GRANT: Category 1C Return Home Grant / CAEN Grant Round - April 2013

PRINCIPAL INVESTIGATOR: Bruno Rezende Souza

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UNIVERSITY: Universidade Federal de Minas Gerais, Brazil

PROJECT TITLE: The regulation of neural development and behaviour by dopamine signalling

RELEVANT BACKGROUND AND RATIONALE FOR PROPOSAL: Schizophrenia (SCZ) is a major psychiatric disease that affects 1% of the worldwide population. Two dysfunctions are well described in the brains of patients with SCZ: DA-mediated neurotransmission imbalances and decreased brain volume (Souza & Tropepe, 2011). Several studies demonstrated decrease in the levels of Akt and DARPP-32, proteins downstream of DA receptor activation, within prefrontal cortex (PFC) neurons in SCZ (Souza & Tropepe, 2011). Moreover, both Akt and DARPP-32 were shown to be involved in the development of the brain (Arguello & Gogos, 2008, Lai, et al., 2006, Viggiano et al., 2003).

Modulation of the Akt and DARPP-32 pathways by DA was demonstrated in adult mice and primates only (Beaulieu et al., 2007; Morissette et al., 2010; Svenningsson et al., 2004). Therefore, the role of DA-dependent Akt and DARPP-32 modulation during neurodevelopment remains unknown. To address this question, we used the zebrafish model to examine the function of this signalling pathway during development *in vivo*. The use of zebrafish as a model to understand human diseases is becoming a prominent feature in biomedical research (Lieschke & Currie, 2007). One of the major advantages of the zebrafish model for the study of neurodevelopmental disorders is that it allows researchers to combine powerful genetic and genomic resources (mutants, transgenics, etc.) with a relatively simple and identifiable pattern of neuronal circuit development in a brain whose fundamental organization is similar to that of the human brain. Furthermore, since the development of zebrafish is external, it is possible to perform pharmacological approaches to assay development and behaviour in a very narrow time window. Finally, neuronal circuit development in the zebrafish brain occurs at a time when simple sensorimotor behaviours are emerging and easily quantifiable (~3-5 days after fertilization).

Our preliminary results demonstrated for the first time that DA modulates the Akt pathway during zebrafish brain development (Souza et al., 2011). We also described the functional roles of different DA receptors in the Akt cascade regulating zebrafish motor behaviour (Souza et al., 2011). Since Akt plays an important role in the development of the brain, we investigated the functions of DA in the development of neural circuits. We demonstrated that DA regulates the development of the GABAergic system, which is suppressed by D2 receptor (Souza et al., 2011). We also observed alterations in motor behaviour associated with abnormalities in the GABAergic system (Souza et al., 2011). With these results, we posited a theoretical inverted-U model for DA effects in zebrafish

behaviour and GABAergic system development, since both decreases and increases of DA levels had the effect of reducing behaviour as well as the number of GABAergic neurons in the pallium. These results showed a fine functional synergism between DA receptors in the development of the GABAergic system, which is abnormal in the brains of schizophrenics.

HYPOTHESIS: Given that a disruption in DA signalling and brain development may underlie some aspects of SCZ, we hypothesize that dopamine has an important function in the development of neural circuits and behavior.

SPECIFIC RESEARCH AIMS, RESULTS AND CONCLUSIONS:

Aim 1: To characterize the DA intracellular pathways in the developing brain

Rationale: DA receptors are expressed in the zebrafish brain by 3 dpf, and our preliminary data showed that DA regulates Akt signalling in the brain of zebrafish larvae at this time. We also demonstrated that this regulation affects larval motor behaviour. However, DA receptors can regulate not only the Akt pathway, but also the cAMP-PKA-DARPP-32 pathway, which is involved in motor behaviour. Therefore, we studied in detail the modulators of dopaminergic Darpp-32 intracellular pathway in the brain of zebrafish larvae.

