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REGULAR RESEARCH ARTICLE

Cortico-Amygdala-Striatal Activation by Modafinil/ Flecainide Combination

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Abstract

Background: Modafinil, a nonamphetaminic wake-promoting compound, is prescribed as first line therapy in narcolepsy, an invalidating disorder characterized by excessive daytime sleepiness and cataplexy. Although its mode of action remains incompletely known, recent studies indicated that modafinil modulates astroglial connexin-based gap junctional communication as administration of a low dose of flecainide, an astroglial connexin inhibitor, enhanced the wake-promoting and procognitive activity of modafinil in rodents and healthy volunteers. The aim of this study is to investigate changes in glucose cerebral metabolism in rodents, induced by the combination of modafinil + flecainide low dose (called THN102).

Methods: The impact of THN102 on brain glucose metabolism was noninvasively investigated using ^{18}F -2-fluoro-2-deoxy-D-glucose Positron Emission Tomography imaging in Sprague-Dawley male rats. Animals were injected with vehicle, flecainide, modafinil, or THN102 and further injected with ^{18}F -2-fluoro-2-deoxy-D-glucose followed by 60-minute Positron Emission Tomography acquisition. ^{18}F -2-fluoro-2-deoxy-D-glucose Positron Emission Tomography images were coregistered to a rat brain template and normalized from the total brain Positron Emission Tomography signal. Voxel-to-voxel analysis was performed using SPM8 software. Comparison of brain glucose metabolism between groups was then performed.

Results: THN102 significantly increased regional brain glucose metabolism as it resulted in large clusters of ^{18}F -2-fluoro-2-deoxy-D-glucose uptake localized in the cortex, striatum, and amygdala compared with control or drugs administered alone. These regions, highly involved in the regulation of sleep-wake cycle, emotions, and cognitive functions were hence quantitatively modulated by THN102.

Conclusion: Data presented here provide the first evidence of a regional brain activation induced by THN102, currently being tested in a phase II clinical trial in narcoleptic patients.

Keywords: modafinil, astroglial connexin, FDG PET imaging, neuroglia, narcolepsy

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Significance Statement

THN102 (modafinil/flecainide) is efficient to reduce excessive daytime sleepiness. The impact of THN102 on rat brain metabolism was investigated with FDG PET imaging. Cortex, striatum, and amygdala were more activated after THN102 compared with modafinil. This study provides insights on the mechanism of action of THN102 innovative product.

Introduction

Modafinil is a nonamphetaminic, wake-promoting compound used as first line treatment of excessive daytime sleepiness (EDS), associated with narcolepsy (Lavault et al., 2011; Thorpy and Dauvilliers, 2015; Barateau et al., 2016). It has also been proposed as a treatment to reduce EDS in Parkinson's disease (Sheng et al., 2013; Rodrigues et al., 2016). Mechanisms involved in wake-promoting and procognitive actions of modafinil are complex, as it modulates multiple monoaminergic and GABAergic neuronal systems (Minzenberg and Carter, 2008).

Early studies using Fos as a marker of neuronal activity in cat (Lin et al., 1996) and rat (Engber et al., 1998) suggested the hypothalamus as main brain target of modafinil. Subsequent studies in rodents indicated that cortex and striatum were activated after wake enhancement by modafinil (Scammell et al., 2000; Willie et al., 2005). Imaging in rodents depicted higher metabolism in hippocampus, thalamus, and amygdala (Engber et al., 1998) and showed activating effects of modafinil in fronto-cortical areas (Gozzi et al., 2012); those areas are involved in both arousal (Duteil et al., 1990; Lin et al., 1992) and cognitive enhancement (Lynch et al.; Béracochéa et al., 2003). Using pharmacological magnetic resonance imaging, or surface electroencephalography, modafinil was found to increase brain activity notably in the hippocampus and frontal cortex in healthy volunteers (Joo et al., 2008a) and narcoleptic patients (Saletu et al., 2007; Joo et al., 2008b). Moreover, positron emission tomography (PET) using ^{18}F -2-fluoro-2-deoxy-D-glucose (FDG) in narcoleptic patients unveiled that modafinil increased the glucose brain metabolism in the hippocampus (Kim et al., 2007).

More recently, modafinil was shown to modulate astrocyte functions by increasing cell-cell communication mediated by connexin channels (Liu et al., 2013), membrane proteins involved in cellular communication (Giaume et al., 2010), and sleep regulation (Franco-Pérez and Paz, 2009; Franco-Pérez et al., 2012; Clasadonte et al., 2017). It was further shown in rodents that modulating astrocyte connexin by flecainide impacted the pharmacological action of modafinil by enhancing its wake-promoting, procognitive, and, notably, antinarcotic effects (Duchêne et al., 2016; Lu and Chen, 2016). The mechanisms of action of the modafinil/flecainide combination (THN102) remain to be further investigated but would likely be based on a restoration of the functionality of astroglial connexins. Using FDG PET in the rat, the aim of the present study was to assess the CNS effects of THN102 at the functional level compared with modafinil or flecainide used alone, both at their effective and clinically relevant doses.

