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O PAPEL DE MARCADORES INFLAMATÓRIOS NO TRANSTORNO DE PÂNICO E CONSIDERAÇÕES SOBRE O TRATAMENTO

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Dissertação de mestrado submetida ao corpo docente do Programa de Pós-Graduação em Psiquiatria e Saúde Mental (PROPSAM) como parte dos requisitos necessários para a obtenção do grau de Mestre em Psiquiatria e Saúde Mental.

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Examinada por:

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DEDICATÓRIA

Aos meus pais.

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LISTA DE SIGLAS

AP- Ataques de pânico

BDNF- *Brain derived neurotrophic factor*

DSM- Manual diagnóstico e estatístico dos transtornos mentais

GABA- Ácido gama-aminobutírico

IDO- Indolamina-2,3-dioxigenase

IL- Interleucina

LABPR- Laboratório de pânico e respiração

NMDA- N-metil-D-aspartato

SNC- Sistema nervoso central

TP- Transtorno de pânico

UFRJ- Universidade Federal do Rio de Janeiro

RESUMO

Quagliato, Laiana Azevedo. O papel de marcadores inflamatórios no Transtorno de Pânico e considerações sobre o tratamento. Rio de Janeiro, 2019. Dissertação (Mestrado em Psiquiatria e Saúde Mental) – Instituto de Psiquiatria da Universidade Federal do Rio de Janeiro, Rio de Janeiro, 2019.

Esta dissertação visa apresentar os trabalhos publicados pela autora a respeito de possíveis associações entre a inflamação e o Transtorno de Pânico (TP). O primeiro artigo consiste em uma revisão sistemática da literatura, na qual foram incluídos estudos que mensuraram citocinas inflamatórias em pacientes com o diagnóstico de TP. Foi demonstrado que as citocinas IL-5, IL-6 e IL-1β estão aumentadas no TP. O segundo artigo, que consiste em um estudo transversal, teve como objetivo avaliar se as citocinas inflamatórias estariam relacionadas com a via da quinurenina no TP. Foram investigadas também possíveis associações desses marcadores inflamatórios com domínios cognitivos em pacientes com diagnóstico de TP. Um aumento na taxa quinurenina/triptofano foi preditor de déficits na memória auditiva verbal de pacientes com TP quando comparados com controles saudáveis. Ademais, apenas os pacientes demonstraram uma associação significativa entre a taxa quinurenina/triptofano e a citocina inflamatória IL-2R, o que poderia indicar um aumento da atividade da enzima IDO nesses pacientes. Nesta dissertação, foram pressupostas razões pelo qual isso poderia acontecer. Uma das hipóteses discutidas é de que a microglia, células imunes do sistema nervoso central, poderiam estar produzindo tais citocinas e marcadores da via da quinurenina nos pacientes com o diagnóstico de TP, caso estivessem com a sua conformação ativada. Dessa forma, o terceiro artigo, que consiste em uma revisão sistemática, investigou o que poderia fazer com que a microglia assumisse uma conformação ativada, com ênfase na discussão sobre os canais iônicos presentes na membrana dessas células. Ademais, evidências mostram que pacientes com TP apresentam um desequilíbrio ácido-básico, que pode estar relacionado a canais sensíveis a ácido. Esses canais são canais iônicos que, portanto, podem contribuir para a ativação da microglia e a produção de citocinas inflamatórias e de metabólitos da quinurenina por parte destas células. Sendo assim, o quarto artigo discorre sobre possíveis associações entre canais sensíveis a ácido na etiologia do TP por meio de uma meta-análise. O quinto

e sexto artigo consistiram em revisões da literatura sobre o tratamento do TP, com destaque para a terapêutica medicamentosa com inibidores seletivos de recaptação de serotonina e benzodiazepínicos. Nos dois artigos, ressaltou-se que ambas as medicações são eficazes no TP. Entretanto, o sexto artigo, que consiste em uma meta-análise de efeitos adversos de tais medicações no tratamento a curto prazo do TP, demonstrou que pacientes apresentaram maior gama de efeitos adversos quando tratados com inibidores seletivos de recaptação de serotonina em comparação com benzodiazepínicos.

Palavras-chave: sistema imune, ansiedade, neurocircuitária do medo, terapêuticas medicamentosas.

ABSTRACT

Quagliato, Laiana Azevedo. The role of inflammatory markers in Panic Disorder and treatment considerations. Rio de Janeiro, 2019. Dissertation (Master in Psychiatry and Mental Health) - Institute of Psychiatry, Federal University of Rio de Janeiro, Rio de Janeiro, 2019.

This dissertation aims to present the work published by the author regarding possible associations between inflammation and Panic Disorder (PD). The first article consists of a systematic review of the literature, which included studies that measured inflammatory cytokines in patients diagnosed with PD. This demonstrated that IL-5, IL-6 and IL-1β cytokines are increased in PD. In the second article, the objective was to evaluate if the inflammatory cytokines would be related to the kynurenine pathway in the PD. We also investigated possible associations of these inflammatory markers with cognitive domains in patients with PD and in healthy controls in a cross-sectional study. An increase in the kynurenine/tryptophan ratio was a predictor of verbal auditory memory deficits in patients with PD when compared to healthy controls. In addition, only patients with PD demonstrated a significant association between the kynurenine/tryptophan ratio and the inflammatory cytokine IL-2R, which could indicate an increase in the activity of the IDO enzyme in these patients. In this dissertation, there were supposed reasons why this could happen. One of the hypotheses discussed is that the microglia, immune cells of the central nervous system, could be producing such cytokines and markers of the kynurenine pathway in patients with the diagnosis of PD if they had their conformation activated. Thus, the third article, wich consists of a systematic review, investigated what could cause the microglia to assume an activated conformation, with emphasis on the discussion of the ion channels present in the membrane of these cells. In addition, evidence shows that patients with PD present an acid-base imbalance, which may be related to acid-sensitive channels. These channels are ionic channels that, therefore, may contribute to the activation of microglia and the production of inflammatory cytokines and kynurenine metabolites by these cells. Thus, the fourth article discusses possible associations between acid-sensitive channels in the etiology of PD in a meta-

analysis. The fifth and sixth articles consisted of reviews of the literature on the treatment of PD, with emphasis on drug therapy with selective serotonin reuptake inhibitors and benzodiazepines. In both articles, it was emphasized that both medications are effective in PD. However, the sixth article, which consists of a meta-analysis of adverse effects of such medications on the short-term treatment of PD, demonstrated that patients had a greater range of adverse effects when treated with selective serotonin reuptake inhibitors compared to benzodiazepines.

Key words: immune system, anxiety, neurocircuitry of fear, drug therapy.

SUMÁRIO

1. INTRODUÇÃO

1.1. APRESENTAÇÃO

Minha formação acadêmica se iniciou na Universidade Federal do Estado do Rio de Janeiro, onde atuei como aluna de iniciação científica em projetos relacionados a meningite meningocócica e tive meu primeiro contato com a disciplina de Psiconeuroimunologia. Entretanto, o maior interesse em pesquisa desenvolveu-se nos últimos anos da graduação de Medicina, nos quais acompanhava o ambulatório de Psiquiatria do Hospital Universitário Gafrée Guinle e me questionava o porquê daquelas alterações comportamentais surgirem nos pacientes. Nessa época, fui introduzida ao mundo da “Psiquiatria Biológica” e iniciei a leitura de artigos científicos nesses moldes. Não é exagero dizer que esse período foi decisivo para a minha escolha da Psiquiatria como especialidade médica.

Após a graduação, ingressei na Residência Médica em Psiquiatria na Universidade do Estado do Rio de Janeiro, em que era proposto que os residentes frequentassem ambulatórios de especialidades no segundo e terceiro ano de residência. Dessa maneira, iniciei um intercâmbio acadêmico com o Instituto de Psiquiatria da UFRJ, aonde tive um primeiro contato com alguns grupos de pesquisa deste local. Nessa época, comecei a “ir atrás” de temáticas que fossem do meu interesse e, logo retornei à Imunologia e sua interface com transtornos mentais.

Ao término da Residência Médica, ingressei no Programa de Pós-Graduação em Psiquiatria e Saúde Mental do Instituto de Psiquiatria da Universidade Federal do Rio de Janeiro (PROPSAM), onde fui incentivada pelo Professor Antonio Egidio Nardi, a trabalhar nas minhas áreas de interesse.

1.2. O SISTEMA NERVOSO CENTRAL E AS CÉLULAS DA GLIA

A interação harmônica que deve ocorrer no sistema nervoso central (SNC) depende fundamentalmente das relações entre as células que o compõem. Neurônios e células da glia mantêm uma estreita relação por meio da troca de fatores secretados por uma ou outra célula que paracrinamente agem nas demais células do organismo (Kimelberg e Norenberg, 1989; Fellin, 2009). Essas células podem ainda realizar um intercâmbio de metabólitos por meio de junções comunicantes (Kimelberg e Norenberg, 1989; Fields e Stevens-Graham, 2002). Dessa forma, podemos caracterizar o SNC como uma imensa rede celular, comunicante e heterogênea. Neurônio e glia representam diferentes componentes de uma unidade funcional, sendo evidente que as funções cerebrais dependem de uma íntima sinalização do neurônio para a glia e da glia para o neurônio (Kimelberg e Norenberg, 1989; Araque e Perea, 2004; Fellin, 2009).

As células da glia são divididas em duas classes principais: microglia e macroglia (Vilhardt, 2005). As células da microglia são as células do sistema imune residentes no SNC. No cérebro adulto, tais células apresentam um fenótipo ramificado (Vilhardt, 2005; Hanisch e Kettenmann, 2007). A microglia ramificada, residente do cérebro adulto, está num estado de permanente “alerta”, sendo capaz de perceber pequenas alterações no ambiente(Davis *et al.*, 1994; Vilhardt, 2005). Essas alterações do ambiente são percebidas com o auxílio de um complexo conjunto de transportadores e canais iônicos (Schilling e Eder, 2007; Nguyen *et al.*, 2017). Estas células organizam-se de forma que cada uma monitora um sítio cerebral, não havendo sobreposição de sítios, o que lhes confere a capacidade de examinar continuamente todo o parênquima cerebral de forma bastante eficiente (Nimmerjahn *et al.*, 2005; Garden e Moller, 2006).

O papel de realizar a fagocitose de microorganismos, de resíduos celulares, assim como de células apoptóticas durante o desenvolvimento, são apenas algumas das muitas funções das células da microglia (Stoll e Jander, 1999;

Vilhardt, 2005; Kaur *et al.*, 2010). Essas células mantém o SNC livre de organismos invasores, exercem o papel de células apresentadoras de antígeno para os linfócitos, e ainda secretam fatores neurotróficos, como o *Brain derived neurotrophic factor* (BDNF), possibilitando, dessa forma, a homeostase ideal do SNC (Vilhardt, 2005).

Quando ocorrem alterações no ambiente em que essas células se encontram, devido à lesões no SNC, presença de microorganismos ou outras condições acidóticas, ou inflamatórias, as células da microglia proliferam-se (Ajami *et al.*, 2007), tornando-se ativas, passando a apresentar um fenótipo ameboide (Stoll e Jander, 1999; Vilhardt, 2005). Neste estado, as células apresentam uma alta taxa metabólica, sintetizando e secretando diversas citocinas, como a interleucina (IL)-6 e a IL-1β (Vilhardt, 2005). Quando as alterações no ambiente celular são interrompidas, a inflamação cessa e estes fatores deixam de ser liberados no meio (Garden e Moller, 2006).

A ativação microglial está associada a inúmeros transtornos mentais, uma vez que a vigilância microglial desempenha um papel importante no monitoramento da função sináptica e na determinação da conectividade cerebral (Stevens *et al.*, 2007; Tremblay *et al.*, 2010; Schafer *et al.*, 2012). Ademais, durante o desenvolvimento pós-natal, as sinapses que devem ser podadas são marcadas com moléculas do complemento e são, assim, removidas pela micróglia (Schafer *et al.*, 2012). O rompimento desse sistema pode levar à alteração da conectividade do SNC, gerando excesso de sinapses excitatórias que podem estar envolvidas na patogênese de vários transtornos, como a epilepsia e o autismo (Zhan *et al.*, 2014; Zhang *et al.*, 2016). Além disso, as células da microglia liberam moléculas como óxido nítrico, ácido quinurênico dentre outras, potencialmente implicadas em um desequilíbrio sináptico inibitório-excitatório, o que também poderia contribuir para a patogênese de diversos transtornos mentais (Villegas-Llerena *et al.*, 2016).

1.3. O TRANSTORNO DE PÂNICO

O Transtorno de Pânico (TP) é classificado pela quinta edição do Manual diagnóstico e estatístico dos transtornos mentais (DSM-5) como um dos transtornos de ansiedade, sendo caracterizado por ataques de pânico (AP) recorrentes (American Psychiatry Association, 2013). Os AP são paroxismos súbitos de ansiedade nos quais os sintomas atingem um pico e diminuem em poucos minutos (American Psychiatry Association, 2013). Pacientes com TP possuem, ainda, medo de ter um novo AP (ansiedade antecipatória) e uma mudança no comportamento em função da presença de AP (sintomas de evitação) (American Psychiatry Association, 2013). A prevalência do TP ao longo da vida está estimada entre 1,5 e 5% (Kessler et al., 2005).

Indivíduos diagnosticados com TP, de maneira geral, possuem uma percepção elevada de perigo ou ameaça (Freeston *et al.*, 1994). Para avaliar uma situação como ameaçadora e gerar uma resposta semelhante à ansiedade, um indivíduo deve primeiro detectar estímulos ambientais através dos sistemas sensoriais e em seguida, identificá-los como aversivos ou potencialmente perigosos (Freeston *et al.*, 1994). As ações combinadas de circuitos neurais distribuídos que emergem da amígdala, núcleo leito da estria terminal, hipocampo ventral e córtex pré-frontal medial resultam na interpretação e avaliação do valor emocional dos estímulos ambientais (Mcdonald, 1998). Se tais estímulos são identificados como ameaçadores com base nessa avaliação, podem resultar em comportamentos defensivos através do recrutamento do tronco cerebral e núcleos hipotalâmicos, resultando em sintomas ansiosos (Adhikari, 2014). Evidências mostram que no TP ocorrem alterações do desenvolvimento cerebral em circuitos relacionados ao medo (Janak e Tye, 2015). Entretanto, fatores determinantes para o surgimento deste transtorno ainda não foram completamente elucidados.

Fatores de base genética, além de modelos neuroquímicos, como o modelo serototinérgico, noradrenérgico e gabaérgico estão entre as hipóteses etiológicas mais difundidas para o TP (Eriksson, 1987). Evidências mostram, ainda, um desequilíbrio na homeostase ácido-base em pacientes com TP (Maddock, 2001). Ademais, marcadores inflamatórios, como citocinas inflamatórias, podem alterar a

atividade da amígdala, originando comportamentos ansiosos em modelos animais (Engler *et al.*, 2011).

Considerando as interações entre neurônio e células da glia, a investigação de marcadores inflamatórios nos diversos transtornos mentais é relevante, uma vez que pode contribuir para achados fisiopatológicos dessas condições. Tendo em vista a alta prevalência do TP, aliada aos prejuízos econômicos e sociais que esse transtorno acarreta (Pollack e Marzol, 2000), a elucidação da fisiopatogenia do mesmo é imperativa. Dessa forma, o objetivo dessa dissertação foi investigar a possível associação entre marcadores inflamatórios e o TP.

Inicialmente, discorreremos sobre as citocinas inflamatórias e o TP, hipotetizando como esses marcadores inflamatórios poderiam se relacionar à etiologia deste transtorno por meio de um artigo de revisão sistemática (artigo 1). A seguir, analisaremos a possível associação de citocinas inflamatórias e da via da quinurenina com a cognição de pacientes com o diagnóstico de TP (artigo 2). Faremos, também, considerações acerca de como canais iônicos poderiam contribuir para a mudança da conformação da microglia, o que poderia estar relacionado à fisiopatologia de diversos transtornos mentais (artigo 3), dentre eles o TP (artigo 4). Posteriormente, falaremos sobre a terapêutica medicamentosa do TP (artigo 5 e 6).

2. DESENVOLVIMENTO

2.1. As citocinas inflamatórias e o TP

Estudos recentes evidenciam associações importantes entre níveis aumentados de IL- 1 e IL-6 com transtornos depressivos (Howren *et al.*, 2009; Dowlati *et al.*, 2010). Considerando a importante comorbidade entre o TP e os transtornos depressivos (Coplan *et al.*, 2015), além de áreas cerebrais comuns muitas vezes envolvidas (Martin *et al.*, 2009; Martin *et al*., 2010), é plausível supor que

mecanismos compartilhados entre estes transtornos possam envolver uma patogenia inflamatória. Dessa forma, foi realizada uma revisão sistemática da literatura, na qual foram incluídos estudos que mensuravam citocinas inflamatórias em pacientes com o diagnóstico de TP.

Artigo 1: Cytokine alterations in panic disorder: A systematic review. *Journal of Affective Disorders*, 2018, 228: 91-96.

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Review article

Cytokine alterations in panic disorder: A systematic review

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ABSTRACT

Background: Panic disorder (PD) occurs in 3.4–4.7% of the general population. Although accumulating evidence suggests that some inflammatory processes play a role in the pathophysiology of mental disorders, very few studies have evaluated cytokine levels in patients with PD. The aim of the present study was to systematically review the characteristic cytokine profile of PD patients and discuss some possibilities for future trials on this common and disabling disorder.

Methods: A comprehensive literature search was carried out in PubMed and Web of Science databases (search terms: "panic disorder" or "panic attacks" and IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-alpha and INF-gamma).

Results: Eleven studies involving measurements of cytokines in PD patients were included in this review article. Increased serum levels of some inflammatory markers such as IL-6, IL-1β and IL-5 were reported in PD patients compared with control subjects. There are some conflicting results regarding IL-2, IL-12, and INF-γ in association with PD.

Limitations: There are discrepant findings in the existing literature regarding PD and cytokines. A significant portion of the recognized heterogeneity may be attributable to variability in assay procedures. The discrepant findings may also have been due to differences in the study populations.

Conclusions: Cytokines induce the production of acute-phase proteins and are linked to neurogenesis, modification of the HPA axis, microglial activation, tryptophan metabolism and an imbalance in excitatory and inhibitory neurotransmission. Investigation of inflammatory biomarkers in PD could contribute to understanding the pathophysiological mechanisms in this debilitating disorder.

1. Introduction

Cytokines are a large group of proteins originally classed together because of their immunologic functions. Each interleukin family plays a variety of important roles in regulating inflammation. The pro-inflammatory interleukins, such as IL-6 and IL-1β, are proteins secreted into the bloodstream in response to immunologic challenge, and elevations of these cytokines in the absence of infection or tissue injury are considered abnormal (Dofferhoff et al., 1991; Koj et al., 1988). The main activities of IL-6 and IL-1β are mediation of acute-phase protein synthesis, regulation of proinflammatory factors, and microglial activation (Audet and Anisman, 2013). Both cytokines have been proven to decrease hippocampal neurogenesis (Goshen et al., 2008; Monje et al., 2003). Meanwhile, IL-2, IL-12, TNF and interferons are highly associated with the growth, development and activity of T- cells (Croft, 2014; Le Page et al., 2000; Liao et al., 2011). IL-3 and IL-5 are also related to the maturation and function of cells, but instead of being associated with T-cells, they are mainly linked to hematopoietic cells (Broughton et al., 2012). Furthermore, IL-5 is a key mediator in eosinophil activation (Broughton et al., 2012). All the previously mentioned markers have inflammatory properties, while IL-10 itself can repress proinflammatory responses (Ouyang et al., 2011).

Proinflammatory cytokines produced peripherally can cross the blood-brain barrier (Banks et al., 1995), and peripheral proinflammatory signals can also be actively propagated across the blood-brain barrier by crosstalk between the peripheral and central immune systems (Maier, 2003; Xiao and Link, 1998). In fact, peripheral cytokines can change amygdaloid activity and increase anxiety-like behavior (Engler et al., 2011). The amygdala, the hub of fear-processing networks, is closely associated with the pathogenesis of panic disorder (PD) as well as panic attack (Kim et al., 2012).

Amygdaloid activity is also associated with the stress response system, which is intricately linked to proinflammatory signaling. Under stressful stimuli, peripheral myeloid cells can produce proinflammatory cytokines and readily circulate to several organs, including the brain. Myeloid cell trafficking in the brain after stress is important because it

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Table 1
Summary of studies evaluating cytokines in PD.

Studies	Sample	Study design	Biomarkers	Main results
Brambilla et al. (1994)	10 PD evaluated before and after treatment with alprazolam × 10 HC	Case-control	IL-1β	Before therapy: PD > IL-1β ($p = 0.0007$) After treatment: PD > IL-1β ($p = 0.04$)
Rapaport and Stein (1994)	15 PD × 19 HC	Case-control	IL-1, IL-2, IL-2R	Trend for PD > IL-2 ($p < 0.07$)
Brambilla et al. (1999)	10 PD evaluated before and after treatment with alprazolam × 10 HC	Case-control	TNF-α	TNF-α did not differ between PD × HC
Weizman et al. (1998)	24 PD × 19 HC	Case-control	IL-2, IL-3	IL-3: negative correlation with severity of state anxiety.
Koh and Lee (2004)	42 PD (21 CBT and 21 CBT + BZD) × 42 HC	RCT	IL-2, lymphocyte proliferation	PD < lymphocyte proliferative response to PHA($p = 0.001$) and < IL-2 production ($p = 0.03$)
Van Duinen et al. (2008)	18 PD × 18 HC (placebo × 35% CO_2 challenge)	RCT	IL-1RA, sIL-6R, sIL-2R, TNF-α, IL-8, haptoglobin	No differences in immune parameters
Hoge et al. (2009)	20 PD or 28 PTSD × 48 HC	Case-control	IL-6, IL-1α, IL-1β, IL-8, MCP-1, MIP-1α, Eotaxin, GM-CSF, and INF-α, IL10	PD or PTSD > peripheral cytokine levels ($p < 0.0025$)
Koido et al. (2010)	312 MDD × 210 PD × 356 HC	Case-control	SNP in 1q32 in IL10 gene	No significant associations
Tükel et al. (2012)	23 PD × 23 HC	Case-control	TNF-α, INF-γ, IL-1β, IL-2, IL-6, IL-12, NK-cell activity	PD associated with IL-12 ($p = 0.01$) and INF-γ ($p = 0.04$) INF-γ predictor of PD ($p = 0.04$)
Ogłodek et al. (2016)	460 PD and/or P × HC	Case-control	CCL-5, CCL-2, CXCR-5, CXCR-4, IL-6	PD + OCPD and PD + APD associated with CCL-5 ($p < 0.0001$)
da Silva et al. (2017)	78 PD (23 current × 55 remission)	Case-control	IL-6, IL-10 and TNF-α	IL-6 ($p = 0.008$) associated with current PD.

PD: Panic Disorder; HC: healthy controls; CBT: cognitive behavioral therapy; BZD: Benzodiazepines; PTSD: Post-traumatic stress disorder; MDD: Major depressive disorder; P: personality disorder; OCPD: obsessive compulsive personality disorder; APD: avoid personality disorder.

coincides with activation of resident microglia and increases proinflammatory cytokine production (Wohleb et al., 2013). Besides this active communication between microglia and the peripheral immune system, there is evidence that peripheral inflammation causes an inflammatory response in the central nervous system characterized by additional synthesis and action of interleukins within the brain (Wohleb et al., 2013). Stimulation of the hypothalamic-pituitary-adrenal (HPA) axis may affect neurogenesis due to the anti-neurogenic properties of glucocorticoids (Cameron and Gould, 1994). The stress response involves the release of proinflammatory cytokines, which increases the release of corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH), and cortisol by acting directly on hypothalamic and pituitary cells (Black, 1994; Vallieres and Rivest, 1999). Dysregulation of the HPA axis is an important finding associated with anxiety behavior (Cowen, 2002).

Not only do stressful stimuli affect neurogenesis, but also treatment of anxiety disorders is implicated in it (Malberg et al., 2000; Revest et al., 2009). The selective serotonin reuptake inhibitors (SSRIs) can upregulate the expression of brain-derived neurotropic factor (BDNF) in the hippocampus, which promotes the survival and proliferation of neural progenitor cells (Nibuya et al., 1995; Sairanen et al., 2005). Furthermore, increased inflammation is associated with less robust antidepressant treatment responses, and treatment-resistant patients exhibit increased inflammatory markers in major depressive disorder (Raison et al., 2013). Acumulated evidence suggests that certain inflammatory processes play a role in the pathophysiology of mood disorders (Eyre and Baune, 2012; Howren et al., 2009). Depression and PD are highly comorbid and share similar first-line treatments, but there is still no certainty as to their shared risk factors or the extent to which the underlying inflammatory mechanisms play a causal role in each disorder.

Although an association between inflammatory response activation and PD has been documented in individual studies of various cytokines (Hoge et al., 2009; Rapaport and Stein, 1994), the association is not consistently significant in all studies or for all cytokines (Brambilla et al., 1999; Van Duinen et al., 2008). Thus, a generalizable pattern of immune dysfunction in PD remains to be defined. The aim of the present study was to systematically review the characteristic cytokine profile in PD patients and discuss some possibilities for future trials in PD.

2. Methods

In this systematic literature review, PubMed and Web of Science databases were searched in June 2017 (last update: 30 June 2017) for randomized control trials, cross-sectional, cohort, and case-control studies, and case series investigating associations between PD and cytokines.

The search terms utilized for the search were “panic disorder” or “panic attacks” and IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-alpha and INF-gamma. The review only included studies published in English in the last 25 years. Articles were reviewed for inclusion by two independent investigators. Disagreements between reviewers were resolved by consensus. Data extraction was performed by one investigator and checked for accuracy by a second investigator. Information was extracted from each included article on: (1) characteristics of research participants (including age, severity of disease, and method of diagnosis), and the paper's inclusion and exclusion criteria; (2) study design; (3) type of outcome measure (including increase or decrease in cytokine levels). The review excluded studies evaluating patients with any other primary clinical or psychiatric disorder and which did not test whether the comorbidity could play a role in the levels of inflammation markers. Studies with other designs, e.g. review articles and meta-analyses, were excluded. The references from selected studies were manually searched for additional articles. The full search queries and search strategies are provided in S1 Appendix. The PRISMA checklist is provided in S2

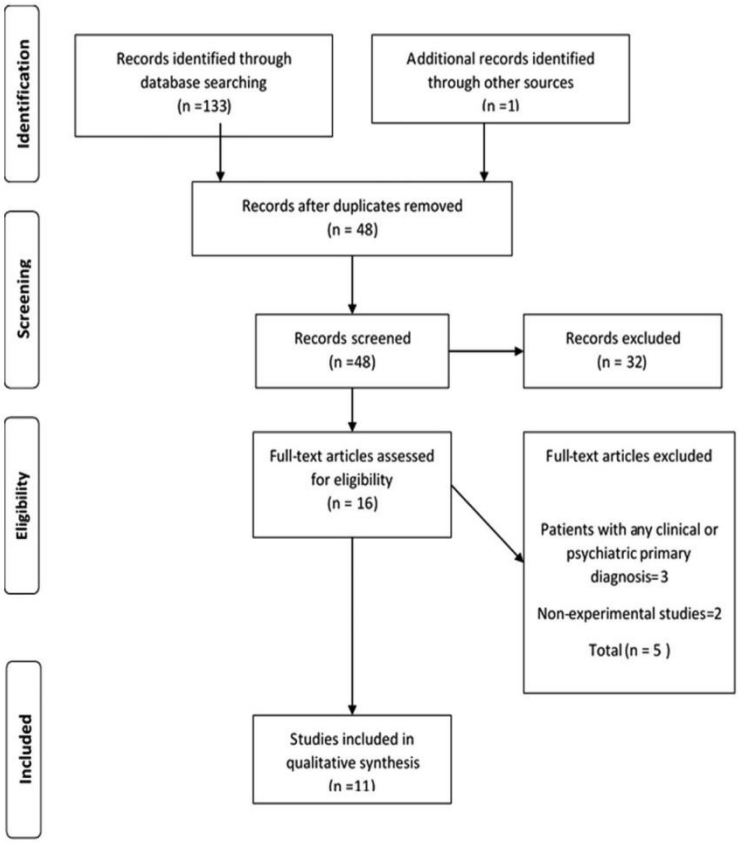

Fig. 1. PRISMA diagram of study identification and selection process.

Checklist.

3. Results

The database search yielded 113 articles. Of these, the final sample excluded 66 duplicate records, 34 articles that evaluated healthy subjects or patients with other primary psychiatric or clinical conditions and which did not evaluate whether the comorbidity could play a role in the levels of inflammation markers, and 3 articles not reporting experimental studies (i.e. commentaries). One additional record was identified by manual search. Thus, eleven studies were included in this review and are briefly described in Table 1. The process of study identification and selection is shown in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Fig. 1) (Moher et al., 2009).

Acumulated evidence suggests that interleukins are associated with the acute phase of some mental disorders (Bai et al., 2014). To investigate whether this association might be true in panic disorder, da Silva et al. (2017) compared IL-6 levels in current and remitted PD patients. Their study demonstrated that current PD was associated with higher mean levels of IL-6 compared to remitted PD ($p = 0.008$) in a sample of chronically affected outpatients. This finding was in the same direction as demonstrated by Hoge et al. (2009), who compared PD patients to matched controls ($p < 0.0005$).

IL-6 could also be associated with severity of mental disorders, as shown by Ogłodek et al. (2016). The authors reported higher IL-6 levels in PD associated with comorbid avoidant personality disorder and obsessive-compulsive personality disorder. On the other hand, Van Duinen et al. (2008) and Tükel et al. (2012) found no difference in IL-6 levels in PD patients.

Tükel et al. (2012) also found no link between the IL-1 family and PD when they compared cytokine levels in PD patients and normal controls, corroborating the work by Rapaport and Stein (1994). Contrasting with these data, Hoge et al. (2009) reported a significant association between IL-1α, IL-1β, and PD ($p < 0.0005$).

Although, the carbon dioxide (CO_2) challenge has been regarded as a safe and noninvasive method to provoke panic attacks in PD patients in research settings (Amaral et al., 2013), Van Duinen et al. (2008) were the only authors to investigate potential differences in immune parameters following 35% CO_2 challenge. They reported no significant association between PD and the IL-1 family.

Asking whether antipanic agents could modify interleukin levels, Brambilla et al. (1994) evaluated IL-1β plasma concentrations before and after therapy with alprazolam. They reported that IL-1β plasma concentrations before therapy with alprazolam were significantly higher in patients than controls ($p = 0.0007$). After regular treatment with alprazolam, IL-1β levels decreased but were still significantly higher than in controls ($p = 0.04$).

The conflicting results regarding the IL-1 family in association with PD may be due to differences in the assay methodologies, since Rapaport and Stein (1994), Tükel et al. (2012), and Van Duinen et al. (2008) used ELISA, while Brambilla et al. (1994) and Hoge et al. (2009) used different assays (bioassays and Luminex, respectively). The discrepant findings may also have been due to differences in the respective study populations.

Some divergence is also reported on publications regarding the IL-2 family. Although various authors like Weizman et al. (1998), Van Duinen et al. (2008), Hoge et al. (2009) and Tükel et al. (2012) found

no associations between IL-2 and PD, Rapaport and Stein (1994) found a trend of higher IL-2 levels in PD patients compared to healthy controls ($p < 0.07$). Meanwhile, seeking to evaluate cell-mediated immunity in PD, Koh and Lee (2004) measured IL-2 production and the lymphocyte proliferative response to phytohemagglutinin (PHA). The authors found that PD patients had a significantly lower lymphocyte proliferative response to PHA and IL-2 production than controls ($p < 0.03$).

In the literature, cell-mediated immunity is highly associated with IL-12, TNF-α and INF-γ (Seruga et al., 2008). However, there are few data associating PD with these inflammatory mediators. For instance, only Tukel and Hodge compared IL-12 levels in PD patients and healthy controls. Tükel et al. (2012) found lower IL-12 levels in the patient group ($p = 0.01$), while Hoge et al. (2009) found no difference in IL-12 levels in PD patients compared to matched controls.

Regarding TNF-α, Brambilla et al. (1994), Van Duinen et al. (2008), Tükel et al. (2012) and da Silva et al. (2017) found no significant association between PD and this inflammatory marker. Meanwhile, Hoge et al. (2009) reported higher TNF-α levels in PD patients compared to matched controls ($p < 0.0005$). The latter study also evaluated INF-γ levels, but did not report a statistically significant result for this marker. On the other hand, according to Tükel et al. (2012), lower INF-γ levels were statistically significant predictors of PD ($p = 0.04$).

Few data are also found in the literature regarding the IL-5 family and PD. Ogłodek et al. (2016) associated IL-5 levels with PD and comorbid personality disorder. They reported higher IL-5 and CXCR-5 levels in PD patients, associated with avoidant personality disorder and obsessive-compulsive personality disorder. Therefore, higher IL-5 levels could be related to the severity of PD. Contrasting with these results, Weizman et al. (1998) reported that higher anxiety levels measured by STAI-trait in PD patients were associated with lower IL-3 production ($p < 0.05$).

The cytokine IL-10 was evaluated by da Silva et al. (2017) and Koido et al. (2010). The authors found no association between PD and IL-10 regarding IL-10 in current PD patients compared to remitted PD patients or in the SNP of the IL-10 gene, respectively. This contrasts with Hoge et al. (2009), who showed higher IL-10 levels in PD patients compared to matched controls ($p < 0.005$).

4. Discussion

This review article reports increased levels of the proinflammatory cytokines IL-1β and IL-6 in PD patients compared to control subjects. Proinflammatory cytokines produced peripherally can cross the blood-brain barrier or can be produced in the brain. Cytokine production by microglia is related to a characteristic morphological change (ramified to amoeboid) associated with their activation state (Galic et al., 2012).

Microglial morphology suffers rapid alteration induced by extracellular acidification (Vollmer et al., 2016). Carbon dioxide (CO_2) inhalation, a biological challenge and pathological marker in PD, evokes intense fear and panic attacks in susceptible individuals. The acid-sensing TDAG8 receptor is located in microglia. It promotes CO_2-evoked behavioral (freezing) and cardiovascular responses via a mechanism involving microglial activation and proinflammatory cytokine IL-1β, as demonstrated by a recent animal study (Vollmer et al., 2016).

Microglia activation is associated with an imbalance in excitatory and inhibitory neurotransmission that could be one of the pathophysiological mechanisms underlying PD. Proinflammatory cytokines can induce the indoleamine-2,3-dioxygenase (IDO) enzyme, which catalyzes the rate-limiting step in the synthesis of kynurenine from tryptophan (Schrocksnadel et al., 2006). Proinflammatory cytokines, including IL-1β and IL-6, have been shown to increase IDO expression in both central and peripheral immune-competent cell types (Tu et al., 2005). Activation of these cell types can degrade tryptophan, which may contribute to anxiety symptoms by reducing the availability of the requisite precursor for the synthesis of serotonin, causing a disrupted serotonergic neurotransmission (Schwarcz et al., 2012). The kynurenine pathway gives rise to metabolites such as quinolinic acid, an endogenous N-methyl-D-aspartate (NMDA) agonist that can disrupt neurotransmission along the glutamatergic network, ultimately contributing to excessive glutamate both within and outside the synapse (Schwarcz et al., 2012).

Not only could inflammatory cytokines alter excitatory neurotransmission through the brain, but these biomarkers could also decrease GABA neurotransmission, which could explain GABA deficit in PD (Goddard et al., 2001). Additionaly, PD patients may have a dysfunction of the $GABA_A$ receptors (Bremner et al., 2000), which play an important role in anti-inflammation via inhibiting the expression of inflammatory cytokines (Palma et al., 2017). $GABA_A$ receptor is found in $CD4^+$ T cells and macrophages and inhibits the proliferation of antigen-specific T cells and the production of IL-6, IL-12, IL-1β, and TNF-α (Wu et al., 2017). Meanwhile, $GABA_B$ receptor is expressed in neutrophils and acts as chemoattractant receptor. Endogenous GABA tonically inhibits plasma IL-6 and IL-1β levels through GABA receptors. Furthermore, GABAergic agents directly affect the function of antigen-presenting cells via $GABA_A$ receptor, and the phosphorylation of MAPK to inhibit the production of inflammatory cytokines from T cells during inflammation (Wu et al., 2017). Therefore, the GABAergic system has great potential to inhibit inflammatory responses. All components of the GABAergic system are related to autoimmune diseases (Wu et al., 2017), and modulation of the GABAergic system could regulate the pathogenesis of various proinflammatory and autoimmune diseases. PD is highly associated with autoimune conditions such as thyroid autoimmunity (Carta et al., 2002). It is plausible to hypothesize that in autoimmune diseases the dysfunction produced in the GABA system or another imbalancing factor could facilitate the onset of panic disorder in a specific group of patients.

PD is also associated with respiratory diseases. Comorbid asthma and PD are quite common (Goodwin et al., 2003; Hasler et al., 2005; Nascimento et al., 2002), and there is reason to believe that the two disorders interact, producing greater morbidity for each (Lehrer et al., 2008). Our review reports higher levels of IL-5 in PD patients compared to matched controls. In humans, interleukin-5 receptor is expressed on eosinophils and basophils. Eosinophils are a prominent feature in the pulmonary inflammation associated with allergic diseases of the airways (Greenfeder et al., 2001). Thus, the investigation of common inflammatory markers such as IL-5 can be of value.

There are some conflicting results regarding IL-2, IL-12 and INF-γ in association with PD. These are proinflammatory cytokines, and some studies (Koh and Lee, 2004; Tükel et al., 2012) have shown low levels of these biomarkers in PD patients compared to matched controls. This fact, as well as the lack of significant associations between some inflammatory markers and PD, could be explained by some limitations of the studies included in this review. Many clinical variables that may have affected the relationship between cytokines and PD still require clarification. According to DSM-5 (APA, 2013), PD is a unitary diagnostic category, although there are diverse clinical presentations for the disorder. High rates of PD in clinical samples of people with autoimmune diseases (Carta et al., 2002) could lead us to postulate that the dysfunction produced in the GABA system could facilitate the onset of a panic disorder in some types of PD patients, which could explain the strong variability of the results. Additionaly, inflammatory markers can be associated with the severity of anxiety symptoms, and differences in symptom severity may account for some of the data heterogeneity between studies. A significant share of such heterogeneity may be attributable to variability in assay procedures both within and between laboratories. As a further limitation, many trials comparing cytokines are not registered with clinical trials databases, so the scope of the unpublished literature cannot be ascertained, and publication bias cannot be ruled out entirely. Replication studies are needed in larger samples and with different methodologies to determine the reasons for discrepant findings in the existing literature.

In conclusion, cytokines induce the production of acute-phase

proteins and are linked to neurogenesis, modification of the HPA axis, microglial activation, GABA dysfunction, and increased tryptophan metabolism as well as the neurotoxic and oxidative consequences. Furthermore, proinflammatory cytokines are modified by several neurotransmitters and administration of psychoactive drugs (Berk et al., 2011). Although there are few reports associating PD and inflammatory markers, it is important to consider PD as a multidimensional syndrome involving both central and peripheral pathologies. Increased activation of glutamate receptors and/or decreased function of GABA receptors may be a pathway by which inflammation causes panic-like behavior. Drugs that act on glutamate receptor signaling, IDO pathway or GABA neurotransmission might have unique applicability to patients with PD and increased inflammation. Therefore, the investigation of inflammatory biomarkers in PD can enhance our understanding of the pathophysiological mechanisms in this common and disabling disorder.

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We have not received contributions from non-authors.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jad.2017.11.094.

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2.2. Citocinas inflamatórias e a via da quinurenina

A via da quinurenina é formada por uma sequência de enzimas localizadas majoritariamente em células da glia (Schwarcz *et al.*, 2012). As citocinas inflamatórias apresentam capacidade de induzir a enzima indolamina -2,3-dioxigenase (IDO), que catalisa a etapa de limitação da síntese da quinurenina advinda do triptofano (Schrocksnadel *et al.*, 2006) (FIGURA 1). O triptofano é o precursor necessário para a síntese da serotonina (Schwarcz *et al.*, 2012; O'mahony *et al.*, 2015). Citocinas pró-inflamatórias aumentam a expressão da IDO tanto em tipos celulares imunes competentes centrais quanto da periferia (Schwarcz *et al.*, 2012).A ativação desses tipos celulares pode degradar o triptofano, reduzindo a concentração desse precursor que é necessário para a síntese da serotonina (Schwarcz *et al.*, 2012). Sabe-se que a serotonina desempenha papel importante na fisiopatologia do TP, sendo que o receptor serotoninérgico 5-HT_{1A} é considerado como modulador da ansiedade nas suas formas normais e patológicas (Maron e Shlik, 2005; Paul *et al.*, 2014). Além disso, de maneira global, os níveis plasmáticos de serotonina estão reduzidos nos diversos transtornos de ansiedade, dentre eles o TP (Maron e Shlik, 2005; Paul *et al.*, 2014).

A via da quinurenina possui diversos metabólitos. A microglia ativa pode converter, por exemplo, a quinurenina em ácido quinolínico (Schwarcz *et al.*, 2012). Este liga-se ao receptor NMDA, um receptor glutamatérgico, ocasionando um aumento na concentração de glutamato, um neurotransmissor excitatório, o que pode culminar em redução do BDNF e excitoxicidade (Stone e Darlington, 2013). Tais achados podem atuar na integridade neural, modificando a neurogênese e potenciação de longo-prazo, afetando o aprendizado e a memória (Miller e Raison, 2016). Além disso, alterações dos metabólitos da rota das quinureninas associam-se a déficits em diferentes domínios cognitivos nos mais diversos transtornos mentais (Schwarcz *et al.*, 2012) (FIGURA 1). Níveis elevados de quinurenina, por exemplo, podem alterar aspectos específicos da flexibilidade cognitiva (Alexander *et al.*, 2013).

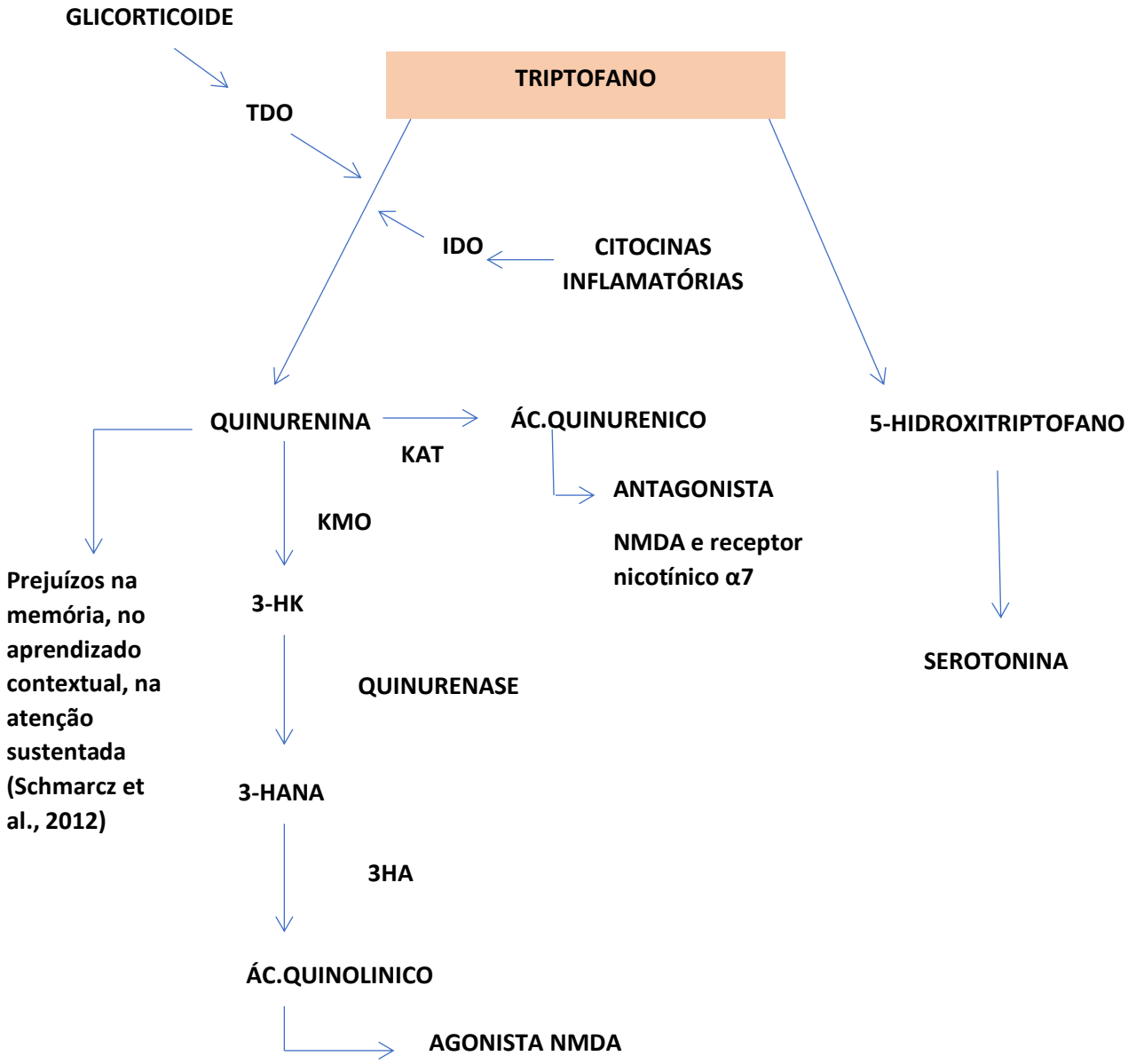

Figura 1-Via da quinurenina

Legenda: IDO, indolamina-2,3-dioxigenase; TDO, triptofano-2,3-dioxigenase; KMO, quinurenina 3-monooxigenase; KTA, quinurenina transaminase; 3-HK, 3-

hidroxi-quinurenina; 3-HANA, 3-ácido- hidroxianthranilico; 3HA, ácido-hidroxianthranilico; NMDA, N-Metil-D-Aspartato.

O segundo artigo dessa dissertação propôs-se a dosar as citocinas IL-2R, IL-10 e IL-1β, além de calcular a relação quinurenina/triptofano, associando tais biomarcadores com diferentes domínios cognitivos em pacientes com TP.

2.2.1. Artigo 2: Elevated peripheral kynurenine/tryptophan ratio predicts poor short-term auditory memory in panic disorder patients: *Journal of Psychiatric Research,* 2019, 113: 159-164

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Elevated peripheral kynurenine/tryptophan ratio predicts poor short-term auditory memory in panic disorder patients

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ABSTRACT

Abnormalities in the kynurenine pathway (KP) have been implicated in the cognitive deficits of psychiatry disorders, possibly through cytokines that increase the activity of indoleamine-2,3 dioxygenase (IDO), a key enzyme for tryptophan-to-kynurenine conversion. Some studies on panic disorder (PD) have detected elevated cytokines in blood. We aimed to determine the extent to which elevated peripheral cytokine levels and kynurenine/tryptophan (kyn/tryp) ratio (1) are biological markers for PD patients and (2) are related to cognition in PD. Seventy-eight PD patients and matched healthy controls were assessed for peripheral serum levels of interleukin (IL)-2R, IL-1β, IL-10, kynurenine and tryptophan. The subjects were evaluated for episodic and short-term memory, selective attention and cognitive flexibility. In patients, IL-2R levels, which are involved in the regulation of IDO, were significantly associated with levels of kynurenine ($p = .029$), but this association was not observed in controls. Importantly, an elevated kyn/tryp ratio significantly predicted poor digit span forward ($p = .004$) and total ($p = .004$) scores in individuals with PD. This study is the first to link blood biomarkers of inflammation and the KP with cognitive deficits in PD subjects, suggesting that those with an elevated kyn/tryp ratio might have short-term auditory memory impairment. These findings indicate that treatments targeting the KP may ameliorate cognitive abnormalities in PD patients.

1. Introduction

Biological abnormalities reported in panic disorder (PD) suggest multiple causative pathways, with no single biological etiology likely to be responsible for all cases (Roberson-Nay and Kendler, 2011). Therefore, improving interventions for PD may depend on identifying particular biological abnormalities that can be targeted with specific treatments.

Group comparisons have indicated abnormalities in specific brain regions in PD patients compared to those in controls, including differences in metabolic activity in the hippocampal and parahippocampal areas (Bisaga et al., 1998) and abnormalities in temporal lobe structures (Vythilingam et al., 2000). Brain abnormalities such as these may contribute to learning and memory deficits (Zhou and Ni, 2017). Furthermore, studies have found associations between PD and impairment in a number of cognitive areas, including executive function and working memory (Airaksinen et al., 2005; Alves et al., 2013; Palomares Castillo et al., 2010; Zhou and Ni, 2017).

Recent evidence suggests that cognitive deficits in psychiatric disorders might be related to the kynurenine pathway (KP) (Schwarcz et al., 2012). The initial and rate-limiting step of the KP is the conversion of tryptophan (tryp) to kynurenine (kyn), catalyzed by indoleamine 2,3-dioxygenase (IDO) or tryp 2,3-dioxygenase (TDO2) enzymes, which, in the brain, are preferentially expressed in immune cells, such as microglia (Guillemin et al., 2003; Schwarcz et al., 2012) (Fig. 1). Kyn can subsequently be metabolized into quinolinic acid (QUIN) or kynurenic acid (KYNA). These neuroactive metabolites may contribute to the cognitive deficits observed in mental disorders, as the kyn/tryp ratio is frequently elevated in these disorders (Birner et al., 2017; Okusaga et al., 2016; Platzer et al., 2017). Thus, the KP is an attractive target for the development of novel treatment strategies (Akagbosu et al., 2012; Chess et al., 2007; Chiappelli et al., 2018; Misztal et al., 1996; Rahman et al., 2018; Schwarcz et al., 2012). In addition, brain kyn is linked to and influenced by the peripheral KP (Schwarcz et al., 2012). As tryp and kyn readily cross the blood-brain barrier, fluctuations in the blood levels of these metabolites directly affect metabolism in the KP (Schwarcz et al., 2012).