Results: We cloned and sequenced the zebrafish *ppp1r1b* gene. We observed that the zebrafish *ppp1r1b* gene is approximately 45% similar to the human, mouse and rat gene orthologues (Table 1). The gene is especially conserved sequence in the PP-1 binding motif and the PKA phosphorylation site. We demonstrated that the *ppp1r1b* gene is expressed in the embryo from the 1st hour post fertilization (hpf) (Figure 1A). We observed a decrease in the Darpp-32 phosphorylation within zebrafish brain after 5 minutes of DA exposure (Figure 1D-G). We then investigated the Darpp-32 expression pattern within 5dpf zebrafish larvae brain by confocal microscopy. We observed that Darpp-32 is highly expressed within pallium, subpallium, preoptic region and rostral hypothalamus (Figure 2).

Conclusion: The *ppp1r1b* gene is relatively conserved during evolution. The zebrafish *ppp1r1b* gene is expressed in the developing brain. Darpp-32 is expressed in dopamine target regions and its activity is regulated by dopamine in the zebrafish larval brain.

Aim 2: Examining the molecular and morphological consequences and resulting behavioural alterations of DA disruption during development

Rationale: We observed that imbalances in DA levels during the 3dpf to 5dpf window affect the morphology of zebrafish larvae brains as well as their motor behaviour, decreasing both the number of GABAergic neurons and the number of movements initiated. We investigated the morphological and behavioral consequence of Darpp-32 downregulation during zebrafish larvae neurodevelopment.

Results: We injected specific Darpp-32 morpholinos (MO) – ATG and Exon-MOs, in the 2-cells stage embryos. We observed a downregulation of Darpp-32 in the brain of 3 dpf larvae (Figure 1B) and a

little downregulation of Darpp-32 expression in the 5 dpf larval brain (Figure 1C). At the age of 3 dpf, we did not see any alterations in the number of dopaminergic and GABAergic neurons within the brain of zebrafish larvae injected with Darpp-32 MOs (Figure 3). On the other hand, there is an increase in the number of dopaminergic neurons within the subpallium (Figure 4) and a decrease in the number of GABAergic neurons within the pallium and subpallium (Figure 5) of 5 dpf zebrafish larvae. We also demonstrated that the decrease of Darpp-32 alters the motor behavior of zebrafish larvae (Figure 6).

Conclusion: The decrease of Darpp-32 affects the development of dopaminergic and GABAergic systems and the motor behavior of zebrafish larvae.

Figures

Map Positions of Zebrafish and Orthologous Human, Rat and Mouse DARPP-32 Genes*

Organism	Accession Number	Location	Similarity to *Danio rerio*[a]
Danio rerio	NC_007130.6	19	100
Mus musculus	Q60829	11	44
Rattus norvegicus	Q6J4I0	10	45
Homo sapiens	Q9UD71	17q12	46

* Source of sequences http://www.ncbi.nlm.nih.gov/gene; [a] Numbers represent % aminoacid similarity to the *Danio rerio* DARPP-32 (http://www.uniprot.org/).

Table 1 – Comparison of the zebrafish *ppp1r1b* gene with mouse, rat and human *ppp1r1b*.

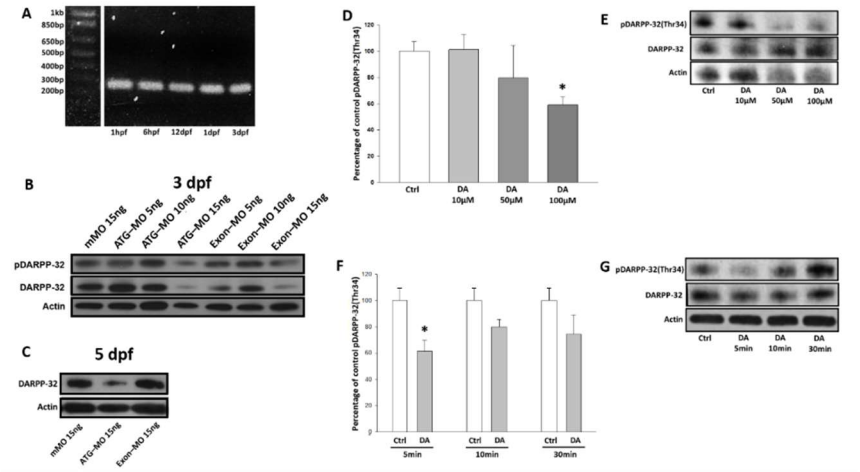

Figure 1 – Expression and regulation of Darpp-32 activity in the zebrafish larvae brain.

