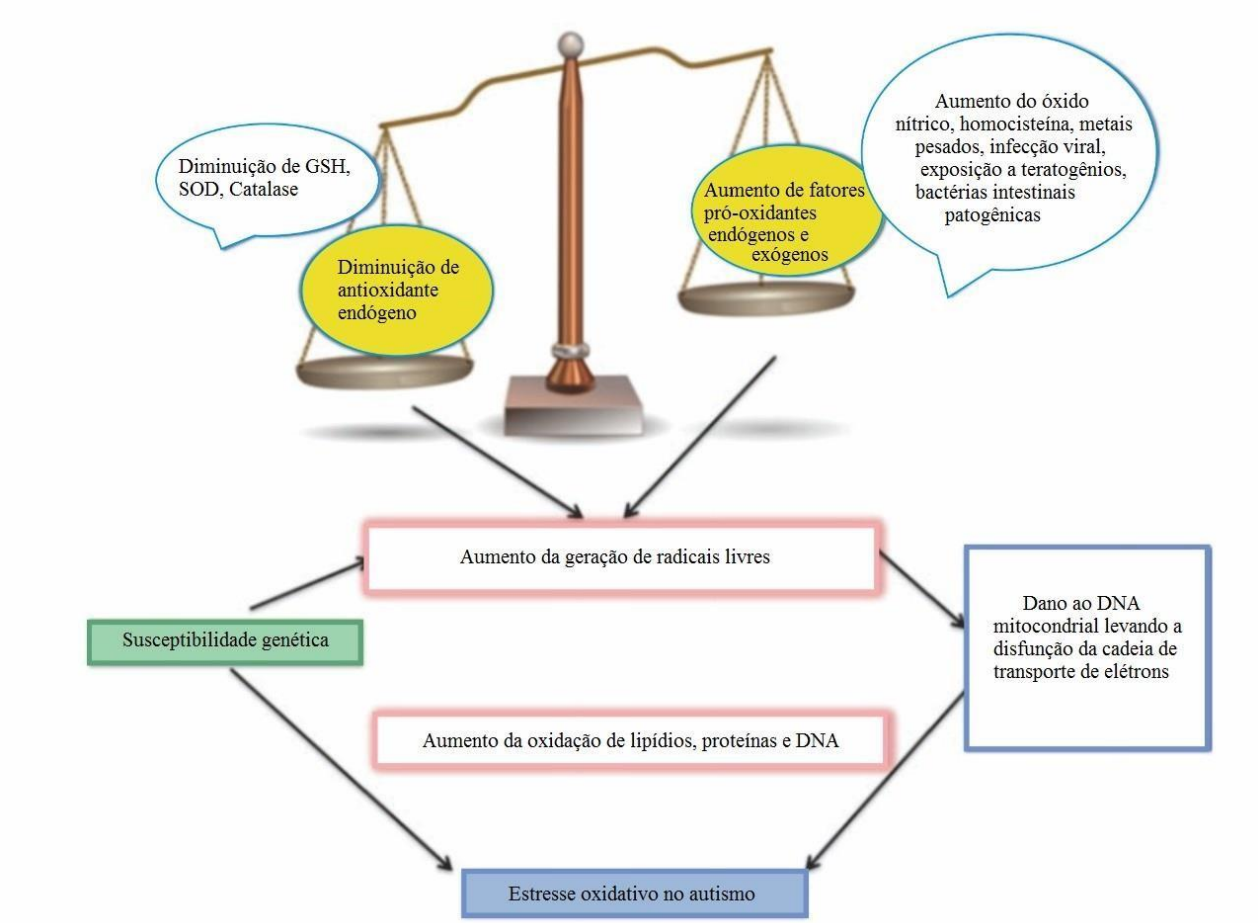



**Figura 4.** Mecanismos de estresse oxidativo no Transtorno do Espectro Autismo Fonte: Adaptado de ESSA & QORONFLEH, (2020).

Inúmeros estudos realizados apontam o estresse oxidativo como um importante contribuinte do TEA e TDAH (AL-AMIN et al., 2015; PARKER et al., 2017; ROSE et al., 2012), no qual a utilização de modelos com alterações genéticas e modelos com exposição a produtos químicos, demonstram alterações em marcadores como substâncias reativas ao ácido tiobarbitúrico (TBARS), ROS, os quais promovem alterações nos níveis de dopamina e na atividade de enzimas como: AChE, GST SOD e CAT.
(HAMILTON et al., 2013; KARVAT; KIMCHI, 2014; SHARMA; RAHI; MEHAN, 2019; WANG et al., 2019).

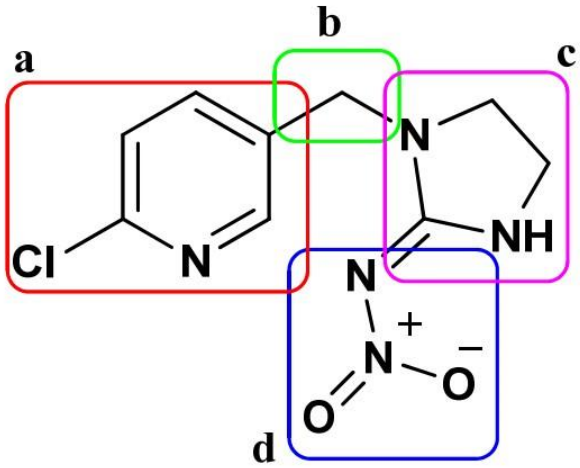
O dano oxidativo causado através da disfunção mitocondrial e auto-oxidação de dopamina, assim como a agregação de α-sinucleina, juntamente com a neuroinflamação e com a ativação de oxidases dependentes de NADPH, possuem importante contribuição para a morte celular dopaminérgica (JUÁREZ OLGUÍN et al., 2016; LABANDEIRAGARCIA et al., 2014). A

dopamina vem sendo largamente associada ao TEA e TDAH, na qual mutações nos genes da sinalização deste neurotransmissor, bem como o desequilíbrio dopaminérgico, foram associados a esses transtornos (NAKAMURA et al., 2010; PAVĂL, 2017).

### 2.1.6 Imidacloprida

Os neonicotinóides atuam como agonistas seletivos de receptores de acetilcolina de insetos, onde a ativação persistente ocasiona uma hiperexcitação do sistema nervoso, culminando em convulsões, paralisia e morte (FFRENCH-CONSTANT et al., 2016) .

A estrutura química dos neonicotinóides é composta por quatro componentes estruturais distintos: (a) um grupo heteroarilmetil ou heterociclilmetil, (b) um ligante flexível, (c) um anel de cinco/seis membros ou um sistema de cadeia aberta e (d) um farmacóforo nitro/ciano (Figura 5). O anel cloro-piridina do imidacloprida é essencial para fornecer fotoestabilidade (YAMAMOTO & CASIDA, 1999).




(a) A fração 6 -cloropiridin-3-ilo, (b) ponte de metileno, (c) anel de imidazolidina, e (d) o nitro -imino eletronegativo.

**Figura 5:** Componentes estruturais dos neonicotinóides (exemplo Imidacloprida).

Fonte: Adaptado de Fusetto, R. (2017).

Desta forma devido a sua ação neurotóxica, os neonicotinoides atuam sobre a neurofisiologia dos insetos, podendo atuar juntamente aos organismos não alvos, como por exemplo, os insetos benéficos, tais como as abelhas (DESNEUX; DECOURTYE; DELPUECH, 2007).

Essa classe de inseticida/pesticida possui como principal representante o imidacloprida, devido ao fato de ter sido o primeiro neonicotinóide introduzido no Japão e na Europa em 1990 e comercializado nos EUA no ano de 1992 (OBANA, et al., 2003). O imidacloprida 1 (6-cloro-

3-piridinil) metil) -N-nitro-2-imidazolidinimina (Figura 6), vem sendo amplamente utilizado mundialmente para proteção de culturas devido à sua baixa persistência no solo, alta atividade inseticida e baixa taxa de aplicação (LEE CHAO; CASIDA, 1997).

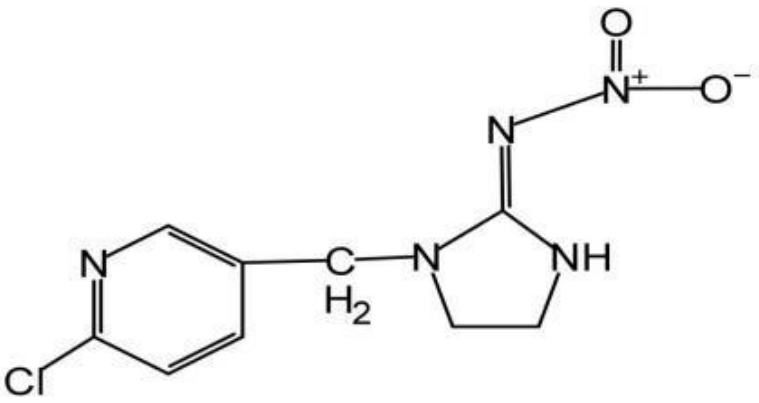



**Figura 6:** Estrutura molecular do imidacloprida.
Fonte: AMARAL, P.O (2017).

Devido à sua toxicidade seletiva para insetos, a utilização deste inseticida vem aumentando globalmente, sendo amplamente utilizado para o controle de insetos sugadores nas agriculturas, bem como injeções de solo e árvores, além do tratamento de sementes revestidas com pesticidas, apresentando assim alta afinidade aos receptores nicotínicos de acetilcolina dos insetos conforme ilustrado na Figura 7, entretanto para humanos aparentemente é considerado seguro (CROSBY et al., 2015; FFRENCHCONSTANT et al., 2016; TOMIZAWA; CASIDA, 2005).

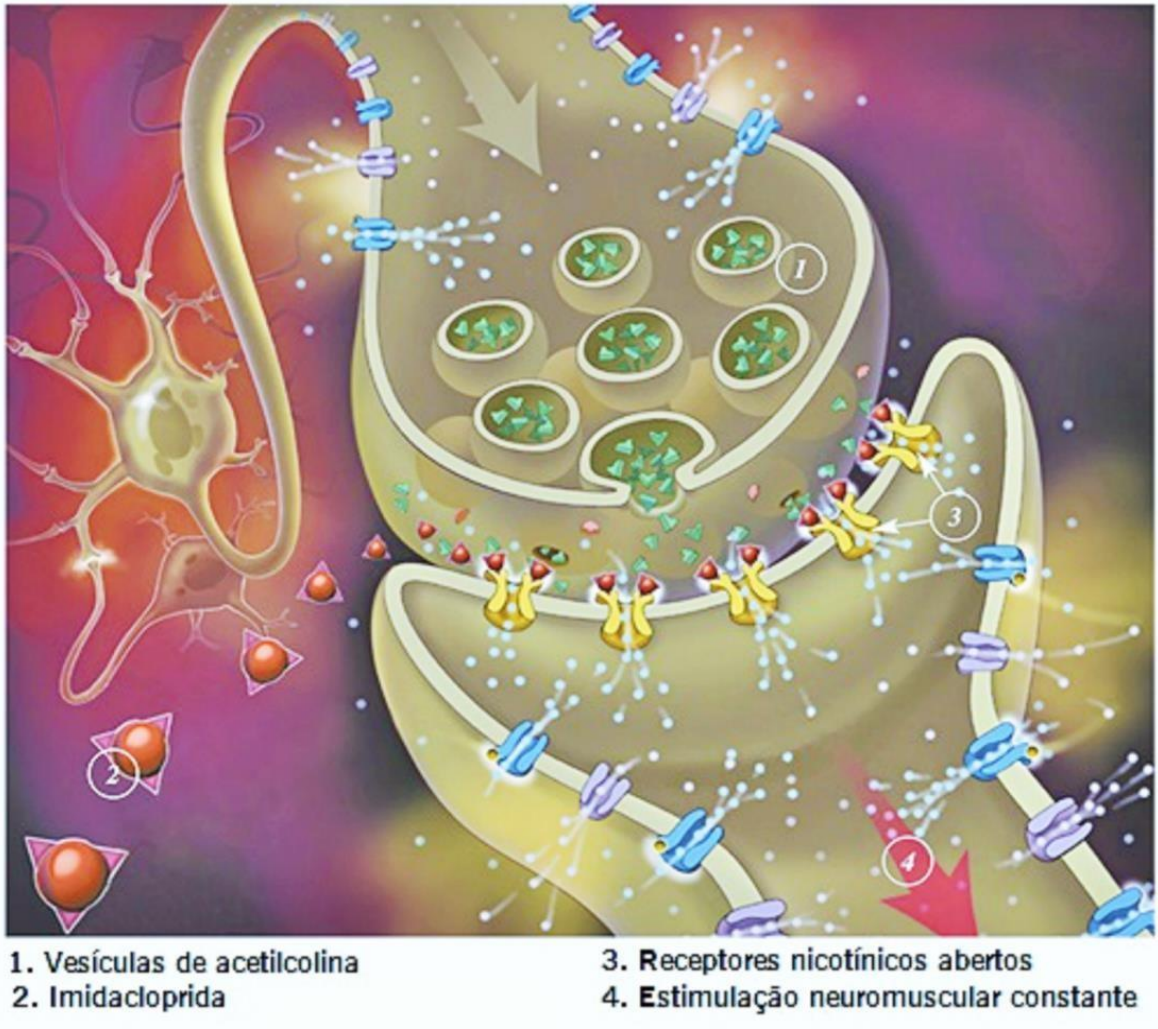



**Figura 7:** Mecanismo de ação do imidacloprida.

Fonte: https://blogparapet.wordpress.com/endectoparasitarios/imidacloprida .

Os neonicotinóides atuam no sistema nervoso central, com exposição pré-natal e pós-natal levando a déficits neurocomportamentais em modelos de peixe-zebras. Entretanto os efeitos dos neonicotinóides no desenvolvimento neurocomportamental em vertebrados não foram bem caracterizados, e suposições sobre sua segurança foram feitas na ausência de uma investigação completa (CROSBY et al., 2015).

Com relação ao imidacloprida, as evidências toxicológicas revelam claramente que a genotoxicidade, as quebras diretas do filamento ácido desoxirribonucleico e a mutação cromossomo/genoma podem ser ocasionadas por esse produto químico, bem como induzir mutagenecidade, estresse oxidativo, imunotoxicidade desenvolvimental e inflamação no sistema nervoso central em organismo não-alvo (FENG et al., 2005; GAWADE et al., 2013; KAPOOR; SRIVASTAVA; SRIVASTAVA, 2011).

Um estudo realizado demonstra que a exposição prolongada a doses baixas de imidacloprida podem causar alterações no fígado, redução do peso corporal, toxicidade reprodutiva além de retardo desenvolvimental e déficits neurocomportamentais em ratos (DUZGUNER; ERDOGAN, 2012).

Uma vez que o imidacloprida é rapidamente absorvido pelo trato gastrointestinal, e uniformemente disseminado nos órgãos e tecidos, sendo as maiores concentrações de imidacloprida encontradas no fígado e rins, sendo estes órgãos responsáveis pela eliminação de substâncias (SHEETS, 2010).

### 2.1.7 Relação entre pesticidas e doenças neurodegenerativas

Os distúrbios neurodegenerativos são definidos a partir de doenças que possuem a perda de células neuronais em inúmeras áreas do córtex, entre elas os gânglios basais, cerebelo, tronco cerebral e sistemas locomotores, acarretando em disfunção do sistema nervoso (BROWN; LOCKWOOD; SONAWANE, 2005). Danos causados a membranas de organelas, bem como disfunção mitocondrial e o estresse oxidativo podem contribuir consideravelmente para a manifestação de doenças neurodegenerativas (KHANAM et al., 2016). A origem da maior parte dos distúrbios neurodegenerativos é multifatorial, consistindo em uma interação entre fatores ambientais e genéticos (KIM; LEE; PARK, 2017). Logo o papel da exposição a pesticidas na ocorrência de distúrbios neurodegenerativos tem sido pressuposta há muito tempo, entretanto os agentes causadores específicos, assim como os mecanismos moleculares até este momento não são completamente compreendidos (PARRÓN et al., 2011).

A exposição a concentrações baixas de pesticidas pode danificar células cerebrais gerando uma perda de neurônios em regiões especificas do cérebro resultando em prejuízos cognitivos e de memória, assim como atenção e função motora (HAYDEN et al., 2010). Os pesticidas possuem mecanismos de neurotoxicidade como processos inflamatórios, estresse oxidativo resultando em apoptose celular (ABDOLLAHI et al., 2004; FRANCO et al., 2009a).

Tem surgido um crescente aumento em estudos relacionados a exposição ambiental a pesticidas durante a gravidez e os primeiros anos de vida, sugerindo essa exposição como um fator de risco ambiental para o desenvolvimento do TEA e TDAH (ROBERTS et al., 2007; SHELTON et al., 2014; VON EHRENSTEIN et al., 2019).

Inúmeros estudos experimentais utilizando exposições a certos pesticidas sugerem alterações em neuroproteínas, alteração na expressão de genes além de anormalidades comportamentais, em diferentes modelos experimentais como ratos, camundongos e

recentemente *Drosophila melanogaster* (DE FELICE et al., 2016; KIM; LEE; PARK, 2017; LEE et al., 2015).

Dois estudos realizados em ratos através da exposição pré natal a pesticidas como dieldrin demonstraram que essa exposição promoveu alterações como redução na capacidade de ligação ao receptor $GABA_{A,}$ além de alterações da composição das subunidades dos seus receptores (BRANNEN et al., 1998; LIU et al., 1998). Outro estudo, utilizando culturas neuronais *in vitro* mostraram que pesticidas como dieldrin e endosulfan produziram aumento da fosforilação da Akt (uma proteína quinase responsável por diversos processos celulares como apoptose, proliferação, entre outras), sendo esse efeito mediado pela ativação do ERβ (um dos dois principais tipos de receptor de estrogênio), os quais ativam o ERK1/2 (quinase 1 e 2 regulada extracelularmente), através de um mecanismo que envolve $GABA_A$ e receptores de glutamato (BRIZ et al., 2011), onde a diminuição de ligações ao GABA em humanos contribui para o tônus muscular impróprio, o qual é observado em uma grande parte de indivíduos com TEA (MING; BRIMACOMBE; WAGNER, 2007).

