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Efecto de la melatonina sobre el sistema nervioso central para disminuir los efectos metabólicos adversos de la olanzapina

Tesis

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Resumen de Tesis

Los pacientes con enfermedades mentales como la esquizofrenia y el trastorno bipolar, muestran una elevada comorbilidad con enfermedades metabólicas. En ellos, el riesgo de problemas metabólicos es hasta 2 o 3 veces mayor que en la población general y la muerte se produce 11 a 20 años antes, causada principalmente por enfermedades cardiovasculares. Algunos medicamentos para tratar estas enfermedades mentales, como los antipsicóticos de segunda generación (ASG), incrementan aún mas el riesgo cardiovascular debido a que inducen efectos metabólicos adversos (EMA). Estos EMA inducidas por ASG se asemejan al síndrome metabólico, para el que se ha propuesto un desequilibrio autonómico central que puede originarse en el núcleo supraquiasmático (NSQ) del hipotálamo.

Recientemente se ha descrito que la melatonina, una hormona secretada en la glándula pineal que sincroniza la actividad de nuestro reloj biológico, atenúa los EMA inducidos por ASG en ratas; lo que sugiere un papel para el reloj biológico en su origen y control.

Por lo tanto, propusimos la hipótesis de que la melatonina podría atenuar los EMA inducidos por ASG en humanos. En este ensayo clínico, surgieron cuatro hallazgos principales: 1) Los pacientes tratados con ASG y melatonina mostraron una disminución de la tensión arterial diastólica en comparación con los que recibieron ASG y placebo; 2) La melatonina mostró un efecto metabólico particularmente benéfico en pacientes con trastorno bipolar en términos de masa grasa y tensión arterial diastólica; cambios que no se observaron en el grupo con diagnóstico de esquizofrenia; 3) La

melatonina mostró efectos metabólicos variados en función del perfil de riesgo metabólico del ASG; 4) La melatonina no altera el efecto de los ASG con respecto a la severidad de la psicopatología.

Hasta donde sabemos, este es el primer ensayo clínico donde se evaluaron los efectos metabólicos de la melatonina en pacientes tratados con ASG y demuestra que el tratamiento con melatonina es particularmente benéfico en el trastorno bipolar.

Debido a que el blanco principal de la melatonina en el cerebro es el NSQ, y que este núcleo está fuertemente involucrado en el control metabólico, estos resultados básicos y clínicos sugieren la participación de mecanismos centrales de regulación metabólica en los EMA inducidos por ASG. Por lo tanto, en un modelo de rata examinamos el efecto de la olanzapina (un ASG) en núcleos hipotalámicos involucrados en la regulación metabólica y encontramos una acción hasta ahora desconocida de la olanzapina sobre el reloj biológico, que resulta en una disminución de la tensión arterial. Este efecto de la olanzapina fue impedido por la melatonina, mientras que su efecto en otras regiones del cerebro asociadas con su acción terapéutica permanecieron intactos. Estos hallazgos proporcionan una base lógica para combinar el tratamiento de ASG con melatonina.

Con estos datos clínicos y básicos, proponemos que el hipotálamo y el sistema nervioso autónomo (SNA) pueden ser un eslabón importante para entender la alta comorbilidad entre los trastornos del estado de ánimo y la enfermedad metabólica. En una revisión, hemos reunido evidencia que muestra que los síndromes afectivos se componen de síntomas y características que indican disfunción hipotalámica y del SNA, la cual también está presente en los trastornos metabólicos comórbidos. Exponemos que los trastornos del estado de ánimo y las enfermedades metabólicas como la

obesidad, el síndrome metabólico, la hipertensión y la diabetes mellitus comparten una interrupción crónica y / o intermitente en la comunicación cerebro / cuerpo donde el hipotálamo y el SNA juegan un papel importante. Nuestros resultados y esta perspectiva podrían favorecer una mejor comprensión de la comorbilidad metabólica en los trastornos mentales, contribuir a implementar acciones preventivas y desarrollar nuevos tratamientos para los millones de pacientes que sufren de ellos.

Thesis Abstract

Patients with mental illness such as schizophrenia and bipolar disorder, show an elevated comorbidity with metabolic illness. Their risk for metabolic abnormalities is up to 2 or 3 times that of the general population and cardiovascular disease is the leading cause of death; which occurs 11 to 20 years earlier. Cardiovascular risk in these patients is greatly increased by the use of drugs to treat them such as second generation antipsychotics (SGA), because they induce adverse metabolic effects (AME). These metabolic abnormalities resemble a metabolic syndrome for which a central autonomic imbalance has been proposed that may originate from the hypothalamic suprachiasmatic nucleus.

Recently it was described that melatonin; a hormone secreted in the pineal that synchronizes the activity of our biological clock, attenuates SGA induced AME in rats; suggesting a role for the biological clock in their origin and control.

Thus, in a clinical trial we hypothesized that melatonin could attenuate SGA induced adverse metabolic effects. Indeed, four main findings emerged; 1) Patients receiving SGA and melatonin showed a decrease in diastolic blood pressure compared to patients allocated in the placebo group, 2) Melatonin showed a particularly beneficial metabolic effect in bipolar disorder patients in terms of fat mass and diastolic blood pressure; changes that were not observed in the schizophrenia group, 3) Melatonin had varied metabolic effects depending on the SGA metabolic risk profile, 4) Melatonin did not affect symptom severity outcome measures in SGA treated subjects. To the best of

our knowledge, this is the first clinical study to evaluate the metabolic effects of melatonin in patients treated with SGA, demonstrating that melatonin treatment is particularly beneficial in bipolar disorder.

Because the main target of melatonin in the brain is the SCN, and this nucleus is strongly involved in metabolic control, these basic and clinical results could suggest the involvement of central mechanisms in the metabolic regulation of SGA induced AME. Therefore, we examined in a rat model the effect of the SGA olanzapine on hypothalamic nuclei relevant to metabolic regulation and uncovered a hitherto unknown action of olanzapine on the biological clock resulting in a decrease in blood pressure. Notably this effect of olanzapine was prevented by melatonin while the effects of olanzapine on other brain regions associated with its anti-psychotic effects remained intact, providing a rationale to combine the treatment of SGA with melatonin.

With our clinical and basic data, we propose that the hypothalamus and the autonomic nervous system are an important link to understand the elevated comorbidity between mood disorders and metabolic illness. In a review, we gathered evidence illustrating that affective syndromes are composed of symptoms and features that indicate hypothalamic and ANS dysfunction; also present in metabolic disorders to which they are comorbid. We set forth that metabolic comorbidity in mood disorders such as obesity, the metabolic syndrome, hypertension, and diabetes mellitus share a chronic and/or intermittent disruption in brain/body communication where the hypothalamus and ANS play a major role. This perspective could favor a better understanding of metabolic comorbidity in mental disorders and contribute to implement preventive actions and develop new treatments for the millions of patients who suffer from them.

Abbreviations

Abbreviations

Adverse Metabolic Effects- AME

Adult Treatment Panel III- ATPIII

Analyses of Covariance- ANCOVA

Analyses of Variance- ANOVA

Autonomic nervous system – ANS

Bipolar Disorder- BPD

Blood Pressure- BP

Body Mass Index-BMI

Calgary Depression Scale- CDSS

cAMP response element binding protein- CREB

Cardiovascular Disease – CVD

Central nervous system - CNS

Cholera Toxin B- CtB

Clinical Global Impression Severity of Illness Scale- CGI-S

Consolidated Standards of Reporting Trials- CONSORT

Dorsal Motor Nucleus of the Vagus- DMV

Fibroblast Growth Factor 21- FGF21

Gastroesophageal Reflux Disease- GERD

Glycogen Kinase Synthase 3 Beta- GSK-3B

Hamilton Depression Rating Scale- HDRS

Hypothalamus- Pituitary- Adrenal – HPA

Intermediolateral column- IML

Intraperitoneal- I.P.

Major depressive disorder- MDD

Manic/hypomanic symptoms- M/h

Melatonin- Mel

Norepinephrine- NE

Nucleus Accumbens- N. Acc

Nucleus of the Tractus Solitarius- NTS

Olanzapine- Olz

Paraventricular Nucleus – PVN

Positive and Negative Syndrome Scale- PANSS

Randomized Clinical Trial - RCT

Standard Deviation - S.D.

Suprachiasmatic nucleus – SCN

Suprachiasmatic Nucleus Bilateral Lesion- SCNxx

Second Generation Antipsychotic – SGA

Serotonin and Noradrenaline Reuptake Inhibitors – SNRI

Vasointestinal Peptide- VIP

Ventral Tegmental Area- VTA

Young Mania Rating Scale- YMRS

Zeitgeber- Zt

1. INTRODUCTION

Schizophrenia and mood disorders such as bipolar disorder are severe and chronic mental disorders. Patients suffering these mental disorders present circadian rhythm dysfunction (Murray and Harvey, 2010; Novakova et al., 2015; Wulff et al., 2012) and suffer an elevated risk for obesity, metabolic syndrome, diabetes mellitus, dyslipidemia and other metabolic disturbances(Bowie et al., 2010; Correll et al., 2014b; Vancampfort et al., 2013b). Second Generation Antipsychotics (SGA) have a demonstrated efficacy in acute and long term treatment of these disorders and are considered a first option on most treatment guidelines.(Davis et al., 2003; Gaebel et al., 2005; Leucht et al., 2013; Yatham et al., 2013) Unfortunately, SGA induce adverse metabolic effects such as weight gain, disturbed glucose, lipid and blood pressure regulation that resemble the metabolic syndrome; which has been associated to hypothalamic and autonomic dysfunction.(Kreier et al., 2003) These drug induced adverse metabolic effects become a cause for non-adherence to treatment and increased cardiovascular risk.(Lieberman et al., 2005b; Vancampfort et al., 2013b) Mortality in these patients often reaches levels that double or triple the rates reported for the general population, and cardiovascular disease is one of the main causes of mortality and morbidity.(Brown, 1997; Garcia-Portilla et al., 2009; Kilbourne et al., 2007) The origin of SGA induced adverse metabolic effects is poorly understood and there is an urgent need to find new options to control them.

1.1 The SGA and their adverse metabolic effects

For more than 20 years, SGA have replaced First Generation Antipsychotic (FGA) treatment for a wide variety of mental disorders. The superiority of SGA over FGA in treatment efficacy has not been demonstrated. However, the change in prescription from FGA and SGA can be explained in part by a SGA decreased risk to induce tardive dyskinesia, hyperprolactinemia, and extrapyramidal symptoms that were frequent with FGA. The other part of the explanation is the marketing effort by the pharmaceutical industry to position the new drugs in the clinical setting. (Naber and Lambert, 2009)

SGA include drugs like olanzapine, clozapine, quetiapine and risperidone among others. They are currently prescribed more than 9 million times each year and each drug has annual sales that exceed $1 billion in the U.S. Their use continues to expand in number of prescriptions and off label use for neuropsychiatric disorders.(Alexander et al., 2011) The SGA show small but consistent differences in their efficacy for the treatment of schizophrenia, bipolar disorder and major depressive disorder and significantly contribute to achieve remission and avoid relapse. (Dossenbach et al., 2005; Yatham et al., 2013) Unfortunately, virtually all of these drugs induce adverse metabolic effects. (McDonagh et al., 2010)

Antipsychotic induced adverse metabolic effects include weight gain(Bak et al., 2014) glucose and insulin disturbances, increase in cholesterol and triglycerides(Rummel-Kluge et al., 2010), as well as cardiovascular side effects and the metabolic syndrome.(Mitchell et al., 2013a) Metabolic side effects are a common cause for non-adherence to treatment (Wong et al., 2011), often indicated for prolonged periods of

time generating an additional tremendous health problem for patients that use SGA acutely or chronically.

The use of SGA almost doubles the risk to suffer the metabolic syndrome(Vancampfort et al., 2013b); A metabolic cluster of risk factors that includes obesity, increases in blood pressure, lipids and glucose.(2001) The metabolic syndrome is associated to a two fold increase in cardiovascular risk.(Mottillo et al., 2010) The origin of the metabolic syndrome is multi-factorial and It has recently been proposed that the hypothalamus and the autonomic nervous system play a fundamental role in its origin.(Kreier et al., 2003) SGA induced metabolic disturbances closely resemble the characteristics of the metabolic syndrome and could share provenience.

SGA induced metabolic disturbances directly correlate with illness and treatment duration. (Correll et al., 2014b) Each SGA has a different risk to generate adverse metabolic effects. Olanzapine for example, is one of the most efficacious SGA but induces severe adverse metabolic effects.

1.2. Olanzapine is one of the most widely used SGA, but induces severe adverse metabolic effects

The first clinical experience with olanzapine was reported in 1997 when it was successfully used to treat patients with schizophrenia. As part of the SGA drug group, olanzapine offered an improved profile over negative symptoms of schizophrenia, as well as extrapyramidal side effects such as dyskinesia and Parkinsonism compared to first generation antispychotics like haloperidol. (Baldwin and Montgomery, 1995) Moreover, olanzapine showed no risk for agranulocytosis, one of the greatest concerns

for the use of clozapine. It did not induce hyperprolactinemia as risperidone; the first choice SGA treatment for schizophrenia at the time. Olanzapine is currently used alone or in combination with other drugs to treat mood disorders such as bipolar disorder and depression and other mental disorders like schizophrenia.(Cristancho and Thase, 2014; Leucht et al., 2013; Yatham et al., 2013)

Olanzapine is a thiobenzodiazepine that has molecular similarities with clozapine, the ruling SGA for treatment resistant schizophrenia. The mechanism of action for olanzapine is complex and still poorly understood. Olanzapine shows high affinity and antagonistic effects for dopamine (D1 and D2), serotonin (5-HT2A, 5-HT2B AND 5-HT2C), histamine (H1), muscarinic (M1, M2, M3 and M5) and adrenergic (alpha1) receptors.(Bymaster et al., 1999; Nyberg et al., 1997) Olanzapine also acts on several brain regions that have been linked to its therapeutic effects such as the nucleus accumbens, striatum, cingulate cortex, ventral tegmental area and the paraventricular nucleus in the hypothalamus.(Kiss et al., 2010; Robertson and Fibiger, 1996; Sebens et al., 1998; Zhao and Li, 2012)

The molecular actions of olanzapine are yet to be unraveled, but several intracellular signaling pathways seem to be involved. A putative intracellular mechanism is through the glycogen kinase synthase 3 beta (GSK-3B), an enzyme where relevant signaling pathways converge for gene regulation, cellular survival, neurogenesis, dendrite formation, neurotransmission and metabolism.(Beaulieu et al., 2007; Freyberg et al., 2010) In mammals, GSK-3B exhibits a circadian oscillation in its activity and plays a role in the organization of circadian clock function. It is most active during the day and least active at night. The inhibition of GSK 3B delays and its activation advances the phase of rhythmic clock gene expression.(Iitaka et al., 2005) GSK-3B plays a fundamental role in

the regulation of the cAMP response element binding protein (CREB) by phoshorylation; which stimulates c-fos transcription (a marker of neuronal activity).(Ginty et al., 1994; Grimes and Jope, 2001) A reduced phosphorylation (increased activity) of GSK-3B is now a focus of research in the pathophysiology of mood disorders.(de Bartolomeis et al., 2014) Interestingly lithium and valproate, two of the most effective and widely used mood stabilizers; as well as olanzapine; increase GSK-3B phosphorylation, decreasing its activity.(Aubry et al., 2009; Ferreira et al., 2015; Iwahana et al., 2004)

Unfortunately, olanzapine induces severe adverse metabolic effects such as weight gain, disturbance in the metabolism of glucose, insulin and lipids. (Leucht et al., 2013; Lieberman et al., 2005a) Children and adolescents that have been treated with SGA including olanzapine also show increased risk for incident diabetes mellitus. (Rubin et al., 2015) Olanzapine induced metabolic abnormalities can be documented in acute administration settings both in human(Choure et al., 2014; Hahn et al., 2013) and animal models (Leung et al., 2014) and cannot be explained solely on the basis of increased food intake and decreased locomotor activity. The causes for SGA induced adverse metabolic effects are still a puzzle to solve.

One of the most investigated putative mechanisms for olanzapine induced adverse metabolic effects is H1 receptor antagonism. H1 antagonism induces food intake in rodents and rodents with H1 knockout are prone to obesity. Also, not only SGA, but also antidepressants (i.e; mirtazapine) that show H1 antagonism properties induce adverse metabolic effects such as weight gain. (He et al., 2013) In rat models, H1 receptor agonist betahistine decreases antipsychotic induced weight gain. Olanzapine increases hypothalamic H1R protein levels, as well as pAMPK-alpha, AMPK-alpha and NPY protein levels, while reducing hypothalamic POMC, and brown adipose tissue UCP1

and PGC-1 alpha protein levels. Co administration of Betahistine reversed changes in hypothalamic H1R,, pAMKalpha, and BAT UCP1 and PGC-1 alpha protein levels. (Lian et al., 2014) Recently, a study found that olanzapine induces activation of the AMP activated protein Kinase in the dorsal motor nucleus of the vagus (DMV) and an H1 receptor agonist reduced olanzapine induced weight gain. These findings indicate that hypothalamic structures and the autonomic nervous system in its parasympathetic branch are involved in olanzapine induced adverse metabolic effects. Unfortunately in humans no randomized controlled clinical trials have been published using betahistine to control SGA induced adverse metabolic effects.

Another suspect for olanzapine induced adverse metabolic effects is the GSK-3B; which as mentioned above, is involved in its therapeutic effect and also plays a role in insulin signaling pathways for metabolic regulation.(Girgis et al., 2008) Unfortunately, this has not been studied. Currently, peripheral and central findings have failed to provide an integrative explanation of how SGA induce metabolic abnormalities and how to control them.

Now there is more evidence that olanzapine alters the functioning of the autonomic nervous system (ANS) and that this effect could be linked to its adverse metabolic effects; supporting the shifting equilibrium hypothesis for metabolic dysruption by Kreier, et al. In which it was proposed that a shifting equilibrium from activity (sympathetic activity) to food (parasympathetic activity) leads to autonomic imbalance and the metabolic syndrome.(Kreier et al., 2003) The proposal is based on the fact that the ANS is compartmentalized throughout the body, namely in the thoracic (cardiovascular), abdominal (visceral and abdominal fat) and movement (muscular) compartments. Each compartment receives a balanced sympathetic/parasympathetic signal to function

according to circadian metabolic needs. The ANS receives the information from pre-autonomic neurons in the autonomic part (parvocellular) of the paraventricular nucleus (PVN), which receives direct and indirect input from the circadian clock localized in the suprachiasmatic nucleus (SCN). The SCN sends output signals to the PVN according to day/night conditions and therefore our metabolism is circadian driven.(Buijs, 2013; Buijs et al., 2013) If the circadian clock is chronically disrupted, the metabolic organization is lost or altered due to a disruption in a SCN-PVN-ANS-Organ circuit. .

Olanzapine can acutely induce a decrease in blood pressure(Choure et al., 2014); which is suggestive of parasympathetic activation. As it turns out, acute vagal (parasympathetic) hyperactivation induces an increase in adiponectin and directly correlates with adiposity.(Suzuki et al., 2014) Adiponectin is an adipokine that has been associated to metabolic risk. Adiponectin levels increase with acute vagal hyperactivation, but low levels are present in chronic metabolic conditions such as obesity, metabolic syndrome, hypertension and diabetes mellitus type 2.(Kim et al., 2013; Song et al., 2015); in which an increased sympathetic activity is reported. (Licht et al., 2010) In the case of olanzapine, a recent meta- analysis showed that its chronic use is associated with low levels of adiponectin. Interestingly, three chronic olanzapine studies reported low levels of adiponectin and were included in the final analysis; but a fourth one, which clearly showed increased levels of adiponectin was excluded because of the short duration of treatment with olanzapine.(Bartoli et al., 2015; Togo et al., 2004) We interpret this not as a discrepancy, but rather as a clear example of the time dependent effects of olanzapine in autonomic activity that also reflects in other metabolic variables such as adiponectin levels. These data supports the possible involvement of the ANS in olanzapine induced adverse metabolic effects.