Materials and Methods

Animals

Male Sprague-Dawley rats (Elevage Janvier) were collectively housed with food and water ad libitum. They were kept in a temperature- and humidity-controlled facility with 12-hour dark-/light cycles (lights on at 8:00 AM). Experiments were performed during the light phase between 9:00 AM and 1:00 PM.

The study was conducted in accordance with the French legislation and European directives on the use of animals in research (EU Directive 2010/63/EU). The protocol has been approved by the local committee for animal use in research (APAFIS#7466-201611 04 1 7049220 v2).

Drugs and Chemicals

Drugs were administered by i.v. injection in the tail vein (1 mL/kg). Treatments included vehicle (VEH), modafinil 10 mg/kg (MOD; Orchid Pharma), flecainide 1 mg/kg (FLE; Sigma-Aldrich), or the combination of both (THN102). FDG for i.v. injection was purchased from Cyclopharma.

FDG PET Imaging: Acquisition Protocol and Imaging Data Analysis

PET imaging study was performed using PET systems coupled with a computerized tomography scanner (Inveon microPET-CT; spatial resolution ~1.6 mm; Siemens) in anesthetized and fasted rats (1.5%–2.5% inhaled isoflurane, weight 250–350 g) (Bao et al., 2009). PET experiments were exclusively performed in the morning. Each day of experiment, 4 rats were randomly assigned to each group: animals were i.v. injected with VEH, MOD, FLE, or THN102 before being placed into the scanner for computerized tomography acquisition. Thirty minutes after the administration, 1 mL FDG (mean dose = 42.2 ± 26.3 MBq) was injected over 1 minute using a syringe pump. Dynamic PET acquisition begun immediately after the start of FDG infusion for 60 minutes. Blood glucose measurement was performed before ^{18}F -FDG injection and at the end of PET acquisition using a portable glucometer (Accu-check Performa, Roche).

PET data were reconstructed using the FORE+OSEM2D algorithm including normalization, attenuation, scatter, and random corrections. To reduce noise and correct for partial volume effect, an iterative deconvolution using the point spread function of the scanner with a temporal based denoising was applied to each image. The method was previously reported and validated for use in small animal PET imaging (Wimberley et al., 2014; Reilhac et al., 2015).

Dynamic and summed (30–60 minutes) FDG PET images were spatially normalized to a standard rat brain FDG Schiffer's template using Pmod software (version 3.6) (Schiffer et al., 2006). The brain kinetics of FDG may depend on its plasma kinetics (input function) and peripheral blood glucose level. Two normalization methods were thus performed to detect any regional change in FDG uptake by the brain. First, summed PET images were normalized by their respective whole-brain activity, thus highlighting the relative FDG uptake by the different brain regions. Then, the absolute metabolic rate of glucose (MRGlu) was estimated using pharmacokinetic modelling. To that end, a volume of interest was drawn on the vena cava to generate an imaged-derived input function of FDG, as previously described (Weber et al., 2002; Lanz et al., 2014). MRGlu Parametric PET images (PXM0D, Pmod software, version 3.6) were then generated for

each animal from the dynamic brain PET images and corresponding imaged-derived input function (from 0 to 60 minutes) using the FDG Patlak model, considering blood glucose level measured immediately before PET (Patlak et al., 1983). The 0.71 lumped constant was used to take the difference between glucose and FDG metabolism into account (Tokugawa et al., 2007).

Comparison of parametric images (FDG brain uptake and MRGlu) obtained in each group ($n=5$ animals/group) was performed using a statistical parametric mapping (SPM) and a voxel-to-voxel analysis (SPM8 software) as previously described (Schiffer et al., 2006; Soto-Montenegro et al., 2009). A brain mask was created from the FDG template and applied to all registered and normalized scans to include only cerebral voxels. Comparison was then performed using an ANOVA design to detect differences between groups. A significance level threshold of .05 (uncorrected for multiple comparisons) and a minimum cluster size of 200 voxels were selected. Only the clusters that were significant at $P < .05$ levels (corrected for multiple comparisons) were considered. The size of the clusters exceeding the threshold and their corrected significance were anatomically located using the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007).