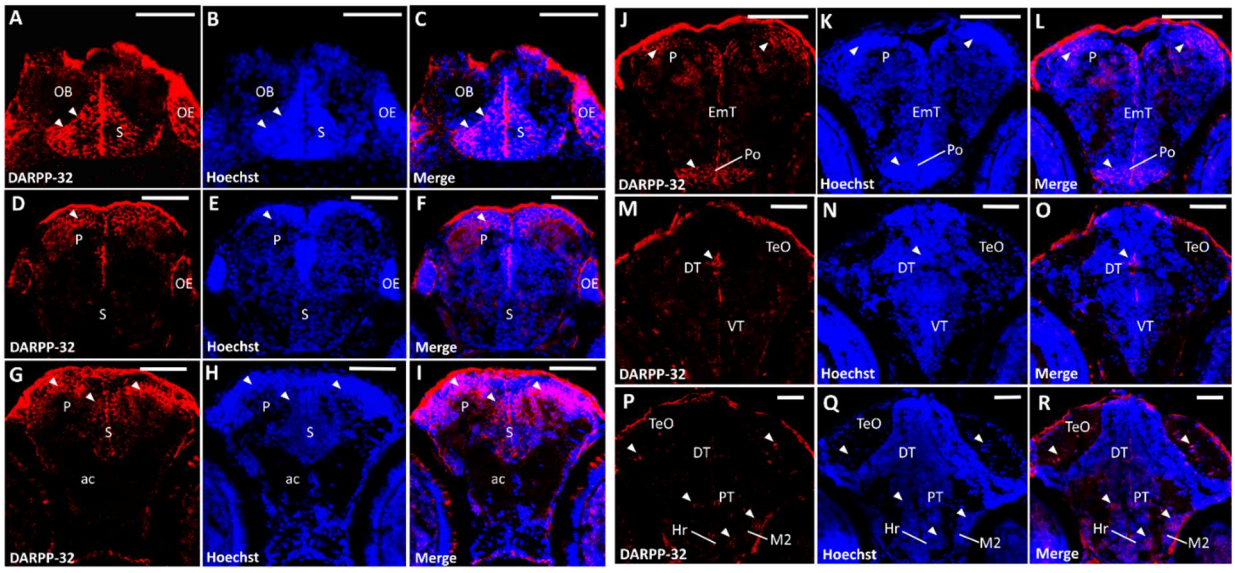

Figure 2 – Detailed expression of Darpp-32 throughout 5dpf zebrafish larvae brain.