Com relação a exposições ao imidacloprida existem dados limitados, devido ao fato desse pesticida ser relativamente novo em comparação a outros químicos utilizados, há na literatura uma pesquisa realizada por Keil; Daniels; Hertz-Picciotto (2014), no qual propõe que essa exposição pode contribuir para o desenvolvimento desses transtornos, e deve ser estudada mais a fundo, outro estudo recentemente realizado com a *Drosophila melanogaster* demonstrou alterações nas interações sociais, aumento da velocidade e distância de vôo e mobilidade quando comparadas às moscas controle, sendo esses indicativos de mudanças comportamentais na mosca semelhantes às mudanças comportamentais observadas em crianças com TEA e TDAH (KEIL; DANIELS; HERTZ-PICCIOTTO, 2014; KIM; LEE; PARK, 2017).

### 2.1.8 *Drosophila melanogaster*

A *Drosophila melanogaster*, popularmente conhecida como mosca da fruta, pertence à subespécie *Diptera* da família *Drosophilidade*, sendo encontrada em diversas partes do mundo. Estudos relacionados a análises genéticas, moleculares e comportamentais realizadas ao longo de um século mostram que a *Drosophila melanogaster* possui uma elevada similaridade com os mamíferos (HIRTH, 2010).

A mosca da fruta dispõe de ferramentas genéticas potentes as quais muitas vezes são impraticáveis em mamíferos, também apresenta um ciclo de reprodução e crescimento rápido conforme demonstrado na Figura 8, e apresenta facilidade de manutenção em laboratório e

baixo custo. Além disto, as moscas são capazes de exercer comportamentos motores complexos, tal como caminhar, escalar e voar, memória e agressividade, apresentando uma complexidade em seu cérebro o suficiente para tornar esses comportamentos relevantes para os seres humanos (MUÑOZ-SORIANO; PARICIO, 2011).

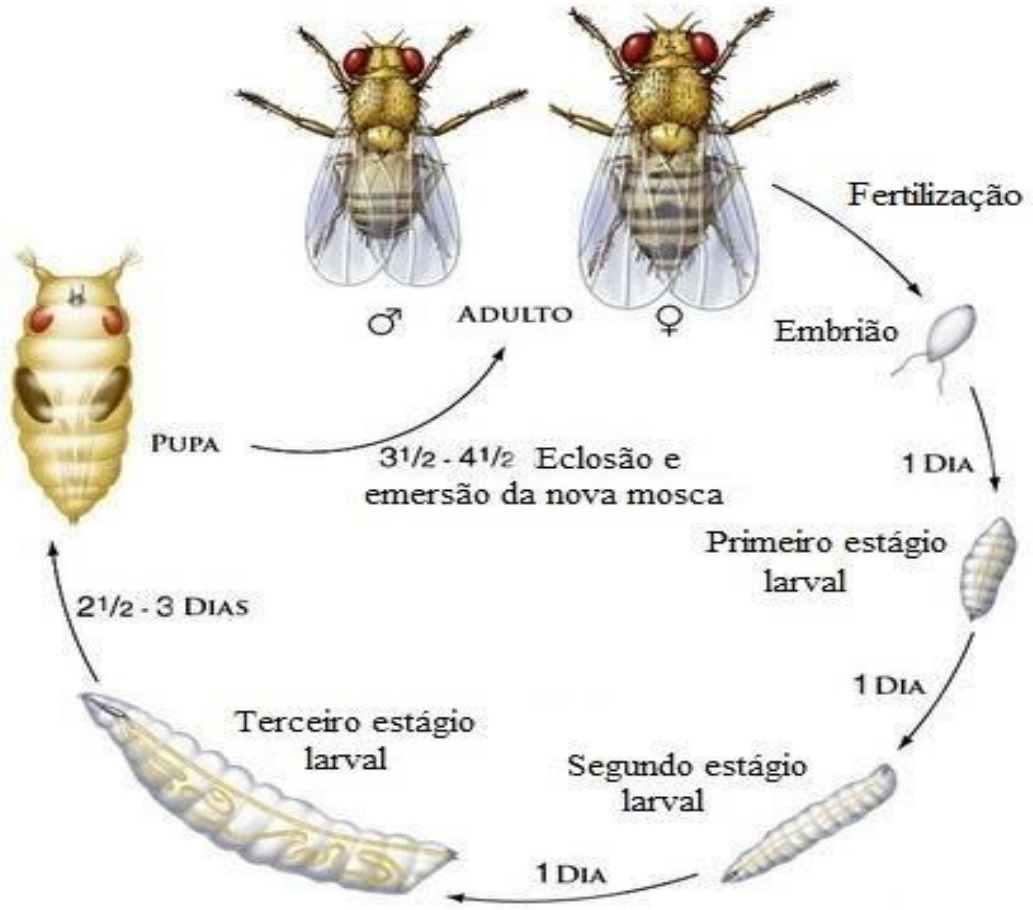



**Figura 8:** Ciclo de vida da *Drosophila melanogaster*.

Fonte: https://dendroterra.jimdofree.com/art%C3%ADculos/droshophila-alimento-vivo

*Drosophila melanogaster* é uma espécie dimórfica, em que machos e fêmeas são distinguidos com base em características morfológicas. O corpo da mosca adulta é dividido em três partes principais: cabeça, tórax e abdômen (Figura 9A). As fêmeas são frequentemente maiores que os machos. Nos machos os segmentos finais do abdômen são completamente escuros, a genitália do macho, conhecida como epandrium, é maior, mais complexa e mais escura do que a da fêmea e em ambos os sexos possuem listras transversais no lado dorsal de cada segmento abdominal. As fêmeas apresentam um abdômen pontiagudo enquanto que o abdômen do macho é arredondado. (Figura 9B) (CHYB e GOMPEL, 2013).

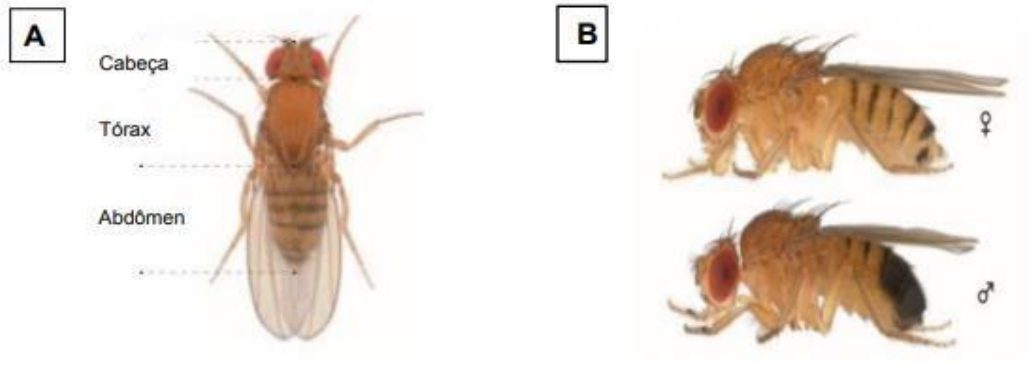



**Figura 9:** *Drosophila melanogaster* . A) Estrutura do corpo da mosca dividido em três partes principais, cabeça, tórax e abdômen. B) Diferenças morfológicas entre machos e fêmeas.

Fonte: Adaptado de Chyb e Gompel (2013).

O sequenciamento do genoma das moscas demonstra que a *Drosophila melanogaster* possui 75% de genes similares relacionados a doenças em humanos, além disso apresenta resposta para muitas drogas que atuam no sistema nervoso, demonstrando efeitos semelhante aos observados em sistemas de mamíferos, tendo assim grande potencial para ser utilizada como modelo de doença humana (PANCHAL; TIWARI, 2017; PANDEY; NICHOLS, 2011). Neste contexto, nos últimos anos a *Drosophila melanogaster* vem sendo utilizada como modelo experimental no estudo de diversas doenças neurodegenerativas, como a doença de Parkinson, Alzheimer, Depressão entre outras (HIRTH, 2010; PANCHAL; TIWARI, 2017).

Estudos envolvendo a exposição de fármacos em *Drosophila melanogaster* são realizados principalmente através da adição ao alimento das moscas. Outras vias como a exposição da mosca a produtos químicos vaporizados, bem como a administração do fármaco através de injeção ou no pescoço de uma mosca decapitada são menos utilizadas (MANEV; DIMITRIJEVIC; DZITOYEVA, 2003; PANDEY; NICHOLS, 2011).

O uso da *Drosophila melanogaster* como modelo experimental ressalta a importância da utilização de modelos alternativos, uma vez que por se tratar de insetos também contribuem para o ensino, onde o estudo dos insetos é abordado em disciplinas presentes na matriz curricular de muitos cursos do ensino fundamental, médio e superior, outra vantagem considerável é o fato de não ser necessário a aprovação pelo Comitê de Ética no Uso de Animais, substituindo assim o uso de camundongos e ratos para estudos experimentais, dessa forma o conjunto de vantagens exemplificam as razões pelas quais a *Drosophila melanogaster*

tem gerado contribuições relevantes para a pesquisa, (MATOS, et al., 2009; MCGURK; BERSON; BONINI, 2015).

## 3. JUSTIFICATIVA

O TEA e o TDAH são transtornos neurodesenvolvimentais que afetam crianças e adolescentes, trazendo inúmeros impactos à vida desses indivíduos, como prejuízos escolar, familiar e social. Tendo-se o conhecimento de que ainda existe pouco entendimento sobre as reais causas desses transtornos e que, os estudos relacionados em sua grande maioria se dão basicamente através da utilização de camundongos ou ratos onde, a maioria dispõe da utilização de animais com alterações genéticas, e que, tem-se relatado a possível contribuição de contaminantes químicos como pesticidas, faz-se necessário a utilização de modelos alternativos para estudar os mecanismos envolvidos nesses transtornos. Assim a utilização da *Drosophila melanogaster* juntamente com a exposição ao imidacloprida podem fornecer dados sobre os mecanismos de ação do imidacloprida, mas principalmente os mecanismos envolvidos nesses distúrbios, além corroborar a utilização da *Drosophila melanogaster* como modelo experimental de TEA e TDAH.

# 4. OBJETIVOS

## 4.1.1 Objetivo geral

O presente estudo tem por objetivo corroborar a ação do imidacloprida como modelo químico de distúrbios neurodesenvolvimentais, no nível comportamental, mas principalmente investigar alterações neuroquímicas induzidas pela exposição ao imidacloprida, que estão relacionadas aos sintomas de TEA e TDAH em *Drosophila melanogaster*.

## 4.1.2 Objetivos específicos

- Avaliar o efeito da exposição ao imidacloprida em análises *in vivo* sobre o comportamento locomotor, exploratório, agressividade, interação social, grooming e ansiedade na progênie de *Drosophila melanogaster*;
- Avaliar a atividade *ex vivo* da AChE na progênie de *Drosophila melanogaster* exposta ao imidacloprida;
- Avaliar os níveis de dopamina *ex vivo* na progênie de *Drosophila melanogaster* exposta ao imidacloprida;
- Avaliar indicadores de estresse oxidativo *ex vivo* na progênie de *Drosophila melanogaster* exposta ao imidacloprida;
- Avaliar a viabilidade celular *ex vivo* na progênie de *Drosophila melanogaster* exposta ao imidacloprida.

## 5. MANUSCRITO CIENTÍFICO

Os resultados que fazem parte desta dissertação estão apresentados sob a forma de artigo científico. Os itens *Materiais e Métodos, Resultados, Discussão* e *Referências Bibliográficas*, encontram-se no próprio artigo. O artigo está disposto conforme as normas da revista "*Food and Chemical Toxicolgy*".

Artigo:

"**Oxidative stress by imidacloprid exposure-induced causes behavioral changes in the neurodevelopmental disorder model in** *Drosophila melanogaster*"

# Oxidative stress by imidacloprid exposure-induced causes behavioral changes in the neurodevelopmental disorder model in *Drosophila melanogaster*

Dieniffer Espinosa Janner[a,b]; Nathalie Savedra Gomes[a,b]; Kétnne Hanna Poleto[a,b]; Márcia Rósula Poetini[a,b]; Elize Aparecida Santos Musachio[a,b]; Francielli Polet de Almeida[a,b]; Elen Caroline de Matos Amador[a]; Bruna Piaia Ramborger[b]; Rafael Roehrs[b], Marina Prigol[a,b]; Gustavo Petri Guerra[a,b,*]

[a]Laboratório de Avaliações Farmacológicas e Toxicológicas Aplicadas às Moléculas Bioativas – LaftamBio, Universidade Federal do Pampa – Campus Itaqui, 97650-000, Itaqui, RS, Brasil.

[b]Programa de Pós-Graduação em Bioquímica, Universidade Federal do Pampa – Campus Uruguaiana, 97508-000, Uruguaiana, RS, Brasil.

*Corresponding author. Adress: Universidade Federal do Pampa, Campus Itaqui. Rua Luiz Joaquim de Sá Britto, s/n, Itaqui, Rio Grande do Sul, CEP 97650-000, Brasil. Phone number: +55 55 98140 9003

E-mail adress: petriguerra@gmail.com (G.P. Guerra)

**Abstract**

Neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) are considered the main constituents of neurological disorders, in which the child exhibits behavior deficits. Imidacloprid is an insecticide that belongs to the neonicotinoid family, it is a nicotinic acetylcholine receptor agonist and it is capable of causing behavioral changes in *Drosophila melanogaster*, which are similar to the ASD and ADHD human phenotypes. Thus, the aim of the present study is to corroborate the action of imidacloprid as a chemical model of neurodevelopmental disorders, at the behavioral level, but mainly to investigate neurochemical changes induced by exposure to imidacloprid, which are related to ASD and ADHD symptoms in *Drosophila melanogaster*. Virgin and male fruit flies of 3 days old were divided into four groups and exposed to either a standard diet (control) or a diet containing imidacloprid (200, 400 or 600 pM) for 7 days. After progeny hatching, the flies were submitted to in vivo (negative geotaxis, open-field, social interaction, aggressiveness, light/dark and grooming) and ex vivo [food consumption, dopamine levels, acetylcholinesterase (AChE) activity, oxidative stress indicators (superoxide dismutase, SOD; catalase, CAT; glutathione S-transferase, GST; reactive oxygen species, ROS; thiobarbituric acid reactive substances, TBARS; and Protein and non-protein thiols, PSH and NPSH) and cell viability (resazurin)] assays. The ones exposed to imidacloprid showed an increase in hyperactivity (geotaxis and open-field task), aggressiveness, anxiety and repetitive movements, as well as, a decrease in social interaction in the flies' progeny. Furthermore, the flies exposed to imidacloprid decreased dopamine levels and cell viability and increased oxidative stress (ROS and TBARS), however, AChE, CAT and GST activity and PSH and NPSH remained unchanged. These results suggest the involvement

of dopamine and oxidative stress as a possible mechanism of action for behavioral damage similar to ASD ADHD induced by imidacloprid in *Drosophila melanogaster*.

**Keywords**: Pesticides, Neonicotinoid, Autism Spectrum Disorder, Attention Deficit Hyperactivity Disorder, Dopamine, Acetylcholinesterase.

## 1. Introduction

Neurodevelopmental disorder refers to a group of disorders that affect the development of the central nervous system, which provoke brain dysfunction with early onset and the most common are Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD) (Luke et al., 2012). The ASD is a clinical condition determined by persistent deficits in communication and social interaction, such as the presence of repetitive patterns and restrictions on use, interests and/or activities (American Psychiatric Association, 2013). While the main feature of ADHD is a persistent pattern of inattention and/or hyperactivity-impulsivity, which is more frequent and severe than the usual in individuals with a comparable level of development (Polanczyk et al., 2007). In both disorders, these symptoms are already present in the early childhood and limit daily functioning (American Psychiatric Association, 2013; Faraone and Mick, 2010). In the last 40 years, the rate of individuals diagnosed with ASD has increased significantly. According to a survey carried out in 2016 in the United States, the prevalence of ASD is approximately 1 in 40 children (Schmidt et al., 2017).

The causes of ASD and ADHD remain unknown, however, several studies consider the involvement of multiple genes (heritability) and environmental factors, making their etiology complex and not yet fully understood (Hallmayer, 2011; Sandin et al., 2014). Increased oxidative stress, as well as changes in dopamine levels are known to be strongly associated with ASD and ADHD (Campbell et al., 2019; Hoyer et al., 2008). Regarding environmental factors, possible culprits include environmental pollutants such as pesticides, heavy metals, particulate materials, among others (Lee et al., 2015; Richardson et al., 2015). Evidence demonstrates that prenatal or early childhood exposure to low doses of different types of pesticides has been associated with brain dysfunction and increases in neurocognitive and behavioral deficits, such as ASD and ADHD (Abou-Donia et al., 2008; Keil et al., 2014; Philippat et al., 2018; Saez et

al., 2018; Schmidt et al., 2017). In line with this view, the offspring from pregnant rats after a single intraperitoneal dose of imidacloprid produces neurobehavioral deficits and an increased expression of GFAP in several brain regions (Abou-Donia et al., 2008), furthermore, neonicotinoid pesticides have been detected in human urine (Taira et al., 2013; Ueyama et al., 2014).