Spontaneous Locomotor Activity Assessment

In an independent experiment, spontaneous locomotor activity was recorded in an open-field device (50x50x50 cm), during 2 hours, starting immediately after administration (VEH, MOD, FLE, and THN102). Cumulated traveled distance per 5-minute time bins was analyzed with ViewPoint. Experiments were performed with 8 drug-naïve animals in each group. The experimenter was blinded to treatment.

Determination of Modafinil Concentration in Serum and Brain

In parallel experiments, performed in another population of rats, brain and serum concentrations of modafinil were determined after MOD and THN102 administration in rats. Animals ($n=8$ rats/group) were anesthetized with isoflurane 30 minutes after i.v. administration and blood samples were collected, centrifuged (3000 $g \times 15$ min, 20°C), and stored at -80°C. Brains were collected immediately after blood sampling, frozen on dry ice, and stored at -80°C. Modafinil and flecainide concentrations in serum and brain lysate were determined using a reverse phase liquid chromatography with tandem mass spectrometry detection technique (Eurofins ADME Bioanalyses).

Statistical Analysis

Results of the locomotion and pharmacokinetic data are expressed as mean \pm SEM. Difference was considered significant at $P < .05$ levels. Statistical analysis was performed using Graphpad software (GraphPad Prism version 7). Cumulative traveled distance over time was compared using a 2-way repeated-measure ANOVA followed by Bonferroni's posthoc test. Finally, serum and brain modafinil concentrations were compared using an unpaired t test.

Results

The goal of this study was to compare the brain metabolism after treatment with THN102, combination between modafinil and flecainide at low dose, to modafinil alone.

THN102 Locally Increases the Brain Glucose Metabolism

Normalization of FDG uptake by whole-brain activity was associated with a low variability. In the VEH group, the coefficient of variation ($CV = SD/mean \times 100$) of the relative uptake of FDG was $CV = 2.73\%$ in the cortex. SPM analysis did not show any significant difference in the distribution of relative FDG brain uptake between FLE or MOD groups compared with the VEH group ($P > .05$). Significant increase in the relative brain uptake of FDG could be regionally observed in the THN102 group compared with the MOD group. The effect was observed bilaterally and was homogeneously distributed in the cortex, amygdala and striatum (Table 1; Figure 1). Notably, a cluster of significant increase in FDG uptake could be observed in the nucleus accumbens. Similar distribution of increased relative FDG uptake could be observed in the THN102 group compared with the FLE or MOD alone ($P < .05$).

Within the significant clusters, further detailed analysis allowed to locate the relevant peak regions. Hence, THN102 enhanced the brain metabolism compared with MOD in the subcortical regions such as the dysgranular and retrosplenial-dysgranular cortices, primary/secondary motor, dorsolateral entorhinal and visual cortices, the retrosplenial-dysgranular area, as well as the layer 3 and basolateral amygdaloid nuclei.

There was a significant increase in blood glucose levels measured before (85.8 ± 4.5 mg/dL) and at the end (116 ± 4.3 mg/dL) of PET acquisitions ($P < .05$). However, initial as well as final glucose concentrations were not different between the 4 treatment groups, suggesting the absence of treatment-induced change in peripheral glucose metabolism. Compared with the relative FDG uptake, absolute quantification of the brain MRGlu was associated with a higher variability ($CV = 19.82\%$ in the cortex of the VEH group). SPM analysis nonetheless highlighted patterns of significantly higher glucose consumption in the THN102 group compared with MOD alone ($P < .05$). The regional increase in MRGlu was consistent with the regional increase in the relative FDG uptake (Table 2; Figure 2). However, difference in MRGlu in the THN102 group compared with the VEH and FLE groups was not significant, which may be due to a higher variability of the MRGlu data.

THN102 Increases Spontaneous Locomotor Activity

To assess whether doses of modafinil allowed to discriminate THN102 compared with modafinil alone, spontaneous motor activity in unrestrained awake rats was monitored for 2 hours after treatment, during the lights-on period (Figure 3). Locomotor activity in the MOD group tends to be increased compared with the VEH group without reaching significance (+15.2% at 120 minutes). THN102 administration induced a significant increase in cumulative locomotor activity compared with VEH-treated animals, beginning at 95 minutes (+22.0% at 120 minutes vs VEH, $P = .0309$). THN102 was more effective in increasing the locomotor activity compared with MOD; however, the difference was not statistically significant (+5.89% at 120 minutes). Moreover, no effect of FLE treatment was found at the 1-mg/kg dose when administered alone.