(FIGURE 1)

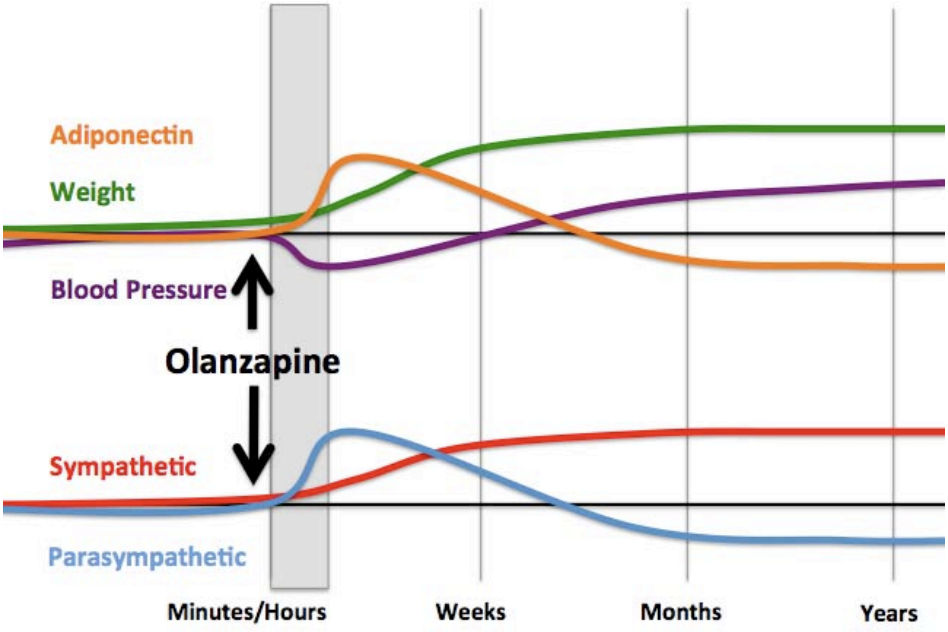

Figure 1. Olanzapine generates time dependent effects on autonomic activity that alter metabolic regulation. Acutely, olanzapine induces parasympathetic activity and consequently decreases blood pressure and induces an increase in adiposity (weight gain) and adiponectin levels. Chronically, the parasympathetic effects by olanzapine are counter regulated by an increase in sympathetic activity and a decrease in adiponectin levels as documented in metabolic disturbances such as obesity, diabetes mellitus and hypertension.

A few years ago, it was reported that melatonin, a hormone that synchronizes the activity of our biological clock, is able to prevent weight increase and adiposity induced by olanzapine. In that study, olanzapine decreased locomotor activity, but the addition of melatonin did not prevent this; suggesting that decreased locomotor activity could contribute to olanzapine induced weight gain, but melatonin prevents this through other

unknown mechanisms. Interestingly, olanzapine decreased the levels of nocturnal plasma melatonin by 55% compared to controls. (Raskind, 2007)

1.3 Melatonin as a regulator of circadian rhythms and metabolic organization

Melatonin is known as the chemical signal for the absence of light. It is produced from tryptophan in many places of our body. In the pineal gland it shows a circadian rhythm with a low production during the day and an increase at night that starts around 8 pm and peaks at 2 am.(Pandi- Perumal, 2006) In mammals, the production of pineal melatonin is mediated by photic signals received by the retinohypothalamic tract and sent to the suprachiasmatic nucleus in the hypothalamus. From there, fibers project to the preautonomic sympathetic neurons in the paraventricular nucleus, and relay sympathetic information by the intermediolateral column to the superior cervical ganglion. Here, the postganglionic sympathetic fibers end at the pinealocytes and regulate the production of melatonin by releasing norepinephrine (NE). (Buijs et al., 1999) The nightly production of NE signals pinealocytes to initiate the production of melatonin via G protein alpha subunit that activates adenylate cyclase. Melatonin is then diffused into capillary blood and cerebrospinal fluid from where it rapidly accesses extrapineal targets.(Reiter et al., 2014)

One of the main targets of melatonin is the SCN, where high concentrations of melatonin receptors are present.(Reppert et al., 1988) As mentioned earlier, the SCN coordinates all the physiological rhythms into day/ night modalities.(Buijs et al., 2013; Reppert and Weaver, 2002) In humans and rodents, melatonin inhibits neuronal activity in the SCN during the night, and in the absence of melatonin during the day, the SCN

shows neuronal activity.(Colwell, 2011; van den Top et al., 2001) The SCN prepares the body to function according to periods of activity/ rest by means of signaling the PVN; which then signals the body to coordinate metabolic activity via the ANS. (Buijs, 2013; Buijs et al., 2013)

Melatonin has been involved in several biological functions. The administration of exogenous melatonin reduces weight gain (Rasmussen et al., 1999) and prevents the appearance of other metabolic syndrome components in rats. (Cardinali et al., 2013) In humans, it plays an important role in the regulation of sleep, blood pressure, mood, weight, metabolism of glucose, insulin, leptin, lipids and the immune system.(Ferracioli-Oda et al., 2013; Kozirog et al., 2011; Radogna et al., 2010; Scheer, 2004; Srinivasan et al., 2006) The mechanisms by which melatonin is able to generate all these metabolic effects is mostly unknown.

As mentioned above, melatonin was recently reported to attenuate olanzapine induced weight gain and adiposity.(Raskind, 2007) These results strongly suggest the involvement of the biological clock in SGA induced adverse metabolic effects and their prevention with the use of exogenous melatonin; and also suggest that the beneficial metabolic effects of melatonin are generated through its action on the SCN.

1.4 The problem

SGAs are complex drugs that act through diverse neurotransmitter systems, intracellular pathways and brain regions that are not fully comprehended. These drugs are effective in controlling psychopathology, but unfortunately induce adverse metabolic effects that further deteriorate the health of patients. Recently, in animals receiving

olanzapine, melatonin prevented to a large extent the body weight increase, indicating a possible role for melatonin in SGA induced adverse metabolic effects.

Melatonin is a hormone secreted by the pineal gland that follows a circadian rhythm with an increased secretion in the middle of the night. This hormone acts importantly on the suprachiasmatic nucleus (SCN) and other areas in the brain and periphery. Melatonin is involved in a series of biological functions such as sleep regulation, blood pressure, regulation of circadian rhythms, mood, behavior, and more recently in the regulation of metabolic processes including insulin, leptin, and lipid regulation. The mechanisms via which melatonin generates these effects are not known.

The problem is that also the mechanisms for SGA induced metabolic disturbances are poorly understood and the options to control them are limited. Our research question is whether melatonin is able to attenuate SGA induced adverse metabolic effects in humans, and if so, what is the mechanism involved?

1.5 General Hypothesis & Aims

Given previous results in experimental animals, the first hypothesis of the present study was that melatonin would be effective and safe in reducing or preventing SGA induced adverse metabolic effects in humans.

Aim: To evaluate in a clinical trial the efficacy and safety of melatonin in reducing or preventing the metabolic disturbances associated with SGA. The results show that melatonin treatment indeed safely attenuates adverse metabolic disturbances in SGA treated patients, particularly in bipolar disorder.

The next hypothesis: Since the main target for melatonin in the brain is proposed to be the SCN, we hypothesized that the SGA olanzapine would act on the SCN and the autonomic nervous system via the PVN.

Aim: To investigate in a rodent experimental model whether SGA could modify the activity of the SCN and one of its main hypothalamic targets: the PVN and its autonomic output. All essential in metabolic and cardiovascular function.

Finally, with our findings as a frame of reference we reviewed current evidence to propose an integrative explanation to the elevated comorbidity between mood and metabolic disorders. We set forth that the hypothalamus and autonomic nervous system play a fundamental role to explain the strong bidirectional relationship between the phenomenology of mood disorders and metabolic dysfunction.

2. CHAPTER 1

Melatonin attenuates antipsychotic metabolic effects: An 8 week randomized, double-blind, parallel group, placebo controlled clinical trial.

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Abstract

Second generation antipsychotics (SGA) are among first line treatments for bipolar disorder and schizophrenia, but have a tendency to generate metabolic disturbances. These features resemble a metabolic syndrome for which a central autonomic imbalance has been proposed that may originate from the hypothalamic suprachiasmatic nuclei. In a clinical trial, we hypothesized that melatonin could attenuate SGA induced adverse metabolic effects.

Design: An 8-week, double-blind, randomized, placebo controlled, parallel group clinical trial. Objective: Evaluate the metabolic effect of melatonin in SGA treated patients in terms of weight, blood pressure, lipid, glucose, body composition and anthropometric measures. Methods: 44 patients with bipolar disorder (n=20) and schizophrenia (n=24), treated with SGA randomly received placebo (n=24) or melatonin 5mg (n=20). Results: The melatonin group showed a decrease in diastolic BP (1.1 vs -5.1 mmHg, p=.003) and attenuated weight gain (2.2 vs 1.5 Kg, F=4.512, p=.040) compared to the placebo group. The strong beneficial metabolic effects of melatonin on fat mass (2.7 vs 0.2 kg,p=.032) and diastolic BP (5.5 vs -5.7 mmHg, p=.001) were observed in the bipolar disorder and not in the schizophrenia group. No adverse events were reported.

Conclusions: Our results show that melatonin is effective in attenuating SGAs adverse metabolic effects, particularly in bipolar disorder. The clinical findings allow us to propose that SGA might disturb a centrally mediated metabolic balance causing adverse metabolic effects and that nightly administration of

melatonin helps to restore. Melatonin could become a safe and cost effective therapeutic option to attenuate or prevent SGA metabolic effects.

Key Words: Melatonin, second generation antipsychotic, metabolic, bipolar disorder, schizophrenia, blood pressure, weight, fat mass

ClinicalTrials.gov Identifier: NCT01811160

2.1 INTRODUCTION

Bipolar disorder and schizophrenia are frequently associated with an elevated risk for obesity, hypertension, diabetes mellitus, dyslipidemia and other metabolic disturbances(Bowie et al., 2010; Vancampfort et al., 2013b). Second Generation Antipsychotics (SGA) have a demonstrated efficacy in acute and long term treatment of these disorders and are considered a first option on most treatment guidelines(Davis et al., 2003; Gaebel et al., 2005; Yatham et al., 2013). Unfortunately the use of SGA is associated to drug induced weight gain, disturbed glucose and lipid regulation and an increase of cardiovascular risk, and these adverse side effects become a cause for non adherence to treatment (Lieberman et al., 2005b). Mortality in this population often reaches levels that double or triple the rates reported for the general population, and cardiovascular disease is one of the main causes of mortality and morbidity in patients suffering from these illnesses(Brown, 1997; Garcia-Portilla et al., 2009; Kilbourne et al., 2007).

Metabolic disturbances induced by SGA vary depending on the drug. For example, the most effective SGA clozapine and olanzapine are more frequently associated with weight gain, increases in triglyceride and cholesterol levels than quetiapine and risperidone (Girgis et al., 2008).

There are several hypotheses attempting to explain the complex pathways that lead to antipsychotic therapeutic effects and their accompanying adverse metabolic effects (Girgis et al., 2008). Melatonin has been shown to be able to reduce weight gain in rats (Rasmussen et al., 1999; Terron et al., 2013; Wolden-Hanson et al., 2000); was also used recently in animals receiving SGA, and prevented to a large extent the body

weight increase indicating a possible role for biological rhythms in SGA induced body weight accumulation (Raskind et al., 2007). Melatonin is a hormone secreted by the pineal gland that follows a circadian rhythm with an increased secretion in the middle of the night (Pandi-Perumal et al., 2006). This hormone acts importantly on the suprachiasmatic nucleus (SCN) and other areas in the brain and periphery (Reppert, 1997). Thus melatonin has been involved in a series of biological functions such as sleep regulation(Laposky et al., 2008; Wyatt et al., 2006), blood pressure(Kozirog et al., 2011; Scheer, 2004), regulation of circadian rhythms, mood, behavior(Srinivasan et al., 2006), and more recently in the regulation of metabolic processes including insulin, leptin, and lipid regulation (Espino et al., 2011; Kozirog et al., 2011; Nduhirabandi et al., 2011).

2.2 AIMS & HYPOTHESIS

Given previous results in experimental animals (Raskind et al., 2007) the purpose of the present study was to test the potential effect of melatonin in reducing or preventing some of the metabolic disturbances associated with SGA. The results show that melatonin treatment indeed prevents to a large extent the adverse metabolic disturbances in SGA treated patients, particularly in bipolar disorder.

2.3 MATERIALS & METHODS

2.3.1. Study setting

The study was conducted in accordance with Good Clinical Practices and the World Medical Association Declaration of Helsinki. The study protocol was registered and approved (Project Registration #144) by the Institutional Review Boards of the Instituto Nacional de Psiquiatría Ramón de la Fuente Muñíz (INPRF) in Mexico City. Written informed consent was obtained after the procedures had been explained in detail to the patients.

2.3.2. Subjects

Patients were recruited from both the inpatient and outpatient services of the INPRF, a highly specialized mental health center dedicated to research, education and treatment of psychiatric patients. Subjects were included in the study if they met the following criteria: 1) Men and non-pregnant, non-lactating women aged between 18 and 45 years; 2) DSM-IV-TR criteria for schizophrenia or bipolar disorder type I; 3) free of concomitant medical or neurological illness (as per review of systems and general physical examination); 4) free of DSM-IV current substance abuse or a history of substance dependence in the last six months; and 5) who were initiated on continuous treatment with SGA (clozapine, olanzapine, quetiapine or risperidone) for a period no greater than the last three months prior to their inclusion to the present study. Patients were excluded if: 1) were diagnosed with hypertension, diabetes mellitus, dyslipidemia, thyroid disorders or hepatic illness; 2) had a history of hypersensitivity to melatonin; 3) exhibited high risk for suicide or high risk for aggressiveness; and 4) women who were not practicing reliable forms of contraception. Patients were eliminated from the study if they suspended SGA or two consecutive doses of the study capsule at any point during the follow up period.

2.3.3. Study design and procedures

This was an 8-week, randomized, double-blind, parallel group, placebo controlled clinical trial. An initial screening interview with eligible patients was performed to determine the fulfillment of inclusion and exclusion criteria. If these criteria were met, voluntary written informed consent was obtained and patients were scheduled for a baseline visit (no more than 7 days after the screening evaluation). After the baseline clinical evaluation, patients were randomly allocated by single randomization procedures (computerized random numbers generated by one investigator: AFO) with a 1:1 allocation ratio to receive daily either a 5mg presentation of slow release melatonin (cronocaps ®, Productos Medix, S.A. de C.V. México City, México) or a physically identical placebo capsule to be administered orally at 20:00hrs. Melatonin administration at 20:00hrs during the duration of the study had the purpose to favor regular sleeping schedules. This prolonged release presentation has a delivery system that allows 12 hrs of melatonin slow release with a Tmax= 2.7± 0.7 hrs and a t1/2=1.5± 0.7 hrs (Unpublished bioavailability study, Productos Medix S.A.. de C. V. México City, México). Therefore a more physiological melatonin distribution is expected if administered 2 hours before bedtime. Similar administration schedules have been reported with positive results (Kozirog et al., 2011). The dose was maintained throughout the end of the follow-up period (8 weeks). Changes in SGA types were prohibited throughout the study, but dose adjustments were allowed and documented. SGA were classified in higher (clozapine and olanzapine) or medium (quetiapine and risperidone) according to their risk for inducing metabolic disturbances. Psychotropic drug administration other than the prescribed study medication and the SGA, such as

antidepressants, hypnotics or mood stabilizers were permissible if clinically indicated. All concomitant medication use was recorded.

2.3.4. Assessments

Patients were asked to fast 12 hours prior to each evaluation. Programmed evaluations began at 7-8 hrs. First, laboratory testing of blood was performed, second, anthropometric measures including body weight, height, body mass index (BMI), fat mass percentage, fat mass, lean mass and total body water measures were obtained using a TANITA 300A Body Composition Analyzer (Tanita Corporation of America, Inc. Ill, USA)**.** Also, waist and hip circumference, waist/hip ratio and blood pressure (BP) were determined. Finally, two trained psychiatrists (FRN and DAI) who were blind to the treatment randomization at all time, performed a clinical evaluation. This included the application of the Positive and Negative Syndrome Scale (PANSS) (30 items, 1-7 severity scale)(Kay et al., 1990), the Clinical Global Impression – Severity of Illness (CGI-S) scale(Guy, 1976), the Hamilton Depression Rating Scale (HDRS)(Hamilton, 1960) and the Young Mania Rating Scale (YMRS)(Apiquian, 1997) for patients diagnosed with bipolar disorder and the Calgary Depression Scale (CDSS)(Addington et al., 1996) for patients with schizophrenia. These assessments were all determined at baseline, after week 3 of treatment and at the end of the study.

2.4. Statistical Procedures

Demographic and clinical characteristics descriptions were analyzed with frequencies and percentages for categorical variables and with means and standard deviations (S.D.) for continuous variables. First, to evaluate group differences, anthropometric and metabolic variable mean change from baseline to endpoint were used as dependent variables and analyzed using T test for independent samples according to treatment group, and diagnostic group comparisons. Second, patients were included in four analyses of covariance (ANCOVA) models of mean change from baseline to endpoint, with baseline values as a covariate, and the SGA metabolic risk in ANCOVA model 1, gender in ANCOVA model 2, mood stabilizers (lithium, valproate, lamotrigine or carbamazepine) in ANCOVA model 3 and baseline BMI in ANCOVA model 4 as the effect of interest. The primary outcome parameters were the changes observed in weight, body composition, anthropometric measures and metabolic variables from baseline to endpoint according to placebo or melatonin treatment allocation. Secondary parameters included changes from baseline to endpoint in the severity symptom scales and the fulfilment of metabolic syndrome criteria during the study (according to ATPIII diagnostic criteria). All statistical tests were two-sided and performed at a 0.05 significance level.

2.5. Results

2.5.1. Patients

A total of 60 patients were assessed for eligibility from October 2008 to November 2011. Fifty patients met inclusion criteria, were recruited and randomized to receive placebo (n=25) or melatonin (n=25); 29 were diagnosed with schizophrenia and 21 with bipolar disorder. Six patients were lost before the third week of the follow-up (placebo n=1;

melatonin n=5) and were excluded from the analysis, as there was no post-baseline assessment in these patients. 44 patients completed the 8-week follow-up (placebo n=24, melatonin n=20) and included for analyses. (see Fig. 1)

(FIGURE 1: CONSORT Patient flow diagram)

2.5.2. Baseline demographic and clinical characteristics

Demographic features of the sample were as follows: 50% of the patients were men, with a mean age of 29.5 years (SD 8.3). Thirty-three patients (75%) were single and unemployed at the time of their recruitment. The educational level was 12.1 years (SD 3.5). Age of illness onset was at 21.9 years (SD 7.3) with a mean length of illness of 412.1 weeks (SD 505.1). There were no significant differences between melatonin and placebo treatment groups on baseline demographic characteristics and were also comparable in terms of illness features at baseline.

At baseline, 15 patients (34.1%) were receiving quetiapine, 14 (31.8%) olanzapine, 13 (29.5%) risperidone and the remaining two patients (4.5%) were treated with clozapine. In this way, 13 patients (54.2%) of the placebo group and 15 (75%) from the melatonin group were treated with medium risk SGA, while 11 (45.8%) and 5 (25%) were under high risk SGA respectively. The mean antipsychotic dose (chlorpromazine equivalence) at baseline was 275.1 (SD 280) mg/day.