One plausible hypothesis for abnormal KP metabolism is that elevated inflammatory activity drives elevations in kyn levels through the activation of IDO (Schwarcz et al., 2012). This enzyme is strongly stimulated by immune activation, including the inflammatory cytokines interleukin (IL)-1β (Zunszain et al., 2012), IL-10 (Yanagawa et al.,

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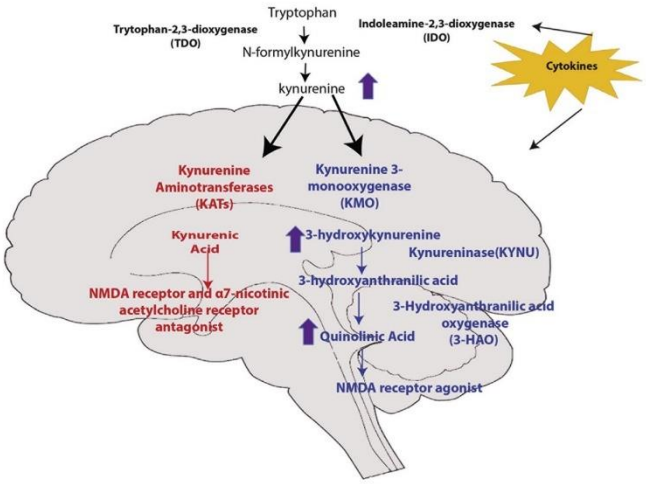

Fig. 1. The kynurenine pathway (KP) of tryptophan (tryp) metabolism. In the brain, kynurenine (kyn) metabolism can begin with the catabolism of tryp to kyn via either tryp 2,3-dioxygenase (TDO) or indoleamine 2,3-dioxygenase (IDO). Kyn is metabolized either via kyn aminotransferases (KATs) to kynurenic acid (KYNA) in astrocytes or via kyn monooxygenase (KMO), kynureninase, and 3-hydroxyanthranilic acid oxygenase (3-HAO) to quinolinic acid (QUIN) in microglia. KYNA is an antagonist of the N-methyl-D-aspartate (NMDA) receptor, and QUIN act as an agonist of this same receptor.

2009) and the IL 2 soluble receptor (IL-2R) (Szymona et al., 2017).

Comparisons of peripheral circulating cytokines between individuals with PD and controls have demonstrated that PD patients have higher mean serum levels of IL-6, IL-1β, IL-2 and other cytokines and even greater elevations in these substances during panic attacks (Quagliato and Nardi, 2018). Therefore, the relationship between peripheral cytokines and KP metabolites and especially their possible relevance for cognitive deficits in PD should be critically examined. Thus, we hypothesized that patients with PD display cognitive deficits in episodic, short-term memory, cognitive flexibility and selective attention, and that these deficits are associated with an elevated kyn/tryp ratio and elevated peripheral cytokine levels compared to those in healthy controls.

2. Materials and methods

2.1. Participants

Seventy-eight people with a diagnosis of PD and 78 age and sex-matched controls were recruited for this study (Supplementary Material). Patient recruitment was via either clinician or self/family referral. All patients were living in the community and had been receiving antidepressant medication, combined or not with a benzodiazepine, for at least 3 months prior to entry into the study (Supplementary Table 1). The diagnosis was determined by a structured clinical interview according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition-Text Revision (DSM-IV-TR) administered by a trained psychiatrist or psychologist and independently confirmed by a research psychiatrist. Antidepressant or benzodiazepine medication doses were obtained from the treating physician or from medical records and were converted to the mean daily fluoxetine or diazepam equivalent dose. The height and weight of all participants were recorded for body mass index (BMI) calculation. Healthy controls were recruited through self/family referral at the local community. The procedures were explained, and written informed consent was obtained from participants prior to participation in the study, which was approved by the research ethics committee of the Federal University of Rio de Janeiro. This study was performed according to the ethical standards of the Declaration of Helsinki.

2.2. Cognitive and symptom assessment

Cognitive function was assessed using the Rey Auditory Verbal Learning Test (RAVLT) for assessing episodic memory and learning (Moradi et al., 2017). Short-term and working memory were evaluated by the digit span task (Richardson, 2007; Sun et al., 2005). Selective attention was analyzed using the Stroop Neuropsychological Screening Test (Streeter et al., 2008). Cognitive flexibility was assessed as the time to complete the Trail Making Test, Part B minus the time to complete the Trail Making Test, Part A (TMT BA) (Hagenaars et al., 2018). The Hamilton Anxiety Rating Scale (HAM-A), Hamilton Depression Rating Scale (HAM-D), Panic and Agoraphobia Scale (PAS) were administered to all patients to obtain measures of general psychopathology and Clinical Global Impression (CGI) Scale were applied to evaluate total symptom severity. All assessments were made by psychiatrists trained in the administration and scoring of the assessments. For further details pertaining to cognitive test scoring, refer to the Supplementary Methods.

2.3. Inflammatory biomarkers

Blood was obtained in the morning (10 a.m. ± 1 h) in EDTA tubes through a catheter after participants had at least 30 min of rest. Blood was immediately centrifuged (1000 g for 10 min), and serum was removed and stored at −80 °C until the batched assay. The method used to measure tryp and kyn was the competitive inhibition immunoassay technique (cloud-Clone, Texas, USA). Concentrations of IL-1β, IL-10 and IL-2R were assessed using the Immulite System (Diagnostic Products Corporation), which is based on a solid phase two-site chemiluminescent enzyme immunometric assay (Berthier et al., 1999). See Supplementary Information for details of the biomarkers assays.

2.4. Statistical analyses

Statistical tests were performed using SPSS (version 17, OSX, IBM, Armonk, NY, USA) (IBM). The normal distribution of variables was tested using the Kolmogorov–Smirnov test. The quantitative cytokine IL-2R, IL-10, and IL-1β levels and kyn/tryp ratio were not normally distributed. We aimed to perform parametric tests, therefore we used log transformation in an intention to transform the data into normally distributed. However, no transformation resulted in satisfactory

normally distributed data of ILs, while log transformation resulted in normal distribution of the kyn/tryp ratio data. Thus, the Mann-Whitney *U* test and independent Student's t tests were performed to identify differences between diagnostic groups on cytokines and kyn/tryp, respectively. Multiple linear regression using stepwise methods were used to assess the relationship of the cytokine levels and kyn/tryp ratio with several potentially confounding variables related to sociodemographic, clinical, and physical characteristics (sex, age, years of education, years of illness, drug equivalents, ethnicity, physical activity, BMI, PAS, HAM-A, HAM-D and CGI) as independent variables. We used Bonferroni correction to control for multiple comparisons (a total of 13 variables were introduced; thus, the p-value threshold was set at 0.05/13 = 0.003). Multiple linear regression using stepwise methods was used to assess the relationship between cognitive scores and several potentially confounding factors related to sociodemographic and clinical characteristics (sex, age, years of education, years of illness, drug equivalents, PAS, HAM-A, HAM-D and CGI) as independent variables. We used Bonferroni correction to control for multiple comparisons (a total of 10 variables were introduced; thus, the p-value threshold was set at 0.05/10 = 0.005). Demographic differences between the groups were tested using t tests or $\chi 2$ tests for continuous and categorical variables, respectively. Student's t or Mann-Whitney U tests were performed on each cognitive measure to identify differences between diagnostic groups. Bonferroni corrections for multiple patient-control group comparisons were applied to analyses of cognitive measures, such that only group comparisons with $p < 0.008$ (0.05/6) for Student's t and $p < 0.01$ (0.05/5) for Mann-Whitney were considered significant. Linear regression using cognitive scores as the independent variable was performed to assess the strength of the relationships among cognition, levels of ILs, the kyn/tryp ratio, and possible cofounders, such as age, educational level and medications (a total of 8 variables were introduced; thus, the p-value threshold was set at 0.05/8 = 0.006). Furthermore, linear regression using kyn and tryp as the dependent variables was performed to assess the strength of the relationships among kyn and tryp expression and IL levels.

3. Results

3.1. Relationship of cytokines and the kyn/tryp ratio to demographic factors

The patient and control groups were not significantly different in educational level (Table 1). However, there was a significant difference between groups in the kyn/tryp ratio (t (2.75) = 95.9; p = 0.007). In addition, IL-1β levels also showed a significant difference between groups (U = 1798; p = 0.017), but this difference did not survive multiple testing corrections. There were no significant associations of cytokine levels and the kyn/tryp ratio with age, sex, years of education, BMI, physical activity, ethnicity, age of onset, fluoxetine or diazepam equivalent dose, PAS, HAM-A, HAM-D or CGI scores.

Table 1
Demographics of study participants.

Demographic	PD (n = 78)	Controls (n = 78)	Difference
Education in years (range)	13.24	12.57	ns
BMI (mean and s.d.)	22.92 (2.89)	23.04 (3.22)	ns
Physical exercise	44 N:34 Y	40 N:38 Y	ns
Age of onset (in years and range)	27.07 (17–46)		
PAS	26.30 (7.1)		
CGI	3.3 (0.69)		
HAM-A	24.25 (13.9)		
HAM-D	5.64 (4.07)		
Antidepressants and benzodiazepines (frequency in total cohort)	Antidepressants (100%) Benzodiazepines (11,5%)		

Abbreviations: ns, not significant; BMI, body mass index; PAS, Panic and Agoraphobia Scale; CGI, Clinical Global Impression Scale; HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale.

3.2. Relationship of cognitive scores to demographic and clinical factors

The multiple regression model showed that educational level and sex were associated with the RAVLT learning score (adjusted $R^2 = 0.83$, F = 16.66, β = 0.91, p = 0.001 and β = 0.69, p = 0.004, respectively) in PD patients. Although age was also associated with RAVLT learning scores (β = −0.36, p = 0.035), this model did not survive multiple test corrections. Years of illness predicted delta Trail Making Test scores (adjusted $R^2 = 0.43$, F = 7.93; β = −0.70, p = 0.023), and HAM-D scores were associated with the digit span backward score (adjusted $R^2 = 0.50$, F = 10.23; β = 0.74, p = 0.013). However, none of these models survived Bonferroni's correction.

3.3. Relationships among kyn, tryp and cytokines

Since cytokines, such as IL-10, IL-1β and IL-2R, increase the activity of IDO, a key enzyme for tryptophan-to-kynurenine conversion, we evaluated the relationships among kyn and tryp expression and cytokines levels. The multiple regression model showed that IL-2R levels explained 7.5% of the variance in kyn levels (adjusted $R^2 = 0.075$, F = 5.058; β = 0.306, p = 0.029) in PD patients; however, IL-2R levels did not predict kyn levels in controls. Tryp was not significantly correlated with any of the cytokines measured in either group examined independently.

3.4. Relationships between the kyn/tryp ratio and cognitive scores

Performance on the majority of cognitive tests was significantly lower in PD patients than in controls (for details see Table 2 and supplementary Table 2 on Supplementary materials). There was a significant difference between PD patients and the control group in digit span forward and total (t (134) = 2.63; p = 0.009; t (135) = 3.0; p = 0.003, respectively), RAVLT learning (t (128) = −5.3; $p < 0.0001$), RAVLT forgetting (t (128) = -7.4; $p < 0.0001$), RAVLT immediate (U = 17; $p < 0.0001$), TMT BA (U = 938, $p < 0.0001$), Stroop A (U = 829; $p < 0.0001$), and Stroop B (U = 1484.5; $p < 0.0001$) scores. The differences in digit span backwards and Stroop C scores were not significantly different between groups after multiple testing correction.

In individuals with PD, an elevated kyn/tryp ratio significantly predicted poor subdigit forward scores (adjusted $R^2 = 0.672$, F = 16.38; β = -0.82; p = 0.004) and subdigit total scores (adjusted $R^2 = 0.673$, F = 15.404; β = -0.811; p = 0.004), which remained statistically significant after Bonferroni's correction (Fig. 2). An elevated blood kyn/tryp ratio was also a biomarker of low Stroop B and C scores (adjusted $R^2 = 0.438$, F = 8.00; β = 0.70; p = 0.022; adjusted $R^2 = 0.389$, F = 6.72; β = 0.67; p = 0.032, respectively). However, this model did not remain statistically significant after multiple testing correction (Table 3). In addition, Stroop A scores were predicted by IL-2R levels (adjusted $R^2 = 0.400$, F = 6.99; β = -0.68; p = 0.030), and RAVLT learning was predicted by educational levels (adjusted $R^2 = 0.360$, F = 6.06; β = 0.657; p = 0.039). Nevertheless, none of these models remained statistically significant after Bonferroni's correction. No significant relationships were found through regression analysis between the kyn/tryp ratio and cognitive scores in healthy controls.

4. Discussion

Our study found evidence that patients with PD presented an elevated peripheral kyn/tryp ratio. An elevated peripheral kyn/tryp ratio

Table 2
PD patients performed worse than controls in short-term and episodic memory, selective attention and cognitive flexibility.

Cognitive domains	Cognitive tests	Patients (mean (sd))	Controls (mean (sd))	p-value
Short-term memory	Digit-span total	12.0 (4.2)	14.06 (3.58)	$p = 0.003$
Selective attention	Stroop B	21.49 (4.72)	18.42 (4.02)	$p < 0.0001$
	Stroop C	32.47 (9.47)	33.92 (9.60)	$p = 0.45$
Cognitive flexibility	TMT B-A	70.63 (12.62)	49.60 (42.78)	$p < 0.0001$
Episodic memory	RAVLT Learning	3.61 (2.51)	6.66 (3.62)	$p < 0.0001$

Abbreviation: TMT B-A, Trail making Test B minus A; RAVLT, Rey's Auditory Verbal Learning Test; sd, standard deviation.
Stroop C score did not remain statistically significant after multiple testing correction.

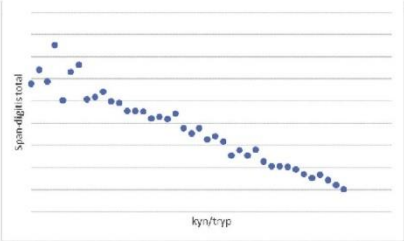

Fig. 2. Increases in kyn/tryp ratio predict decreases in span-digits total score in individuals with PD ($\beta = -0.811$, $p = 0.004$).

Table 3
Relationship between the kyn/tryp ratio and cognitive measurements in PD patients.

Cognitive test	β	Adjusted R Squared	p value
Subdigits total	- .811	.673	.004
Subdigits forward	- .82	.672	.004
Stroop B	.70	.438	.022
Stroop C	.67	.389	.032

kyn/tryp ratio, dependent variable. Associations between kyn/tryp and Stroop B and C tests did not remain statistically significant after multiple testing correction.

has been associated with cognitive deficits in a variety of mental disorders (Birner et al., 2017; Schwarcz et al., 2012). In PD, this increase in kyn metabolism was associated with poor short-term verbal memory. In addition, IL-2R levels predicted kyn levels in PD patients but not in controls. These observations may be indicative of abnormal peripheral IDO activation in PD patients, since cytokines, such as IL-2R, increase the activity of IDO, a key enzyme for kyn conversion.

In the current study, a high peripheral kyn/tryp ratio in PD patients but not healthy subjects was correlated with cognitive deficits, namely, low short-term verbal memory. Deficits in short-term memory, the ability to keep a small amount of information available for a short period of time, have been related to PD (Alves et al., 2013; Gordeev, 2008; Palomares Castillo et al., 2010) and might be associated with glia cells (Azevedo et al., 2013; Li et al., 2014). Activated microglia mediate synapse loss and short-term memory deficits in animal models (Azevedo et al., 2013). Furthermore, although the hippocampus has long been thought to be exclusively involved in long-term memory processes (Scoville and Milner, 1957; Squire et al., 1993), several studies have raised doubt on this traditional view by emphasizing its role within an integrated operational network that also covers short-term memory functions (Beyer et al., 2013; Finke et al., 2008; Henke, 2010; Nee and Jonides, 2008; von Allmen et al., 2014; von Allmen et al., 2013). In the hippocampal region of the central nervous system, the birth of new neurons occurs throughout life, and the amount of neurogenesis correlates closely with the hippocampal functions of learning and memory (Monje et al., 2003). Impaired adult neurogenesis is associated with short-term memory deficits (Denis-Donini et al., 2008). Proinflammatory cytokines have been shown to decrease adult hippocampal neurogenesis possibly through the KP (Zunszain et al., 2012). In the hippocampus, peripheral inflammation induces brain region-dependent changes in the balance of kyn metabolites, favoring neurotoxic metabolite production (Parrott et al., 2016). Specifically, microglia in the hippocampus are more responsive to proinflammatory stimuli, earning them the label 'immunovigilant' (Parrott et al., 2016). This hyperresponsive state of hippocampal microglia may underlie the region-specific elevation in neurotoxic kyn metabolism (Parrott et al., 2016). Therefore, metabolites of the KP and activation of glial cells could contribute to the observed deficits in short-term memory in PD (Fig. 3).

Cognitive deficits described in PD patients might also be related to hippocampal pattern separation (Hu and Dolcos, 2017). Hippocampal pattern separation is the process of encoding details of an environment in an intention to allow individuals to distinguish between similar memories (Lange et al., 2017), and is the resultant ability to resolve memory interference, or to discriminate previously encoded stimuli from highly similar stimuli (Bernstein and McNally, 2018; Lange et al., 2017). Pattern separation is related to the neuron suppression of the excitability of the dentate gyrus so that stimuli trigger unique patterns of activation in this region, decreasing the likelihood that two stimuli activate overlapping representations (Bernstein and McNally, 2018). Without this inhibitory action, two cues may be encoded as insufficiently distinct, and consequently interpreted as overly similar (Bernstein and McNally, 2018). Impaired pattern separation may be a risk factor for PD (Lange et al., 2017). Indeed, patterns of encoding ambiguous cues as more threatening could influence cognitive measurements and might be associate to a deficient pattern separation (Bernstein and McNally, 2018). Thus, it is possible that a non-functional behavioral pattern separation could be underlying the cognitive deficits demonstrated in PD patients.

Although PD patients performed worse than controls in almost all cognitive tests, not all cognitive measures were associated with an increase in kyn metabolism, which may be explained by the differential

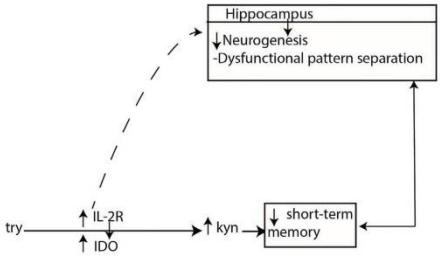

Fig. 3. IL-2R increases the activity of IDO, a key enzyme for kyn conversion, therefore contributing to an increase in kyn. This increase in kyn is associated with poor short-term verbal memory in PD patients. Poor short-term memory might be related to reduced hippocampal neurogenesis and dysfunctional hippocampal pattern separation.

expression of functional markers in microglia across brain regions (Chhor et al., 2013). Kyn metabolism may also differ across brain regions (Parrott et al., 2016). Microglial phenotypes are shaped by the local environment (Grabert et al., 2016), and the microenvironment is not uniform throughout the brain (Grabert et al., 2016). Variations in neuronal subtypes, neurotransmitter profiles, hemodynamics and metabolism could all influence and be influenced by the local microglial phenotype (Grabert et al., 2016; Quagliato et al., 2018).

There are several potential confounding factors common to case-control studies of PD that are relevant to the present study. The correlations between the kyn/tryp ratio and cognitive function may not be directly related in a causal manner, but, rather, both may change as a consequence of other factors. A worse performance on cognitive tests, for instance, could be related to the chronicity and severity of an illness, educational levels of a patient or even medication treatments. In the current study, medication treatments were not associated with cognitive deficits. However, this must be interpreted with caution since the small PD population may have contributed to this matter. Thus, since benzodiazepines are associated with cognitive deficits in specific populations (Nardi et al., 2018), the fact that some PD patients were on benzodiazepines medications, could have influenced memory scores tests of PD patients. The current data showed that only RAVLT learning scores in PD patients were associated with educational levels and sex, and other demographic and clinical factors were not related to any cognitive scores. One important potential confound is that all of the individuals with PD in our study were receiving antidepressants. Recent studies of cytokine measures have suggested that antidepressants may result in decreases in peripheral cytokine levels (Wiedlocha et al., 2018). Because all patients in the current study were on antidepressants, the antiinflammatory effects of these drugs cannot be ruled out. PD patients may display an infiammatory profile if not on antidepressants. Regarding the KP, different antidepressants exert a variety of effects on kyn and tryp levels (Reus et al., 2015). Treatment with selective serotonin reuptake inhibitor (SSRI) antidepressants inhibits IDO, while treatment with tricyclic antidepressants, such as imipramine, decreases the kyn/tryp ratio (Reus et al., 2015). As exposure to antidepressants could suppress IDO and therefore decrease the kyn/tryp ratio, one would expect more subjects with an altered KP profile in an unmedicated sample; therefore, exposure to antidepressants is unlikely to explain our findings of many PD patients presenting an elevated kyn/tryp ratio. There are several other limitations to the current study; recent research has indicated that aerobic exercise can reduce kyn levels in humans (Schlittler et al., 2016). Although we did account for levels of physical exercise, which were not significantly different between patients and controls (Table 1), we did not control for exercise type, which could have interfered with the results. Another potentially relevant limitation is that up to 80% of kyn in the plasma appears to be bound to albumin or other circulating binding proteins (Fukui et al., 1991). As only total plasma levels of this metabolite were measured in the present study, we may have missed a higher group difference caused by the possible differential availability of free kyn, which can readily penetrate the blood-brain barrier and then function as a highly effective bioprecursor of KYNA and QUIN within the brain (Schwarcz et al., 2012).

The challenges of determining to what extent blood biomarkers vary across the course of the illness and in response to factors such as symptom status emphasize the importance of studying the relationship among brain structural changes, clinical features and blood biomarkers of the KP and inflammation using a longitudinal design that includes subjects after first-episode panic attacks and in acute relapses. Future studies should also systematically obtain data on lifestyle factors to aid in the interpretation of the potentially elevated kyn/tryp ratio. An interesting question raised by our study is the extent to which the elevated peripheral kyn/tryp ratio reported here is indicative of the elevated brain kyn, KYNA and QUIN levels found in postmortem samples of PD patients. The main boundary between peripheral circulation and the brain is the blood-brain barrier (Schwarcz et al., 2012). Increased kyn can cross the blood-brain barrier, be converted to QUIN and KYNA and lead to neurotoxicity, potentially resulting in alterations in brain morphology (Schwarcz et al., 2012). Therefore, further in vivo and postmortem research into a possible KP alteration-based mechanism could contribute to the pathophysiology of PD. Furthermore, because KP enzymes are mostly localized in glia cells (Schwarcz et al., 2012), the observed impaired short-term auditory memory related to the increase in the kyn/tryp ratio suggests that targeted treatment of individuals with PD displaying the elevated kyn biomarker with inhibitory KP agents may be beneficial for short-term memory deficits. Nevertheless, very few therapeutic agents specifically targeting glia cells and the KP have entered clinical studies (Biber et al., 2016), likely due to several unresolved issues, including challenges related to glia target specificity, central nervous system penetrance and the profound differences between mouse and human glia (Biber et al., 2016).

This study is the first to link blood biomarkers of infiammation and KP with cognitive deficits in people with PD. Independent replication of these findings would support further clinical trials of inhibitory KP drugs in PD patients presenting poor cognitive function, which could lead to effective novel treatments for people with PD. Importantly, future studies might focus on an estimation of the kyn/tryp ratio in parallel with a determination of other immune factors to confirm the involvement of IDO activity in deregulated tryp breakdown. These might be a valid tool to monitor the progression of cognitive deficits and has great potential to be applied to check treatments and estimate the necessity of psychiatric interventions before aggravation of symptoms, thus serving to personalize therapeutic strategies.

Conflicts of interest

The authors have no conflict of interest to disclose.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jpsychires.2019.03.027.

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2.3. A via da quinurenina e a microglia

Enzimas da via da quinurenina localizam-se predominantemente em células gliais. A microglia, em sua conformação ativada, produz citocinas inflamatórias e contribui para uma elevação da taxa quinurenina/triptofano. Isso nos leva a questão: O que faz com que a microglia saia da sua morfologia ramificada e passe a exibir uma configuração ameboide/ativa?

Evidências indicam que mudanças na morfologia da microglia dependem de que essas células detectem alterações no meio ambiente por meio da extensão e retração repetidas de seus processos microgliais (Nimmerjahn *et al.*, 2005). A microglia interage com seu meio ambiente com o auxílio de um conjunto de transportadores e canais iônicos (Schilling e Eder, 2007). A variedade de canais expressos pela microglia mostra padrões espaço-temporais complexos de acordo com as mudanças no ambiente das células imunes, o que pode contribuir para os diferentes fenótipos expressos pela mesma (Kettenmann *et al.*, 2011).

No artigo 3 discorremos sobre como os canais iônicos podem auxiliar na mudança da conformação da microglia e hipotetizamos de que modo isso poderia estar implicado nos mais variados distúrbios psiquiátricos e neurológicos.

2.3.1. Artigo 3: The role of convergent ion channel pathways in microglial phenotypes: a systematic review of the implications for neurological and psychiatric disorders. *Translational psychiatry*, 2018, 8.1: 259.

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Translational Psychiatry

REVIEW ARTICLE Open Access

The role of convergent ion channel pathways in microglial phenotypes: a systematic review of the implications for neurological and psychiatric disorders

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Abstract

Increases in the activated state of microglia, the main neuroimmune cells, are widely reported in the brains of patients with neurological and psychiatric disorders. Microglia transform from the resting to the activated state by sensing their environment, aided by a variety of ion channels. To examine the effect of ion channels on microglial phenotypes, we conducted a systematic review of immunohistochemical analyses of these neuroimmune cells in animal models following administration of ion channel antagonists, compared to control conditions. A systematic search of the PubMed and Web of Science electronic databases using the PRISMA and WHO methodologies for systematic reviews yielded 15 original peer-reviewed studies. The majority (13 out of 15) of these studies reported a decrease in microglial activated state after ion signaling pharmacological blockade. The studies provide evidence that acute administration of ion channel antagonists leads to a reduction in microglial activation in rodent brains in the models for epilepsy, Parkinson's disease, inflammation, pain, ischemia, and brain and spinal cord injury. Future research should explore microglial-specific druggable targets for neurological and psychiatric disorders. The investigation of acute and chronic administration of ion channel antagonists in microglial phenotypes in primates and the development of microglia-like cells derived from human stem cells could be valuable sources in this direction.

Introduction

Microglia, the resident immune cells of the central nervous system (CNS), can differentiate into distinct phenotypes, including resting and activated cells[1]. In vivo resting microglial cells exhibit a ramified morphology, characterized by several branched processes arising from an elongated and flattened cell body. Microglial cells undergo shape changes following activation, i.e., cells transform from ramified to ameboid morphology in response to neuronal injury and during inflammation or infection[1]. Activated microglia are capable of proliferation, migration, and antigen presentation and release a variety of substances that can be either neuroprotective or neurotoxic[1].

Evidence indicates that changes in microglial morphology depend on the cells sensing the environment by repeatedly extending and retracting their processes, but the factors regulating microglial surveillance are unknown[2]. Microglia interact with their environment with the aid of a complicated ensemble of transporters and ion channels[3]. The latter include purinergic metabotropic P2Y receptors and ionotropic P2X receptors, the transient receptor potential (TRP) channels such as TRPC6, and the K^+ channels kir 2.1, KV 1.3 and KCa 3.1[1,4]. The variety of channels expressed by microglia shows complex spatiotemporal patterns according to changes in the immune cells' microenvironment, which may contribute to the different phenotypes expressed by

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microglia[5]. Furthermore, these various molecules play different roles in microglial function. For instance, K^+ channels such as kir, KV, and KCa act by regulating microglial membrane potential[6]. TRP channels are associated with microglial activation[1,7], and the purinergic receptors P2X and P2Y are related to microglial surveillance and phagocytic activity[8]. Additionally, all the channels may maintain the resting potential of microglia and thus contribute to microglial ramification and continuous surveillance of the brain via process motility[1].

In recent decades, an increasingly compelling body of evidence has emerged linking microglial activation to neurological and psychiatric disorders. Broadly, this evidence stems from the observations that microglial surveillance plays an important role in monitoring synaptic function and determining brain connectivity[9,10]. During postnatal development, synapses that are to be pruned become tagged with complement molecules and are thus removed by microglia[11]. Disruption of this system may lead to altered CNS connectivity, generating excess excitatory synapses that may be involved in the pathogenesis of various disorders, such as epilepsy[12] and autism[13]. Furthermore, microglial cells release different molecules that are potentially implicated in an excitatory-inhibitory imbalance, which may also contribute to the pathogenesis of psychiatric and neurological disorders[14]. Based on the above, we review the evidence of current preclinical literature on ion signaling in microglial phenotypes, providing evidence for the role of ion channels in microglial state and identifying gaps in the literature to inform future research.

Our primary outcome

- Does ion channel pharmacological blockade modify microglial phenotype?

Our secondary outcomes

- How does interaction between microglial ion channels occur?
- Can ion signaling contribute to the development of neurological and psychiatric disorders?

Materials and methods

The systematic search was conducted in PubMed and Web of Science, covering articles published up to 31 December 2017. The search protocol was developed based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and World Health Organization (WHO) Review Protocol Template Guidelines where applicable for this systematic review, as provided in Supplementary Materials Section 1. We also manually checked references cited in the systematically searched articles. To avoid publication bias, non-english language studies and gray literature (for example, conference abstracts) were included. A broad but highly structured search strategy was used, based on the PICOS framework. The study population was microglia, the intervention/exposure was ionic channel antagonism, comparison was with absence of ion channel blockade, outcome was resting versus activated microglia, and study design included any type of design. Keywords for the search included various combinations of terms for microglia and the nervous system, including both historical and contemporary ion channel names. A full list of terms used for the search strategy can be found in Supplementary Materials Section 2.

Study selection

Studies were selected for data extraction and analysis based on the following inclusion criteria: (1) ion channel antagonists administered in vivo and (2) studies evaluating microglial activated or resting state. Exclusion criteria were (1) studies that lacked a baseline condition or control group, (2) studies that did not report original data, (3) studies without immunohistochemical analysis of microglia, (4) studies evaluating genetic ionic channel deficiencies, (5) studies that investigated only in vitro ion channel blockade. Due to the highly reactive nature of microglia, which substantially alters their morphology and functional properties when exposed to culture conditions[15], in vitro studies should be interpreted with caution.

Data extraction

A standard data extraction template adapted from the Cochrane checklist of items (Supplementary Materials Section 3) was used. As the type of outcome reporting was extremely heterogeneous, results were reported as higher, lower, or unchanged for ion channel antagonism relative to control conditions as identified. Meta-analyses and other summary statistics were not used because of the wide variation between studies in assessment techniques and brain regions examined.

Quality assessment

This systematic review was performed according to preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines[16] (Supplementary Materials Section 4). Quality assessment used SYRCLES Bias Tool[17] (Supplementary Materials Section 5).

Results

The literature search identified 6362 potentially relevant articles for initial screening. Duplicates ($N = 1475$) were identified using a function in Endnote and confirmed by manual screening of the titles. A total of 4605 studies were

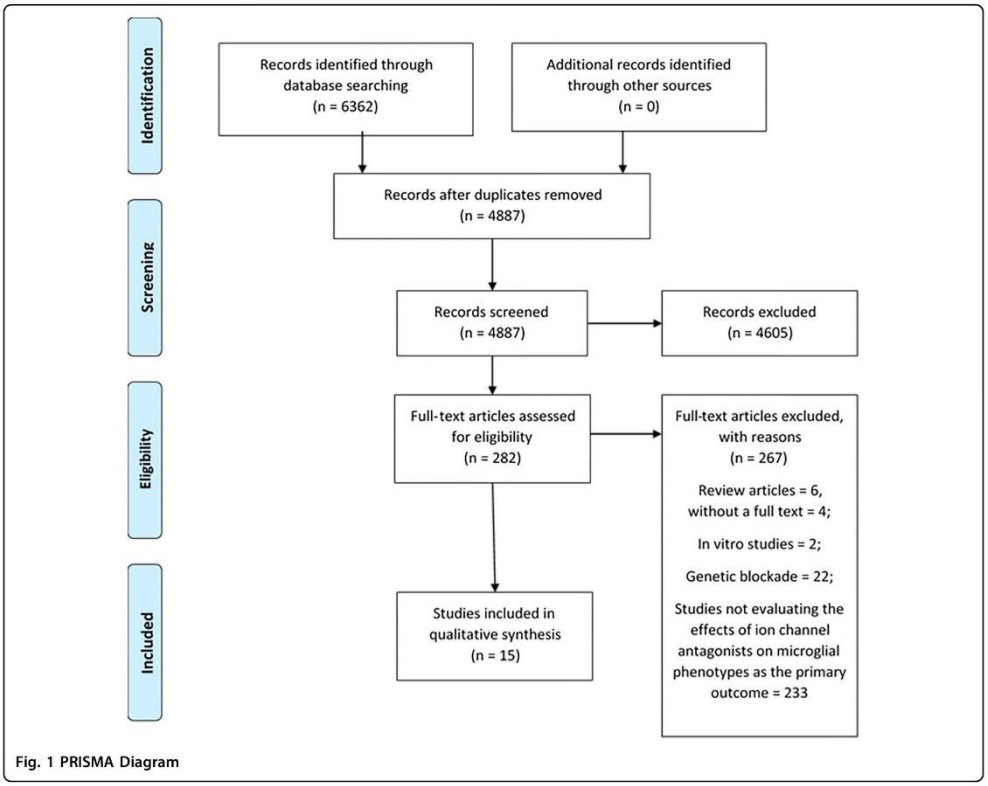

Fig. 1 PRISMA Diagram

excluded from initial assessment of titles and abstracts. In all, 282 abstracts were classified for possible inclusion, and their full texts were obtained. In all, 267 papers were excluded from further analysis. In all, 282 full texts were reviewed, of which 15 met the inclusion criteria for our systematic review (see Fig. 1 for a PRISMA diagram of the literature search). Table 1 summarizes the included studies. Additional data regarding the included studies can be found in Supplementary Materials Section 6. Reasons for the exclusion of studies can be found in Supplementary Material Section 7.

Microglial ion channel antagonists in epilepsy animal models

Three studies assessed the effect of ion signaling blockade on microglial phenotypes in animal models for epilepsy. Two studies evaluated P2X7[18,19], while only one investigated TRPC6[20]. These studies were performed on the hippocampus of corialactone and pilocarpine seizure models and showed an increase in microglial channels after induction of status epilepticus. Furthermore, all of the studies demonstrated that P2X7 and TRPC6 antagonism were related to a decrease in microglial activated state.

Microglial ion channel antagonists in ischemic animal models

The majority of studies (4 out of 6) on ischemic models associated a decrease in microglial activation with purinergic channel blockade. Of these, three evaluated P2X7 channels[21–23], and two of the three demonstrated a reduction in microglial activation[21,22]. Although the investigation of reactive blue 2 (RB2) in P2X7 blockade showed an increase in reactive microglia, a protective effect against the severity of post ischemic neurological impairment was seen in RB2-treated rats[23]. Studies evaluating P2X4 and P2Y12 antagonists also demonstrated a reduction in the activated state of CNS-resident immune cells[24,25].

Table 1 Studies of microglial phenotypes following administration of ion channels antagonists

Condition studied	Receptor	Study	Animal model	*n*	Antagonist/ deficiency	Duration of intervention	Intervention dose	Timepoint for outcome measurement	Immunoreactivity	Region of interest	Change in ion/ neurotransmitter channel measure after injury/lesion	Change in microglial activation after antagonist infusion compared to control condition
Epilepsy	P2X7	Choi et al. 2012[18]	Pilocarpine	30 × 37	OxATP	1 week	0,5 mmL/h	1, 4, and 8 weeks after SE	Iba-1	Hippocampus	na	↓
		Huang et al. 2017[19]	Corialactone	30/group	BBG; A-438079 and A-740003	30 min prior to and 60 min post injection	BBG: 1, 5, 10 μg; A-438079 and A-740003: 10 μG	0, 8, 24, 2 days, 4 days, 1 week, 2 weeks after SE	Iba-1	Hippocampus	↑	↓
	TRPC6	Lee et al. 2014[20]	Pilocarpine	12/group	Hyperforin	1 week	6 μM	3 days	Iba-1	Piriform cortex	↑	↓
Ischemia	P2X7	Yu et al. 2013[21]	Four-vessel occlusion method	5/group	BBG and A-740003	3 days	BBG: 50 mg/Kg and A-740003: 100 mml/Kg	After 12 h, 24 h, 48 h, 4 days, 7 days of I/R injury	IB4	Hippocampus	↑	↓
		Chu et al. 2012[22]	Four-vessel occlusion method	4/group	BBG and OxATP and A-438079	Right before cerebral injury	BBG 10 μg, OxATP 1 μg, A-438079 3 μg	After 3 days of reperfusion	Iba-1	Hippocampus	na	↓
		Melani et al. 2006[23]	MCAo	14/group	RB2	5 min after sham operation or MCAo	100 mg/Kg	24 h after surgical procedures	OX-42	Striatum, hippocampus	↑	↑
	P2X4	Wixey et al. 2009[24]	Hypoxia–ischemia model	8 × 12	Minocycline	9 days	45 mg/kg i.p. 2 h post insult then 22.5 mg/ kg i.p. every 24 h until day 9	10 days after the insult	Iba-1	Corpus callosum, cingulum	↑	↓
	k-atp channel	Ortega et al. 2012[26]	MCAo	15/group	Glibenclamide	6, 12, and 24 h after reperfusion	0.06 or 0.6 or 6 mcg	72 h after MCAo	IB4	Subcortical and cortical region	↑	-----
	P2Y12	Gelosa et al. 2014[26]	MCAo	6/group	Ticagrelor	10 min, and then 22 and 36 h after MCAo	3 mg/Kg or 30 mg/Kg	2 h after MCAo	Iba-1 and ED-1	Dorsal and ventral areas	↑	↓
TBI	P2X7	Liu et al. 2017[28]	Modified weight drop technique	6/group	A804598	5 days	A804598: 10 mg/Kg	6,12, 24 h	Iba-1	Cortex	↑	↓
Spinal cord injury	P2X4	Zhou et al. 2014[29]	Method of Decosterd and Woolf	4/group	Dexmedetomidine	14 days	40 mcg/kg	1, 3, 5, 7, 14 h after surgery	Iba-1	Spinal cord	↑	↓
Pain	P2X7	He et al. 2012[30]	Chronic constriction injury model	7/group	BBG	14 days	10 mcl	14 days after nerve injury	OX-42	Spinal cord	↑	↓
Parkinson	P2X7	Wang et al. 2017[31]	LPS injected to the right substantia nigra	12 × 6	BBG	15 days	50 mg/ Kg	15 days	Iba-1	Substantia nigra	↑	↓
	kir2.1		LPS injection	4/group	E2	1 h before LPS			Iba-1		na	↓

Table 1 continued

Condition studied	Receptor	Study	Animal model	*n*	Antagonist/ deficiency	Duration of intervention	Intervention dose	Timepoint for outcome measurement	Immunoreactivity	Region of interest	Change in ion/ neurotransmitter channel measure after injury/lesion	Change in microglial activation after antagonist infusion compared to control condition
		Wu et al. 2016[32]					0.1 or 100 μg/ Kg	30 min before, together and 30 min after		Striatum, hippocampus and motor cortex		
Inflammation	P2X7	Choi et al. 2007[22]	LPS animal model for inflammation	5/group	oxATP	30 min before LPS injection	1 μ	1, 4, 8, 12, 24, 48 h	OX-42	Striatum	↑	↓

MCAo middle cerebral artery occlusion, *SE* status epilepticus, *I/R* ischemia/reperfusion, *LPS* lypopolysaccharide, *oxATP* oxidized ATP, *BBG* brilliant blue G, *RB2* reactive blue 2, *E2* estrogen, *Iba-1* ionized calcium binding adaptor molecule 1, *OX-42* ntegrin alpha M antibody, *IB4* isolectin IB4, *na* not available, ↑ increase,↓ decrease ------ no significant change

Regarding K^+ channels in ischemic models, it was shown that K^+ channel blockade by glibenclamide did not modify microglial state[26]. However, glibenclamide decreased neurological deficits and reduced neuronal death in preclinical studies[26].

Microglial ion channel antagonists and inflammation in animal models

One study assessed ion channel blockade and microglial cell function in an inflammatory model[27] and demonstrated a time-dependent increase in P2X7, to a maximum level at 12 h, after injection of lipopolysaccharide (LPS). Furthermore, P2X7 antagonism with oxATP pointed to a decrease in microglial activated state[27].

Animal models for microglial ion channel blockade in traumatic brain injury and spinal cord injury

There was only one study evaluating P2X7 blockade and microglial activation in traumatic brain injury (TBI)[28]. According to the study, purinergic antagonism attenuated microglial activated state and improved neurobehavioral outcomes after TBI. Moreover, P2X7 blockade decreased IL-1β expression and p38 phosphorylation, increasing the survival of neurons in the injured cerebral cortex[28].

In a study on P2X4 antagonism in microglia after spinal cord injury (SCI), Zhou et al. found that dexmedetomidine, a highly selective α2 adrenergic agonist with sedative properties, decreased microglial activation and reversed mechanical hyperalgesia[29]. In addition, spared nerve injury rats presented high levels of p38 and brain-derived neurotropic factor (BDNF) expression in the dorsal horn compared to controls, downregulated by dexmedetomidine treatment[29].

Microglial ion channel antagonists in pain models

One study assessed P2X7 blockade in microglial phenotype in preclinical pain models[30]. This channel expression was increased in the ipsilateral spinal cord after nerve injury. Temporal evolution in P2X7 levels in the dorsal horn of the spinal cord and the difference in P2X7 levels matched the emergence of mechanical allodynia and thermal hypersensitivity. The study showed that nerve injury-induced mechanical allodynia and thermal hypersensitivity was reversed by intrathecal administration of Brilliant Blue G (BBG)[30].

Microglial ion channel antagonists in animal models for Parkinson's disease

In animal models for Parkinson's disease, P2X7 and kir 2.1 blockade decreased microglial activation[31,32]. In rats treated with BBG, a P2X7 antagonist, p38-MAPK activation was reversed, microglial activation was attenuated, and a reduction in the loss-of-dopaminergic neurons was observed in the substantia nigra[31]. Increases in neuron

Box 1 Suggested future research directions

1. Further studies on primates to determine the effect of acute and chronic administration of ion channel antagonists on microglial function.
2. Studies comparing males and females to investigate potential gender differences.
3. Studies needed to investigate whether there are strain differences.
4. Studies to investigate the effect of acute and chronic ion channel blockade on neuronal firing and how it relates to measures of neurotransmitter release and synthesis capacity.
5. Studies to investigate correlations between microglial channels and different brain regions at different time points.
6. Studies to investigate correlations between microglial ion channel modulation by different types of drugs, such as antipsychotics, anti-epileptics, and antidepressants.
7. Development of microglia-like cells derived from human stem cells.

survival was also demonstrated by estrogenic blockade of kir 2.1. Estrogen incremented anti-apoptotic genes and pro-survival PI3K-Akt signaling[32].

Discussion

Our review shows that pharmacological blockade of ionic signaling in microglia in preclinical models for epilepsy, ischemia, Parkinson, pain, TBI, SCI, and inflammation modify microglial phenotypes when compared to controls, thereby decreasing microglial activation. There is evidence of reduced microglial activation following blockade of purinergic channels, such as P2X7, P2X4, and P2Y12[18,19,24,25,27–31]. Specifically, the majority (8 out of 9) of studies investigating P2X7 channels in preclinical models for epilepsy, ischemia, TBI, SCI, Parkinson, pain, and inflammation demonstrated a decrease in microglial activated state[18,19,21,22,27,28,30,31]. However, there was an increase in microglial activation in an ischemic animal model following P2X7 blockade[23]. Variations between studies in the range of doses of ion channel antagonists and the time elapsed from the last drug treatment to microglial evaluation may explain the differences between studies. All the P2X4 and P2Y12 studies showed a decline in immune cell activation after blockade of these channels (3 out of 3 studies)[24,25,29].

Studies with animal models for epilepsy and Parkinson's disease showed a reduction in microglial activated state after TRPC6 and kir 2.1 blockade[20,32]. However, in an ischemic animal model, K^+-channel antagonism with glibenclamide showed no differences in microglial state[26]. This variety in the results may occur since glibenclamide does not reach significant concentrations in the brain unless the plasma concentration is extremely high[33]. The drug binds to plasma proteins, which impairs its entry into the brain, as plasma proteins are unable to cross the blood–brain barrier[33]. Additionally, glibenclamide appears to be removed from the brain rapidly by a highly efficient efflux system when injected directly into the CNS[33].

Taken together, these findings indicate that ion channel blockade elicits a significant decrease in microglial activated state in preclinical models for epilepsy, ischemia, TBI, SCI, inflammation, Parkinson, and pain. Nevertheless, further in vivo studies are needed to determine the effects of ion channel antagonists on microglial function (see Box 1 for suggested future directions).

General methodological considerations

Importantly, despite substantial evidence that microglial dysfunction is part of the core pathology of many neurological and psychiatric disorders, evidence remains limited for a direct causal link between a specific and druggable target in microglial cells and a specific disease. Under physiological conditions, microglia reside in the brain parenchyma, so a drug targeting microglia needs to be able to enter the CNS[1,34]. In recent years, various ion channel antagonists have been developed as potential drugs. However, most of these compounds were not designed to enter the CNS and would therefore lack the properties to be effective in CNS disorders mediated by microglial channels[34].

Additionally, microglial cells are highly plastic. Therefore, it is not always clear whether the molecule targeted by a drug is indeed expressed by the microglia[34]. For example, various P2 receptors have been suggested as drug targets in microglia. However, the expression pattern of P2 receptors strongly depends on the cell phenotype[35]. For example, microglia may lose P2Y12 receptors in the disease state, whereas P2X4 and P2X7 expression is often increased in microglia in disease[35–37]. Thus, the spatial-temporal effect of microglial channel antagonists still needs to be tested directly (Box 1).

General limitations of the selected studies were that the strain- and sex-specific effects of acute and repeated ion channel blockade on microglial function have still not been tested directly (Box 1). There is evidence for sexual dimorphism in microglial function and a sex bias in CNS disorders with microglial pathology[37].