Brain and Serum Concentrations of Modafinil

Serum and brain were collected 30 minutes after treatment with modafinil alone (MOD) or combined with flecainide (THN102; Figure 4). Quantification of modafinil concentration levels treated with MOD or THN102 demonstrated no significant difference between both groups in serum (2.12 ± 0.472 ng/mL and 2.14 ± 0.419 ng/mL, respectively) or in brain (1.37 ± 0.262 ng/g and

Table 1. SPM Results Showing Clusters, Peak Coordinates, and Significant Levels of Brain Regions with an Increase in ^{18}F -FDG Uptake When Comparing THN102 to MOD, FLE, or VEH

Intergroup Comparisons	Significant Clusters	Significant Peak Coordinates within Clusters			Peak Regions
		x	y	z	
THN102 > VEH	Cortex	0.5	4.6	3.6	R pre-limbic cortex
	Caudate and putamen (Striatum)	6.7	4.6	-5.6	R primary auditory cortex
	Amygdala	-5.7	3	-8.8	L temporal association cortex
		-0.1	1.2	-0.2	L cingulate cortex area 1
		-5.5	8.2	-0.6	L layer 3 of cortex
THN102 > FLE	Cortex	6.5	4.6	-5.8	R primary auditory cortex
	Caudate and putamen (Striatum)	4.3	2.8	2.0	R primary somatosensory
	Amygdala	-5.5	8.6	-4.8	L layer 3 of cortex
		-2.1	3.6	3.8	L forceps minor corpus callosum
		-3.1	0.4	-7.8	L primary visual cortex monocular
THN102 > MOD	Cortex	5.7	7.2	-1.6	R dysgranular cortex
	Caudate and putamen (Striatum)	2.1	1.4	4.4	R secondary motor cortex
	Amygdala	2.5	0.8	-5.2	R secondary visual cortex
		-0.7	0.4	-4.2	L retrosplenialdysgranular cortex
		-3.7	6.8	3.0	L layer 3 of cortex
		-5.5	8.6	-4.8	L basolateral amygdaloïd nucleus, posterior

Voxels comparison-based analysis was performed using SPM8 ($n=5$) rats per group using 1-way ANOVA followed by multiple comparison test. A significance level threshold of .05 and a minimum cluster size of 200 voxels were selected to identify significant clusters. Coordinates of significant peak ($P < .05$) were given according to the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007). FLE, flecainide 1 mg/kg; MOD, modafinil 10 mg/kg; THN102, modafinil 10 mg/kg + flecainide 1 mg/kg; VEH, vehicle.