Imidacloprid is an insecticide that belongs to the neonicotinoid family, which is widely used for crop protection due to its low soil persistence, high insecticide activity and low application rate (Lee Chao and Casida, 1997). Imidacloprid is capable of causing neurobehavioral deficits due to the fact that it belongs to the family of neonicotinoids, which acts on the central nervous system as a potent nicotinic acetylcholine receptor agonist (Mengoni and Farina, 2015). In this sense, interestingly, the exposure to imidacloprid causes behavioral changes in *Drosophila melanogaster* similar to the ASD and ADHD human phenotypes (Kim et al., 2017).

The use of *Drosophila melanogaster* as a study model has increased over the past few years due to some important points. Firstly, the fly's genome has been fully sequenced, presenting a genetic similarity of approximately 75% related to diseases in humans. Secondly, it presents a response to various drugs that act on the nervous system, showing effects similar to those observed in mammalian systems. Thirdly, researchers were able to demonstrate innumerable human behaviors in *Drosophila melanogaster*, such as repetitive behavior, learning, memory, aggressiveness and others (Roberts et al., 2019; Tauber et al., 2011; Tully et al., 1994). Due to the complexibility of ASD and ADHD, as well as, the neurochemical dosing considered unethical, it has been difficult to carry out mechanistic studies in humans (Roberts et al., 2019). This makes the search for alternative models for ASD and ADHD,such as *Drosophila melanogaster*, even more important..

Thus, Imidacloprid can be a useful tool for a chemical model that allows the assessment of environmental factors on ASD and ADHD in *Drosophila melanogaster*, beyond the evaluations with genetic models already described. However, there is only the study by Kim et al., (2017), about the effects of imidacloprid, requiring a greater understanding of its action as a chemical model for neurodevelopmental disorders in *Drosophila melanogaster*. Thus, the aim of the present study is to corroborate the action of imidacloprid as a chemical model of neurodevelopmental disorders, at the behavioral level, but mainly to investigate neurochemical changes induced by exposure to imidacloprid, which are related to ASD and ADHD symptoms in *Drosophila melanogaster*. Thus, throughout the present study, the effect of imidacloprid exposure at different concentrations on behavior and biochemical markers was evaluated, in order to generate knowledge about the action of imidacloprid to consolidate an experimental model of ASD and ADHD in *Drosophila melanogaster* in the future.

## 2. Materials and Methods

### 2.1 Materials

The imidacloprid was obtained from Sigma-Aldrich (St. Louis, MO, USA) and diluted in 0.0001% dimethyl sulfoxide (DMSO). All the other reagents used were of analytical grade.

### 2.2 *Drosophila melanogaster* Stock and Culture

The fruit flies (*Drosophila melanogaster* - wild-type - Harwich strain) of both sexes (1 to 3 days old) used were obtained from the National Species Center (Bowling Green, Ohio, USA), maintained under controlled light conditions (12 hours light / dark cycle), temperature

(25 ± 1°C) and 60% humidity and fed with a standard diet (76.59% cornmeal, 8.51% wheat germ, 7.53% sugar, 7.23% milk powder, 0.43% salt and 0.08% methylparaben).

### 2.3 Exposure treatment with Imidacloprid

A response-concentration curve of imidacloprida was performed from the lowest effect concentration observed (LOEC) by (Charpentier et al., 2014), to evaluate the effect and to define the concentration that causes behavioral and neurochemical changes similar to ASD and ADHD human symptoms. To obtain progeny (F1), virgin and male flies up to 3 days old were used, in the proportion of 5: 1, kept in a standard diet as described by (Kaur et al., 2015) with modifications. The flies were divided into four groups (50 flies each) exposed to: (1) control (standard diet only); (2) Imidacloprid 200 pM; (3) Imidacloprid 400 pM; (4) Imidacloprid 600 pM for 7 days. After the exposure, the progenitors (F0) were discarded and the vials were kept under observation at controlled temperature until the progeny hatched. The progeny of both sexes up to 3 days of age was submitted to in vivo (negative geotaxis, open-field, social interaction, aggressiveness, light/dark and grooming) and ex vivo [food consumption, dopamine levels, AChE activity, oxidative stress indicators (superoxide dismutase, SOD; catalase, CAT; glutathione S-transferase, GST; reactive oxygen species, ROS; thiobarbituric acid reactive substances, TBARS; and Protein and non-protein thiols, PSH and NPSH) and cell viability (resazurin)] assays. The treatment scheme is shown in Figure 1.

## 2.4 In vivo assays

### 2.4.1 Negative geotaxis assay

The negative geotaxis test was performed to evaluate the fly's ability to climb, as described by (Bland et al., 2014), with minor modifications. Briefly, for each assay, 5 flies from each group were individually immobilized on ice and placed separately in a vertical glass test tube with a diameter of 1.5 cm. After 10 minutes, the flies were gently tapped to the bottom of the tube and the time required to climb up to the mark of 8 cm of the tube wall was recorded. The test was repeated five times each during 120 seconds and a 1 minute interval. Data were analyzed according to the average time of each fly.

### 2.4.2 Open field test

To evaluate locomotor and exploratory activity the open field test was performed as described by Connolly, (1966), with modifications by (Musachio et al., 2020). Fifteen flies per group were used in five independent experiments, totaling 75 flies per group for the test. Each fly was immobilized on ice and transferred to a Petri dish divided by squares measuring 1x1 cm, after 5 minutes of recovery, the number of crossing of each fly was determined during 60 seconds. The test was performed in duplicate and the average values were calculated.

### 2.4.3 Grooming

The repetitive behavior test was determined by *Drosophila melanogaster´s* cleaning activity, as described by Tauber et al. (2011), with modifications. Five individual flies of both sexes were used to perform the test. The time each individual fly performed "self-cleaning" movements was recorded for 2 minutes. The test was performed in duplicate, and the data were analyzed according to the average "self-cleaning" movement time.

### 2.4.4 Social interaction

To assess the sociability of the flies, the social interaction test was performed using vertical triangular chambers as described by Simon et al., (2012), with some modifications. Ten female flies per group were immobilized on ice, transferred to the triangular chambers and after 30 minutes of adaptation an image was recorded with a digital camera. Digital images were imported into ImageJ software (NIH, rsbweb.nih.gov/ij) and analyzed for distances (cm) from the nearest neighbors (Burg et al., 2013).

### 2.4.5 Aggressiveness test

The aggressiveness test was performed with a pair of male flies from each group, with ten flies per group evaluated, totaling 50 flies representing four independent experiments. The flies were left in test tubes for 90 minutes without food before the start of the test. They were then transferred to a circular combat chamber with a radius of 45 mm and a height of 12 mm containing a drop of food. The flies were allowed to acclimate for 2 minutes and afterwards, they were observed during 5 minutes. The following behaviors were considered aggressive

encounters: leg extension from one fly to another resulting in physical contact, stalking, rapid loading approach leading to direct orientation, wing raising in response to the proximity/approach of both flies. Data were recorded according to the number of encounters that exhibited aggressive behavior among the observed flies (Edwards et al., 2006; Machado et al., 2018).

#### 2.4.6 Light/Dark test

This experiment was conducted as described by (Edwards et al., 2018; Neckameyer e R. Nieto-Romero, 2015), with some adaptations. To observe the anxiety behavior in the flies, the light/dark test was performed using a box, which consisted of a dark compartment made of black material and a light compartment made of white material. The box was 12 cm length, 8.2 cm wide and 42 cm high and the two compartments were connected by an opening. The light intensity in the light compartment was 16 W. Five flies from each group were used for the test. At the beginning of the experiment, each fly was placed in the center of the lighted box facing the opening. During the 3 minutes test, a fly was considered to have entered the lighted or dark compartment when both front legs were within the compartment. The amount of time spent in the dark compartment was recorded.

### 2.5 Ex vivo assays

#### 2.5.1 Homogenate preparation

Sample preparation was performed immediately after the behavioral tests, where flies from each experimental group were separated and immobilized by freezing on ice. Thereafter,

the head and body regions were carefully separated, homogenized in a HEPES buffer (20 mM, pH 7.0), 10:1 (flies/volume μL) for 2 minutes and centrifuged according to each analysis protocol. After the centrifugation, the supernatant was removed and used for biochemical assays. The protein content was verified colorimetrically using the method by Bradford, (1976), with bovine serum albumin (1 mg/mL) as standard. All experiments were performed in duplicate.

### 2.5.2 Food consumption

Food consumption was assessed according to Lushchak, Rovenko, Gospodaryov, & Lushchak, (2011), with adaptations. Groups of 15 flies were fasted for 30 minutes prior to the test, and afterwards they were exposed to the aforementioned media with the addition of 0.5% FD & C Blue Nº1 (FCF Brilliant Blue) dye and allowed to feed on this medium for 2 hours. After the feeding period, each group of flies was immediately frozen in ice. The 15 headless flies were homogenized in 200 μL of 20 mM HEPES, pH 7.5, centrifuged at 14,288-x g for 15 minutes, and the supernatant was measured in a 96-well microplate reader at 629 nm. The optical density of the flies' homogenates that consumed the corresponding diets without the dye was used as a blank. A total of 480 flies were used, with 120 flies for each treatment group. The consumption test is performed to eliminate the bias that the flies may not be consuming the diet containing the damage causing agent.

### 2.5.3 Determination of Dopamine levels by HPLC-DAD

To determine dopamine levels using high performance liquid chromatography (HPLC), 30 head of flies per treatment group were homogenized in 0.9% NaCl (288 μL) and 0.5 M HCl (12 μL), the homogenates were centrifuged at 7840-x g for 10 minutes at 4°C. In 0.22-μm PTFE

filters the supernatant was filtered and then stored at 80°C until it was used. The supernatant of the samples (20 µL) was injected into the HPLC system by an auto sampler device. The YL9100 HPLC system consisted of a vacuum degasser and a quaternary pump connected to a reverse phase column (Synergi 4 µm Fusion-RP 80 Å 4.6 x 250 mm; Phenomenex) coupled to a diode array detector (DAD). The mobile phase consisted of methanol and water (12:88 v/v) adjusted to pH 3.0 with phosphoric acid and the flow rate was maintained at 0.8 mL $min^{-1}$ (Bianchini et al., 2019). The detection was performed at 198 nm, and the results of the dopamine levels were expressed as µg/mg protein.

### 2.5.4 Acetylcholinesterase (AChE) activity

AChE activity was measured according to the method described by (Courtney and Francisco, 1961), using acetylthiocholine iodide as substrate. The head and body samples were centrifuged at 78-x g for 5 minutes. The reaction was monitored and carried out for 2 minutes at 412 nm by the release of thiol compounds, which reacted with 5,5′-dithiobis- (2-nitrobenzoic acid) (DTNB), producing the thionitrobenzoic acid colored product. Enzyme activity was expressed in nmol protein/mg/min.

### 2.5.5 Determination of Superoxide Dismutase (SOD) Activity

The determination of SOD activity was performed as described by (Kostyuk & Potapovich, 1989), by homogenizing ten flies from each group that had their heads and bodies separated and adding 100 µL and 400 µL of 20 mM HEPES buffer (pH 7.0) on the head and

body respectively. Then, the samples were centrifuged at 15,366-x g for 10 minutes at 4°C. The reaction mixture contained sodium phosphate buffer (0.025M/0.1mM EDTA, pH 10.0), N, N, N, Ntetramethylethylenediamine (TEMED) and 10 µL of sample and it was started by adding 0.15% quercetin dissolved in dimethylformamide. At the moment of sample reading quercetin was added and monitored for 2 minutes at 406 nm. SOD activity was measured by monitoring quercetin auto-oxidation inhibition, as described by Franco et al. (2009), with modifications. Results were expressed in terms of the amount of protein required for 50% of quercetin oxidation inhibition. Four independent experiments were performed (10 flies per group). Enzyme activity was expressed in mU/mg protein.

### 2.5.6 Determination of Catalase (CAT) Activity

Catalase activity was measured following the method described by (Aebi, 1984), with modifications. Ten flies from each group had their heads and bodies separated, homogenized in 100 µL and 400 µL 20 mM HEPES buffer (pH 7.0) respectively, and centrifuged at 15,366-x g for 10 minutes at 4°C. Subsequently, a potassium phosphate buffer solution (0.25M/2.5mM EDTA, pH 7.0), 30% hydrogen peroxide ($H_2O_2$) and Triton X-100 were prepared. This solution was added to the supernatant and the samples were monitored at 240 nm for 2 minutes. Four independent experiments (10 flies per group) were performed. Enzyme activity was expressed in mU/mg protein.

### 2.5.7 Determination of Glutathione S-Transferase (GST) Activity

For the evaluation of GST activity ten flies from each group had their heads and bodies separated and homogenized in 100 µL and 400 µL 20 mM HEPES buffer (pH 7.0) respectively.

They were then centrifuged at 15,366-x g for 10 minutes at 4°C. After the centrifugation, the supernatant removal was performed, samples were prepared containing 0.25 M Kpi/EDTA buffer (2.5 mM, pH 7.0), distilled water and 100 mM GSH, and 1-chloro-2-4-dinitrobenzene (50 mM CDNB) was added as substrate and monitored at 340 nm for 2 minutes. The GST activity assay was performed as described by (Habig, W. H., Pabst, M. J., Jakoby, 1974). Four independent experiments were performed (10 flies per group). Enzyme activity was expressed as GST activity (nmol/mg protein).

### 2.5.8 Levels of Reactive Oxygen Species (ROS)

Fifteen flies per group were used and homogenized in 1000 µL10 mM Tris buffer, pH 7.0 and centrifuged at 999-x g for 5 minutes at 4°C. The quantification of the DCF-DA oxidation assay was monitored as a general index of oxidative stress according to the protocol proposed by Pérez-severiano et al., (2004). After one hour, the fluorescence emission resulting from DCF-DA oxidation was monitored, the reading was performed in excitation at 485 nm and 530 nm emission in the spectrophotometer using an EnsPireR multimode microplate reader (Perkin Elmer, USA). The rate of DCF formation was calculated as a percentage of fluorescence treatment relative to the control group. Three independent experiments were performed (20 flies per group).

### 2.5.9 Determination of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by (Ohkawa et al., 1979) with minor modifications. Twenty flies from each group had their heads and bodies separated and

homogenized in 120 µL 20 mM HEPES buffer (pH 7.0) and the 20 bodies in 400 µL 20 mM HEPES buffer (pH 7.0) were subsequently centrifuged at 78-x g for 10 minutes at 4°C. The supernatant was removed and then thiobarbituric acid (TBA 0.8%, pH 3.2), acetic acid/HCl (20%, pH 3.4) and sodium sulfate (SDS 8.1%) were added. Then the samples were incubated for two hours at 95°C and the absorbance was measured in a 532 nm microplate reader. TBARS values were normalized by protein concentration and expressed in nmol MDA/mg protein.

### 2.5.10 Content of Protein (PSH) and non-protein (NPSH) thiols

The determination of non-protein (NPSH) and protein (PSH) thiol was estimated as described by (Ellman, 1959). Briefly, 35 flies' heads were homogenized in 350 µL Tris buffer (pH 8.0) and 35 flies' bodies were homogenized in 1400 µL Tris buffer (pH 8.0) and centrifuged at 7,840-x g. For protein thiol measurements, the supernatant was used (the pellet was reserved for later use) and 5'M 5.5 'dithiobis-2-nitrobenzoic acid (DTNB) was added for 15 minutes at room temperature protected from light and the reading was performed by a spectrophotometer at 412 nm. For non-protein thiol measurements from previous samples, the pellet was resuspended in 0.5 M Tris/HCl pH 8.0 buffer, the supernatant was removed and added to the 5 mM DTNB, and left15 minutes at room temperature protected from light and reading was performed by spectrophotometry at 412 nm.

### 2.5.11 Cell viability

Cell viability was measured using a method based on the ability of viable cells to reduce rezasurin to resorufin, a fluorescent molecule (Franco et al., 2009). Twenty flies per group had their heads and bodies separated and homogenized in 100 µL 20 mM Tris buffer (pH 7.0), and

400 µL 20 mM Tris buffer (pH 7.0) respectively. Afterwards, they were centrifuged at 999-x g for 10 minutes at 4°C. Subsequently, the samples were incubated in ELISA plates with 180 µL 20 mM Tris buffer (pH 7.0) and 10 µL rezasurine for 1 hour. The absorbance was recorded using a microplate reader at a wavelength of 573 nm. Four independent experiments were performed (20 flies per group). Data were expressed as resazurin reduction (% control).

### 2.6 Statistical Analysis

GraphPad Prism 6 software was used for statistical analysis and plotting graphs. The statistical analyzes were performed by a one-way analysis of variance (ANOVA), followed by Tukey's *post hoc* test, depending on the experiment. In addition, the Kruskal-Wallis test was used, followed by the *Post-Hoc* Dunnet test for nonparametric data that did not follow a normal distribution. All data were expressed as mean ± S.E.M. or median and interquartile range. We considered statistically significant values $P < 0.05$.

## 3. Results

### 3.1 Behavioural tests and food consumption

Figure 2 (A-G) shows the progeny effect of the flies exposed to imidacloprid (200, 400 or 600 pM) for 7 days on climbing time, crossing number, social interaction, light/dark, aggressiveness, grooming and food consumption respectively. Statistical analysis (one-way ANOVA) revealed that exposure to imidacloprid significantly decreased the climbing time [$F_{(3,16)} = 5.26$; $P < 0.05$] and increased the crossing number [$F_{(3,16)} = 7.35$; $P < 0.05$], the distance from the nearest fly neighbour ($P < 0.05$), the time spent in the dark compartment [$F_{(3,16)} =$

14.77; $P < 0.05$], the aggressiveness [$F_{(3,16)} = 40.92$; $P < 0.05$], and time for self-cleaning movements [$F_{(3,16)} = 7.91$; $P < 0.05$]. However, the statistical analysis did not show a significant difference between groups for food consumption. *Post hoc* comparisons showed that the progeny of the flies exposed to imidacloprid at the concentration of 400 pM, increased locomotor and exploratory activity in the geotaxis (Fig. 2A) and open field task (Fig. 2B) and increased the time of self-cleaning movements(Fig. 2F). Furthermore, the progeny of the flies exposed to imidacloprid at the concentration of 200 and 400 pM, decreased the social interaction (the distance from the nearest fly neighbour ) (Fig. 2C), compared to the control group. Likewise, an increase in anxiety was observed, since the flies stayed longer in the dark compartment (Fig. 2D), as well as an increase in aggressive behaviour (Fig. 2E) in the progeny of the flies exposed to imidacloprid at the concentrations of 200, 400 and 600 pM, compared to the control group.