Prior to baseline comparison of demographic characteristics and illness features between treatment groups, some differences emerged between schizophrenia and bipolar patients; a higher percentage of patients with schizophrenia were single when

compared to bipolar patients (91.7% vs. 55%; χ^2=7.8, df 1, p=0.005), while bipolar were more frequently employed (40% vs. 12.5%; χ^2=4.4, df 1, p=0.03). Bipolar patients reported an earlier age of illness onset (19.5 vs. 23.9 years; t=2.0, df 42, p=0.04) and a prolonged duration of illness (642 vs. 220.6 weeks; t=-3.4, df 42, p=0.005). **(Table 1).**

2.5.3. Concomitant treatment

The comparisons of concomitant medication between patients allocated in the placebo or in the melatonin group showed no significant differences in the use of each medication with the exception of mood stabilizers; a higher percentage of patients in the melatonin group (n=13, 65%) when compared to the placebo group (n=8, 33.3%) were using this type of medication (χ^2=4.3, df 1, p=0.03). Most patients in the bipolar group were treated with mood stabilizers (n=18, 80%) during the study and only two patients in the schizophrenia group (8%). No differences in concomitant treatment with mood stabilizers, antidepressants or benzodiazepines were observed between patients that received placebo or melatonin in each diagnostic group as shown in Table 1 and 2.

(TABLE 1 AND 2)

2.5.4. Baseline Anthropometric, body composition, metabolic and symptom severity variables

At baseline, melatonin-treated patients had significantly higher cholesterol (t-2.2, df 42, p=0.02) and triglyceride (t=-2.0, df 42, p=0.04) levels while at the end of the study differences remained in cholesterol (t=-2.2, df 42, p=0.03) but not in triglyceride (t=-0.7,

df 42, p=0.44) levels as compared to placebo treated patients. Changes reported from baseline to endpoint were significant (<0.05) in all variables with the exception of waist circumference (p=0.06) and triglyceride levels (p=0.77).

Although patients with schizophrenia in the placebo group showed a higher score on the CDSS at baseline when compared to the melatonin group (t=2.27, df 22, p=0.03), no statistically reliable differences emerged between these groups at the end of the follow up (2.0 SD 3.1 vs 1.4, SD 2.0; t=0.5, df 22, p=0.56).

2.5.5. Primary Outcome Measures:

2.5.5.1. Melatonin vs Placebo

A significant difference in mean diastolic blood pressure change was observed between the placebo and melatonin groups (1.1 vs -5.1 mmHg, p=.003). **(Figure 2-A)**

In the ANCOVA model 1, when baseline values were used as a covariate and the metabolic risk for SGA interaction was considered, the difference in mean weight change was significant between the placebo and melatonin groups (2.2 SD 3.0 vs 1.5 SD 2.9 kg, F=4.512, p=.040) with a higher increase in patients receiving medium risk SGA (1.9 SD 2.1 vs 0.6 SD 2.5) and a lower increase in patients treated with high risk SGAs that received placebo compared to patients that received melatonin (2.4 SD 3.9 vs 4.3 SD 2.4 kg). A significant difference was also observed for waist circumference differences between the placebo and melatonin groups (1.9 SD 3.8 vs 2.2 SD 3.0, p=.05), a trend to significance was observed in BMI (0.78 vs 0.60 kg/mts^2, F=3.235, p=.08) and total body water mean change (0.6 vs 0.95 kg, F=3.329, p=0.076). Baseline values and changes from baseline to endpoint in anthropometric and metabolic

variables are shown in Table 3 and Table 4; statistics of the effect of treatment with melatonin (M), metabolic risk of SGA (AP) and the combined effect of both (M x AP) are included in the tables 4 and 5.

(TABLES 3, 4 AND 5)

In ANCOVA model 2, when baseline values were used as a covariate and gender interaction was considered, diastolic blood pressure showed a significant difference in mean change between the placebo and the melatonin groups (1.1 SD 7.1 vs -5.1 SD 5.9 mmHg, F=6.37, p=.016). A stronger effect was noted in female (3.8 SD 6.6 vs. -8.3 SD 6.1mmHg) than in male (-1.5 SD 6.7 vs -2.0 SD 4.0 mmHg) subjects.

In ANCOVA model 3, when baseline values were used as a covariate and the use of a mood stabilizer interaction was considered, a significant difference in mean change between the placebo and the melatonin groups in fat mass was observed (1.2 SD 2.4 vs -0.005 SD 2.8 kg, p=.011), where a stronger effect was noted in patients treated with mood stabilizers (2.8 SD 2.9 VS -.28 SD 2.9 Kg) than patients without mood stabilizers (.48 SD 1.9 VS .5 SD 2.9 Kg). In this ANCOVA model, a significant difference in mean change between the placebo and melatonin groups in diastolic blood pressure was observed (1.12 SD 7.1 vs -5.15 SD 5.9 mmHg, p=.005). Patients using mood stabilizers showed a stronger effect (6.2 SD 5.8 vs -6.3 SD 6.2 mmHg) than patients without a mood stabilizer (-1.4 SD 6.4 vs -2.8 SD 5.0 mmHg). In ANCOVA model 4, no other significant differences were observed.

Other similar ANCOVA models using baseline blood pressure, cholesterol, triglyceride, glucose, body mass index and current antidepressant treatment, as effects of interest were performed, and no significant findings were observed.

2.5.5.2. SGA metabolic risk

Patients treated with Medium risk SGA showed a significant difference in diastolic blood pressure mean change if treated with placebo or melatonin (1.8 vs - 4.6mmHg ,p=.008). High risk SGA treated patients showed a significant mean change difference in lean mass (0.8 vs 2.7 kg, p=.007) and total body water (0.6 vs 2.0, p=.008).

2.5.5.3. Schizophrenia and Bipolar disorder groups.

In the bipolar disorder group, a significant difference in mean fat mass % (2.8 vs -.03 %, p=.004), fat mass (2.7 vs 0.2 kg,p=.032), and diastolic blood pressure (5.5 vs -5.7 mmHg, p=.001) mean change was observed, and a trend was observed for triglycerides mean change (50.1 vs -20 mg/dl, p=.08). (see Fig. 2 B, C and D)

In the Schizophrenia group, no significant differences were present in anthropometric, body composition and metabolic variables.

(FIGURES 2-B, C AND D)

2.5.6. Secondary outcome measures

2.5.6.1. Symptom severity

Mean baseline and change from baseline to endpoint efficacy rating scale scores in both treatment groups are shown in Table 5. In this analysis, placebo and melatonin groups were related to similar improvement as measured by changes from baseline on the total PANSS, PANSS subscales, CGI-S, and depressive symptoms assessed by the CDSS in patients with schizophrenia and the HDRS and the YMRS for patients with bipolar disorder. There were no serious adverse events reported for placebo or melatonin groups.

(TABLE 5)

2.5.6.2. Metabolic Syndrome

At baseline, seven patients (15.9%) met criteria for metabolic syndrome and at the end of week 8, these same patients met the criteria. Nevertheless, at the end of the study four additional patients had metabolic syndrome. All of them were diagnosed with bipolar disorder; 3 were under treatment with quetiapine and 1 with olanzapine and three of them received placebo.

2.6. Discussion

In the present study four main findings emerged; 1) Patients receiving SGA and melatonin showed a decrease in diastolic blood pressure compared to patients allocated in the placebo group, 2) Melatonin showed a particularly beneficial metabolic

effect in bipolar disorder patients in terms of fat mass and diastolic blood pressure mean changes that were not observed in the schizophrenia group, 3) Melatonin had varied metabolic effects depending on the SGA metabolic risk profile, 4) Melatonin did not affect symptom severity outcome measures in SGA treated subjects.

To the best of our knowledge, this is the first clinical study to evaluate the metabolic effects of melatonin in patients treated with SGA demonstrating that melatonin treatment is particularly beneficial in bipolar disorder. We observed that SGA metabolic risk profile was associated with a differential effect on weight gain. Melatonin showed a greater weight gain attenuating effect on medium risk SGA that was not apparent in the high risk SGA treated patients where melatonin seems to be associated with a greater weight gain. This should be examined carefully, since weight is not only composed by fat, but also by non-fat components (lean mass and total body water). In this sense, we observed that High risk SGA treated patients that received melatonin had a significantly greater increase in total body water compared to the placebo group, an effect that was not observed in the medium metabolic risk SGA treated groups and could account for the apparent weight and waist increase in high metabolic risk SGA treated subjects that received melatonin. A particular interaction of melatonin with high metabolic risk SGA in the modulation of corporal water and lean mass balance should be studied further.

It is known that patients with a bipolar disorder or schizophrenia have a disrupted circadian cycle(Murray and Harvey, 2010; Wulff et al., 2012; Wulff et al., 2010) and several metabolic disturbances(Guan et al., 2010; Vancampfort et al., 2013b) that could precede their onset(Ritter et al., 2012) and the initiation of treatment(Forest et al., 2007). For example, recently a study showed that melatonin receptor type 1 and AVP and/or VIP positive cells were increased in the hypothalamic SCN and PVN of patients

with mood disorders, further indicating a disturbance in mood disorders (Wu et al., 2013)that can be linked with melatonin. A baseline circadian rhythm disturbance and metabolic vulnerability associated to these disorders could be exacerbated by SGA treatment. We propose that the SGA induced metabolic adverse effects are generated at least in part by altering the functioning of hypothalamic structures which disturbs the central regulation of metabolism while SGA also modulate other neurotransmitter systems and achieve therapeutic effects. The observation that melatonin attenuates fat mass increase, decreases diastolic blood pressure and possibly triglyceride mean changes in bipolar disorder but not in schizophrenia patients suggests that melatonin could help reestablish a damaged circadian rhythm in bipolar disorder, but could have more difficulty in restoring the function of the biological clock in schizophrenia.

There is scarce knowledge regarding the central effect of SGA and their relationship to their adverse metabolic effects. It has been reported that antipsychotics have differential effects in hypothalamic structures (Weston-Green et al., 2012). An immunohistochemistry study, for example, showed that clozapine and olanzapine induced a greater activation than risperidone in the paraventricular nucleus (PVN), a fundamental structure in the execution of hypothalamic signals to the body by hormones and the autonomic nervous system (ANS) and its regulation of metabolic functions (Kiss et al., 2010). These functions of the PVN are strongly under the influence of the SCN giving a circadian rhythm to autonomic and hormonal output (Buijs et al., 2006).

Since the SCN is one of the main targets of melatonin in the brain and melatonin inhibits the neuronal firing of SCN neurons(Liu et al., 1997; Reppert et al., 1994; van den Top et al., 2001) these results suggest that through the diminished activity of SCN neurons those in the PVN are also inhibited. Based on our clinical results, we propose that a

SCN mediated mechanism could contribute to explain SGA induced adverse metabolic effect, and the attenuating effects of melatonin. (Kreier et al., 2002)

The administration of exogenous melatonin has shown beneficial effects in essential hypertension(Grossman et al., 2011) the metabolic syndrome (Cardinali et al., 2011; Kozirog et al., 2011), as well as diabetes mellitus (Kadhim et al., 2006), all diseases with evidence for disturbed circadian pacemaker function. The effects of melatonin in lowering blood pressure have been attributed to the enhancement of the functioning of the biological clock by repeated melatonin administration(Scheer, 2004). Hereby melatonin's blood pressure lowering effect is consistent with previous clinical studies(Grossman et al., 2011; Kozirog et al., 2011; Lusardi et al., 1997; Scheer, 2004) and is a desired, possibly prophylactic effect in subjects treated with SGA (Anderson and Maes, 2012; Henderson et al., 2004; Kinon et al., 2001).

The beneficial metabolic effects of melatonin were first described in rats in a study where it was observed that the chronic exogenous administration of melatonin suppressed increases in visceral fat, insulin and leptin associated with aging (Rasmussen et al., 1999). Later, it was also reported in animal experiments that melatonin reduced weight gain independent of food intake and total body fat (Terron et al., 2013; Wolden-Hanson et al., 2000) and that melatonin increased locomotor activity during the activity period (Terron et al., 2013). This effect has also been observed under various obesity generating conditions like high fat diet (Puchalski et al., 2003) or a prolonged photoperiod (Bartness and Wade, 1985).

In humans, evidence regarding the effect of melatonin on weight is still missing and basic research theories need yet to be proven in clinical trials. We provide clinical data that aligns to those theories.

Limitations to this study include the small sample, and the inclusion of four different SGA with different metabolic risk profiles, and the concomitant use of other psychotropic drugs that can generate metabolic disturbances or modify circadian rhythm by themselves (ie, valproate and lithium) as a possible source of bias (Abe et al., 2000; Aichhorn et al., 2006; Chengappa et al., 2002; Dokucu et al., 2005; Iwahana et al., 2004).

SGA induced weight gain and other metabolic disturbances are very frequently unavoidable landmarks to the pharmacologic treatment of chronic mental disorders, for which they currently represent the best therapeutic option. This study shows that the administration of exogenous melatonin could become a benign, relatively innocuous, inexpensive and effective alternative to the attenuation of SGA induced adverse metabolic effects, particularly in bipolar disorder, without affecting the beneficial therapeutic effect.

2.7. ACKNOWLEDGEMENTS

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2.8. Conflict of Interest: The authors declare that no conflict of interest exist for this study.

2.9. Figures and Tables

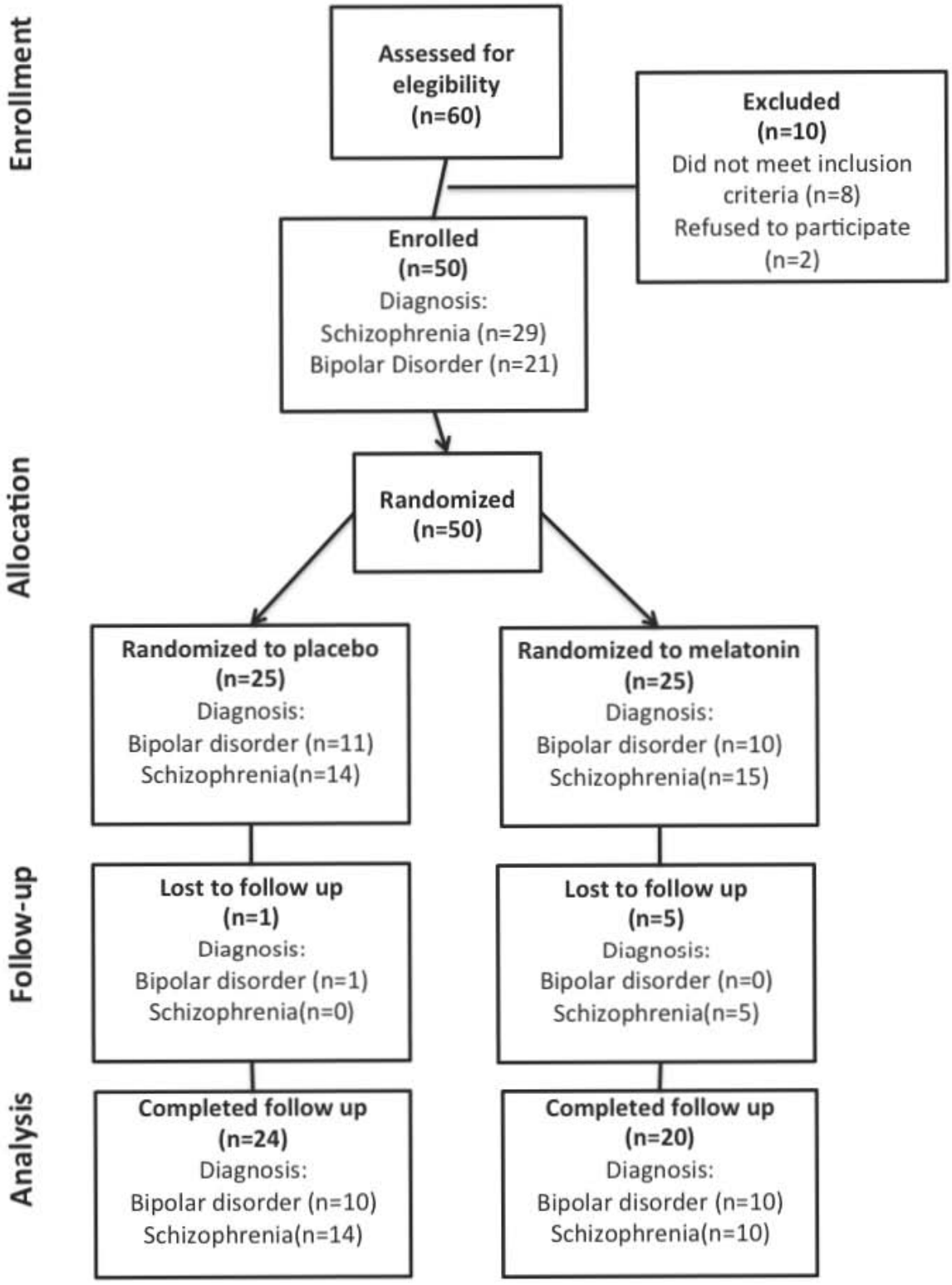

Figure 1. CONSORT PATIENT FLOW DIAGRAM

FIGURE 2-A

Diastolic blood pressure (DBP) mean change melatonin vs. placebo groups.

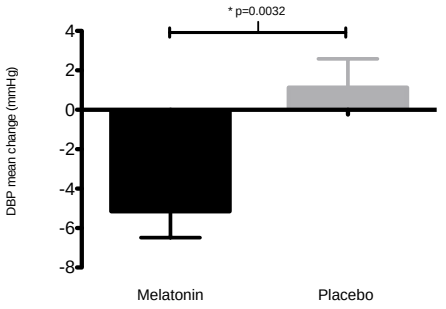

FIGURE 2-B

DBP mean change Melatonine vs. Placebo according to diagnostic groups.

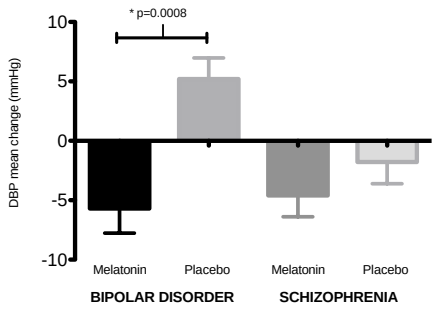

FIGURE 2-C

Fat mass mean change according to diagnostic groups.

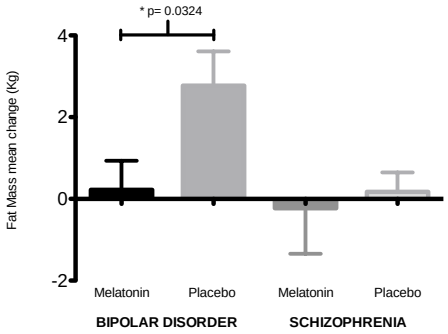

FIGURE 2-D

Triglyceride mean change comparison according to diagnostic groups.

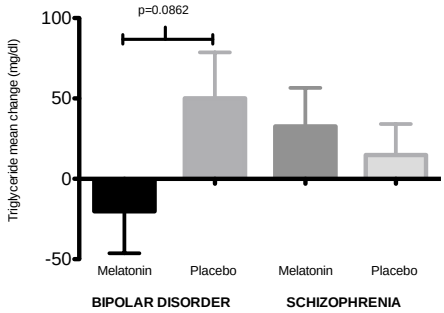

Figure 2. Blood pressure and metabolic effects of melatonin treatment.

Shows student-T comparison graphs; Patients randomized to receive melatonin showed a decrease in diastolic BP (-5.1 vs. 1.1mmHg, p=.003). **(FIGURE 2-A)** Melatonin showed a particularly beneficial metabolic effect in BIPOLAR DISORDER patients in terms of DIASTOLIC BLOOD PRESSURE (5.5mmHg vs. -5.7 mmHg, p=.001), FAT MASS (2.7 vs. 0.2 kg, p=.032) and TRIGLYCERIDE mean changes (50.1 vs. -20 mg/dl, p=.08) that was not observed in the SCHIZOPHRENIA group **(FIGURES 2-B,C,D).**

DBP: Diastolic blood pressure

* Statistically significant results (p<.05)

Table 1. Baseline demographic characteristics between treatment groups.