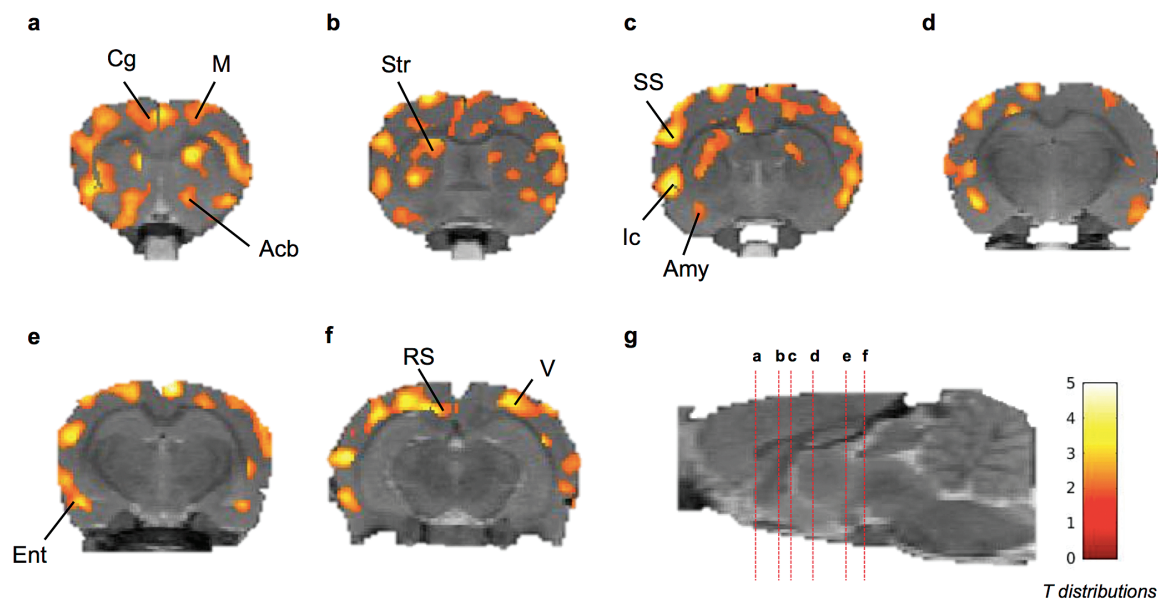


Figure 1. Coronal brain section showing statistical parametric map for increase in relative ^{18}F -fluoro-2-deoxy-D-glucose (^{18}F -FDG) uptake after Modafinil (MOD) + flecainide (THN102) treatment in comparison to MOD alone in rats. Following a cranio-caudal orientation centered on bregma (anterior is positive), coronal brain sections were at (a) 1.05, (b) -0.68, (c) -1.29, (d) -3.07, (e) -4.34, and (f) -5.76 millimeters from the bregma and (g) sagittal view of the brain with the marked coronal sections (red dotted lines) from a to f. Color scale represents all T distributions achieving statistical significance (see SPM statistical analysis). Acb, accumbens; Amy, amygdala; Cg, cingulate cortex; Ent, entorhinal cortex; Ic, insular cortex; M, motor cortex; RS, retrosplenial cortex; SS, somatosensory cortex; Str, striatum; V, visual cortex.

1.50 ± 0.277 ng/g, respectively). Therefore, there is no apparent pharmacokinetic interaction between MOD and FLE on MOD metabolism.

Discussion

In this study, we reported that THN102 significantly activates the cortico-amygdala-striata regions compared with MOD used alone.

Modafinil has been largely proposed in sleep medicine to treat EDS associated with narcolepsy (Lavault et al., 2011; Thorpy and Dauvilliers, 2015; Barateau et al., 2016), Parkinson's disease (Sheng et al., 2013; Rodrigues et al., 2016), idiopathic hypersomnia (Lavault et al., 2011; Lopez et al., 2017), and obstructive sleep apnea/hypopnea syndrome (Black and Hirshkowitz, 2005). Numerous preclinical studies have generated a wealth of experimental data, which lead to many hypotheses regarding the mode of action of modafinil (Gerrard and Malcolm, 2007;

Table 2. SPM Results Showing Clusters, Peak Coordinates, and Significant Levels of Brain Regions with an Increase in MRGlu When Comparing THN102 to MOD in Rats

Intergroup Comparisons	Significant Clusters	Significant Peak Coordinates within Clusters			Peak Regions
		x	y	z	
THN102 > MOD	Cortex	4.1	0.8	-5.2	R primary visual cortex
	Caudate and putamen (Striatum)	0.7	2.2	3.8	R secondary motor cortex
	Amygdala	5.7	8.6	-4.6	R layer 3 of cortex
	Accumbens	-1.5	0.6	-0.4	L primary motor cortex
		-4.9	9.0	0.0	L layer 3 of cortex
		-6.5	6.4	-8.8	L dorsolateral enthorinal cortex

Voxels comparison based analysis was performed using SPM8 ($n=5$) rats per group using 1-way ANOVA followed by multiple comparison test. A significance level threshold of .05 and a minimum cluster size of 200 voxels were selected to identify significant clusters. Coordinates of significant peak ($P < .05$) were given according to the Paxinos and Watson rat brain atlas (Paxinos and Watson, 2007). MOD, modafinil 10 mg/kg; MRGlu, metabolic rate of glucose; THN102, modafinil 10 mg/kg + flecainide 1 mg/kg.