### 3.2 Dopamine levels

Figure 3 shows the effect of the progeny of the flies exposed to imidacloprid (200, 400 or 600 pM) for 7 days on the dopamine levels of *Drosophila melanogaster*. Statistical analysis (one-way ANOVA), followed by *Post hoc* comparisons, revealed that exposure to imidacloprid, at concentrations of 200 and 400 pM, significantly decreased the dopamine levels [$F_{(3,12)} = 5.39$; $P < 0.05$], compared to the control group.

### *3.3* AChE activity

Figure 4 shows the progeny effect of the flies exposed to imidacloprid (200, 400 or 600 pM) for 7 days on AChE activity in the head and body of *Drosophila melanogaster*. Statistical

analysis (one-way ANOVA) did not show a significant difference between groups for AChE activity in the head and body, compared to the control group.

### *3.6* Oxidative stress indicators

Figures 5 (A-J) and 6 (A-D) show the progeny effect of the flies exposed to imidacloprid (200, 400 or 600 pM) for 7 days on oxidative stress indicators (SOD, CAT, GST, PSH, NPSH, ROS and TBARS) in the head and body of *Drosophila melanogaster*. Statistical analysis (one-way ANOVA) revealed that exposure to imidacloprid significantly decreased SOD activity in the head [$F_{(3,12)} = 10.23$; $P < 0.05$] and body [$F_{(3,12)} = 23.92$; $P < 0.05$]. *Post hoc* comparisons showed that the progeny of the imidacloprid exposed flies, at 200 and 600 pM concentrations, decreased SOD activity in the head (Fig. 5A), and body at all the imidacloprid concentrations (200, 400 and 600 pM) (Fig. 5B), compared to the control group. Imidacloprid did not induce any alterations for CAT, GST, PSH and NPSH in the head and body (Fig. 5C-J).

Statistical analysis (one-way ANOVA) also revealed that exposure to imidacloprid significantly increased the ROS levels in the head [$F_{(3,12)} = 11.50$; $P < 0.05$] and body [$F_{(3,12)} = 9.68$; $P < 0.05$], as well as, increased the TBARS levels in the head [$F_{(3,24)} = 5{,}78$; $P < 0.05$]. *Post hoc* comparisons demonstrated that the progeny of the flies exposed to imidacloprid, at 200 and 400 pM concentrations, increased the ROS levels in the head (Fig. 6A) and body (Fig. 6B) and, at the 400 pM concentration, it increased the TBARS levels only in the head (Fig. 6C), compared to the control group. Imidacloprid did not induce any alterations for TBARS levels in the flies´ body (Fig. 6D).

### 3.8 Cell Viability

Figure 7 shows the progeny effect of the flies exposed to imidacloprid (200, 400 and 600 pM) for 7 days on cell viability by the reduction of rezasurin in the head and body of *Drosophila melanogaster*. Statistical analysis (one-way ANOVA) revealed that exposure to imidacloprid significantly decreased rezasurin levels in the head [$F_{(3,12)} = 10.52$; $P < 0.05$], however, it did not show a significant difference in the body. *Post hoc* comparisons demonstrated that the progeny of the flies exposed to imidacloprid, at the concentrations of 400 and 600 pM, decreased the rezasurin levels in the head (Fig. 7A), compared to the group control.

## 4. Discussion

In the present study we evaluated the progeny effect of imidacloprid exposure on possible neurochemical mechanisms involved in a developmental disorder model in *Drosophila melanogaster*. Exposure to imidacloprid (400 pM) showed to increase locomotor and exploratory activity and grooming in *Drosophila melanogaster* (Fig 2A, B and F), which suggests it causes hyperactivity and stimulates repetitive movements in the flies. It was also observed that exposure to imidacloprid was capable of causing behavioral changes, such as a decrease in social interaction (Fig. 2C) and an increase in aggressiveness and anxiety (Fig. 2D and E). As expected, our results corroborate with the important previous report by Kim et al. (2017), in which they also observed behavioral changes, which are similar to the human symptoms of ASD and ADHD in the progeny of the flies exposed to imidacloprid. Thus, as it has already been demonstrated, a prenatal exposures to several types of pesticides has been associated with impaired neurodevelopment of ASD and ADHD types in different species such as, humans, rats and mice (Abou-Donia et al., 2008; De Felice et al., 2016; Roberts et al., 2007). The present results contribute to the consolidation of such disorders as a neurodevelopmental disorder model in *Drosophila melanogaster*.

However, the most important finding is that this study provides evidence for the alterations in the dopamine levels (Fig. 3) and oxidative stress (Fig. 5 and 6) in a neurodevelopmental disorder model induced by imidacloprid in *Drosophila melanogaster*. Interestingly, it has been shown that decreased dopamine levels, increased AChE activity along with oxidative stress and antioxidant enzymes are strongly associated with the ASD and ADH neurodevelopmental disorders (Al-Amin et al., 2015; Campbell et al., 2019; Gadow et al., 2008; Karvat and Kimchi, 2014a). This is the first report to describe that behavioral alterations in *Drosophila melanogaster*, similar to the symptoms of ASD and ADHD in humans, are associated with changes in dopamine levels and oxidative stress, after exposure to imidacloprid.

Dopamine is a monoaminergic neurotransmitter that plays a very important role in the central nervous system, as it is responsible for the regulation of numerous functions such as cognition, emotion, motor activity and motivation. Changes in dopaminergic signaling are linked to several neurological disorders, including ASD and ADHD (Hamilton et al., 2013). Our findings corroborate with previous studies that found lower levels of dopamine in both patients and rodents with ASD, suggesting that this decrease in neurotransmitters may contribute to behavioral abnormalities (Gadow et al., 2008; Hara et al., 2015; Lake, 1977; Nguyen et al., 2018). Furthermore, it has been shown that mutations in the genes of the dopamine transporter, receptors and enzymes involved in dopamine metabolism are associated with ASD (Anderson et al., 2008; DiCarlo et al., 2019; Gadow et al., 2008; Hansen et al., 2014). Regarding this point, mutation in the dopamine transporter gene associates dopamine dysfunction with hyperlocomotion and ASD in *Drosophila melanogaster* (Bowton et al., 2014; Campbell et al., 2019; Cartier et al., 2015; Hamilton et al., 2013).

The oxidative stress is considered responsible for the dopamine production system dysfunction, consequently it decreased the dopamine levels and dopaminergic system activation, contributing significantly to the development of a series of disorders (Arel-Dubeau

et al., 2014; Ortiz et al., 2016). The present results showed that a neurodevelopmental disorder model induced by the exposure to imidacloprid was able to promote an increase in oxidative stress and lipid peroxidation on ROS and TBARS indicators, as shown in previous studies with others neurodevelopmental disorders models that simulates ASD and ADHD in humans and rodents (Mirza and Sharma, 2019; Pamies et al., 2018; Wang et al., 2019). Furthermore, evidence has shown that exposure to or administration of imidacloprid induces oxidative stress in different species (Chang et al., 2020; Li et al., 2020, 2019; Mohany, 2011). In this sense, oral administration of imidacloprid causes oxidative stress and lipid peroxidation in pregnant female rats and their offspring (Ndonwi et al., 2019). Oxidative stress is an imbalance between the production of free radicals and antioxidant defenses (Birben et al., 2012). Although most studies show changes in the activity of the antioxidant enzymes, such as SOD, CAT and GST, after imidacloprid exposure (EL-Gendy et al., 2010; Emam et al., 2018; Lafi et al., 2018; Wang et al., 2016), we did not find any changes in the activity of these enzymes or in the non-enzymatic antioxidant, with the exception of SOD. In line with this view, SOD, GST and CAT activities are notably increased during initial imidacloprid exposure, however, SOD and GST activities are inhibited, whereas CAT returns to the control level towards the final period of the exposure (Ge et al., 2015). Similarly, Christen et al. (2016) recently showed that honey bees exposed to imidacloprid did not exhibit alterations in the expression of catalase.

We believe that imidacloprid, under the conditions used, such as concentration, form and time of administration, has an effect on oxidative stress through the generation of reactive oxygen species and not through the inhibition of endogenous antioxidant defenses. Moreover, it is necessary to take into account that the antioxidant barriers were not able to express an effect that compensates for this oxidative imbalance, showing no changes in its activity. Acetylcholine is a neurotransmitter involved in locomotor activity because it acts on the neuromuscular junction between the motor nerve and the skeletal muscle in the central nervous

system by AChE action (Colovic et al., 2013). Once this enzyme is deregulated together with the cholinergic receptors (muscarinic and nicotinic acetylcholine receptors), numerous neurological disorders occur, such as Alzheimer's disease, Parkinson's disease, schizophrenia and, recently, this was expanded to ASD (Kim et al., 2014). Surprisingly, our results showed that the progeny of the flies exposed to imidacloprid did not alter AChE activity. Studies carried out with experimental models of neurodevelopmental disorders in mice and rats have shown that AChE activity is increased in offspring exposed to valproic acid (VPA) (Karvat and Kimchi, 2014b; Sharma et al., 2019), however, a post-mortem study conducted in humans revealed that AChE activity had not been altered in the cerebral cortex tissues (Perry et al., 2001). Furthermore, regarding imidacloprid action on AChE activity there are some conflicting results. Imidacloprid exposure can significantly increase (Miao et al., 2016), decrease (Topal et al., 2017) or even have no effect (Gauthier et al., 2018; Rios et al., 2017) on AChE activity. However, this evidence makes comparing challenging due to the differences in species, exposure period and concentrations of imidacloprid. Although exposure to imidacloprid did not alter AChE activity, we cannot rule out that the neonicotinoid had bound continuously to the receptor, since it is an agonist nicotinic acetylcholine receptor, causing central nervous system hyperexcitability consequently causing neurological toxicity (Gibbons et al., 2015). Thus, these events, together with the change in dopamine levels and oxidative stress, may be responsible for the behavioral damage found.

As expected, our data of food consumption (Fig. 2G) showed that the intake of the standard diet containing imidacloprid at different concentrations (200, 400 or 600 pM) did not differ significantly, suggesting that the flies did not have aversion to imidacloprid and fed normally during the exposure period. This also demonstrated that any effect found does not occur due to a higher or lower consumption of imidacloprid.

In summary our results suggest that exposure to imidacloprid may contribute to the emergence of a progeny with characteristics similar to those observed in neurodevelopmental disorders, demonstrating that imidacloprid triggers a series of locomotor and behavioral alterations that are mainly associated with a decrease in dopamine levels and an increase in oxidative stress. The data above support the hypothesis that exposure to imidacloprid is a useful tool for a chemical model that allows the assessment of behavioral alterations similar to ASD and ADHD in *Drosophila melanogaster*. But, mainly, the evidence from the present study demonstrated the involvement of dopamine and oxidative stress as possible mechanisms of action for behavioral damages similar to ASD and ADHD induced by imidacloprid in *Drosophila melanogaster*. However, further studies are needed to clarify the mechanisms of action involved in the changes caused by imidacloprid, as well as the behavioral changes observed in neurodevelopmental disorders in *Drosophila melanogaster*.

**Conflict of interest**: The authors declare that there are no conflicts of interest.

**Acknowledgments**

This study was supported by CNPq (421769/2018-1). Dieniffer Espinosa Janner; Nathalie Savedra Gomes; Márcia Rósula Poetini; Elize Aparecida Santos Musachio; Francielli Polet de Almeida; Bruna Piaia Ramborger; are the recipient of a FAPERGS or CAPES fellowship. All of the experiments comply with the current laws of Brazil.

## References

Abou-Donia, M.B., Goldstein, L.B., Bullman, S., Tu, T., Khan, W.A., Dechkovskaia, A.M., Abdel-Rahman, A.A., 2008. Imidacloprid Induces Neurobehavioral Deficits and Increases Expression of Glial Fibrillary Acidic Protein in the Motor Cortex and Hippocampus in Offspring Rats Following in Utero Exposure. J. Toxicol. Environ. Heal. Part A 71, 119–130. https://doi.org/10.1080/15287390701613140

Aebi, H., 1984. Catalase in vitro. Methods Enzym. 105 105, 121–126.

Al-Amin, M.M., Rahman, M.M., Khan, F.R., Zaman, F., Mahmud Reza, H., 2015. Astaxanthin improves behavioral disorder and oxidative stress in prenatal valproic acid-induced mice model of autism. Behav. Brain Res. 286, 112–121. https://doi.org/10.1016/j.bbr.2015.02.041

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, Journal of Experimental Biology. American Psychiatric Association. https://doi.org/10.1176/appi.books.9780890425596

Anderson, B.M., Schnetz-Boutaud, N., Bartlett, J., Wright, H.H., Abramson, R.K., Cuccaro, M.L., Gilbert, J.R., Pericak-Vance, M.A., Haines, J.L., 2008. Examination of association to autism of common genetic variationin genes related to dopamine. Autism Res. 1, 364–369. https://doi.org/10.1002/aur.55

Arel-Dubeau, A.-M., Longpré, F., Bournival, J., Tremblay, C., Demers-Lamarche, J., Haskova, P., Attard, E., Germain, M., Martinoli, M.-G., 2014. Cucurbitacin E Has Neuroprotective Properties and Autophagic Modulating Activities on Dopaminergic Neurons. Oxid. Med. Cell. Longev. 2014, 1–15. https://doi.org/10.1155/2014/425496

Bianchini, M.C., Gularte, C.O.A., Nogara, P.A., Krum, B.N., Gayer, M.C., Bridi, J.C., Roos,

D.H., Roehrs, R., Fachinetto, R., Pinton, S., Avila, D.S., Hirth, F., Rocha, J.B.T., Puntel, R.L., 2019. Thimerosal inhibits Drosophila melanogaster tyrosine hydroxylase (DmTyrH) leading to changes in dopamine levels and impaired motor behavior: implications for neurotoxicity. Metallomics 11, 362–374. https://doi.org/10.1039/c8mt00268a

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., Kalayci, O., 2012. Oxidative Stress and Antioxidant Defense. World Allergy Organ. J. 5, 9–19. https://doi.org/10.1097/WOX.0b013e3182439613

Bland, N.D., Robinson, P., Thomas, J.E., Shirras, A.D., Turner, A.J., Isaac, R.E., Charpentier, G., Louat, F., Bonmatin, J., Marchand, P.A., Vanier, F., Locker, D., Decoville, M., 2014. Peptides Locomotor and geotactic behavior of Drosophila melanogaster over-expressing neprilysin 2 30, 571–574. https://doi.org/10.1016/j.peptides.2008.10.020

Bowton, E., Saunders, C., Reddy, I.A., Campbell, N.G., Hamilton, P.J., Henry, L.K., Coon, H., Sakrikar, D., Veenstra-VanderWeele, J.M., Blakely, R.D., Sutcliffe, J., Matthies, H.J.G., Erreger, K., Galli, A., 2014. SLC6A3 coding variant Ala559Val found in two autism probands alters dopamine transporter function and trafficking. Transl. Psychiatry 4, e464. https://doi.org/10.1038/tp.2014.90

Bradford, M.M., 1976. A Rapid and Sensitive Method for the Quantitation Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding 254, 248–254.

Burg, E.D., Langan, S.T., Nash, H.A., 2013. Drosophila social clustering is disrupted by anesthetics and in narrow abdomen ion channel mutants 1–10. https://doi.org/10.1111/gbb.12025

Campbell, N.G., Shekar, A., Aguilar, J.I., Peng, D., Navratna, V., Yang, D., Morley, A.N., Duran, A.M., Galli, G., O'Grady, B., Ramachandran, R., Sutcliffe, J.S., Sitte, H.H., Erreger, K., Meiler, J., Stockner, T., Bellan, L.M., Matthies, H.J.G., Gouaux, E., Mchaourab, H.S., Galli, A., 2019. Structural, functional, and behavioral insights of

dopamine dysfunction revealed by a deletion in SLC6A3. Proc. Natl. Acad. Sci. 116, 3853–3862. https://doi.org/10.1073/pnas.1816247116

Cartier, E., Hamilton, P.J., Belovich, A.N., Shekar, A., Campbell, N.G., Saunders, C., Andreassen, T.F., Gether, U., Veenstra-Vanderweele, J., Sutcliffe, J.S., Ulery-Reynolds, P.G., Erreger, K., Matthies, H.J.G., Galli, A., 2015. Rare Autism-Associated Variants Implicate Syntaxin 1 (STX1 R26Q) Phosphorylation and the Dopamine Transporter (hDAT R51W) in Dopamine Neurotransmission and Behaviors. EBioMedicine 2, 135–146. https://doi.org/10.1016/j.ebiom.2015.01.007

Chang, Y., Mao, L., Zhang, L., Zhang, Y., Jiang, H., 2020. Combined toxicity of imidacloprid, acetochlor, and tebuconazole to zebrafish (Danio rerio): acute toxicity and hepatotoxicity assessment. Environ. Sci. Pollut. Res. 1. https://doi.org/10.1007/s11356-020-07653-3

Charpentier, G., Louat, F., Bonmatin, J., Marchand, P.A., Vanier, F., Locker, D., Decoville, M., 2014. Lethal and Sublethal E ff ects of Imidacloprid, After Chronic Exposure, On the Insect Model Drosophila melanogaster.