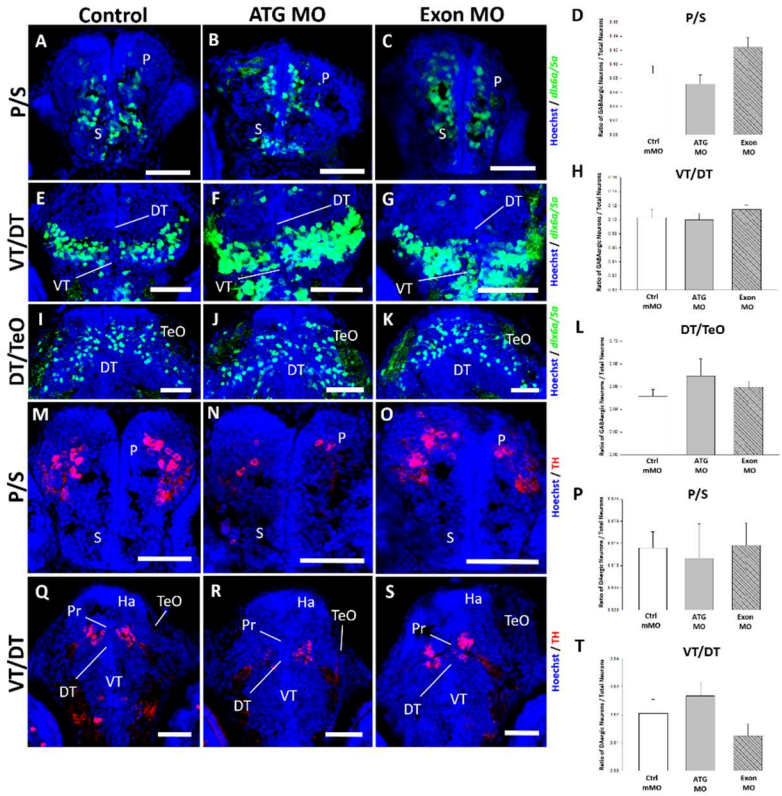

Figure 3 - Darpp-32 downregulation did not change the development of dopaminergic and GABAergic systems in the brain of 3 dpf larvae.

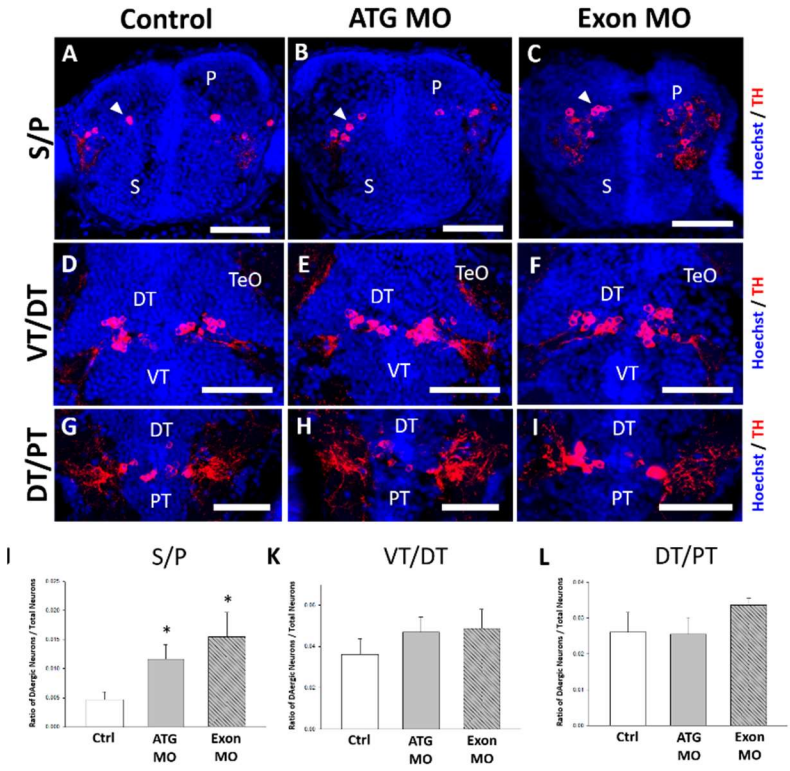

Figure 4 – Darpp-32 downregulation increase the number of dopaminergic neurons within 5 dpf zebrafish pallium.