In an attempt to produce more homogeneous outcomes and better understand how pharmacological blockade of microglial ion channels alters microglial state, our search strategy only included studies that evaluated microglia in the activated state (ameboid morphology) or resting state

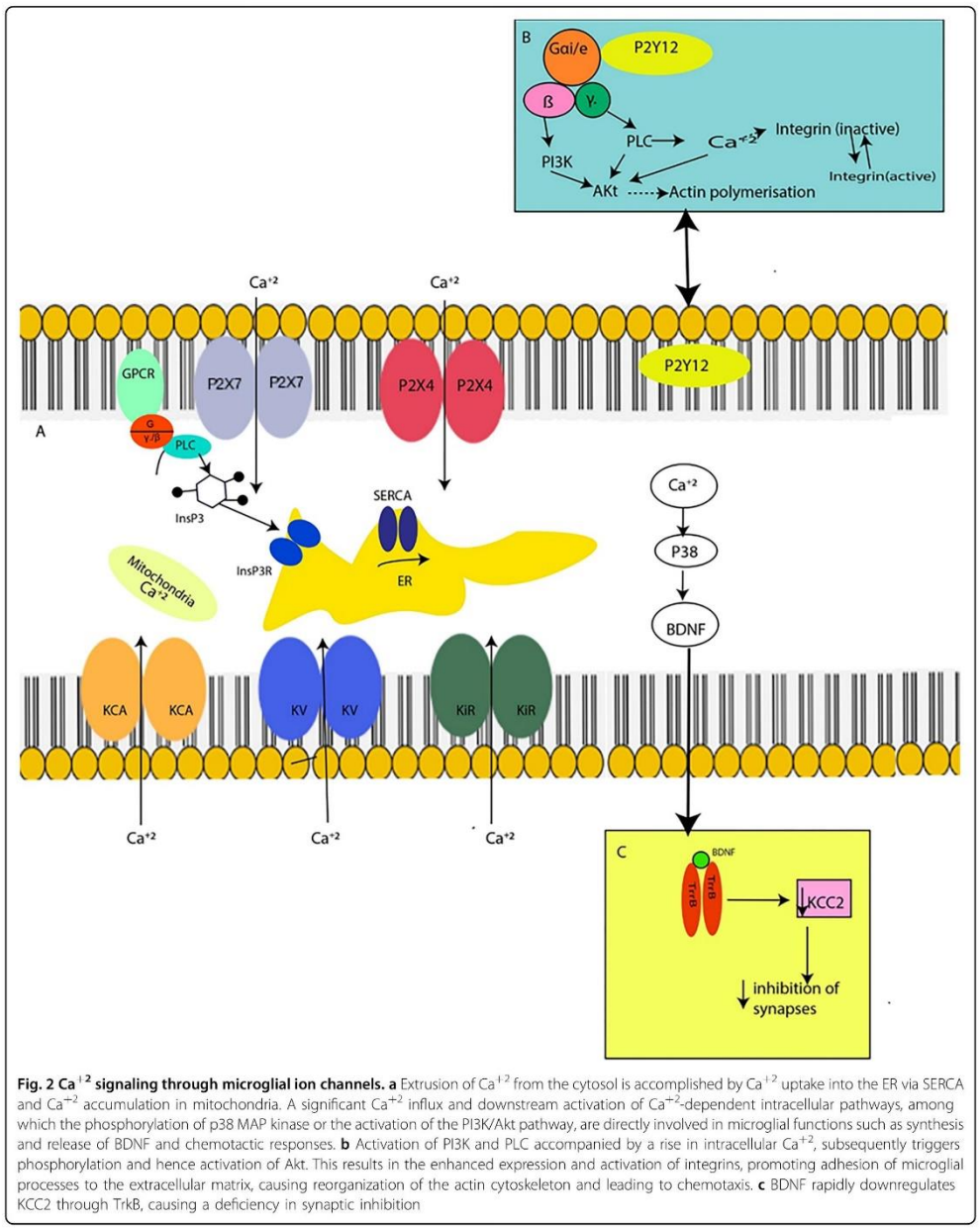

Fig. 2 Ca^{+2} signaling through microglial ion channels. a Extrusion of Ca^{+2} from the cytosol is accomplished by Ca^{+2} uptake into the ER via SERCA and Ca^{+2} accumulation in mitochondria. A significant Ca^{+2} influx and downstream activation of Ca^{+2}-dependent intracellular pathways, among which the phosphorylation of p38 MAP kinase or the activation of the PI3K/Akt pathway, are directly involved in microglial functions such as synthesis and release of BDNF and chemotactic responses. **b** Activation of PI3K and PLC accompanied by a rise in intracellular Ca^{+2}, subsequently triggers phosphorylation and hence activation of Akt. This results in the enhanced expression and activation of integrins, promoting adhesion of microglial processes to the extracellular matrix, causing reorganization of the actin cytoskeleton and leading to chemotaxis. **c** BDNF rapidly downregulates KCC2 through TrkB, causing a deficiency in synaptic inhibition

(ramified morphology) as evidenced by microglial immunohistochemical analysis. Moreover, studies that evaluated only in vitro ion channel blockade were also excluded. This is justified due to the highly reactive nature of microglia, which substantially alters their morphology and functional properties when exposed to culture conditions[15]. The two restrictions significantly reduced the number of studies included in this review.

Our review also included different types of microglial ion channels. Although we found that ion channel blockade was associated with a decrease in microglial activated state in 13 of 15 studies, caution is necessary when interpreting this result. Importantly, microglia have different types of ion channels that play a variety of roles[1]. It is thus plausible that some ion channel antagonists decrease microglial function while others increase it. Our search criteria yielded studies showing that P2X7, P2X4, P2Y12, kir 2.1, and TRPC6 blockade were related to a decrease in microglial activated state. However, future studies should be performed on different types of ion channels and their association with microglial phenotypes. Furthermore, although our review demonstrated that ionic signaling blockade decreases microglial activation, the majority of studies were conducted in rodent models. Thus, extrapolation of microglial ion channel modulation to primates should be treated with caution, and further studies are needed (Box 1).

Hypothesis on the effects of ion channel antagonists on microglial function

The underlying mechanism of ion channel action in microglia remains to be fully established. However, several lines of evidence indicate that the mechanism involves Ca^{+2} signaling[38–40]. Microglial activation occurs via a cascade of extracellular and intracellular signaling events beginning with cell surface receptor and ligand-gated ion channel activation followed by short-term and long-term changes in intracellular Ca^{+2}[40]. These Ca^{+2} signals can cause release of other factors in autocrine-paracrine feedback loops and are necessary for various subsequent cellular responses in microglia[39,40] (Fig. 2).

Microglia contain at least two types of intracellular Ca^{+2} stores: the mitochondria and the endoplasmic reticulum (ER)[1]. The main pathway for generation of intracellular Ca^{+2} signaling is associated with inositol 1,4,5-trisphosphate (InsP3) receptors on the ER membrane[1,40]. Stimulation of G protein-coupled metabotropic receptors results in the activation of phospholipase C (PLC), production of two secondary messengers, including diacylglycerol and InsP3, and Ca^{+2} release from the ER[1]. Importantly, ER depletion activates store-operated Ca^{+2} entry (SOCE), known as a capacitive Ca^{+2} influx, mediated by plasmalemmal channels such as calcium release-activated Ca^{+2} (CRAC) channels and/or transient receptor potential (TRP) channels[1]. In addition, STIM1, one of the ER membrane proteins, senses the filling state of ER Ca^{+2} and delivers the ER to the plasma membrane, where it directly activates Orai1/CRAC channels, thereby facilitating the reuptake of Ca^{+2} to ER through the endoplasmic reticulum Ca^{+2}-ATPases (SERCA). Ca^{+2} concentration in the ER is precisely controlled by SERCA[1]. The influx of Ca^{+2} through ion channels plays an important role in many inflammatory processes, including microglial activation[40].

In response to an injury, various extracellular signals may activate microglia and upregulate ion channels. For instance, proinflammatory cytokines upregulate kir, TRPC6, and KCa expression, which may cause hyperpolarization of microglial cells[41]. Additionally, not only cytokines but also ATP can cause an increase in purinergic receptors, which are highly Ca^{+2} permeable[1]. A significant Ca^{+2} influx and the downstream activation of Ca^{+2}-dependent intracellular pathways, among which the phosphorylation of p38 MAP kinase or the activation of the PI3K/Akt pathway, are directly involved in microglial functions such as synthesis and release of BDNF and chemotactic responses[1].

Under pathological conditions, one of the first activities at the injury site is ATP release, activating microglia[40]. ATP binds to P2X7, a "sensor of danger", driving resting microglial cells into the activated form, forming large pores and allowing Ca^{+2} entry[40]. Ca^{+2} entry is also increased by the inward rectifier K currents (ikir), an early marker for activated microglia[4]. Furthermore, extracellular ATP induces membrane ruffling and microglial chemotaxis mediated by the Gi/o protein-coupled P2Y12 receptor[1]. P2Y12 receptor-mediated activation of the PI3K pathway and increased Akt phosphorylation are required for microglial chemotaxis in response to ATP[1].

Microglia exert phagocytic activity at the site of injury. Microglial phagocytosis can initiate respiratory burst, a key function of activated microglia that produces toxic reactive oxygen species[42]. The K^+-channels are involved in controlling microglial respiratory burst[42], whereas P2X7 channels are associated with NADPH-oxidase and consequently with oxidative stress[1]. Phagocytic activity of microglial cells is felt to be involved in synapse removal during development and potentially in pruning synapses in the postnatal brain, which is associated with different psychiatric and neurological disorders[9,11]. Microglial phagocytosis is intimately associated with cytokine modulation and may contribute to synaptic plasticity[43].

Microglial action on synaptic plasticity

Microglia may regulate synaptic plasticity by modulating Cl^- gradient in neurons through microglial BDNF release and purinergic signaling[44]. The purinergic

receptors P2X7, P2Y12, and P2X4 are related to p38 MAP kinase, which is directly involved in BDNF synthesis and release[45]. BDNF release occurs through Ca^{+2}-regulated exocytosis, and regulation of BDNF synthesis involves the p38-MAPK signaling pathway[46]. BDNF rapidly downregulates K^+- Cl^- cotransporter 2 (KCC2) through receptor tyrosine kinase B (TrkB)[46]. KCC2 activity maintains a low intracellular Cl^- concentration, a prerequisite for effective GABA/Gly-mediated inhibition in the nervous system[47]. Thus, since microglial BDNF release decreases KCC2 activity, there is a deficiency in the inhibition of synapses, increasing neuronal hyperexcitability. However, microglial channels could also be linked to neuronal hyperexcitability in a different way: BDNF secreted following stimulation of microglial purinergic receptors may induce phosphorylation of NR1 subunit of NMDA neuronal receptors. This may contribute to neuronal hyperexcitability[48].

Implications for neurological and psychiatric disorders

Microglial activation is related to a variety of neurological and psychiatric disorders[49]. This activated state is related to an excitatory-inhibitory imbalance, generating oxidative stress[50], modifying BDNF release[51], and changing molecular signaling[52,53]. Additionally, in the brain, Cl^- homeostasis and the reduction of KCC2 activity has been associated with several neurological and psychiatric disorders, such as epilepsy, neuropathic pain, autism spectrum disorders, affective disorders, and schizophrenia[54–57]. Several lines of evidence accumulated during the last decade have indeed demonstrated that the increase in excitability in these pathological conditions can be largely explained by a loss of inhibition, and KCC2 has been recognized as an important molecular target underlying this loss[55,56].

It is thus plausible that Ca^{+2} entry through microglial ion channels may activate microglia and influence MAPK and AKT pathways. This could alter microglial phagocytosis functions, modify BDNF release, and increase neuronal hyperexcitability through KCC2 inhibition, contributing to the pathogenesis of mental disorders. Furthermore, first-line treatments for neurological and psychiatric disorders have potential inhibitory effects on microglia. To name a few, antipsychotics such as haloperidol, risperidone, olanzapine, clozapine, and chlorpromazine inhibit microglial proton currents, suppressing reactive oxygen production, acting as anti-inflammatory modulators[58]. Proton currents are also inhibited by the SSRIs paroxetine and sertraline, for example, attenuating the mobilization of intracellular Ca^{+2} [59]. Aripiprazole also downregulates microglial Ca^{+2} signaling[59], while carbamazepine suppresses microglial activation via p38-MAPK. Additionally, these drugs interfere with neurotransmitters, such as dopamine, serotonin, acetylcholine, and norepinephrine. This could contribute to further alterations in microglial phenotypes. Norepinephrine inhibits microglial inflammatory reactions through activation of cyclic AMP and suppression of downstream MAPK[60]. Dopamine and serotonin could interfere with microglial chemotaxis, and acetylcholine may alter Ca^{+2} signaling[60]. These effects could additionally contribute to a decrease in microglial activated state in neurological and psychiatric disorders.

Calcium-channel blockers might also contribute to a decrease in microglial activated state[61,62], providing potential utility in the treatment of neurological and psychiatric disorders[63–65]. In fact, calcium-channel blockers have been used successfully to treat absence seizures[63], and are emerging as potential therapeutic targets for pathologies, such as Parkinson disease, pain, mood disorders, and anxiety[63]. For instance, centrally acting calcium-channel blockers targeting Ca^{2+} channels of dopaminergic neurons might decrease risk of Parkinson disease[66], the calcium-channel blocker isradipine could be efficacious for bipolar disorder[65], and the drug nifedipine might have an antiepileptic effect[67]. Nevertheless, clinical trials with calcium-channel blockers in neurological and psychiatric disorders present inconsistent results[64]. One possible reason for this is the pharmacokinetic variability among different calcium-channel blockers[68]. Dihydripyridines, such as isradipine, have more favorable brain–blood barrier penetration and binding to calcium-channel compared to other calcium-channel blockers, such as diltiazem and verapamil[68]. Therefore, although there is evidence supporting the utility of calcium channel blockers in a variety of psychiatric and neurological disorders, what remains to be understood is how to develop new types of calcium channel inhibitors that specifically target calcium channels that are involved in pathophysiological processes, while sparing those that contribute to normal physiological function[63]. This requires an extensively understanding of how calcium channels participate in the function of specific brain circuits that are implicated in pathophysiology and how these channels may be dysregulated in pathological states[63]. This represents an enormous challenge in finding compounds that effectively cross the blood–brain barrier, have high affinity and target selectivity[63].

Clinical trials with drugs targeting-specific ion channels are currently underway. Some P2X7 receptor antagonists have been developed as potential drugs. For example, AZD9056[69] and CE-224,535[70] have been tested in clinical trials in rheumatoid arthritis, while GSK1482160[71] was developed to target peripheral pain. These compounds were not designed to enter the CNS and would therefore most likely lack the properties to be effective in nervous system disorders that could be associated with microglial

channels. Nevertheless, as our understanding of the role of ion channels on microglia in human diseases grows, the development of therapeutics targeting-specific microglial molecules will probably occur. However, some challenges remain for the development of microglial channel antagonists for use in humans, such as target specificity, CNS penetrance, and differences between animal and human microglia. New research strategies such as the development of microglia-like cells derived from human stem cells might help overcome the current limitations (Box 1).

Conclusion

The administration of ion channel antagonists leads to a decrease in microglial activation in different preclinical models. These findings suggest that ion channels have convergent pathways that potentially contribute to differentiation of microglial phenotypes and functions, which could be altered in a variety of neurological and psychiatric disorders. Further studies are required to explore whether the same antagonists' effects are seen in primate brain microglia and to investigate-specific druggable targets in microglial cells.

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Conflict of interest
The authors declare that they have no conflict of interest.

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2.4. Os canais sensíveis à ácido, a microglia e o TP

Canais sensíveis à ácido são expressos pela microglia e podem ser classificados como canais iônicos (Holzer, 2009). Esses canais contribuem para a manutenção do potencial e dos fenótipos da membrana microglial, colaborando para variações morfológicas dessas células (Thei *et al.*, 2018). Ambientes inflamatórios ocasionam a estimulação da microglia, que aumenta a expressão de canais sensiveis à ácido em sua membrana, promovendo a elevação de cálcio intracelular e induzindo a liberação de citocinas inflamatórias (Kweon e Suh, 2013; Yu *et al.*, 2015).

Estudos de neuroimagem em pacientes com TP evidenciam um papel de um distúrbio do pH na fisiopatologia do TP (Kim *et al.*, 2012; Magnotta *et al.*, 2014). Os achados são consistentes com um modelo de desregulação metabólica do pH cerebral associado à alteração da função dos circuitos de medo que são sensíveis ao ácido como um fator de vulnerabilidade ao TP (Wemmie, 2011; Leibold *et al.*, 2015).

No quarto artigo desta dissertação foi realizada uma revisão sistemática da literatura e uma meta-análise a fim de melhor compreender o papel de canais sensíveis à ácido no TP e na neurocircuitária do medo.

2.4.1. Artigo 4: The role of acid-sensitive ion channels in panic disorder: a systematic review of animal studies and meta-analysis of human studies. *Translational psychiatry*, 2018, 8.1: 185.

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Translational Psychiatry

REVIEW ARTICLE Open Access

The role of acid-sensitive ion channels in panic disorder: a systematic review of animal studies and meta-analysis of human studies

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Abstract
Acid-sensitive ion channels, such as amiloride-sensitive cation channel (ACCN), transient receptor potential vanilloid-1 (TRPV1), and T-cell death-associated gene 8 (TDAG8) are highly related to the expression of fear and are expressed in several regions of the brain. These molecules can detect acidosis and maintain brain homeostasis. An important role of pH homeostasis has been suggested in the physiology of panic disorder (PD), with acidosis as an interoceptive trigger for panic attacks. To examine the effect of acid-sensitive channels on PD symptoms, we conducted a systematic review and meta-analysis of these chemosensors in rodents and humans. Following PRISMA guidelines, we systematically searched the Web of Science, Medline/Pubmed, Scopus, Science Direct, and SciELO databases. The review included original research in PD patients and animal models of PD that investigated acid-sensitive channels and PD symptoms. Studies without a control group, studies involving patients with a comorbid psychiatric diagnosis, and in vitro studies were excluded. Eleven articles met the inclusion criteria for the systematic review. The majority of the studies showed an association between panic symptoms and acid-sensitive channels. PD patients appear to display polymorphisms in the *ACCN* gene and elevated levels of TDAG8 mRNA. The results showed a decrease in panic-like symptoms after acid channel blockade in animal models. Despite the relatively limited data on this topic in the literature, our review identified evidence linking acid-sensitive channels to PD in humans and preclinical models. Future research should explore possible underlying mechanisms of this association, attempt to replicate the existing findings in larger populations, and develop new therapeutic strategies based on these biological features.

Introduction

Acid-sensitive channels are highly related to the expression of fear. In mice, deleting or inhibiting acid-sensitive ion channels (ASICs), such as ASIC1a, transient receptor potential (TRP) vanilloid-1 (TRPV1), or proton-sensing G protein-coupled receptors, such as T-cell death-associated gene 8 (TDAG8) can render the animal less fearful to conditioned and/or unconditioned fear[1–4].

Fear is produced by CO_2 inhalation, which also generates autonomic and respiratory responses that can evoke panic attacks in individuals with panic disorder (PD)[5]. For this reason, CO_2 is frequently used as a biological challenge and biomarker for PD[6]. In addition to CO_2, other agents such as lactate can cause pH shifts and evoke panic attacks[7]. Furthermore, neuroimaging studies suggest the presence of dysregulated acid-base buffering[8] and increased plasma and brain lactate responses to metabolic challenges in patients with PD[9].

Brain extracellular pH is a fundamental signal for regulating homeostatic arousal, such as in behavior and breathing. In the intact brain in vivo, interstitial pH

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generally ranges from 7.1 to 7.25, but this balance can be endangered by numerous conditions, including metabolic acidosis, inflammation, and hypoventilation, in which pH can drop to 6.5[10]. Monitoring systems, such as molecular acid sensors, deal with these challenges by detecting harmful acidosis, and initiating appropriate emergency reactions, thereby limiting any resulting tissue damage[11]. Among the molecular acid sensors, ASICs, also known as amiloride-sensitive cation channel (ACCN), TRPV1, and TDAG8 are the most extensively studied[11].

ASIC1a, which can be activated at pH 7.0[12], is reportedly involved in the amygdala's control of learning mechanisms[13]. This function is also related to the TRPV1 channel. TRPV1-expressing neurons are activated by threatening stimuli[1], and this channel does not begin to open until pH reaches 6.4[14]. The pH range for TDAG8 stimulation and signaling is 5.2–5.7[15], and its deficiency leads to attenuated CO_2-evoked freezing in animal models[2,3,16].

Taken together, these observations support the hypothesis of defective homeostatic acid-base regulatory systems in PD. We thus aimed to review the evidence that acid-sensitive channels are involved in PD pathophysiology and to identify gaps in the literature to inform future research.

Primary outcome:

- Verify the presence of an association between acid-sensitive channels and PD symptoms.

Secondary outcomes:

- Ascertain whether deletions or antagonisms of acid-sensitive channels decrease PD symptoms.
- Identify single nucleotide polymorphisms (SNPs) in acid-sensitive channel genes related to PD.
- Elucidate the molecular basis linking acid-sensitive channels and PD.

Materials and methods

Data sources

We searched Web of Science, Medline, Pubmed, Scopus, Science Direct, and SciELO up to 10 June 2018. The references cited in the systematically searched articles were checked manually. In an effort to avoid publication bias, the search also included non-English language studies and gray literature (for example, conference abstracts). The search used a broadly structured strategy based on the Problem, Intervention, Comparator, Outcome, Setting (PICOS) framework, where the problem was PD symptoms, the intervention/exposure was polymorphisms, antagonisms, and deletions of acid-sensitive channels, the comparison was absence of PD symptoms, the outcome was an improvement or worsening of PD symptoms, and any type of study design was allowed. Search terms included various combinations of terms for panic and acid-sensitive channels, such as "panic disorder" OR "panic attacks" AND ASIC, "acid-sensing ion channel", ACCN, ACCN2, ACCN1, "amiloride-sensitive cation channel", TDAG8, "GPR65 protein, human", "GPCR25 protein, mouse", "TDAG8 protein, rat", "transient receptor potential vanilloid-1 ion channel", TRPV1, "TRPV cation channels", "two-pore domain K+", K2P, "ionotropic purinoceptors", and P2X. The full search strategy is available in Supplementary Material 1.

Study selection

Studies were selected for data extraction and analysis based on the following inclusion criteria: (1) original research studies in humans and/or animals associating acid-sensitive channels with PD symptoms; (2) in human studies, subjects met PD criteria based on a conventional psychiatric classification system, while control subjects did not meet criteria for PD; (3) in animal studies, only studies with recognized preclinical models of PD[17], such as elevated T-maze and/or escape behavior induced by electrical/chemical stimulation of the periaqueductal gray matter, and a control group were included. Escape behavior was characterized by jumps and crossings. The following exclusion criteria were used in the search: (1) participants had a comorbid or additional psychiatric diagnosis that would exclude or confound PD; (2) studies that lacked a baseline condition or control group; (3) human studies with prepubertal participants; and (4) in vitro studies. The same search criteria were used to identify non-human and human studies.

Data extraction and quality score

The following variables were extracted from all the studies: authors, year of publication, subject characteristics in the affected and control groups, and characteristics of experiments involving acid-sensitive channels. The main outcome change in panic symptoms in humans and non-humans was the presence of a polymorphism, deletion, or antagonism in acid-sensitive channels. The current review was conducted according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[18] (Supplementary Material 2). Quality assessment used the OHAT Risk of Bias Tool (Supplementary Material 3).

Statistical analyses

A meta-analysis was performed when there were at least three studies investigating antagonism of acid channels in a specific human polymorphism. Animal studies were not statistically analyzed since their outcomes presented high heterogeneity. The threshold of a minimum of three studies for the meta-analysis was selected so that there were at least two replication attempts of the original finding[19]. Statistical analyses of the extracted data were

conducted using the Comprehensive Meta-Analysis Program, version 3.

Pooled odds ratios (ORs) with their 95% confidence intervals (95% CIs) were calculated to evaluate the strength of the association between the ACCN2 rs685012 polymorphism and PD symptoms based on the allele model (C vs. T). Statistical heterogeneity between eligible studies was evaluated by using the Cochran's Q statistic and I^2-test. A p-value above 0.1 or I^2 below 50% indicated substantial homogeneity across studies[20]. Therefore, the fixed-effects model using the Mantel–Haenszel weighting method was selected[21] to perform the meta-analysis. Otherwise, the DerSimonian and Laird random effects model was chosen[22]. The study intended to assess publication bias using funnel plot techniques, Begg's rank test, and Egger's regression test, as appropriate, given the known limitations of these methods[23].

Results

The use of PRISMA guidelines and a systematic search of electronic databases yielded a total of 376 studies. No additional studies were identified through manual searching of references. After elimination of duplicates, 247 titles were reviewed, of which 197 were excluded. In all, 50 full texts were reviewed, of which 11 met the inclusion criteria for our systematic review (Fig. 1). Table 1 summarizes the studies associating acid-sensitive channels with panic-like symptoms in non-humans, and Table 2 summarizes the findings in humans.

TRPV channels

We found no human study on TRPV channels that met the search criteria. Five studies assessed how TRPV1 antagonism affected escape behavior in male rats[24–28]. The majority of studies reported that TRPV1 blockers decreased escape responses in animals[24,26–28]. Three out of five studies evaluated the effects of capsazepine, a TRPV1 receptor antagonist, in the dorsolateral periaqueductal gray (dlPAG). These studies were conducted in different animal models and demonstrated that blockade of TRPV1 receptors in the dlPAG decreased escape responses ($p < 0.05$)[24,26,28]. One study investigated the effects of the TRPV1 antagonist 6-iodonordihydrocapsaicin (6-I-CPS) in the ventromedial hypothalamus and confirmed that blocking this receptor decreased jumping and crossing responses[27] ($p > 0.05$). Another study found no effect of TRPV1 blockade on escape behavior[25].

ASIC/ACCN channels

A meta-analysis of four studies involving 1981 participants (742 PD patients and 1239 controls) evaluated ACCN2, the human homologue to the rodent ASIC1a[29–32]. These studies assessed the C and T allele in the rs685012 SNP of the *ACCN2* gene and showed a significant increase in C allele in PD patients compared to its presence in controls (effect size: 1.275; 95% CI: 1.048–1.552; $p = 0.015$; Fig. 2). Although no significant heterogeneity was detected ($p = 0.112$), the inconsistency was moderate ($I^2 = 50.01\%$). Publication bias was not assessed as there were inadequate numbers of included studies to properly assess a funnel plot or to perform more advanced regression-based assessments. Interestingly, the C allele of SNP rs685012 in the *ACCN2* gene was related to early-onset PD and prominent respiratory symptoms in one study[31].

In addition, we examined the association with ACCN2 variants using neuroimaging measures of amygdala structure and function. The PD risk allele C at rs10875995 was associated with increased amygdala volume as well as task-evoked amygdala reactivity to angry and fearful faces[31]. Furthermore, the ACCN2 rs10875995 T/T genotype was related to higher fear scores in PD patients[30]. Examination of SNPs within the *ACCN1* gene revealed a nominal association between this gene and PD[33].

TDAG8

A recent study evaluating blood samples of 15 individuals 15–44 years of age with a diagnosis of PD and 17 healthy controls showed significantly higher TDAG8 mRNA expression in mononuclear cells from PD patients than in those from controls ($p = 0.008$), as well as an association between TDAG8 mRNA expression and PD symptom severity ($p < 0.001$). There are concerns related to the generalizability of these findings, since this pilot study evaluated only a small sample of PD patients. Nevertheless, elevated TDAG8 mRNA expression was found even in young patients close to the onset of their illness, and a trend suggested a relationship between this receptor and treatment response in PD patients previously treated with antidepressants ($p = 0.08$)[34].

Discussion

Our review shows that acid-sensitive channels are related to PD and panic symptoms. Specifically, there was evidence of a reduction in panic-like symptoms after TRPV1 blockade in the dlPAG and hypothalamus in the majority of studies (4 out of 5) in rodents[24,26–28]. These studies were performed with injections directly targeting-specific brain regions, while the only study that failed to show any association between TRPV1 antagonism and panic symptoms used intravenous administration of arachidonoyl-serotonin[25]. Although systemic injection may be more appropriate for testing a potential therapeutic effect, the pharmacokinetic properties of the drug are important to consider. For example, arachidonoyl-serotonin is catalyzed by a cytochrome P450 enzyme that is widely expressed[35], potentially limiting the availability

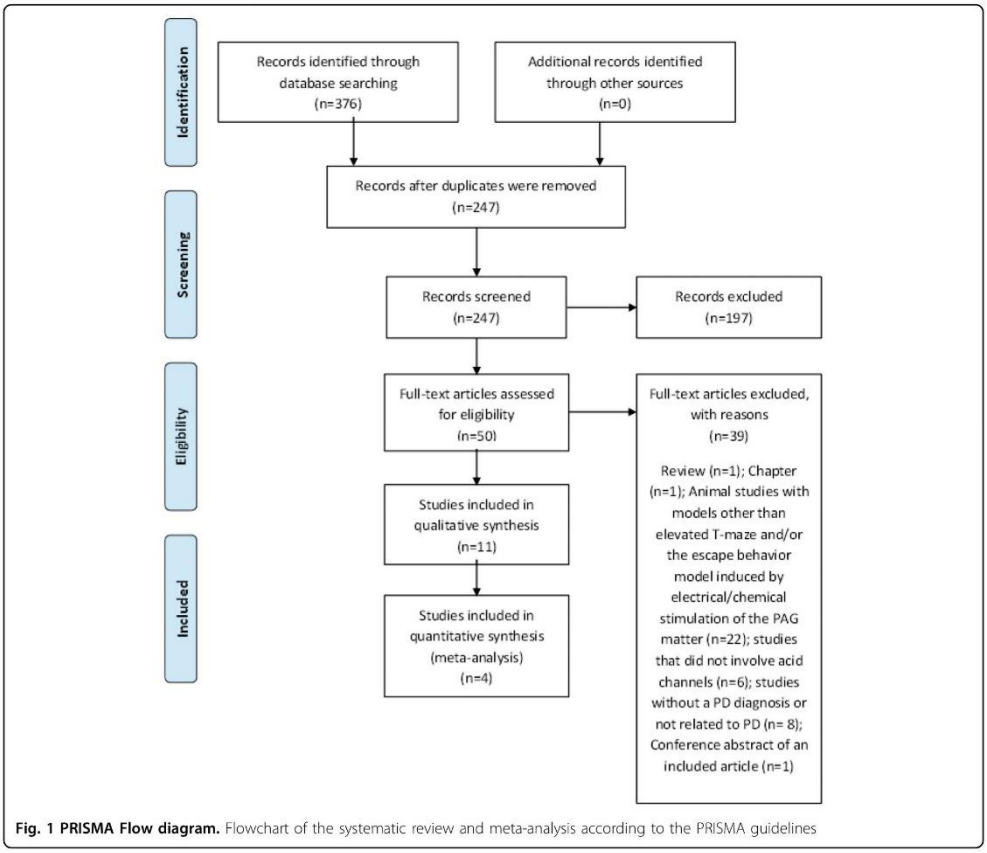

Fig. 1 PRISMA Flow diagram. Flowchart of the systematic review and meta-analysis according to the PRISMA guidelines

of the drug in the tissue and contributing to the negative findings of the study.

A meta-analysis involving four studies identified the C allele of the rs685012 SNP in the *ACCN2* gene as a significant risk factor for PD symptoms[29–32]. Interestingly, the C allele of SNP rs685012 in the *ACCN2* gene was related to PD cases with prominent respiratory symptoms[31]. The C allele of rs10875995 in the *ACCN2* gene was associated with increased amygdala volume, as well as task-evoked amygdala reactivity to fearful and angry faces[31], while the T/T genotype was associated with higher fear scores in PD patients[30]. This variation could potentially be attributed to differences in endophenotypes, since the C allele was shown to be associated with PD diagnosis and amygdala volume and function, i.e., largely anxiety-related endophenotypes, whereas the T allele was associated with experimentally provoked fear/panic sensations, i.e., specific fear-related endophenotypes[30].

Another case control study investigated TDAG8 mRNA in PD and demonstrated higher mRNA expression in PD patients than in controls[34]. Taken together, these results suggest that acid-sensitive channels are related to PD symptoms. Nevertheless, further studies are needed to confirm these findings and determine the potential mechanisms associating acid-sensitive channels with PD.

A hypothesis on the mechanism of action of acid-sensitive channels in the central nervous system (CNS) and its potential effect on PD symptoms

The underlying mechanism of the effect of acid-sensing ion channels on PD remains to be fully established. However, this mechanism could be elucidated by rodent

Table 1 Non-human studies on acid-sensitive channels and panic-like symptoms

Study	Species	Strain	Sex	Weight	Acid channel	Control (*n*)	Panic (*n*)	Drugs	Area	*F*-value	*p*-value	Outcome
Almeida-Santos et al.[24]	Rats	Wistar	Male	220–240 g	TRPV1	9	7	Capsazepine	dlPAG	$F_{(8,33)} = 2.81$	<0.05	Blockade of TRVP1 receptors in the dlPAG decreases escape responses in animals exposed to the ETM test
Batista et al.[25]	Rats	Wistar	Male	250–350 g	TRPV1	14	29	Arachidonoyl-serotonin	IV route	$F_{(3,39)} = 1.07$	0.37	No effect of blockade on escape behavior
Casarotto et al.[26]	Rats	Wistar	Male	300–330 g	TRPV1	9	20	Capsazepine	dlPAG	$F_{(3,25)} = 16.58$	<0.05	Capsazepine increases the threshold of electric current required to induce a panic-like response
dos Anjos et al.[27]	Rats	Wistar	Male	230–270 g	TRPV1	8	8	6-I-CPS	Ventromedial hypothalamus	NA	>0.05	Pretreatment with 6-I-CPS prevented escape behavior
Lisboa et al.[28]	Rats	Wistar	Male	230–270 g	TRPV1	35	22	Capsazepine	dlPAG	$F_{(1,53)} = 10.6$	<0.005	Capsazepine reduced flight reactions

TRPV1 transient receptor potential vanilloid receptor-1, *6-I-CPS* 6-iodonordihydrocapsaicin, *dlPAG* dorsolateral periaqueductal gray, *IV* intravenous, *ETM* elevated T-maze, *NA* not available

models of panic-like behaviors. In rats, both electrical and chemical stimulation of the dlPAG induces fight and flight behaviors, along with cardiovascular changes[36]. Since these responses resemble those observed in humans with PD, stimulation of this region has been suggested to serve as an experimental model of panic attack[37]. Likewise, the elevated T-maze (ETM) has been proposed as an animal model to study panic-related behavior[17]. In this model, a rat must perform a one-way escape test in which the animal is positioned at the end of one of the open arms, and the escape latency is measured three times[38]. The latter is associated with the escape response and has been associated with PD[38]. In our review, studies evaluated the effects of a TRPV1 receptor antagonist in the dlPAG and demonstrated that TRPV1 receptor blockade in this region decreased escape responses. TRPV1 inhibition in the ventromedial hypothalamus (where electrical stimulation leads to tachycardia and panic in humans[39]) also decreased panic-like behavior in animals.

Findings in translational rodent models of panic could provide information on potential ion channels and receptors that may contribute to the pathophysiology of PD in humans. Clinical studies over the years have shown that an imbalance in acid-base homeostasis may exist in PD patients, thus pointing to the relevance of pH sensing as well as the underlying circuits that contribute to pathophysiological responses. As an interoceptive stimulus, CO_2 inhalation can evoke panic attacks[5]. In humans, CO_2-sensitivity lies on a continuum[40], with PD subjects being highly sensitive to low CO_2, while healthy volunteers only experience panic-like symptoms at higher concentrations[41,42]. A study in twins demonstrated high concordance for CO_2 sensitivity[43], suggesting a genetic etiology for this interoceptive stimulus. Our findings suggest that SNP in the *ACCN* gene is associated with PD symptoms, while the C allele of SNP rs685012 in the *ACCN2* gene was more prevalent in respiratory-subtype PD patients[31]. Nevertheless, PD does not develop in all individuals with CO_2 hypersensitivity. Therefore, a combination of genetic and environmental factors may determine hypersensitivity to CO_2 and PD symptoms[44,45].

Environmental factors as well as various cognitive processing errors also likely play a part in the development of panic attacks[46]. Even if a threat or danger is perceived by a cognitive process, the pituitary gland produces adrenocorticotropic hormone, which in turn stimulates the adrenal cortex to produce the hormone cortisol[46]. Drug-naive PD patients show higher baseline cortisol levels than controls[46]. Cortisol may significantly increase the acid secretion capacity of H^+-ATPase at the cellular level, which could contribute to systemic acidosis in PD patients[47].

Systemic acidosis may also occur through a combination of CO_2 with water, a reaction that may be responsible for the panicogenic effects of CO_2[48]. Evidence indicates

Table 2 Studies on acid-sensitive channels in panic disorder patients

Study	Design	Country	Patients	Controls	Dominant ethnicity	Diagnostic criteria	Experiment	Candidate genes /RNA	SNP	Genotypes	Patients (%)	Controls (%)	*p*-value	Outcome
Gugliandolo et al.[29]	Case control	Italy	71	100	Sicilian	SCID	TaqMan	ACCN2	rs685012	Allele C	64.7	47	0.030	PD was associated with the SNP rs685012
										Allele T	35.2	53		
Leibold et al.[30]	Case control	The Netherlands	183	107	Caucasian	MINI	TaqMan	ACCN2	rs10875995	Allele C	49.18	51.4	0.032	T allele of rs10875995 was associated with higher fear scores in PD
									rs685012	Allele T	50.81	48.59	0.061	
										Allele C	49.7	52.33		
										Allele T	88.5	47.66		
Smoller et al.[31]	Case control	US	414	846	Caucasian	SCID	MassArray system	ACCN2	rs685012	Allele C	61.83	53.9	0.011	PD associated was associated with the SNPs rs685012 and rs10875995
										Allele T	39.13	45.8		
									rs10875995	Allele C	58.68	51.88	0.46	
										Allele T	41.30	48.10		
Hettema et al.[32]	Case control	US	188	188	Caucasian	SCID	TaqMan	ACCN2	rs685012	Allele C	34.5	89.3	0.077	No significant associations were found
										Allele T	6.54	10.6		
Gregersen et al.[33]	Case control	Denmark	305	969	Caucasian	ICD-10	Sequenom platform	ACCN1	rs9915774	Allele A	12	17	0.006	A nominally significant allelic association was observed

Table 2 continued

Study	Design	Country	Patients	Controls	Dominant ethnicity	Diagnostic criteria	Experiment	Candidate genes /RNA	SNP	Genotypes	Patients (%)	Controls (%)	*p*-value	Outcome
														between PD and rs9915774
										Allele G	88	83		
Strawn et al.[34]	Case control	US	15	17	Caucasian	SCID	TaqMan	TDAG8 mRNA		NA	NA	NA	0.008	Higher expression of TDAG8 mRNA in PD

SCID structured clinical interview for *DSM*, *MINI* mini international neuropsychiatric interview, *ICD-10* international statistical classification of diseases and related health problems 10th revision, *SNP* single nucleotide polymorphism, *ACCN2* amiloride-sensitive cation channel 2, *ACCN1* amiloride-sensitive cation channel 1, *TDAG8* G protein-coupled receptor T-cell death-associated gene 8, *NA* not available

that ASIC1a channels located in the amygdala detect a reduced pH arising from increased CO_2 or from direct injection of acid, initiating a fear response[2]. However, studies in patients with Urbach–Wiethe disease indicate that the amygdala is not required for the expression of panic and fear in response to CO_2 inhalation[49], suggesting that distinct chemosensors in other brain regions may be responsible for fear and panic responses in response to interoceptive stimuli.

In addition to the amygdala, acid-sensitive circuits are present in other brain regions potentially relevant to PD, including the bed nucleus of the stria terminalis, periaqueductal gray (PAG), hypothalamus, and circumventricular organs[9,50,51]. Acidosis sensed by acid channels may be translated to autonomic, respiratory, and behavioral symptoms of a panic attack[52]. Respiratory symptoms may be controlled by the parabrachial nucleus (PBN) via inputs from the hypothalamus[53] and indirectly from the subfornical organ (SFO) [54], while the amygdala, PAG, and hypothalamus may regulate the autonomic and behavioral manifestations of panic[55] (Fig. 3).

The SFO, which is a sensory circumventricular organ (CVO), has access to systemic and CNS compartments for the maintenance of homeostasis[56]. Recent studies associate the SFO with panic-like responses to intravenous lactate[57] and CO_2[3]. PD patients are also susceptible to the induction of panic attacks not only by CO_2 but also by systemic administration of a variety of agents, such as lactate, cholecystokinin, and norepinephrine. Many of these agents do not cross the blood-brain barrier easily[57]. Therefore, regions lacking a blood-brain barrier can be exposed to these circulating substances and in turn stimulate other areas, such as the amygdala or the dorsomedial hypothalamus, thereby eliciting a panic response. Such a mechanism involving CVOs could also provide a single unifying explanation for the existence of multiple, apparently unrelated, agents that appear to induce panic attack in PD patients[57].

The acid-sensitive channels TRPV1[58], ASIC[59], and TDAG8[3] are expressed in microglial cells in several regions of the brain, including CVOs[8,51]. Microglia, innate immune cells of the CNS, are recruited in physiological responses to homeostatic fluctuations, transforming from a resting to a proinflammatory activated state[60,61]. Extracellular acidification induces rapid alteration in microglial morphology[62], suggesting a potential role of microglia in the effects of acidotic stimuli. Furthermore, recent findings associated alterations in the immune system with PD[63]. Considering the close relationship between acid-sensitive channels and microglia, an active engagement of these cells in the detection of an acidotic pH threat is tempting to be considered as a potential mechanism in the genesis of panic attacks.

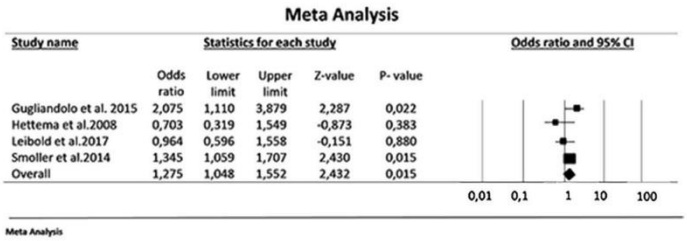

Fig. 2 Meta-analysis of the strength of the association between the ACCN2 rs685012 polymorphism and PD symptoms. The C allele of the rs685012 polymorphism was associated with a significant increase in the risk of PD (summary effect size = 1.275; $p = 0.015$)

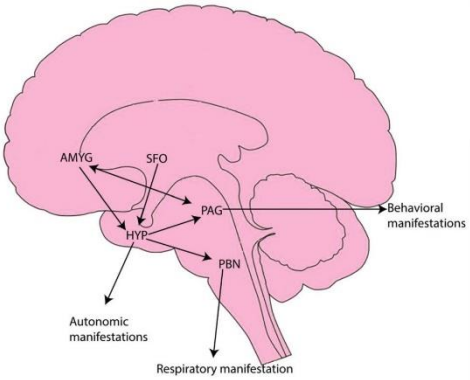

Fig. 3 Acidosis sensed by acid channels may be translated as autonomic, respiratory, and behavioral symptoms of a panic attack. Respiratory manifestations could be controlled by the PBN via direct inputs from the hypothalamus and indirect inputs from the SFO. The hypothalamus may regulate autonomic expression through inputs from the amygdala and SFO. Additionally, the hypothalamus, amygdala, and PAG could be involved in behavioral symptoms. AMYGD amygdala, HYP hypothalamus, PAG periaqueductal gray, SFO subfornical organ, PBN parabrachial nucleus

Additionally, acid-sensitive channels can be classified as proton channels and may contribute to the maintenance of the microglial membrane potential and phenotypes[64]. Panic attacks triggered by agents such as caffeine, cholecystokinin, and norepinephrine could be explained by modifications in microglial ion currents[64], altering the membrane potential of the cells and potentially transforming microglia from a resting to an active state[64]. However, the relationship between PD symptoms and acid chemosensors in microglia has not been fully investigated but is an important future direction for understanding the contribution of microglia to PD pathophysiology.

Translational applicability of acid-sensitive channel mechanisms: from an animal model to PD patients

While there is a clear need for more studies in primates addressing the issues discussed above, our findings that pharmacological blockade of acid channels in animal models of PD implies that these channels may contribute to PD treatment. PD has been treated primarily with drugs that have anxiolytic properties, including benzodiazepines and selective serotonin reuptake inhibitors[65]. Nevertheless, 20–40% of patients do not achieve full remission with the recommended medicine[65]. Thus, new therapeutic targets and drug development for PD patients should be further investigated.

In humans, dlPAG stimulation produces emotional and autonomic responses strikingly similar to those of panic attacks, whether spontaneous or provoked by intravenous infusions of lactate[66]. In addition, dlPAG-evoked panic-like behaviors are attenuated by clinically effective panicolytics given at doses and regimens similar to those given for panic therapy[67]. One animal model of PD is the escape behavior induced by electrical/chemical stimulation of dorsal portions of the PAG matter. After stimulation of the dlPAG, a vigorous reaction is observed, with piloerection, miosis, vertical jumps, and strong flight reactions represented by an increase in locomotion and average speed[17]. Another preclinical model for PD is the ETM animal test. The ETM test assumes that a panic attack is a reaction to a proximal threat when no threat is present. Therefore, this test summons neural mechanisms that underpin proximal defense, which can be investigated by experimentally analyzing the escape task in the ETM[17].

Findings in translational rodent models of panic could provide information that may contribute to our understanding of the pathophysiology of this disorder in humans. Our review shows that pharmacological blockade of TRPV1 channels decreases panic symptoms in animal models with injections directly targeting-specific brain regions. In humans, antagonists of TRPV1 channels are being studied in clinical trials on osteoarthritis and atopic dermatosis, conditions where there is a pH

imbalance[68,69]. In none of these trials does the drug need to enter the brain. Thus, future studies are needed to investigate drugs that potentially block TRPV1 channels and cross the blood-brain barrier.

Blockade of amiloride ion channels may also be a promising therapeutic target for PD, since a SNP in the *ACCN* gene is related to PD symptoms in humans. The C allele of SNP rs685012 in the *ACCN2* gene is related to PD cases with prominent respiratory symptoms[31]. In addition, PD patients with the respiratory subtype of the disorder are more sensitive to CO_2 challenge[70]. Mirroring human studies of PD, animal studies have shown that inhalation of CO_2 evokes fear behavior in mice, an effect that is reduced by deletion or blockade of ASIC1a, which is homologous to the ACCN human gene, within the amygdala. Overexpression of ASIC1a in the amygdala is sufficient to trigger CO_2-induced fear behavior[2]. The ACCN2 allele associated with both PD and amygdala volume is associated with increased amygdala reactivity to emotional faces, a phenotype linked to PD[31]. The observed association between allelic variants and amygdala reactivity has been suggested to potentially reflect enhanced sensitivity to reduced pH secondary to neuronal activity that mediates the processing of emotional stimuli[31]. Therefore, ASIC antagonists such as amiloride are being studied and have shown promise for acidosis-associated conditions, such as stroke, migraine, pain, spinal cord injury, and multiple sclerosis[71]. Recent studies have shown neuroprotective effects of amiloride and corroborated the efficacy of this agent for alleviating pH-associated pathophysiology[71].

A role for pH and chemosensory mechanisms in panic physiology is increasingly appreciated on the basis of the recent data from animal studies. However, the degree to which these systems may be targeted by psychopharmacologic interventions in PD is still unexplored. Thus, the lack of studies related to pH-modulating therapies is of public health significance, given that the therapeutic options for PD are limited.

Limitations and strengths

There are a number of limitations to this systematic review and meta-analysis. Importantly, as part of our search criteria in preclinical data, we only included studies associating acid-sensitive channels with ETM and/or escape behavior tests. Although no animal model to date has perfectly mimicked panic symptoms, the relationship between these models and PD is recognized in the literature[17]. Other preclinical models such as elevated plus maze and predator encounter-based models could also evaluate some panic symptoms such as freezing or fear conditioning[17]. However, we chose not to expand our search criteria, since these models are also related to other anxiety disorders such as generalized anxiety disorder and post traumatic stress disorder. Although these disorders are commonly comorbid with PD, we opted not to include preclinical models evaluating exteroceptive threat response systems since our main goal in this review was to evaluate internal triggers and interoceptive chemosensory pathways of particular relevance to PD. In addition, preclinical models investigating nociception and long-term potentiation were also excluded. Supplementary Material 4 lists the full-text articles that were excluded and the reasons for their exclusion.

Regarding human studies, although PD is highly comorbid with other conditions, especially depressive disorder, we excluded articles involving participants with a comorbid or additional psychiatric diagnosis and/or those that were prepubertal. Nevertheless, some comorbidities may have been unintentionally included in our review, since even the best-designed studies may not properly assess all comorbidities. Although this exclusion may have limited the number of studies included in the review, this exclusion was justified since the variety of acid channels investigated can be expressed by microglia, the innate immune cells of the CNS. Evidence has shown that the majority of mental health disorders can be related to neuroinflammation[72]. Inflammation per se may contribute to an acidic environment. Based on changes in the microglial microenvironment, the investigated acid channels can display a variety of complex spatiotemporal patterns[64]. The same is true for age-related alterations in the microglial microenvironment[64]. We thus excluded studies with participants that had a comorbid psychiatric diagnosis or were prepubertal, since they might present a different pattern of acid-sensitive channels in the microglia.

Finally, one general limitation to the included studies was that only a few patients were evaluated for polymorphisms in genes for acid-sensitive channels, which greatly limits the statistical power to detect actual SNP associations with phenotype.

In addition, the preclinical studies only included male rats. Research has shown higher numbers of acid-sensing ion channels in female mice than in males[73]. There are also differences in acid-sensitive channels in the brains of mice when compared to those in the brains of rats[74]. Thus, the extrapolation of findings on acid-sensitive channels in animal models of panic-like symptoms should be viewed with caution, and studies in female animals and non-rat species are needed.

Although pH imbalance is commonly accepted to be linked to PD and acid-sensitive channels are thought to play a role in PD pathophysiology, no authors to date have systematically summarized the literature to determine whether this relationship is backed by the totality of evidence. The current article reports the results of a systematic review examining acid-sensitive channels in

human and animal studies. To our knowledge, this is the first systematic review and meta-analysis that has evaluated acid channels and PD based on PRISMA guidelines and after evaluating the quality of the included articles. One strength of the review is that by including multiple databases and following PRISMA guidelines, relevant studies were unlikely missed. The findings indicate that TRPV1, TDAG8, and ACCN channels are related to PD pathophysiology. However, more studies are needed to corroborate these results, which could represent an important step towards elucidating PD pathophysiology and contributing to new therapeutic options for the disorder.

Future directions

A number of steps are necessary to fully understand the associations between acid-sensing ion channels and the neurobiology of PD. Antagonists of acid-sensitive channels in humans are a promising therapeutic target for PD. To date, there have been no clinical studies in PD patients on interventions targeting control of pH imbalance or acid-sensitive ion channel blockers. Nevertheless, ASIC antagonists such as amiloride have shown promise for other acidosis-associated conditions, such as pain and multiple sclerosis[71]. Therefore, clinical trials evaluating PD symptoms and acid channel blockade are needed, since acid channels could represent new therapeutics options for this disorder. Moreover, future genetic studies of SNPs in genes for acid-sensitive channels should consider replicating the initially reported findings through well-powered studies.

Much of the research to date has focused on the dysregulation of central fear circuitry, including the limbic network, which involves connections between the amygdala, anterior cingulate cortex, and PAG, during panic symptoms[67]. The potential role of areas devoid of a blood-brain barrier in PD is important to investigate, especially given their connectivity to downstream sites responsible for the expression of behavioral and physiological responses.