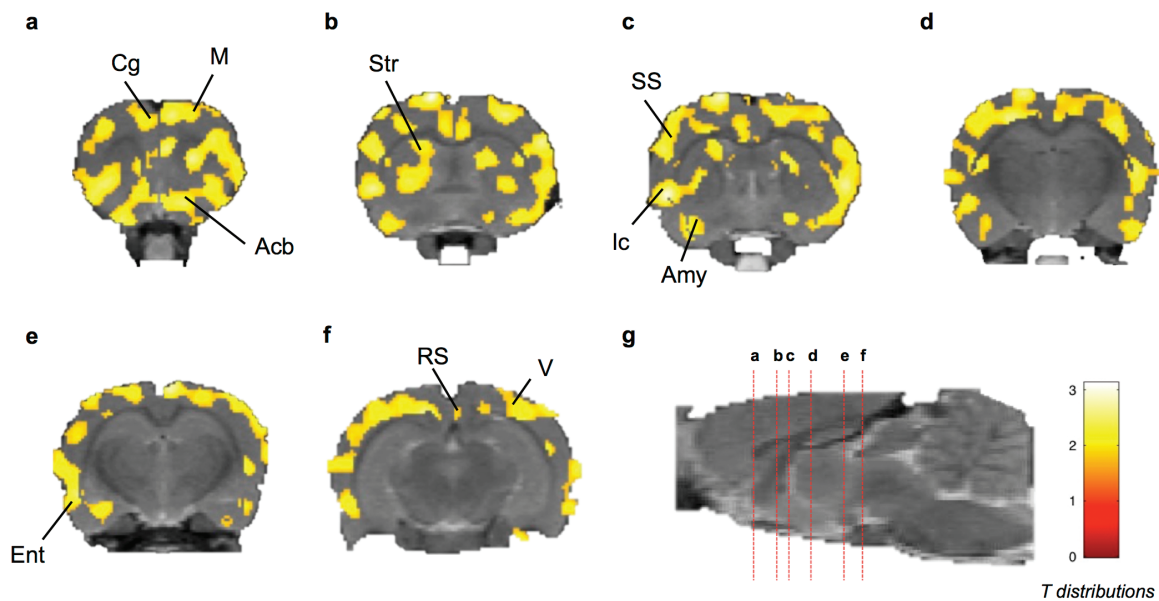


Figure 2. Coronal brain section showing statistical parametric map for increase in absolute metabolic rate of glucose (MRGlu) after Modafinil (MOD) + flecainide (THN102) treatment in comparison to MOD in rats. Following a cranio-caudal orientation centered on bregma (anterior is positive), coronal brain sections were at (a) 1.05, (b) -0.68, (c) -1.29, (d) -3.07, (e) -4.34, and (f) -5.76 millimeters from the bregma; and (g) sagittal view of the brain with the marked coronal sections (red dotted lines) from a to f. Color scale represents all T distributions achieving statistical significance (see SPM statistical analysis). Acb, accumbens; Amy, amygdala; Cg, cingulate cortex; Ent, entorhinal cortex; Ic, insular cortex; M, motor cortex; RS, retrosplenial cortex; SS, somatosensory cortex; Str, striatum; V, visual cortex.

Minzenberg and Carter, 2008). The central noradrenergic hypothesis has been supported by data showing that inhibition of catecholamine synthesis or antagonism of adrenergic receptors is able to attenuate the wake-promoting effects of modafinil (Duteil et al., 1991; Lin et al., 1992). Recent studies using mice lacking the noradrenaline synthesis or $\alpha 1\beta$ -adrenoceptor (Stone et al., 2002a, 2002b; Hou et al., 2005) or brain imaging in humans (Minzenberg and Carter, 2008) also support the critical involvement of the locus cœruleus noradrenergic system in modafinil profile. The dopaminergic hypothesis has been very attractive and prevailing since the identification of an affinity of modafinil with dopamine transporter (Mignot et al., 1994; Wisor et al., 2001; Wisor and Eriksson, 2005; Korotkova et al., 2007; Qu et al., 2008). The brain disinhibitory hypothesis (Lin et al., 1996, 2000) has received less attention, yet it is supported by the fact that modafinil induces a significant decrease in GABA outflow (Ferraro et al., 1996) in many brain areas, notably those critically involved in sleep-wake cycle control.

Recent data indicated that not only neurons but also glial cells, in particular astrocytes, were modulated by modafinil, as it enhanced astrocyte coupling and the expression of one of their connexins, Cx30 (Liu et al., 2013). These data suggest that astroglial connexins might be involved in modafinil mode of action. Indeed, flecainide, an astroglial connexin inhibitor, was able to enhance modafinil procognitive and wake-promoting activities when coadministered to modafinil in rodents (Duchêne et al., 2016; Lu and Chen, 2016). THN102 is currently in phase II clinical trial on narcoleptic patients (NCT02821715). In this context, we compared the impact on brain functions between THN102 and modafinil in the rat by assessing their drug-induced changes in brain glucose metabolism.

The route of administration and dose of modafinil (10 mg/kg, i.v.) was based on a previous functional magnetic resonance imaging (fMRI) study in rats (Gozzi et al., 2012). Flecainide dose was chosen according to previous studies in rodents (Duchêne et al., 2016). Additionally, at selected doses, THN102 significantly increased locomotor activity, whereas modafinil alone