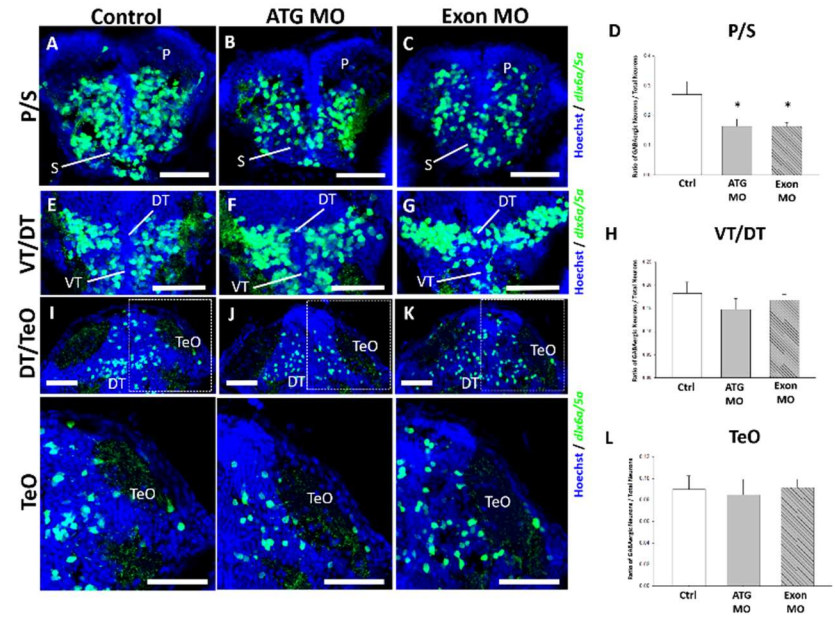

Figure 5 – Downregulation of Darpp-32 decrease the number of GABAergic neurons within 5 dpf zebrafish pallium and subpallium.

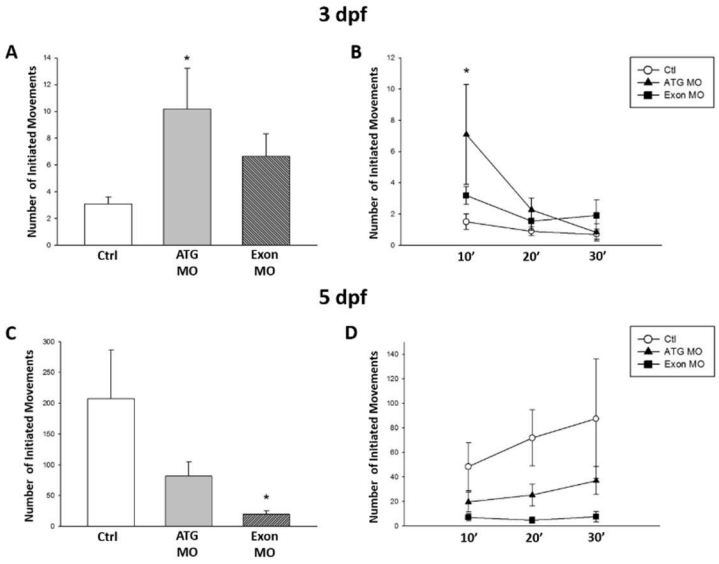

Figure 6 – Darpp-32 downregulation changes the motor behavior of zebrafish larvae.

OTHER PROJECTS SUPPORTED BY CATEGORY 1C RETURN HOME GRANT

To evaluate the role of dopamine in the postnatal brain development, we injected L-Dopa + Benserazide intraperitoneally in male and female mice in the first 5 days of life. We evaluated motor behavior, anxiety-like behavior, depression-like behavior and reward behavior when they were 4 and 8 weeks old. We observed a sexually dimorphic role of dopamine in the development of mice behaviors.