Preclinical animal models are also needed to evaluate pH chemosensory interoceptive stimulus processing. These replicas could elucidate the interaction between different pH chemosensory molecules in the brain. A variety of sensory mechanisms in distinct areas can provide a highly sensitive pH detection system, which can be relevant to PD. Such animal models are also important for testing new drugs. Additionally, studies are needed on the effect of gender and species.

While there is significant evidence for the role of pH homeostasis and impaired acid-base buffering in patients with PD[8], not all data support the link between acidosis and panic attacks, since not all panicogens cause acidosis, and hyperventilation (which produces alkalosis) can also produce panic attacks. Future studies are required to further clarify these inconsistencies.

More fundamental mechanistic research is essential if we are to truly understand the role that acid plays in the context of PD. Acid chemosensors on microglia could provide further insights into the integration of interoceptive pH fluctuations leading to behavioral and respiratory arousal. Future studies focusing on the crosstalk among acid chemosensors and inflammatory and neuromodulatory functions and the relationship among them will provide further insight into the pathophysiological mechanisms of PD.

Conclusion

According to this systematic literature review, acid-sensitive channel antagonists decreased escape behavior in preclincal animal models of PD. In humans, an acid-sensitive channel SNP was shown to be associated with PD, PD symptoms, and a subtype of PD patients. Acid-sensitive channels may play an important role in the pathophysiological mechanisms of PD and provide a promising therapeutic target for the disorder. Future research should explore possible mechanisms underlying this association, attempt to replicate existing findings in larger populations, and aim to develop new therapeutic strategies based on these biological features.

Conflict of interest

The authors declare that they have no conflict of interest.

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3. RELEVÂNCIA

A melhor compreensão da fisiopatologia dos transtornos mentais torna-se imperativa para o desenvolvimento de melhores estratégias terapêuticas direcionadas aos mesmos.

Inibidores seletivos de recaptação da serotonina e benzodiazepínicos apresentam eficácia comprovada no tratamento do TP (Baldwin *et al.*, 2005; Stein e Mcintyre, 2010). Entretanto, estudos demonstraram que 30 a 48% dos pacientes com TP continuam a apresentar AP, apesar do tratamento farmacológico (Pollack e Marzol, 2000). Dessa forma, torna-se essencial a necessidade de propor tratamentos com o objetivo de obter a remissão completa do TP, uma vez que a presença de sintomas residuais está associada a maior chance de recaídas, maior custo socioeconômico e pior qualidade de vida para pacientes e seus familiares (Pollack e Marzol, 2000; Stein e Seedat, 2004; Bystritsky, 2006).

Os artigos 5 e 6 dissertam sobre terapêuticas medicamentosas atuais e bem estabelecidas no TP.

3.1. Artigo 5: Risks and benefits of medications for panic disorder: a comparison of SSRIs and benzodiazepines. *Expert opinion on drug safety*, 2018, 17.3: 315-324

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REVIEW

Risks and benefits of medications for panic disorder: a comparison of SSRIs and benzodiazepines

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ABSTRACT

Introduction: Panic disorder (PD) is a prevalent and disabling anxiety disorder that can be treated effectively. Selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines are among the most frequently prescribed drugs for PD. In this article, the authors review the current evidence on efficacy, adverse events, and limitations of these two treatment options.

Areas covered: MEDLINE/Pubmed and Web of Science databases were searched for open or placebo-controlled trials on SSRIs and/or benzodiazepines in PD treatment.

Expert opinion: The literature search yielded 4,957 articles related to the theme. Of these, 24 articles were included in this review. Despite their usefulness in PD, SSRIs are associated with a delay of several weeks in onset of therapeutic effect and have the potential to exacerbate anxiety and panic early in the treatment course. Benzodiazepines present rapid onset of action, but can cause tolerance and dependence. Despite strong evidence of the effectiveness of SSRIs and benzodiazepines in the treatment of PD, few trials have performed head-to-head comparisons of these two drug classes. Future studies on the pharmacological treatment of PD should make direct comparisons of risks, benefits, and limitations of each group. This could help improve the evidence-based pharmacotherapy of PD.

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1. Introduction

Panic disorder (PD) is a prevalent psychiatric disorder, affecting 1.6%–2.2% of the world population [1]. PD is characterized by recurrent unexpected panic attacks and persistent worry about future panic attacks and their consequences. The longer PD patients remain untreated, the higher the risk of nonresponse to pharmacotherapy [1].

According to the neuroanatomical hypothesis of Gorman et al. [2], panic attacks originate from a dysfunction in the brain's fear network that integrates various structures of the brainstem, amygdala, medial hypothalamus, and cortical regions. The serotoninergic (5-HT) system is well-positioned to influence these areas with its neuronal cell bodies in the brainstem raphe nuclei and widespread axonal projections to the forebrain regions [3]. In symptomatic PD patients, studies have demonstrated decreases in midbrain 5-HTT and 5-HT1A receptor binding. This could reflect a compensatory process attempting to increase 5-HT neurotransmission, particularly in the dorsal periaqueductal gray-amygdala pathway, in order to inhibit hyperactivity or spontaneous neuronal discharge in this region [4].

Apart from the 5-HT neurotransmitter system, there is increasing evidence that the γ-aminobutyric acid (GABA) system is important in the pathophysiology of PD. GABA is the most important inhibitory neurotransmitter in the central nervous system. The rapid inhibitory action of GABA is mediated mostly through $GABA_A$ receptors. Several studies have demonstrated that patients with PD have a dysfunction of the $GABA_A$ receptors and/or altered brain GABA concentrations. Single-photon emission computed tomography studies have shown that patients with PD have decreased benzodiazepine receptor binding [5], and a magnetic resonance spectroscopy study revealed a significant overall reduction in total occipital GABA concentrations in patients with PD compared to normal controls [6], which supports the hypothesis of GABA deficit in PD patients.

PD has been treated primarily with drugs that have anxiolytic properties, including benzodiazepines [7]. Benzodiazepines increase the potency of GABA by modulating the function of $GABA_A$ receptors. Over time, many therapeutic strategies for PD emerged, including selective serotonin reuptake inhibitors (SSRIs) [1]. SSRIs increase synaptic availability of 5-HT by blocking its transport into neurons. The psychopharmacology of fear and its neuroanatomical substrates predict that drugs from both pharmacological classes should have independent therapeutic actions in anxiety disorders. The purpose of this review is to assess the relative place of benzodiazepines and SSRIs, the most widely used medications in the treatment of PD.

2. Method

2.1. Search strategy

The authors used the following selection criteria for articles in this review. First, the article had to report on an empirical study in which the pharmacological treatment was applied

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Article highlights

- Panic disorder is a prevalent and disabling anxiety disorder that can be treated effectively.
- There is evidence of the effectiveness of SSRIs and benzodiazepines in PD treatment.
- SSRIs are recommended as first-line treatments for PD and can assist in the treatment of comorbidities in PD, but they have slower onset of action, and have the potential to exacerbate anxiety and panic early in the treatment course.
- Benzodiazepines present rapid onset of action, but can cause withdrawal symptoms, tolerance and dependence.
- A relatively common practice is to combine SSRIs with benzodiazepines to provide more rapid, and potentially additive, clinical efficacy
- Evaluating risks and benefits of the most frequently prescribed drugs in PD could help improve evidence-based pharmacotherapy for this disorder.

This box summarizes key points contained in the article.

to a sample of adult subjects diagnosed with PD with or without agoraphobia based on diagnostic criteria recognized by the scientific community. Second, the study had to be an open or placebo-controlled clinical trial. Articles published in English were searched in Medline, PubMed and Web of Science databases, using the following search words: (1). PD or panic attacks and (2). SSRI or citalopram or escitalopram or paroxetine or fluoxetine or fluvoxamine or sertraline, and (3). Benzodiazepines or clonazepam or alprazolam or lorazepam or bromazepam or clobazam or cloxazolam or diazepam. The time period for the literature search was 1997–2017. Articles were excluded if they involved any modality of psychotherapy, medications other than SSRIs and benzodiazepines, studies with no placebo group, and/or included individuals with clinical or psychiatric comorbidities associated with PD. Also, studies with fewer than 10 patients and articles on augmentation treatment or other non-pharmacological treatments such as transcranial magnetic stimulation were excluded. Meeting abstracts, congress proceedings, case reports, reviews, book reviews, corrections, editorials, news items, and reprints were excluded. References cited in the selected papers were also searched manually to ensure that no relevant study on this topic was left out.

3. Results

The search yielded 4957 articles related to the theme. Two additional studies were identified through manual searching of references. After removal of duplicates, 559 titles and abstracts were reviewed, of which 482 were excluded. In all, 77 full texts were reviewed. Of these, 24 articles were included in this review according to the inclusion/exclusion criteria described above. The primary outcome in all 24 studies was the reduction of panic attack frequency or the percentage of patients free of panic attacks at the end of the study. The process of study identification and selection is shown in a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram (Figure 1) [8]. The excluded articles and the reasons for exclusion are presented on supplementary material 1.

3.1. Selective serotonin reuptake inhibitors

The increasing acceptance of the role of the serotonergic system in the etiology of PD has led to the investigation of selective 5-HT agents in the treatment of panic symptoms, and studies with currently available SSRIs have shown them to be effective in the treatment of PD. The anxiolytic function of SSRIs is already recognized in clinical practice and scientific research, and they are considered the first choice of treatment for PD [9]. In the last 20 years, a variety of studies have confirmed the efficacy of SSRIs as a drug class for PD. Table 1 summarizes the findings from these studies.

Paroxetine was the first member of the SSRI class to be recommended for PD treatment. In a fixed-dose, double-blind study reported by Ballenger et al. [10], 278 patients with PD were randomized to receive paroxetine (10, 20, or 40 mg/day) or placebo for 10 weeks. Paroxetine 40 mg/day, but not 10 or 20 mg/day, produced a statistically significant reduction in frequency of panic attacks when compared to placebo ($p = 0.019$), starting at week 4 of treatment. During the final 2 weeks of the study, 86% of patients treated with paroxetine 40 mg/day were free of panic attacks, compared to 50% of patients in the placebo group. Similar results were reported by Sheehan et al. [11]. The authors conducted a 10-week placebo-controlled trial of controlled-release paroxetine (25–75mg/day) in PD patients. By week 10, 73% and 60% of controlled-release paroxetine and placebo-treated patients, respectively, were panic-free ($p < 0.005$).

Michelson et al. [12] reported the first large-scale, placebo-controlled study of fluoxetine in PD. A total of 243 patients with PD were randomized to receive either fluoxetine (10–20 mg/day) or placebo for 10 weeks. Fluoxetine, particularly the 20 mg/day dose, produced statistically greater improvement than placebo on multiple measures including Clinical Global Impression (CGI) improvement ($p = 0.006$), total panic attack frequency ($p = 0.04$), phobic symptoms ($p = 0.002$), and overall functioning ($p = 0.006$). In a 24-week extension to the study by Michelson et al. [12], patients who had responded to acute fluoxetine treatment were randomized to continue on the same dose of fluoxetine or switch to placebo with no tapering period [13]. Patients who remained on fluoxetine experienced continuing clinical improvement over the 24 weeks, with significant decreases from randomization to endpoint in panic attack frequency ($p = 0.02$) and phobia rating scale score ($p = 0.01$). In contrast, those who switched to placebo experienced worsening of their symptoms over the course of the study, as evidenced by statistically worse scores on the Hamilton Anxiety Rating Scale and Hamilton Depression Rating Scale. In 2001, Michelson et al. conducted a 12-week placebo-controlled trial with 180 PD patients. The authors reported that fluoxetine 20 mg/day is effective in reducing PD symptoms ($p = 0.02$) [14].

Not only paroxetine and fluoxetine were associated with improvement in PD, but sertraline also showed some significant results compared to placebo. Londborg et al. [15] used fixed doses of 50, 100, and 200 mg/day of sertraline or placebo in 177 patients for 12 weeks. All three doses showed statistically significant improvement over placebo. At endpoint, the pooled sertraline treatment group showed a 65% reduction in the ratio of panic attacks to baseline compared to a 39% reduction in the placebo group.

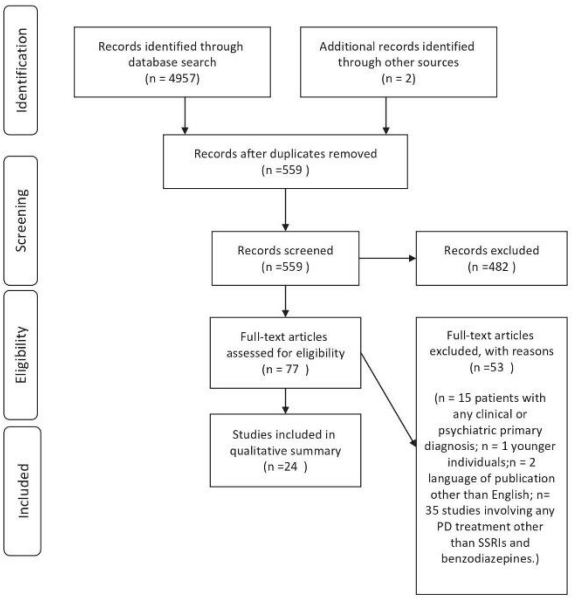

Figure 1. PRISMA diagram of study identification and selection process.

Table 1. Clinical studies of SSRIs in panic disorder.

Study	Drugs	Daily dose(mg)	Duration	n	Effect size (Cohen's d)	Panic-free at endpoint or reduction in panic attack frequency
Ballenger et al. [10]	Paroxetine x placebo	10, 20, 40 mg	10 weeks	278	0.96	Paroxetine 40 mg/day: 86.0% + Paroxetine 20 mg/day: 65.2% Paroxetine 10 mg/day: 67.4% Placebo: 50.0%
Londborg et al. [15]	Sertraline x placebo	50–200mg	12 weeks	177	0.74	Sertraline 50 mg/day: 71% Sertraline 100 mg/day: 83% Sertraline 200 mg/day: 42% Placebo: 39%
Pohl et al. [16]	Sertraline x placebo	25–200mg	10 weeks	166	0.47	Sertraline: 77%† Placebo: 51%
Pollack et al. [17]	Sertraline x placebo	50–200mg	10 weeks	176	NA	Sertraline: 0.21 ± 0.57† Placebo: 0.41 ± 0.73 (log ratio: endpoint to baseline)
Sandmann et al. [19]	Fluvoxamine x placebo	Average 160 mg	6 weeks	46	NA	Fluvoxamine: 47.1% + Placebo: 33.3%
Michelson et al. [12]	Fluoxetine x placebo	10–20 mg	10 weeks	243	NA	Fluoxetine: superior to placebo for panic frequency, phobia, disability, global improvement +
Michelson et al. [13]	Fluoxetine x placebo	10–20 mg	24 weeks	165	0.32	Fluoxetine was associated with improved clinical outcomes compared with placebo during continuation therapy.
Asnis et al. [20]	Fluvoxamine x placebo	100–300 mg	8 weeks	188	1.43	Fluvoxamine: 69% + Placebo: 45.7%
Michelson et al. [14]	Fluoxetine x placebo	10–20mg	12 weeks	180	0.79	Fluoxetine 42% + Placebo: 28%
Stahl et al. [23]	Citalopram x escitalopram x placebo	Citalopram (dose 20–40 mg) Escitalopram (10–20 mg)	10 weeks	351	NA	Escitalopram: 1.61 ± 0.1 Citalopram: 1.43 ± 0.1 Placebo: 1.32 ± 0.1 (log ratio: endpoint to baseline)
Kamijima et al. [18]	Sertraline x placebo	12.5mg–100mg	8 weeks	240	1.75	Sertraline: 89.9% + placebo:74.4%
Sheehan et al. [11]	Paroxetine CR x placebo	25–75mg	10 weeks	889	1.48	Paroxetine controlled-release: 73% + Placebo: 60%
Bertani et al. [22]	Citalopram	10 mg	1 week	18	0.73	Reduction in PASS score +
Choi et al. [21]	Escitalopram	5–20 mg	24 weeks	119	NA	73.1% +

+ Statistically significant result compared to placebo; PASS: Panic-Associated Symptom Scale; NA: Not available

Pohl [16] and Pollack [17] conducted 10-week trials with 166 and 176 PD patients, respectively, randomized to receive either sertraline (50–200 mg/day) or placebo. Pohl reported that the mean number of panic attacks per week dropped by 88% in sertraline-treated patients, compared to 53% in the placebo group. Pollack reported similar results, with sertraline patients showing greater improvement than placebo controls ($p = 0.01$).

A double-blind, long-term study by Kamijima et al. [18] evaluated the efficacy and safety of sertraline for 8 weeks in Japanese patients with PD. Three hundred and ninety-four patients were initially treated with 8 weeks of open-label sertraline followed by 8 weeks of double-blind treatment with either placebo or sertraline (50–100mg/day). Responders during the open-label phase were eligible to enter the double-blind phase. Two hundred and forty patients entered the double-blind phase and were randomly assigned to receive sertraline or placebo. PD Severity Scale total score was significantly lower (p = 0.012) in the sertraline group compared to the placebo group.

Although most randomized controlled trials have reported the efficacy of SSRIs compared to placebo, Sandmann et al. [19] reported contrasting results with the SSRI fluvoxamine. The authors conducted a 6-week study with 46 PD patients treated with fluvoxamine (mean dose 160 mg/day) or placebo. Fluvoxamine was not significantly superior to placebo at reducing the number of panic attacks, but it was significantly more effective at reducing the number of limited panic attacks. These results were not corroborated by Asnis [20] in a 6-week study of 87 PD patients randomized to receive either fluvoxamine (100–300mg/day) or placebo. In the fluvoxamine group, 69% patients were panic-free at endpoint (p = 0.002).

Regarding escitalopram treatment, Choi [21] studied 119 PD patients in an open-label trial. Ninety-six patients (80.7%) showed treatment response, and 87 patients (73.1%) had achieved remission after 24 weeks of treatment.

Citalopram was the drug selected for a study by Bertani et al. [22]. In an open trial involving carbon dioxide (CO_2) challenge, the authors reported that 18 PD patients treated with citalopram 10 mg/day for 1 week showed a significant decrease in anxious reactivity to CO_2 (p = 0.01).

Stahl et al. [23] compared escitalopram and citalopram versus placebo in 351 patients. Escitalopram 10–20 mg/day and citalopram 20–40 mg/day were administered for 10 weeks. The primary outcome was the reduction in frequency of panic attacks, evaluated with the Sheehan Panic and Anticipatory Anxiety Scale, using a log scale. Patients receiving escitalopram showed a reduction of 1.61 times in frequency of panic attacks, compared to 1.43 for citalopram and 0.32 for placebo.

3.2. Benzodiazepines

Modern guidelines for psychopharmacological treatment of anxiety disorders have shifted from GABAergic benzodiazepines to serotonergic agents as first-line drugs [9]. Nevertheless, benzodiazepines are still prescribed more often than antidepressants for the treatment of anxiety disorders [24], although from 1997 to 2017 only placebo-controlled or open-label studies involving clonazepam were found. It is northworthy that given the time period from our search strategy, we missed some important reports in benzodiazepines field, for instance studies involving extended-release alprazolam, which contributed to an improvement in treatment of PD [25]. Table 2 summarizes our findings from studies involving benzodiazepines in PD treatment.

Table 2. Clinical studies of benzodiazepines in panic disorder.

Study	Drugs	Daily dose(mg)	Duration	n	Effect size (Cohen d)	Reduction in panic attack frequency
Rosenbaum et al. [49]	Clonazepam x placebo	0,5–4 mg clonazepam titrated to optimal dose over 3 weeks, optimal dose maintained for 3 weeks, 7 weeks discontinuation phase.	16 weeks	413	NA	Clonazepam 1 mg: 73%+ Placebo:55%
Moroz and Rosenbaum [48]	Clonazepam x placebo	0,5–4 mg clonazepam titrated to optimal dose over 3 weeks, optimal dose maintained for 3 weeks, 7 weeks discontinuation phase.	13 weeks	438	NA	Clonazepam: 61.9% + Placebo: 36.8%
Nardi et al. [42]	Clonazepam x placebo	2 mg	Single dose	22	1.57	After CO_2 challenge: Clonazepam: 18% mild attack Placebo: 82% moderate-severe attack
Valença et al. [50]	Clonazepam x placebo	2 mg	6 weeks	24	NA	Placebo:11.1%
Valença et al. [41]	Clonazepam x placebo (Placebo >CO_2 challenge > clonazepam or clonazepam. CO_2 challenge>placebo)	2 mg	6 weeks	34	NA	Clonazepam: 61.5% + Panic attacks after 6 weeks treatment and CO_2 challenge: Clonazepam: 11% Placebo: 67%
Valença et al. [46]	Clonazepam x placebo	2 mg	6 weeks	34	NA	Clonazepam: 78% + Placebo: 8%
Valença et al. [37]	Clonazepam	2 mg	6 weeks	14	NA	Of patients with panic attack after CO_2 challenge at baseline, 14% had panic attacks after treatment
Nardi et al. [47]	Clonazepam	1–4 mg	3 years	67	0.69	>95% +

+ Statistically significant result compared to placebo; NA: Not available

A variety of trials induced acute panic attacks in PD patients under laboratory conditions [26]. These experiments used different methods, such as oral administration of caffeine [26,27], breath-holding [28–30], hyperventilation [29–36], and respiratory challenge tests with CO_2 inhalation [37–40].

CO_2 increases anxiety and induces panic attacks in PD patients [41]. A true anti-panic agent should block CO_2-induced panic as well as spontaneous attacks [37]. Therefore, Nardi [42] aimed to determine if an acute dose of clonazepam 2 mg attenuates panic attacks induced by inhalation of 35% CO_2 in PD patients. Twenty-two patients who had been off medication for 1 week participated in a CO_2 challenge test 1 h after a dose of either 2 mg of clonazepam or placebo, using a randomized double-blind design. In double-blind fashion, during the tests patients inhaled either atmospheric compressed air ('placebo control') or the CO_2 mixture. All patients participated in both tests. Anxiety levels and panic symptoms were assessed immediately before and after inhalation. In the clonazepam group, 18.2% had a mild panic attack, while in the placebo group 81.8% had a moderate to severe panic attack in the CO_2 challenge test. After the CO_2 test, anxiety levels were significantly higher in the CO_2 group ($p = 0.013$). These findings were confirmed in a double-blind study of 34 PD patients, where the panicogenic effect of CO_2 was significantly attenuated after an acute dose of clonazepam (2 mg/day) and after 2 and 6 weeks of treatment, compared to placebo [41].

In an open trial also involving CO_2 challenge, 86% of PD patients who had a panic attack after CO_2 challenge at baseline did not have a panic attack after CO_2 challenge following 6 weeks of treatment with clonazepam 2 mg/day [37]. These results corroborate a pilot study with PD patients in which CO_2-induced attacks were attenuated after approximately 10 days of clonazepam 2 mg/day [43].

According to DSM-5, PD is a single diagnostic category, but the disorder has diverse clinical presentations. Therefore, alternative classification schemes have been devised. Some authors have postulated subtypes like the respiratory subtype (RS) and the non-RS [44,45].Valença et al. found that 93.7% of RS patients had panic attacks during 35% CO_2 inhalation, compared to only 43.4% of non-RS patients, indicating higher sensitivity to CO_2 in RS patients [46]. Nardi et al. [47] described the therapeutic response of RS PD patients versus non-RS treated openly with clonazepam (1–4 mg/day) for 3 years. In the first 8 weeks of treatment, the RS group had a significantly faster response on CGI Severity and Improvement Scales, PD Severity Scale, anticipatory anxiety, overall phobic avoidance, and Hamilton anxiety. During the follow-up (weeks 12–156), there was no difference in the scores, and the reduction in panic attacks from baseline to endpoint did not differ significantly between the two groups. From 1997 to 2017, we found no studies on SSRI treatment of RS PD. Meanwhile, research comparing benzodiazepines to tricyclic antidepressants showed that the latter may be more effective than benzodiazepines in RS patients [46].

Two multicenter studies showed higher efficacy of clonazepam compared to placebo. Moroz and Rosenbaum [48] conducted a 6-week dose-optimization study (clonazepam 0.5–4 mg/day titrated over 3 weeks and then maintained at the optimal dose for 3 weeks). Rosenbaum [49] performed a 9-week dose–response study (clonazepam dose escalated over 3 weeks to 0.5–4 mg/day followed by a 6-week fixed-dose phase). Both studies used a 7-week discontinuation phase. At the therapeutic endpoint, 61.9% of patients were panic-free, compared to 36.8% with placebo ($p < 0.001$). In the dose–response study, 9 weeks of clonazepam 1 mg/day (minimum effective dose) achieved panic-free rates of 73%, compared to 55% with placebo ($p < 0.05$). The 1 mg dose showed the most consistently significant differences from placebo in panic attack frequency.

In a 6-week placebo-controlled trial, Valença et al. [50] treated 24 PD patients with clonazepam 2 mg/day or placebo. At the therapeutic endpoint, 11.1% of placebo patients were free of panic attacks, compared to 61.5% of clonazepam patients ($p = 0.031$). The results are corroborated by Valença [46], who studied 34 PD patients receiving either placebo or clonazepam 2 mg/day. After 6 weeks, the clonazepam group showed statistically significant clinical improvement, i.e. remission of panic attacks ($p < 0.001$) and decrease in anxiety ($p = 0.024$).

4. The clinical strategies in pharmacotherapy

4.1. Advantages and disadvantages of SSRIs

SSRIs are recommended as first-line treatments for PD, and a variety of randomized, controlled trials have demonstrated their efficacy in PD treatment [1,9]. Regarding the SSRIs, no evidence suggests a differential efficacy within the class, whereas differences exist in side effects profiles (Table 3), half-life and drug interaction [4].

Even though a withdrawal syndrome has been observed when an SSRI is abruptly discontinued, this drug class is not associated with tolerance or physical dependence [51]. Moreover, SSRIs should be preferred in PD patients with other comorbid disorders, such as depression and/or obsessive- compulsive disorder [10].

The SSRIs are a heterogeneous drug class. Since serotonin transporter inhibition is the common mechanism of action of all drugs in this class, secondary binding properties to other sub-receptor sites more likely account for the observed

Table 3. Some differences in side effects profile among the SSRIs.

	Citalopram	Escitalopram	Fluoxetina	Fluvoxamina	Paroxetina	Sertraline
Sedation	+	0	0	+	+	+
Insomnia	+	+	+	+	+	+
Hyperstimulation	+	+	++	+	++	+
Nausea	+	+	+	+	+	+
Weight gain	+	0	0	0	+	+
Sexual dysfunction	+	+	+	+	++	+

differences between the different drugs. The plethora of well-documented biological substrates, receptors, and pathways for serotonin are candidates to mediate the therapeutic actions and side effects of SSRIs. Across published studies, adverse events reported more frequently by patients receiving SSRIs than those receiving placebo were sudoresis, diarrhea, nausea, vomiting, and sexual dysfunction [10,14,16]. Dry mouth, dyspepsia, headache [10,14,15], tremor, asthenia, drowsiness, and insomnia [14,20] were also reported as side effects of SSRIs in PD.

There are some hypothetical biological substrates for the different side effects observed with SSRIs. For example, central control of nausea and vomiting is known to be mediated both in the hypothalamus and in the brainstem. The acute increase in serotonin in such areas could cause these side effects. Alternatively, nausea may result from peripheral signals. Antagonism of 5HT3 receptors blocks the nausea produced by SSRIs. Gastrointestinal cramps and diarrhea can result from increased serotonin at 5HT3 receptors in the gut [52]. Another adverse effect, the anxiogenic initiating actions of SSRIs, may be mediated by the hypothalamus and limbic cortex [52]. Brainstem sleep centers are controlled by serotonergic input. Increased serotonin in these areas may be responsible for the insomnia and sleep disruption seen with SSRIs [52]. Sexual function is probably mediated by a large number of neuronal pathways. However, orgasm and ejaculation are mediated in part by sympathetic and parasympathetic pathways and are spinal reflexes [52]. Serotonergic pathways descending the spinal cord with synapses on 5HT2 receptors may thus mediate some of the observed sexual dysfunctions associated with SSRI administration, although other 5HT receptors may also be involved [52].

A low dose of an SSRI is recommended at the beginning of the treatment to minimize the onset and severity of side effects [9,10]. The dose should then be slowly titrated upward to reach the effective dose. Usually, in the first 4–8 weeks of treatment with an SSRI, a concomitant administration of benzodiazepines may be useful to improve clinical condition or to reduce the anxiety symptoms that may get worse at the beginning of an SSRIs treatment [9]. Furthermore, patients should be informed about the frequency and the time of onset of side effects, and about the decrease of their severity as treatment is continued.

Moreover, even though SSRI administration is not associated with physical dependence, an abrupt discontinuation of the drug may induce a withdrawal syndrome [51]. The withdrawal syndrome begins a few days after discontinuation and is characterized by nausea, insomnia, incoordination, headache, irritability, and tends to resolve spontaneously within 1–2 weeks [51]. This syndrome is more likely to affect patients treated for several months with a short half-life compound, and after a short period of tapering [51].

4.2. Advantages and disadvantages of benzodiazepines

High-potency benzodiazepines are effective in treating PD and panic attacks with or without agoraphobia. In the past decade, this class remained one of the most prescribed medications for the treatment of PD [24]. Although benzodiazepines act rapidly and are well tolerated, their use presents clinical issues such as dependence, memory impairment rebound anxiety, and discontinuation syndrome [53]. In the benzodiazepines class there are differences in half-life and drug interaction (Table 4). Furthermore, the use of these drugs presents some disadvantages compared to the prescription of other effective medications (Table 5).

Benzodiazepines are positive allosteric modulators of γ-aminobutyricacid type-A receptors ($GABA_A$). $GABA_A$ are ligand-gated chloride-selective ion channels that are physiologically activated by GABA, the main inhibitory neurotransmitter in the brain. Pharmacological and behavioral studies have shown that $GABA_A$ containing the a1 subunit mediate the sedative, anterograde amnesic, and in part the anticonvulsive effects of benzodiazepines. $GABA_A$ containing a2 mediate the anxiolytic actions and to a large degree the myorelaxant effects. $GABA_A$ containing a3 and a5 also contribute to benzodiazepines' myorelaxant actions, whereas $GABA_A$ comprising the a5 subunit were shown to modulate the temporal and spatial memory effects of benzodiazepines. The addictive properties of benzodiazepines have been shown to require the a1-containing $GABA_A$ [54]. Side effect profiles for benzodiazepines in the treatment of PD are very similar and are mostly related to $GABA_A$. Across all published studies, the most common side effects were ataxia or incoordination, fatigue, slurred speech, drowsiness, memory problems, sexual

Table 4. Some differences among benzodiazepines prescribed in clinical practice.

Drug	Half-life range (hr)	Time to peak plasma concentration (hr)	Primary elimination
Chlordiazepoxide	5–30	0.5–4	Renal (1–2), 3–6% as conjugate
Clorazepate	30–100	0.5–2	Renal; fecal
Diazepam	20–80	1–2 (Injection: intramuscular, 0.5–1.5; intravenous, within 0.25) (Sterile emulsion: intramuscular, >2; intravenous, 0.13–0.25) (Rectal gel: 1.5)	Renal
Flurazepam	2.3	0.5–1	Renal
Alprazolam	6.3–26.9	1–2	Renal
Bromazepam	8–19	1–4	Renal
Clonazepam	18–50	1–2	Renal (<2)
Lorazepam	10–20	1–6 (Intramuscular, 1–1.5; sublingual, 1)	Renal
Oxazepam	5–15	1–4	Renal; fecal
Temazepam	8–15	1–2	Renal (<1)

Table 5. SSRIs vs. benzodiazepines.

	SSRIs	Benzodiazepines
Advantages	Treatment of comorbidities like depression, obsessive- compulsive disorder, and social anxiety disorder	Rapid onset of action
Disadvantages	Slower onset of action	Risk of abuse or dependence
	Adverse events such as sudoresis, diarrhea, nausea, vomiting, weight gain and sexual dysfunction	Adverse events such as ataxia, fatigue, slurred speech, sedation, drowsiness, and memory problems

dysfunction (decreased libido, or anorgasmia), dry mouth, constipation, and lightheadedness [47,49].

A withdrawal syndrome may appear after short-term administration of benzodiazepines and its severity depends not only on the half-life and dose of the benzodiazepine, but also on the duration of the treatment and of the taper period [54]. Finally, abuse of benzodiazepines may be found in patients, particularly those with a history of alcohol or substance abuse [54]. Therefore, in these patients, the use of benzodiazepines should be evaluated with caution and, if possible, avoided [54]. Furthermore, benzodiazepines are less effective than SSRIs in treating comorbidities, such as depression associated with PD [53].

4.3. SSRIs vs. benzodiazepines

In an open-label study comparing the SSRI paroxetine to the benzodiazepine clonazepam, the adverse events associated with paroxetine were appetite and weight change, dry mouth, excessive sudoresis, and sexual dysfunction, while clonazepam did not have such effects. It is northworthy that most of the treatment-emergent adverse events with paroxetine were rated as mild to moderate in severity and occurred early in the study. The most common side effects reported in the clonazepam group were difficulties in memory and concentration [55,56]. In a 3-year follow-up of continued monotherapy with either clonazepam or paroxetine in PD, patients on paroxetine experienced more sexual dysfunction, and insomnia when compared to clonazepam [57]. The most frequently reported adverse effects in the clonazepam group were drowsiness and lapse in memory/concentration [57]. Other side effects, such as sedation, somnolence, and impairment of motor coordination are frequently associated with benzodiazepines treatment [9,48,49,58]. Furthermore, the risk of developing tolerance and dependence are limitations for the use of this class [58,59]. In view to this, the available guidelines do not recommend benzodiazepines as the drug of first choice in the treatment of PD [9].

Although recent meta-analysis demonstrated that there was no difference between antidepressants and benzodiazepines in terms of efficacy on panic symptoms, frequency of panic attacks, agoraphobia, and general anxiety [58,60] few head-to-head comparisons have been conducted. Analysis of remission rates showed a benefit for benzodiazepines compared to antidepressants, even if the effect was very small and close to no difference [60,61]. In terms of participants who dropped out due to any cause, it was demonstrated a small difference in favor of benzodiazepines [60]. This could be explained since an SSRI compound takes usually 4–6 weeks to become effective and some PD patients are very sensitive to the 'activation' side effect of this class of drugs. Therefore, anxiety, agitation, and insomnia may increase or appear after administration of the SSRI [52], inducing patients to discontinue treatment because medication could worsen his symptoms. Therefore, the guidelines recommend the combination of SSRIs and benzodiazepines in the first weeks of treatment [9]. The administration of benzodiazepines in the first weeks of treatment may alleviate the 'activation' induced by the SSRI and reduce frequency and severity of panic attacks, before the SSRI become effective [9]. In studies evaluating benzodiazepines and SSRIs combination treatment, such as clonazepam-sertraline or clonazepam-paroxetine [59], it was demonstrated that the combination treatment induced a more rapid response than the SSRI treatment alone [60]. Therefore, an initial combined treatment followed by benzodiazepines tapering after a few weeks may provide early benefit while avoiding the potential adverse consequences of longterm benzodiazepine use [54].

5. Conclusion

PD is a prevalent and disabling anxiety disorder that can be treated effectively. In recent years, a progressive change in the prescribing pattern from benzodiazepines to newer antidepressants such as SSRIs has been observed in PD patients. SSRIs are recommended as first-line treatments for PD and can assist in the treatment of comorbidities in PD, but they have slower onset of action, and have the potential to exacerbate anxiety and panic early in the treatment course. On the other hand, benzodiazepines present rapid onset of action, but can cause withdrawal symptoms, tolerance, and dependence. Therefore, a relatively common practice is to combine SSRIs with benzodiazepines to provide more rapid, and potentially additive, clinical efficacy. Nevertheless, few head-to-head comparisons have been conducted comparing SSRIs to benzodiazepines. In view to this, further research comparing SSRIs and benzodiazepines is needed to advance the evidence-based pharmacotherapy of PD.

6. Expert opinion

PD is a chronic, recurrent anxiety disorder. Inadequate treatment of PD entails serious loss to the patient and high cost to society. SSRIs and benzodiazepines are the most widely prescribed drugs for PD. Table 5 summarizes the advantages and disadvantages of benzodiazepines versus antidepressants in the treatment of PD.

Despite guidelines supporting the use of SSRIs [9],benzodiazepines are the most commonly used medication for PD [24]. The main advantage of benzodiazepines in the treatment

of PD is the rapid therapeutic onset [53]. SSRIs often take weeks before beneficial effects occur, and some patients express an urgent need to reduce their panic attacks. Benzodiazepines may thus be preferable when very rapid control of symptoms is critical [9].

A disadvantage of benzodiazepines for PD is the risk of withdrawal symptoms. In comparison to patients with generalized anxiety, PD patients are more likely to be unsuccessful in discontinuing benzodiazepines [62]. In Klein et al. [63], 36 patients with PD and 35 patients with generalized anxiety disorder entered a controlled discontinuation phase after 2 months of alprazolam. Fifty-two percent of all patients were able to discontinue alprazolam, but only 37% completed the study in terms of maintaining alprazolam-free status for 4 weeks. Eighty-eight percent of PD patients and 56% of generalized anxiety disorder patients withdrew from the discontinuation phase, due either to the development of withdrawal symptoms or failure to adhere to the discontinuation schedule ($p = 0.02$). The authors concluded that PD patients are more likely to experience re-emergence of their symptoms triggered by physiological withdrawal symptoms. To avoid withdrawal symptoms, Nardi et al. [64] developed a protocol for safe tapering-off of clonazepam in patients with PD who had been receiving treatment for at least 3 years. A specific scale for judging withdrawal was developed, called the Composite Benzodiazepine Discontinuation Symptom Scale. In 81 PD patients receiving clonazepam, the authors demonstrated a tapering scheme of 0.25 mg/week in 4 months. The main reported symptoms during clonazepam discontinuation were anxiety, irritability, nausea, vomiting, insomnia, nightmares, excessive sudoresis, tachycardia, headache, weakness, shaking, tremor, muscle aches, stiffness, hyperventilation, panic attacks, memory or concentration difficulties, muscle fasciculation, agoraphobia, phobias, depersonalization, and depressive mood [64].

Although it is undisputed that benzodiazepines can lead to withdrawal symptoms after cessation, little is known about the residual cognitive effects after benzodiazepine treatment in PD. Three and a half years after suspending alprazolam, 15 alprazolam-treated PD patients and 16 placebo-treated PD patients answered some tests, including Free Word Recall, Cancellation of 4s, Symbol Copying Test, Subjective Memory Questionnaire, and National Adult Reading Test. There was no evidence of impaired function on any of the objective tests [65].

Treatment with SSRIs is less likely to lead to withdrawal symptoms compared to benzodiazepines, but carries a risk of discontinuation symptoms if treatment is stopped abruptly. Some symptoms seen with SSRI discontinuation are nausea, lethargy, insomnia, headache, dizziness, sensory abnormalities, anxiety, and palpitations [51]. Along with other side effects, long-term treatment with SSRIs can cause increased lipid levels in PD patients [66,67]. Abnormal bleeding [68] and hyponatremia [69] are also associated with SSRI use. Nevertheless, benzodiazepines are also related to adverse events, such as sedation, drowsiness, impairment of motor coordination, cognitive impairment, and the risk of developing tolerance and dependence [54].

Furthermore, some PD patients are very sensitive to the 'activation' side effect of SSRI medications, which could induce patients to discontinue treatment [52]. Therefore, the co-administration of benzodiazepines in the first weeks of treatment may lower the 'activation' induced by the SSRI and reduce severity and frequency of panic attacks before the SSRI become effective [57].

PD is a chronic condition with relevant effects on patients' quality of life, and requires long-term management. For the individual patient, the goal of therapy is complete cessation of panic attacks and associated anticipatory anxiety, along with the treatment of any comorbidity and reduction in functional disability. The review of the benefits and the risks associated with a drug is basically an evaluation of two dimensions. The dimension of benefits is measured primarily in terms of the successful treatment of the condition for which the drug is indicated. The dimension of risks includes the safety profile observed in the form of the sum of all adverse drug reactions. The current review showed strong evidence of the effectiveness of SSRIs and benzodiazepines in the treatment of PD. Few studies to date have performed head-to-head comparisons of these two drug classes. When looking at the comparison between individual benzodiazepines, the available evidence, suggests that there is no significant difference between individual benzodiazepines in terms of response rate and dropout due to any reason [60]. Furthermore, no evidence suggests a differential efficacy within the SSRIs class [60]. Therefore, the main question of whether there are differences between antidepressants and benzodiazepines, and between individual antidepressants and individual benzodiazepines, remains unanswered. Future studies on the pharmacological treatment of PD should include direct comparison of risks and benefits of these medications. This could help improve the evidence-based pharmacotherapy of PD. Although extremely important, evidence-based medicine is population-based medicine, whereas clinical medicine is practiced one patient at a time. What is right for the average patient is not always right for the individual patient. Therefore, knowing the risks and benefits of the most widely prescribed medications in PD is not only crucial for evolving guidelines, but is also highly important for individual physicians to choose the most adequate drug for each patient and thus achieve the most successful treatment.

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3.2. Artigo 6: Selective serotonin reuptake inhibitors and benzodiazepines in Panic Disorder: a meta-analysis of common side effects in acute treatment

(Artigo em submissão.)

Selective serotonin reuptake inhibitors and benzodiazepines in Panic Disorder: a meta-analysis of common side effects in acute treatment

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ABSTRACT

Background: Benzodiazepines (BZs) and selective serotonin reuptake inhibitors (SSRIs) are effective in the pharmacologic treatment of panic disorder (PD). However, treatment guidelines favor SSRIs over BZs based on the belief that BZs are associated with more adverse effects than SSRIs. This belief, though, is currently supported only by opinion and anecdotes.

Aim: The aim of this review and meta-analysis is to determine if there truly is evidence that BZs cause more adverse effects than SSRIs in acute PD treatment.

Methods: We systematically searched Web of Science, PubMed, Cochrane Central Register of Controlled Trials and clinical trials register databases. Short-randomized clinical trials of at least 4 weeks and maximum of 12 weeks that studied SSRIs or BZs compared to placebo in acute PD treatment were included in a meta-analysis. The primary outcome was all-cause adverse event rate in participants who received SSRIs, BZs, or placebo.

Results: Overall, the meta-analysis showed that SSRIs cause more adverse events than BZs in short-term PD treatment. Specifically, SSRI treatment was a risk factor for diaphoresis, fatigue, nausea, diarrhea, and insomnia, whereas BZ treatment was a risk factor for memory problems, constipation, and dry mouth. Both classes of drugs were associated with somnolence. SSRIs were associated with abnormal ejaculation, while BZs were associated with libido reduction. BZs were protective against tachycardia, diaphoresis, fatigue and insomnia.

Conclusion: Randomized, blinded studies comparing SSRIs and BZs for short term treatment of PD should be performed. Clinical guidelines based on incontrovertible evidence are needed.

INTRODUCTION

Treatment guidelines for panic disorder (PD) have favored selective serotonin reuptake inhibitors (SSRIs) over benzodiazepines (BZs) (American Psychiatric Association, 1998; Nielsen et al., 2012) for short- and long-term treatment. Considering that BZs have not been demonstrated to be inferior to SSRIs in terms of efficacy (American Psychiatric Association, 1998), there are two main reasons for this position: 1.the assertion that BZs induce more drug dependence than SSRIs after long-term use;(Roy-Byrne et al., 2006; Baldwin et al., 2014)2. The notion that BZs cause a higher risk of discontinuation symptoms than SSRIs(Bandelow et al., 2008; Roy-Byrne et al., 2006). In addition, the notion that BZs are associated with more adverse effects than SSRIs, restricts the prescription of this drug class (Nielsen et al., 2012).

Some authors advocate that the key signs of BZs dependence are withdrawal symptoms, such as tremor, dizziness, anxiety and insomnia, on dosage reduction or discontinuation(Ashton, 2005). Indeed, withdrawal symptoms and misuse of BZs may occur, especially in the context of other substance abuse(Tvete et al., 2013). However, it is important to distinguish between a physiologic dependency to and a natural adaptation of a body system long accustomed to the presence of any drug, including BZs and SSRIs (O'Brien, 2005). A predictable and natural adaptation of any body system long accustomed to the presence of a drug may occur in patients taking therapeutic doses of BZs(O'Brien, 2005). However, this situation, which generally manifests itself in withdrawal symptoms upon the abrupt discontinuation of the medication, may be controlled and ended through dose tapering, medication switching, or medication augmentation(O'Brien, 2005). There is a subgroup of PD patients comorbid with substance use disorders that when exposed without BZs, are more likely to present a dose escalation and to exhibit a dependency syndrome, with BZs withdrawal symptoms(Ait-Daoud et al., 2018). However, withdrawal reactions may ensue with either BZs or SSRIs (Nielsen et al., 2012; Fava, 2006; Fava et al., 2015; Greenblatt et al., 1990; Rickels et al., 1990; Schweizer et al., 1990; Nardi et al., 2010; Bhanji et al., 2006; Belaise et al., 2012). In both classes, this syndrome is characterized by symptoms such as anxiety, crying, dizziness, headache, increased dreaming, insomnia, irritability, myoclonus, nausea, paresthesia and tremor (Starcevic, 2012b; Nielsen et al., 2012).

The supposition that BZs are more addictive than SSRIs is largely related to ill-informed conceptualizations of addiction. The term addiction is commonly related to a diagnostic of dependence of a substance. The

diagnostic criteria in psychiatry are subjective and not based on evidence, and it is well known that the panel of experts at the front of the DSM often receives great incentives from the pharmaceutical industry (Cosgrove et al., 2012; Neuman et al., 2011). In DSM-III revision (American Psychiatric Association,1987), the criteria for dependence was modified and it became widely accepted that BZs as a class caused dependence (Nielsen et al., 2012). In this same period, the SSRIs entered the market, and were market as drugs that did not cause this problem (Nielsen et al., 2012). However, as the years goes by, it became clear that SSRIs might cause a discontinuation syndrome (Haddad, 1998; Rosenbaum et al., 1998). This syndrome is quite similar to BZs withdrawal syndrome, and is characterized by symptoms such as anxiety, crying, dizziness, headache, increased dreaming, insomnia, irritability, myoclonus, nausea, paresthesia and tremor (Starcevic, 2012b; Nielsen et al., 2012). In this discontinuation syndrome, SSRIs with a shorter half-life (e.g., paroxetine) seem more likely to be associated with the discontinuation symptoms than SSRIs with a longer half-life (e.g., fluoxetine), just like BZs with a shorter half-life (e.g., alprazolam) are more likely to be associated with the withdrawal symptoms than BZs with a longer half-life (e.g., clonazepam) (Starcevic, 2009).Therefore, we argue how much the discontinuation syndrome from SSRIs differ from the withdrawal syndrome of BZs? The fact that the discontinuation symptoms occur with SSRIs does not mean that these agents are addictive (Fava et al., 2015). Why should the occurrence of the withdrawal symptoms with BZs be taken as evidence that BZs are addictive? PD patients treated with BZs, in the absence of misuse of other substances, rarely exhibit an addictive behavior (American Psychiatric Association, 2013), with an all-encompassing preoccupation with BZs, craving for these medications, uncontrollable BZs-seeking behavior or financial or legal problems due to BZs use (Starcevic, 2012b; Nielsen et al., 2012). There is no evidence that general, non-substance abusing psychiatric patients are likely to be euphoric from BZs use (Griffin et al., 2013). However, BZs have immediate effects on patients, which make then more likely to reinforce their own administration than SSRIs (Griffin et al., 2013). Therefore, BZs have higher liability for misuse than SSRIs (Evans et al., 2014). Nevertheless, addiction is not usually an applicable term to describe the effects of these agents during appropriate treatment of PD with BZs. Furthermore, a commonly exhibited opinion that addiction is a moral failing and that substance use is a choice(Stanbrook, 2012) coupled with a general tendency towards an under recognition and undertreatment of PD(Bandelow and Michaelis, 2015) might contribute to deprecated BZs in short-term PD treatment.

This article will focus on the notion embedded in PD treatment guidelines that BZs produce more adverse effects than SSRIs and are therefore less tolerable in short-term PD treatment. We conducted a systematic review of the literature and meta-analysis of adverse effects associated with use of SSRIs and BZs in short-term PD treatment.

METHODS

This systematic review was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement extension for harms (Zorzela et al., 2016) (Supplementary Material 1) and was conducted following an *apriori*–established protocol registered with PROSPERO (CRD42018104339).

Search Strategy

The databases included PubMed, Web of Science, and Cochrane Central Register of Controlled Trials which were searched from inception to January 20, 2019. Clinical trial registries (http://www.clinicaltrials.gov and http: //www.clinicaltrialsregister.eu) and published systematic reviews were screened for additional studies. To avoid publication bias, non-English language studies, specifically Chinese language manuscripts, and grey literature (for example, conference abstracts) were not included (Wu et al., 2006). A broad but highly structured search strategy was used, based on the PICOS framework (Richardson et al., 1995).

Studies were included if they met the following criteria: a) they enrolled adult (> 18 years) acute PD patients, with or without agoraphobia, without any other comorbidities; b) medications (BZs and SSRIs) approved for acute PD treatment by North American (American Psychiatric Association, 1998) or European (National Institute for Health and Clinical Excellence, 2011) regulatory agencies were administered at the most effective recommended doses for at least 4 weeks and a maximum of 12 weeks; c) there was a placebo comparison group; d) adverse events associated with SSRIs or BZs short-term PD treatment were reported, and e) study design was a randomized controlled trial.