		Placebo (n=24)		Melatonin (n=20)	
		n	%	n	%
Gender	Male	12	50	10	50
	Female	12	50	10	50
Marital Status	Single	18	75	15	75
	Married	6	25	5	25
Employment Status	Unemployed	19	79.2	14	70
	Employed	5	20.8	6	30
Antipsychotic					
High Metabolic Risk SGA					
	Clozapine	1	4.2	1	5
	Olanzapine	10	41.7	4	20
Medium Metabolic Risk SGA					
	Risperidone	6	25	7	35
	Quetiapine	7	29.2	8	40
Concomitant Medication					
Bipolar Disorder					
	Mood Stabilizers				
	Lithium	8	80	10	100
	Valproate	2	20	1	10
	Carbamazepine	6	60	7	70
	Lamotrigine		-	1	10
	SSRI		-	1	10
	Benzodiazepines		-		-
Schizophrenia		1	10	4	40
	Mood Stabilizers				
	Lithium		-	1	10
	Valproate		-		-
	Carbamazepine		-	1	10
	Lamotrigine		-		-
			-		-
	SSRI	3	21.4	2	20
	Benzodiazepines	4	28.5	4	40

No significant differences were observed between placebo or melatonin groups.

Table 2. Baseline clinical characteristics between treatment groups.

	Placebo (n=24)		Melatonin (n=20)	
	Mean	**S.D.**	**Mean**	**S.D.**
Age (years)	28.6	9.0	30.6	7.5
Years of education	12.4	3.3	11.8	3.7
Age of illness onset (years)	21.0	5.6	23.0	8.9
Length of illness (weeks)	458.1	544.7	357.0	460.8
Antipsychotic dose (equivalency)	256.1	307.9	297.9	248.3

No significant differences were observed between placebo or melatonin groups.

Table 3. Mean change from baseline to endpoint in anthropometric variables.

Variable	Placebo (n=24)				Melatonin (n=20)				Statistic*
	SGA medium risk		SGA high risk		SGA medium risk		SGA high risk		
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	
Body weight (kgs)									
Baseline	70.0	11.0	66.1	14.7	72.2	15.8	75.7	14.3	**M** F=0.71, p=0.40
Endpoint	72.0	10.7	68.6	15.0	72.9	13.9	80.1	13.2	
Mean Change	**1.9**	**2.1**	**2.4**	**3.9**	**0.6**	**2.5**	**4.3**	**2.4**	**AP** F=5.52, p=0.02
									MxAP p=0.04
Body mass index									
Baseline	26.7	5.4	24.6	6.1	26.1	4.2	26.2	5.3	**M** F=0.18, p=0.67
Endpoint	27.4	5.3	25.5	5.9	26.4	3.6	27.7	4.7	
Mean Change	**0.7**	**0.8**	**0.8**	**1.6**	**0.3**	**0.8**	**1.4**	**0.8**	**AP** F=2.70, p=0.10
									MxAP p=0.08
Waist circumference									
Baseline	96.2	13.4	92.1	16.8	95.4	10.9	97.6	12.7	**M** F=0.95, p=0.33
Endpoint	99.0	12.5	93.2	17.1	97.0	9.4	101.6	12.7	
Mean Change	**2.7**	**4.1**	**1.1**	**3.4**	**1.6**	**3.1**	**4.0**	**2.0**	**AP** F=0.06, p=0.79
									MxAP p=0.05
Hip circumference									
Baseline	100.0	9.1	93.5	12.7	99.3	10.1	99.3	8.1	**M** F=0.008, p=0.93
Endpoint	101.6	7.5	95.5	13.6	99.1	6.2	101.3	7.1	
Mean Change	**1.5**	**4.5**	**2.0**	**4.5**	**-0.1**	**7.7**	**2.0**	**1.5**	**AP** F=0.07, p=0.79
									MxAP p=0.29
Fat percentage									
Baseline	27.2	10.5	22.6	13.6	28.6	8.8	25.1	14.1	**M** F=0.50, p=0.48
Endpoint	28.0	10.1	24.2	13.3	28.5	7.2	26.2	12.5	**AP** F=0.74, p=0.39
Mean Change	**0.8**	**1.8**	**1.6**	**3.1**	**-0.10**	**2.2**	**1.1**	**2.7**	**MxAP** p=0.732
Fat mass									
Baseline	19.8	9.8	16.1	12.5	21.6	9.0	20.4	13.1	**M** F=0.30, p=0.58
Endpoint	20.7	9.5	17.7	12.5	21.12	7.4	22.1	12.1	**AP** F=2.29, p=0.13
Mean Change	**0.9**	**1.6**	**1.6**	**3.3**	**-0.5**	**2.8**	**1.6**	**2.4**	**MxAP** p= 0.294
Lean mass									
Baseline	50.0	6.5	49.9	8.6	51.0	10.4	55.3	6.5	**M** F=2.45, p=0.125

Endpoint	50.0	7.4	50.8	9.3	51.8	9.8	58.1	6.8	**AP** F=2.43, p=0.12
Mean Change	**0.007**	**4.7**	**0.8**	**1.2**	**0.8**	**1.4**	**2.7**	**0.9**	**MxAP** p= 0.486
Total body water									
Baseline	36.9	4.4	36.6	6.3	37.3	7.6	40.5	4.7	**M** F=3.32, p=0.07
Endpoint	37.5	4.9	37.2	6.8	37.9	7.1	42.5	5.0	**AP** F=3.41, p=0.07
Mean Change	**0.6**	**1.5**	**0.6**	**0.9**	**0.6**	**1.0**	**2.0**	**0.6**	**MxAP** p= 0.076

*Based on analysis of covariance adjusted for baseline score, treatment with melatonin or placebo (M) and antipsychotic metabolic risk (AP) as effects of interest. MxAP refers the interaction between M and AP.

Abbreviations: SGA; Second generation antipsychotic, **M**; Treatment effect (melatonin or placebo), **AP**; Effect according to antipsychotic metabolic risk type, **MxAP**; Effect according to interaction of treatment (melatonin or placebo) and antipsychotic metabolic risk type.

Table 4. Mean change from baseline to endpoint in metabolic variables.

Variable	Placebo (n=24)				Melatonin (n=20)				Statistic *
	SGA medium risk		SGA high risk		SGA medium risk		SGA high risk		
	Mean	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	**Mean**	**S.D.**	
Systolic blood pressure									
Baseline	109.4	10.1	112.0	10.5	109.2	9.4	115.6	11.2	**M** F=1.61, p=0.21
Endpoint	109.8	10.6	108.3	8.7	104.1	10.5	107.4	18.3	**AP** F=0.17,
Mean Change	**0.3**	**6.4**	**-3.6**	**10.3**	**-5.0**	**14.4**	**-8.2**	**9.6**	p=0.67
									MxAP p=0.68
Diastolic blood pressure									
Baseline	71.2	3.9	72.3	11.4	73.4	4.9	81.6	10.1	**M** F=4.11, p=0.04
Endpoint	73.0	6.3	72.6	5.1	68.8	7.7	74.8	11.0	**AP** F=0.009,
Mean Change	**1.8**	**5.8**	**0.2**	**8.6**	**-4.6**	**5.9**	**-6.8**	**6.2**	p=0.92
									MxAP p=0.53
Fasting glucose									
Baseline	90.2	8.2	84.1	5.7	88.8	8.4	87.4	13.1	**M** F=0.08, p=0.76
Endpoint	90.3	7.1	86.9	3.8	88.5	6.7	87.8	3.9	**AP** F=0.65, p=0.42
Mean Change	**0.1**	**10.7**	**2.7**	**5.5**	**-0.2**	**8.1**	**0.4**	**15.6**	**MxAP** p=0.59
Triglyceride levels									
Baseline	144.4	87.4	114.7	62.8	174.2	88.6	197.2	41.6	**M** F=0.47, p=0.49
Endpoint	177.2	125.9	140.3	113.2	180.1	111.4	204.2	43.0	**AP** F=0.01, p=0.90
Mean Change	**32.7**	**89.3**	**25.6**	**72.4**	**5.9**	**89.2**	**7.0**	**62.8**	**MxAP** p=0.84
HDL									
Baseline	43.6	11.1	48.3	12.3	45.2	12.9	39.2	5.4	**M** F=0.003,
Endpoint	41.8	9.4	48.3	11.2	43.6	11.5	41.8	2.5	p=0.95
Mean Change	**-1.7**	**5.8**	**0.01**	**8.5**	**-1.6**	**9.2**	**2.6**	**4.6**	**AP** F=1.52, p=0.22
									MxAP p=0.80
LDL									
Baseline	93.8	26.4	95.2	23.5	113.0	25.7	97.1	42.6	**M** F=2.60, p=0.11
Endpoint	92.9	24.9	96.8	22.5	112.0	30.5	115.7	41.2	**AP** F=1.18, p=0.28
Mean Change	**-0.9**	**28.2**	**1.6**	**21.4**	**-0.9**	**25.8**	**18.6**	**19.5**	**MxAP** p=0.50
Total Cholesterol									
Baseline	163.2	43.4	166.3	29.1	193.1	35.1	181.0	43.8	**M** F=1.13, p=0.29
Endpoint	168.9	28.8	173.1	27.0	198.4	57.1	198.4	36.8	**AP** F=0.23, p=0.63
Mean Change	**5.6**	**31.0**	**6.8**	**22.8**	**5.3**	**44.9**	**17.4**	**20.2**	**MxAP** p=0.77

*Based on analysis of covariance adjusted for baseline score, treatment with melatonin or placebo (M) and antipsychotic metabolic risk (AP) as effects of interest. MxAP refers the interaction between M and AP.

Abbreviations: SGA; Second generation antipsychotic, M; Treatment effect (melatonin or placebo), AP; Effect according to antipsychotic metabolic risk type, MxAP; Effect according to interaction of treatment (melatonin or placebo) and antipsychotic metabolic risk type.

Table 5. Mean change from baseline to Endpoint in clinical rating scale.

Test	Placebo (n=24) Mean	SD	Melatonin (n=20) Mean	SD	Statistic*
Positive PANSS					
Baseline	19.6	10.3	17.3	8.2	Change F=33.8, p<0.001
Endpoint	13.5	7.4	12.5	6.4	Group F=0.03, p=0.86
Mean Change	**-6.1**	**7.2**	**-4.7**	**6.3**	
Negative PANSS					
Baseline	17.9	9.6	15.7	8.6	Change F=11.9, p=0.001
Endpoint	16.0	8.5	13.6	7.4	Group F=0.2, p=0.61
Mean Change	**-1.9**	**5.0**	**-2.0**	**2.9**	
Cognitive PANSS					
Baseline	17.1	8.3	16.1	7.1	Change F=19.6, p<0.001
Endpoint	14.5	6.6	13.1	6.1	Group F=0.4, p=0.50
Mean Change	**-2.5**	**4.6**	**-3.0**	**4.5**	
Excitement PANSS					
Baseline	7.9	4.6	7.2	3.4	Change F=128.7, p<0.001
Endpoint	5.9	2.5	5.1	1.2	Group F=1.3, p=0.25
Mean Change	**-2.0**	**3.0**	**-2.0**	**3.1**	
Depression & Anxiety PANSS					
Baseline	9.5	3.9	10.3	9.5	Change F=174.7, p<0.001
Endpoint	7.0	3.2	6.9	3.1	Group F=0.05, p=0.81
Mean Change	**-2.5**	**4.0**	**-3.4**	**9.9**	
Total PANSS					
Baseline	72.3	33.3	64.6	25.7	Change F=24.8, p<0.001
Endpoint	57.0	25.8	50.9	21.2	Group F=0.06, p=0.80
Mean Change	**-15.3**	**18.6**	**-13.7**	**14.5**	
Clinical Global Impression – Severity					
Baseline	3.5	1.5	3.4	1.1	Change F=10.3, p=0.003
Endpoint	2.9	1.3	2.8	1.1	Group F=0.03, p=0.86
Mean Change	**-0.5**	**1.1**	**-0.6**	**0.9**	
Calgary Depression Scale (n=24)					
Baseline	4.0	3.6	1.8	1.5	Change F=8.4, p=0.008
Endpoint	2.0	3.1	1.4	2.0	Group F=0.2, p=0.62
Mean Change	**-2.0**	**3.1**	**-0.2**	**2.2**	
Hamilton Depression Rating Scale (n=20)					
Baseline	10.7	9.8	7.8	3.8	Change F=34.7, p<0.001
Endpoint	4.4	4.8	4.2	3.6	Group F=0.1, p=0.75
Mean Change	**-4.7**	**8.1**	**-3.1**	**5.3**	
Young Mania Rating Scale (n=20)					
Baseline	5.0	5.9	6.2	8.2	Change F=121.1, p<0.001
Endpoint	1.3	1.9	1.7	3.0	Group F=0.06, p=0.79
Mean Change	**-3.7**	**5.7**	**-4.5**	**8.1**	

*Based on analysis of covariance adjusted for baseline score. "Change F" refers to ANCOVA F statistic for change in time, while "Group F" refers to between group difference.

3. CHAPTER 2

Prevention of cardiovascular effects of antipsychotic drugs by melatonin: A novel neural pathway

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3.1. Abstract

Recent clinical studies showed that melatonin, a hormone synchronizing the activity of the biological clock, attenuates antipsychotic drug induced adverse metabolic effects. We investigated the acute effects of olanzapine and melatonin in male Wistar rats. The suprachiasmatic (SCN) an important target area of melatonin was activated by olanzapine followed by the paraventricular nucleus and dorsal motor nucleus of the vagus indicating an important parasympathetic tone induced by olanzapine. This was confirmed by an Olanzapine induced decrease in blood pressure and heart rate. Melatonin abolished the neuronal activation in the SCN as well as in the parasympathetic pathway; simultaneously melatonin prevented the cardiovascular side effects. The SCN as main target for olanzapine cardiovascular effects was confirmed since without the SCN, olanzapine did not decrease blood pressure. Areas in the brain activated by olanzapine associated with its anti-psychotic effect were unchanged in their activity after melatonin. This identifies a novel mechanism for olanzapine metabolic side effects.

Key words: Olanzapine, Melatonin, suprachiasmatic nucleus, paraventricular nucleus, cardiovascular.

3.2. Introduction

Patients with mental illness such as schizophrenia and bipolar disorder, show chronic disturbances in circadian rhythms. (Etain et al., 2011; Wulff et al., 2012) Their risk of metabolic abnormalities is up to 2 or 3 times that of the general population and the leading cause of death is cardiovascular disease; which occurs 11 to 20 years earlier. Cardiovascular risk in these patients is greatly increased by the use of drugs to treat them such as second generation antipsychotics (SGA), because they induce adverse metabolic effects (AME). (Vancampfort et al., 2013c; Westman et al., 2013) Additionally, AME generate poor adherence to treatment and currently there are few effective options to control them. (Girgis et al., 2008)

The SGA induced AME are present in acute administration studies in healthy individuals, indicating metabolism changes independent from caloric intake and disease related factors.(Hahn et al., 2013) Up till now no explanation is accepted providing a mechanism for these metabolic adverse effects.(Leucht et al., 2013)

A recent study demonstrated that melatonin, the hormone of the pineal, attenuates olanzapine induced weight gain in rats. Olanzapine is currenty one of the most widely used SGA which induces more severe AME.(Raskind et al., 2007) Hereafter in a controlled randomized clinical trial, we demonstrated that melatonin attenuated SGA induced AME in patients diagnosed with bipolar disorder and schizophrenia without affecting the psychopathological outcome.(Romo-Nava et al., 2014a) In another study, melatonin was able to attenuate olanzapine induced AME in patients diagnosed with schizophrenia.(Modabbernia et al., 2014)

Aim & hypothesis

Because the main target of melatonin in the brain is the SCN(Reppert et al., 1988), and the SCN is strongly involved in metabolic control(Buijs et al., 2013); these basic and clinical results could suggest the involvement of central mechanisms in the metabolic regulation of SGA induced AME. Therefore, we examined in a rat model the effect of olanzapine on hypothalamic nuclei relevant to metabolic regulation and uncovered a hitherto unknown action of olanzapine on the biological clock resulting in a decrease in blood pressure. Notably this effect of olanzapine was prevented by melatonin while the effects of olanzapine on other brain regions associated with its anti-psychotic effects remained intact, providing a rationale to combine the treatment of SGA with melatonin.

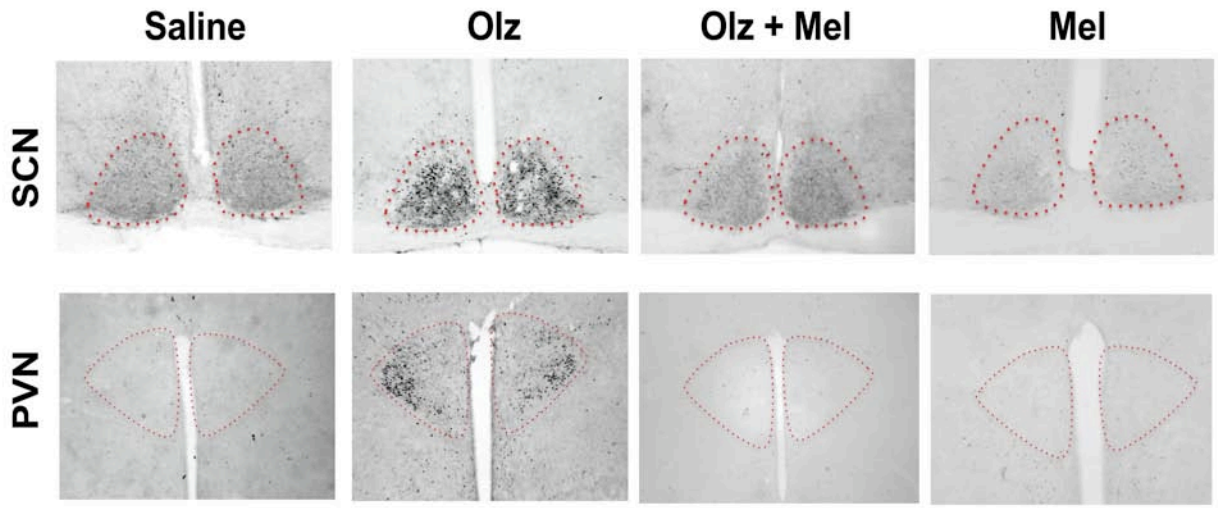
3.4. Results

Olanzapine induces the activation of the SCN at night; melatonin prevents this.

To assess the role of olanzapine on the brain at night, we administered a single dose of olanzapine and analyzed neuronal activity using c-fos. The acute administration of olanzapine induced a three times higher activation of the SCN as compared to saline. The co administration of melatonin with olanzapine completely prevented this effect. The administration of melatonin alone showed a significantly lower SCN activation as compared to the other experimental groups. **(Figure 1-A and B)** In the PVN **(Figure 1-A and C)** and DMV **(Figure 2-A and B)** the acute administration of olanzapine also induced a significantly greater activation compared to the saline group and similarly to the SCN, the co administration of olanzapine and melatonin prevented this effect. In the spinal cord however, olanzapine did not induce an activation of sympathetic motor

neurons. **(Figure 2-A and C)** This olanzapine- induced activation indicates that olanzapine selectively increases the activity of the parasympathetic and not the sympathetic branch of the ANS. Since the SCN strongly signals to the PVN and modulates its autonomic output(Buijs et al., 2003) and melatonin receptors are present in the SCN and not in the PVN and DMV these results indicate that olanzapine activates only the SCN and that the PVN and DMV activation is consequence of it.