Christen, V., Mittner, F., Fent, K., 2016. Molecular Effects of Neonicotinoids in Honey Bees ( Apis mellifera ). Environ. Sci. Technol. 50, 4071–4081. https://doi.org/10.1021/acs.est.6b00678

Colovic, M.B., Krstic, D.Z., Lazarevic-Pasti, T.D., Bondzic, A.M., Vasic, V.M., 2013. Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. Curr. Neuropharmacol. 11, 315–335. https://doi.org/10.2174/1570159X11311030006

Connolly, K., 1966. Locomotor activity in Drosophila. II. Selection for active and inactive strains. Anim. Behav. 14, 444–449. https://doi.org/10.1016/S0003-3472(66)80043-X

Courtney, K.D., Francisco, S., 1961. A NEW AND RAPID COLORIMETRIC OF ACETYLCHOLINESTERASE DETERMINATION 7, 88–95.

De Felice, A., Greco, A., Calamandrei, G., Minghetti, L., 2016. Prenatal exposure to the

organophosphate insecticide chlorpyrifos enhances brain oxidative stress and prostaglandin E2 synthesis in a mouse model of idiopathic autism. J. Neuroinflammation 13, 149. https://doi.org/10.1186/s12974-016-0617-4

DiCarlo, G.E., Aguilar, J.I., Matthies, H.J.G., Harrison, F.E., Bundschuh, K.E., West, A., Hashemi, P., Herborg, F., Rickhag, M., Chen, H., Gether, U., Wallace, M.T., Galli, A., 2019. Autism-linked dopamine transporter mutation alters striatal dopamine neurotransmission and dopamine-dependent behaviors. J. Clin. Invest. 129, 3407–3419. https://doi.org/10.1172/JCI127411

Edwards, A.C., Rollmann, S.M., Morgan, T.J., Mackay, T.F.C., 2018. Genômica Quantitativa do Comportamento Agressivo em Drosophila melanogaster 1–13.

Edwards, A.C., Rollmann, S.M., Morgan, T.J., Mackay, T.F.C., 2006. Quantitative Genomics of Aggressive Behavior in Drosophila melanogaster 2. https://doi.org/10.1371/journal.pgen.0020154

EL-Gendy, K.S., Aly, N.M., Mahmoud, F.H., Kenawy, A., El-Sebae, A.K.H., 2010. The role of vitamin C as antioxidant in protection of oxidative stress induced by imidacloprid. Food Chem. Toxicol. 48, 215–221. https://doi.org/10.1016/j.fct.2009.10.003

Ellman, G.L., 1959. Tissue Su ~ yd ~ l Groups 70–77.

Emam, H., Ahmed, E., Abdel-Daim, M., 2018. Antioxidant capacity of omega-3-fatty acids and vitamin E against imidacloprid-induced hepatotoxicity in Japanese quails. Environ. Sci. Pollut. Res. 25, 11694–11702. https://doi.org/10.1007/s11356-018-1481-9

Faraone, S. V., Mick, E., 2010. Molecular Genetics of Attention Deficit Hyperactivity Disorder. Psychiatr. Clin. North Am. 33, 159–180. https://doi.org/10.1016/j.psc.2009.12.004

Franco, J.L., Posser, T., Dunkley, P.R., Dickson, P.W., Mattos, J.J., Martins, R., Bainy, A.C.D., Marques, M.R., Dafre, A.L., Farina, M., 2009. Free Radical Biology & Medicine Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme

glutathione peroxidase. Free Radic. Biol. Med. 47, 449–457. https://doi.org/10.1016/j.freeradbiomed.2009.05.013

Gadow, K.D., Roohi, J., DeVincent, C.J., Hatchwell, E., 2008. Association of ADHD, tics, and anxiety with dopamine transporter ( DAT1 ) genotype in autism spectrum disorder. J. Child Psychol. Psychiatry 49, 1331–1338. https://doi.org/10.1111/j.1469-7610.2008.01952.x

Gauthier, M., Aras, P., Paquin, J., Boily, M., 2018. Chronic exposure to imidacloprid or thiamethoxam neonicotinoid causes oxidative damages and alters carotenoid-retinoid levels in caged honey bees ( Apis mellifera ). Sci. Rep. 1–11. https://doi.org/10.1038/s41598-018-34625-y

Ge, W., Yan, S., Wang, Jinhua, Zhu, L., Chen, A., Wang, Jun, 2015. Oxidative Stress and DNA Damage Induced by Imidacloprid in Zebrafish ( Danio rerio ). J. Agric. Food Chem. 63, 1856–1862. https://doi.org/10.1021/jf504895h

Gibbons, D., Morrissey, C., Mineau, P., 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environ. Sci. Pollut. Res. 22, 103–118. https://doi.org/10.1007/s11356-014-3180-5

HABIG, W. H., PABST, M. J., JAKOBY, W.B., 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, (1974), 7130-7139. 7130–7140.

Hallmayer, J., 2011. Genetic Heritability and Shared Environmental Factors Among Twin Pairs With Autism. Arch. Gen. Psychiatry 68, 1095. https://doi.org/10.1001/archgenpsychiatry.2011.76

Hamilton, P.J., Campbell, N.G., Sharma, S., Erreger, K., Herborg Hansen, F., Saunders, C., Belovich, A.N., Sahai, M.A., Cook, E.H., Gether, U., Mchaourab, H.S., Matthies, H.J.G., Sutcliffe, J.S., Galli, A., 2013. De novo mutation in the dopamine transporter gene

associates dopamine dysfunction with autism spectrum disorder. Mol. Psychiatry 18, 1315–1323. https://doi.org/10.1038/mp.2013.102

Hansen, F.H., Skjørringe, T., Yasmeen, S., Arends, N. V., Sahai, M.A., Erreger, K., Andreassen, T.F., Holy, M., Hamilton, P.J., Neergheen, V., Karlsborg, M., Newman, A.H., Pope, S., Heales, S.J.R., Friberg, L., Law, I., Pinborg, L.H., Sitte, H.H., Loland, C., Shi, L., Weinstein, H., Galli, A., Hjermind, L.E., Møller, L.B., Gether, U., 2014. Missense dopamine transporter mutations associate with adult parkinsonism and ADHD. J. Clin. Invest. 124, 3107–3120. https://doi.org/10.1172/JCI73778

Hara, Y., Takuma, K., Takano, E., Katashiba, K., Taruta, A., Higashino, K., Hashimoto, H., Ago, Y., Matsuda, T., 2015. Reduced prefrontal dopaminergic activity in valproic acid-treated mouse autism model. Behav. Brain Res. 289, 39–47. https://doi.org/10.1016/j.bbr.2015.04.022

Hoyer, S.C., Eckart, A., Herrel, A., Zars, T., Fischer, S.A., Hardie, S.L., Heisenberg, M., 2008. Octopamine in Male Aggression of Drosophila. Curr. Biol. 18, 159–167. https://doi.org/10.1016/j.cub.2007.12.052

Karvat, G., Kimchi, T., 2014a. Acetylcholine Elevation Relieves Cognitive Rigidity and Social Deficiency in a Mouse Model of Autism. Neuropsychopharmacology 39, 831–840. https://doi.org/10.1038/npp.2013.274

Karvat, G., Kimchi, T., 2014b. Acetylcholine Elevation Relieves Cognitive Rigidity and Social Deficiency in a Mouse Model of Autism. Neuropsychopharmacology 39, 831–840. https://doi.org/10.1038/npp.2013.274

Kaur, K., Simon, A.F., Chauhan, V., Chauhan, A., 2015. Effect of bisphenol A on Drosophila melanogaster behavior – A new model for the studies on neurodevelopmental disorders. Behav. Brain Res. 284, 77–84. https://doi.org/10.1016/j.bbr.2015.02.001

Keil, A.P., Daniels, J.L., Hertz-Picciotto, I., 2014. Autism spectrum disorder, flea and tick

medication, and adjustments for exposure misclassification: the CHARGE (CHildhood Autism Risks from Genetics and Environment) case–control study. Environ. Heal. 13, 3. https://doi.org/10.1186/1476-069X-13-3

Kim, J.-W., Seung, H., Kwon, K.J., Ko, M.J., Lee, E.J., Oh, H.A., Choi, C.S., Kim, K.C., Gonzales, E.L., You, J.S., Choi, D.-H., Lee, J., Han, S.-H., Yang, S.M., Cheong, J.H., Shin, C.Y., Bahn, G.H., 2014. Subchronic Treatment of Donepezil Rescues Impaired Social, Hyperactive, and Stereotypic Behavior in Valproic Acid-Induced Animal Model of Autism. PLoS One 9, e104927. https://doi.org/10.1371/journal.pone.0104927

Kim, S., Lee, H., Park, Y., 2017. Perinatal exposure to low-dose imidacloprid causes ADHD-like symptoms: Evidences from an invertebrate model study. Food Chem. Toxicol. 110, 402–407. https://doi.org/10.1016/j.fct.2017.10.007

Lafi, B., Chaâbane, M., Elwej, A., Grati, M., Jamoussi, K., Mnif, H., Boudawara, T., Ketata Bouaziz, H., Zeghal, N., 2018. Effects of co-exposure to imidacloprid and gibberellic acid on redox status, kidney variables and histopathology in adult rats. Arch. Physiol. Biochem. 124, 175–184. https://doi.org/10.1080/13813455.2017.1371195

Lake, C.R., 1977. Increased Norepinephrine Levels and Decreased Dopamine-β-Hydroxylase Activity in Primary Autism. Arch. Gen. Psychiatry 34, 553. https://doi.org/10.1001/archpsyc.1977.01770170063005

Lee Chao, S., Casida, J.E., 1997. Interaction of Imidacloprid Metabolites and Analogs with the Nicotinic Acetylcholine Receptor of Mouse Brain in Relation to Toxicity. Pestic. Biochem. Physiol. 58, 77–88. https://doi.org/10.1006/pest.1997.2284

Lee, I., Eriksson, P., Fredriksson, A., Buratovic, S., Viberg, H., 2015. Developmental neurotoxic effects of two pesticides: Behavior and neuroprotein studies on endosulfan and cypermethrin. Toxicology 335, 1–10. https://doi.org/10.1016/j.tox.2015.06.010

Li, A.J., Martinez-Moral, M.-P., Kannan, K., 2020. Variability in urinary neonicotinoid

concentrations in single-spot and first-morning void and its association with oxidative stress markers. Environ. Int. 135, 105415. https://doi.org/10.1016/j.envint.2019.105415

Li, Z., Yu, T., Chen, Y., Heerman, M., He, J., Huang, J., Nie, H., Su, S., 2019. Brain transcriptome of honey bees (Apis mellifera) exhibiting impaired olfactory learning induced by a sublethal dose of imidacloprid. Pestic. Biochem. Physiol. 156, 36–43. https://doi.org/10.1016/j.pestbp.2019.02.001

Luke, L., Clare, I.C.H., Ring, H., Redley, M., Watson, P., 2012. Decision-making difficulties experienced by adults with autism spectrum conditions. Autism 16, 612–621. https://doi.org/10.1177/1362361311415876

Lushchak, O. V., Rovenko, B.M., Gospodaryov, D. V., Lushchak, V.I., 2011. Drosophila melanogaster larvae fed by glucose and fructose demonstrate difference in oxidative stress markers and antioxidant enzymes of adult flies. Comp. Biochem. Physiol. Part A Mol. Integr. Physiol. 160, 27–34. https://doi.org/10.1016/j.cbpa.2011.04.019

Machado, S., Rósula, M., Cardoso, V., Freitas, S. De, Cabral, F., Barreto, L., Polet, F., Almeida, D., Aparecida, E., Musachio, S., Trindade, M., Paula, D., 2018. Chronic unpredictable mild stress-induced depressive-like behavior and dysregulation of brain levels of biogenic amines in Drosophila melanogaster. Behav. Brain Res. 351, 104–113. https://doi.org/https://doi.org/10.1016/j.bbr.2018.05.016

Mengoni, C., Farina, W.M., 2015. Effects of Sublethal Doses of Imidacloprid on Young Adult Honeybee Behaviour 1–15. https://doi.org/10.1371/journal.pone.0140814

Miao, J., Reisig, D.D., Li, G., Wu, Y., 2016. Sublethal Effects of Insecticide Exposure on Megacopta cribraria (Fabricius) Nymphs: Key Biological Traits and Acetylcholinesterase Activity. J. Insect Sci. 16, 99. https://doi.org/10.1093/jisesa/iew083

Mirza, R., Sharma, B., 2019. Benefits of Fenofibrate in prenatal valproic acid-induced autism spectrum disorder related phenotype in rats. Brain Res. Bull. 147, 36–46.

https://doi.org/10.1016/j.brainresbull.2019.02.003

Mohany, M., 2011. Immunological and histological effects of exposure to imidacloprid insecticide in male albino rats. African J. Pharm. Pharmacol. 5, 2106–2114. https://doi.org/10.5897/AJPP11.625

Musachio, E.A.S., Araujo, S.M., Bortolotto, V.C., de Freitas Couto, S., Dahleh, M.M.M., Poetini, M.R., Jardim, E.F., Meichtry, L.B., Ramborger, B.P., Roehrs, R., Petri Guerra, G., Prigol, M., 2020. Bisphenol A exposure is involved in the development of Parkinson like disease in Drosophila melanogaster. Food Chem. Toxicol. 137, 111128. https://doi.org/10.1016/j.fct.2020.111128

Ndonwi, E.N., Atogho-Tiedeu, B., Lontchi-Yimagou, E., Shinkafi, T.S., Nanfa, D., Balti, E. V., Indusmita, R., Mahmood, A., Katte, J.-C., ArmMbanya, Matsha, T., Mbanya, J.C., Shakir, A., Sobngwi, E., 2019. Gestational Exposure to Pesticides Induces Oxidative Stress and Lipid Peroxidation in Offspring that Persist at Adult Age in an Animal Model. Toxicol. Res. 35, 241–248. https://doi.org/10.5487/TR.2019.35.3.241

Neckameyer, W.S., R. Nieto-Romero, A., 2015. Response to stress in Drosophila is mediated by gender, age and stress paradigm. Stress 18, 254–266. https://doi.org/10.3109/10253890.2015.1017465

Nguyen, H.T.N., Kato, H., Masuda, K., Yamaza, H., Hirofuji, Y., Sato, H., Pham, T.T.M., Takayama, F., Sakai, Y., Ohga, S., Taguchi, T., Nonaka, K., 2018. Impaired neurite development associated with mitochondrial dysfunction in dopaminergic neurons differentiated from exfoliated deciduous tooth-derived pulp stem cells of children with autism spectrum disorder. Biochem. Biophys. Reports 16, 24–31. https://doi.org/10.1016/j.bbrep.2018.09.004

Ohkawa, H., Ohishi, N., Yagi, K., 1979. Assay for Lipid Peroxides in Animal Tissues Thiobarbituric Acid Reaction 358, 351–358.

Ortiz, G.G., Pacheco-Moisés, F.P., Mireles-Ramírez, M.A., Flores-Alvarado, L.J., González-Usigli, H., Sánchez-López, A.L., Sánchez-Romero, L., Velázquez-Brizuela, I.E., González-Renovato, E.D., Torres-Sánchez, E.D., 2016. Oxidative Stress and Parkinson's Disease: Effects on Environmental Toxicology, in: Free Radicals and Diseases. InTech. https://doi.org/10.5772/63794

Pamies, D., Block, K., Lau, P., Gribaldo, L., Pardo, C.A., Barreras, P., Smirnova, L., Wiersma, D., Zhao, L., Harris, G., Hartung, T., Hogberg, H.T., 2018. Rotenone exerts developmental neurotoxicity in a human brain spheroid model. Toxicol. Appl. Pharmacol. 354, 101–114. https://doi.org/10.1016/j.taap.2018.02.003

Pérez-severiano, F., Santamaría, A., Pedraza-chaverri, J., Medina-campos, O.N., Ríos, C., Segovia, J., 2004. Increased Formation of Reactive Oxygen Species , but No Changes in Glutathione Peroxidase Activity , in Striata of Mice Transgenic for the Huntington ' s Disease Mutation 29, 729–733.