In flexible-dose studies we assume that the doctors would titrate the medication to find the ideal dose for the individual patient. In studies that examined several fixed doses we will include only the optimum

doses as they were recommended by North American (American Psychiatric Association, 1998) or European guidelines (National Institute for Health and Clinical Excellence, 2011).

Observational studies, trials of PD short-term treatment non-approved by European (National Institute for Health and Clinical Excellence, 2011) or North American (American Psychiatric Association, 1998) regulatory agencies, trials of fixed-dose combination drugs (e.g., alprazolam-sertraline), trials in which drugs were used as an augmentation strategy to any other psychotropic medication, and investigations that involved any modality of psychotherapy or other non-pharmacological treatments, such as transcranial magnetic stimulation, were excluded. Additionally, studies with less than 10 patients were excluded because of the lack of statistical power.

The search queried the following terms with numerous synonyms and related words: Panic Disorder AND (selective serotonin reuptake inhibitors OR benzodiazepines) AND adverse events. A full list of terms used for each search can be found in Supplementary Material 2.

Data Abstraction and Quality Assessment

Data on study-, patient- and treatment-related characteristics were abstracted onto a standardized form. The following variables were extracted from all studies: authors, year of publication, subject characteristics of the group, drugs measure characteristics (duration and dose of drugs treatment). When patients were randomized to different doses of the active intervention, only data for the highest approved dosage of the medication were used. The risk of bias of individual studies was assessed using the Cochrane Risk of Bias assessment tool (Higgins et al., 2011). AEN and LAQ agreed with the final inclusion of studies into the systematic review, data abstraction, and quality assessment. Details of the data abstraction and the quality assessment are reported in the Supplementary Material 3.

Statistical analyses

Separate meta-analyses were performed for each individual adverse event for each class, SSRIs or BZs (versus placebo). P-values were considered statistically significant at the $\alpha = 0.05$ level. Random-effects pairwise meta-analyses, using the DerSimonian and Laird random effects model (DerSimonian and Laird, 1986), were conducted in the R statistical programming language version 3.2.2, using the R studio

software, with the 'meta' package, through the metabin command (R ConsortiumTeam, 2013). The main outcome measure was all-cause adverse event rate in acute PD patients who received SSRIs, BZs, or placebo. The process of grouping the adverse events observed during the evaluation of the eligible and selected studies for the metanalytic step was performed by extracting all the dichotomous outcome measures linked to the population of interest (intervention group, placebo-control group), through the presentation of the number of participants who reported some adverse event and, subsequently, the incidence of a certain adverse event for measurement and analysis of outcomes related to these events. We calculated the effect measure (OR) from the raw numbers from a 2x2 table for dichotomous outcomes. An odds ratio greater than 1.0 indicates that the exposure to a drug might be a risk factor for the adverse event, while an odds ratio less than 1.0 indicates that the exposure to a drug might be a protective factor against the adverse effect. The results of the meta-analysis and the comparison of the intervention group and the control group with respect to the reported adverse events were presented by the Forest Plot, grouped by adverse reaction and drug class.

Heterogeneity was estimated using the I^2 value (I^2 values <50% indicate low to moderate heterogeneity, whereas I^2 >50% indicate moderate to high heterogeneity). The meta-analysis procedure also calculates a χ^2 value for the between-study heterogeneity in effect size estimates, using Cochran's Q-statistic (Huedo-Medina et al., 2006). If heterogeneity was significant, we intended to perform a sensitivity analysis, carried out by removing one study at a time and repeating the meta-analysis procedure, to examine its impact on the odds ratio and between-study heterogeneity(Huedo-Medina et al., 2006). In addition, in order to explain possible heterogeneity, we aimed to conduct meta-regression analysis, when there was at least ten studies(Higgins et al., 2011), using industry sponsorship, dose-response and age as covariates. We intended to assess publication bias using funnel plot techniques, Begg's rank test and Egger's regression test, as appropriate given the known limitations of these methods (Higgins et al., 2011).

RESULTS

The literature search identified 6,577 potentially relevant articles for initial screening. Duplications (N=1,811) were identified using a function in Endnote and confirmed by manual screening of the titles. We excluded 4,766 studies from first assessment of titles and abstracts, since they did not meet the selection criteria mentioned above (see Figure 1 for a PRISMA diagram of literature search). Full texts were obtained on 198 abstracts that were classified as possible for inclusion. One hundred seventy seven papers were excluded from further analysis, since they did not compare drugs with a placebo, or quantify SSRIs or BZs adverse events in short-term PD treatment. In addition, studies without adverse events as an outcome, investigated psychotherapy or other non-pharmacological treatments, evaluated augmentation strategies in PD or included PD patients with comorbidities were excluded. Of the 21 studies included in meta-analyses, 10 investigated adverse events in BZs and 11 in SSRIs (see Supplementary Material 2 for the search results and Supplementary Material 3 for the subject characteristics for the included studies identified from our searches). The adverse events were assessed in studies by different methods, such as inventories, as Symptoms and Side Effects Checklist, or interviews (Supplementary Material 3). Publication bias was not assessed as there were inadequate numbers of included trials to properly assess a funnel plot or more advanced regression-based assessments. Figure 2 shows forest plots of the adverse events diaphoresis, fatigue, somnolence. Figure 3 exhibits forest plots of sexual dysfunction (abnormal ejaculation and decrease of libido), insomnia, and diarrhea. Figure 4 shows forest plots of nausea, constipation, tachycardia, dry mouth, and memory problems. Reported unwanted effects are discussed next.

[insert Figure 1.]

Diaphoresis

Two studies including a total 623 PD patients evaluated diaphoresis as an adverse event in 404 SSRI and 219 placebo treated patients (Lecrubier et al., 1997; Wade et al., 1997). SSRIs were associated with an increased risk for diaphoresis (OR=2.09; $p<0.01$; CI= 1.23-3.56). There was no evidence of statistical heterogeneity across the studies (I^2= 0%). Regarding BZs, three studies evaluating 1,383 PD patients assessed for differences in diaphoresis between 690 BZ and 693 placebo treated patients (Andersch et

al., 1991; Cross-National Collaborative Panic Study, 1992; Noyes et al., 1988). BZs showed a reduction in diaphoresis (OR=0.54; p<0.01; CI=0.33-0.86; I^2= 0).

Fatigue

Fatigue, defined as a sensation in which the individual feels weak and with lack of motivation, was evaluated in SSRI and BZ trials. SSRIs and BZs were compared to placebo across 1,381 and 2,155 PD patients, respectively (Asnis et al., 2001; Ballenger et al., 1998; Cassano et al, 1994; Cross-national Collaborative Panic Study, 1992; Lecrubier et al., 1997; Londborg et al., 1998; Lydiard et al, 1992; Noyes et al, 1998; Pollack et al., 1998; Sheikh et al., 2000). Fatigue was reported in 880 SSRI and 501 placebo treated patients. SSRIs demonstrated a risk to cause fatigue (OR=1.76; p=0.02; CI=1.07-2.87; I^2= 36%). One thousand and eighty-eight BZ and 1,067 placebo treated patients were evaluated for fatigue. BZs exhibited a protective effect (OR=0.37; p<0.01; CI=0.21-0.64; I^2=52%).

Somnolence

Daytime somnolence was compared in 903 SSRI and 524 placebo treated patients. It was also evaluated in 799 patients comparing 399 BZ to 400 placebo acute treated PD patients (Asnis et al., 2001; Ballenger et al., 1998; Cassano et al, 1994; Lecrubier et al., 1997; Londborg et al., 1998; Pollack et al, 1998; Sandmann et al., 1995; Sheikh et al., 2000; Valença et al., 2000). Both drugs increase somnolence during the day (BZs: OR=4.59; p=0.03; CI=1.12-18.72; I^2=32%; SSRIs: OR=2.03; p<0.01; CI=1.36-3.02; I^2=4%).

Nausea

Ten studies investigated the adverse event nausea in acute PD patients comparing 1,353 acute PD patients treated with SSRIs and 797 acute PD patients treated with placebo (Asnis et al., 2001; Ballenger et al., 1998; Lecrubier et al., 1997; Londborg et al., 1998; Michelson et al., 2000; Pohl et al., 1998; Pollack et al., 1998; Sandmann et al., Sheik et al, 2000; 1995; Wade et al., 1997). SSRIs increased risk of nausea (OR=1.68; p<0.01; CI=1.34-2.10) with no evidence of statistical heterogeneity across the studies (I^2= 0%). Although five studies evaluated nausea comparing 1,121 BZs and 1,097 placebo treated patients, (Beauclair et al, 1994; Cassano et al, 1994; Cross-National Collaborative Panic Study, 1992; Lydiard et al., 1992; Noyes et al., 1988) we could not find a risk or a protective effect for this class of drugs in this adverse event (CI=0.37-1.14).

Diarrhea and constipation

The SSRIs investigation on diarrhea were conducted in 1,405 PD patients (Ballenger et al., 1998; Lecrubier et al., 1997; Londborg et al., 1998; Pohl et al., 1998; Pollack et al., 1998; Sandmann et al., 1995; Sheikh et al., 2000b). Eight hundred and eighty-nine acute PD patients were treated with SSRIs and 516 were taking placebo. SSRIs were associated with an increased risk of diarrhea (OR=2.71; p<0.01; CI=1.83-4.02; I^2=0%). Meanwhile, only one study investigated BZs and diarrhea, in 263 BZs and 262 placebo treated patients, with no significant result (Noyes et al., 1988). Acute BZ treatment of 1,129 PD patients was compared to administration of placebo to 1,108 patients to evaluate constipation. BZs presented as a risk factor for constipation (OR=2.52; p<0.01; CI=1.84-3.46; I^2=0%) (Andersch et al, 1991; Cassano et al, 1994; Cross-National Collaborative Panic Study, 1992; Lydiard et al., 1992; Noyes et al., 1988). Although three studies evaluated this adverse event comparing 613 SSRIs with 288 placebo treated patients (Ballenger et al., 1998; Lecrubier et al., 1997; Wade et al., 1997), we could not find a risk or a protector effect for this class of drugs (CI=0.56-1.98).

Sexual dysfunction

Seven studies assessed SSRIs and placebo regarding sexual dysfunction (Ballenger et al., 1998; Lecrubier et al., 1997; Londborg et al., 1998; Pohl et al., 1998; Pollack et al., 1998; Sheik et al, 2000; Wade et al., 1997), one of them evaluated anorgasmia (Wade et al., 1997) in 281 SSRIs and 96 placebo acute PD treated patients, while the others investigated abnormal ejaculation in 866 SSRIs and 493 short-term placebo treated patients (Ballenger et al., 1998; Lecrubier et al., 1997; Londborg et al., 1998; Pohl et al., 1998; Pollack et al., 1998; Sheikh et al., 2000b). These studies demonstrated that SSRIs increased the risk for abnormal ejaculation (OR=15.13; p<0.01; CI=5.40-42.34; I^2=0%). In our review we did not find SSRIs trials evaluating libido reduction, an adverse event only investigated in BZs trials of short-term PD treatment. This event was investigated in 652 patients comparing 342 BZs with 310 placebo treated patients (Beauclair et al., 1994; Lydiard et al., 1992; Noyes et al., 1988; Valença et al., 2000). BZs use was associated with a risk for libido reduction (OR=4.28; p<0.01; CI=2.23-8.23; I^2=0%).

Insomnia

Nine studies evaluated insomnia in 1,274 SSRIs acutely treated patients compared to 710 placebo acutely treated patients (Asnis et al., 2001; Ballenger et al., 1998; Lecrubier et al., 1997; Londborg et al., 1998; Michelson et al., 2000; Pollack et al., 1998; Sandmann et al., 1995; Sheik et al, 2000; Wade et al., 1997) and four investigations assessed 1,088 BZs and 1,067 placebo treated (Cassano et al, 1994; Cross-National Collaborative Panic Study 1992;Lydiard et al., 1992; Noyes et al., 1988). The findings from these studies demonstrated that treatment with SSRIs constitute a risk factor for insomnia (OR=1.54; p<0.01; CI=1.15-2.05; I^2=2%) while BZs showed a protective effect (OR=0.37; p<0.01; CI=0.21-0.64; I^2=52%).

Tachycardia

Only one study (Stahl et al., 2003) assessed tachycardia in 128 SSRIs users contrasted to 119 placebo treated patients with no significant result. Five studies compared 1,228 BZs to 1,136 placebo short-term treated PD patients regarding tachycardia (Cassano et al, 1994; Cross-National Collaborative Panic Study 1992; Lydiard et al., 1992; Noyes et al., 1988; Pecknold et al., 1994). BZs demonstrated a protective effect in this event (OR= 0.43; p<0.01; CI=0.30-0.61; I^2=0%).

Dry mouth

Although eight studies (Ballenger et al., 1998; Hoehn-Saric et al., 1993; Lecrubier et al., 1997; Londborg et al., 1998; Pohl et al., 1998; Pollack et al., 1998; Wade et al., 1997; Sheikh et al., 2000) evaluated dry mouth comparing 1,172 SSRIs with 614 placebo acute treated patients, we could not find a risk or protective effect for this class of drugs (CI=0.87-2.72). Six investigations were conducted evaluating dry mouth in 2,446 PD patients comparing 1,269 BZs to 1,177 placebo treated patients (Andersch at al, 1991; Cassano et al, , 1994; Cross-National Collaborative Panic Study 1992; Lydiard et al., 1992; Noyes et al., 1988; Pecknold et al., 1994). BZs were associated with an increased risk (OR=1.54; p<0.01; CI=1.19-2.00; I^2=0%).

Memory problems

Only one investigation (Stahl et al., 2003) compared 128 SSRIs to 119 placebo treated patients concerning memory retrieval complaints (OR=4.80, CI=1.9-12.13). Seven studies assessed memory recall, comparing 1,254 BZs to 1,161 placebo acute treated patients (Beauclair et al, 1994; Cassano et al, 1994; Cross-National Collaborative Panic Study 1992; Lydiard et al., 1992; Noyes et al., 1988; Pecknold et al., 1994

Valença et al., 2000). It was shown that BZs increased the risk for memory problems in acute PD treatment (OR=2.11; p<0.01; CI=1.40-3.18; I^2=43%).

[insert Figure 2.]

[insert Figure 3.]

[insert Figure 4.]

Other adverse events

Nervousness, headache, dizziness, and tremor were mentioned in the studies included. No significant differences were observed between SSRIs and BZs. Details on the calculated differences can be seen in Supplementary Figures 5 and 6.

DISCUSSION

Our systematic review of the literature and meta-analysis evaluated short-term PD treatment and showed that SSRIs are associated with diaphoresis, fatigue, nausea, diarrhea, and insomnia, whereas BZs are associated with constipation, memory problems, and dry mouth. Both classes of drugs were associated with daytime somnolence. SSRIs were associated with a higher risk of sexual dysfunction than BZs. Only BZs appear to be protective in PD treatment against tachycardia, diaphoresis, fatigue, and insomnia.

Adverse events are considered one important factor for treatment dropout rates in mental health disorders (Zagmutt and Tarrants, 2012). Considering that PD patients presents the interpretation of bodily sensations as threatening, since the primary fear in PD is the fear of physical sensations, adverse events in the begging of a PD treatment are especially troublesome(Clark et al., 1997). The interpretation of body sensations as dangerous and threatening would facilitate chronic apprehension and hypervigilance of individuals with the diagnosis of PD(Clark et al., 1997).

Our systematic review demonstrated that SSRIs are related to adverse events, namely diaphoresis, fatigue, nausea, diarrhea, insomnia, and sexual side effects in short-term PD treatment. It is important to note that the risk for some events have a relationship only to the first days of a treatment(Predictable,

2006). Symptoms, such as nausea and diarrhea, caused by SSRIs are common transient effects of short-term treatment with SSRIs, not lasting more than 12 weeks(Predictable, 2006). Indeed, many of these symptoms associated are related to SSRIs activation syndrome, that usually occurs at the beginning of treatment with SSRIs (Predictable, 2006). Although this activated symptoms may reduce over time, they might increase hypervigilance and anxiety in acute PD patients, contributing to a worse disease scenario (Clark et al., 1997).

Meanwhile, BZs appear to be protective in symptoms, such as tachycardia, diaphoresis, fatigue, and insomnia. BZs allows a rapid reduction in anxiety, and a decrease of sympathetic symptoms(Vemulapalli and Barletta, 1984). In fact, some BZs, such as Clonazepam, may lower blood pressure(Dmitriev et al., 2001) and could contribute to atrial fibrillation treatment in PD patients (Kahn et al., 2018). Nevertheless, in our review, BZs were associated with an increase in the risk of memory problems, constipation, and dry mouth. Memory problems are associated with BZs use and could affect a patient's quality of life. However, some BZs may cause more memory problems than others (Beracochea, 2006). In addition, other factors may contribute to BZ's memory impairment in PD patients, such as elderly patient (Verdoux et al., 2005), substance abuse disorder (Gould, 2010) and/or sleep disturbances (Cellini, 2017) , or use of concomitant medications with anticholinergic properties (Carriere et al., 2009). Impairments in memory registration or recall require relatively high drug levels of BZs in serum of patients(Griffin III et al., 2013). The cognitive impairment due to BZs is usually low grade and not apparent to patients. However, it may persist, to some degree, even after medication withdrawal (Stewart, 2005).

Our review demonstrated that SSRIs and BZs were associated with an increased risk for day time somnolence. However, this adverse event had different incident proportions, since different drugs presented different rates of day time somnolence. When comparing to placebo BZs, as a class, presented a higher risk of causing somnolence than SSRIs. However, some SSRIs presented some particularities regarding the risk of causing day time somnolence. For instance, the SSRIs sertraline and fluvoxamine presented approximately two times more risk of causing somnolence than other SSRIs in PD patients(Sheikh et al., 2000a; Asnis et al., 2001).

Many clinicians consider cotherapy of SSRIs and BZs as first-line in acute PD treatment (Nardi et al., 2018). It offsets disadvantages of SSRIs adverse effects that can be quite severe, especially at the beginning of

treatment(Nardi et al., 2018). Most PD patients will need not only adequate medication treatment, but also psychotherapy to prevent recurrence of panic attacks(Nardi et al., 2018). The most empirically supported psychosocial treatment, with the largest effect size for PD is cognitive behavioral therapy (Tolin, 2017). Particularly, exposure therapy, of which the central focus is repeated exposure to feared situations and sensations, supported by a set of control-based coping skills, has a fast onset of effect on PD(Meuret et al., 2012).

Our systematic review only evaluated adverse events in short-term PD treatment. Therefore, a variety of adverse events, such as cardiovascular events and weight gain, were not addressed in this systematic review and should be assess in future studies. Nevertheless, it is important to note that the literature regarding adverse events of SSRIs and BZs in long-term PD treatment is insufficient, and our search strategy only found long-term studies highly heterogeneous, and open label studies that were not homogeneous enough to enter a statistical analysis (Supplementary Material 2). Therefore, future research should focus on comparisons between BZs and SSRIs regarding long-term treatment, and dependence and misuse in different anxiety populations.

A rational use of BZs is needed, taking into account that not all BZs are the same (Cosci et al., 2015) since some (e.g., alprazolam) may be more likely to induce dependence than others (e.g., clonazepam) (Ait-Daoud et al., 2018). Dependence and tolerance are related to the profile of the BZs used and occurs at different rates for different patients. There is a subgroup of patients that are at risk of from substance use disorders that when are exposed to BZs, are more likely to escalate their dose (Ait-Daoud et al., 2018). In addition, a realistic view of the potential for dependence, toxicity, and abuse of BZs is warranted, but the percentage of individuals abusing them is low in relation to the total number of people using these medications (American Psychiatric Association, 1990). As Greenblatt et al (1983) pointed out, since BZs do not permanently cure anxiety or insomnia, symptom recurrence can be anticipated after their discontinuation, and many critics may have confounded symptoms of withdrawal with recurrence of the anxiety disorder symptomatology. Furthermore, the withdrawal phenomena that occur with SSRIs (Chouinard and Chouinard, 2015; Nielsen et al., 2012;) have been frequently ignored. In the case of SSRIs, these phenomena have been termed “discontinuation syndromes”, but are in no way milder or less troublesome than those entailed by BZs (Nielsen et al., 2012). Although persistent withdrawal syndromes

from BZs have been described(Ashton, 1987), BZs mainly induce short-lasting withdrawal symptoms (Chouinard and Chouinard, 2015) (the so called new withdrawal symptoms and rebound according to Chouinard & Chouinard diagnostic criteria (Chouinard and Chouinard, 2015), and SSRI discontinuation or tapering can trigger persistent post-withdrawal syndromes which are long lasting, severe, and reduce the working, social, and family functioning of the patients(Csoka and Shipko, 2006).

The present study has several limitations. Since there is a paucity of head-to-head trials between SSRIs and BZs in PD, our meta-analysis did not compare directly adverse events caused by SSRIs, BZs, and placebos in PD treatment, and it is possible that the populations in SSRIs and BZs studies comparing different adverse events were not completely homogeneous. Furthermore, the adverse events were not associated with an overall measure of a patient's quality of life or satisfaction with a treatment. It is important to note that more adverse events are not equal to more serious side effects and that some side effects might affect a patient's quality of life more than others.

Sexual dysfunction caused by SSRIs is troublesome and may reduce a patient's quality of life. Our review found evidence for abnormal ejaculation with SSRIs. Although, SSRIs were related to a high risk of causing ejaculatory dysfunction in male PD patients, also because delayed ejaculation is one side effect of SSRIs, they are sometimes used off-label to treat premature ejaculation(Linton and Wylie, 2010). However, long-acting SSRIs require chronic dosing, which can cause other side effects, including decreased libido and erectile dysfunction(Hsu and Shen, 1995). Although libido reduction was associated with BZs in our review, comparing to placebo this adverse event is much less related to BZs than with SSRIs (Montejo et al., 2001; Montejo-Gonzalez et al., 1997; Simoes et al., 2010). BZ's reduction of sexual performance anxiety may in some instances increase libido (Kalmbach et al., 2012). In our review, studies that evaluated sexual dysfunction were heterogeneous and included both sexes, which might contribute to the sexual dysfunction's conclusions.

A limitation in our review is that only published trials were included. Using only published reports may alter the apparent risk–benefit ratio. There may be selective bias in publication of safety issues. This factor has been reported with the use of SSRIs for depression in children (Whittington et al., 2004). Furthermore, since a substantial proportion of trials with SSRIs do not get published (Turner et al., 2012), we may have missed some studies by only including published trials. Finally, the studies included in the present meta-

analysis were not designed to investigate rare events, such as suicidality and suicide, and as such are unlikely to have sufficient statistical power to detect potential risk for these events. Therefore, studies designed to identify rare adverse events in PD are needed.

Questions have arisen about the quality of evidence on first line treatments for the treatment of PD, especially with respect to whether data exist to suggest that BZs could be considered worst and, therefore, second line in PD treatment (Nardi et al., 2018; Offidani et al., 2013; Nielsen et al., 2012; Starcevic, 2012b; Bruce et al., 2003). In fact, the superiority of SSRIs over BZs in terms of efficacy and tolerability was not supported by the available evidence (Offidani et al., 2013). BZs trials resulted in comparable or greater improvements and fewer adverse events compared to antidepressants (mostly tricyclic antidepressants) in PD (Offidani et al., 2013). The findings of the present systematic review of the literature and meta-analysis show that both SSRIs and BZs cause more adverse effects than placebo in PD patients when used as short-term treatment. The difference between SSRIs and BZs is mainly in the type of associated adverse effects associated, not in the number of adverse effects. In addition, BZs seem to be protective against diaphoresis and tachycardia. Thus, our findings provide no support to the notion that SSRIs should *always* be favored over BZs in the short-term treatment of PD based on their allegedly more favorable adverse effect profile. Instead, SSRIs may be a preferable option in certain clinical situations (e.g., patients with cognitive impairment and significant constipation or those for whom sedation might jeopardize their everyday functioning), whereas BZs may be more appropriate in others (e.g., patients with prominent insomnia or a history of problematic sweating, prominent gastrointestinal symptoms or sexual dysfunction). In addition, the combination of SSRIs and BZs in the first weeks of PD treatment is recommended (Quagliato et al., 2018). The administration of BZs in the first weeks of treatment may alleviate some adverse events triggered by SSRIs, such as the 'activation' induced by the SSRI in the beginning of a treatment (Quagliato et al., 2018). Indeed, it may be the time to reconsider the role of BZs as first-line treatment of PD (Balon et al., 2018; Nardi et al., 2018; Starcevic, 2014; Dell'Osso et al., 2015), especially in situations where there is a need for rapid relief of anxiety (Nardi et al., 2018; Starcevic, 2012a) and in patients who are not at risk of substance use disorders (Starcevic, 2014).

In conclusion, on the basis of the present review of the literature and meta-analysis, treatment guidelines for short-term treatment for panic disorder need to be assessed to reflect literature-based evidence. Our

findings show that BZs are not associated with more adverse effects than SSRIs in the course of short-term treatment of PD. Consequently, an increasing role of BZs in short-term PD treatment should be evaluated. To better choose the medication more directly randomized controlled trials need to be performed between SSRIs and BZs.

Conflicts of interest

Dr. Balon, Dr. Cosci, Dr. Freire, Dr. Nardi, Dr. Salzman, Dr. Shader, Dr. Silberman, Dr. Starcevic, Dr. Weintraub, and Dr. Quagliato have no conflicts of interest.

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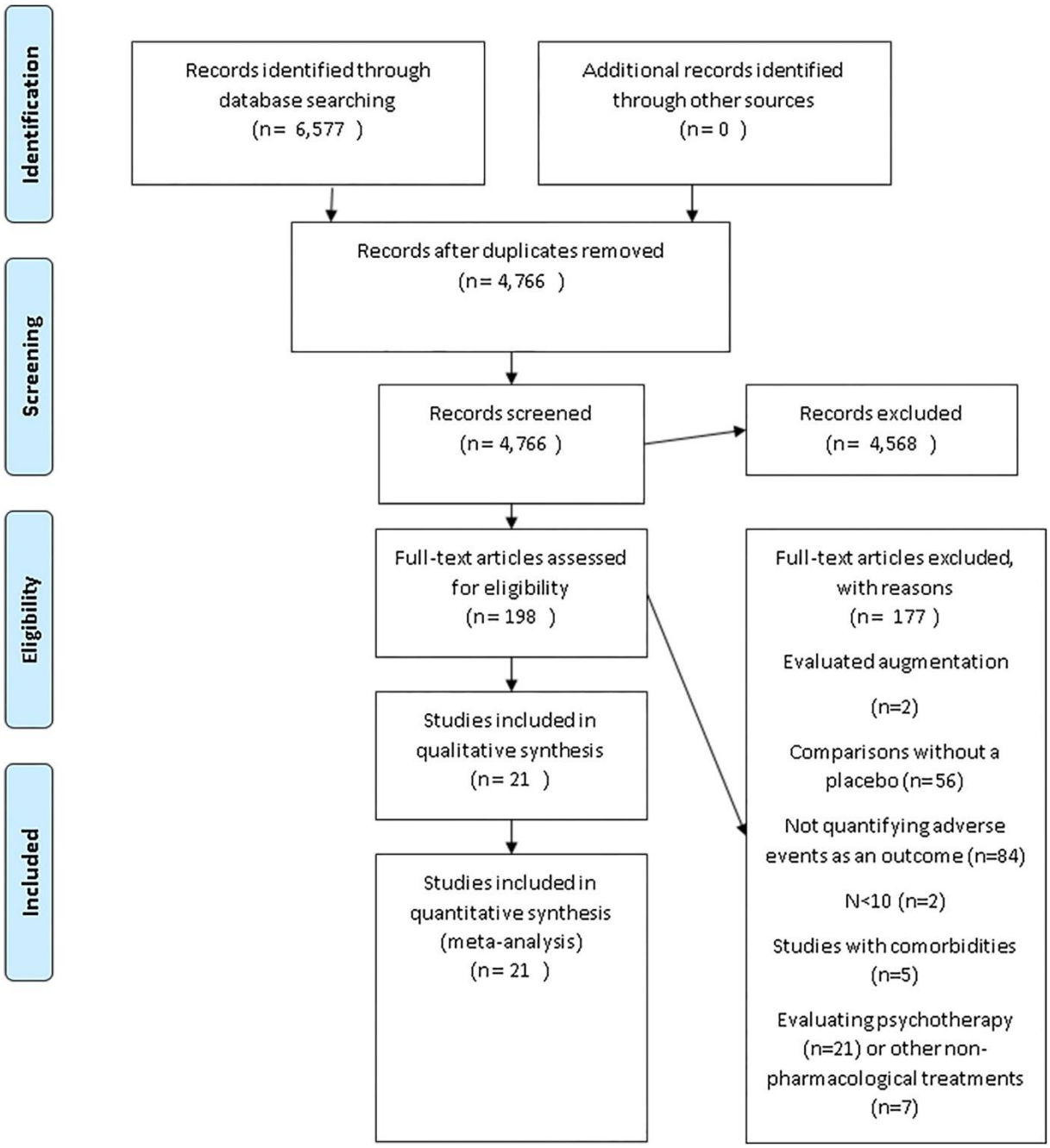

Figure 1. PRISMA Flow Diagram.

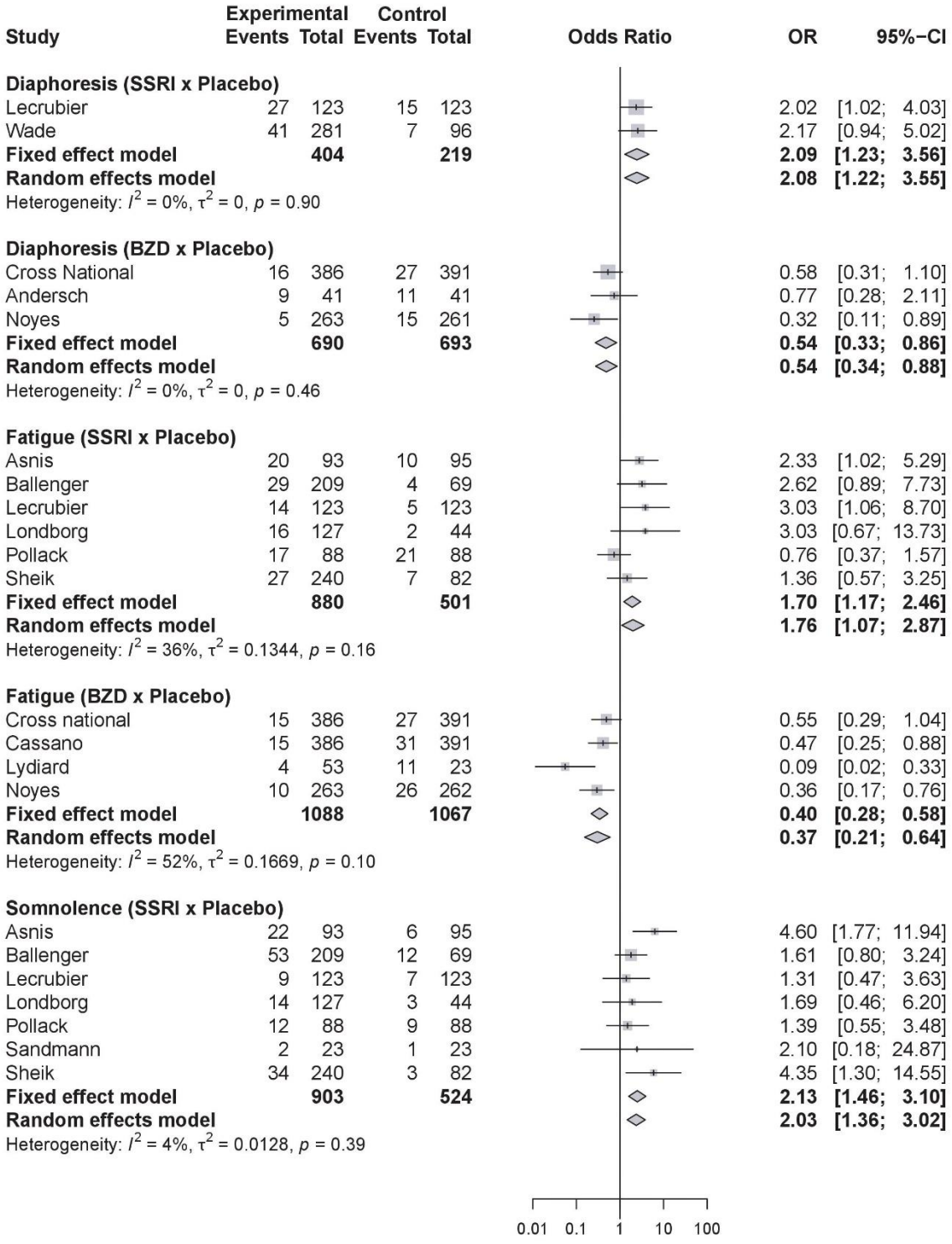

Figure 2. Forest plots of diaphoresis, fatigue, somnolence.

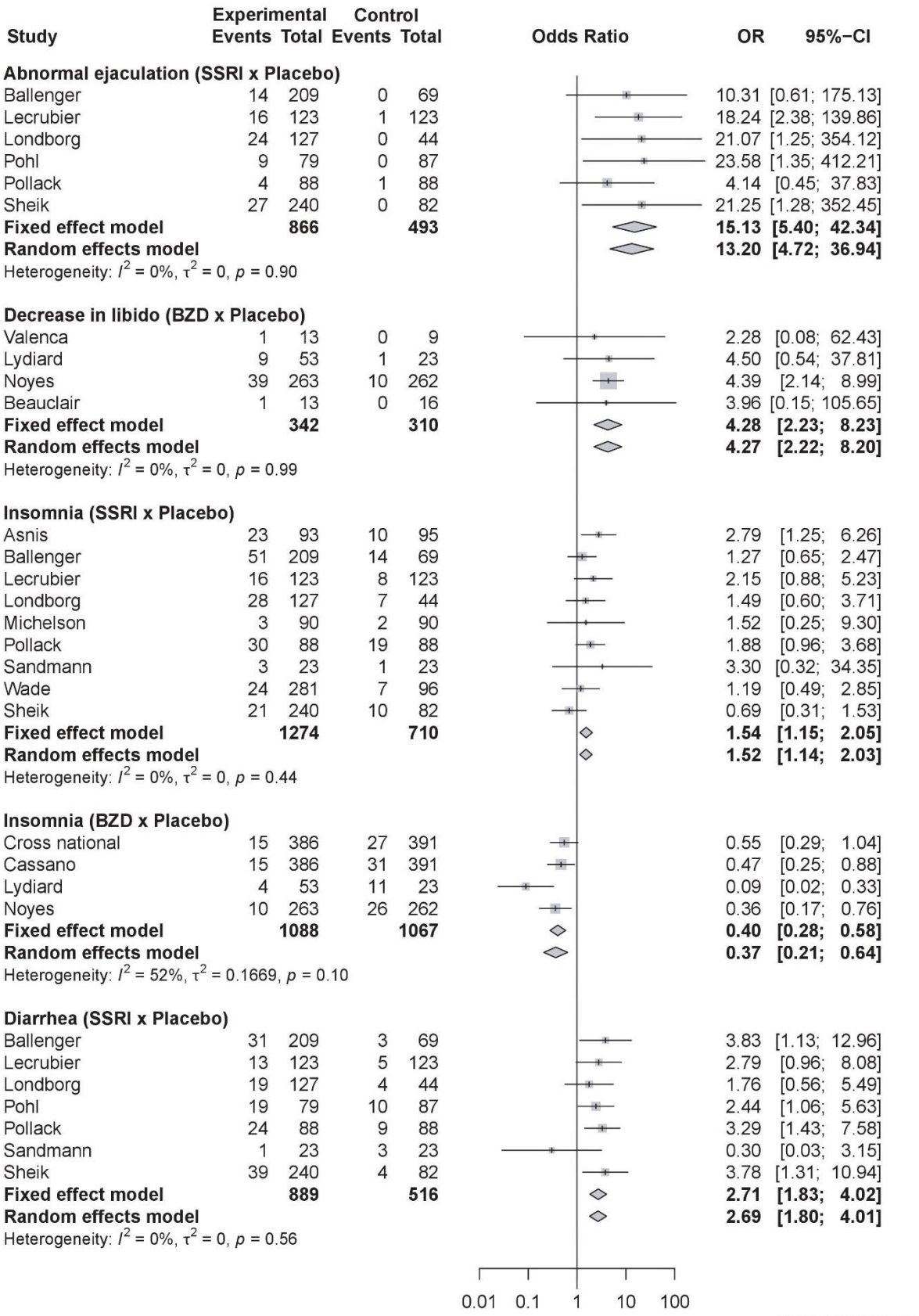

Figure 3. Forest plots of abnormal ejaculation, decrease in libido, insomnia, and diarrhea.

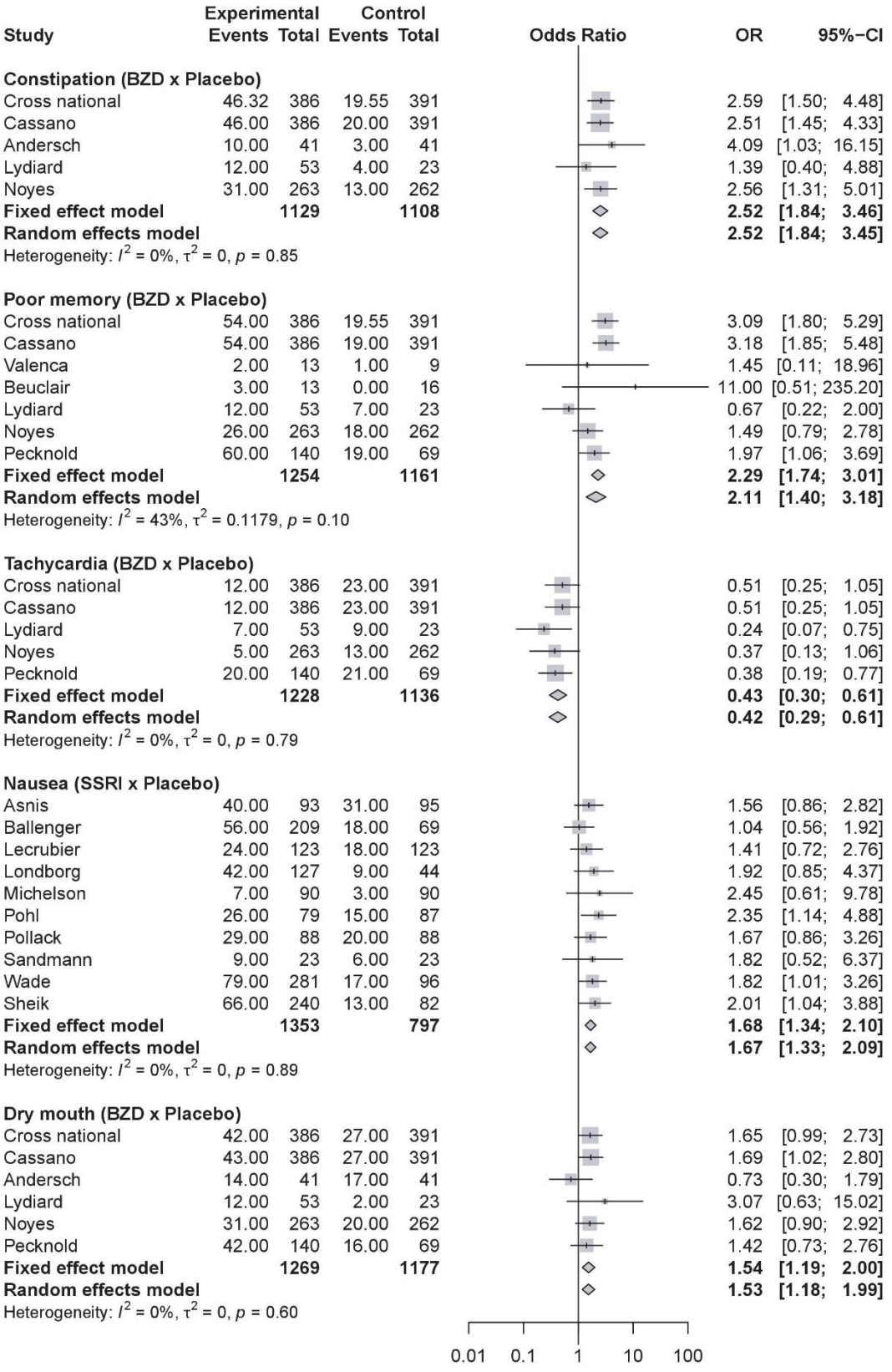

Figure 4. Forest plots of nausea, constipation, tachycardia, dry mouth, and memory problems.

4. CONCLUSÃO

O TP tem grande importância clínica e alta prevalência por isso, a pesquisa sobre esse transtorno é relevante e deve ser incentivada no campo da psiquiatria. Os achados desta dissertação demostram que nos pacientes com diagnóstico de TP, os níveis de IL-2R, que estão envolvidos na regulação da enzima IDO, estavam significativamente associados aos níveis de quinurenina. Essa associação não foi observada em controles saudáveis. Ademais, uma relação elevada de quinurenina/triptofano foi um preditor significativo de prejuízos na memória auditiva de curto prazo nos pacientes com diagnóstico de TP. As revisões sistemáticas da literatura que se encontram nesta dissertação corroboram e levantam hipóteses para os resultados do artigo original. A primeira revisão sistemática confirma um aumento de citocinas inflamatórias nos pacientes com TP. A segunda, disserta sobre como canais ionicos presentes na microglia interagem com o meio extracelular, podendo fazer com que o fenótipo dessas células sofra modificações para uma conformação ativada. A terceira, demonstra uma relação entre canais sensiveis à acido, que são canais ionicos, e estão em grande parte localizados na microglia, no TP. A microglia ativa pode produzir citocinas inflamatorias, como a IL-2R, que ativaria a enzima IDO da cascata da quinurenina, aumentando a degradação desta e, ocasionando, prejuizos na memória auditiva de curto prazo nos pacientes com o diagnostico de TP.

Nossos resultados sugerem que intervenções objetivando especificamente a redução de citocinas inflamatórias e a inibição da via da quinurenina e/ou da microglia, poderiam criar novas perspectivas terapêuticas para pacientes com TP que apresentam disfunções cognitivas. Entretanto, tais hipóteses devem ser validadas em modelos pré-clínicos e replicadas em estudos controlados em humanos.

Pesquisas direcionadas para o TP serão capazes de nos auxiliar na compreensão dos mecanismos fisiopatogênicos envolvidos nesse complexo transtorno. Isso possibilitará o surgimento de estratégias terapêuticas mais eficazes para os pacientes com esse diagnóstico.

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6. APÊNDICE

6.1. Material suplementar artigo 2

Supplementary Information

Supplementary Methods

Participants: recruitment, inclusion and exclusion criteria.

Sample size was determined on the basis of mean group differences between patients and controls from published studies (Young et al., 2016). Seventy-eight people with a diagnosis of panic disorder and 78 age and sex-matched controls were recruited for this study. Patient recruitment was via either clinician or self/family referral. Subjects were excluded for a number of medical conditions that might confound study interpretation as confirmed by medical history. Patients were excluded for uncontrolled cardiovascular, endocrinologic, hematologic, hepatic, renal, or neurologic disease, autoimmune conditions, chronic infection (i.e. HIV, hepatitis B or C), history of liver abnormalities, or evidence of infection within one month of screening. Participants were also excluded for a history of cancer, pregnancy or lactation; a history of schizophrenia (determined by SCID-IV); active psychotic or depressive disorder; substance abuse and/or dependence within the past 6 months (determined by SCID-IV); an active eating disorder or obsessive compulsive disorder; and/or a score of less than 28 on the Mini-Mental State Examination(Folstein et al., 1983). Blood was collected at the Institute of Psychiatry at one screening visit for analysis of IL-2R, IL-10, IL-1β, kynurenine and tryptophan.

Objective measures of cognitive performance.

Trail Making Test (TMT): TMT performance was calculated taking the time needed to perform TMT-B minus time needed for TMT-A. Trail Making Test Part A is a timed task that provides information on psychomotor processing speed.(Marvel and Paradiso, 2004; Shindo et al., 2013) Trail Making Test A requires an individual to draw lines sequentially connecting 25 encircled numbers distributed on a sheet of paper.(Spreen, 1991) A lower score indicates better performance. Trail Making Test Part B is also a timed task. TMT B is similar to TMT A, but letters (A–L) and numbers (1–13) had to be selected in alternating ascending order, for example, 1A 2 B 3 C and so on. A lower score also indicates better performance (Spreen, 1991). The delta TMT value "removes" eventual bias due to differences in upper extremity motor speed, simple sequencing, visual scanning, and psychomotor functioning and is considered a measure of cognitive flexibility relatively independent of manual dexterity (Vazzana, 2010).

Stroop Task: The Stroop task is comprised of three subtests, designed to assess the subject's ability to suppress interfering stimuli. In the color-naming subtest, the subject is asked to report the color of randomly sequenced color rectangles, establishing the tendency to respond to color. In the word-reading subtest, the subject is asked to read color words randomly printed in black ink, establishing a response set to reading color words. In the interference subtest, the subject is given color words, which are printed in an incongruent ink color. The subject is asked to report the ink color, and therefore has to suppress the

tendency to read the color word. The score is measured by these three different subtests (color naming, reading and interference). The Stroop task test cognitive processes associated with selective attention, cognitive control, and goal-oriented behavior that supports the ability to select a weaker, task-relevant response in the face of competition from a potentially stronger, task-irrelevant one (MacDonald et al., 2000; Pardo et al., 1990).

Digit- Span Test: The Digit- Span Test, a subtest of the Wechsler Adult Intelligence Scale-Revised (Wechsler, 2008), measures immediate memory span to assess attention, concentration, and working memory . The subset consists of forward and backward conditions in which the examiner reads aloud series of numbers which increase in length; the participant is requested to repeat the numbers in the same or reverse order after each series is presented. Digit Span scores may be calculated using the total number of items correctly repeated, which is based on the sum of the Digits Forward and Digits Backward raw scores or the longest list length correctly recalled (Pisoni and Cleary, 2003; Wechsler, 2008). Digit Span forward and backward are related because they share a component of short-term verbal memory (Baddeley, 2003). However, measures of Digit Span backward require not only rote immediate verbal memory but also include an additional processing component of concurrent mental operations (i.e., reordering). (Baddeley, 2003; Baddeley, 2007).

Rey's Auditory Verbal Learning Test (RAVLT): is used for assessing episodic memory by providing scores for evaluating different aspects of memory. The RAVLT is sensitive to, for instance, verbal memory deficits. Briefly, the RAVLT consists of presenting a list of 15 words across five consecutive trials. The list is read aloud to the participant, and then the participant is immediately asked to recall as many as words as he/she remembers. This procedure is repeated for 5 consecutive trials (Trials 1 to 5). After that, a new list (List B) of 15 new words is read to the participant, who then is immediately asked to recall the words. After the List B trial, the examiner asks participant to recall the words from the first list (Trial 6). After30-minutes of interpolated testing, the participant is again asked to recall the words from the first list (delayed recall). Different summary scores are derived from raw RAVLT scores. These include RAVLT Immediate (the sum of scores from 5 first trials (Trials 1 to 5), RAVLT Learning (the score of Trial 5 minus the score of Trial 1), and RAVLT Percent Forgetting (RAVLT Forgetting divided by the score of Trial 5) (Rey, 1958; Van der Elst et al., 2005).

Laboratory assays: measurement of kynurenine, tryptophan, and cytokine levels

The method used to measure tryptophan and kynurenine was the competitive inhibition immunoassay technique (Cloude-Clone, Texas, USA), as it follows: a competitive inhibition reaction was launched between biotin labeled tryptophan and unlabeled tryptophan (standards or samples) with the pre-coated antibody specific to tryptophan. After incubation the unbound conjugate is wash off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of tryptophan in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of tryptophan in the

sample. Similar technique occurred concerning kynurenine measurements, in which standards or samples are added with a biotin-conjugated antibody specific to kynurenine. Next, avidin conjugated to HRP is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain kynurenine, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibited a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength at 450nm plus or minus 10 nm. The concentration of kynurenine in the samples is then determined by comparing the O.D. of the samples to the standard curve. Concentrations of cytokines and their soluble receptors were assessed using Immulite System (Diagnostic Products Corporation) (Berthier et al., 1999). This technology is based on a solid phase two-site chemiluminescent enzyme immunometric assay. The solid phase, a polystyrene bead, is coated with either a monoclonal specific antibody or an anti-ligand. Patient serum and alkaline phosphatase-conjugated monoclonal antibody or, depending on the technique, a ligand-labeled antibody is incubated for 30 to 60 min at 37 °C. Unbound conjugate is then removed by a centrifugal wash (x3), after which a chemiluminescent substrate (a phosphate ester of adamantyl dioxetane) is added, and the test unit is incubated for a further 10 minutes. The chemiluminescent substrate undergoes hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate with an emission of light. The bound complex, and thus also the photon output, as measured by the luminometer, is proportional to the concentration of cytokine in the sample. For each cytokine calibration, a master curve is constructed by the manufacturer using a material calibrated against the National Institute for Biological Standards and Control standards (Berthier et al., 1999).

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6.2. Material suplementar artigo 3

Appendix 1: Search Protocol

Systematic Search Protocol

Written based on the WHO Review Protocol Template, 2011

Title: **The role of ion channels convergent pathways on microglial phenotypes: a systematic review of the implications for neurological and psychiatry disorders.**

1. Background: Microglia activation and ion signaling are linked characteristic of neuropsychiatric disorders. However, it is not clear specifically the molecular mechanisms that exist between these conditions.
2. Objective: This review aims to bring together all the research on how ionic channels interacts with microglia cells to identify a pattern of immune response activation.