OTHER GRANTS

Universal CNPq - 480260/2012-5 – R$21,000

Universal FAPEMIG - APQ-01896-13 – R$28,000

PRPq UFMG – R$7,000

MENTORSHIP

Doctorate students

Name: Muiara Aparecida Moraes

Date: 2015 / present

Topic: Investigation of the effects of L-Dopa chronic treatment in neonate mice on subsequent cannabinoid system in adulthood

Masters students

Name: Ana Luiza Araujo Lima Reis

Date: 2015 / present

Topic: Investigation of the intracellular pathways regulated by dopaminergic receptors in the brain of embryo mice

Name: Ana Carolina Monteiro de Souza Lima

Date: 2014 / present

Topic: Study of the effects of dopaminergic imbalance in early development of zebrafish larvae on subsequent behavior in later stages

Name: Lorena Oliveira de Matos

Date: 2014 / 2016

Topic: Investigation of the effects of L-Dopa chronic treatment in neonate mice on subsequent behavior in adulthood

Undergraduates training

Name: Larissa de Oliveira
Date: 2015 / present

Name: Laila Árabe
Date: 2015 / present

Name: Beatriz Codo
Date: 2015 / present

Name: Lorena Terene
Date: 2014 / present

Name: Luiza Elias Coutinho
Date: 2015 / 2016

Name: Iago Souza Wolf
Date: 2013 / 2015

Name: Lilia Gama de Pinho
Date: 2014 / 2015

Name: Ana Luiza Araujo Lima Reis
Date: 2014

Name: Tomaso Zanato Francia
Date: 2013

Name: Nair Gyselle Gonçalves
Date: 2013 / 2014

Name: Iago Junqueira Simoes
Date: 2013 / 2014

Name: Lucas de Oliveira Ribeiro

Date: 2013 / 2014

BOOK CHAPTER

Souza, RP; **Souza, BR**. Lewy Body Dementia and Frontotemporal Dementia. In: Glaucia Noeli Maroso Hajj. (Org.). Young Perspectives for Old Diseases. 1ed.: Bentham Books, v. , p. 251-267. 2015

MANUSCRIPT SUBMITTED OR IN PREPARATION FOR PEER REVIEW

Souza, BR; Tropepe, V.; Romano-Silva, MA. DARPP-32 is regulated by dopamine and it regulates the development od GABAergic system. Submitted.

Wolff, IS; Pinho, LG; Sacramento, EK; Rosa, DV; Romano-Silva, MA; **Souza, BR**. Zebrafish larvae submitted to Haloperidol chronic treatment shows a decrease of motor behavior. In preparation.

Matos, LO; Guarnieri, LO; Reis, ALAL; Terene, L; Pereira, GS; **Souza, BR**. Behavioral changes in young and adult mice undergoing dopaminergic insult after birth. In preparation.

PAPERS PRESENTED AT MEETINGS OR SYMPOSIA:

Matos, L.O.; Lima, A.L.A.; Guerra, L.T.L; Guarnieri, L.O.; Pereira, G.S.; **Souza, B.R**. Evaluation of postnatal stress and L-Dopa treatment in the development of mice behavior. II International Symposium on Brain Origins of Diseases, Ouro Preto, 2015

Lima, A.L.A.; Matos, L.O.; Guerra, L.T.L.; Guarnieri, L.O.; Pereira, G.S.; **Souza, B.R.** Sexually dimorphic role of dopamine in the development of mice motor behavior. II International Symposium on Brain Origins of Diseases, Ouro Preto, 2015

MORAES, M. A.; ARABE, L. B.; MOREIRA, F. A.; **SOUZA, B. R**. L-Dopa treatment decreases motor behavior in female mice but not males. II International Symposium on Brain Origins of Diseases, Ouro Preto, 2015

SOUZA, B. R. ; TROPEPE, V. ; ROMANO-SILVA, M.A. . DARPP-32 is regulated by dopamine in the developing zebrafish brain. In: IBRO Conference, 2015, Rio de Janeiro.

MATOS, L. O. ; GUARNIERI, L. O. ; PEREIRA, G. S. ; **SOUZA, B. R**. . Behavioral changes in young and adult mice undergoing dopaminergic insult after birth. In: I Jornada de Neurociências da UFMG, 2014, Belo Horizonte. I Jornada de Neurociências, 2014.