A



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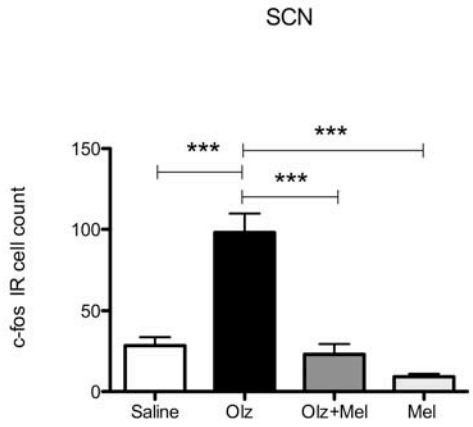

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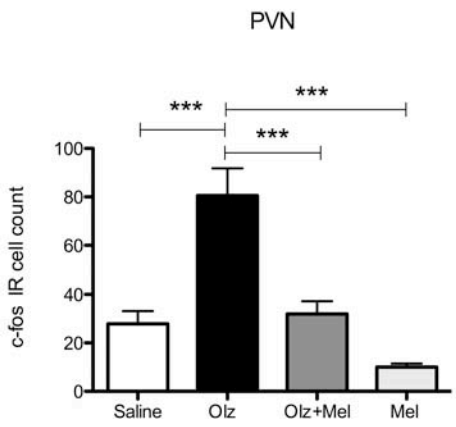


Figure 1. Olanzapine induces neuronal activation in the SCN and PVN that melatonin prevents. Panel with representative microphotographs of the SCN (top row) and PVN (bottom row) with c -Fos immunoreactivity as a marker of neuronal activity.(A) The left column is for rats treated with saline, followed by a column of rats treated with olanzapine, olanzapine + melatonin and melatonin only. Bars represent the mean ± SEM of c –Fos IR nuclei count in the SCN (B) and the PVN (C). ANOVA´s Bonferroni´s post hoc test pair-wise comparisons; *** p<.0001.

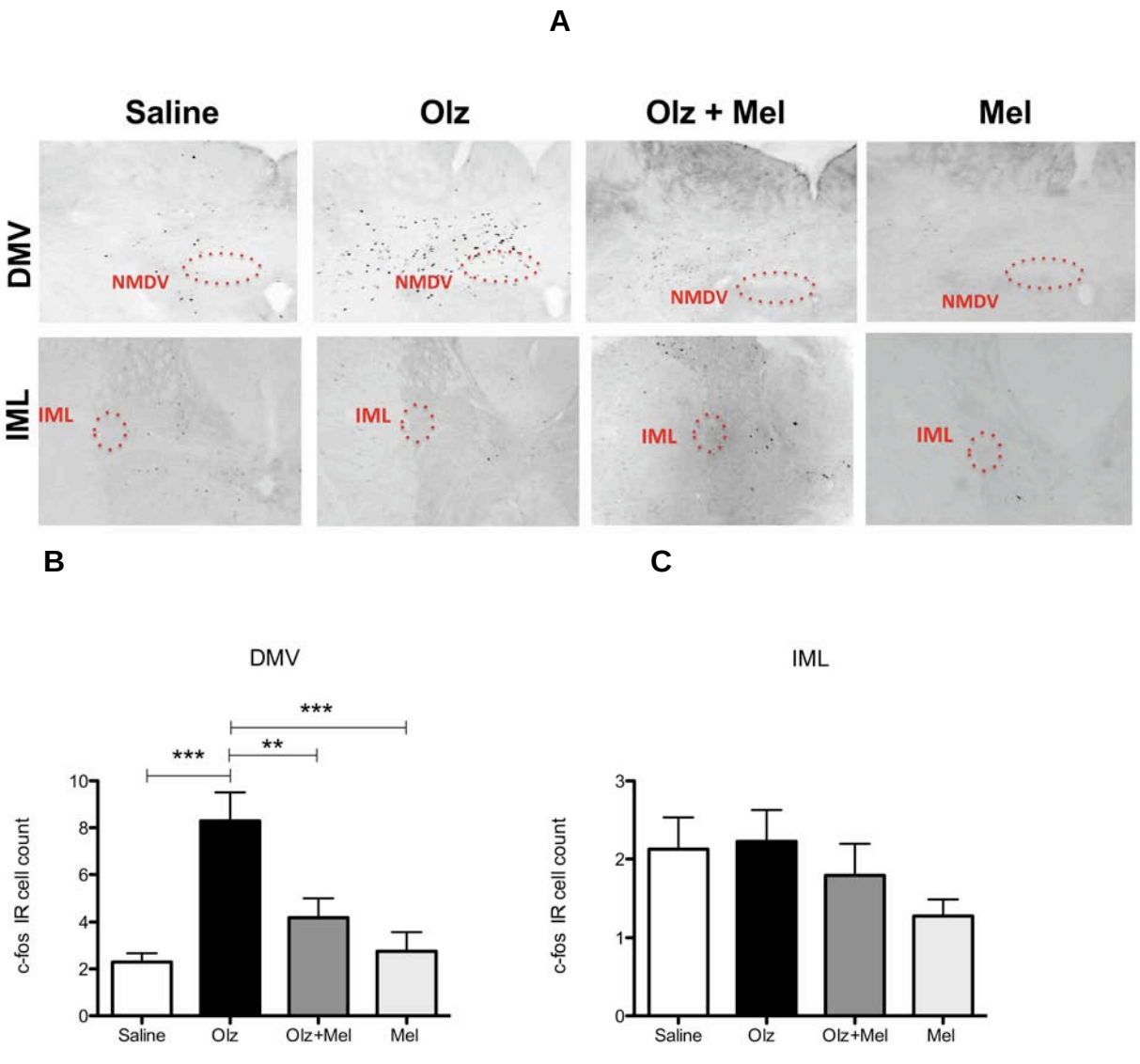


Figure 2. Olanzapine also induces an activation of the dorsal motor nucleus of the vagus (DMV) that melatonin prevents. Microphotograph example panel (Fig 4-A) of the DMV and intermediolateral column (IML) with c-Fos immunoreactivity as a marker of neuronal activity.(A) The left column is for rats treated with saline, followed by a column of rats treated with olanzapine, olanzapine + melatonin and melatonin alone. The top row corresponds to DMV and the bottom row corresponds to the IML. Bars represent the mean ± SEM of c-fos IR nuclei count in the DMV counted (B) and the IML (C). ANOVA Bonferroni´s post hoc test pair-wise comparisons; ** p <.005; *** p<.0005.

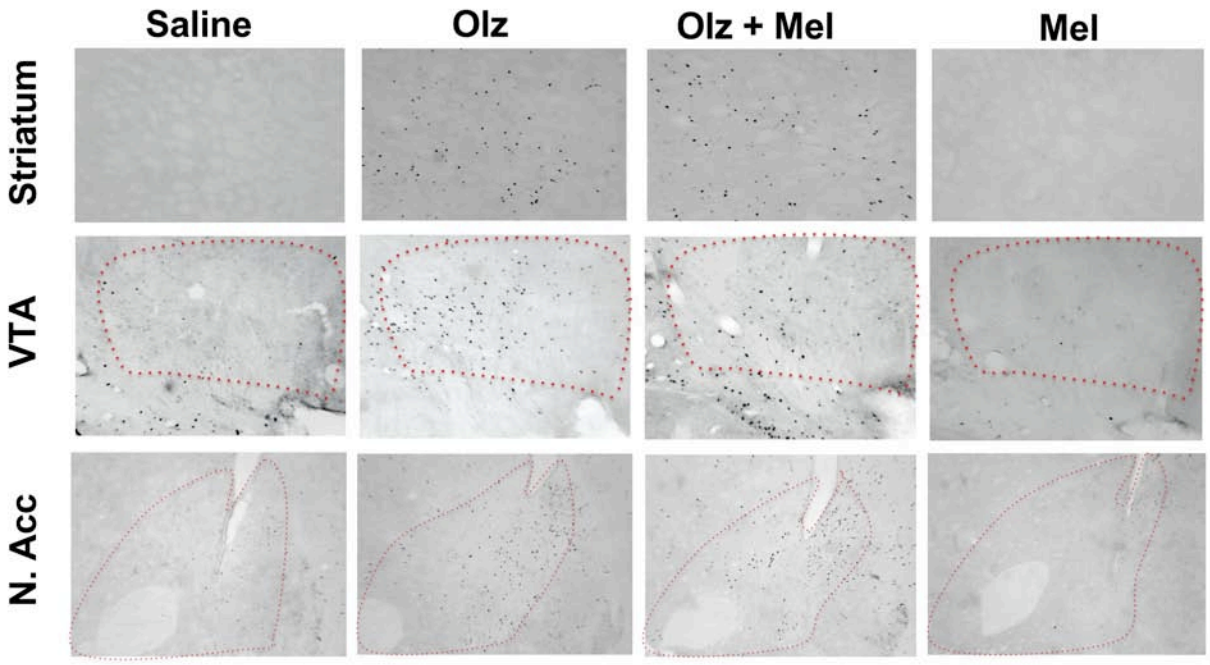
Olanzapine induced activation in brain areas associated to its therapeutic effect is not inhibited by melatonin.

As expected we observed that the administration of olanzapine induces also an activation of the nucleus accumbens, striatum and VTA; brain areas associated with its therapeutic effect, but that do not receive direct input from the SCN. (Robertson and Fibiger, 1996; Sebens et al., 1998) In contrast with the observation in the SCN the co administration of melatonin with olanzapine shows a similar activation pattern in the striatum and VTA as compared to that with olanzapine alone. In the nucleus accumbens the co administration of melatonin with olanzapine induced a significant increase of activity as compared to the administration of olanzapine alone. These results confirm that olanzapine induces activation of brain regions involved in its therapeutic action and the etiology of mental disorders for which it is prescribed; whereas melatonin does not affect or as seen in the nucleus accumbens, even potentiates the effect. **(FIGURE 3-A to D)** This observation agrees with the absence of melatonin receptors in these brain

areas while it also concurs with the clinical observations that the co administration of melatonin with SGA does not impair their beneficial effects. (Modabbernia et al., 2014; Romo-Nava et al., 2014a).

Further, we observed that olanzapine induces an activation of Lamina I to IV sensory pathways in the spinal cord and the tractus of the nucleus solitarius which receives input from the sensory pathways; but melatonin did not prevent these effects. (Supplementary material Figure -) This indicates that olanzapine also has a peripheral effect that melatonin does not prevent; supporting a central, rather than peripheral action of olanzapine and melatonin over the SCN, PVN and the DMV.

A



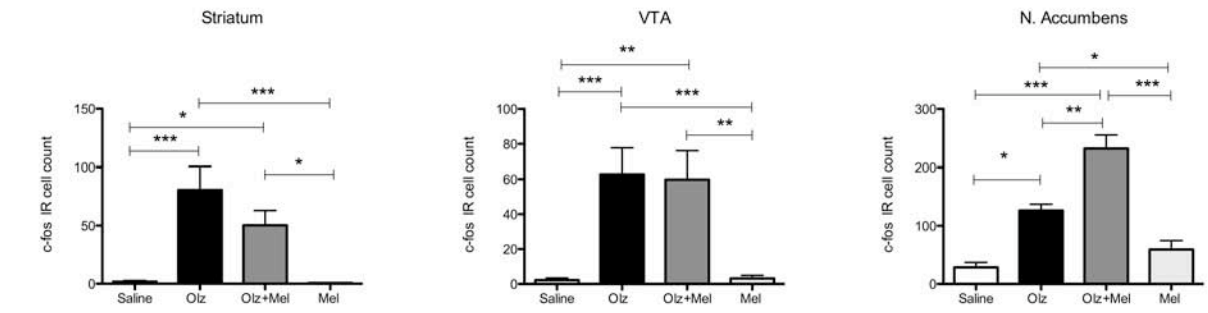


Figure 3. Olanzapine induces an activation of brain regions associated with its therapeutic effect that melatonin does not prevent. Panel with microphotograph examples of c- fos immunorreactivity (A) from top to bottom; the striatum, ventral tegmental area (VTA) and nucleus accumbens (N. acc). The left column is for rats treated with saline, followed by a column of rats treated with olanzapine (Olz), olanzapine + melatonin (Olz+mel) and melatonin (Mel) only. Bars represent the mean ± SEM count for c-Fos IR nuclei in the striatum (B), VTA (C) and N. acc.(D) ANOVA Bonferroni´s post hoc test pair-wise comparisons; *p<.01; ** p <.001; *** p<.0001.

Olanzapine activates pre-autonomic neurons in the PVN that project to the DMV.

The pattern of c-Fos activation in the PVN suggested that mainly autonomic neurons might be activated, in view of the activation of the NTS and DMV we decided to place a retrograde tracer into the DMV complex and analyzed the activation of the retrogradely labeled neurons. CtB labeled neurons in the PVN showed a high coincidence with c-Fos when olanzapine was given as 37% of the neurons in the parvocellular PVN retrogradely labeled from the DMV were activated. Magnocellular cells showed few CtB

labeled neurons and co-localization with c-fos was significantly lower as compared to the parvocellular region; only 14%. (FIGURE & Supplementary material table -) Moreover some of these activated CtB labeled neurons showed input from SCN neurons as visualized by vasoactive intestinal peptide (VIP) immunohistochemical staining.

(FIGURE 4)

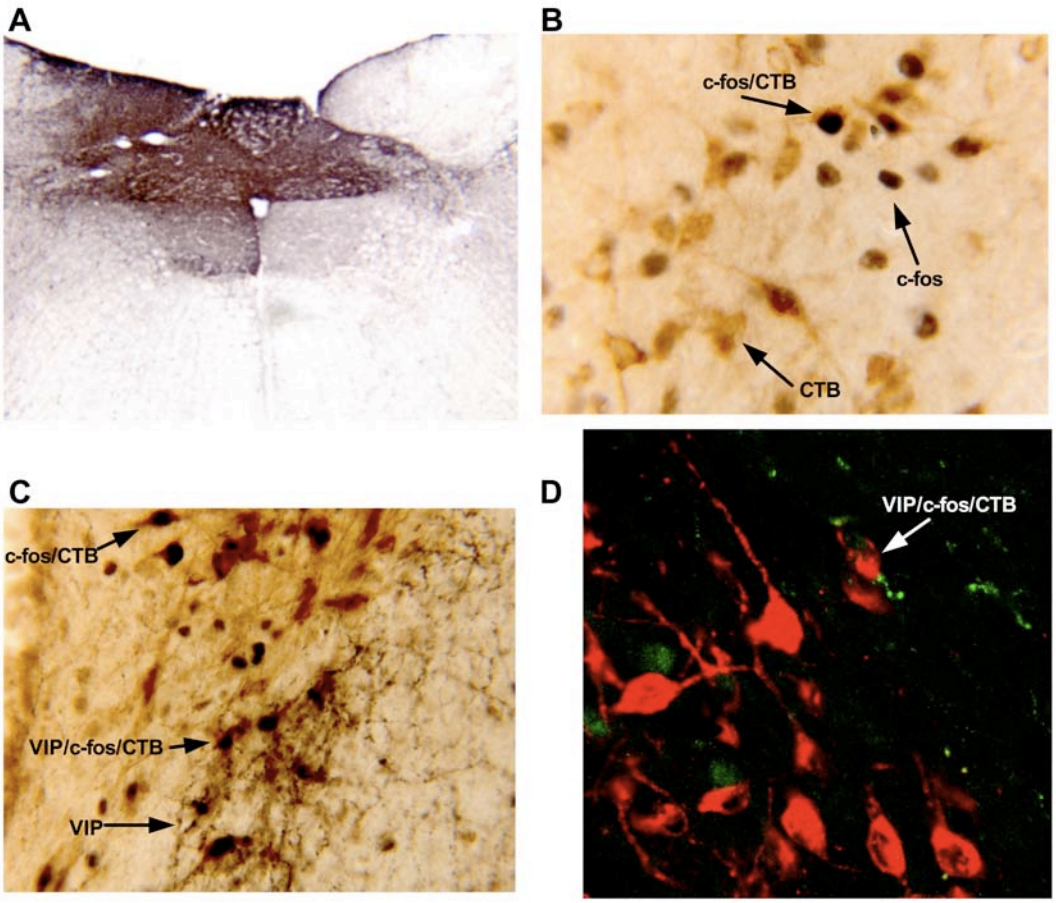


Figure 4. Olanzapine induces activation of pre-autonomic neurons in the PVN that receive input from the SCN and project to the DMV. Immunohistochemical confirmation of CtB injection in the DMV complex 14 days after injection.(A) Confocal microphotograph shows strong colocalization of c-fos (black nuclei)/CTB (red-brown

cytoplasm) in the dorsal parvocellular PVN (40x). (B) Confocal microphotograph shows preautonomic neurons IR to c-fos (black nuclei) in the dorsal parvocellular PVN after olanzapine injection and retrogradely filled with CTB (red cytoplasm) that receive VIP (dark projections) input from the SCN (6x). (C) Immunofluorescence microphotograph showing VIP (Cy2-green projections)/c-fos (Cy2-green nuclei)/CtB (red cytoplasm) colocalization in preautonomic neurons at the dorsal parvocellular PVN (40x). (D)

Olanzapine decreases blood pressure and heart rate, while melatonin attenuates this effect; an intact SCN is necessary to observe the effect of olanzapine.

Parasympathetic activity plays an important part in blood pressure regulation(Shaffer et al., 2014), adiposity(Kreier et al., 2002) and metabolic activity (Kreier et al., 2006). Its hyperactivation could contribute to the appearance of the metabolic syndrome, obesity (Suzuki et al., 2014) or the SGA adverse metabolic effects. Our anatomical analysis showed that olanzapine induces an activation of the parasympathetic branch of the ANS, and melatonin attenuates this effect. To evaluate a functional implication of this finding, we measured the acute effects of olanzapine and melatonin on blood pressure and heart rate; two variables influenced by autonomic function and modified by olanzapine.(Choure et al., 2014; Leung et al., 2014; Markowitz et al., 2002) In agreement with the observed increase in activity of DMV neurons we observed that olanzapine also induced a great decrease in systolic, diastolic, mean arterial blood pressure (MAP) and heart rate. The administration of melatonin ten minutes before the injection of olanzapine significantly decreased its effects on systolic, diastolic and MAP; but not heart rate. These findings show that the olanzapine induced hyperactivation of

the parasympathetic nervous system is translated into a decrease in blood pressure and heart rate which is largely avoidable by the use of melatonin.

By the presence of melatonin receptors on the SCN and their absence in the PVN and DMV we hypothesized that the cardiovascular effects of Olanzapine and their prevention by melatonin were due to the effect of olanzapine on the SCN. To evaluate the involvement of the SCN in the hemodynamic effects of olanzapine, we performed bilateral SCN lesions (SCNxx) and after two weeks of recovery, we measured the effects of olanzapine on blood pressure and heart rate. The injection of olanzapine in SCNxx animals induced a decreased effect on hemodynamic parameters similar to that observed in intact animals treated with melatonin and olanzapine. This observation confirms that Olanzapine mainly acts on the SCN to induce its cardiovascular effects.