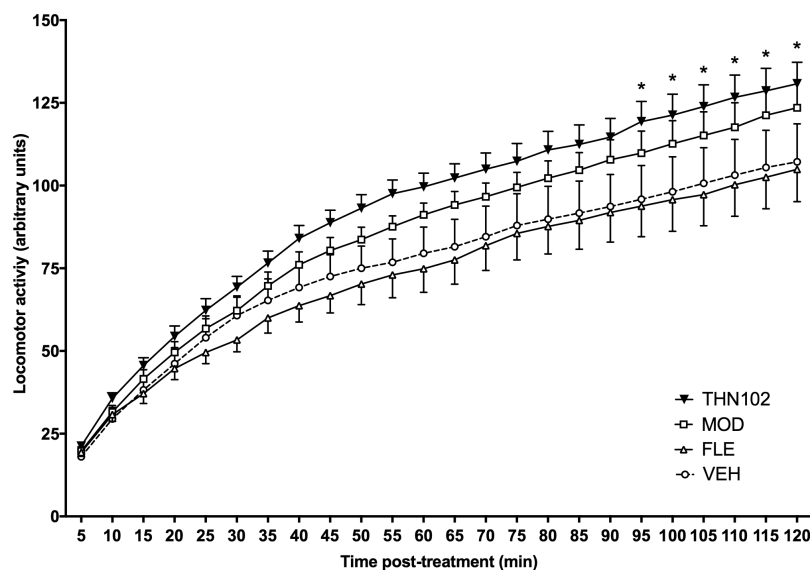


Figure 3. Cumulative traveled distance over 2 hours after vehicle (VEH), flecainide 1 mg/kg (FLE), modafinil 10 mg/kg (MOD), or MOD 10 mg/kg+FLE 1 mg/kg (THN102) treatment in awake rats. Locomotor activity of rats was measured during 2 hours after treatment. Data are expressed as means \pm SEM ($n=8$ rats/group) and compared using 2-way ANOVA followed by Bonferroni's multiple comparison test: * $P < .05$ THN102 vs VEH.

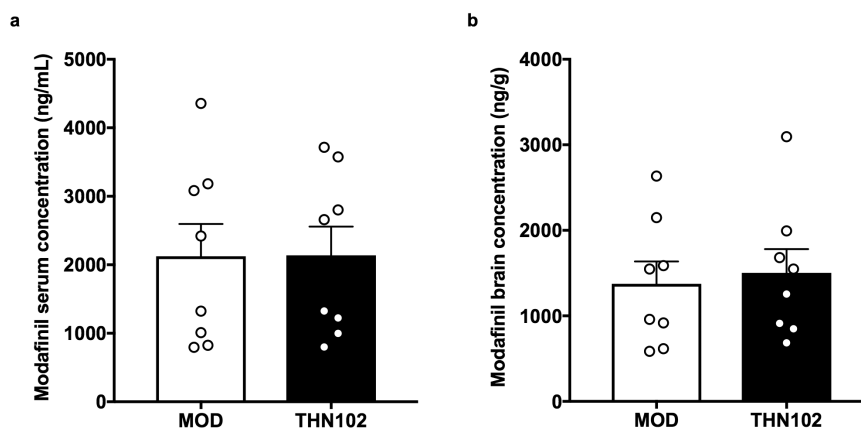


Figure 4. Serum (a) and brain (b) concentrations of modafinil after modafinil 10 mg/kg+flecainide 1 mg/kg (THN102) and modafinil 10 mg/kg (MOD) treatment. Thirty minutes after the treatment injection, serum and brain levels of MOD were sampled and quantified by liquid chromatography with tandem mass spectrometry. Data are expressed as means with scattered plots \pm SEM ($n=8$ rats/group) and compared using an unpaired t test.

showed only a tendency to increase it (nonsignificant), as partially described elsewhere (Simon et al., 1996; Edgar and Seidel, 1997; Zolkowska et al., 2009). As previously reported in mice (Duchêne et al., 2016), assessment of modafinil concentration in the serum and brain confirmed that flecainide did not increase the brain distribution of modafinil. Therefore, pharmacokinetic interaction between flecainide and modafinil can unlikely explain the brain effect of THN102 compared with vehicle and modafinil alone. Serum concentrations were close to modafinil levels found in subjects following dosing of clinically effective doses (McClellan and Spencer, 1998; Wong et al., 1999), suggesting potential relevance of the presented data to clinical conditions.

Several methods have been proposed to investigate the CNS effects of modafinil in human (Ellis et al., 1999; Spence et al., 2005; Hunter et al., 2006; Thomas and Kwong, 2006; Kim et al., 2007; Joo et al., 2008a; Minzenberg et al., 2008; Rasetti et al., 2010; Ghahremani et al., 2011; Minzenberg et al., 2011; Goudriaan et al., 2013; Schmaal et al., 2013; Funayama et al., 2014; Schmaal et al., 2014; Ikeda et al., 2017; Schmidt et al., 2017) and animals (Engber et al., 1998; van Vliet et al., 2008; Dawson et al., 2012;

Gozzi et al., 2012), including fMRI, 2-deoxyglucose autoradiography, FDG PET, and cerebral blood flow assessment using single-photon emission computed tomography. These data tend towards a consensus over cortical and subcortical brain activations induced by modafinil including hypothalamic and thalamic regions (Thomas and Kwong, 2006; Gozzi et al., 2012), which are highly involved in the regulation of sleep-wake cycle (Szabadi, 2006; Lin et al., 2011). Other major structures are highlighted following modafinil administration as the caudate putamen (striatum), the amygdala, and the hippocampus (Engber et al., 1998; Ghahremani et al., 2011), known for their implication in driving emotions and cognitive functions (Joo et al., 2008a).

