

INDEX

GENERAL INTRODUCTION	28
<i>Schizophrenia: a connectivity disorder.....</i>	<i>28</i>
<i>The strong genetic component in the schizophrenia disorder: The NRG1 risk gene.....</i>	<i>30</i>
<i>NRG1 hypotheses of schizophrenia</i>	<i>31</i>
<i>NRG1 and ERBB genes and proteins</i>	<i>32</i>
<i>Nrg1 and ErbB4 signaling pathways</i>	<i>35</i>
Nrg1 backward signaling	35
Nrg1/ErbB canonical forward signaling.....	37
Nrg1/ErbB non-canonical forward signaling.....	37
<i>Nrg1 functions in the Central Nervous System.....</i>	<i>38</i>
Neuronal migration	38
Axon guidance.....	38
Axon myelination	39
Dendrite development	40
Synapse formation and excitatory-inhibitory circuitry homeostasis	40
Neuronal survival.....	42
<i>Nrg1 as a potential therapeutic target</i>	<i>43</i>
OBJECTIVES	45
MATERIALS AND METHODS	46
<i>Animals.....</i>	<i>46</i>
<i>Nrg1 and astrocyte reprogramming constructs.....</i>	<i>46</i>
<i>Primary neuronal culture and neurons transfection by electroporation.....</i>	<i>47</i>
<i>Neuronal transfection and mitochondrial staining</i>	<i>49</i>

<i>Primary astrocyte culture</i>	49
<i>Astrocytes retroviral transduction for direct neuronal reprogramming</i>	50
<i>Immunofluorescence</i>	50
<i>Nes-Cre mice characterization (E15 and P0): RNA extraction and RT-qPCR</i>	51
<i>Protein extraction from neuronal cultures, Western blot and quantification</i>	53
<i>Anterograde tracing of the callosal projections and histology</i>	54
<i>In utero electroporation and histology</i>	55
<i>Imaging</i>	56
<i>Image analysis</i>	57
<i>Statistical analysis</i>	59

CHAPTER 1: PRIMARY NEURONAL CULTURES FOR NEUROPATHOLOGIES

STUDIES..... 61

<i>Introduction</i>	62
Primary neuronal cultures: internal controls and sparse labeling.....	62
Primary neuronal cultures to study neuronal survival.....	64
Mutations in PRDX3 cause an infantile onset ataxia: a case study as an example of applicability	64
<i>Results</i>	66
Mutations in PRDX3 cause an infantile onset ataxia: a case study as an example of applicability	66
<i>Discussion</i>	69

CHAPTER 2: NRG1 INTRACELLULAR SIGNALING REGULATES THE DEVELOPMENT OF INTERHEMISPHERIC CALLOSAL CONNECTIONS..... 71

<i>Introduction</i>	73
Schizophrenia and Nrg1	73
The corpus callosum is the main interhemispheric connection in the brain .	74
<i>Results</i>	76

Nrg1 is required for the development of cortico-cortical projecting axons in vivo.....	76
Nrg1 is cell-autonomously required for the growth of cortical axons in vitro.	79
The Nrg1 intracellular signaling is sufficient to promote axonal growth in vitro	82
Nrg1 signaling enhances the elongation of callosal axons in vivo	85
<i>Discussion</i>	89
 CHAPTER 3: GAP43 IS A PUTATIVE EFFECTOR OF NRG1 SIGNALING IN AXONAL GROWTH.....	92
<i>Introduction</i>	93
Main signaling pathways involved in axon development and regeneration ...	93
Growth-Associated Protein 43: a key protein in neuron development.....	94
<i>Results</i>	96
Effect of Nrg1 loss on axonal growth pathways: GAP43 protein level is impaired in Nrg1 KO mice	96
Nrg1 does not regulate GAP43 mRNA expression	98
GAP43 expression is decreased in the axons of Nrg1-deficient neurons in vitro	99
GAP43 expression rescues axonal growth in Nrg1-deficient neurons	100
<i>Discussion</i>	103
 CHAPTER 4: NRG1 ROLE IN REPROGRAMMED NEURONS AS A POTENTIAL THERAPEUTIC TARGET	105
<i>Introduction</i>	106
Brain damage: etiology and treatments	106
Direct neuronal reprogramming as a novel therapeutic approach following brain injury	106
Nrg1 as an emerging target in CNS repair: Nrg1 function in the Neuronal Stem Cells.....	108
<i>Results</i>	110

Nrg1 role in astrocyte-to-neuron reprogramming efficiency.....	110
Nrg1 role in reprogrammed neurons development.....	112
<i>Discussion</i>	114
GENERAL DISCUSSION	117
<i>Cortical wiring in the etiology of SZ</i>	117
<i>The role of the SZ-risk gene NRG1</i>	117
<i>Nrg1 intracellular signaling in the excitatory neurons and corpus callosum....</i>	118
<i>Nrg1 downstream mechanisms: a role for GAP43</i>	120
<i>Nrg1 signaling in astrocyte-to-neuron reprogramming</i>	120
<i>Main conclusions</i>	121
CONCLUSIONS	122
ANEXES	123
REFERENCES	129