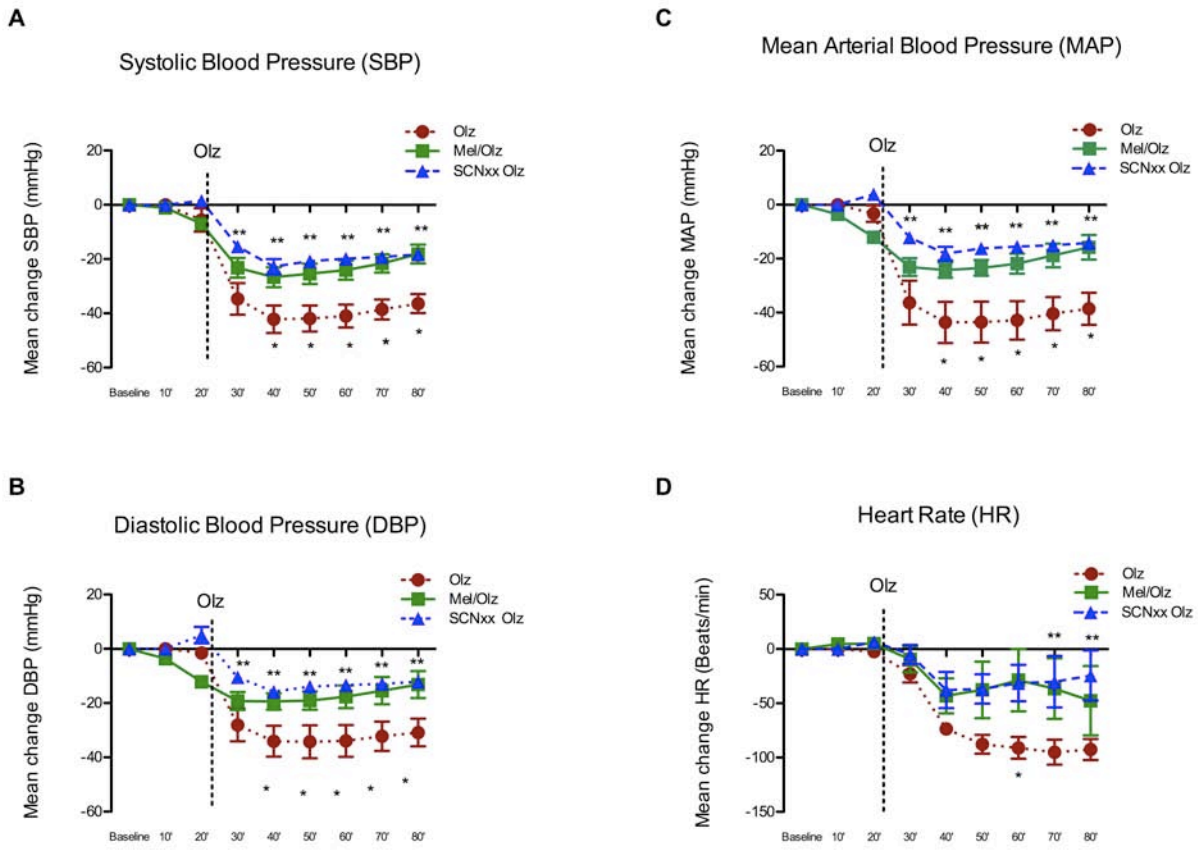


Figure 5. Olanzapine induced a decrease in systolic blood pressure (A), diastolic (B) and mean arterial blood pressure (C) that melatonin and a bilateral SCN lesion prevent. Olanzapine also induces a decrease in heart rate that is decreased by SCNxx, but not melatonin.(D) Olanzapine (Olz); Melatonin and Olanzapine (Mel/Olz) (Olz); SCN lesioned animals injected with olanzapine (SCNxx). N=5 animals per group; Repeated measures ANOVA with Bonferroni´s post hoc test; * p< .01 Olz vs. Mel/Olz; ** p< .01 Olz vs. SCNxx Olz.

3.5 Materials & Methods

Experimental Strategy I: Acute subcutaneous administration of olanzapine and olanzapine + melatonin.

To evaluate the effect of acute adiminstration of olanzapine (Zyprexa ® powder for solution, Lilly USA , LLC , Indianapolis , IN 46285 , USA) and melatonin (Sigma-Aldrich product No. M5250, Saint Louis, MO 63103 , USA) were used in male Wistar rats (200 - 250g) in day / night cycles of 12 h with ad libitum food and water. Rats received a single subcutaneous dose of olanzapine (0.5mg/kg), olanzapine (0.5mg/kg) + melatonin (2.5 mg), melatonin (2.5mg) or saline (NaCl 0.9%) Zt11 (one hour before starting the night) and sacrificed in Zt14. Olanzapine was administered in 0.4 ml diluted saline solution and 0.1 ml of ethanol. Melatonin was first diluted in 0.1 ml of ethanol and 0.4 ml of saline, in 0.1 ml of ethanol and then 0.4 ml of saline / olanzapine solution and finally the saline group received 0.1 ml of ethanol 0.4 ml of saline.

(FIGURE 7)

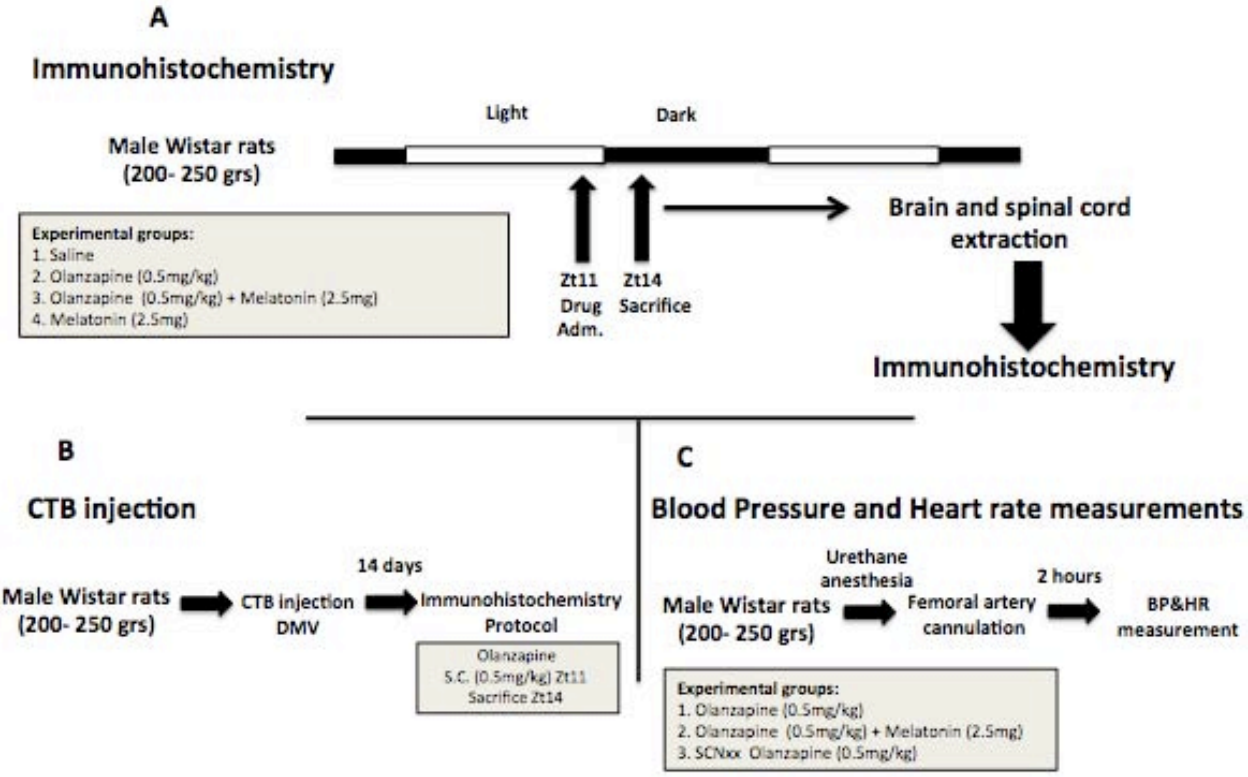


Figure 7. Experimental settings.

Immunohistochemistry for c- Fos: Animals were sacrificed (Zt14), 3 hours after drug injection. To obtain brains, the animals were anesthetized with an overdose of pentobarbital injected intraperitoneally (150 mg/ Kg) and perfused with an intracardiac infusion of 250 ml of saline (NaCl 0.9 %) followed by 200ml of 4% paraformaldehyde prepared in phosphate buffer 0.1 M pH of 7.2, and saline (NaCl 0.9 %). Brain and spinal cord were removed and placed in 4% paraformaldehyde for 24 hrs and subsequently cryo-preserved in 30% sucrose for 72 hrs. The brains were frozen and cut in 40 um coronal plane slices and maintained in culture dishes with a 0.01M phosphate buffer pH 7.2. The primary antibody for c-Fos (anti rabbit–c-Fos) in a 1:40,000 dilution buffer

(PBS + 0.1% BSA and 0.2% Triton X100) was used as a marker of neural activity; was incubated for 1 hour at room temperature and 48 hours at 4 °C. Donkey-antirabbit biotinylated secondary antibody at 1:200 in dilution buffer (Jackson Immunoresearch , West Grove , PO , USA) was applied and incubated for 2 hrs and then avidin -biotin complex (Vector laboratories) 1:500 in dilution buffer was incubated 1 hr and finally the reaction was visualized with diaminobenzidine 10mg/100 ml TBS PH 7.4) combined with 0.003 % H_2O_2 and 0.05% nickel. The sections were mounted on gelatinized slides and cover slipped with Entellan.

Experimental Strategy III :

Retrograde tracers (Cholera toxin-B) were injected into the DMV and co-localization with c-Fos after acute subcutaneous administration of olanzapine, following the procedure as described above, to document involvement of pre-autonomic neurons in the PVN and ANS output to the DMV.

Neuronal tracer injection. After anesthesia the rats were placed in a David Kopf stereotaxic frame with the head fixed at 45º. Dissection of the dura and arachnoid to expose the dorsal surface of the bone at the level of the area postrema was performed. The head of the rat was placed so that the micropipette aligned perpendicularly to the medulla oblongata. The anterograde and retrograde CTB (Cholera Toxin B))(Invitrogen) 0.5% neuronal tracer was injected by means of a glass micropipette with a 0.02ul tip to inject 50ul in the DMV complex. The CTB tracer was injected unilaterally into the DMV complex by pressure (10 mbar, for 5 seconds). After 10 days of recovery,

the animals were sacrificed and injection accuracy was confirmed by immunohistochemistry with CTB. **(Figure 4-A)**

Immunohistochemistry in PVN coronal sections was performed with c -fos as described above. Immunohistochemistry for vasointestinal peptide (VIP) to observe SCN projections to the PVN pre-autonomic neurons was then performed using the primary antibody for (anti VIP in rabbit) 1:2000 in dilution buffer for 1 hour at room temperature and 24 hours at 4 °C. Donkey-antirabbit biotinylated secondary antibody at 1:200 in dilution buffer (Jackson Immunoresearch , West Grove , PO , USA) incubated for 2 hrs and then avidin -biotin complex (1:500) was applied and finally the reaction was visualized with diaminobenzidine tetrahydrochloride (10mg/100 ml TBS PH 7.4) combined with 0.003 % H_2O_2 and 0.05% nickel. . On the same PVN sections, CtB immunohistochemistry was performed to show PVN neurons that were retrogradely filled after CtB injection in the DMV complex using polyclonal rabbit anti- CTB at 1:1000 dilution for 24 hrs at 4°C. Secondary antibody incubation was performed using Donkey antirabbit biotinylated antibody at 1:200 dilution (Jackson Immunoresearch , West Grove , PO , USA) for 2 hrs, followed by the application of avidin-biotin complex (Vector laboratories) 1:500 in dilution buffer and finally the reaction was visualized with diaminobenzidine tetrahydrochloride 10mg/100 ml TBS PH 7.4) in combination with 0.003 % H_2O_2 and 0.05% nickel.

For immunofluorescence, primary antibodies for c-Fos and VIP were incubated as described above. Sections were then incubated in Donkey anti-rabbit Cy2 at 1:200 dilution (Jackson Immunoresearch , West Grove , PO , USA) for 2 hrs.

Experimental strategy IV

In order to evaluate the functional relevance of the effect of olanzapine and melatonin on the parasympathetic nervous system, we measured their acute effects on blood pressure and heart rate. To evaluate the involvement of the SCN over the cardiovascular effects of olanzapine, we included a group of bilaterally SCN lesioned (SCNxx) animals and measured the acute effects of olanzapine on blood pressure and heart rate.

Blood Pressure Meassurements

Blood pressure measurements were made through a femoral artery catheter. Cannulation of the femoral artery was performed as described elsewhere. (Jespersen et al., 2012) Briefly, rats were anesthetized with urethane (1.5g/kg) diluted in 2ml of saline (NaCl 0.9%) I.P. The rat was placed in supine position and fur on inguinal surgical region was shaved. A small 1- 2 cm incision along the natural angle of the leg was performed, and connective tissue blunt dissected until exposure of femoral vein and artery. The femoral artery was separated from the vein, nerve and surrounding tissue and retractors into incision placed to fully view the artery and vein. Folded sterile 4.0 silk was placed under the femoral artery and cut to obtain proximal and distal silk pieces. The distal silk piece was pulled caudally and the proximal piece as cranially as possible to allow haemostatic control of the artery. A small incision was made on the artery section between the silk pieces. Fine tip forceps were inserted into the incision and used to allow the insertion of the catheter. The catheter was pushed into the artery and proximally fixed. The functionality of the catheter was checked and two hours after surgery, connected to a blood pressure transducer.

Bilateral lesion of the suprachiasmatic nucleus.

The SCN lesion technique has been previously described.(Buijs et al., 1993) In brief, animals were anesthetized with a ketamine /xylazine (80 mg and 8 mg / kg) i.p. and placed on stereotaxic surgery frame (Model 900, David Kopf) coordinates to lesion the SCN were: 2.2mm posterior to bregma, 8.1mm ventral, and 0.2mm lateral. Lesion was performed with epoxy insulated (except at the tip) insect pins (0.20mm). Direct electrical current for 30 seconds with a marker lesion developed by our group was applied bilaterally. Rats recovered from surgery for 2 weeks, and successful lesion confirmed by actigraphic registration and SCN imunohistochemical staining with VIP as described above. **(Supplementary material)** The protocol for acute administration of olanzapine was then followed as described previously **(Figure 7-A)**

Blood pressure and heart rate measurements were recorded from the artery catheter by a pressure transducer (P23 XL, Grass Instrument, Quincy, MA, USA.) connected to a MP150 Research System (Biopac System Inc., CA,, USA) and the data were analyzed using AcqKnowledge software. For the purpose of this experiment, olanzapine and melatonin were administered intraperitoneally (I.P.), to ensure rapid effects on hemodynamic parameters. Tracing was obtained for three groups of animals. Group 1) olanzapine only; Baseline tracing (10 min), .5ml saline (NaCl 0.9%) I.P. tracing (10 min), followed by a single olanzapine I.P. injection and tracing (60 min). In Group 2) Melatonin + olanzapine; Baseline tracing (10 min), .5ml saline (NaCl 0.9%) I.P. tracing (10 min), followed by a single melatonin (2.5mg) I.P. injection tracing (10min) and finally

olanzapine I.P. injection tracing (60 min). Group 3) Bilateral SCN lesion animals (SCNxx) injected with olanzapine; Baseline tracing (10 min), .5ml saline (NaCl 0.9%) I.P. tracing (10 min), followed by a single olanzapine I.P. injection tracing (60 min).

Ethical statement for animal experimentation: All experiments were carried out with the approval of the research ethics committee of the Institute of Biomedical Research at the UNAM and were conducted in strict accordance with current legislation and technical specifications for production, care and use of laboratory animals. (Norma Oficial Mexicana NOM- 062 -ZOO- 1999).

Statistical Analysis

The number of immunoreactive nuclei for c-fos were calculated by semi-automatic quantification of the region of interest with 1.46r software ImageJ (NIH, USA, http://imagej.nih.gov/ij) using two or three microphotographs of coronal sections in. jpg format of each side in each nucleus studied. For comparison between groups (n=4 per group), we considered the average count immunoreactive to c-fos in each region and analysis by ANOVA Bonferroni´s post hoc test for pair-wise comparisons according. Statistical tests were two-tailed and considered a significance level of 0.05.

Blood pressure and heart rate analysis were performed using AcqKnowledge software. Ten minute blood pressure tracing mean values for baseline, saline, olanzapine or melatonin injections were obtained. Mean change values from baseline in systolic, diastolic and mean arterial blood pressure were calculated for each experimental group tracing segment of ten minutes. Heart rate analysis was performed using maximum

values in four 2 minute segments for each ten minute tracing to clear from noise in registration, and the mean for each ten minute tracing was calculated using these values. Mean change from baseline in heart rate was then calculated for saline, olanzapine and/or melatonin injections. Repeated measures ANOVA Bonferroni´s post-hoc test was used for pair-wise comparisons at each time point (10 min segments).

3.6 Discussion

The results of our animal experiments provide a plausible and comprehensive explanation for cardiovascular side effects of olanzapine and the beneficial effects of melatonin with the following observations; 1) olanzapine activates neurons in the SCN, PVN and DMV; 2) Melatonin prevents this effects; 3) Pre-autonomic neurons in the PVN that are activated by olanzapine project to the DMV and receive input from the SCN; 4) olanzapine induces a decrease in blood pressure that melatonin prevents; and 5) an intact SCN is necessary for the cardiovascular effects of olanzapine.

We observed that olanzapine activates neurons in the SCN, PVN and DMV at a time point where the SCN is normally inactive in rodents and humans. The SCN signals pre-autonomic neurons in the PVN influencing autonomic output via glutamate and GABA.(Cui et al., 2001) We propose that the effects of olanzapine which is a medication with a diverse spectrum of action, could induce direct SCN activity via a reverse agonistic action on the melatonin receptor in the SCN. Or by shared intracellular signaling cascades that modify neuronal activity in the SCN. In this sense, it is already known that olanzapine and melatonin act on similar signaling pathways. Examples are

the Akt1, Wnt and PKC signaling pathways that converge in relevant biochemical actors such as the glycogen synthase kinase 3 b (GSK3B); important for neurogenesis, dendrite formation and metabolic processes. Olanzapine induces phosphorylation of the GSK3b at least through the Akt, Wnt and PKC pathways, both linked to antipsychotic induced metabolic adverse effects and therapeutic effects.(Aubry et al., 2009; Girgis et al., 2008; Lee et al., 2010; Pavan et al., 2010) Melatonin in contrast is able to decrease phosphorylation of GSK3B through Akt1.(Ge et al., 2013) Through these intracellular crossroads it is possible that olanzapine induces an activation of the SCN that melatonin is able to prevent.

Previous studies have shown how altering the output signal of the biological clock affects selective balance of the ANS to different parts of the body that over time could generate metabolic problems such as the metabolic syndrome.(Kreier et al., 2003) Acutely, olanzapine favors a shift to an increased parasympathetic tone translated to a decrease in blood pressure in rats and postural hypotension in humans.(Choure et al., 2014) In time, the use of olanzapine and other SGA is associated to an elevated prevalence of obesity, metabolic syndrome, glucose disturbance and hypertension(Mitchell et al., 2013b; Vancampfort et al., 2013b); In which an increased sympathetic tone is reported. These we interpret as a sympathetic effort of the ANS to counterbalance the chronic parasympathetic stimulus induced first by olanzapine and followed by the progressive appearance of metabolic disturbances. Melatonin seems to favor metabolic balance via the ANS according to context and in a time dependent manner, instead of just favoring the sympathetic or parasympathetic outputs. (Pechanova et al., 2014) Acutely we have demonstrated that it prevents the increased

parasympathetic output induced by olanzapine, but in a chronic context melatonin prevents an increase in blood pressure and has antihypertensive effects. (Romo-Nava et al., 2014a; Scheer, 2004)

With this background, our previous clinical data and the present basic results, we propose a new SCN based model that could explain drug induced adverse metabolic effects and how it is that melatonin prevents them.

(Figure 8)

Considering that melatonin is safe, inexpensive and effective in preventing drug induced metabolic adverse effects, this study provides evidence for the neurobiological pathways involved and support for widespread use in the clinical setting in parallel to the initiation of drugs prone to generate adverse metabolic effects.