3. Review Question (Population Intervention Comparison Outcome)

Population: microglia cells
Intervention: activation and /or inhibition of ion channels
Comparison: without activation and /or inhibition of ion channels
Outcome: microglia cells active or at resting stage
Evidence Gathering and Study Selection: Web of Science and Pubmed databases will be searched. Full search terms for each database and results found per database are listed in the Appendix 2.
Reference searches: Bibliographics of papers deemed eligible for this review will be hand searched to identify any additional eligible references, which will then be screened for title, abstract or full text as appropriate.

4. Eligibility Criteria

The results of these searches will be combined and deduped using Endnote. They will then be screened for title and abstract, and then full text using the following eligibility criteria.

i) Type of study included: any type of study design.
ii) Types of participants: Studies that examine the relationship between ion channels and microglia will be included
iii) Types of outcome measures: microglia in an activate or at resting state.

7. Exclusion Criteria

Reviews, studies which lacked a baseline condition or control group, studies that did not report original data, studies without an immunohystochemical analyses of microglia, studies evaluating genetic deficiency of ionic channels, in vitro studies will be excluded.

8. Data extraction

Data extracted will include:

- Citation information
- Subject age, sex and species, duration in fixative solution, brain region examined, method of measuring microglia state used and results. The final decision on what to include in the published tables will be made by the systematic review author team based on importance and variability within the studies.

9. Data Synthesis

Narrative synthesis is planned, with the same disorders/conditions grouped together as appropriate depending on number of papers and commonalities in measurement. Statistical synthesis is not expected to be possible as there is wide variability in the types of measures, types of outcomes and brain regions investigated, but will be considered if feasible given the data.

10. Dissemination

A manuscript will be prepared for submission to a peer reviewed journal in the neuroscience field.

	Appendix 2: Search terms and results
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or nucleus ventralis posteriors or ventral posterior thalamic nucleus or ventral posterior medial nucleus or ventral posteroinferior nucleus or posteroinferior nucleus ventral or posteriors nucleus ventralis or arcuate nucleus-3 or thalamus nucleus ventrolateralis or nucleus ventralis or posteromediali or complex ventrobasal or ventral lateral thalamic nucleus or ventral thalamic nuclei or ventral lateral thalamic nuclei or ventral posteromedial nucleus or posteromedialis nucleus ventralis or ventral anterior nucleus or ventral posterolateral nucleus or nuclear masses ventral or ventral posterior inferior thalamic nucleus or thalamic nucleus ventrolateral or ventral nuclear group or thalamus ventrolateral or thalami nucleus ventrolateralis or posteromediali nucleus ventralis or posterolateralis nucleus ventralis or posterior nucleus ventralis or nucleus ventral posteromedial or nucleus ventralis laterali or ventral posterolateral thalamic nucleus or nucleus ventral thalamic or ventralis lateralis nucleus or ventral nuclear mass or ventralis posteriors nucleus or ventralis laterali nucleus or nucleus ventral posterior or ventral thalamic nucleus or ventrolateral thalamus or nucleus ventralis lateralis or Telencephalon or telencephalon or endbrain or endbrains or Cerebrum or cerebrum or cerebral hemisphere left or cerebral hemisphere right or cerebral hemispheres or right cerebral hemisphere or cerebral hemisphere or left cerebral hemisphere or Basal Ganglia or ganglia basal or nuclei basal or basal ganglia or ganglion basal or basal nuclei or claustrum or Corpus Striatum or lenticular nucleus or nucleus lentiform or lentiformis nucleus or lentiform nucleus or corpus striatum or nucleus lenticular or nucleus lentiformis or lentiform nuclei or striatum corpus or nuclei lentiform or Globus Pallidus or pallidum or paleostriatum or globus pallidus or pallidums or Neostriatum or Caudate Nucleus or nucleus caudatus or caudate nucleus or caudatus nucleusor nucleus caudate or caudatus or High Vocal Center or Putamen or putamens or nucleus putamens or putamens nucleus or putamen nucleus or nucleus putamen or putamen or Ventral Striatum or Nucleus Accumbens or nucleus accumbens or accumbens septus nucleus or accumbens septi nucleusor nucleus accumbens septi or septi nucleus accumbens or accumbens nucleus or septus nucleus accumbens or nucleus accumbens septus or Olfactory Tubercleor Islands of Calleja or Basal Nucleus of Meynert or nucleus basalis of meynert or meynert basal nucleus or nucleus basalis magnocellularis or basal nucleus of meynert or meynert nucleus basalisor Cerebral Cortex or plates cortical or insular cortex or cerebral cortices or archipalliums or paleocortex or allocortices or periallocortices or plate cortical or cerebri cortex or	

	cortices cerebral or paleocortices or cortices insular or insular cortices or cortex insular or periallocortex or archipallium or cortical plates or cortex cerebral or cortex cerebri or reil insula or cortex cerebrus or cortical plate or Frontal Lobe or gyrus anterior centralor central gyrus anterior or lobe frontalor frontal lobeor cortex frontal or gyrus precentralis or frontal eye fieldor supplementary eye field or gyrus precentrali or frontali lobusor precentrali gyrus or frontal lobes or frontal cortex or field supplementary eye or lobes frontal or eye field supplementary or lobus frontali or supplementary eye fields or frontalis lobus or gyrus precentral or eye fields supplementary or eye fields frontal or anterior central gyrus or fields frontal eye or lobus frontalis or Motor Cortex or motor area or primary motor cortex or motor area precentral or strip motor or somatomotor areas or strips motor or motor cortices primary or premotor areas or motor area secondary or cortex precentral motor or motor area somatic or supplementary motor areas or area primary motoror area premotor or secondary motor area or motor cortices secondary or area motor or secondary motor areas or area somatomotor or motor areas or motor cortex secondary or precentral motor areas or cortices secondary motor or area supplementary motor or motor areas supplementary or area precentral motor or cortices primary motor or precentral motor cortices or areas somatic motor or area somatic motor or areas motor or motor cortex precentral or motor areas precentral or motor strips or cortex primary motor or somatomotor area or premotor area or precentral motor cortex or primary motor area or somatic motor area or motor areas somatic or areas premotor or areas somatomotor or areas precentral motor or areas supplementary motor or motor cortex primary or cortex secondary or motor primary motor cortices or motor cortex or motor cortices precentral or motor area supplementary or cortices precentral motor or somatic motor areas or cortex motor or areas secondary motor or Prefrontal Cortex or orbital gyrus)	
#13	(hippocampal mossy fibers or mossy fiber hippocampal or Fornix, Brain or hippocampal commissure or hippocampal commissures or commissures dorsal hippocampal or fornix commissures or fornices or brain fimbrias or fornical commissures or fornical commissure or fornix or hippocampal commissures dorsal or commissures hippocampal or fornix-fimbria or hippocampal commissure dorsal or fimbria or fornix fimbria or fimbria of hippocampus or brain fornices or dorsal hippocampal commissure or commissure fornical or commissure dorsal hippocampal or commissure of fornix or commissures fornical or commissure hippocampal or fornix commissure or fimbria-fornix or fimbria fornix or fimbria brain or hippocampus fimbrias or hippocampus fimbria or brain fimbria or Hypothalamus or preoptico-hypothalamic areas or preoptico hypothalamic area or lamina terminalis or hypothalamus or areas preoptico-hypothalamic or area preoptico-hypothalamic or preoptico-hypothalamic area or Hypothalamic Area, Lateral or area hypothalamica laterali or hypothalamica laterali area or hypothalami area lateralis or lateralis area hypothalamica or hypothalamus area lateralis or laterali area hypothalamica or areas lateral hypothalamic or lateralis hypothalami area or lateral hypothalamic areas or accessory nucleus of the ventral horn or lateral tuberal nuclei or tuberal nucleus lateral or lateral hypothalamus or area hypothalamica lateralis or hypothalamus lateral or tuberomammillary nucleus or hypothalamic area lateral or nucleus tuberomammillary or nuclei lateral tuberal or nucleus lateral hypothalamic or lateralis hypothalamus area or area lateral hypothalamic or hypothalamic nucleus lateral or area lateralis hypothalamus or nucleus lateral tuberal or Hypothalamus, Anterior or commissures anterior hypothalamic or anterior hypothalamic decussation of ganser or hypothalamic commissures anterior or anterior hypothalamic commissures or commissure anterior hypothalamic or periventricular nucleus anteroventral or nucleus anteroventral periventricular or anterior hypothalamic commissure or hypothalamic commissure anterior or hypothalamus anterior or hypothalamus supraoptic or anteroventral periventricular nucleus or anterior hypothalamus or supraoptic hypothalamus or Anterior Hypothalamic Nucleus or areas anterior hypothalamic or hypothalamic area anterior or nucleus anterior hypothalamic or anterior hypothalamic nucleus or hypothalami nucleus anterior or hypothalamic areas anterior or anterior hypothalami nucleus or anterior hypothalamic area or area anterior hypothalamic or nucleus anterior hypothalamus or hypothalamus nucleus anterior or anterior hypothalamic areas or anterior hypothalamus nucleus or nucleus anterior hypothalami or hypothalamic nucleus anterior or Organum Vasculosum or Paraventricular Hypothalamic Nucleus or hypothalamic paraventricular nucleus or paraventricular hypothalamic nucleus or nucleus paraventricular hypothalamic or nucleus hypothalamic paraventricular or nucleus paraventricular or paraventricular nucleus or hypothalamic nucleus paraventricular or paraventricular nucleus hypothalamic or Preoptic Area or area medial preoptic or preoptic area medial or preoptic nucleus or nuclei preoptic or lateral preoptic area or preoptic areas lateral or area preoptic or areas medial preoptic or area lateral preoptic or preoptic areas medial or lateral preoptic areas or preoptica area or nucleus preoptic or medial preoptic areas or areas lateral preoptic or area preoptica or areas preoptic or preoptic nuclei or medial preoptic area or preoptic area or preoptic areas or Suprachiasmatic Nucleus or nucleus suprachiasmatic or suprachiasmatic nucleus or Supraoptic Nucleus or hypothalamus supraoptic nucleus or supraoptic group accessory or accessory supraoptic groups or supraoptic nucleus of hypothalamus or supraopticus nucleus or groups accessory supraoptic or nucleus supraoptic or group accessory supraoptic or accessory supraoptic group or nucleus supraopticus or supraoptic groups accessory or supraoptic nucleus or Hypothalamus, Middle or regions intermediate hypothalamic or hypothalamic region intermediate or region intermediate hypothalamic or middle hypothalamus or hypothalamus medial or hypothalamic regions intermediate or intermediate hypothalamic regions or intermediate hypothalamic region or hypothalamus middle or medial hypothalamus or Arcuate Nucleus of Hypothalamus or nucleus arcuate or arcuate nucleus or hypothalamus arcuate nucleus or nucleus infundibular or infundibular nucleus or arcuate nucleus of hypothalamus or Dorsomedial Hypothalamic Nucleus or nucleus arcuate or arcuate nucleus or hypothalamus arcuate nucleus or nucleus infundibular or infundibular nucleus or arcuate nucleus of hypothalamus or Hypothalamo-Hypophyseal System or hypothalamic pituitary unit or hypothalamo hypophyseal system or hypothalamo-hypophyseal system or hypothalamic-pituitary unit or Median Eminence or eminentia medianas or median eminence or eminences medial or eminence medial or medial eminences or medianas eminentia or eminentia mediana or mediana eminentia or eminence median or medial eminence or Pituitary Gland or hypophyseal infundibulum or infundibular hypothalamus or pituitary glands or infundibulum or stalk infundibular or hypothalamus infundibular or infundibulums or pituitary stalks or pituitary gland or hypophysis or pituitary stalk or infundibular stem or stalks infundibular or glands pituitary or hypophysis cerebri or hypophyseal stalks or cerebri hypophysis orstalk hypophyseal or infundibular stalk or infundibular stalks or hypophysis cerebrus or hypophyseal stalk or Pituitary Gland, Anterior or lobus anteriors or anterior lobe of pituitary or anterior lobus or pituitary pars distalis or anterior pituitary glands or anteriors lobus or lobus anterior or pituitary gland anterior or adenohypophyses or pituitary glands anterior or adenohypophysis or pituitary anterior lobe or anterior pituitary gland or pars distalis of pituitary or Corticotrophs or Gonadotrophs or lh producing cells or lh-secreting cells or fsh cells or gonadotrophs or lh cell or fsh-secreting	

	cellsor fsh secreting cells or fshproducing cells or fsh-producing cell or fsh cell or lh-producing cells or fsh producing cells or lh secreting cells or fsh-secreting cell or gonadotroph or lh-producing cell or lh-secreting cell or lh cell or Lactotrophs or pituitary prolactin-secreting cells or lactotrophs or pituitary prolactin cell or prolactin-secreting cell pituitary or prolactin-secreting cells pituitary or lactotroph or prolactin cell pituitary or prolactin cells pituitary or pituitary prolactin cells or pituitary prolactin-secreting cell or pituitary prolactin secreting cells or Somatotrophs or gh cell pituitary or somatotrophs or gh cells pituitary or pituitary growth hormone-secreting cells or pituitary gh cell or pituitary growth hormone secreting cells or pituitary gh cells or somatotroph or Thyrotrophs or Pituitary Gland, Intermediate or Melanotrophs or Pituitary Gland, Posterior or lobes neural or posterior pituitary glands or neural lobe or pituitary pars nervosa or infundibular processes or infundibular process or process infundibular or neurohypophysis or lobe neural or gland posterior pituitary or pituitary posterior lobe or pars nervosa of pituitary or posterior lobe of pituitary or neural lobes or nervosus lobus or lobus nervosus or pituitary gland posterior or processes infundibular or Tuber Cinereum or cinereums tuber or cinereum tuber or tuber cinereum or tuber cinereums or Ventromedial Hypothalamic Nucleus or nucleus ventromedial hypothalamic or hypothalamic nucleus ventromedial or ventromedial hypothalamic nucleus or Hypothalamus, Posterior or posteriors area hypothalamica or area hypothalamica posterior or mammillary regions or region mammillary or nucleus posterior periventricular or hypothalamic regions posterior or hypothalamus posteriors or mammillary region or posterior area hypothalamica or posterior hypothalamic regions or supramammillary commissures or region posterior hypothalamic or supramammillary commissure or regions posterior hypothalamic or posterior hypothalamus or commissures supramammillary or premammillary nucleus or hypothalamic region posterior or posterior hypothalamic region or commissure supramammillary or hypothalamus posterior or hypothalamica posteriors area or periventricular nucleus posterior or nucleus premammillary or Mammillary Bodies or mammillary bodies ormammillary body or body mammillary or mamillary bodies or body mamillary or bodies mamillary or bodies mammillary or mamillary body or Limbic Lobe or Gyrus Cinguli or gyrus cingular or anterior cingulate gyrus or cingulate gyri posterior or cortex anterior cingulate or posterior cingulate gyrus or cinguli anteriors gyrus or mesial region superior or gyrus cingulate or cingulate cortex anterior or cingulate cortex or superior mesial regions or regions cingulate or cortex posterior cingulate or anterior cingulate cortices or posterior cingulates or cingulate bodies or cingulates anterior or cortices anterior cingulate or posterior cingulate cortices or mesial regions superior or posterior cingulate cortex or regions posterior cingulate or cingulate posterior or posterior cingulate region or region posterior cingulate or body cingulate or cortex cingulate or posterior cingulate regions or cingulate gyrus anterior or cingulate gyrus or cingulate gyrus posterior or cingular gyrus or bodies cingulate or cingulate area or anterior cingulates or area cingulate or cingulate regions or regions superior mesial or ingulates posterior or areas cingulate or cingulate cortices anterior or anterior gyrus cinguli or gyri posterior cingulate or gyrus anterior cingulate or gyrus cinguli anteriors or cinguli anterior gyrus or superior mesial region or anterior cingulate or gyrus cinguli anterior or cingulate anterior or region cingulate or cingulate areas or Parahippocampal Gyrus or gyrus parahippocampal or gyri parahippocampal or parahippocampal gyri posterior or hippocampal gyrus or gyri posterior parahippocampal or posterior parahippocampal gyrus or gyrus parahippocampalis or parahippocampal gyrus uncus or presubiculums or posterior parahippocampal gyri or gyrus posterior parahippocampal or parahippocampal gyrus posterior or uncus of parahippocampal gyrus or gyri hippocampal or parahippocampal gyrus or presubiculum or gyrus hippocampi or uncus parahippocampal gyrus or gyrus uncus parahippocampal or gyrus hippocampal or parahippocampal gyri or Entorhinal Cortex or area entorhinali or areas entorhinal or entorhinalis area or entorhinal area or area entorhinal or entorhinal cortices or area entorhinalis or cortices entorhinal or entorhinali area or olfactory cortices secondary or secondary olfactory cortex or cortex secondary olfactory or cortices secondary olfactory or entorhinal cortex or olfactory cortex secondary or secondary olfactory cortices or cortex entorhinal or entorhinal areas or Olfactory Pathways or olfactory pathways or pathways olfactory or olfactory pathway or pathway olfactory or Perforant Pathway or pathway perforant or pathways perforant or perforant paths or perforant pathways or perforant pathway or fasciculus perforating or paths perforant or perforant path or perforating fasciculus or path perforant or Septum of Brain or paraterminal body or brain septums or brain septum or septum of brain or paraterminal bodies or area septal or bodies paraterminal or body paraterminal or septal area or region septal or septal region or Septal Nuclei or nucleus of the stria terminalis or septi lateralis nucleus or septal nuclear complices or nucleus of anterior commissure or terminali nucleus striae or laterali nucleus septalis or nucleus lateralis septi or nucleus lateralis septus or nuclear complices septal or septofimbrial nucleus or diagonal band nucleus or nucleus septofimbrial or nucleus septi lateralis or laterali nucleus septi or nucleus triangular septal or medial septal nucleus or nucleus of diagonal band or nucleus septalis lateralis or nucleus striae terminali or nuclear complex septal or septum nucleus lateral or lateral septal nucleus or lateralis nucleus septalis or septal nucleus lateral or septalis laterali nucleus or nuclei septal or anterior commissure nucleus or septus nucleus lateralis or septi laterali nucleus or nucleus medial septum or nucleus septalis laterali or triangularis septus nucleus or lateralis nucleus septi or complex septal nuclear or nucleus striae terminalis or nucleus of stria terminalis or septalis lateralis nucleus or dorsal septal nucleus or nucleus triangularis septus or nucleus lateral septumor nucleus triangularis septi or nucleus lateral septal or septal nucleus triangular or terminalis nucleus striae or septi nucleus lateralis or septi nucleus triangularis or triangular septal nucleus or septus nucleus triangularis or nucleus medial septal or complices septal nuclear or lateralis septus nucleus or medial septum nucleus or lateralis septi nucleus or Substantia Innominata or innominata substantia or substantia innominata or Prosencephalon or prosencephalon or forebrains or forebrain or Diencephalon or diencephalon or interbrain or interbrains or Optic Chiasm or chiasmas optic or optic chiasms or decussation optic or chiasma optic or optic chiasm or optic chiasma or optic decussation or opticums chiasma or optic decussations or chiasma opticum or decussations optic or opticum chiasma or optic chiasmas or chiasma opticums or chiasms optic or chiasm optic or Optic Tract or Subthalamus or subthalamus or fasciculus thalamic or field h nucleus or campi forelus nucleus or fasciculus lenticular or field h1 forel's or campi foreli nucleus or enticular fasciculus or forels field h2 or forel field h2 or thalamicus fasciculus or fasciculus thalamicus or thalamic fasciculus or forelus nucleus campi or nucleus of ansa lenticularis or foreli nucleus campi or nucleus campi forelus or nucleus of field h or forels field h1 or forel's field h2 or field h1 of forel or forel field h1 or Entopeduncular Nucleus or Subthalamic Nucleus or nucleus of luys or luys subthalamic nucleus or corpus luysi or luys body or subthalamic nucleus of luys or subthalamicus nucleus or luys nucleus or nucleus subthalamic or luysi corpus or body of luys or nucleus subthalamicus or subthalamic nucleus or Zona Incerta or Thalamus or thalamencephalon or thalamencephalons or thalamus or Thalamic Nuclei or nuclei thalamic or thalamic nuclei or Anterior Thalamic Nuclei or nucleus anterodorsal thalamic or anterior nuclear group or nucleus anteromedial thalamic or nucleus anteroventral thalamic or thalamus anterior nucleus or anterior thalamic nucleus or nucleus anteroventral or anteroventral nucleus or	

	thalamic nucleus anterodorsal or nuclei anterior thalamic or thalamic nuclei anterior or anteromedial nucleus or anteromedial thalamic nucleusor thalamus anterior or nucleus anteromedial or anterodorsal nucleus or anterior thalamus or anterior thalamic nuclei or anterodorsal thalamic nucleus or nucleus anterodorsal or thalamic nucleus anteroventral or Geniculate Bodies or nucleus geniculate or medial geniculate nucleus or geniculate complex medial or geniculatum mediales corpus or bodies geniculate or nucleus lateral geniculate or mediales corpus geniculatum or geniculate bodies medial or mediale corpus geniculatum or geniculate body or geniculatum mediale corpus or geniculate nucleus lateral or geniculate bodies or geniculate bodies lateral or metathalamus or corpus geniculatum mediale or geniculate body lateral or complex medial geniculate or nucleus geniculatus lateralis pars dorsalis or geniculate body medial or geniculate complices medial or geniculate nucleus or complices medial geniculate or medial geniculate body or medial geniculate bodies or geniculate nucleus medial or Intralaminar Thalamic Nuclei or nucleus paracentrali or centrum medianum or paracentrali nucleus or centromedian thalamic nucleus or central lateral nucleus or thalamic nucleus parafascicular or central lateral thalamic nucleus or parafascicular thalamic nucleus or thalamic nucleus intralaminar or nucleus central dorsal or parafascicular nucleus of the thalamus or centromedian nucleus or intralaminar nuclei rostral or intralaminar nuclear group or thalamic nucleus centromedian or parafascicularis nucleus or nucleus central lateral or thalamic nuclei intralaminar or central dorsal thalamic nucleus or interlaminar nuclei of thalamus or rostral intralaminar nuclei or thalamus nucleus parafascicularis or centrum medianums nucleus or medianum centrum or thalamic nucleus paracentral or thalamus reticulate nucleus or nucleus paracentral or nucleus central medial or paracentral thalamic nucleus or median nucleus centre or nuclei intralaminar thalamic or nuclei rostral intralaminar or central medial nucleus or nucleus centrum medianums or nucleus centre median or medianum nucleus centrum or nucleus paracentral thalamic or nucleus centromedian thalamic or nucleus parafascicularis thalamus or nucleus intralaminar thalamic or nucleus centrum medianum or nucleus parafascicularis thalami or parafascicularis thalami nucleus or parafascicularis thalamus nucleus or reticulate nuclei of thalamus or nucleus parafasciculari or centrum medianums or centrum medianum nucleus or paracentralis nucleus or lateral nucleus central or parafascicular nucleus or central medial thalamic nucleus or nucleus centromedian or Lateral Thalamic Nuclei or medial pulvinar nucleus or Pulvinar or anterior pulvinar nucleus)	
#12	(Brain* or hippocamp* or encephalon or Blood Brain Barrier or hemato-encephalic barriers or barriers brain-blood or hemato encephalic barrier or barriers hemato-encephalic or barrier hemato-encephalic or hemato-encephalic barrier or truncus cerebrus or truncus cerebri or cerebri truncus or brainstems or cerebrus truncus or Mesencephalon or mesencephalon or mesencephalons or midbrains or midbrain or Cerebral Peduncle or Cerebral Crus or Substantia Nigra or nigras substantia or nigra substantia or substantia nigras or Pars Compacta or Pars Reticulata or Tegmentum Mesencephali or midbrain trigeminal nucleus or nucleus peripeduncular or annulari nucleus or nervi trochlearis nucleus or midbrain tegmentum or mesencephalus tegmentum or tegmental nucleus ventral or mesencephalic tegmentums or midbrain tegmentums or trigeminal nucleus mesencephalic or tegmentums midbrain or trochlearis nucleus nervi or nucleus annularis or trigeminal nucleus midbrain or nucleus annular or mesencephali tegmentum or darkshevichs nucleus or tegmentums mesencephalic or ventral tegmental nucleus or mesencephalic trigeminal nucleus or nervi trochleari nucleus or nucleus darkshevich's or darkschewitsch nucleus or tegmentum of midbrain or nucleus annulari or cajal interstitial nucleus or mesencephalic tegmentum or nuclei accessory oculomotor or trochlear nucleus or annularis nucleus or nucleus mesencephalic trigeminal or nucleus of darkschewitschor peripeduncular nucleus or oculomotor nuclei accessory or tegmentum midbrain or tegmentum mesencephali or nucleus nervi trochlearis or darkshevich nucleus or nucleus tractus mesencephalici nervi trigemini or interstitial nucleus of cajal or Cerebral Aqueduct or ducts mesencephalic or mesencephalic ducts or aqueduct mesencephalic or sylvian aqueducts or duct mesencephalic or sylvius aqueduct or cerebrus aqueductus or aqueductus cerebrus or cerebral aqueduct or aqueduct sylvian or aqueduct of sylvius or mesencephalic duct or cerebral aqueducts or aqueducts sylvian or aqueduct cerebral or sylvian aqueduct or aqueductus cerebri or aqueducts mesencephalic or cerebri aqueductus or mesencephalic aqueduct or Midbrain Reticular Formation or Pedunculopontine Tegmental Nucleus or nucleus tegmentalis pedunculopontinus or nucleus pedunculopontine tegmental or tegmental nucleus pedunculopontine or pedunculopontine tegmental nucleus or Oculomotor Nuclear Complex or Edinger-Westphal Nucleus or Periaqueductal Grey or greys central periaqueductal or griseum centrales or central grey substance of midbrain or periaqueductal greys central or grey matter periaqueductal or grey central periaqueductal or substantia grisea centralis or periaqueductal grey matter or central periaqueductal grey or grisea centralis substantia or periaqueductal grey or centrale mesencephali griseumor centrale mesencephalus griseum or centrale griseum or grey matters periaqueductalor centrales griseum or periaqueductal grey central or substantia grisea centralis mesencephali or mesencephalus griseum central or midbrain central grey or central grey mesencephalic or central periaqueductal greys or central grey midbrain or griseum centrale mesencephali or Raphe Nuclei or nucleus incertus or nucleus superior central ornuclei raphe ornucleus interfascicular or superior central nucleus or raphe nuclei or interfascicular nucleus or raphe nucleus or incertus nucleus or central nucleus superior or rostral linear nucleus of the raphe or caudal linear nucleus of the raphe or rostral linear nucleus of raphe or nucleus rapheor Dorsal Raphe Nucleus or Interpeduncular Nucleus or Midbrain Raphe Nuclei or Red Nucleus or nucleus ruber or red nucleus or nucleus red or Ventral Tegmental Area or tegmentalis ventralis area or tegmentalis ventrali area or area tegmentalis ventralis or ventral tegmental area of tsai or ventral tegmental area or tegmental area ventral or area tegmentalis ventrali or Locus Coeruleus or coeruleus complex locus or complices locus coeruleus or locus caeruleus or complex locus ceruleus or complices locus ceruleus or coeruleus complices locus or ceruleus complex locus or locus ceruleus complex or complex locus coeruleus or locus ceruleus complices or locus ceruleus or nucleus pigmentosus pontis or locus coeruleus complices or pontis nucleus pigmentosus or ceruleus complices locus or locus coeruleus or locus coeruleus complex or Tectum Mesencephali or corpora quadrigemina or inferior colliculus commissures or colliculus commissures superior or colliculus commissures inferior or quadrigeminal plates or superior colliculus commissure or plate quadrigeminal or commissure of superior colliculus or quadrigemina corpora or commissure of inferior colliculus or lamina quadrigemina or inferior colliculus commissure or colliculus commissure inferior or quadrigeminal plate or tectum mesencephalus or mesencephalus tectum or plates quadrigeminal or quadrigemina lamina or colliculus commissure superior or Inferior Colliculi or colliculi inferior or inferior colliculi or inferiors colliculus or posterior colliculus or brachial nucleus of the inferior colliculus or caudal colliculus or colliculus inferiors or colliculus caudal or inferior colliculus or colliculus posterior or colliculus inferior or Subcommissural Organ or subcommissural organs or subcommissural organ or organs subcommissural or organ subcommissural or Superior Colliculi or mammalian optic lobesor optic lobe	

	mammalian or optic tectums or superior colliculi or optic tectum or anterior colliculus or colliculus superior or human optic lobes or superior colliculus or optic lobes human or optic lobes mamalian or optic lobe human or colliculi superior or tectum optic or tectums optic or mammalian optic lobe or human optic lobe or colliculus anterior or Reticular Formation or formations reticular or reticular formation or reticular formations or formation reticular or edunculopontine Tegmental Nucleus or nucleus tegmentalis pedunculopontinus or nucleus pedunculopontine tegmental or tegmental nucleus pedunculopontine or pedunculopontine tegmental nucleus or Respiratory Center or centers respiratory or respiratory centers or center respiratory or respiratory center or hombencephalon or hind brains or brains hind or rhombencephalons or hindbrain or hindbrains or brain hind or rhombencephalon or hind brain or Medulla Oblongata or medulla oblongata or nucleus ambiguous or arcuate nucleus-1 or accessory cuneate nucleus or nucleus external cuneate or cuneate nucleus accessory or nucleus ambiguous or medulla oblongatas or arcuate nucleus of the medulla or cuneate nucleus lateralor nucleus lateral cuneate or ambiguous nucleus or cuneate nucleus external or arcuate nucleus 1 or external cuneate nucleus or ambiguus nucleus or arcuate nucleus-1s or lateral cuneate nucleus or Area Postrema or area postremas or trigger zone chemoreceptor or chemoreceptor trigger zone or chemoreceptor trigger zones or trigger zones chemoreceptor or zone chemoreceptor trigger or postrema area or zones chemoreceptor trigger or area postrema or Olivary Nucleus ornucleus basalis olivary or nucleus olivary or basalis olivary nucleus or nucleus olivary basal or olivary basal nucleus or basal nucleus olivary or olivary nucleus or Raphe nuclei or nucleus incertus or nucleus superior central or nuclei raphe or nucleus interfascicular or superior central nucleus or raphe nuclei or interfascicular nucleus or raphe nucleus or incertus nucleus or central nucleus superior or rostral linear nucleus of the raphe or caudal linear nucleus ofthe raphe or rostral linear nucleus of raphe or nucleus raphe or Nucleus Raphe Obscurus or Nucleus Raphe Pallidus or Solitary Nucleusor solitary nuclear complices or nucleus of tractus solitaries or complex solitary nuclear or tractus solitarii nuclei or nucleus solitaries or solitarius nucleus tractus or tractus solitarius nucleus or solitariusnuclei tractus or solitary tract nucleus or nucleus solitary tract or solitary nuclear complex or tractus solitarius nuclei or nuclear complices solitary or nuclei tractus solitarii or solitary nucleus ornucleus solitaryor nucleus of the solitary tract or nuclear complex solitary or complices solitary nuclear or nucleus of solitary tract or nucleus tractus solitaries or Trigeminal Nucleus, Spinal or trigeminal nucleus spinal or nucleus spinal trigeminal or spinal trigeminal nucleus or Trigeminal Caudal Nucleus or caudal nucleus trigeminal or nucleus trigeminal caudal or trigeminal caudal nucleus or Metencephalon or Cerebellumor corpus cerebellus or parencephalons or cerebellus corpus or cerebellum or cerebellums or corpus cerebelli or parencephalon or cerebelli corpus or Cerebellar Cortex or cerebelli cortex or cortex cerebellus or cerebellar cortex or cortex cerebelli orcerebellus cortex orcortex cerebellar or Cerebellar Vermis or Purkinje Cells or purkinje cells or cells purkinje or Cerebellar Nuclei or nucleus dentatus or Cerebellopontine Angle or central nucleus or central nucleus or interposed nucleus anterior or nucleus globosus or medial cerebellar nucleus or emboliformis nucleus or nuclei cerebellar or intracerebellar nuclei or nucleus fastigii or nucleus fastigial orfastigii nucleus or central nuclei or nuclei central or deep cerebellar nucleus or intracerebellar nucleus or nucleus fastigial cerebellar or nucleus anterior interposed or nucleus intracerebellar oranterior interposed nucleus or nucleus anterior interpositus or nucleus medial cerebellar or nuclei intracerebellar or nucleus dentate or dentate nucleus or interpositus nucleus anterior or globosus nucleus orcerebellar nucleus deep or nucleus central or nucleus cerebellar or cerebellar nuclei deep or nucleus dentate cerebellar or anterior interpositus nucleus or cerebellar nucleus medial or cerebellar nuclei or fastigial cerebellar nucleus or Pons or pons or varolii ponsor pontes or pons varolius or varolius pons or pons varolii or ponte or Barrington's Nucleusor Cochlear Nucleus or cochlear nucleus or nuclei cochlear or cochlear nuclei or nucleus cochlear or Kolliker-Fuse Nucleus or Middle Cerebellar Peduncle or Pontine Tegmentum or Abducens Nucleus or Facial Nucleus or Parabrachial Nucleus or Nucleus Raphe Magnus or Superior Olivary Complex or Trapezoid Body or Trigeminal Motor Nucleus or Vestibular Nuclei or schwalbes nucleus or nucleus schwalbe or vestibular nuclei or vestibular nucleus medial or nuclei vestibular or schwalbe's nucleus or nucleus schwalbe's or medial vestibular nucleus or schwalbe nucleus or nucleus medial vestibular or Vestibular Nucleus, Lateral or deiters nucleus or deiter's nucleus or nucleus of deiters or lateral vestibular nucleus or nucleus lateral vestibular or vestibularis laterali nucleus or nucleus vestibularis laterali or vestibular nucleus lateral or vestibularis magnocellulari nucleus or vestibularis magnocellularis nucleus or deiter nucleus or nucleus vestibularis magnocellularis or nucleus vestibularis magnocellulari or nucleus deiter or nucleus vestibularis lateralis or vestibularis lateralis nucleus or nucleus deiter's or Tectospinal Fibers or Trigeminal Nuclei or trigeminal nucleus or trigeminal nuclear complices or trigeminal nuclear complex or nuclei trigeminal or trigeminal nuclei or nucleus trigeminal or nuclear complices trigeminal or nuclear complex trigeminal or Grey Matter or grey matter or grey matters cerebellar or grey matter cerebellar 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ventricles fourth or ventricle fourth or 4th ventricle or quarto ventricolos or ventricle 4th or ventricles 4th or fourth ventricle or ventricolos quarto or fourth ventricles or 4th ventricles or quarto ventricolo or Lateral Ventricles or lateral ventricle orsubventricular zones or lateral ventricles or ventricle lateral or zone subventricular or ventricles lateral or subventricular zone or zones subventricular or Septum Pellucidum or septum supracommissural or pelusidum septum or septum pellucidum or lucidums septum or supracommissural septum or pellucidum septum or septum pelusidums or septum pelusidum or pelusidums septum or septum lucidums or supracommissural septums or septums supracommissural or lucidum septum or septum lucidum or Third Ventricle or ventricle or ventricles third or ventricles 3rd or third ventricle or ventricle 3rd or 3rd ventricles or third ventricles or ventricle third or Limbic System or limbic system or system limbic or systems limbic or limbic systems or Amygdala or amygdaloid bodies or corpus amygdaloideums or nucleus intercalated amygdaloid or corpus amygdaloideum or amygdaloid body or complex amygdaloid nuclear or amygdaloid nuclear complices or amygdaloid nucleusor intercalata massa or amygdaloideums corpus or intercalatas massa or amygdaloid nucleus intercalated or nuclear complices amygdaloid or archistriatums or amygdala or massa intercalates or nucleus amygdaloid or amygdaloideum corpus or amygdalae nucleus or nuclear complex amygdaloid or archistriatum or nucleus amygdalae or amygdaloid nuclear complex or Basolateral Nuclear Complex or Central Amygdaloid Nucleus or Corticomedial Nuclear Complex or	

	Periamygdaloid Cortex or epithalamus or Habenula or commissure habenular or habenula complex or habenulas or complices habenula or nucleus habenularis or habenular commissures or complex habenula or habenula complices or nucleus habenular or nucleus habenulari or commissures habenular or habenula or habenularums commissura or commissura habenularum or habenularis nucleus or habenular nuclei or commissura habenularums or nuclei habenular or habenulari nucleus or habenular nucleus or Pineal Gland or pineales corpus or body pineal or glands pineal or pineal glands or pineal body or cerebri epiphysis or corpus pineales or gland pineal or pineale corpus or bodies pineal or corpus pineale or pineal gland or pineal bodies or epiphysis cerebri or Hippocampus or hippocampal formation or propers hippocampus or hippocampus propers or formations hippocampal or horn ammon's or schaffer collateral or ammon horn or hippocampus or horn ammon or cornu ammonis or hippocampus proper or proper hippocampus or collaterals schaffer or formation hippocampal or hippocampal formations or subiculum or subiculums or ammon's horn or CA1 Region, Hippocampal or regio superior of hippocampus or field hippocampus ca1 or ca1 stratum radiatum or stratum radiatum ca1 or hippocampal sector ca1 or hippocampus ca1 field or hippocampus regio superior or ca1 stratum radiatums or sector ca1 hippocampal or ca1 hippocampus or radiatums ca1 stratum or stratum radiatums ca1 or ca1 hippocampal sector or ca1 pyramidal cell area or ca1 region hippocampal or ca1 pyramidal cell layer or ca1 stratum pyramidale or stratum pyramidale ca1 or cornu ammonis 1 area or radiatum ca1 stratum or CA2 Region, Hippocampal or ca2 stratum pyramidale or radiatums ca2 stratum or cornu ammonis 2 area or ca2 field hippocampus or stratum pyramidale ca2 or stratum radiatum ca2 or ca2 stratum radiatums or radiatum ca2 stratum or sector ca2 hippocampal or region hippocampal ca2 or ca2 field of hippocampus or stratum radiatums ca2 or ca2 region hippocampal or hippocampal sector ca2 or hippocampal ca2 region or hippocampus ca2 field or ca2 pyramidal cell layer or field hippocampus ca2 or ca2 pyramidal cell area or CA3 Region, Hippocampal or stratum lucidum ca3 or ca3 stratum lucidum or stratum lucidums ca3 or lucidum ca3 stratum or ca3 region hippocampal or ca3 pyramidal cell area or hippocampus ca3 field or ca3 hippocampal sector or sector ca3 hippocampal or ca3 stratum radiatum or ca3 stratum lucidums or hippocampal ca3 regions or cornu ammonis 3 area or ca3 field of hippocampus or radiatum ca3 stratum or field hippocampus ca3 or stratum radiatums ca3 or ca3 pyramidal cell layer or lucidums ca3 stratum or region hippocampal ca3 or radiatums ca3 stratum or ca3 stratum pyramidale or ca3 field hippocampus or Dentate Gyrus or ca4 region hippocampal or dentate fascia or cornu ammonis 4 area or hilus gyri dentate or ca4 field of hippocampal formation or ca4 hippocampal sector or gyrus dentate or sector ca4 hippocampal or hippocampal ca4 region or area dentata or region hippocampal ca4 or dentata area or field hippocampal ca4 or gyrus dentatus or hilus of the fascia dentata or hilus of dentate gyrus or dentate gyrus or area dentatas or dentata fascia or hippocampal sector ca4 or hippocampal ca4 field or ca4 of lorente de no or Mossy Fibers, Hippocampal or hippocampal mossy fiber or mossy fibers hippocampal)
#11	(IClH OR "Cl channels" OR "acid-activated Cl channels")
#10	(GJA1 OR GJA3 OR GJA4 OR GJA5 OR GJA6P OR GJA8 OR GJA9 OR GJA10 OR GJB1 OR GJB2 OR GJB3 OR GJB4 OR GJB5 OR GJB6 OR GJB7 OR GJC1 OR GJC2 OR GJC3 OR GJD2 OR GJD3 OR GJD4 OR GJE1 OR "gap junction protein epsilon 1" OR "gap junction protein delta 4" OR "gap junction protein delta 3" OR "gap junction protein delta 2" OR "gap junction protein gamma 3" OR "gap junction protein gamma 2" OR "gap junction protein gamma 1" OR "gap junction protein beta 7" OR "gap junction protein beta 6" OR "gap junction protein beta 5" OR "gap junction protein beta 4" OR "gap junction protein beta 3"OR "gap junction protein beta 2" OR "gap junction protein beta 1" OR "gap junction protein alpha 10"OR "gap junction protein alpha 9" OR "gap junction protein alpha 8" OR "gap junction protein alpha 5" OR "gap junction protein alpha 4" OR "gap junction protein alpha 3" OR "gap junction protein alpha 1" OR "gap junction protein alpha 6 pseudogene" OR CX43 OR ODD OR SDTY3OR CX46 OR CX37 OR CX40 OR CX50 OR CX58 OR CX59 OR CX62 OR CX32 OR CX26 OR CX31 OR CX30.3 OR CX31.1 OR CX45 OR CX47 OR CX46.6 OR CX30.2 OR CX36 OR CX31.9 OR CX40.1 OR CX23)
#9	("cyclic nucleotide gated channel alpha 1" OR "cyclic nucleotide gated channel alpha 2" OR "cyclic nucleotide gated channel alpha 3" OR "cyclic nucleotide gated channel alpha 4" OR CNGA1 OR CNGA2 OR CNGA3 OR CNGA4 OR "cyclic nucleotide gated channel beta 1" OR "cyclic nucleotide gated channel beta 3" OR CNGB1 OR CNGB3 OR HCN1 OR HCN2 OR HCN3 OR HCN4 OR "hyperpolarization activated cyclic nucleotide gated potassium channel 1" OR "hyperpolarization activated cyclic nucleotide gated potassium channel 3" OR "hyperpolarization activated cyclic nucleotide gated potassium channel 4" OR "hyperpolarization activated cyclic nucleotide gated potassium and sodium channel 2" OR RCNC1 OR RCNCa OR CNG1 OR RP49 OR CNG2 OR OCNC1 OR OCNCa OR OCNCALPHA OR OCNCalpha OR FLJ46312 OR CCNC1 OR CCNCa OR CNG3 OR OCNC2 OR OCNCb OR CNG5 OR RCNC2 OR RCNCb OR GARP OR GAR1 OR CNGB1B OR RP45 OR BCNG-1 OR BCNG-2 OR HAC-2 OR HAC-1 OR KIAA1535)
#8	("potassium voltage-gated channel modifier subfamily V member 2" OR "potassium voltage-gated channel modifier subfamily V member 1" OR Kv8.2 OR Kv8.1 OR "potassium voltage-gated channel modifier subfamily S member 3" OR "potassium voltage-gated channel modifier subfamily S member 2" OR "potassium voltage-gated channel modifier subfamily S member 1" OR Kv9.3 OR Kv9.2 OR Kv9.1 OR "potassium voltage-gated channel subfamily Q member 5" OR "potassium voltage-gated channel subfamily Q member 4" OR "potassium voltage-gated channel subfamily Q member 3" OR "potassium voltage-gated channel subfamily Q member 2" OR "potassium voltage-gated channel subfamily Q member 1" OR Kv7.5 OR Kv7.4 OR Kv7.3 OR Kv7.2 OR Kv7.1 OR ENB1 OR BFNC OR KCNA11 OR HNSPC OR KCNA8 OR KVLQT1 OR JLNS1 OR LQT1 OR KCNA9 OR LQT OR EBN OR DFNA2 OR EBN2 OR "potassium voltage-gated channel subfamily H member 8" OR "potassium voltage-gated channel subfamily H member 7" OR "potassium voltage-gated channel subfamily H member 6" OR "potassium voltage-gated channel subfamily H member 5" OR "potassium voltage-gated channel subfamily H member 4" OR "potassium voltage-gated channel subfamily H member 3" OR "potassium voltage-gated channel subfamily H member 2" OR "potassium voltage-gated channel subfamily H member 1" OR LQT2 OR Kv12.1 OR elk3 OR Kv11.3 OR HERG3 OR erg3 OR Kv11.2 OR erg2 OR HERG2 OR Kv10.2 OR H-EAG2 OR eag2 OR Kv12.3 OR Kv12.2 OR Kv11.1 OR elk1 OR elk2 OR BEC1 OR erg1 OR HERG OR Kv10.1 OR eag OR h-eag OR Kv6.4 OR Kv6.3 OR Kv6.2 OR Kv6.1 OR KCNF2 OR kH2 OR K13 OR "potassium voltage-gated channel modifier subfamily G member 4" OR "potassium voltage-gated channel modifier subfamily G member 3" OR "potassium voltage-gated channel modifier subfamily G member 2" OR "potassium voltage-gated channel modifier subfamily G member 1" OR "potassium voltage-gated channel modifier subfamily F member 1" OR KCNF OR Kv5.1 OR "potassium voltage-gated channel subfamily D member 3" OR "potassium voltage-gated channel subfamily D member 2" OR "potassium voltage-gated channel subfamily D member 1" OR Kv4.3 OR Kv4.2 OR Kv4.1 OR "potassium voltage-gated channel subfamily C member 4"