Perry, E.K., Lee, M.L.W., Martin-Ruiz, C.M., Court, J.A., Volsen, S.G., Merrit, J., Folly, E., Iversen, P.E., Bauman, M.L., Perry, R.H., Wenk, G.L., 2001. Cholinergic Activity in Autism: Abnormalities in the Cerebral Cortex and Basal Forebrain. Am. J. Psychiatry 158, 1058–1066. https://doi.org/10.1176/appi.ajp.158.7.1058

Philippat, C., Barkoski, J., Tancredi, D.J., Elms, B., Barr, D.B., Ozonoff, S., Bennett, D.H., Hertz-Picciotto, I., 2018. Prenatal exposure to organophosphate pesticides and risk of autism spectrum disorders and other non-typical development at 3 years in a high-risk cohort. Int. J. Hyg. Environ. Health 221, 548–555. https://doi.org/10.1016/j.ijheh.2018.02.004

Polanczyk, G., de Lima, M.S., Horta, B.L., Biederman, J., Rohde, L.A., 2007. The Worldwide Prevalence of ADHD: A Systematic Review and Metaregression Analysis. Am. J. Psychiatry 164, 942–948. https://doi.org/10.1176/ajp.2007.164.6.942

Richardson, J.R., Taylor, M.M., Shalat, S.L., Guillot, T.S., Caudle, W.M., Hossain, M.M., Mathews, T.A., Jones, S.R., Cory-Slechta, D.A., Miller, G.W., 2015. Developmental pesticide exposure reproduces features of attention deficit hyperactivity disorder. FASEB J. 29, 1960–1972. https://doi.org/10.1096/fj.14-260901

Rios, F.M., Wilcoxen, T.E., Zimmerman, L.M., 2017. Effects of imidacloprid on Rana catesbeiana immune and nervous system. Chemosphere 188, 465–469. https://doi.org/10.1016/j.chemosphere.2017.08.155

Roberts, E.M., English, P.B., Grether, J.K., Windham, G.C., Somberg, L., Wolff, C., 2007. Maternal Residence Near Agricultural Pesticide Applications and Autism Spectrum Disorders among Children in the California Central Valley. Environ. Health Perspect. 115, 1482–1489. https://doi.org/10.1289/ehp.10168

Roberts, J.R., Dawley, E.H., Reigart, J.R., 2019. Children's low-level pesticide exposure and associations with autism and ADHD: a review. Pediatr. Res. 85, 234–241. https://doi.org/10.1038/s41390-018-0200-z

Saez, M., Barceló, M.A., Farrerons, M., López-Casasnovas, G., 2018. The association between exposure to environmental factors and the occurrence of attention-deficit/hyperactivity disorder (ADHD). A population-based retrospective cohort study. Environ. Res. 166, 205–214. https://doi.org/10.1016/j.envres.2018.05.009

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C.M., Reichenberg, A., 2014. The Familial Risk of Autism. JAMA 311, 1770. https://doi.org/10.1001/jama.2014.4144

Schmidt, R.J., Kogan, V., Shelton, J.F., Delwiche, L., Hansen, R.L., Ozonoff, S., Ma, C.C., McCanlies, E.C., Bennett, D.H., Hertz-Picciotto, I., Tancredi, D.J., Volk, H.E., 2017. Combined Prenatal Pesticide Exposure and Folic Acid Intake in Relation to Autism Spectrum Disorder. Environ. Health Perspect. 125, 097007.

https://doi.org/10.1289/EHP604

Sharma, R., Rahi, S., Mehan, S., 2019. Neuroprotective potential of solanesol in intracerebroventricular propionic acid induced experimental model of autism: Insights from behavioral and biochemical evidence. Toxicol. Reports 6, 1164–1175. https://doi.org/10.1016/j.toxrep.2019.10.019

Simon, A.F., Chou, M., Salazar, E.D., Nicholson, T., Saini, N., Metchev, S., Krantz, D.E., 2012. A simple assay to study social behavior in Drosophila : measurement of social space within a group 1 243–252. https://doi.org/10.1111/j.1601-183X.2011.00740.x

Taira, K., Fujioka, K., Aoyama, Y., 2013. Qualitative Profiling and Quantification of Neonicotinoid Metabolites in Human Urine by Liquid Chromatography Coupled with Mass Spectrometry. PLoS One 8, e80332. https://doi.org/10.1371/journal.pone.0080332

Tauber, J.M., Vanlandingham, P.A., Zhang, B., 2011. Elevated Levels of the Vesicular Monoamine Transporter and a Novel Repetitive Behavior in the Drosophila Model of Fragile X Syndrome. PLoS One 6, e27100. https://doi.org/10.1371/journal.pone.0027100

Topal, A., Alak, G., Ozkaraca, M., Yeltekin, A.C., Comaklı, S., Acıl, G., Kokturk, M., Atamanalp, M., 2017. Neurotoxic responses in brain tissues of rainbow trout exposed to imidacloprid pesticide: Assessment of 8-hydroxy-2-deoxyguanosine activity, oxidative stress and acetylcholinesterase activity. Chemosphere 175, 186–191. https://doi.org/10.1016/j.chemosphere.2017.02.047

Tully, T., Preat, T., Boynton, S.C., Del Vecchio, M., 1994. Genetic dissection of consolidated memory in Drosophila. Cell 79, 35–47. https://doi.org/10.1016/0092-8674(94)90398-0

Ueyama, J., Nomura, H., Kondo, T., Saito, I., Ito, Y., Osaka, A., Kamijima, M., 2014. Biological Monitoring Method for Urinary Neonicotinoid Insecticides Using LC-MS/MS and Its Application to Japanese Adults. J. Occup. Health 56, 461–468. https://doi.org/10.1539/joh.14-0077-OA

Wang, Juan, Wang, Jinhua, Wang, G., Zhu, L., Wang, Jun, 2016. DNA damage and oxidative stress induced by imidacloprid exposure in the earthworm Eisenia fetida. Chemosphere 144, 510–517. https://doi.org/10.1016/j.chemosphere.2015.09.004

Wang, X., Lu, J., Xie, W., Lu, X., Liang, Y., Li, M., Wang, Z., Huang, X., Tang, M., Pfaff, D.W., Tang, Y.-P., Yao, P., 2019. Maternal diabetes induces autism-like behavior by hyperglycemia-mediated persistent oxidative stress and suppression of superoxide dismutase 2. Proc. Natl. Acad. Sci. 116, 23743–23752. https://doi.org/10.1073/pnas.1912625116

**Figures caption:**

**Figure 1.** Experimental design of imidacloprid exposure at the concentrations of 200, 400 and 600 pM in *Drosophila melanogaster*.

**Figure 2.** Effect of the exposure to imidacloprid (200, 400 or 600 pM) for 7 days on the behavioral activities of *Drosophila melanogaster's* progeny. (A) Negative geotaxis test; (B) Open field test; (C) Social interaction test; (D) Light / dark test; (E) Agressiveness test; (F) Grooming test; (G) Food consumption in the progeny of *Drosophila melanogaster*. Data are mean ± SEM, median and interquartile range, for n = 5-6 in each group. *Indicates a significant difference ($P < 0.05$) compared to the control group.

**Figure 3**. Effect of exposure to imidacloprid (200, 400 or 600 pM) for 7 days on the dopamine levels in the progeny of *Drosophila melanogaster*. Data are mean ± SEM, for n = 6 in each group. *Indicates a significant difference ($P < 0.05$) compared to the control group.

**Figure 4.** Effect of exposure to imidacloprid (200, 400 or 600 pM) for 7 days on AChE activity in the (A) head and (B) body in the progeny of *Drosophila melanogaster*. Data are mean ± SEM, for n = 11 in each group.

**Figure 5.** Effect of exposure to imidacloprid (200, 400 or 600 pM) for 7 days on SOD activity in the (A) head and (B) body; CAT in the (C) head and (D) body; GST in the (E) head and (F) body; PSH in the (G) head and (H) body; NPSH in the (I) head and (J) body in the progeny of *Drosophila melanogaster*. Data are mean ± SEM, for n = 4-5 in each group. *Indicates a significant difference ($P < 0.05$) compared to the control group.

**Figure 6.** Effect of exposure to imidacloprid (200, 400 or 600 pM) for 7 days on ROS levels in the (A) head and (B) body; TBARS levels in the (C) head and (D) body in the progeny of *Drosophila melanogaster*. Data are mean ± SEM for n = 4-7 in each group. *Indicates a significant difference ($P < 0.05$) compared to the control group.

**Figure 7.** Effect of exposure to imidacloprid (200, 400 or 600 pM) for 7 days on cell viability in the (A) head and (B) body in the progeny of *Drosophila melanogaster*. Data are mean ± SEM for n = 4 in each group. *Indicates a significant difference ($P < 0.05$) compared to the control group.

**Figures:**

**Figure 1**

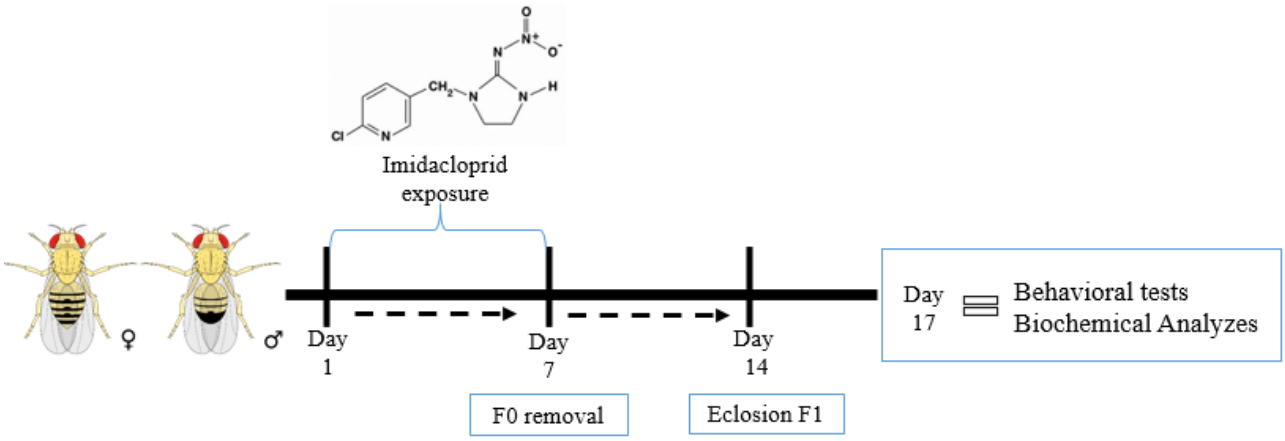


**Figure 2**

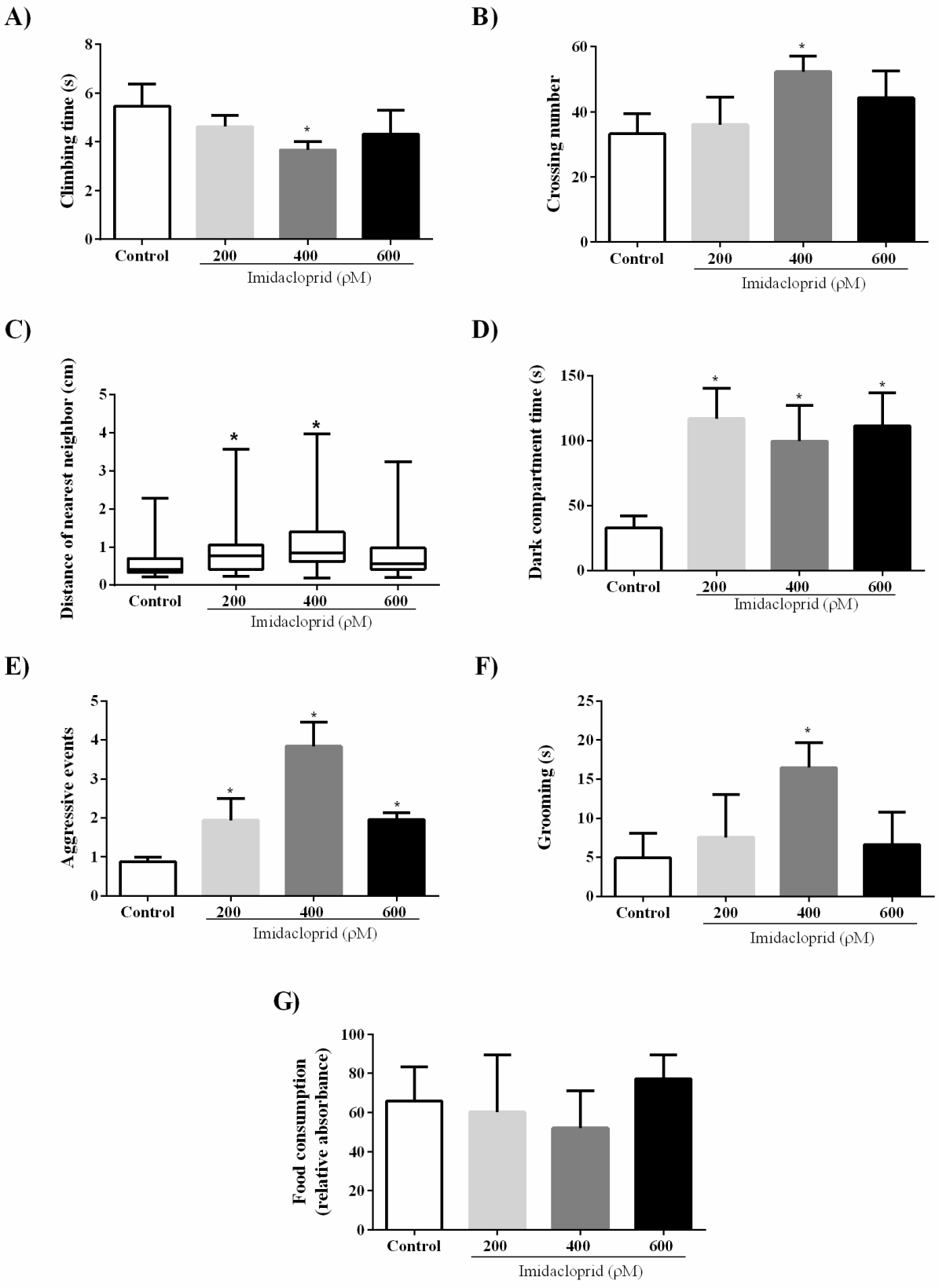

A)
Climbing time (s)
Control
200
400
600
Imidacloprid (ρM)
B)
Crossing number
C)
Distance of nearest neighbor (cm)
D)
Dark compartment time (s)
E)
Aggressive events
F)
Grooming (s)
G)
Food consumption (relative absorbance)