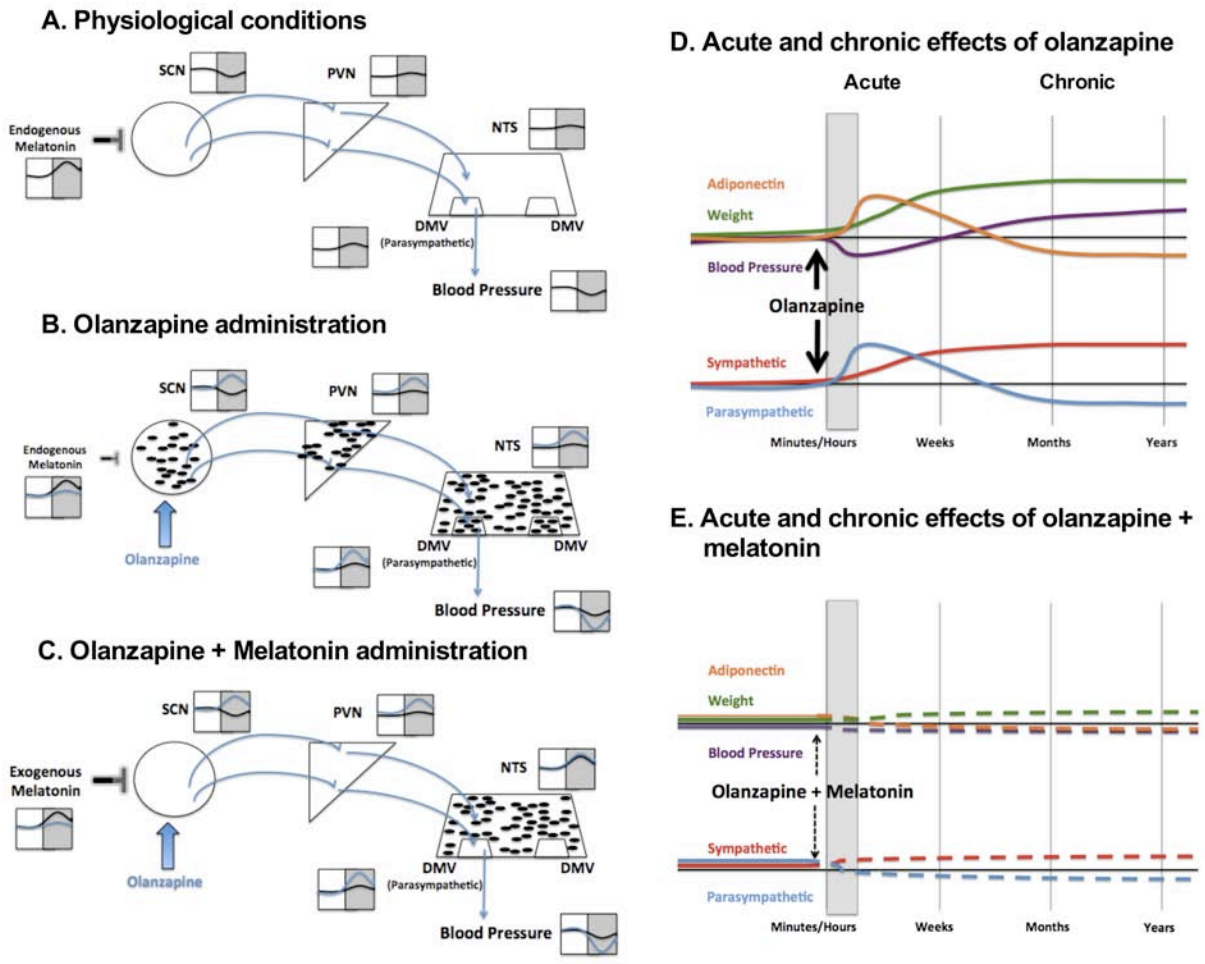


Figure 8. A proposed neural pathway for SGA induced metabolic effects and their attenuation by melatonin.

In physiological conditions (A), the activity of the SCN prepares the body to function according to activity/rest periods by signaling the PVN that will then execute accordingly through the autonomic nervous system (ANS). The ANS works through two antagonistic branches, the sympathetic nervous system (SNS) with motor neurons in the intermediolateral column (IML), and the parasympathetic nervous system (PNS) in the dorsal motor nucleus of the vagus (DMV). The SNS activity is predominant in activity (fight, fright and flight), and the PNS activity is predominant during inactive (rest and

digest) periods. The ANS discriminates between different compartments throughout the body (thoracic, abdominal or muscular), and maintains a sympathetic- parasympathetic balance according to each compartment.(Kreier et al., 2002) Olanzapine disrupts this balance by activating the SCN at a time point in which it is normally inactive and decreasing the production of endogenous melatonin.(Raskind, 2007) The SCN then signals the pre-autonomic neurons in the PVN and increases the parasympathetic activity through the DMV inducing a decrease in blood pressure. (B) The administration of melatonin prevents these effects by inhibiting the activation of the SCN. (Figure C) Acute parasympathetic activation favors a decrease in blood pressure and is associated to adiposity and an increase in adiponectin. (Suzuki et al., 2014) Such effects are also observed with the short- term treatment with olanzapine. (Togo et al., 2004) With time, an increased parasympathetic activity induced by olanzapine favors the appearance of a myriad of metabolic consequences like obesity, lipid, insulin and glucose disturbances similar to those observed in the metabolic syndrome. (Kreier et al., 2003; Lieberman et al., 2005b) These gradually trigger an increase in sympathetic tone as a counter measure to reinstate an autonomic balance. These dynamic adjustments in autonomic activity could explain the increased sympathetic tone, increased blood pressure and decreased levels of adiponectin found in patients with obesity, diabetes, hypertension (Canale et al., 2013; Li et al., 2009) and after chronic use of SGAs such as olanzapine.(Bartoli et al., 2015) (D) Melatonin would prevent the acute and chronic(Modabbernia et al., 2014; Raskind, 2007; Romo-Nava et al., 2014a) hypothalamic and ANS disturbances induced by olanzapine thus attenuating its adverse metabolic effects. (E) Arrows indicate activation; capped lines indicate inhibition and in both cases width and font size indicate magnitude. In A, B and C small squares

illustrate circadian activity divided in day (white) and night (gray); Black lines (melatonin + olanzapine); Blue lines (olanzapine). Black dots inside the schematic nuclei illustrate neuronal activity according to c-fos IR. In D and E continuous lines refer to the effects of olanzapine and dashed lines to the effect of olanzapine + melatonin.

Supplementary material

A **B**

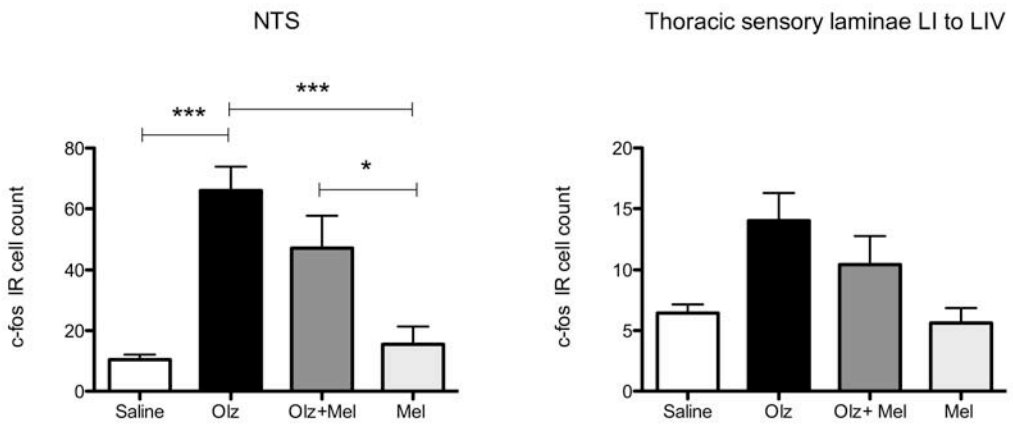


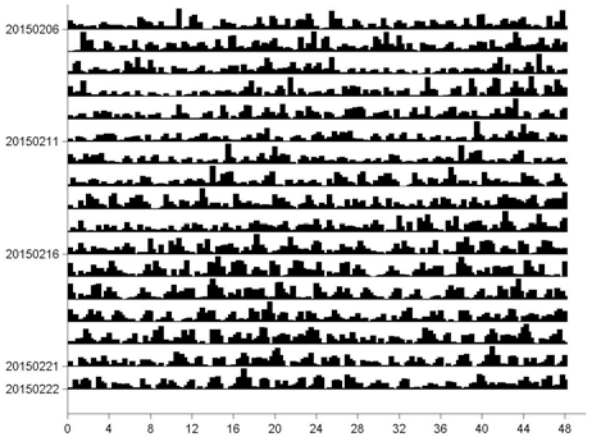
Figure S2. Melatonin does not prevent olanzapine induction of c-fos in spinal sensory pathways and the nucleus of the tractus solitarius (NTS). Bars represent the mean ± SEM count for c-Fos IR nuclei in the NTS (A) and thoracic sensory laminae LI to LIV (B). ANOVA Bonferroni´s post hoc test pair wise comparisons; *p<.01; *** p<.0001.

CtB injection in the DMV complex

PVN Region	CtB Mean(SD)	c-fos Mean(SD)	CtB & c-fos Mean(SD)	(CtB & c-fos)/CtB %
Ventral parvocellular	25.8(17.8)	23.0(5.1)	10.5 (6.4)	43.2
Medial parvocellular	13.5 (10.2)	12.5 (1.9)	4.0 (2.7)	37.0
Dorsal parvocellular	4.8 (4.1)	4.5 (3.5)	1.0 (0.8)	31.5
Mean parvocellular	14.6 (9.8)	13.3 (0.4)	5.1 (3.0)	37.2 *
Magnocellular	6.8 (7.8)	17.8 (17.4)	1.3 (2.8)	14.7

Table S-1. c-fos and CtB co- localization in the PVN. * p<0.0001. Mean Parvocellular vs. Magnocellular Chi-square test.

A **B**



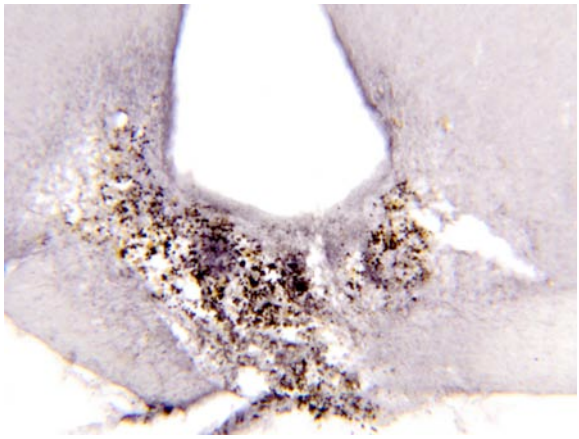


Figure S-3 . Shows actigraphic confirmation for arrhytmicity after SCN lesion. (A) VIP immunohystochemical confirmation of bilateral SCN lesion. (B)

4. CHAPTER 3

Mood disorders and their metabolic comorbidities: A shared disruption in brain-body communication.

To be submitted

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4.1 Abstract

Mood disorders are highly comorbid with metabolic illness such as obesity, metabolic syndrome, hypertension, diabetes and hyperlipidemia. This comorbidity increases the cardiovascular risk to patients and contributes to early mortality that occurs 10 to 20 years earlier than in the general population. The drugs that are currently used to treat these patients further increase their cardiovascular risk by generating adverse metabolic effects. The connection between these metabolic entities and drug induced adverse metabolic effects is not random; and there is overwhelming evidence that supports the involvement of a shared central neurobiological mechanism. The hypothalamus via the autonomic nervous system plays a fundamental part in the functional organization of our body, enabled by corporal information that is constantly sent back to the brain where it is interpreted to respond accordingly in order to maintain a dynamic physiological balance. Here we propose that If the brain-body communication is disturbed, deleterious consequences such as mental disorders and their comorbidities occur. The phenomenology of mood disorders and other accompanying symptoms are evidence for hypothalamic and autonomic dysfunction. Metabolic disorders are entities that also show hypothalamic and autonomic disturbances. With the current evidence, we propose a hypothalamic and ANS based integrative view for such comorbid phenomena. The challenge now is to document further the hypothalamic and ANS involvement in mood disorders and their non- psychiatric comorbidities. New integrative models for brain/body interaction need to be developed to favor an enhanced and wider view

for clinical and research purposes in the context of psychiatric and non psychiatric comorbidity prevention, diagnosis and treatment.

4.2 Introduction

Mood disorders such as major depressive disorder (MDD) and bipolar disorder (BPD) are highly comorbid with other non- psychiatric medical illness such as, obesity(McElroy and Keck, 2012), metabolic syndrome, hypertension, diabetes mellitus type 2, and hyperlipidemia.(Vancampfort et al., 2013b) Patients with mood disorders die up to 10 years earlier than people in the general population, and the main cause of death is non-psychiatric illness; particularly cardiovascular disease. (Crump et al., 2013; Miller and Bauer, 2014). In fact, early mortality is greatly explained by cardiovascular risk, which is further increased by drugs like second generation antipsychotics (SGA) used to treat patients; which produce adverse metabolic effects that resemble the metabolic syndrome. Associated with these “non-psychiatric” comorbidities is the overwhelming evidence for the involvement of the hypothalamus and autonomic nervous system (ANS) in their etiology. Hypothalamic changes in mood disorders are extensively investigated with a focus on the hypothalamic pituitary adrenal (HPA) axis and stress response.(Naughton et al., 2014) However, the causality of mood disorders relying solely on a HPA axis explanation has not been established.(Bao et al., 2008) At the other hand, circadian rhythm disruptions and ANS involvement in depression and bipolar disorder have attracted relatively limited and intermittent attention. In view of the strong relationship between the hypothalamus and the non-psychiatric comorbidities and their consequences we suggest a shared physiopathology with mood disorders. Bearing in mind that the feedback of disturbed physiology will affect more processes in

the brain than only hypothalamic function we propose that the link between psychiatric and non-psychiatric comorbidities is via the hypothalamus and/or the ANS.

4.3. The hypothalamus- body axis

The hypothalamus is a complex neurological entity that represents just 0.3% (4cm^3) of the adult brain. It controls systems essential in many physiological, endocrine, and behavioral processes. Among them, the control of sleep/wake cycles, reproductive behavior, feeding, drinking, blood pressure, glucose/insulin and temperature regulation.(Hofman and Swaab, 1992) These processes are tightly linked to higher cortical functions such as emotional regulation, fear related responses, executive function, memory, pain and sensory perception that allow brain- body organization. The hypothalamus uses several communication pathways to signal to the body and other brain areas. The outgoing hormonal and autonomic signals need to be adequately synchronized in order to have an optimal effect. Hereto the autonomic signals prepare the organs of the body for the coming hormones associated with the correct time of the day, with the correct moment for the reproduction cycle, with the correct feeding status and the correct temperature. Thus the hypothalamus not only has pre-autonomic neuronal systems connected to sympathetic and parasympathetic motor nuclei in brain stem and spinal cord it also has hormonal 'motor' neurons able to release their content into the circulation. In addition the hypothalamus receives information from the body about the level of metabolites and hormones, blood pressure and the physiological state of the organs. At the same time the hypothalamus is via its lateral part richly connected to the cortex. Thus via these elaborate pathways the hypothalamus maintains a balance

for optimal functioning of brain and body. If this balance is lost or changed, several deleterious consequences are expected to occur.(Buijs, 2013)

Clearly this communication between hypothalamus and body is bidirectional in order that the balance in physiology can be maintained. Therefore the body is constantly sending information to the brain updating the status of the body. Pain, somatic and sensory visceral information travels through the spinal dorsal horn and vagal afferents up to the nucleus of the tractus solitarius (NTS) and parabrachial nucleus, which relay this information in two streams; one directly to the hypothalamus and the other to integrative brain regions such as the thalamus.(Ruggiero et al., 1998) The thalamus sends the relevant information to cortical areas like the insula, providing consciousness of the corporal situation “interoceptive awareness” which is essential for emotional processing and regulation as well as other cognitive processes such as motivational behavior, decision making, etc. (Craig, 2009; Egeland et al., 2012) The insula also plays an essential role in autonomic control by providing input to the hypothalamus.(Nagai et al., 2010) The information that is sent directly from the NTS to the hypothalamus allows it to act accordingly in order to adapt bodily functions. At the same time, the NTS sends and receives information to and from the prefrontal cortex. (Pessoa, 2010) This complex neuronal crosstalk between body and brain is essential to coordinate autonomic and physiological processes associated with emotional regulation, to implement fear responses, brain and body circadian organization, feeding and sexual behavior, blood pressure, energy metabolism and temperature regulation, etc… Hereto essentially the hypothalamus needs also to be informed about the state of the body by means of hormonal and humoral feedback. Hereby we need not to think only of "classical" steroid hormones such as cortisol or gonadal hormones; also growth factors such as FGF21

secreted from the liver have an important signaling function towards the hypothalamus.(Bookout et al., 2013; Owen et al., 2014) Clearly we are just at the beginning of the understanding of the various ways and mechanisms the brain and body use to communicate with each other (see also Buijs et al., 2015 in press). Consequently disease in organs of the body may result in disturbed signaling to the brain with adaptations of the physiology as consequence but in addition with the possibility of severe mood disturbances.

(FIGURE 1)

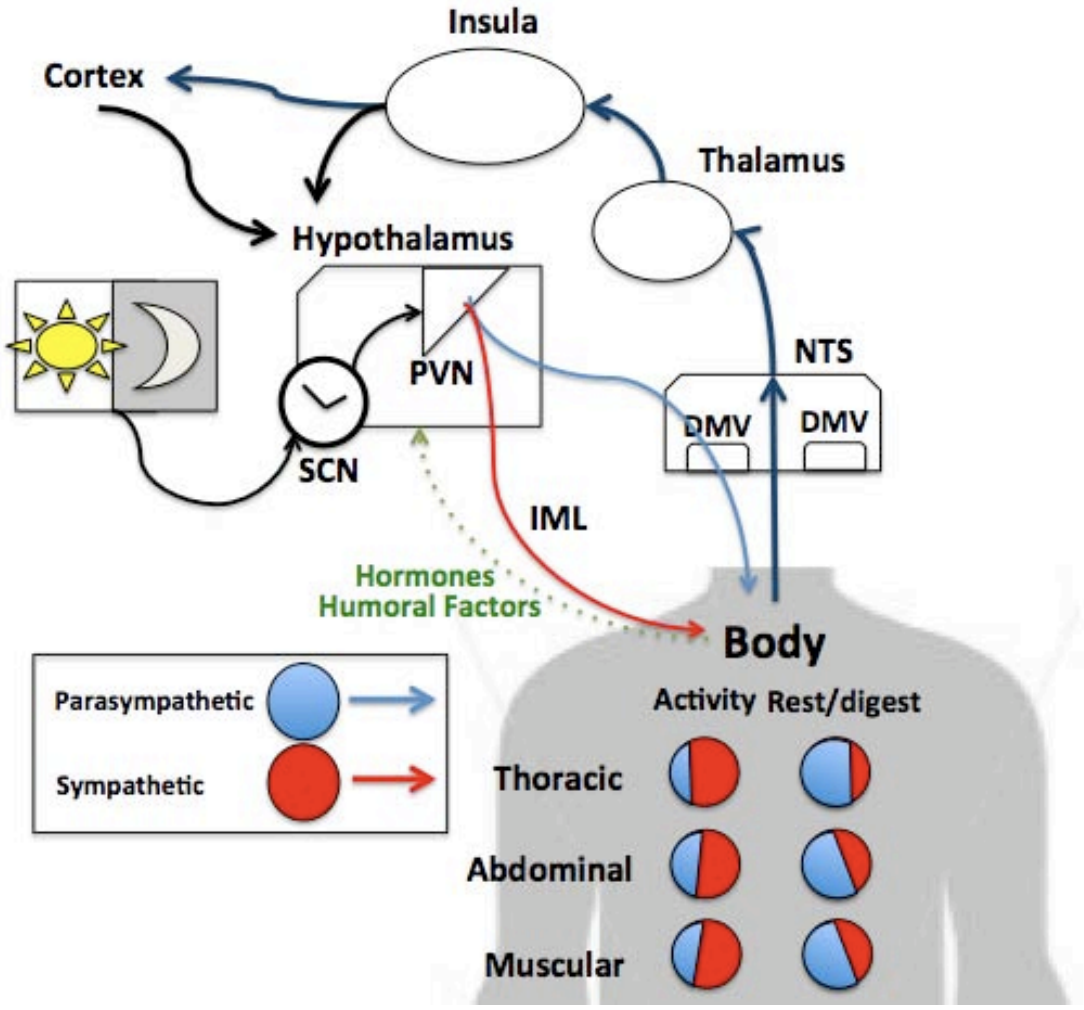

Figure 1. The hypothalamus-body axis. The hypothalamus is circadian driven. It organizes metabolic function according to day (activity) or night (rest/digest) by receiving light/dark information through our biological clock located in the SCN (suprachiasmatic nucleus). The SCN signals the PVN (paraventricular nucleus) to coordinate autonomic output through its parasympathetic (rest/ digest) or sympathetic (activity) branches via the DMV (dorsal motor nucleus of the vagus) or the IML (intermediolateral column) respectively. This information arrives to systems and organs that are compartmentalized in thoracic (cardiovascular), abdominal (visceral and fat) or muscular compartments. Feedback from the resulting corporal information is then sent back to the central nervous system through sensory pathways via the dorsal horn to the NTS (nucleus of the tractus solitarius), the insula, and other cortex areas or directly to the hypothalamus. The hypothalamus also receives input of bodily states via hormones and humoral factors (green dashed line).