	OR "potassium voltage-gated channel subfamily C member 3" OR "potassium voltage-gated channel subfamily C member 2" OR "potassium voltage-gated channel subfamily C member 1" OR Kv3.4 OR Kv3.3 OR Kv3.2 OR Kv3.1 OR "potassium voltage-gated channel subfamily B member 2" OR "potassium voltage-gated channel subfamily B member 1" OR Kv2.2 OR Kv2.1 OR "potassium voltage-gated channel subfamily A member 10" OR "potassium voltage-gated channel subfamily A member 7" OR "potassium voltage-gated channel subfamily A member 6" OR "potassium voltage-gated channel subfamily A member 5" OR "potassium voltage-gated channel subfamily A member 4" OR "potassium voltage-gated channel subfamily A member 3" OR "potassium voltage-gated channel subfamily A member 2" OR "potassium voltage-gated channel subfamily A member 1" OR Kv1.8 OR Kv1.7 OR Kv1.6 OR Kv1.5 OR Kv1.4 OR Kv1.3 OR Kv1.2 OR Kv1.1)	
#7	(KCNJ1 OR KCNJ2 OR KCNJ3 OR KCNJ4 OR KCNJ5 OR KCNJ6 OR KCNJ8 OR KCNJ9 OR KCNJ10 OR KCNJ11 OR KCNJ12 OR KCNJ13 OR KCNJ14 OR KCNJ15 OR KCNJ16 OR KCNJ18 OR "potassium voltage-gated channel subfamily J member 1" OR "potassium voltage-gated channel subfamily J member 2" OR "potassium voltage-gated channel subfamily J member 3" OR "potassium voltage-gated channel subfamily J member 4" OR "potassium voltage-gated channel subfamily J member 5" OR "potassium voltage-gated channel subfamily J member 6" OR "potassium voltage-gated channel subfamily J member 8" OR "potassium voltage-gated channel subfamily J member 9" OR "potassium voltage-gated channel subfamily J member 10" OR "potassium voltage-gated channel subfamily J member 11" OR "potassium voltage-gated channel subfamily J member 12" OR "potassium voltage-gated channel subfamily J member 13" OR "potassium voltage-gated channel subfamily J member 14" OR "potassium voltage-gated channel subfamily J member 15" OR "potassium voltage-gated channel subfamily J member 16" OR "potassium voltage-gated channel subfamily J member 18" OR KCNJ7 OR KCNJN1 OR ROMK1 OR Kir1.1 OR Kir2.1 OR IRK1 OR LQT7 OR Kir3.1 OR GIRK1 OR KGA OR Kir2.3 OR HIR OR HRK1 OR hIRK2 OR IRK3 OR Kir3.4 OR CIR OR KATP1 OR GIRK4 OR LQT13 OR Kir3.2 OR GIRK2 OR KATP2 OR BIR1 OR hiGIRK2 OR Kir6.1 OR GIRK3 OR Kir3.3 OR Kir4.1 OR Kir1.2 OR BIR OR Kir6.2 OR Kir2.2 OR Kir2.2v OR IRK2 OR hIRK1 OR Kir7.1 OR Kir1.4 OR LCA16 OR Kir2.4 OR IRK4 OR Kir4.2 OR Kir1.3 OR IRKK OR Kir5.1 OR BIR9 OR KIR2.6 OR TTPP2 OR "Inwardly rectifying potassium channels" OR Kir OR IRK OR "Voltage-gated potassium channels" OR KCNA1 OR KCNA2 OR KCNA3 OR KCNA4 OR KCNA5 OR KCNA6 OR KCNA7 OR KCNA10 OR KCNB1 OR KCNB2 OR KCNC1 OR KCNC2 OR KCNC3 OR KCNC4 OR KCND1 OR KCND2 OR KCND3 OR KCNF1 OR KCNG1 OR KCNG2 OR KCNG3 OR KCNG4 OR KCNH1 OR KCNH2 OR KCNH3 OR KCNH4 OR KCNH5 OR KCNH6 OR KCNH7 OR KCNH8 OR KCNQ1 OR KCNQ2 OR KCNQ3 OR KCNQ4 OR KCNQ5 OR KCNS1 OR KCNS2 OR KCNS3 OR KCNV1OR KCNV2)	
#6	(FLJ14471 OR FEX OR HG38 OR GPR49 OR GPR67 OR GPR48 OR EBI2 OR "G protein-coupled receptor 183" OR "G protein-coupled receptor 182" OR "G protein-coupled receptor 176" OR "G protein-coupled receptor 174" OR "G protein-coupled receptor 173" OR "G protein-coupled receptor 171" OR "G protein-coupled receptor 162" OR "G protein-coupled receptor 161" OR "G protein-coupled receptor 160" OR "G protein-coupled receptor 153" OR "G protein-coupled receptor 152" OR "G protein-coupled receptor 151" OR "G protein-coupled receptor 150" OR "G protein-coupled receptor 149" OR "G protein-coupled receptor 148" OR "G protein-coupled receptor 146" OR "G protein-coupled receptor 142" OR "G protein-coupled receptor 141" OR "G protein-coupled receptor 139" OR "G protein-coupled receptor 135" OR "G protein-coupled receptor 132" OR "G protein-coupled receptor 119" OR "G protein-coupled receptor 101" OR "G protein-coupled receptor 88" OR "G protein-coupled receptor 87" OR "G protein-coupled receptor 85" OR "G protein-coupled receptor 84" OR "G protein-coupled receptor 82" OR "G protein-coupled receptor 79, pseudogene" OR "G protein-coupled receptor 78" OR "G protein-coupled receptor 75" OR "G protein-coupled receptor 68" OR "G protein-coupled receptor 65" OR "G protein-coupled receptor 63" OR "G protein-coupled receptor 62" OR "G protein-coupled receptor 61" OR "G protein-coupled receptor 55" OR "G protein-coupled receptor 52" OR "G protein-coupled receptor 50" OR "G protein-coupled receptor 45" OR "G protein-coupled receptor 42" OR "G protein-coupled receptor 39" OR "G protein-coupled receptor 37" OR "G protein-coupled receptor 35" OR "G protein-coupled receptor 34" OR "G protein-coupled receptor 33" OR "G protein-coupled receptor 32" OR "G protein-coupled receptor 31" OR "G protein-coupled receptor 27" OR "G protein-coupled receptor 26" OR "G protein-coupled receptor 25" OR "G protein-coupled receptor 22" OR "G protein-coupled receptor 21" OR "G protein-coupled receptor 20" OR "G protein-coupled receptor 19" OR "G protein-coupled receptor 18" OR "G protein-coupled receptor 17" OR "G protein-coupled receptor 15" OR "G protein-coupled receptor 12" OR "G protein-coupled receptor 6" OR "G protein-coupled receptor 4" OR "G protein-coupled receptor 3" OR "G protein-coupled receptor 1" OR ACCA OR PPP1R84 OR GPCR21 OR "G protein-coupled receptor 37 like 1" OR SREB1 OR 12-HETER OR HETER1 OR RVDR1 OR EDNRBL OR hET(B)R-LP OR PAELR OR ETBR-LP-2 OR GPR42P OR GPR41L OR FFAR3L OR PSP24 OR PSP24A OR H9 OR Mel1c OR BALGR OR PSP24B OR PSP24(beta) OR hTDAG8 OR TDAG8 OR OGR1 OR WI-31133 OR GPR79P OR GPR72 OR EX33 OR SREB2 OR GPR95 OR hGPCR2 OR GPCR2 OR G2A OR PAFR OR HUMNPIIY20 OR PGR3 OR PGR13 OR PGR2 OR PGR8 OR PGR6 OR PGR10 OR PGR11 OR PGR7 OR PGR5 OR PGR1 OR IEDA OR R35 OR GALR4 OR GPCR150 OR GPCR1 OR RE2 OR A-2 OR GRCA OR H963 OR SREB3 OR FKSG79 OR Gm1012 OR hrhAMR OR G10D OR AM-R OR ADMR OR "G protein-coupled receptor") ;	
#5	(P2RX1 OR P2RX2 OR P2RX3 OR P2RX4 OR P2RX5 OR P2RX6 OR P2RX7 OR "purinergic receptor P2X 1" OR "purinergic receptor P2X 2" OR "purinergic receptor P2X 3" OR "purinergic receptor P2X 4" OR "purinergic receptor P2X 5" OR "purinergic receptor P2X 6" OR "purinergic receptor P2X 7" OR P2X1 OR P2X2 OR P2X3 OR P2X4 OR P2X5 OR P2X6 OR P2X7 OR DFNA41 OR P2RXL1 OR LRH-1 OR P2XM OR MGC129625 OR MGC20089 OR "purinergic receptors" OR "ionotropic purinoceptors" OR GPR1 OR GPR3 OR GPR4 OR GPR6 OR GPR12 OR GPR15 OR GPR17 OR GPR18 OR GPR19 OR GPR20 OR GPR21 OR GPR22 OR GPR25 OR GPR26 OR GPR27 OR GPR31 OR GPR32 OR GPR33 OR GPR34 OR GPR35 OR GPR37 OR GPR37L1 OR GPR39 OR GPR42 OR GPR45 OR GPR50 OR GPR52 OR GPR55 OR GPR61 OR GPR62 OR GPR63 OR GPR65 OR GPR68 OR GPR75 OR GPR78 OR GPR79 OR GPR83 OR GPR82 OR GPR84 OR GPR85 OR GPR87 OR GPR88 OR GPR101 OR GPR119 OR GPR132 OR GPR135 OR GPR139 OR GPR141 OR GPR142 OR GPR146 OR GPR148 OR GPR149 OR GPR150 OR GPR151 OR GPR152 OR GPR153 OR GPR160 OR GPR161 OR GPR162 OR GPR171 OR GPR173 OR GPR174 OR GPR176 OR GPR183 OR GPR182 OR LGR4 OR LGR5 OR LGR6 OR MAS1 OR MAS1L OR MRGPRD OR MRGPRE OR MRGPRF OR MRGPRG OR MRGPRX1 OR MRGPRX2 OR MRGPRX3 OR MRGPRX4 OR P2RY8 OR P2RY10 OR "P2Y receptor family member 10" OR "P2Y receptor family member 8" OR P2Y10 OR P2Y8 OR MRGX4 OR MRGX3 OR MRGX2 OR MRGX1 OR "MAS related GPR family member X4" OR "MAS related GPR family member X3" OR "MAS related GPR family member X2" OR "MAS related GPR Family member X1" OR "MAS related GPR family member G" OR "MAS related GPR Family member F" OR "MAS related GPR family member E" OR "MAS related GPR family member D" OR GPR169 OR GPR168 OR GPR167 OR GPR140 OR mrgG OR mrgF OR mrgE OR mrgD OR MGC21621 OR dJ994E9.2 OR MRG OR MAS-L OR "MAS1 proto-oncogene like, G protein-coupled	

	receptor" OR "MAS1 proto-oncogene, G protein-coupled receptor" OR "leucine rich repeat containing G protein-coupled receptor 6" OR "leucine rich repeat containing G protein-coupled receptor 5" OR "leucine rich repeat containing G protein-coupled receptor 4")	
#4	(KCNK1 OR KCNK2 OR KCNK3 OR KCNK4 OR KCNK5 OR KCNK6 OR KCNK7 OR KCNK9 OR KCNK10 OR KCNK12 OR KCNK13 OR KCNK15 OR KCNK16 OR KCNK17 OR KCNK18 OR "potassium two pore domain channel subfamily K member 1" OR "potassium two pore domain channel subfamily K member 2" OR "potassium two pore domain channel subfamily K member 3" OR "potassium two pore domain channel subfamily K member 4" OR "potassium two pore domain channel subfamily K member 5" OR "potassium two pore domain channel subfamily K member 6" OR "potassium two pore domain channel subfamily K member 7" OR "potassium two pore domain channel subfamily K member 9" OR "potassium two pore domain channel subfamily K member 10" OR "potassium two pore domain channel subfamily K member 12" OR "potassium two pore domain channel subfamily K member 13" OR "potassium two pore domain channel subfamily K member 15" OR "potassium two pore domain channel subfamily K member 16" OR "potassium two pore domain channel subfamily K member 17" OR "potassium two pore domain channel subfamily K member 18" OR KCNK11 OR KCNK14 OR K2p1.1 OR K2p2.1 OR DPK OR TWIK-1 OR TREK-1 OR K2p3.1 OR TASK OR TASK-1 OR K2p4.1 OR TRAAK OR K2p5.1 OR TASK-2 OR K2p6.1 OR TWIK-2 OR K2p9.1 OR K2p7.1 OR TASK3 OR TASK-3 OR K2p10.1 OR TREK-2 OR TREK2 OR PPP1R97 OR THIK-2 OR THIK2 OR K2p12.1 OR K2p13.1 OR THIK-1 OR THIK1 OR K2p15.1 OR dJ781B1.1 OR KT3.3 OR KIAA0237 OR TASK5 OR TASK-5 OR K2p16.1 OR TALK-1 OR TALK1 OR K2p17.1 OR TALK-2 OR TALK2 OR TASK4 OR TASK-4 OR K2p18.1 OR TRESK-2 OR TRESK2 OR TRESK OR TRIK OR "two-pore-domain potassium channel")	
#3	(TRPA1 OR "transient receptor potential cation channel subfamily A member 1" OR ANKTM1 OR TRPC1 OR "transient receptor potential cation channel subfamily C member 1" OR HTRP-1 OR TRPC2 OR "transient receptor potential cation channel subfamily C member 2, pseudogene" OR TRPC3 OR "transient receptor potential cation channel subfamily C member 3" OR "transient receptor potential cation channel subfamily C member 4" OR "transient receptor potential cation channel subfamily C member 5" OR "transient receptor potential cation channel subfamily C member 6" OR "transient receptor potential cation channel subfamily C member 7" OR TRPC4 OR TRPC5 OR TRPC6 OR TRPC7 OR FSGS2 OR HTRP4 OR TRP4 OR PPP1R159 OR TRP6 OR MCOLN1 OR MCOLN2 OR MCOLN3 OR mucolipin 1 OR mucolipin 2 OR mucolipin 3 OR TRPM-L1 OR MSTP080 OR MST080 OR MLIV OR TRPML1 OR ML4 OR FLJ36691 OR TRP-ML2 OR TRPML2 OR TRP-ML3 OR TRPML3 OR FLJ11006 OR TRPM1 OR TRPM2 OR TRPM3 OR TRPM4 OR TRPM5 OR TRPM6 OR TRPM7 OR TRPM8 OR "transient receptorpotential cation channel subfamily M member 1" OR "transient receptor potential cation channel subfamily M member 2" OR "transient receptor potential cation channel subfamily M member 3" OR "transient receptor potential cation channel subfamily M member 4" OR "transient receptor potential cation channel subfamily M member 5) OR "transient receptor potential cation channel subfamily M member 6" OR "transient receptor potential cation channel subfamily M member 7" OR "transient receptor potential cation channel subfamily M member 8" OR MLSN1 OR LTRPC1 OR CSNB1C OR TRPC7 OR KNP3 OR LTRPC2 OR NUDT9L1 OR NUDT9H OR EREG1 OR KIAA1616 OR LTRPC3 OR GON-2 OR FLJ20041 OR LTRPC5 OR MTR1 OR HOMG OR HSH OR CHAK2 OR FLJ22628 OR CHAK1 OR LTRPC7 OR TRP-PLIK OR PKD2 OR PKD2L1 OR PKD2L2 OR "polycystin 2, transient receptor potential cation channel" OR "polycystin 2 like 1, transient receptor potential cation channel" OR "polycystin 2 like 2, transient receptor potential cation channel" OR PKD2L OR PKDL OR TRPP3 OR PCL OR TRPP5 OR VR1 OR TRPV1 OR TRPV2 OR TRPV3 OR TRPV4 OR TRPV5 OR TRPV6 OR "transient receptor potential cation channel subfamily V member 1" OR "transient receptor potential cation channel subfamily V member 2" OR "transient receptor potential cation channel subfamily V member 3" OR "transient receptor potential cation channel subfamily V member 4" OR "transient receptor potential cation channel subfamily V member 5" OR "transient receptor potential cation channel subfamily V member 6" OR VRL OR VRL-1 OR VRL1 OR VRL3 OR OTRPC4 OR TRP12 OR VROAC OR VRL-2 OR VR-OAC OR CMT2C OR ECAC1 OR CaT2 OR ECAC2 OR CaT1 OR Transient receptor potential channels OR TRP channels OR "vanilloid receptor subtype 1" OR "amiloride-sensitive cation channel 1, neuronal" OR ""amiloride-sensitive cation channel" OR acid chemosensors)	
#2	("acid sensing ion channels" OR ASIC* OR ASIC2 OR "acid-sensing ion channels" OR ASIC 2 OR ASIC3 OR ASIC 3 OR ASIC1 OR ASIC 1 OR ASIC4 OR ASIC 4 OR ASIC5 OR ASIC 5 OR BNaC2 OR hBNaC2 OR ASIC2a OR BNC1 OR BNaC1 OR hBNaC1 OR MDEG OR TNaC1 OR DRASIC OR BNAC4 OR INAC OR HINAC OR ACCN2 OR ACCN OR ACCN1 OR ACCN3 OR ACCN4 OR ACCN5 OR "acid sensing ion channel subunit 1" OR "acid sensing ion channel subunit 2" OR "acid sensing ion channel subunit 3" OR "acid sensing ion channel subunit family member 4" OR "acid sensing ion channel subunit family member 5")	
#1	(microglia* OR gliosis OR glia* OR nissl OR macrophage* OR monocyte* OR CD11 OR CD68 OR CD40 OR CD45 OR Ox-42 OR Ox42 OR ed-1 OR ed1 OR cd200 OR cd 200 OR Iba1 OR Iba1 OR ly6g OR cd3 OR mpo OR mcp1 OR mcp-1 OR ccr2 OR arg1 OR arg 1 OR mhc OR major histocompatibility complex OR aldh1 OR aldh 1 OR hla dr OR cd20 OR HLA OR neuroglia OR leukocytes OR antibody-producing cells OR antigen-presenting cells OR neurogenic inflammation OR astrocyte*)	

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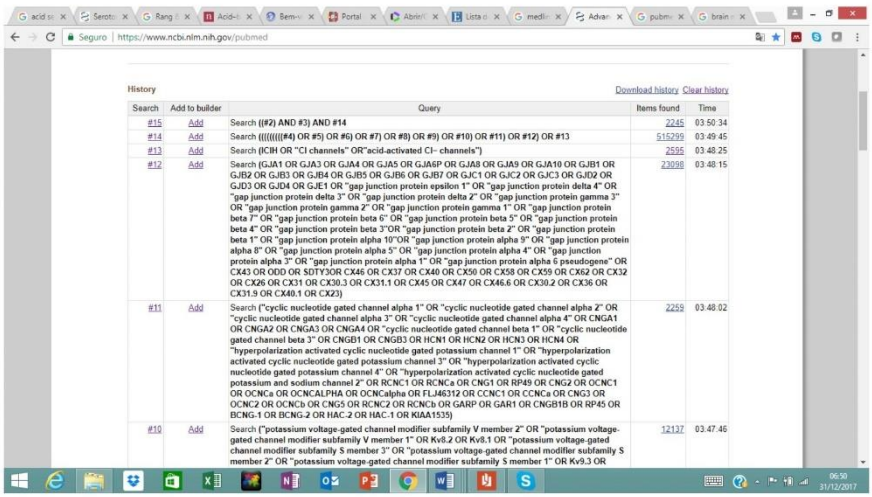

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#15	Add	Search ((#2) AND #3) AND #14	2245	03:50:34
#14	Add	Search (((((((#4) OR #5) OR #6) OR #7) OR #8) OR #9) OR #10) OR #11) OR #12) OR #13	515299	03:49:45
#13	Add	Search (ICIH OR "Cl channels" OR"acid-activated Cl- channels")	2595	03:48:25
#12	Add	Search (GJA1 OR GJA3 OR GJA4 OR GJA5 OR GJA6P OR GJA8 OR GJA9 OR GJA10 OR GJB1 OR GJB2 OR GJB3 OR GJB4 OR GJB5 OR GJB6 OR GJB7 OR GJC1 OR GJC2 OR GJC3 OR GJD2 OR GJD3 OR GJD4 OR GJE1 OR "gap junction protein epsilon 1" OR "gap junction protein delta 4" OR "gap junction protein delta 3" OR "gap junction protein delta 2" OR "gap junction protein gamma 3" OR "gap junction protein gamma 2" OR "gap junction protein gamma 1" OR "gap junction protein beta 7" OR "gap junction protein beta 6" OR "gap junction protein beta 5" OR "gap junction protein beta 4" OR "gap junction protein beta 3"OR "gap junction protein beta 2" OR "gap junction protein beta 1" OR "gap junction protein alpha 10"OR "gap junction protein alpha 9" OR "gap junction protein alpha 8" OR "gap junction protein alpha 5" OR "gap junction protein alpha 4" OR "gap junction protein alpha 3" OR "gap junction protein alpha 1" OR "gap junction protein alpha 6 pseudogene" OR CX43 OR ODD OR SDTY3OR CX46 OR CX37 OR CX40 OR CX50 OR CX58 OR CX59 OR CX62 OR CX32 OR CX26 OR CX31 OR CX30.3 OR CX31.1 OR CX45 OR CX47 OR CX46.6 OR CX30.2 OR CX36 OR CX31.9 OR CX40.1 OR CX23)	23098	03:48:15
#11	Add	Search ("cyclic nucleotide gated channel alpha 1" OR "cyclic nucleotide gated channel alpha 2" OR "cyclic nucleotide gated channel alpha 3" OR "cyclic nucleotide gated channel alpha 4" OR CNGA1 OR CNGA2 OR CNGA3 OR CNGA4 OR "cyclic nucleotide gated channel beta 1" OR "cyclic nucleotide gated channel beta 3" OR CNGB1 OR CNGB3 OR HCN1 OR HCN2 OR HCN3 OR HCN4 OR "hyperpolarization activated cyclic nucleotide gated potassium channel 1" OR "hyperpolarization activated cyclic nucleotide gated potassium channel 3" OR "hyperpolarization activated cyclic nucleotide gated potassium channel 4" OR "hyperpolarization activated cyclic nucleotide gated potassium and sodium channel 2" OR RCNC1 OR RCNCa OR CNG1 OR RP49 OR CNG2 OR OCNC1 OR OCNCa OR OCNCALPHA OR OCNCalpha OR FLJ46312 OR CCNC1 OR CCNCa OR CNG3 OR OCNC2 OR OCNCb OR CNG5 OR RCNC2 OR RCNCb OR GARP OR GAR1 OR CNGB1B OR RP45 OR BCNG-1 OR BCNG-2 OR HAC-2 OR HAC-1 OR KIAA1535)	2259	03:48:02
#10	Add	Search ("potassium voltage-gated channel modifier subfamily V member 2" OR "potassium voltage-gated channel modifier subfamily V member 1" OR Kv8.2 OR Kv8.1 OR "potassium voltage-gated channel modifier subfamily S member 3" OR "potassium voltage-gated channel modifier subfamily S member 2" OR "potassium voltage-gated channel modifier subfamily S member 1" OR Kv9.3 OR	12137	03:47:46

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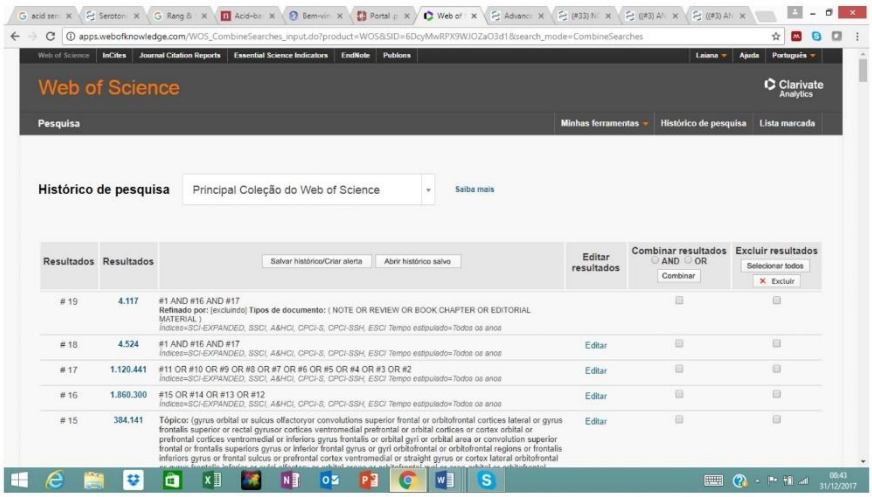

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Web of Science — Clarivate Analytics

Pesquisa | Minhas ferramentas | Histórico de pesquisa | Lista marcada

Histórico de pesquisa — Principal Coleção do Web of Science — Saiba mais

Resultados	Resultados	Salvar histórico/Criar alerta / Abrir histórico salvo	Editar resultados	Combinar resultados (AND / OR) Combinar	Excluir resultados Selecionar todos Excluir
# 19	4.117	#1 AND #16 AND #17 **Refinado por:** [excluindo] **Tipos de documento:** (NOTE OR REVIEW OR BOOK CHAPTER OR EDITORIAL MATERIAL) *Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Tempo estipulado=Todos os anos*			
# 18	4.524	#1 AND #16 AND #17 *Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Tempo estipulado=Todos os anos*	Editar		
# 17	1.120.441	#11 OR #10 OR #9 OR #8 OR #7 OR #6 OR #5 OR #4 OR #3 OR #2 *Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Tempo estipulado=Todos os anos*	Editar		
# 16	1.860.300	#15 OR #14 OR #13 OR #12 *Índices=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI Tempo estipulado=Todos os anos*	Editar		
# 15	384.141	Tópico: (gyrus orbital or sulcus olfactoryor convolutions superior frontal or orbitofrontal cortices lateral or gyrus frontalis superior or rectal gyrusor cortices ventromedial prefrontal or orbital cortices or cortex orbital or prefrontal cortices ventromedial or inferiors gyrus frontalis or orbital gyri or orbital area or convolution superior frontal or frontalis superiors gyrus or inferior frontal gyrus or gyri orbitofrontal or orbitofrontal regions or frontalis inferiors gyrus or frontal sulcus or prefrontal cortex ventromedial or straight gyrus or cortex lateral orbitofrontal	Editar		

Appendix 3: Custom data extraction form used for included studies

Study ID:		
Coder initials:		
Date started:		
Date completed:		
Title of the paper:		
Year of publication:		
Journal:		
Location (country):		
Study design		
1. Randomised controlled trial		
2. Controlled trial / pseudo-randomised / quasi-experimental		
3. Cohort		
4. Clinical audit		
5. Case-control		
6. Case series		
7. Case report		
8. Other design (specify)		
Population description (Strain):		
Sample size:		
Sex of animals		
***n* (males)**		
***n* (females)**		
Age of animals		
Mean		
Range		
Primary aim of study:		
Intervention / level of exposure	**Prescription**	**Reported**
Number of participants		
Duration of treatment		
How did the study define the intervention?		

How did the study deliver the intervention?		
Antagonist characteristics		
Total daily dosage		
Other treatment (additional to antagonists of ion channels)		
Type of comparison		
Microglia activate vs. microglia at a resting state		
Comparator	**Prescription**	**Reported**
Number of participants		
Duration of treatment		
How did the study define the comparator?		
How did the study deliver the comparator?		
Total daily antagonist dosage		
Other treatment (additional to the antagonist)		
Outcome : microglia in an activate or at resting state.		
Is outcome tool validated?		
Time points measured		
Between Groups		
Effect size (difference between groups)		
Effect size (%) (if different units)		
Level of significance (P-value or CI)		
Direction of result	**1. Favours intervention**	**2. Favours comparator**
Sponsorship / funding (verbatim):		
Authors conflicts of interest		

Appendix 5: Supplementary table 1. Quality score.

	1	2	3	4	5	6	7	8	9	10
Study	**Selection bias 1**	**Selection bias 2**	**Selection bias 3**	**Performance bias 1**	**Performance bias 2**	**Detection bias 1**	**Detection bias 2**	**Attrition bias**	**Reporting bias**	**Other potential bias**
Choi et al. 2012	?	✓	✓	?	x	x	x	✓	✓	✓
Huang et al. 2017	x	✓	✓	?	x	✓	x	✓	✓	✓
Lee et al. 2014	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Yu et al. 2013	x	✓	✓	?	x	x	x	✓	✓	✓
Chu et al. 2012	x	✓	✓	?	x	x	x	✓	✓	✓
Melani et al. 2006	✓	✓	✓	✓	x	✓	x	✓	✓	✓
Wixey et al. 2009	✓	✓	✓	✓	x	✓	x	✓	✓	✓
Ortega et al. 2012	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Gelosa et al. 2014	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Liu et al. 2017	✓	✓	✓	✓	x	✓	x	✓	✓	✓
Zhou et al. 2014	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
He et al. 2012	✓	✓	✓	✓	x	✓	x	✓	✓	✓
Wang et al. 2017	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Wu et al. 2016	✓	✓	✓	✓	x	✓	x	✓	✓	✓
Choi et al. 2007	x	✓	✓	?	x	x	x	✓	✓	✓

Appendix 6: Supplementary table 2. Additional information about included studies

Study	species	sex	weight	age
Choi et al. 2012	Sprague-Dawle rats	male	260-320	9-11 weeks
Huang et al. 2017	Sprague- Dawle rats	male	280-320 g	na
Lee et al. 2014	Sprague- Dawle rats	male	na	7 weeks
Yu et al. 2013	Sprague- Dawle rats	male	250 -300 g	na
Chu et al. 2012	Sprague- Dawle rats	male	260 - 320 g	na
Melani et al. 2006	Wistar rats	male	270 -290 g	na
Wixey et al. 2009	Sprague- Dawle rats	mixed sexes	na	Post-natal day 3
Ortega et al. 2012	Wistar rats	male	250 - 300 g	3 months
Gelosa et al. 2014	Sprague- Dawle rats	male	200 - 250 g	na
Liu et al. 2017	Sprague–Dawley rats	male	250-300 g	na
Zhou et al. 2014	case control	Sprague- Dawle rats	180-220 g	na
He et al. 2012	Sprague- Dawle rats	na	180-220 g	na
Wang et al. 2015	Sprague- Dawle rats	male	250 - 300 g	na
Choi et al. 2007	Sprague- Dawle rats	male	240 - 260 g	na
Wu et al. 2016	C57BL/6J and BALB/c mice	mixed sexes	na	3, 6, 9, and 12 months

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	01
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	02
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	03
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	04
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	04
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	04
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	04
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary material 2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	04
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	05 and Supplementary material 3
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	04
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	05
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	na
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	na

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	05
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	na
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	05/ Figure 01
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Supplementary material 3
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Supplementary table 1
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	na
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	na
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	na
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	na
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	05 and 06
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	06 and 07
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10 and 11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Title	Reason for excluson
Influence of extracellular zinc on M1 microglial activation	without ion channels blockage
Microglial Zinc Uptake via Zinc Transporters	without ion channels blockage
Ca2+ spiking activity caused by the activation of store-operated Ca2+ channels mediates INF-	without ion channels blockage
Activation of P2X7 receptors induces CCL3	without ion channels blockage
ATP selectively suppresses the synthesis of the inflammatory protein microglial response factor	without ion channels blockage
Uridine 5 '-Diphosphate Induces Chemokine Expression in Microglia and Astrocytes through	without ion channels blockage
P2X(7) signaling promotes microsphere embolism-triggered microglia activation by	without ion channels blockage
ATP release during cell swelling activates a Ca2+-dependent Cl- current by autocrine mechanism	not evaluating microglia state
Inwardly rectifying K+ channels influence Ca2+	without ion channels blockage
Integration of K+ and Cl- currents regulate steady-state and dynamic membrane potentials	without ion channels blockage
A systems pharmacology-based approach to identify novel Kv1.3 channel-dependent	without ion channels blockage
Microglial Kv1.3 Channels and P2Y12 Receptors Differentially Regulate Cytokine and Chemokine	without ion channels blockage
ACTIVATION OF MICROGLIAL P2Y12 RECEPTOR IS REQUIRED FOR OUTWARD POTASSIUM	without ion channels blockage
P2Y(13) Receptor-Mediated Rapid Increase in Intracellular Calcium Induced by ADP in Cultured	without ion channels blockage
Hydrogen peroxide and ADP-ribose induce	without ion channels blockage
Expression and Contributions of TRPM7 and KCa2.3/SK3 Channels to the Increased Migration	without ion channels blockage
Differential activation of subtype purinergic receptors modulates Ca(2+) mobilization and	without ion channels blockage
Microglia-derived purines modulate mossy fibre synaptic transmission and plasticity through	without ion channels blockage
Extracellular ATP differentially modulates Toll-	without ion channels blockage
Purinergic receptors activating rapid intracellular	without ion channels blockage
P2X4-Receptor-Mediated Synthesis and Release of Brain-Derived Neurotrophic Factor in	without ion channels blockage
Endogenous hydrogen sulphide attenuates NLRP3 inflammasome-mediated	without ion channels blockage
P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic	without ion channels blockage
Microglial P2X(7) receptor expression is accompanied by neuronal damage in the	without ion channels blockage
CLIC1 Function Is Required for beta-Amyloid-	without ion channels blockage
The ketone body metabolite beta-hydroxybutyrate induces an antidepression-	without ion channels blockage
A Kv1.5 to Kv1.3 switch in endogenous	without ion channels blockage
The potassium channels Kv1.5 and Kv1.3	without ion channels blockage

TGF-beta and LPS modulate ADP-induced migration of microglial cells through P2Y1 and	without ion channels blockage
Pharmacological blockage and P2X7 deletion	not evaluating microglia state
Activation of P2X7 receptor and NLRP3 inflammasome assembly in hippocampal glial	without ion channels blockage
Evidence for alterations of the glial syncytial	not evaluating microglia
G-Protein-Coupled Receptor Screen Reveals a Role for Chemokine Receptor CCR5 in	CCR5 and microglia function
Purinergic (P2X7) receptor activation of microglia induces cell death via an interleukin-1-	without ion channels blockage
The role of microglial P2X7: modulation of cell	genetic
The P2X7 receptor drives microglial activation	genetic
Interleukin-1beta has trophic effects in microglia	genetic
Altered microglial phagocytosis in GPR34-	genetic
Kir6.1 Knockdown Aggravates Cerebral	genetic
The potassium channel KCa3.1 constitutes a pharmacological target for neuroinflammation	genetic
Transient receptor potential melastatin 2 channels (TRPM2) mediate neonatal hypoxic-	genetic
Protective effects of P2X7 receptor deletion on	genetic
The human-specific CASP4 gene product	genetic
GPR84 deficiency reduces microgliosis, but accelerates dendritic degeneration and cognitive	genetic
GRK5 deficiency exaggerates inflammatory	genetic
S100a9 knockdown decreases the memory impairment and the neuropathology in Tg2576 mice, AD animal model	genetic
Loss of TMEM106B Ameliorates Lysosomal and Frontotemporal Dementia-Related Phenotypes	genetic
Adenosine A2A receptor antagonism reverses inflammation-induced impairment of microglial	genetic
Lack of neuroprotection in the absence of P2X7	genetic
ASIC1a Deficient Mice Show Unaltered	genetic
P2x7 deficiency suppresses development of	genetic
Sociocommunicative and Sensorimotor	genetic
Defective microglial development in the	genetic
Lack of functional P2X7 receptor aggravates	genetic
Deletion of the P2X4 receptor is neuroprotective acutely, but induces a depressive phenotype	genetic
CD44 deficiency in mice protects brain from	genetic
Role of P2X7 Receptor in an Animal Model of	without antagonism
Microglial P2X(7) receptor in the hypothalamic paraventricular nuclei contributes to	without antagonism
The phenothiazine-class antipsychotic drugs prochlorperazine and trifluoperazine are potent	not directly associating ion channels and microglia
Extracellular ATP enhances radiation-induced brain injury through microglial activation and	not directly associating ion channels and microglia
Nerve growth factor (NGF) expression in rat microglia is induced by adenosine A(2a)-	not directly associating ion channels and microglia
Evidence for functional adenosine A(3) receptors	not directly associating ion channels and microglia

The extracellular calcium-sensing receptor is	not directly associating ion channels and microglia
Sphingosine kinase 2-deficiency mediated	not directly associating ion channels and microglia
Inhibitory effects of U73122 and U73343 on Ca2+ influx and pore formation induced by the	not directly related to ion channels
ATP-P2X7 receptor signaling controls basal and	not directly related to ion channels
Elevation of basal intracellular calcium as a central element in the activation of brain	not directly related to ion channels
Onset of microglial entry into developing quail retina coincides with increased expression of	not directly related to ion channels
Involvement of ERK1/2, cPLA(2) and NF-kappa B in microglia suppression by cannabinoid	not directly related to ion channels
SDF-1alpha and LPA modulate microglia	not directly related to ion channels
Mechanisms Underlying Interferon-gamma-	not directly related to ion channels
Charge compensation for NADPH oxidase activity	not directly related to ion channels
The Sur1-Trpm4 channel regulates NOS2	not directly related to ion channels
Releasing factors from mature neurons	not directly related to ion channels
Upregulation of P2RX7 in Cx3cr1-Deficient Mononuclear Phagocytes Leads to Increased	not directly related to ion channels
Glia contribute to the purinergic modulation of	not directly related to ion channels
Microglia release ATP by exocytosis	not directly related to ion channels
The azetidine derivative, KHG26792 protects against ATP-induced activation of NFAT and	not directly related to ion channels
Microglial alpha 7 nicotinic acetylcholine receptors drive a phospholipase C/IP3 pathway	not directly related to ion channels
Lysophospholipids and ATP mutually suppress maturation and release of IL-1 beta in mouse	not directly related to ion channels
Microglia at brain stab wounds express connexin 43 and in vitro form functional gap junctions	not directly related to ion channels
Microglial CX3CR1 promotes adult neurogenesis	not directly related to ion channels
Microglial inhibition of neuroprotection by	not directly related to ion channels
Activation of EP2 prostanoid receptors in human	not directly related to ion channels
NLR members NLRC4 and NLRP3 mediate sterile	not directly related to ion channels
Cytosolic phospholipase A(2) plays a crucial role in ROS/NO signaling during microglial activation	not directly related to ion channels
Spontaneous Ca2+ transients in mouse microglia	not directly related to ion channels
Role of iPLA(2) in the Regulation of Src	not directly related to ion channels
Regulation of Integrin 6 Recycling by Calcium-independent Phospholipase A(2) (iPLA(2)) to	not directly related to ion channels
Inhibitory effect of carbamazepine on	not directly related to ion channels
Pro-inflammatory cytokines and lipopolysaccharide induce changes in cell	not directly related to ion channels
Inhibitory effect of a 2,4-bis(4-hydroxyphenyl)-2-butenal diacetate on neuro-inflammatory	not directly related to ion channels
Toll-like receptor 4 deficiency impairs microglial	not directly related to ion channels
Altered cerebellar development in nuclear receptor TAK1/ TR4 null mice is associated with	not directly related to ion channels
Edaravone alleviates hypoxia-	not directly related to ion channels
Dietary Sutherlandia and Elderberry Mitigate Cerebral Ischemia-Induced Neuronal Damage	not directly related to ion channels
Phospholipase A2 of Peroxiredoxin 6 Plays a	not directly related to ion channels

Neuroprotective effects of a nanocrystal formulation of sPLA(2) inhibitor PX-18 in	not directly related to ion channels
Reactive glia express cytosolic phospholipase	not directly related to ion channels
Presenilin 2 deficiency facilitates A beta-induced	not directly related to ion channels
Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the	not directly related to ion channels
Dexibuprofen (S(+)-isomer ibuprofen) reduces microglial activation and impairments of spatial	not directly related to ion channels
Neuroprotective effects of bee venom	not directly related to ion channels
Montelukast targeting the cysteinyl leukotriene receptor 1 ameliorates A beta(1-42)-induced	not directly related to ion channels
Opening of microglial K(ATP) channels inhibits	not directly related to ion channels
Bee venom phospholipase A2 ameliorates motor dysfunction and modulates microglia activation	not directly related to ion channels
4-hydroxy-2-nonenal upregulates and phosphorylates cytosolic phospholipase A(2) in	not directly related to ion channels
Reduction of cytosolic phospholipase A(2)alpha upregulation delays the onset of symptoms in	not directly related to ion channels
Non-phosphorylated FTY720 Induces Apoptosis	not directly related to ion channels
Glia and epilepsy: Experimental investigation of antiepileptic drugs in an astroglia/microglia co-	not directly related to ion channels
Selective Activation of Microglia Facilitates	not directly related to ion channels
TUDCA: An Agonist of the Bile Acid Receptor	not directly related to ion channels
Thromboxane A2 receptor antagonist SQ29548 suppresses the LPS-induced release of	not directly related to ion channels
Thromboxane A(2) Receptor Stimulation Enhances Microglial Interleukin-1 beta and NO	not directly related to ion channels
Protective effect of 3-(naphthalen-2-yl(propoxy)methyl) azetidine hydrochloride on	not directly related to ion channels
TNP-ATP is Beneficial for Treatment of Neonatal	not directly related to ion channels
Aminoguanidine inhibits caspase-3 and calpain activation without affecting microglial activation	not directly related to ion channels
Thromboxane A2 receptor antagonist SQ29548 reduces ischemic stroke-induced	not directly related to ion channels
Inhibition of inflammatory mediator release	not directly related to ion channels
Neuroprotective Effects of Lycium barbarum	not directly related to ion channels
Basal CD38/cyclic ADP-ribose-dependent signaling mediates ATP release and survival of	not directly related to ion channels
P2X4 Receptor Regulates Alcohol-Induced	not directly related to ion channels
Huanglian-Jie-Du-Tang Extract Ameliorates Depression-Like Behaviors through BDNF-TrkB-	not directly related to ion channels
Administration of 2-arachidonoylglycerol ameliorates both acute and chronic	not directly related to ion channels
Microglial Activation Enhances Associative Taste Memory through Purinergic Modulation of	not directly related to ion channels
Complex molecular and functional outcomes of	not directly relating ion channels to microglia
Inhibition of interleukin-1 beta production by extracellular acidification through the	not evaluating microglia state
ASIC1A in neurons is critical for fear-related	not evaluating microglia state
Kv1.1 deletion augments the afferent hypoxic	not evaluating microglia state

Acid-sensing T cell death associated gene-8	not evaluating microglia state
Microglial Acid Sensing Regulates Carbon	not evaluating microglia state
TRP channels are involved in mediating hypercapnic Ca2+ responses in rat glia-rich	not evaluating microglia state
The bed nucleus of the stria terminalis is critical	not evaluating microglia state
Interaction of NG2(+) Glial Progenitors and	not ion channel
P2 receptor-mediated stimulation of the PI3-	not microglia
Differential changes in GPR55 during microglial	not microglia
P2X7 Participates in Intracerebral Hemorrhage-Induced Secondary Brain Injury in Rats via	not microglia
P2X7 Receptor Suppression Preserves Blood-Brain Barrier through Inhibiting RhoA Activation	not microglia
Up-regulation of P2X2, P2X4 receptor and	not microglia
P2X7 receptor modulation of the viability of	not microglia
Neuroglial activation and Cx43 expression are reduced upon transplantation of human	not microglia
TDAG8 activation attenuates cerebral ischaemia-	not microglia
THE NEW P2Y-LIKE RECEPTOR G PROTEIN-COUPLED RECEPTOR 17 MEDIATES ACUTE	not microglia
Activated microglia in ischemic stroke penumbra upregulate MCP-1 and CCR2 expression in	not microglia
Changes in lipid-sensitive two-pore domain potassium channel TREK-1 expression and its	not microglia
Fluoxetine Protection in Decompression Sickness in Mice is Enhanced by Blocking TREK-1	not microglia
Block of purinergic P2X(7) receptor is	not microglia
Alzheimer's amyloid-beta peptide disturbs P2X7	not microglia
Abnormality of G-protein-coupled receptor kinases at prodromal and early stages of	not microglia
Akebia Saponin D attenuates amyloid beta-induced cognitive deficits and inflammatory	not microglia
On the role of P2X(7) receptors in dopamine nerve cell degeneration in a rat model of	not microglia
Electroconvulsive therapy: a novel hypothesis	not microglia
TGR5 signaling reduces neuroinflammation	not microglia
Increased neocortical expression of the P2X7 receptor after status epilepticus and	not microglia
The effect of P2X7 receptor activation on nuclear factor-kappa B phosphorylation induced by	not microglia
P2X7 receptor regulates leukocyte infiltrations in	not microglia
P2X7 receptor differentially modulates astroglial apoptosis and clasmatodendrosis in the rat brain	not microglia
P2X7 receptor activation ameliorates CA3 neuronal damage via a tumor necrosis factor-	not microglia
P2X7 receptor inhibition interrupts the progression of seizures in immature rats and	not microglia
Effects of P2X7 receptor antagonists on hypoxia-	not microglia
Increased Expression and Cellular Localization of P2X7R in Cortical Lesions of Patients With Focal	not microglia
Reduction of K+ uptake in glia prevents long-	not microglia

Tenidap, an agonist of the inwardly rectifying K+ channel Kir2.3, delays the onset of cortical	not microglia
Changes in TWIK-related acid sensitive K+-1 and -3 channel expressions from neurons to glia in	not microglia
P2X7 receptors are a potential novel target for	not microglia
Block of Purinergic P2X(7)R Inhibits Tumor	not microglia
Surface expression of ASIC2 inhibits the	not microglia
Neonatal Maternal Deprivation Enhances Presynaptic P2X7 Receptor Transmission in	not microglia
Central sensitization of nociceptive neurons in	not microglia
A novel P2X4 receptor-selective antagonist	not microglia
P2X7 receptor inhibition improves recovery after	not microglia
Inhibition of the MEK/ERK pathway reduces microglial activation and interleukin-1-beta	not microglia
Dexamethasone attenuates early expression of three molecules associated with	not microglia
ATP mediates rapid microglial response to local	not microglia
Changes of the GPR17 receptor, a new target for neurorepair, in neurons and glial cells in patients	not microglia
Targeting Kv1.3 channels to reduce white matter	not microglia
Microglial phagocytosis attenuated by short-	without pharmacological antagonist
Methamphetamine alters microglial immune	without pharmacological antagonist
Involvement of P2X and P2Y receptors in	not directly associating ion channels and microglia
3,4-Methylenedioxymethamphetamine (MDMA, ecstasy) disrupts blood-brain barrier integrity	not directly associating ion channels and microglia
P2X4 Receptors Control the Fate and Survival of	without pharmacological antagonist
Anandamide, Acting via CB2 Receptors, Alleviates LPS-Induced Neuroinflammation in Rat	in vitro
Anti-inflammatory property of the cannabinoid agonist WIN-55212-2 in a rodent model of	without pharmacological antagonist
The G Protein-Coupled Receptor 55 Ligand L-alpha-Lysophosphatidylinositol Exerts Microglia-	without pharmacological antagonist
Hypoxia induced amoeboid microglial cell	not directly associating ion channels and microglia
Cannabinoid receptor stimulation is anti-	not directly associating ion channels and microglia
Brilliant blue G attenuates lipopolysaccharide-	in vitro
Involvement of P2X4 receptors in hippocampal	not directly associating ion channels and microglia
Predominant functional expression of Kv1.3 by	without pharmacological antagonist
Status epilepticus induces a particular microglial	without pharmacological antagonist
Neuronal CCL21 up-regulates microglia P2X4	without pharmacological antagonist
Spinal P2X(7) receptor mediates microglia activation-induced neuropathic pain in the	without pharmacological antagonist
Duloxetine Inhibits Microglial P2X4 Receptor Function and Alleviates Neuropathic Pain after	without immunohystochemical analysis
Inhibition of P2X4 function by P2Y6 UDP	without pharmacological antagonist
Agonists for G-protein-coupled receptor 84 (GPR84) alter cellular morphology and motility	not directly associating ion channels and microglia
Expression of G-protein-coupled receptor kinase	without pharmacological antagonist
Suppression of the rat microglia Kv1.3 current by	evaluating oxidative stress
Store-Operated Ca2+ Entry (SOCE) and Purinergic Receptor-Mediated Ca2+ Homeostasis	not directly associating ion channels and microglia

Pathophysiological roles of extracellular nucleotides in glial cells: differential expression	not directly associating ion channels and microglia
Mechanism of microglia neuroprotection:	not directly associating ion channels and microglia
Chloride Channel Blockers Suppress Formation of Engulfment Pseudopodia in Microglial Cells	the primary outcome is not the activation/ resting microglia state
Microglia Processes Block the Spread of Damage	not directly associating ion channels and microglia
Expression of Kv1.2 in microglia and its putative roles in modulating production of	without pharmacological antagonist
Upregulation of Kv1.3 K+ channels in microglia	without pharmacological antagonist
Kv1.1 expression in microglia regulates production and release of proinflammatory	without pharmacological antagonist
TRPM2 contributes to LPS/IFN gamma-induced	without pharmacological antagonist
Targeting P(2)X(7) receptor for the treatment of	not directly associating ion channels and microglia
P2X7 receptor activation regulates microglial cell	not directly associating ion channels and microglia
Purinergic receptor P2RY12-dependent	not directly associating ion channels and microglia
Upregulated expression of purinergic P2X(7) receptor in Alzheimer disease and amyloid-beta	not directly associating ion channels and microglia
Potassium channel Kv1.3 is highly expressed by	without pharmacological antagonist
Broad-spectrum effects of 4-aminopyridine to modulate amyloid beta1-42-induced cell	not directly associating ion channels and microglia
P2Y(4) Receptor-Mediated Pinocytosis	not directly associating ion channels and microglia
Lysophosphatidic acid-induced membrane ruffling and brain-derived neurotrophic factor	not directly associating ion channels and microglia
Morphine hyperalgesia gated through microglia-	not directly associating ion channels and microglia
Involvement of TRPM2 in Peripheral Nerve Injury-Induced Infiltration of Peripheral Immune Cells	not directly associating ion channels and microglia
Purinergic signaling induces cyclooxygenase-1-dependent prostanoid synthesis in microglia:	without pharmacological antagonist
The ectonucleotidase cd39/ENTPDase1	without pharmacological antagonist
Microglia: Proliferation and activation driven by	review
Purinergic receptors in microglia: Functional	review
Purinergic receptors modulate MAP kinases and transcription factors that control microglial	review
Microglial Voltage-Gated Proton Channel Hv1 in	review
P2X4R(+) microglia drive neuropathic pain	review
Molecular mechanisms of microglial activation	without pharmacological antagonist
TRPM2 is elevated in the tMCAO stroke model, transcriptionally regulated, and functionally	without pharmacological antagonist
Tanshinone IIA increases levels of NeuN, protein disulfide isomerase, and Na+/K+-ATPase and	not directly associating ion channels and microglia
THE MICROGLIAL SUICIDE RECEPTOR P2X7 IS PRESENT AT ADULT NEURAL PRECURSOR CELLS	not directly associating ion channels and microglia
P2X7 as a new target for chrysophanol to treat	not directly related to microglia
Spinal microglial P2X4 receptor-brain-derived neurotrophic factor signaling regulates nicotine	not directly related to microglia
N-Substituted Phenoxazine and Acridone Derivatives: Structure-Activity Relationships of	not directly related to microglia
The effects of general anesthetics on P2X(7) and	not directly associating ion channels and microglia
Synergy of TRIF-dependent TLR3 and MyD88-dependent TLR7 in up-regulating expression of	not directly related to microglia and ion channels

Purinergic receptors on microglial cells: functional expression in acute brain slices and modulation of microglial activation in vitro	the primary outcome is not the activation/ resting microglia state. The study intention is to evaluate different ranges of ATP on purinergic receptors
Effect of Methamphetamine on the Microglial	review
Microglial SK3 and SK4 Currents and Activation	not directly associating ion channels and microglia
Aripiprazole inhibits polyI: C-induced microglial	not directly associating ion channels and microglia
Ketamine Alleviates Depressive-Like Behaviors via Down-Regulating Inflammatory Cytokines	not directly related to microglia
P2X(7) receptors control 2-arachidonoylglycerol	without antagonism
MODULATION OF POTASSIUM CURRENTS IN CULTURED MURINE MICROGLIAL CELLS BY	without antagonism
Arachidonic acid-induced inhibition of microglial	without antagonism
Lysophosphatidy1choline potentiates Ca2+ influx, pore formation and p44/42 MAP kinase	without antagonism
Microglia Kv1.3 channels contribute to their	without antagonism
Selective Activation of KCa3.1 and CRAC Channels by P2Y2 Receptors Promotes Ca2+	without antagonism
Signalling mechanisms mediating Zn2+-induced	without antagonism
COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial	without antagonism
Regulation of a TRPM7-like current in rat brain	without antagonism
The Neuroprotective Effect of a Specific P2X7 Receptor Antagonist Derives from its Ability to	without immunohystochemical analysis
Ion channel expression in resting and activated	without antagonis
P2X4 receptors in activated C8-B4 cells of	without antagonism
Responses of rat and mouse primary microglia to pro- and anti-inflammatory stimuli: molecular	without antagonism
Expression and contributions of the Kir2.1 inward-rectifier K+ channel to proliferation,	without antagonism
Expression of purinergic P2X7 receptor in rat brain during the symptomatic phase of	without antagonism
Activation of the P2X(7) receptor induces migration of glial cells by inducing cathepsin B	without antagonism
P2X7-dependent, but differentially regulated	without antagonism
P2X(7) receptors on microglial cells mediate	without antagonism
Production and release of neuroprotective	without antagonism
Two different ionotropic receptors are activated	without antagonism
Migration and Phagocytic Ability of Activated Microglia During Post-natal Development is	not presenting P2X blocked
Minocycline decreases in vitro microglial	witout specific microglial receptors antagonism
Activation of P2X7 promotes cerebral edema and	without ion channels blockage
Inhibitory Effects of Isoquinoline Alkaloid Berberine on Ischemia-Induced Apoptosis via	not directly associating ion channels and microglia
Downregulation of Kir4.1 inward rectifying potassium channel subunits by RNAi impairs	not directly associating ion channels and microglia
Connexin expression by radial glia-like cells is	not directly associating ion channels and microglia