MATOS, L. O. ; GUARNIERI, L. O. ; PEREIRA, G. S. ; **SOUZA, B. R**. . Behavioral changes in young and adult mice undergoing dopaminergic insult after birth. In: XXII Encontro em Fisiologia e Farmacologia, 2014, Jaboticatubas. XXII Encontro em Fisiologia e Farmacologia, 2014.

SOUZA, B. R. ; TROPEPE, V. ; ROMANO-SILVA, M.A. . DARPP-32 is regulated by dopamine in the developing zebrafish brain. In: XXXVIII Reunião Anual da SBNeC, 2014, Búzios. XXXVIII Reunião Anual da SBNeC, 2014.

PINHO, L. G. ; WOLFF, I. S. ; SACRAMENTO, E. K. ; ROMANO-SILVA, M. A. ; **SOUZA, B. R**. . Efeito do tratamento crônico com Haloperidol no desenvolvimento de larvas de zebrafish. 2014, Belo Horizonte. Semana do Conhecimento e Semana de Iniciação Científica da UFMG, 2014.

WOLFF, I. S. ; PINHO, L. G. ; SACRAMENTO, E. K. ; ROMANO-SILVA, M. A. ; **SOUZA, B. R.** . Efeitos do tratamento crônico com Haloperidol no comportamento motor da larva de zebrafish. Semana do Conhecimento e Semana de Iniciação Científica da UFMG, 2014.

RIBEIRO, L. O. ; SACRAMENTO, E. K. ; ROMANO-SILVA, M. A. ; **SOUZA, B. R**. . Efeitos do tratamento crônico com DMSO no desenvolvimento de larvas de zebrafish. Semana do Conhecimento e Semana de Iniciação Científica da UFMG, 2014.

SIMOES, I. J. ; SACRAMENTO, E. K. ; ROMANO-SILVA, M. A. ; **SOUZA, B. R**. . Efeitos do tratamento com Metilfenidato na regulação da sinalização da via da Akt e da DARPP-32 em um cérebro em desenvolvimento. Semana do Conhecimento e Semana de Iniciação Científica da UFMG, 2014.

SOUZA, B. R. ; TROPEPE, V. ; ROMANO-SILVA, M. A. . The role of dopamine signalling in the GABAergic neuron development and motor behavior in zebrafish larvae. In: Congresso da Sociedade Brasileira de Neurociências e Comportamento (SBNeC), 2013, Belo Horizonte. Congresso da Sociedade Brasileira de Neurociências e Comportamento (SBNeC), 2013.

INVITED LECTURES:

2015 – “Dopamine, Neurodevelopment and Behavior”
II International Symposium on Brain Origins of Diseases
Ouro Preto, Brasil

2015 – “Dopamine, Neurodevelopment and Behavior”
Center for Addiction and Mental Health (CAMH)
Toronto, Canadá

2015 – “Dopamine, Development and Behavior”
Universidade Federal do Rio Grande do Norte
Natal, Brazil

2015 – “Dopamine: from development to behavior”
Universidade Federal de Minas Gerais
Belo Horizonte, Brazil

2014 - “Dopamine and Neurodevelopment: One swam under the zebrafish nest”
VII International Symposium of UFMG
Belo Horizonte, Brazil

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Lieschke, G. J., & Currie, P. D. (2007). Animal models of human disease: zebrafish swim into view. *Nat Rev Genet, 8*(5), 353-367

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Souza BR, Tropepe V. 2011. The role of dopaminergic signalling during larval zebrafish brain development: a tool for investigating the developmental basis of neuropsychiatric disorders. *Rev Neurosci* 22, 1:107-19.

Souza BR, Romano-Silva MA, Tropepe V. 2011. Dopamine D2 receptor activity modulates Akt signaling and alters GABAergic neuron development and motor behavior in zebrafish larvae. *J Neurosci* 31, 14:5512-25

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Viggiano D, Ruocco LA, Sadile AG. 2003. Dopamine phenotype and behaviour in animal models: in relation to attention deficit hyperactivity disorder. Neurosci Biobehav Rev. 2003 Nov;27(7):623-37.