4.4. Hypothalamus and the ANS in mood disorders

In mood disorders, hypothalamic and ANS involvement is conspicuous in their phenomenology. Sleep, appetite and libido disturbances during affective episodes as well as other neuro-vegetative symptoms (hyperhidrosis, palpitations, gastrointestinal or urinary symptoms) indicate a hypothalamic and ANS origin. The answer to the question what is cause or consequence seems to point to early hypothalamic and ANS dysfunction that appears years before the “first affective episode” or the so called “age of onset”. Evidence of early dysfunction in brain-body communication is illustrated by studies showing that children of bipolar parents present early sleep disturbances

(Faedda et al., 2014; Jones et al., 2006) and anxiety symptoms.(Duffy et al., 2007) An increased autonomic response to stressful tasks as early as 6 months of age has been documented in infants; who share this autonomic response with their mothers diagnosed with bipolar disorder.(Johnson et al., 2014) Autonomic arousal (i.e; increased vagal tone) has also been consistently documented in subjects at increased risk for mania(Gruber et al., 2008) and bipolar disorder patients both in adolescence(Egeland et al., 2012) and adulthood(Levy, 2013) and has been found to correlate inversely with the age of onset.(Latalova et al., 2010) Another expression of autonomic dysregulation could be present in the form of emotional temperamental traits such as “sensitivity”; thought to reflect how someone reacts to common stressors like interpersonal events (rejection, criticism, offense) or general situations (frustration, pressure, trauma, and loss) (Lara et al., 2012). Sensitivity is increased in remitted bipolar disorder patients(Romo-Nava et al., 2014b) and state rejection sensitivity increased during depressive episodes in bipolar disorder predicting the presence of headaches and corporal pain during depressive episodes for both bipolar and unipolar patients. (Ehnvall et al., 2014) Interpersonal sensitivity has also been reported to be increased in bipolar patients(Fletcher et al., 2012) and their offspring in whom it correlates with elevated cortisol levels; an indicator of increased hypothalamic/autonomic and or HPA axis hyperactivity.(Ostiguy et al., 2011) The offspring of bipolar disorder patients also show elevated levels of cortisol during adolescence that persist through adulthood.(Ellenbogen et al., 2010) All these findings strongly argue for the involvement of the hypothalamus and the ANS in the etiology of bipolar disorder and depression.

Bouts of increased energy, mood swings and other specific subsyndromal manic symptoms such as hyper sexuality and decreased need for sleep in youths precede the

onset of bipolar disorder for several months.(Correll et al., 2014a) As individuals age, the intensity of these prodromic symptoms gradually increases and eventually transforms into recurrent overt clinical affective episodes (Correll et al., 2014a; Egeland et al., 2012; Zeschel et al., 2013) along with the progressive appearance of comorbid entities such as obesity and the metabolic syndrome; followed by the appearance of diabetes mellitus type 2, dyslipidemias, hypertension and their cardiovascular consequences.

(FIGURE 2)

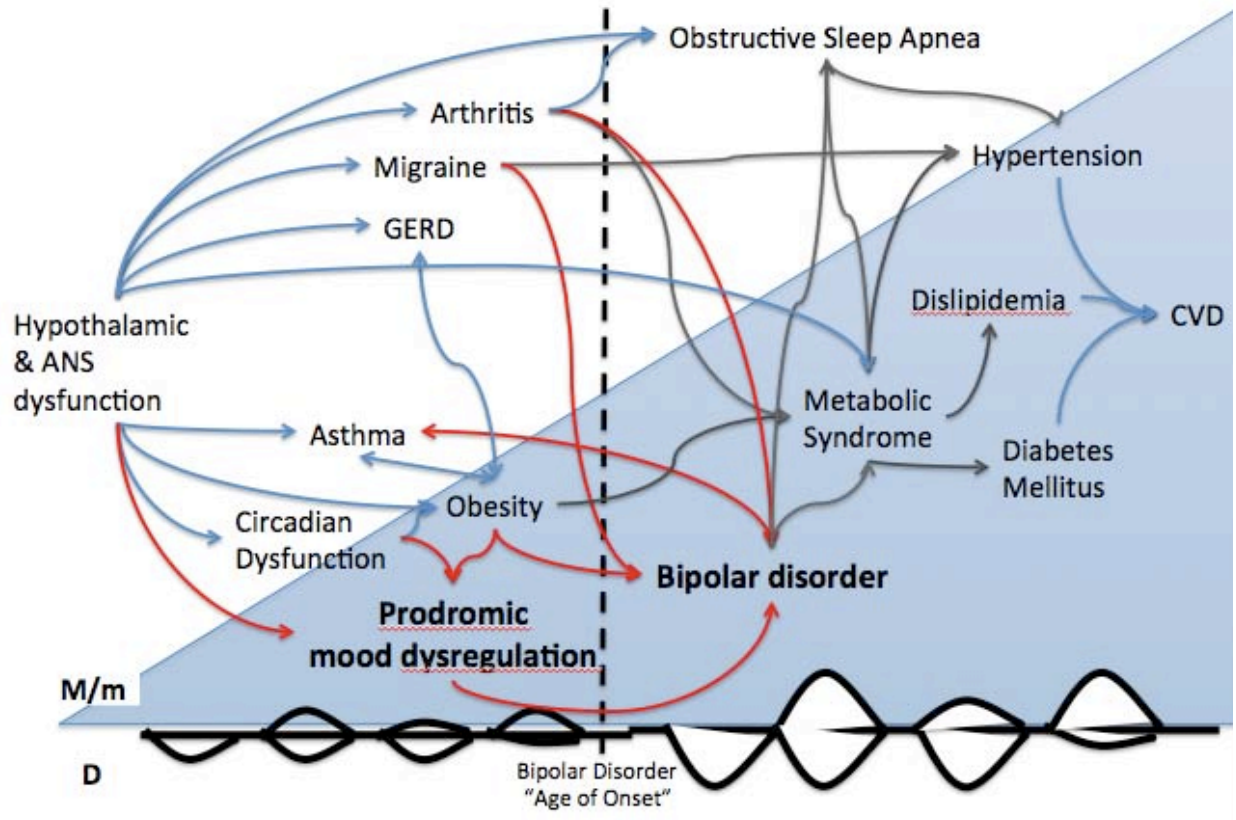

Figure 2. Illustrates the association between bipolar disorder and non-psychiatric comorbidities. The blue background shows metabolic comorbidities that appear

gradually during the course of bipolar disorder. Arrows represent evidence of association (directional or bidirectional). Blue arrows are used to indicate association between hypothalamic/ ANS dysfunction and illness. Red arrows are used to indicate association between non-psychiatric illness and bipolar disorder. Gray arrows are used to indicate association between non-metabolic illness and metabolic illness. M/m: Manic/hypomanic symptoms; D: Depressive symptoms; GERD: Gastroesophageal reflux disease; CVD: Cardiovascular disease

4.5. Mood disorders and their association to metabolic comorbidity: The hypothalamic and ANS link

Obesity, the metabolic syndrome, hypertension, diabetes mellitus type 2, and dyslipidemias in mood disorders are highly prevalent and have been documented in drug naïve (Maina et al., 2008) and treated (Gurpegui et al., 2012; McElroy et al., 2002) patients.(Goldstein et al., 2011) Depression during childhood is associated to an increased risk to suffer obesity in adolescence.(Rottenberg et al., 2014) Obesity has been linked to an increased risk to suffer from depression or bipolar disorder; and mood disorders have been linked to an increased risk to suffer from obesity. Further, obesity in depressed patients has been associated to bipolarity(Goldstein et al., 2011).

If we look at mood disorders as a gradual process, we could also look at other comorbidities as gradual events, with one event preceding the next. Obesity could represent one of the first indicators of metabolic disruption and the door of entrance for metabolic comorbidities. In bipolar disorder, obesity independently predicts the accumulation of medical conditions like hypertension, diabetes, hyperlipidemia in adult

patients.(Goldstein et al., 2009) In that way, the elevated prevalence of obesity in bipolar disorder and depressed unipolar patients(Hung et al., 2014; Vancampfort et al., 2013a; Vancampfort et al., 2013b) could represent a step further towards the development of other well-established metabolic illness. Even though patients diagnosed with bipolar disorder can develop obesity, hypertension, diabetes mellitus, and hyperlipidemia during adolescence or well into adulthood, there is evidence that such comorbid entities can even precede the diagnosis of early onset bipolar disorder in children and most probably accompany prodromal mood symptoms.(Jerrell et al., 2010)

(FIGURE 2)

It has been reported that depressed patients without evidence of HPA activation show metabolic disturbances, supporting the argument of an alternative pathway such as the ANS. More than a decade ago it was proposed that a shifting equilibrium from activity to food leads to autonomic imbalance and the metabolic syndrome.(Kreier et al., 2003) Kreier et al, proposed that the ANS is compartmentalized throughout the body, namely in the thoracic (cardiovascular), abdominal (visceral and abdominal fat) and movement (muscular) compartments. Each compartment receives a balanced sympathetic/parasympathetic signal to function according to circadian metabolic needs. The ANS receives the information from pre-autonomic neurons in the autonomic part of the hypothalamus and cortex, the preautonomic neurons of the paraventricular nucleus (PVN), which receive direct and indirect input from the circadian clock localized in the suprachiasmatic nucleus (SCN). The SCN sends output signals to the PVN according to day/night conditions and therefore our metabolism is circadian driven. If the circadian clock is chronically disrupted, as is the case in shift workers or patients diagnosed with

depression or bipolar disorder, the metabolic organization is lost or altered due to a disruption in a SCN-PVN-ANS-Organ circuit. Thus, the early, intermittent and/or progressive loss of sympathetic/parasympathetic autonomic balance in the corporal compartments could explain the metabolic disturbances present in mood disorders.

Currently, it is possible to document ANS activity in the thoracic compartment (heart rate, blood pressure, etc…) in humans, but is more difficult to do the same in the abdominal and muscular compartments. However, it has been possible to document ANS activity disturbances in metabolic conditions that support the shifting ANS hypothesis. For example, it was recently reported that in non affectively ill subjects, an increased sympathetic and decreased parasympathetic activity in the thoracic compartment, rather than changes in HPA axis, are associated to the metabolic disturbances present in the metabolic syndrome, obesity, diabetes mellitus type 2 and hypertension. (Licht et al., 2010) In the case of obesity for example, this seems counterintuitive; since acute parasympathetic activation is present during food ingestion(D'Alessio et al., 2001) and linked to increased adiposity(Kreier et al., 2002); and sympathetic activity to decreased adiposity. The brain and body are dynamic entities that share the constant effort to achieve a metabolic balance. At this point, it is necessary to understand that the body does not function as a block of uniform responses, nor the brain sends only one signal to adapt the whole body at once. It sends diverse signals to coordinate with multiple organs at different time points to respond to internal and external demands. Thus, the existence of corporal compartments which distinctly receive and send information to the brain. Therefore, the “when” and “where (in which corporal compartment)” over the course of an enduring metabolic disturbance, the measurements of ANS activity are obtained is of extreme

importance to understand varying results. If there is a condition in which parasympathetic activation is chronically present, it is expected that a counter measure is implemented resulting in an increased sympathetic tone.(Canale et al., 2013) With time, balance in such compartmentalized parasympathetic/sympathetic struggle becomes increasingly more difficult to achieve as metabolic disturbances establish. Hence, the measurements of a thoracic ANS compartment indicate that when obesity, the metabolic syndrome, diabetes mellitus type 2, and/or hypertension are established; the sympathetic tone is increased and a parasympathetic tone decreased. (Pavlov and Tracey, 2012) It is important to highlight that far less is known about the early changes (acute) in the ANS balance compared to what is documented in well-established metabolic conditions (chronic).

Mood disorders seem to fast forward the appearance of hypertension and CVD by ten years or more compared to the general population, and also increase their prevalence. (Goldstein et al., 2009) Hypertensive patients with depression (treated or untreated) have lower systolic BP levels (Mejia-Lancheros et al., 2014), and antidepressant treatment with tryciclic, and serotoninergic and noradrenergic (SNRI) agents has been associated to an increased risk for hypertension(Licht et al., 2009), whereas selective serotonin reuptake inhibitors have not. Women with preexisting hypertension, but not pregnancy induced hypertension have an increased risk to develop depression.(Katon et al., 2012) Depression during early pregnancy increases the risk for preeclampsia.(Kurki et al., 2000) Very recently the American Heart Association has recognized depression as an independent cardiovascular risk factor for patients with acute coronary syndromes(Fiedorowicz, 2014; Lichtman et al., 2014), and bipolar

disorder is not shy in gathering credits to follow.(Goldstein et al., 2015) Therefore unsurprisingly since obesity, the metabolic syndrome, hypertension, dyslipidemias and diabetes mellitus in bipolar disorder patients show a prevalence of more than double than in the general population(Vancampfort et al., 2013b); the one of the most frequent causes of early mortality in bipolar disorder is cardiovascular disease that occurs ten years earlier than in the general population.(Westman et al., 2013)

4.6 Autonomic modulation as treatment for mood disorders and their comorbidities: Vagus nerve stimulation and blocking

With such strong associations between autonomic disturbance, mood disorders and metabolic comorbidities, it is not surprising that vagus nerve stimulation has been used to treat depression while interestingly it also has been used to treat obesity. Vagus nerve stimulation has been used to induce weight loss in obesity. Open label clinical trials have shown beneficial effects, but RCT have failed to do so.

More recently, vagus nerve block (VNB) was used first in the EMPOWER trial with limited results, but a correlation between time of VNB and weight loss was found.(Sarr et al., 2012) Therefore, in the ReCHARGE trial 12 hrs of VNB were used and a greater and significant weight loss was achieved in the active vs sham group.(Ikramuddin et al., 2014)

Although controlled randomized data is negative (Rush et al., 2005), there are controlled (Aaronson et al., 2013) and long term open clinical trials with vagus nerve stimulation (VNS) showing its efficacy in unipolar and bipolar treatment resistant depression (TRD) (Berry et al., 2013; Nahas et al., 2005), rapid cycling bipolar

disorder(Nahas et al., 2005), as well as case reports on VNS induced manic symptoms (Gerson et al., 2011) which also point to a hypothalamic and ANS involvement in mood disorders. For the outcome of those studies it might be advantageous to select patients with metabolic syndrome or autonomic disturbance. The inconsistencies of results in mood disorders are similar to those observed in VNS to treat obesity, and we wonder if circadian oriented intermittent VNB would also improve results in the treatment of mood disorders. Unfortunately, no VNB studies in mood disorders have been published.

4.7. Melatonin attenuates drug induced adverse metabolic effects: A hypothalamic clue.

Patients with mental disorders are treated pharmacologically with drugs that include antipsychotics (i.e, clozapine, olanzapine, quetiapine, risperidone), antidepressants (i,e. paroxetine, mirtazapine), or mood stabilizers (lithium or valproate). Unfortunately several of these drugs induce an important problem that presents itself in parallel to the mental disorders for which they are used. It turns out that these drugs increase the cardiovascular risk by generating adverse metabolic effects that include weight gain, glucose intolerance, dyslipidemia, long- term risk for hypertension, diabetes, and other CVD.(Torrent et al., 2008) This illustrates that many of the most efficacious and widely used drugs to treat mood disorders induce adverse metabolic effects show ANS related side effects.(Leung et al., 2012) Surprisingly, the study of the effects of these drugs on relevant hypothalamic nuclei and the regulation of the ANS is scarce.

In particular, Second Generation Antipsychotics (SGA) are commonly used for the acute and chronic treatment in bipolar disorder, depression and other mental disorders like

schizophrenia. The group of SGA includes clozapine, olanzapine, quetiapine and risperidone among others. They are effective in the control of psychopathology and have significantly improved the wellbeing, global functioning and quality of life for the millions of patients that have received treatment with these drugs. Unfortunately SGA are amongst the group of drugs that induce more adverse metabolic effects and can double the risk of metabolic syndrome in patients that use them.(Lieberman et al., 2005a; Vancampfort et al., 2013b)

Recently, we published a clinical trial were the administration of melatonin was useful in attenuating the adverse metabolic effects of second generation antipsychotics in bipolar disorder patients.(Romo-Nava et al., 2014a) In parallel, an independent group from Iran reported similar results in a group of treatment naïve schizophrenia patients that were started on olanzapine.(Modabbernia et al., 2014) Since melatonin acts importantly in the SCN to synchronize its activity, these clinical results strongly suggest a role of the biological clock in SGA induced adverse metabolic effects. Thus, in a rat experiment we investigated the effects of olanzapine and melatonin in the biological clock and the autonomic output. We demonstrated that olanzapine induces cardiovascular effects through its action in the SCN and altering the function of the PVN and its autonomic output to the body. Melatonin prevents SGA cardiovascular effects by acting on the SCN; possibly through shared intracellular mechanisms that include the GSK-3B. These results strongly suggest the involvement of the biological clock in SGA metabolic adverse effects; and provide a clue to the hypothalamic and ANS origin of metabolic comorbidities in mood disorders.

4.9. Conclusions

Mood disorders are complex entities that show a high risk for metabolic comorbidity. In this review, we gathered evidence illustrating that affective syndromes are composed of symptoms and features such as cognitive dysfunction, that indicate hypothalamic and ANS dysfunction. We also reviewed recent evidence that suggests that metabolic comorbidity in mood disorders such as obesity, the metabolic syndrome, hypertension, diabetes mellitus, could share a chronic and/or intermittent disruption in brain/body communication where the hypothalamus and ANS could play a major role and explain their association to mood disorders. An example of these complex interactions is illustrated in figure 2.

Pharmacologic treatment with drugs such as SGA, further increases the metabolic vulnerability that accompanies patients suffering from mood and other mental disorders. Evidence that melatonin is able to prevent the appearance of SGA induced adverse metabolic effects in animals supports the hypothesis that SGA somehow disrupt metabolic organization through their effect over hypothalamic structures and underscores the importance of adequate brain-body communication to maintain mental and physical health.

The challenge now is to document further the hypothalamic and ANS involvement in mood disorders and their non- psychiatric comorbidities. The development of new integrative models for the understanding of brain/body interaction will favor an enhanced and wider view for clinical and research purposes in the context of psychiatric and non-psychiatric comorbidity prevention, diagnosis and treatment.

5. General Discussion

Throughout this study, we have used drug induced adverse metabolic effects to underscore the relevance of hypothalamic and ANS in metabolic regulation. We provide hitherto clinical data showing that drug induced metabolic adverse effects have a hypothalamic and ANS origin that can be attenuated through the use of melatonin; A hormone that synchronizes our biological clock to light/dark activity and is fundamental in the metabolic organization of our body. We also provide new basic evidence showing that it is through our biological clock in the suprachiasmatic nucleus that olanzapine (one of the most widely used SGA) disturbs the autonomic output and generates its cardiovascular effects; and that melatonin can prevent this. Together, our results also allow us to propose a new neural mechanism through which drug induced adverse metabolic effects are generated and can be controlled.

Patients with mood disorders suffer metabolic comorbidities that result in early mortality as much as 20 years earlier than people from the general population. There is enough evidence now to propose that the link between mood disorders and metabolic illness is via the hypothalamus and autonomic nervous system. Cardiovascular risk is greatly increased by the use of drugs to treat mental disorders such as second generation antipsychotics (SGA); which can generate adverse metabolic effects that include weight gain, metabolic syndrome, hypertension, dyslipidemias and diabetes mellitus. The use of SGA can double the cardiovascular risk of these patients and contributes substantially to early mortality.

Our findings open a new possibility to improve the health of millions of patients that receive pharmacologic treatment prone to induce adverse metabolic effects. This by preventing the appearance of metabolic illness and reducing cardiovascular risk by administering melatonin; A safe and non expensive substance.

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