**Figure 3**

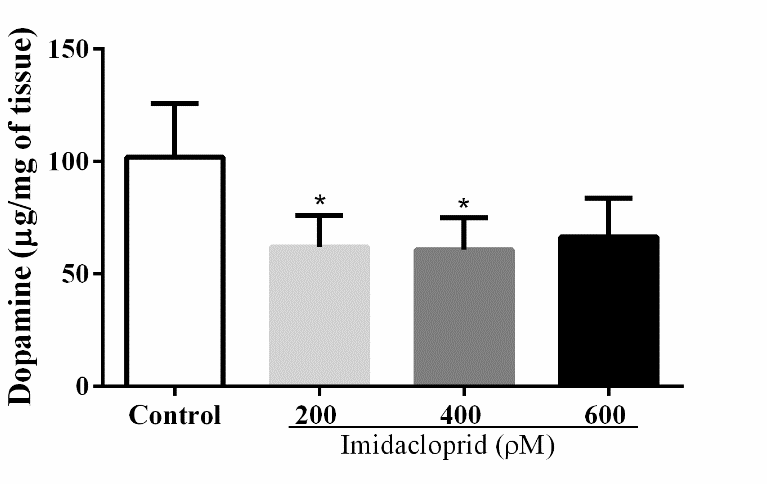



**Figure 4**

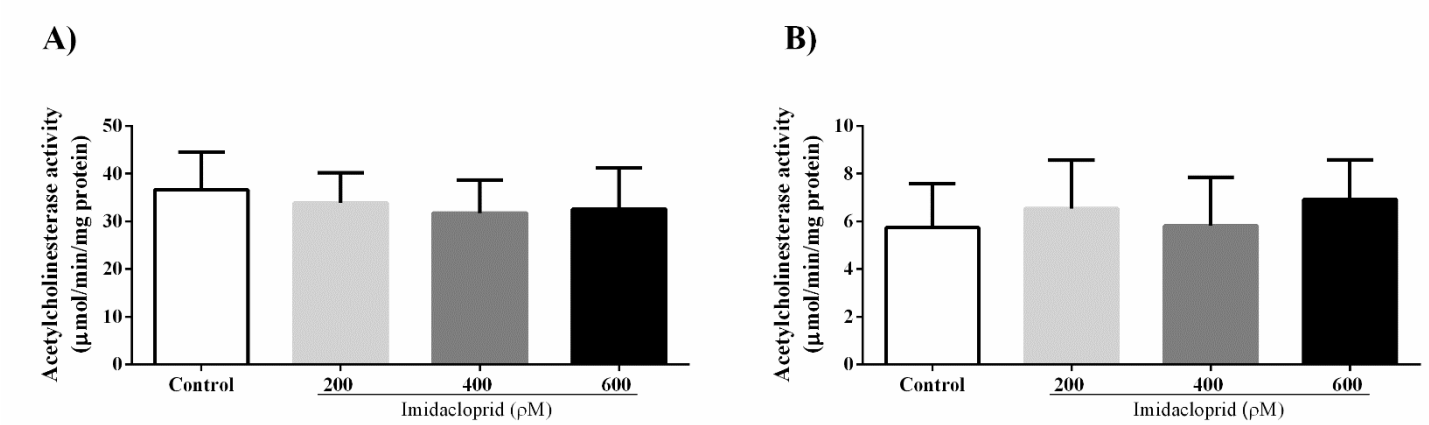


**Figure 5**

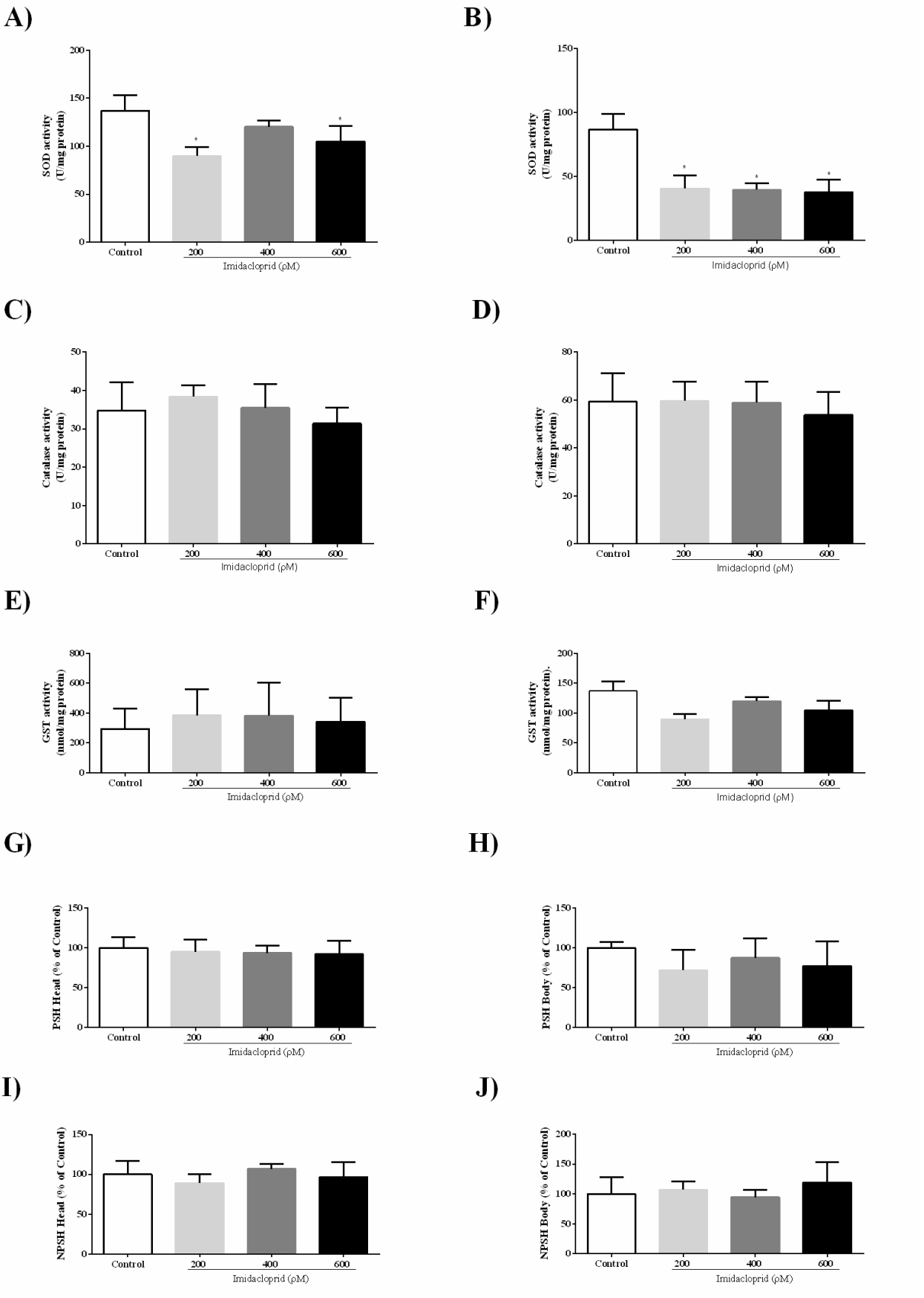

A)
SOD activity (U/mg protein)
Control
200
400
600
Imidacloprid (ρM)
B)
SOD activity (U/mg protein)
Control
200
400
600
Imidacloprid (ρM)
C)
Catalase activity (U/mg protein)
Control
200
400
600
Imidacloprid (ρM)
D)
Catalase activity (U/mg protein)
Control
200
400
600
Imidacloprid (ρM)
E)
GST activity (nmol/mg protein)
Control
200
400
600
Imidacloprid (ρM)
F)
GST activity (nmol/mg protein).
Control
200
400
600
Imidacloprid (ρM)
G)
PSH Head (% of Control)
Control
200
400
600
Imidacloprid (ρM)
H)
PSH Body (% of Control)
Control
200
400
600
Imidacloprid (ρM)
I)
NPSH Head (% of Control)
Control
200
400
600
Imidacloprid (ρM)
J)
NPSH Body (% of Control)
Control
200
400
600
Imidacloprid (ρM)

**Figure 6**

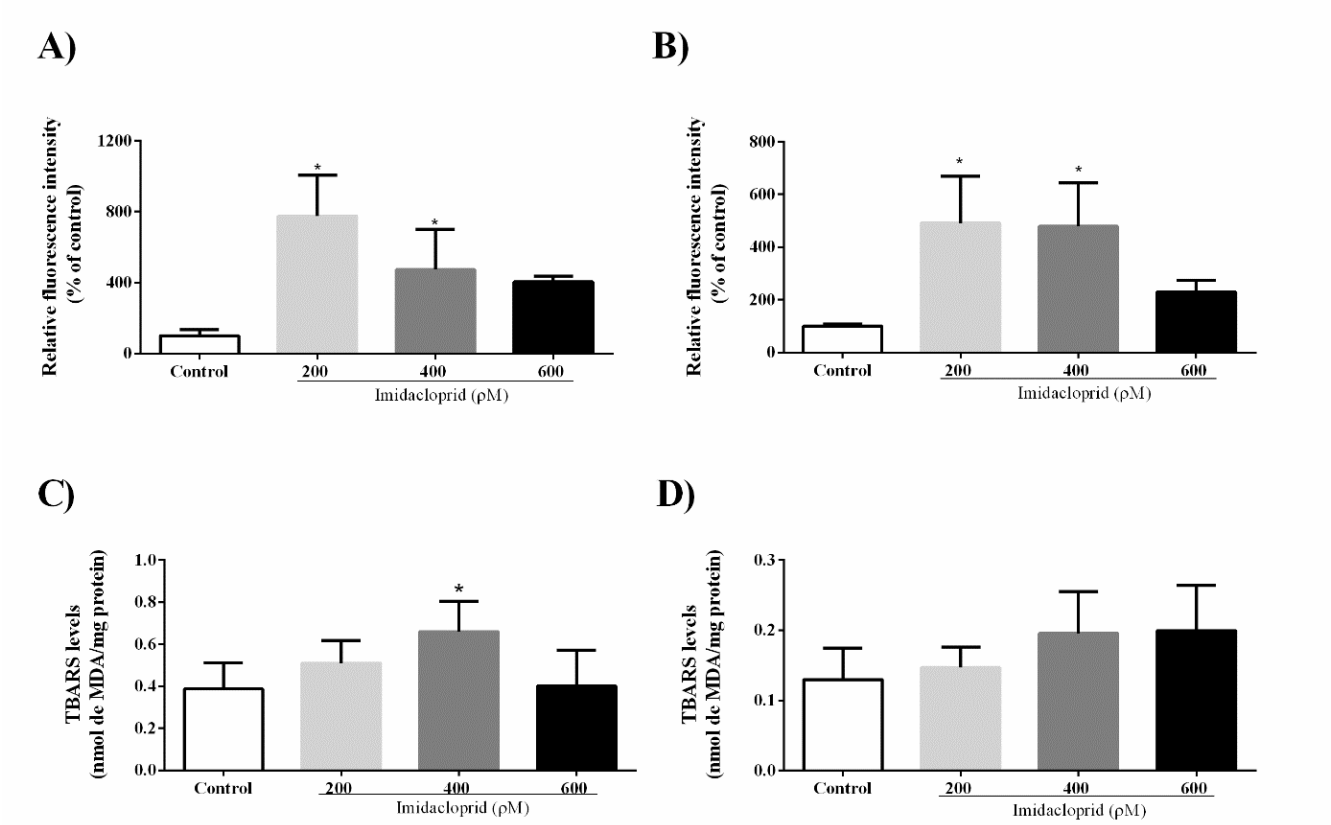
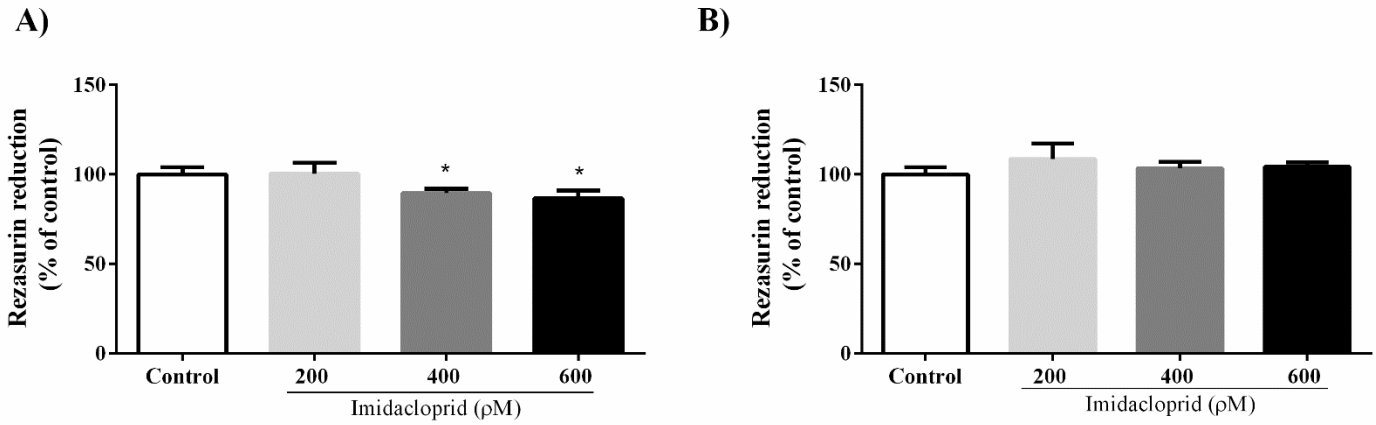



**Figure 7**



## 6. CONSIDERAÇÕES FINAIS

Baseando-se nos resultados apresentados nesta dissertação, pode-se concluir que:

- A progênie de *Drosophila melanogaster* exposta ao imidacloprida apresentou alterações comportamentais nos testes de geotaxia, open-field, grooming, agressividade, claro/escuro e interação social, semelhantes as observadas nos transtornos TEA e TDAH em modelos experimentais.

- Inesperadamente a progênie de moscas expostas ao imidacloprida não apresentou alteração significativa sobre a atividade da enzima AChE, diferentemente do que se observa nos transtornos TEA e TDAH.

- A progênie de moscas expostas ao imidacloprida também apresentou alterações nos níveis de dopamina havendo uma diminuição significativa, assim como se observa nos transtornos TEA e TDAH.

- A progênie de moscas expostas ao imidacloprida apresentou aumento dos níveis de ROS e TBARS indicando que gerou-se estresse oxidativo, sem alterar as defesas antioxidantes.

- Também foi possível observar que a progênie de moscas expostas ao imidacloprida apresentou uma diminuição da viabilidade celular assim como ocorre nos transtornos TEA e TDAH.

Assim nossos resultados sugerem que a exposição ao imidacloprida pode vir a ser utilizada como modelo experimental químico para transtornos neurodesenvolvimentais como TEA e TDAH em *Drosophila melanogaster*, demonstrando que o imidacloprida desencadeia uma série de alterações locomotoras e comportamentais que podem estar associadas à diminuição dos níveis de dopamina e um aumento no estresse oxidativo. No entanto faz-se necessários mais estudos para esclarecer os mecanismos de ação

envolvidos nas alterações causadas pelo imidacloprida, bem como o comportamento observado nos distúrbios do desenvolvimento neurológico em *Drosophila melanogaster*.

## 7. PERSPECTIVAS

A partir dos resultados apresentados nessa dissertação, poderíamos realizar estudos com o seguinte propósito:

Avaliar a atividade antioxidante de moléculas bioativas na progênie de *Drosophila melanogaster*, sobre aos danos causados pela exposição ao imidacloprida.

Avaliar mecanismos genéticos envolvidos nos transtornos neurodesenvolvimentais como TEA e TDAH, sendo um exemplo o gene da família SHANK.

Avaliar o possível envolvimento da via do BDNF relacionada ao TEA e TDAH na progênie da *Drosophila melanogaster* exposta ao imidacloprida.

## 8. REFERÊNCIAS BIBLIOGRÁFICAS


ABDOLLAHI, M. et al. Pesticides and oxidative stress: A review. **Medical Science Monitor**, v. 10, n. 6, p. 141–148, 2004.

AL-AMIN, M. M. et al. Astaxanthin improves behavioral disorder and oxidative stress in prenatal valproic acid-induced mice model of autism. **Behavioural Brain Research**, v. 286, p. 112–121, 2015.

AMERICAN PSYCHIATRIC ASSOCIATION. Diagnostic and Statistical Manual of Mental Disorders. **American Psychiatric Association**, 2013. v. 217

BAIO, J. ET AL. Prevalence of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, United States, 2014. **Morbidity and Mortality Weekly Report Surveillance Summaries**, 2018 APR 27; 67(6): 1–23.

BERNARDI, M. M.; KIRSTEN, T. B.; TRINDADE, M. O. Sobre o autismo, neuroinflamação e modelos animais para o estudo de autismo: Uma revisão. **Revista Neurociencias**, v. 20, n. 1, p. 117–127, 2012.

BIEDERMAN, J. Attention-Deficit/Hyperactivity Disorder: A Selective Overview. **Biological Psychiatry**, v. 57, n. 11, p. 1215–1220, jun. 2005.

BIEDERMAN, J.; FARAONE, S. V. Attention-deficit hyperactivity disorder. **The Lancet**, v. 366, n. 9481, p. 237–248, jul. 2005.

BLOOM B, COHEN R, F. G. Summary Health Statistics for U . S . Children : National Health Interview Survey. **Vital and Health Statistics** v. 218, 1-81, Dec 2013.

BOLDUC, F. V et al. An assay for social interaction in *Drosophila* fragile X mutants. **Fly**, v. 4, n. 3, p. 216–225, 27 jul. 2010.

BOURGERON, T. From the genetic architecture to synaptic plasticity in autism spectrum disorder. **Nature Reviews Neuroscience**, v. 16, n. 9, p. 551–563, 20 set. 2015.

BRANNEN, K. C. et al. Prenatal Exposure to Neurotoxicants Dieldrin or Lindane Alters tert-ButylbicyclophosphorothionateBinding to GABA $_A$ Receptors in Fetal Rat Brainstem. **Developmental Neuroscience**, v. 20, n. 1, p. 34–41, 1998.

BRIZ, V. et al. Differential Estrogenic Effects of the Persistent Organochlorine Pesticides Dieldrin, Endosulfan, and Lindane in Primary Neuronal Cultures. **Toxicological Sciences**, v. 120, n. 2, p. 413–427, abr. 2011.

BROWN, R. C.; LOCKWOOD, A. H.; SONAWANE, B. R. Neurodegenerative Diseases: An Overview of Environmental Risk Factors. **Environmental Health Perspectives**, v. 113, n. 9, p. 1250–1256, set. 2005.

CALABRESEC, V. et al. Hormesis, cellular stress response, and redox homeostasis in autism spectrum disorders. **Journal of Neuroscience Research**, v. 94, n. 12, p. 1488–1498, dez. 2016.

CHIRUMBOLO, S.; BJØRKLUND, G. PERM Hypothesis: The Fundamental Machinery Able to Elucidate the Role of Xenobiotics and Hormesis in Cell Survival and Homeostasis. **International Journal of Molecular Sciences**, v. 18, n. 1, p. 165, 15 jan. 2017.

CHRISTENSEN, D.L. ET AL. Prevalence and characteristics of autism spectrum disorder among children aged 4 years - early autism and developmental disabilities monitoring network, seven sites, united states, 2010, 2012, and 2014. **Morbidity and Mortality Weekly Report Surveillance Summaries**, 2019 APR 12;68 (2):1-19.

CROSBY, E. B. et al. Neurobehavioral impairments caused by developmental imidacloprid exposure in zebrafish. **Neurotoxicology and Teratology**, v. 49, p. 81–90, maio 2015.

DE FELICE, A. et al. Prenatal exposure to the organophosphate insecticide chlorpyrifos enhances brain oxidative stress and prostaglandin E2 synthesis in a mouse model of idiopathic autism. **Journal of Neuroinflammation**, v. 13, n. 1, p. 149, 14 dez. 2016.

DE LA TORRE-UBIETA, L. et al. Advancing the understanding of autism disease mechanisms through genetics. **Nature Medicine**, v. 22, n. 4, p. 345–361, 6 abr. 2016.

DESNEUX, N.; DECOURTYE, A.; DELPUECH, J. The Sublethal Effects of Pesticides on Beneficial. **Arthropods**. 2007.

DOCKENDORFF, T. C. et al. *Drosophila* Lacking dfmr1 Activity Show Defects in Circadian Output and Fail to Maintain Courtship Interest. **Neuron**, v. 34, n. 6, p. 973–

984, jun. 2002.

DOLL, C. A.; BROADIE, K. Impaired activity-dependent neural circuit assembly and refinement in autism spectrum disorder genetic models. **Frontiers in Cellular Neuroscience**, v. 8, n. FEB, p. 1–26, 2014.

DUZGUNER, V.; ERDOGAN, S. Chronic exposure to imidacloprid induces inflammation and oxidative stress in the liver & central nervous system of rats. **Pesticide Biochemistry and Physiology**, v. 104, n. 1, p. 58–64, set. 2012.

ESSA, M. M. Personalized Food Intervention and Therapy for Autism Spectrum Disorder Management. Cham: **Springer International Publishing**, 2020. v. 24

FARAONE, S. V.; MICK, E. Molecular Genetics of Attention Deficit Hyperactivity Disorder. **Psychiatric Clinics of North America**, v. 33, n. 1, p. 159–180, mar. 2010.

FENG, S. et al. Assessing the genotoxicity of imidacloprid and RH-5849 in human peripheral blood lymphocytes in vitro with comet assay and cytogenetic tests. **Ecotoxicology and Environmental Safety**, v. 61, n. 2, p. 239–246, jun. 2005.

FFRENCH-CONSTANT, R. H. et al. Ion channels as insecticide targets. **Journal of Neurogenetics**, v. 30, n. 3–4, p. 163–177, 2 out. 2016.

FRANCO, J. L. et al. Free Radical Biology & Medicine Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. **Free Radical Biology and Medicine**, v. 47, n. 4, p. 449–457, 2009a.