In our conditions, modafinil alone did not modulate glucose brain metabolism assessed by FDG PET compared with vehicle. Even though the dosage and the route were identical to a previous fMRI study (Gozzi et al., 2012), the observed cortical activation after treatment was not detected in our analysis. This difference highlights the discrepancies that may exist between the drug-induced response on blood oxygenation levels-dependent response and on energy consumption (Di et al., 2012;

Cabrera et al., 2016). Another explanation for such discrepancies may be the presence of anesthesia. In the present study, animals were exposed to <2.5% isoflurane during drug administration and subsequent PET acquisition. It was reported that isoflurane may modulate the brain glucose uptake and may thus limit the sensitivity of the method to detect the CNS response to investigated compounds in vivo (Spangler-Bickell et al., 2016; Park et al., 2017). Working on awake rodents was shown feasible, although technically challenging (Spangler-Bickell et al., 2016; Park et al., 2017). In the present study, we chose to administer investigated treatments under isoflurane anesthesia to facilitate animal handling, i.v. administration, and avoid any stress due to the experimental procedure. Moreover, we showed that the locomotor activity was different between groups, which may non-specifically impact FDG uptake by the brain in awake animals. Therefore, the whole procedure was performed under isoflurane anesthesia to highlight the intrinsic effect of each treatment on brain function and allow for dynamic PET acquisition for 60 minutes in immobile animals.

Some brain structures such as the locus coeruleus, thalamus, and hypothalamus, modulated by modafinil (Minzenberg et al., 2008; Gozzi et al., 2012; Schmaal et al., 2013) were not activated by THN102 compared with modafinil alone. More interestingly, this study reported that THN102 significantly increased the relative glucose brain uptake in the whole cortex, amygdala, and striatum compared with modafinil alone, hence suggesting a different activity on glucose metabolism between both treatment groups. Similar effects could be observed using pharmacokinetic modelling and estimation of the absolute MRGlu. Despite higher variability in this parameter, patterns of increased metabolic rate of glucose were detected in the cortex, striatum, amygdala, and accumbens of rats in the THN102 group compared with the MOD group. Such brain regions may be considered as substrates for the enhancement of modafinil effects by flecainide. They notably process attention states and motivation and code behavioral responses (Cardinal et al., 2002; Cho et al., 2013), functions that are altered in narcolepsy and that are potentially modulated by THN102 (Bayard et al., 2012).

The amygdala, cortex, and striatum are dopamine- and glutamine-rich brain areas (Darvas et al., 2011; Oikonomou et al., 2014). Data presented in this study point toward a putative role of THN102 on those dopaminergic and glutamatergic neurotransmission systems, potential targets of modafinil (Minzenberg and Carter, 2008). Furthermore, plasticity of astrocytes has also been demonstrated at least in the amygdala (Johnson et al., 2010) and cortex (Sims et al., 2015), providing potential evidence for the involvement of astrocyte networks in modafinil pharmacological profile (Duchêne et al., 2016).

Furthermore, our data confirm, for the first time, the greater effects of THN102 compared with modafinil in terms of brain activation, supporting the hypothesis that a modulation of connexins, and notably astroglial connexins Cx30 and Cx43 by flecainide, can impact modafinil mechanism of action. Recent PET studies in narcoleptic patients evidenced hypermetabolism in cortical regions compared with healthy controls, further pointing out the role of those areas in this disorder (Dauvilliers et al., 2010, 2017). Clinical FDG PET is feasible and may be useful to highlight the impact of THN102 on brain function in healthy volunteers and patients in the absence of anesthesia. Such studies might encompass dose-effect range and address the impact of flecainide on the neuropharmacology of modafinil in humans.

Taking together, we demonstrated here that THN102 enhanced both locomotor activity and glucose metabolism in cortical and subcortical areas involved in the regulation of

sleep-wake cycle and behaviors. Our results further support the hypothesis that astrocyte connexins are involved in pharmacological responses of psychoactive drugs such as modafinil (Duchêne et al., 2016; Jeanson et al., 2016; Charvériat et al., 2017).

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Statement of Interest

F.M., M.C., and A.D. are full-time employees of Theranexus company. Y.D. served as consultant for Bioprojet Pharma, Flamel Technologies, Jazz Pharmaceuticals, Theranexus, Takeda, and UCB. The other authors declare no financial conflict of interest.

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