FRANCO, R. et al. Environmental toxicity, oxidative stress and apoptosis: Ménage à Trois. **Mutation Research/Genetic Toxicology and Environmental Mutagenesis,** v. 674, n. 1–2, p. 3–22, mar. 2009b.

GAWADE, L. et al. A detailed study of developmental immunotoxicity of imidacloprid in Wistar rats. **Food and Chemical Toxicology**, v. 51, n. 1, p. 61–70, jan. 2013.

GENRO, J. P. et al. Attention-deficit/hyperactivity disorder and the dopaminergic hypotheses. **Expert Review of Neurotherapeutics**, v. 10, n. 4, p. 587–601, 9 abr. 2010.

GENRO, J. P. et al. The Brazilian contribution to Attention-Deficit/Hyperactivity Disorder molecular genetics in children and adolescents. **Genetics and Molecular Biology**, v. 35, n. 4 suppl 1, p. 932–938, 2012.

GESCHWIND, D. H.; LEVITT, P. Autism spectrum disorders: developmental disconnection syndromes. **Current Opinion in Neurobiology**, v. 17, n. 1, p. 103–111, fev. 2007.

GIZER, I. R.; FICKS, C.; WALDMAN, I. D. Candidate gene studies of ADHD: a meta-analytic review. **Human Genetics**, v. 126, n. 1, p. 51–90, 9 jul. 2009.

HALLMAYER, J. Genetic Heritability and Shared Environmental Factors Among Twin Pairs With Autism. **Archives of General Psychiatry**, v. 68, n. 11, p. 1095, 1 nov. 2011.

HAMILTON, P. J. et al. De novo mutation in the dopamine transporter gene associates dopamine dysfunction with autism spectrum disorder. **Molecular Psychiatry**, v. 18, n. 12, p. 1315–1323, 27 dez. 2013.

HARICH, B. et al. From man to fly – convergent evidence links FBXO25 to ADHD and comorbid psychiatric phenotypes. **Journal of Child Psychology and Psychiatry**, 2019.

HAYDEN, K. M. et al. Occupational exposure to pesticides increases the risk of incident AD: The Cache County Study. **Neurology**, v. 74, n. 19, p. 1524–1530, 11 maio 2010.

HIRTH, F. *Drosophila melanogaster* in the Study of Human Neurodegeneration. **CNS & Neurological Disorders** - Drug Targets, v. 9, n. 4, p. 504–523, 1 ago. 2010.

JONES, W.; KLIN, A. Attention to eyes is present but in decline in 2–6-month-old infants later diagnosed with autism. **Nature**, v. 504, n. 7480, p. 427–431, 6 dez. 2013.

JUÁREZ OLGUÍN, H. et al. The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. **Oxidative Medicine and Cellular Longevity**, v. 2016, p. 1–13, 2016.

KAPOOR, U.; SRIVASTAVA, M. K.; SRIVASTAVA, L. P. Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats. **Food and Chemical Toxicology**, v. 49, n. 12, p. 3086–3089, dez. 2011.

KARAHAN, A. et al. Sublethal imidacloprid effects on honey bee flower choices when foraging. **Ecotoxicology**, v. 24, n. 9, p. 2017–2025, 28 nov. 2015.

KARVAT, G.; KIMCHI, T. Acetylcholine Elevation Relieves Cognitive Rigidity and Social Deficiency in a Mouse Model of Autism. **Neuropsychopharmacology,** v. 39, n. 4, p. 831–840, 7 mar. 2014.

KEIL, A. P.; DANIELS, J. L.; HERTZ-PICCIOTTO, I. Autism spectrum disorder, flea and tick medication, and adjustments for exposure misclassification: the CHARGE (CHildhood Autism Risks from Genetics and Environment) case–control study. **Environmental Health**, v. 13, n. 1, p. 3, 23 dez. 2014.

KHANAM, H. et al. Neurodegenerative diseases linked to misfolded proteins and their therapeutic approaches: A review. **European Journal of Medicinal Chemistry**, v. 124, p. 1121–1141, nov. 2016.

KIM, K. C. et al. The critical period of valproate exposure to induce autistic symptoms in Sprague–Dawley rats. **Toxicology Letters**, v. 201, n. 2, p. 137–142, mar. 2011.

KIM, K. C. et al. Clinical and Neurobiological Relevance of Current Animal Models of Autism Spectrum Disorders. **Biomolecules & Therapeutics**, v. 24, n. 3, p. 207–243, 1 maio 2016.

KIM, S.; LEE, H.; PARK, Y. Perinatal exposure to low-dose imidacloprid causes ADHD-like symptoms: Evidences from an invertebrate model study. **Food and Chemical Toxicology**, v. 110, n. October, p. 402–407, dez. 2017.

KLEIN, M. ET AL. Contribution of intellectual disability-related genes to adhd risk and to locomotor activity in *drosophila*. **The American Journal of Psychiatry**, 2020.

KLIN, A. Autismo e síndrome de Asperger: uma visão geral. **Revista Brasileira de Psiquiatria**, v. 28, n. suppl 1, p. s3–s11, maio 2006.

KOLOZSI, E. et al. Prenatal exposure to valproic acid leads to reduced expression of synaptic adhesion molecule neuroligin 3 in mice. **Neuroscience**, v. 163, n. 4, p. 1201–1210, nov. 2009.

LABANDEIRA-GARCIA, J. L. et al. Brain renin-angiotensin system and

dopaminergic cell vulnerability. **Frontiers in Neuroanatomy**, v. 8, n. 3, p. 226–244, 8 jul. 2014.

LANDRIGAN, P. J.; LAMBERTINI, L.; BIRNBAUM, L. S. A Research Strategy to Discover the Environmental Causes of Autism and Neurodevelopmental Disabilities. **Environmental Health Perspectives,** v. 120, n. 7, p. 2002–2004, jul. 2012.

LEE CHAO, S.; CASIDA, J. E. Interaction of Imidacloprid Metabolites and Analogs with the Nicotinic Acetylcholine Receptor of Mouse Brain in Relation to Toxicity. **Pesticide Biochemistry and Physiology,** v. 58, n. 1, p. 77–88, maio 1997.

LEE, I. et al. Developmental neurotoxic effects of two pesticides: Behavior and neuroprotein studies on endosulfan and cypermethrin. **Toxicology**, v. 335, n. 2015, p. 1–10, 2015.

LEE, S. Y. et al. Ube3a, the E3 ubiquitin ligase causing Angelman syndrome and linked to autism, regulates protein homeostasis through the proteasomal shuttle Rpn10. **Cellular and Molecular Life Sciences**, v. 71, n. 14, p. 2747–2758, 1 jul. 2014.

LIU, J. et al. Prenatal Exposure to the Pesticide Dieldrin or the $GABA_A$ Receptor Antagonist Bicuculline Differentially Alters Expression of $GABA_A$ Receptor Subunit mRNAs in Fetal Rat Brainstem. **Developmental Neuroscience**, v. 20, n. 1, p. 83–92, 1998.

LUKE, L. et al. Decision-making difficulties experienced by adults with autism spectrum conditions. **Autism**, v. 16, n. 6, p. 612–621, 16 nov. 2012.

MACHADO-VIEIRA, R. et al. Neurobiologia do transtorno de humor bipolar e tomada de decisão na abordagem psicofarmacológica. **Revista de Psiquiatria do Rio Grande do Sul**, v. 25, n. suppl 1, p. 88–105, abr. 2003.

MANDY, W.; LAI, M.-C. Annual Research Review: The role of the environment in the developmental psychopathology of autism spectrum condition. **Journal of Child Psychology and Psychiatry**, v. 57, n. 3, p. 271–292, mar. 2016.

MANEV, H.; DIMITRIJEVIC, N.; DZITOYEVA, S. Techniques: fruit flies as models for neuropharmacological research. **Trends in Pharmacological Sciences**, v. 24, n. 1, p. 41–43, jan. 2003.

MARKRAM, H. The intense world syndrome – an alternative hypothesis for

autism. **Frontiers in Neuroscience**, v. 1, n. 1, p. 77–96, 1 nov. 2007.

MATOS, C. H. C.; OLIVEIRA, C. R. F.; SANTOS, M. P. F.; FERRAZ, C. S.. Utilização de modelos didáticos no ensino de entomologia. **Revista de Biologia e Ciências da Terra**, v. 9, n.1, p.19-23, 2009.

MCGURK, L.; BERSON, A.; BONINI, N. M. *Drosophila* as an In Vivo Model for Human Neurodegenerative Disease. **Genetics**, v. 201, n. 2, p. 377–402, 7 out. 2015.

MENGONI, C.; FARINA, W. M. Effects of Sublethal Doses of Imidacloprid on Young Adult Honeybee Behaviour. **Plos One**. p. 1–15, 2015.

MING, X.; BRIMACOMBE, M.; WAGNER, G. C. Prevalence of motor impairment in autism spectrum disorders. **Brain and Development**, v. 29, n. 9, p. 565–570, out. 2007.

MORALES, J. et al. *Drosophila* Fragile X Protein, DFXR, Regulates Neuronal Morphology and Function in the Brain. **Neuron**, v. 34, n. 6, p. 961–972, jun. 2002.

MUÑOZ-SORIANO, V.; PARICIO, N. *Drosophila* Models of Parkinson's Disease: Discovering Relevant Pathways and Novel Therapeutic Strategies. **Parkinson's Disease**, v. 2011, p. 1–14, 2011.

NAKAMURA, K. et al. Brain Serotonin and Dopamine Transporter Bindings in Adults With High-Functioning Autism. **Archives of General Psychiatry**, v. 67, n. 1, p. 59, 1 jan. 2010.

NEALE, B. M. et al. Genome-wide association scan of ADHD. **American Journal Of Medical Genetics Part B Neuropsychiatric Genetics**, v. 147B, n. 8, p. 1337–1344, 2008.

NEALE, B. M. et al. Case-Control Genome-Wide Association Study of Attention-Deficit/Hyperactivity Disorder. **Journal of the American Academy of Child & Adolescent Psychiatry**, v. 49, n. 9, p. 906–920, set. 2010.

NEWSCHAFFER, C. J. National Autism Prevalence Trends From United States Special Education Data. **Pediatrics**, v. 115, n. 3, p. e277–e282, 1 mar. 2005.

NORTOX. Imidacloprid Nortox. Disponível em: <http://www.nortox.com.br/wp-content/uploads/2018/12/Imidacloprid-Nortox-Bula-VER-14-17.07.2018.pdf> acesso

em 06/02/2020.

OBANA, K.; OKIHASHI, M.; AKUTSU, K.; KITAGAWA, Y.; HORI, S. Determination of Neonicotinoid Pesticide Residues in Chromatography Mass Spectrometry. **Journal of Agricultural and Food Chemistry**. p. 2501–2505, 2003.

PALMER, E. D.; FINGER, S. An Early Description of ADHD (Inattentive Subtype): Dr Alexander Crichton and 'Mental Restlessness' (1798). **Child Psychology and Psychiatry Review**, v. 6, n. 2, p. 66–73, 4 maio 2001.

PANCHAL, K.; TIWARI, A. K. *Drosophila melanogaster* "a potential model organism" for identification of pharmacological properties of plants/plant-derived components. **Biomedicine & Pharmacotherapy**, v. 89, p. 1331–1345, maio 2017.

PANDEY, U. B.; NICHOLS, C. D. Human Disease Models in *Drosophila melanogaster* and the Role of the Fly in Therapeutic Drug Discovery. **Pharmacological Reviews**, v. 63, n. 2, p. 411–436, jun. 2011.

PARKER, W. et al. The role of oxidative stress, inflammation and acetaminophen exposure from birth to early childhood in the induction of autism. **Journal of International Medical Research**, v. 45, n. 2, p. 407–438, 16 abr. 2017.

PARRÓN, T. et al. Association between environmental exposure to pesticides and neurodegenerative diseases. **Toxicology and Applied Pharmacology**, v. 256, n. 3, p. 379–385, nov. 2011.

PAVĂL, D. A Dopamine Hypothesis of Autism Spectrum Disorder. **Developmental Neuroscience**, v. 39, n. 5, p. 355–360, 2017.

POLANCZYK, G. et al. The Worldwide Prevalence of ADHD: A Systematic Review and Metaregression Analysis. **American Journal of Psychiatry**, v. 164, n. 6, p. 942–948, jun. 2007.

QUIRÓS-ALCALÁ, L.; MEHTA, S.; ESKENAZI, B. Pyrethroid pesticide exposure and parental report of learning disability and attention deficit/hyperactivity disorder in U.S. children: NHANES 1999–2002. **Environmental Health Perspectives**, v. 122, n. 12, p. 1336–1342, 2015.

ROBERTS, E. M. et al. Maternal Residence Near Agricultural Pesticide Applications and Autism Spectrum Disorders among Children in the California Central

Valley. **Environmental Health Perspectives**, v. 115, n. 10, p. 1482–1489, out. 2007.

ROBERTS, J. R.; DAWLEY, E. H.; REIGART, J. R. Children's low-level pesticide exposure and associations with autism and ADHD: a review. **Pediatric Research**, v. 85, n. 2, p. 234–241, 8 jan. 2019.

ROBERTS, J. R.; KARR, C. J. Pesticide Exposure in Children. **Pediatrics**, v. 130, n. 6, p. e1765–e1788, 1 dez. 2012.

ROHLMAN, D. S.; ANGER, W. K.; LEIN, P. J. Correlating neurobehavioral performance with biomarkers of organophosphorous pesticide exposure. **NeuroToxicology**, v. 32, n. 2, p. 268–276, mar. 2011.

ROSE, S. et al. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. **Translational Psychiatry**, v. 2, n. 7, p. e134–e134, 10 jul. 2012.

ROSSIGNOL, D. A.; FRYE, R. E. Mitochondrial dysfunction in autism spectrum disorders: a systematic review and meta-analysis. **Molecular Psychiatry**, v. 17, n. 3, p. 290–314, 25 mar. 2012.

ROULLET, F. I.; CRAWLEY, J. N. Mouse Models of Autism: Testing **Hypotheses About Molecular Mechanisms**. In: [s.l: s.n.]. p. 187–212.

SADOCK, B. J.; SADOCK, V. A.; RUIZ, P. **Compêndio de psiquiatria**: ciência do comportamento e psiquiatria clínica. 11. ed. Porto Alegre: Artmed, 2017.

SANDIN, S. et al. The Familial Risk of Autism. **JAMA**, v. 311, n. 17, p. 1770, 7 maio 2014.

SCHLICKMANN, E.; FORTUNATO, J. J. O uso de ácido valproico para a indução de modelos animais de autismo: uma revisão. **Jornal Brasileiro de Psiquiatria**, v. 62, n. 2, p. 151–159, jun. 2013.

SCHMIDT, R. J. et al. Combined Prenatal Pesticide Exposure and Folic Acid Intake in Relation to Autism Spectrum Disorder. **Environmental Health Perspectives**, v. 125, n. 9, p. 097007, 22 set. 2017.

SHARMA, R.; RAHI, S.; MEHAN, S. Neuroprotective potential of solanesol in intracerebroventricular propionic acid induced experimental model of autism: Insights from behavioral and biochemical evidence. **Toxicology Reports**, v. 6, n. July, p. 1164–

1175, 2019.

SHATTUCK, P. T. The Contribution of Diagnostic Substitution to the Growing Administrative Prevalence of Autism in US Special Education. **Pediatrics**, v. 117, n. 4, p. 1028–1037, 1 abr. 2006.

SHEETS, L. P. Imidacloprid. In: Hayes' Handbook of Pesticide Toxicology. **Elsevier**, 2010. p. 2055–2064.

SHELTON, J. F. et al. Neurodevelopmental Disorders and Prenatal Residential Proximity to Agricultural Pesticides: The CHARGE Study. **Environmental Health Perspectives**, v. 122, n. 10, p. 1103–1109, out. 2014.

TAUBER, J. M.; VANLANDINGHAM, P. A.; ZHANG, B. Elevated Levels of the Vesicular Monoamine Transporter and a Novel Repetitive Behavior in the *Drosophila* Model of Fragile X Syndrome. **Plos One**, v. 6, n. 11, p. e27100, 2 nov. 2011.

TOMIZAWA, M.; CASIDA, J. E. NEONICOTINOID INSECTICIDE TOXICOLOGY: Mechanisms of Selective Action. **Annual Review of Pharmacology and Toxicology**, v. 45, n. 1, p. 247–268, 22 set. 2005.

VON EHRENSTEIN, O. S. et al. Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: population based case-control study. **BMJ**, p. l962, 20 mar. 2019.

WAN, L. et al. Characterization of dFMR1, a *Drosophila melanogaster* Homolog of the Fragile X Mental Retardation Protein. **Molecular and Cellular Biology**, v. 20, n. 22, p. 8536–8547, 15 nov. 2000.

WANG, X. et al. Maternal diabetes induces autism-like behavior by hyperglycemia-mediated persistent oxidative stress and suppression of superoxide dismutase 2. **Proceedings of the National Academy of Sciences**, v. 116, n. 47, p. 23743–23752, 19 nov. 2019.

WING, L.; POTTER, D. The epidemiology of autistic spectrum disorders: is the prevalence rising? **Mental Retardation and Developmental Disabilities Research Reviews**, v. 8, n. 3, p. 151–161, 2002.

WÖHR, M.; SCATTONI, M. L. Behavioural methods used in rodent models of autism spectrum disorders: Current standards and new developments. **Behavioural Brain**

**Research**, v. 251, p. 5–17, ago. 2013.

YAMAMOTO, I.; CASIDA, J. E. **Nicotinoid insecticides and the nicotinic acetylcholine receptor**. Springer, ed 1999.

YAMAMOTO, S. et al. A *Drosophila* Genetic Resource of Mutants to Study Mechanisms Underlying Human Genetic Diseases. **Cell**, v. 159, n. 1, p. 200–214, set. 2014.