6.3. Material suplementar artigo 4

Search Strategy

Search	Query
1	“panic disorder”
2	“panic attacks”
3	ASIC or “acid sensing ion channel” or ACCN or ACCN2 or ACCN1 or “amiloride-sensitive cation channel”
4	TDAG8 or “GPR65 protein, human” or "GPCR25 protein, mouse" or "TDAG8 protein, rat"
5	TRPV1 or “transient receptor potential vanilloid-1 ion channel” or "TRPV Cation Channels"
6	“Two-pore domain K+” or K2P
7	“ionotropic purinoceptors” or P2X
8	1 OR 2
9	3 OR 4 OR 5 OR 6 OR 7
10	8 AND 9

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	01
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	02
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	03
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	03/04
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	na
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	04
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	04
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	S1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	04
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	04
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	04
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	04
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	05
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	05

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	04/05
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	na
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	05/Fig. 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1 and 2
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	S3
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Fig. 2
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Fig. 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	06
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	na
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	06
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	09/10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11/12
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	13

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Table 1- Quality Assessment case-control studies

Study	comparison group	confounding	missing outcome data	exposure characterization	outcome assessment	outcome reporting
Gugliandolo et al. 2015	++	++	++	++	NR	++
Hettema et al. 2008	+	++	++	++	NR	++
Leibold et al.2017	++	++	++	++	++	++
Smoller et al.2014	++	++	++	++	NR	++
Gregersen et al. 2012	++	+	++	++	NR	++
Strawn et al.2017	++	++	++	++	NR	++

Table 2- Quality Assessment experimental animal studies

study	randomization	allocation concealment	identical experimental conditions	performance bias	bliding	missing outcome data	exposure characterization, confidence	outcome assessment	outcome reporting
Almeida-Santos et al.2013	+	++	++	++	++	++	++	++	++
Batista et al.2017	NR	++	++	++	++	++	++	++	++
Casarotto et al. 2011	+	++	++	++	++	++	++	++	++
Lisboa et al.2012	+	++	++	++	NR	++	++	+	++
dos Anjos-Garcia et al.2016	+	++	++	++	NR	++	++	+	++

Article	Reason
ASIC-dependent LTP at multiple glutamatergic synapses in amygdala network is required for fear memory	Evaluating fear conditioning
Neurobiology of panic and pH chemosensation in the brain	Review
Peptide receptor ligands to treat anxiety disorders	Chapter
Involvement of TRPV1 channels in the periaqueductal grey on the modulation of innate fear responses	predator exposure
Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats	Evaluating fear conditioning
Medial prefrontal cortex Transient Receptor Potential Vanilloid Type 1 (TRPV1) in the expression of contextual fear conditioning in Wistar rats	Evaluating fear conditioning
Role of TRPV1 in consolidation of fear memories depends on the averseness of the conditioning procedure	Memory consolidation
N-arachidonoyl-serotonin, a dual FAAH and TRPV1 blocker, inhibits the retrieval of contextual fear memory: Role of the cannabinoid CB1 receptor in the dorsal hippocampus	Evaluating fear conditioning
Possible involvement of TRPV1 and TRPV4 in nociceptive stimulation induced nocifensive behavior and neuroendocrine response in mice	Evaluating nociception
Effects of single swim stress on changes in TRPV1-mediated plasticity in the amygdala	Evaluating long- term potentiation in a animal model for depression
N-arachidonoyl-serotonin in the basolateral amygdala increases anxiolytic behavior in the elevated plus maze	Without fulfilling animal search criteria (elevated-plus maze test)
2-AG promotes the expression of conditioned fear via cannabinoid receptor type 1 on GABAergic neurons	Evaluating fear conditioning
Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice	Evaluating fear conditioning
Role of TRPV1 channels of the dorsal periaqueductal gray in the modulation of nociception and open elevated plus maze-induced antinociception in mice	Evaluating nociception
Altered responses of dopamine D3 receptor null mice to excitotoxic or anxiogenic stimuli: Possible involvement of the endocannabinoid and endovanilloid systems	Not directly associated with PD

Capsaicin-induced changes in LTP in the lateral amygdala are mediated by TRPV1	Learning mechanisms
Involvement of central TRPV1 receptors in pentylenetetrazole and amygdala-induced kindling in male rats	Not directly associated with PD
Polymodal activation of the endocannabinoid system in the extended amygdala	Not directly associated with PD
Histone Modifications in a Mouse Model of Early Adversities and Panic Disorder: Role for Asic1 and Neurodevelopmental Genes	Epigenetic
Validation of candidate anxiety disorder genes using a carbon dioxide challenge test	Without a PD diagnosis
Functional and pharmacological characterization of two different ASIC1a/2a heteromers reveals their sensitivity to the spider toxin PcTx1	Not directly associated with PD
Overexpression of acid-sensing ion channel 1a in transgenic mice increases acquired fear-related behavior	Evaluating fear conditioning
Localization and behaviors in null mice suggest that ASIC1 and ASIC2 modulate responses to aversive stimuli	Not directly associated with PD
P.4.b.001 Role of transient receptor potential vanilloid type 1 channels in brain regions related to anxiety in rats	without fulfilling animal search criteria (elevated-plus maze test)
Acid-sensing by the T cell death-associated gene 8 (TDAG8) receptor cloned from rat brain	Not directly associated with PD
Microglial Acid Sensing Regulates Carbon Dioxide-Evoked Fear	Without fulfilling animal search criteria
Involvement of serotonin-mediated neurotransmission in the dorsal periaqueductal gray matter on cannabidiol chronic effects in panic-like responses in rats	no direct relation to acid sensitive channels
Enhanced anandamide signaling reduces flight behavior elicited by an approaching robo-beetle	Without fulfilling animal search criteria
The role of 5-HT 1A receptors in the anti-aversive effects of cannabidiol on panic attack-like behaviors evoked in the presence of the wild snake Epicrates cenchria crassus (Reptilia, Boidae)	no direct relation to acid sensitive channels
Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptor	no direct relation to acid sensitive channels
Anti-aversive role of the endocannabinoid system in the periaqueductal gray stimulation model of panic attacks in rats	no direct relation to acid sensitive channels
Anxiolytic-like effects induced by blockade of transient receptor potential vanilloid type 1 (TRPV1) channels in the medial prefrontal cortex of rats	without fulfilling animal search criteria (elevated-plus maze test and Vogel confilct test)

P.2.003 CB1 and TRPV1 receptors located in periaqueductal gray matter mediate opposite effects in panic-like responses in rats	Conference abstract of an original article that was included
Anxiolytic-like effect of cannabinoids injected into the rat dorsolateral periaqueductal gray	no direct relation to acid sensitive channels
Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors	no direct relation to acid sensitive channels
Modulation of defensive behavior by Transient Receptor Potential Vanilloid Type-1 (TRPV1) Channels	without fulfilling animal search criteria
Association of the polimorphisms in P2RX7 and CaMKKb with anxiety disorders	Evaluating panic attacks on the spectrum of anxiety disorders
The Amygdala is a Chemosensor that Detects Carbon Dioxide and Acidosis to Elicit Fear Behavio	Without fulfilling animal search criteria (evaluating freezing)
Targeting ASIC1a reduces innate fear and alters neuronal activity in the fear circuit	Witout fulfilling animal serch criteria

6.4. Material suplementar artigo 5

year	title	reason for exclusion
2017	Impact of Proactive Diazepam Loading Protocol on Clinical Outcomes Among Patients with History of Complicated Alcohol Withdrawal Syndrome	Not only PD
2004	5-HT1A receptors, gene repression, and depression: Guilt by association	Not related to PD
2014	Benzodiazepine use and aggressive behaviour: A systematic review	review
2005	Basilar artery blood flow velocity changes in patients with panic disorder following 35% CO2 challenge	meeting abstract
2007	Basilar artery blood flow velocity changes in patients with panic disorder following 35% carbon dioxide challenge	Not related to PD
2007	Benzodiazepines and psychotherapy in panic disorder	meeting abstract
2004	Efficacy of sertraline in a 12-week trial for generalized anxiety disorder	evaluating generalized anxiety disorder
2017	Benzodiazepines intake at youth-experience from adolescent consultation at centro hospitalar Lisboa Norte	Not only PD
2015	Pharmacokinetic evaluation of fluvoxamine for the treatment of anxiety disorders	not only PD
2003	A 15-year follow-up study of patients with panic disorder	article including tricycles antidepressants
2006	The new guidelines from the British Association for Psychopharmacology for anxiety disorders	not only PD
2013	Newer antidepressants and panic disorder: a meta-analysis	meta-analysis
2014	Factors affecting discontinuation of initial treatment with paroxetine in panic disorder and major depressive disorder	not only PD patients were evaluated
2004	Neurobiology and pharmacotherapy of social phobia	noy related to PD
2004	Citalopram, a selective serotonin reuptake inhibitor augments harmaline-induced tremor in rats	animal study
2008	An investigation of clinical factors influence the therapeutic response of paroxetine in patients with panic disorder	meeting abstract
2000	Comparison of Fluvoxamine alone, Fluvoxamine and cognitive psychotherapy and psychotherapy alone in the treatment of panic disorder in Kelantan--implications for management by family doctors	article including psychological treatment
2003	Effects of selective serotonin reuptake inhibitors on cholesterol levels in patients with panic disorder	not examining direct improvement of PD.
2011	Validating the inhalation of 7.5% CO2 in healthy volunteers as a human experimental medicine: a model of generalized anxiety disorder (GAD)	not only PD
2008	GABA-A receptors and the response to CO2 inhalation - A translational trans-species model of anxiety?	not only PD
2011	Preliminary evidence of anxiolytic effects of the CRF1 receptor antagonist R317573 in the 7.5% CO2 proof-of-concept experimental model of human anxiety	not only PD
2001	Correlates of panic disorder response to placebo and clonazepam for sleep measures and sodium lactate infusion	meeting abstract
1999	Paroxetine, clomipramine, and cognitive therapy in the treatment of panic disorder	article including tricycles antidepressants
2004	Anxiety disorders: does one treatment fit all?	not only PD
2005	Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology	not only PD
2004	Remission rates in patients with anxiety disorders treated with paroxetine	not only PD patients were evaluated
2004	Benzodiazepines versus antidepressants for panic disorder	review
2005	The advantages of SSRI treatment of panic disorder	review
2005	The advantages of SSRI treatment of panic disorder	meeting abstract
2002	Sertraline versus paroxetine in the treatment of panic disorder: A multinational randomized double-blind 15 week study	meeting abstract
2004	Sertraline versus paroxetine in the treatment of panic disorder: an acute, double-blind noninferiority comparison	without a placebo
2007	Drug treatment of generalized anxiety disorder (GAD) - a consensus statement	not only PD

2004	Treatment-resistant panic disorder	review
2007	Improvement of quality of life in panic disorder with escitalopram, citalopram, or placebo	primary outcome quality of life
2008	World Federation of Societies of Biological Psychiatry (WFSBP) Guidelines for the Pharmacological Treatment of Anxiety, Obsessive-Compulsive and Post-Traumatic Stress Disorders - First Revision	guidelines
2004	Paroxetine controlled release	review
2007	The use of escitalopram beyond major depression: pharmacological aspects, efficacy and tolerability in anxiety disorders	not only PD
2005	Self-management and pregnancy-safe interventions for panic, phobia and other anxiety-disorders might include over-the-counter (OTC) 'SSRI' antihistamines such as diphenhydramine and chlorpheniramine - Reply	not only PD
2010	Effects of venlafaxine and fluoxetine on lymphocyte subsets in patients with major depressive disorder: A flow cytometric analysis	Not related to PD
2012	Evidence-based pharmacotherapy of panic disorder: an update	review
2008	QT interval variability and cardiac norepinephrine spillover in patients with depression and panic disorder	not examining direct improvement of PD.
2007	Anxiolytic therapy with alprazolam increases muscle sympathetic activity in patients with panic disorders	not examining direct improvement of PD.
2007	Anxiety disorders: Sex differences in prevalence, degree, and background, but gender-neutral treatment	Not related to PD
2002	Does 5-HT restrain panic? A tryptophan depletion study in panic disorder patients recovered on paroxetine	not examining direct improvement of PD.
2008	Use of benzodiazepines and selective serotonin reuptake inhibitors in middle-aged and older adults with anxiety disorders: a longitudinal and prospective study	not only PD
2012	Potential role of cortical 5-HT2A receptors in the anxiolytic action of cyamemazine in benzodiazepine withdrawal	Not related to PD

2017	Benzodiazepines and frail elderly people: optimization proposals in a geriatric day-hospital	Not only PD
2009	Systematic Review and Quality Assessment of Economic Evaluations and Quality-of-Life Studies Related to Generalized Anxiety Disorder	review
2006	Lack of effects on core obsessive-compulsive symptoms of tryptophan depletion during symptom provocation in remitted obsessive-compulsive disorder patients	Not related to PD
1998	Stressful reactions and panic attacks induced by flumazenil in chronic benzodiazepine users	evaluating flumazenil
1997	Pharmacologic effect of imipramine, paroxetine, and sertraline on 35% carbon dioxide hypersensitivity in panic patients: a double-blind, random, placebo-controlled study	article including tricycles antidepressants
2004	Comparison of the treatment with paroxetine and reboxetine in panic disorder: a randomized, single-blind study	other class of antidepressants than SSRI
2017	Benzodiazepines in detention: the key role of the pharmacist to reduce addiction and misuse	Not only PD
2016	Antidepressants and benzodiazepines for panic disorder in adults	review
2011	Awareness Under General Anesthesia	Not related to PD
2017	Prevalence and Correlates of Benzodiazepine Use, Misuse, and Use Disorders Among Adults in the U.S	Not only PD
2006	Outcomes of late-life anxiety disorders during 32 weeks of citalopram treatment	not only PD
2007	A randomized, open-label comparison of paroxetine (rexetine) and cognitive-behavioral therapy in management of panic disorder	meeting abstract
1998	Antipanic effect of fluoxetine measured by CO2 challenge test	less than 10 PD patients
2008	Analytical methodologies for the determination of sertraline	procedure
2001	Changes in rCBF of panic disorder patients due to effective treatment with sertraline	meeting abstract
2005	Efficacy of acute and extension treatment with self-administered cognitive behaviour therapy and sertraline in panic disorder	meeting abstract

2000	SPECT [I-123]iomazenil measurement of the benzodiazepine receptor in panic disorder	not examining direct improvement of PD.
2004	Effects of myo-inositol versus fluoxetine and imipramine pretreatments on serotonin 5HT(2A) and muscarinic acetylcholine receptors in human neuroblastoma cells	evaluating tricycles antidepressants
2003	Are benzodiazepines still the medication of choice for patients with panic disorder with or without agoraphobia?	review
1999	Cognitive-behavioral therapy helps prevent relapse and recurrence of panic disorder following alprazolam discontinuation: a long-term follow-up of the Peoria and Dartmouth studies	article including psychological treatment
2010	Slow vs standard up-titration of paroxetine in the treatment of panic disorder: a prospective randomized trial	without placebo
2009	Paroxetine in the treatment of panic disorder: a comparison between slow and standard up-titrations	meeting abstract
2004	Lack of pharmacologic interaction between paroxetine and alprazolam at steady state in healthy volunteers	healthy volunteers
2007	Reduced gamma-aminobutyric Acid(A)-benzodiazepine binding sites in insular cortex of individuals with panic disorder	not direct evaluating PD symptoms
2003	Clomipramine and fluoxetine effects in the treatment of panic disorder	article including tricycles antidepressants
2017	An online survey on misuse of benzodiazepines and "Z drugs" in Singapore	Not only PD
2005	Self-management and pregnancy-safe interventions for panic, phobia and other anxiety-disorders might include over-the-counter (OTC) 'SSRI' antihistamines such as diphenhydramine and chlorpheniramine	not only PD
2004	Issues in the clinical use of benzodiazepines: Potency, withdrawal, and rebound	review
2006	The search for new off-label indications for antidepressant, antianxiety, antipsychotic and anticonvulsant drugs	not related to PD
2007	Three-year medication prophylaxis in panic disorder: to continue or discontinue? A naturalistic study	not examining direct improvement of PD.

2010	Protocol for a randomised controlled trial investigating the effectiveness of an online e-health application compared to attention placebo or sertraline in the treatment of generalised anxiety disorder	not only PD
2004	Clonazepam and milnacipran in the treatment of patients with panic disorder and comorbid major depression - Preliminary	meeting abstract
2004	Diagnosis and treatment of depression during pregnancy	Not related to PD
2012	Effect of treatment with selective serotonin reuptake inhibitors on lipid profile: state of the art	noy related to PD
1997	Clinical improvement with fluoxetine therapy and noradrenergic function in patients with panic disorder	article involving clonidine
2015	Evidence for serotonin function as a neurochemical difference between fear and anxiety disorders in humans?	not examining direct improvement of PD.
2009	Effects of escitalopram on anxiety and respiratory responses to carbon dioxide inhalation in subjects at high risk for panic disorder: a placebo-controlled, crossover study	subjects at high risk for PD
2015	Well-Being Therapy in a Patient with Panic Disorder Who Failed to Respond to Paroxetine and Cognitive Behavior Therapy	Case report
2016	Establishment of a treatment for generalized anxiety	review
1997	Determinants of pharmacologic treatment failure in panic disorder	
2014	Assessment and management of anxiety disorders in children and adolescents	children
2002	Paroxetine and respiration in panic disorder: preliminary results	meeting abstract
2004	Environmental risk assessment of paroxetine	not only PD
1997	Adrenocorticotropic hormone and cortisol responses to corticotropin-releasing hormone: changes in panic disorder and effects of alprazolam treatment	article including tricycles antidepressants
2004	Cognitive behavioral group therapy in panic disorder patients: the efficacy of CBGT versus drug treatment	article including psychological treatment
2002	Psychoeducation in panic disorder patients: effect of a self-information booklet in a randomized, masked-rater study	not examining direct improvement of PD.

2006	Recurrence of panic disorder during pregnancy: a 7-year naturalistic follow-up study	not examining direct improvement of PD.
2004	Paroxetine in panic disorder: clinical management and long-term follow-up	review
2015	Psychosocial interventions for benzodiazepine harmful use, abuse or dependence	noy related to PD
2017	CONCOMITANT USE OF OPIOID AND BENZODIAZEPINES AND THE RISK OF OPIOID OVERDOSE REQUIRING HOSPITALIZATIONS: A RETROSPECTIVE COHORT STUDY	Not only PD
2004	Escitalopram in the treatment of generalized anxiety disorder: double-blind, placebo controlled, flexible-dose study	not only PD patients were evaluated
1998	Pivotal studies of clonazepam in panic disorder	review
2006	Treatment of posttraumatic stress disorder with venlafaxine extended release - A 6-month randomized controlled trial	Not related to PD
2006	Depleting serotonin enhances both cardiovascular and psychological stress reactivity in recovered patients with anxiety disorders	not examining direct improvement of PD.
2014	Differential impact of anxiety symptoms and anxiety disorders on treatment outcome for psychotic depression in the STOP-PD study	evaluation of depression
2013	Benzodiazepines in Combination with Opioid Pain Relievers or Alcohol: Greater Risk of More Serious ED Visit Outcomes	not PD related
2013	IS THERE A ROLE FOR AGOMELATINE IN THE TREATMENT OF ANXIETY DISORDERS? A REVIEW OF PUBLISHED DATA	review
1999	Long-term outcome of pharmacological and psychological treatment for panic disorder with agoraphobia: a 2-year naturalistic follow-up	article including psychological treatment
2006	Effects of fluoxetine and buspirone on the panicolytic-like response induced by the activation of 5-HT1A and 5-HT2A receptors in the rat dorsal periaqueductal gray	animal study
2008	Alprazolam potentiates the antiaversive effect induced by the activation of 5-HT(1A) and 5-HT (2A) receptors in the rat dorsal periaqueductal gray	not examining direct improvement of PD.

2017	Use of benzodiazepines in relation to onset and duration of untreated illness in psychotic and affective disorders	Not only PD
2002	Memory performance in panic disorder patients after chronic use of clomipramine	article including tricycles antidepressants
1999	[Clinical use of sustained release of alprazolam: A naturalistic study]	article in spanish
2011	Memory dysfunction in panic disorder: an investigation of the role of chronic benzodiazepine use	not PD
2004	Serotonin-mediated cyclic AMP inhibitory pathway in platelets of patients affected by panic disorder	not examining direct improvement of PD.
2014	Agomelatine in treating generalized anxiety disorder	review
2005	Predictors of pharmacotherapy response in anxiety disorders	not only PD
2017	Evaluation of the effects of etifoxine (100 mg) and lorazepam (2 mg) on alertness and cognitive functions in elderly subjects	Not only PD
2010	Second-generation antipsychotics for anxiety disorders	review
2016	Immunomodulatory effects of fluoxetine: A new potential pharmacological action for a classic antidepressant drug?	Not related to PD
2016	Benzodiazepines as novel psychoactive substances (NPS): characterization of the use in a sample of subjects asking for counselling	Not only PD
2017	Xenon in the treatment of panic disorder: an open label study	not SSRI or benzodiazepine evaluation
2005	Post-traumatic stress disorder in patients with coronary artery disease: Screening and management implications	Not related to PD
2017	Clinical characteristics and treatment outcomes of patients with major depressive disorder and comorbid anxiety disorders - results from a European multicenter study	not only PD
2008	GABAergic and Endocannabinoid Dysfunction in Anxiety - Future Therapeutic Targets?	not related to PD
2017	Healthy Human Male Dopamine D3 Receptor Uptake of 18F Fluortriopride (FTP) Before and After Lorazepam Challenge	Not only PD
2006	A randomized controlled trial of paroxetine for noncardiac chest pain	not examining direct improvement of PD.

2005	Recent advances in animal models of chronic antidepressant effects: The novelty-induced hypophagia test	Not related to PD
2017	Patterns of use and diversion of benzodiazepines and related subtances in France: results of the OSIAP survey	Not only PD
2017	Benzodiazepines and related citations on the suspicious prescription recorded in the OSIAP survey	Not only PD
2015	Vaginal bleeding associated with antidepressants	not PD related
2007	Which panic disorder patients benefit from which treatment: cognitive therapy or antidepressants?	article including psychological treatment
1999	Once-weekly dosing of fluoxetine in the maintenance of remission in panic disorder	dose comparision as primary outcome
2007	Increased brain serotonin turnover in panic disorder patients in the absence of a panic attack: reduction by a selective serotonin reuptake inhibitor	not examining direct improvement of PD.
1997	Open fluoxetine treatment of mixed anxiety disorders in children and adolescents	children
2007	Effects of gradual discontinuation of selective serotonin reuptake inhibitors in panic disorder with agoraphobia	not examining direct improvement of PD.
2015	Withdrawal Symptoms after Selective Serotonin Reuptake Inhibitor Discontinuation: A Systematic Review	review
2011	The mechanisms of tolerance in antidepressant action	Not related to PD
2001	Long-term outcome of panic disorder with agoraphobia treated by exposure	article including psychological treatment
2010	VERTIGO - COMORBIDITY WITH PSYCHIATRIC DISORDERS	Not related to PD
1997	Effects of clomipramine on plasma amino acids and serotonergic parameters in panic disorder and depression	article including tricycles antidepressants
2007	Relapse prevention of panic disorder in adult outpatient responders to treatment with venlafaxine extended release	evaluating venlafaxine
2005	Sertraline and alprazolam in the treatment of panic disorder	other language than English
2013	Selective serotonin reuptake inhibitor exposure	not only PD
2005	Randomized, placebo-controlled trial of exposure and ritual prevention, clomipramine, and their combination in the treatment of obsessive-compulsive disorder	evaluating clomipramine

2017	DOES THE COMMERCIAL AVAILABILITY OF CLOBAZAM AFFECT HEALTHCARE RESOURCE UTILIZATION AND COSTS AMONG PATIENTS WITH LENNOX-GASTAUT SYNDROME?	Not only PD
2000	Influence of panic-agoraphobic spectrum symptoms on treatment response in patients with recurrent major depression	not PD patients
2017	Will early intervention with SSRI versus benzodiazepines reduce the risk of developing posttraumatic stress disorder?	Not only PD
2004	Panic attacks with spontaneous ejaculation successfully treated with citalopram and clonazepam	Case report
2009	The Benzodiazepine Stigma Persists	review
2011	New treatment options for panic disorder: clinical trials from 2000 to 2010	review
2014	Current pharmacological interventions in panic disorder	review
2014	Dependence on benzodiazepines in patients with panic disorder: A cross-sectional study	meeting abstract
2007	Effects of genetic polymorphism of cytochrome P450 enzymes on the pharmacokinetics of benzodiazepines	not direct evaluating PD symptoms
2006	Psychotherapy plus antidepressant for panic disorder with or without agoraphobia - Systematic review	review
2006	Efficacy of typical and atypical antipsychotics for primary and comorbid anxiety symptoms or disorders: A review	review
2009	Effect of medication and psychotherapy on heart rate variability in panic disorder	article including psychological treatment
2000	Long-term treatment of panic disorder with agoraphobia in private practice	article including psychological treatment
1998	Combined pharmacotherapy and cognitive behavior therapy in the treatment of panic disorder	article including psychological treatment
2010	NEURODEVELOPMENTAL EFFECTS OF PRENATAL EXPOSURE TO PSYCHOTROPIC MEDICATIONS	Not related to PD
2001	Absence of neuropsychologic deficits in patients receiving long-term treatment with alprazolam-XR for panic disorder	not examining direct improvement of PD.

2005	A randomized double-blind comparison of sertraline with early alprazolam XR Co-administration vs. sertraline/placebo for panic disorder	meeting abstract
2001	Early coadministration of clonazepam with sertraline for panic disorder	augmentation treatment
1998	SSRIs in the treatment of panic disorder	review
2015	A controlled trial of quetiapine XR coadministration treatment of SSRI-resistant panic disorder	combinated treatment
2004	Impaired GABA neuronal response to acute benzodiazepine administration in panic disorder	not examining direct improvement of PD.
2006	Sertraline with early alprazolam XR co-administration vs sertraline/placebo for panic disorder	meeting abstract
2008	SSRI/benzodiazepine (Alprazolam XR) coadministration in panic disorder: Results from a multisite, controlled trial	meeting abstract
2017	Characterization of the Ability of Diazepam to Reverse Prescription Opioid Tolerance	Not only PD
2017	New designer benzodiazepines use in Barcelona	Not only PD
2007	Risk of adverse behavioral effects with pediatric use of antidepressants	not only PD
2000	Different modes of action of alprazolam in the treatment of panic attacks	review
2017	Prepubertal parvalbumin protein loss precedes adult parvalbumin interneuron loss in MAM-treated rats: rescue by peripubertal diazepam	Not only PD
2017	PREPUBERTAL STRESS LEADS TO PARVALBUMIN PROTEIN LOSS IN ADOLESCENCE THAT PRECEDES ADULT PARVALBUMIN INTERNEURON LOSS: RESCUE BY PERIPUBERTAL DIAZEPAM	Not only PD
2015	New Findings on the Neurotransmitter Modulation of Defense in the Dorsal Periaqueductal Gray	not only PD related
2001	[Cortico-subcortical electrophysiological study during the effects of benzodiazepines in patients with panic disorders]	not examining direct improvement of PD.
2015	Ghrelin and lipid levels in panic disorder before and after treatment and their relationship with agoraphobia	not examining direct improvement of PD.

2007	Effect of dissociative experiences on drug treatment of panic disorder	not examining direct improvement of PD.
2008	Is it justified an extended treatment with benzodiacepines?	not only PD
2014	A prospective naturalistic study of antidepressant-induced jitteriness/anxiety syndrome	not only PD
2008	Incidence and predictors of activation syndrome induced by antidepressants	not PD related
2009	Effects of acute citalopram on the expression of conditioned freezing in naive versus chronic citalopram-treated rats	animal studies
2008	Altered cerebral gamma-aminobutyric acid type A-benzodiazepine receptor binding in panic disorder determined by [11C]flumazenil positron emission tomography	not examining direct improvement of PD.
2017	Correlation between cytochrome P450 2C19 genetic polymorphism and treatment response to escitalopram in panic disorder	not examining direct improvement of PD.
2011	Anxiety sensitivity and illicit sedative use among opiate-dependent women and men	Not related to PD
2009	Benzodiazepine Dependence: Causalities and Treatment Options	review
2012	Predictors of outcome of pharmacological and psychological treatment of late-life panic disorder with agoraphobia	article including psychological treatment
2010	A randomized controlled study of paroxetine and cognitive-behavioural therapy for late-life panic disorder	article including psychological treatment
2005	To study the effectiveness of paroxetine or cognitive behavioural therapy (cbt) in the treatment of panic disorder in elderly people	meeting abstract
2002	Serotonin, 5-hydroxyindoleacetic acid and serotonin transporter in blood peripheral lymphocytes of patients with generalized anxiety disorder	not only PD patients were evaluated
2006	Panic disorder, treatment with selective serotonin reuptake inhibitors, and cholesterol levels	comment
2005	The acute phase response in panic disorder	not examining direct improvement of PD.
2002	Depression and panic disorder after heart transplantation--treatment with sertraline	not only PD patients were evaluated

2000	Pindolol augmentation in patients with treatment-resistant panic disorder: A double-blind, placebo-controlled trial	pindolol on PD
2008	Anxiety disorders: A comprehensive review of pharmacotherapies	review
2014	Effect of pharmacotherapy for anxiety disorders on quality of life: a meta-analysis	meta-analysis
2008	Aripiprazole as augmentation treatment of refractory generalized anxiety disorder and panic disorder	augmentation treatment
2017	Intravaginal diazepam for the treatment of pelvic pain among women with pelvic floor hypertonic disorder: A double blind, randomized, placebo controlled trial	Not only PD
2008	Central CRH system in depression and anxiety - Evidence from clinical studies with CRH1 receptor antagonists	not related to PD
2016	Don't panic. A guide to tryptophan depletion with disorder-specific anxiety provocation	not only PD
2010	Effects of acute tryptophan depletion in serotonin reuptake inhibitor-remitted patients with generalized anxiety disorder	not only PD
2014	Benzodiazepine dependence and its treatment with low dose flumazenil	not only PD
2014	Anxiety Associated with Asthma Exacerbations and Overuse of Medication: The Role of Cultural Competency	Not related to PD
2002	Different profiles of the therapeutic response in panic disorder: A re-analysis of two naturalistic studies with alprazolam extended-release and citalopram	meeting abstract
2016	PHENOBARBITAL VERSUS BENZODIAZEPINES IN THE TREATMENT OF ALCOHOL WITHDRAWAL SYNDROME	Not only PD
2010	Moderators and mediators among panic, agoraphobia symptoms, and suicidal ideation in patients with panic disorder	not only PD
2017	Lorazepam as an adjuvant to haloperidol for agitated delirium at the end of life: A double-blind randomized controlled trial	Not only PD
2008	Combined therapy vs. CBT or SSRIs alone in patients with panic disorder	article including psychological treatment
2014	Azapirones versus placebo for panic disorder in adults	evaluating azapirone

2016	Psychological therapies versus pharmacological interventions for panic disorder with or without agoraphobia in adults	article including psychological treatment
2014	Patient-reported outcomes of quality of life, functioning, and depressive symptom severity in major depressive disorder comorbid with panic disorder before and after SSRI treatment in the star*d trial	comorbid conditions
2011	FGF2 nor BDNF affect the therapeutic response of paroxetine in panic disorder measured by serotonergic gene polymorphisms and plasma levels of paroxetine	meeting abstract
2011	Determinants of pharmacodynamic trajectory of the therapeutic response to paroxetine in Japanese patients with panic disorder	not examining direct improvement of PD.
1997	The effects of clonazepam on quality of life and work productivity in panic disorder	main outcome was quality of life
2009	An Integrated Approach to the Diagnosis and Treatment of Anxiety Within the Practice of Cardiology	not only PD
2012	An animal model of panic vulnerability with chronic disinhibition of the dorsomedial/perifornical hypothalamus	animal studies
2002	Further evidence for the predictive validity of the unstable elevated exposed plus-maze, a behavioural model of extreme anxiety in rats: differential effects of fluoxetine and chlordiazepoxide	animal study
2017	Keeping pace in the NPS race: opioids and benzodiazepines in samples submitted to a large reference laboratory	Not only PD
2016	Attitudes Toward Use of Benzodiazepines among US Hospice Clinicians: Survey and Review of the Literature	review
2005	A placebo-controlled, randomized withdrawal study of sertraline for panic disorder in Japan	not examining direct improvement of PD.
2002	A randomized, double-blind, placebo-controlled study of the effects of adjunctive paroxetine in panic disorder patients unsuccessfully treated with cognitive-behavioral therapy alone	article including psychological treatment
2017	Association between the use of benzodiazepines and the occurrence of acute angle-closure glaucoma in the elderly: A population-based study	Not only PD

2007	Increased low-density lipoprotein cholesterol levels after citalopram treatment in panic disorder patients	meeting abstract
2012	Regional brain metabolism and treatment response in panic disorder patients: an [18F]FDG-PET study	not examining direct improvement of PD.
2010	Platelet serotonin transporter function after short-term paroxetine treatment in patients with panic disorder	not examining direct improvement of PD.
2005	Benzodiazepines and anxiety disorders: a review for the practicing physician	review
2000	Differences in pharmacodynamics but not pharmacokinetics between subjects with panic disorder and healthy subjects after treatment with a single dose of alprazolam	less than 10 PD patients
2001	Paroxetine in panic disorder with agoraphobia	case report
2006	Anxiety disorders: under-diagnosed and insufficiently treated	review
2001	Panic disorder: the place of benzodiazepines and selective serotonin reuptake inhibitors	review
2006	Time to response in panic disorder in a naturalistic setting: combination therapy with alprazolam orally disintegrating tablets and serotonin reuptake inhibitors compared to serotonin reuptake inhibitors alone	combinated treatment
2009	Current Considerations in the Treatment of Generalized Anxiety Disorder	review
2009	Epidemiology and Management of Anxiety in Patients with Bipolar Disorder	not only PD
2015	Determining factors for discontinuation of initial treatment with paroxetine in panic disorder and major depressive disorder	meeting abstract
2015	Prognostic subgroups for remission and response in the Coordinated Anxiety Learning and Management (CALM) trial	not only PD related
1998	Clinical utility of the selective serotonin reuptake inhibitors in the spectrum of anxiety	evaluating not only PD
2004	Benzodiazepines versus antidepressants for panic disorder	review
2012	Recurrence of Panic Attacks After Brucellosis Treatment-Highly Probable Citalopram and Rifampin Drug Interaction	Case report

2005	Patient-reported quality of life and functionality in panic disorder: Venlafaxine, paroxetine, and placebo	meeting abstract
2005	The effect of venlafaxine, paroxetine, and placebo on health-related work productivity in panic disorder patients	meeting abstract
2012	The long-term efficacy of escitalopram for the treatment of korean panic disorder patients : A prospective, open-labeled, multi-center trial	meeting abstract
2010	The changes of cerebral cortical and limbic brain function after short-term paroxetine treatment in panic disorder : An 18F FDG-PET study	meeting abstract
2004	Influences of treatment with paroxetine on cholesterol levels in patients with panic disorder	meeting abstract
2005	Increased cholesterol levels after paroxetine treatment in patients with panic disorder	not examining direct improvement of PD.
2004	Changes in lymphocyte subsets after short-term pharmacotherapy in patients with panic disorder	not examining direct improvement of PD.
2002	The role of extended-release benzodiazepines in the treatment of anxiety: a risk-benefit evaluation with a focus on extended-release alprazolam	not only PD patients were evaluated
2004	Benzodiazepines versus antidepressants for panic disorder	review
2011	A randomized trial of sertraline, self-administered cognitive behavior therapy, and their combination for panic disorder	article including psychological treatment
2013	[The effect of mexidol in the combination with antidepressants on sleep disturbance in young patients with panic disorder]	combination treatment/young patients
2010	Benzodiazepines: good or bad medicine?	review
2012	Escitalopram increased gray matter and white matter in a first-episode drug-naive panic disorder patient within 6 weeks	not examining direct improvement of PD.
2013	Changes in regional homogeneity of parieto-temporal regions in panic disorder patients who achieved remission with antidepressant treatment	not examining direct improvement of PD.
2016	The changes in the low-frequency fluctuations of cingulate cortex and postcentral gyrus in the treatment of panic disorder: The MRI study	not examining direct improvement of PD.

2013	Improvements in white matter micro-structural integrity of right uncinate fasciculus and left fronto-occipital fasciculus of remitted first-episode medication-naive panic disorder patients	not examining direct improvement of PD.
2006	Challenges in the treatment of anxiety disorders: beyond guidelines	review
2012	Commentary on 'The role of alprazolam for the treatment of panic disorder in Australia'	commentary
2017	Proper use of benzodiazepines: study of prescriptions' prevalence	Not only PD
2006	Comparison of high-potency benzodiazepines vs. combination of high potency-benzodiazepines and fluoxetine in treatment of panic disorder with agoraphobia	cobinated treatment
2006	Benzodiazepines vs. combination of benzodiazepines and SSRI in treatment of panic disorder with agoraphobia	combined treatment
2006	Comparison of high-potency benzodiazepines vs. combination of high potency-benzodiazepines and fluoxetine in treatment of panic disorder with agoraphobia	meeting abstract
2006	Benzodiazepines vs. combination of benzodiazepines and SSRI in treatment of panic disorder with agoraphobia	meeting abstract
2006	[Relationship of psychiatric comorbidity and treatment of panic disorder and agoraphobia]	not examining direct improvement of PD.
2002	Combination of benzodiazepines and SSRI in treatment of panic disorder	meeting abstract
2008	Transcriptional regulation at a HTR1A polymorphism associated with mental illness	not only PD
2009	Paroxetine-induced increase in LDL cholesterol levels	not examining direct improvement of PD.
1997	A comparison of paroxetine, clomipramine and placebo in the treatment of panic disorder. Collaborative Paroxetine Panic Study Investigators	article including tricycles antidepressants
2002	Remission analysis of patients with panic disorder treated with paroxetine	meeting abstract
1997	Long-term evaluation of paroxetine, clomipramine and placebo in panic disorder. Collaborative Paroxetine Panic Study Investigators	article including tricycles antidepressants

2008	beta-adrenoceptor affinity as a biological predictor of treatment response to paroxetine in patients with acute panic disorder	not examining direct improvement of PD.
2006	The effectiveness of 6-month treatment with citalopram in Korean panic disorder patients: a prospective, open-labelled, multi-centre trial	meeting abstract
2000	Citalopram controls phobic symptoms in patients with panic disorder: randomized controlled trial	article including tricycles antidepressants
1998	A controlled, prospective, 1-year trial of citalopram in the treatment of panic disorder	article including tricycles antidepressants
2003	Sertraline versus imipramine treatment of comorbid panic disorder and major depressive disorder	article including tricycles antidepressants
2003	Venlafaxine XR and paroxetine in the short-term treatment of panic disorder	meeting abstract
1997	Long-term citalopram treatment in panic disorder	meeting abstract
2008	Sertraline in treatment of depression, panic disorder, OCD and PTSD at dailly hospital, psychiatric clinic Sarajevo university	meeting abstract
2009	A double-blind, placebo-controlled, parallel-group, flexible-dose study of venlafaxine extended release capsules in adult outpatients with panic disorder	evaluating venlafaxine
2016	Facing the fear--clinical and neural effects of cognitive behavioural and pharmacotherapy in panic disorder with agoraphobia	article including psychological treatment
2010	Attenuation of fear-like response by escitalopram treatment after electrical stimulation of the midbrain dorsolateral periaqueductal gray	animal study
2017	Identifying Diazepam Induced Alterations in GABA(A)R Synaptic Plasticity	Not only PD
2016	Neurobiological markers predicting treatment response in anxiety disorders: A systematic review and implications for clinical application	review
1998	Efficacy studies of paroxetine in panic disorder	review
2010	The Role of Benzodiazepines in the Treatment of Anxiety Disorders: A Clinical Review	review

1998	Acute effects of low-dose flumazenil in panic disorder	evaluating flumazenil
2006	Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats	animal studies
2004	Monotherapy with benzodiazepines or SSRIs versus combined treatment in a sample of primary care patients with panic disorder	meeting abstract
2005	Emerging treatments for child and adolescent social phobia: A review	review
2009	Radio electric treatment vs. Es-Citalopram in the treatment of panic disorders associated with major depression: an open-label, naturalistic study	comorbid patients
2015	Neurobiological correlates of social anxiety disorder: an update	review
2012	Treatment Strategies of Obsessive-Compulsive Disorder and Panic Disorder/Agoraphobia	review
1999	Decreased platelet 3H-paroxetine binding in untreated panic disorder patients	not evaluating as a primary outcome the antid
1997	The treatment of panic disorder in a clinical setting: a 12-month naturalistic study	The treatment of panic disorder in a clinical se
2005	The effect of pharmacotherapy on personality disorders in panic disorder: a one year naturalistic study	not examining direct improvement of PD.
2006	Predictors of symptom resolution in panic disorder after one year of pharmacological treatment: a naturalistic study	not only PD
2006	The effect of temperament and character on response to selective serotonin reuptake inhibitors in panic disorder	not examining direct improvement of PD.
2006	Personality disorders and response to medication treatment in panic disorder: a 1-year naturalistic study	not examining direct improvement of PD.
2008	Effect of pharmacological treatment on temperament and character in panic disorder	not examining direct improvement of PD.
2005	Relationship between alexithymia and panic disorder: a longitudinal study to answer an open question	not examining direct improvement of PD.
2016	Clinical management of perinatal anxiety disorders: A systematic review	review
2002	Panic disorder patients have reduced cyclic AMP in platelets	not examining direct improvement of PD.

2008	Paroxetine: safety and tolerability issues	review
2010	Brain serotonin transporter availability in panic disorder: Gender differences and implications for treatment response to escitalopram	meeting abstract
2011	Efficacy of alprazolam sublingual tablets in the treatment of the acute phase of panic disorders	without placebo
2017	D-PRESCRIBE overtakes EMPOWER in patient-centered deprescribing of benzodiazepines: Preliminary results from a pragmatic cluster-randomized community-based trial in Canada	Not only PD
2006	Pharmacological treatment options for panic disorder in children and adolescents	children
2001	Paroxetine in child and adolescent outpatients with panic disorder	children
2003	Imipramine vs. sertraline in panic disorder: 24-week treatment completers	article including tricycles antidepressants
2003	Sertraline in panic disorder: initial treatment versus switch strategy	article including tricycles antidepressants
2004	Switching from imipramine to sertraline in panic disorder	article including tricycles antidepressants
2002	Switching from imipramine to sertraline in panic disorder with agoraphobia	meeting abstract
2017	Evaluation of dependence among benzodiazepines in population of elderly subjects followed in psychiatric service in Sfax	Not only PD
2000	Increased left posterior parietal-temporal cortex activation after D-fenfluramine in women with panic disorder	not evaluating SSRIs or benzodiazepines on PD
2001	Fluoxetine in panic disorder: A randomized, placebo-controlled study	meeting abstract
1998	Fluoxetine treatment of panic disorder: A randomized, placebo-controlled, multi-center trial	meeting abstract
1998	Long-term treatment in panic disorder: Randomization of acute fluoxetine responders to continued treatment with fluoxetine or placebo	meeting abstract
2002	Comparison of paroxetine and reboxetine in panic disorder	meeting abstract

2002	Efficacy of Alprazolam monotherapy vs. Alprazolam plus Fluvoxamine in long term treatment for panic disorder	meeting abstract
2006	Valerian for anxiety disorders	review
2007	Passiflora for anxiety disorder (Review)	review
2010	Selective serotonin-reuptake inhibitors in the treatment of panic disorder: a systematic review of placebo-controlled studies	review
2016	BENZODIAZEPINES AND TRANSITION TO DELIRIUM IN CRITICALLY ILL CHILDREN	Not only PD
2005	Mirtazapine versus paroxetine in panic disorder: an open study	evaluating mirtazapine
2017	Benzodiazepines abstinence syndrome with psychotic symptoms: Case report	Case report
2000	[Clinical effect and tolerance of paxil (paroxetine) in management of panic disorders]	article in russian
2012	The role of alprazolam for the treatment of panic disorder in Australia	review
2011	The efficacy and safety of alprazolam versus other benzodiazepines in the treatment of panic disorder	meta- analysis
2017	Scheduled Diazepam Does Not Add To Midazolam/lorazepam Symptom-Titrated Regimens For Alcohol Withdrawal Syndrome	Not only PD
2014	Panic disorder and serotonin reuptake inhibitors predict coupling of cortical and cardiac activity	not examining direct improvement of PD.
2005	Long-term use of benzodiazepines in participants with comorbid anxiety and alcohol use disorders	not only PD
2005	Where are the guidelines for the treatment of asthma with panic spectrum symptoms?	review
2015	A schedule for tapering out clonazepam in panic disorder patients after long-term treatment	meeting abstract
2013	Clonazepam for the treatment of panic disorder	review
2011	Treatment of the acute phase of panic disorder: an eight-week randomised, naturalistic trial with clonazepam and paroxetine	meeting abstract
2008	Demographic and clinical features of panic disorder comorbid with bipolar I disorder: a 3-year retrospective study	comorbid patients
2006	Clonazepam in the treatment of psychiatric disorders: an update	review

2009	A RANDOMIZED NATURALISTIC OPEN LONG-TERM TREATMENT OF PANIC DISORDER WITH CLONAZEPAM OR PAROXETINE	meeting abstract
2016	High relapse rate after efficacious ultra-long term treatment of panic disorder with clonazepam or paroxetine	meeting abstract
2014	Ultra-long-term outcome of panic disorder with clonazepam or paroxetine: A randomized, open, systematic treatment for 3 years and a follow-up for 6 years after	meeting abstract
2012	Long-term treatment of panic disorder : Tapering out clonazepam and paroxetine	meeting abstract
2010	Treatment of Panic Disorder with Clonazepam or Paroxetine: a Long-Term Randomized Naturalistic Open Study	meeting abstract
2008	Serotonin 5-HT1A receptor binding in people with panic disorder: positron emission tomography study	not examining direct improvement of PD.
2006	Posttraumatic stress disorder: A state-of-the-science review	review
2017	COMPARING THE EFFICACY OF SODIUM VALPROATE AND LEVETIRACETAM FOLLOWING INITIAL LORAZEPAM IN GENERALIZED CONVULSIVE STATUS EPILEPTICUS (GCSE) IN ELDERLY: A PROSPECTIVE RANDOMIZED CONTROLLED STUDY	Not only PD
2002	Effect of citalopram treatment on relationship between platelet serotonin functions and the Karolinska scales of personality in panic patients	not examining direct improvement of PD.
2000	The effect of citalopram treatment on platelet serotonin function in panic disorders	not examining direct improvement of PD.
2014	Ketamine for chronic pain: risks and benefits	Not related to PD
2001	Pharmacokinetically induced benzodiazepine withdrawal	not PD related
2016	Use of selective serotonin reuptake inhibitors reduces fertility in men	not related to PD
2012	Recent Developments in Potential Anxiolytic Agents Targeting GABA(A)/BzR Complex or the Translocator Protein (18kDa) (TSPO)	Not only PD
2015	Anxiolytic-Like Effects of 7H-Benzo e perimidin-7-One Derivatives through Elevated Plus-Maze Test in Mice	animal studies
2017	Healthcare Utilization Characteristics for Intranasal Midazolam Versus Rectal Diazepam	Not only PD

2004	Anxiety and depression: individual entities or two sides of the same coin?	review
2005	Overview of diagnosis and drug treatments of anxiety disorders	review
2013	Efficacy and Tolerability of Benzodiazepines versus Antidepressants in Anxiety Disorders: A Systematic Review and Meta-Analysis	review
2015	Plasma catecholamine levels before and after paroxetine treatment in patients with panic disorder	not examining direct improvement of PD.
2002	Paroxetine influences respiration in rats: Implications for the treatment of panic disorder	meeting abstract
2017	Impact of Lorazepam on Chemotherapy-induced Nausea and Vomiting in Patients with ALL	Not only PD
2011	Current status of research on cognitive therapy/cognitive behavior therapy in Japan	cognitivetherapy
2010	Two Cases of Panic Disorder Responsive to Low-Dose Sertraline	Case report
1999	Predictors of individual differences in alprazolam self-medication	not only PD patients were evaluated
2005	Benzodiazepine use, cognitive impairment, and cognitive-behavioral therapy for anxiety disorders: issues in the treatment of a patient in need	article including psychological treatment
2005	Cognitive-behavioral therapy and the treatment of panic disorder: efficacy and strategies	article including psychological treatment
2002	Benzodiazepine discontinuation difficulties in panic disorder: conceptual model and outcome for cognitive-behavior therapy	article including psychological treatment
2010	Efficacy of CBT for benzodiazepine discontinuation in patients with panic disorder: Further evaluation	article including psychological treatment
2004	Posttraumatic stress disorder in patients with bipolar disorder: a review of prevalence, correlates, and treatment strategies	review
1999	Group cognitive-behavior therapy for patients failing to respond to pharmacotherapy for panic disorder: a clinical case series	article including psychological treatment
2001	An effect-size analysis of the relative efficacy and tolerability of serotonin selective reuptake inhibitors for panic disorder	effect-size analysis

2004	Blood-injury related phobic avoidance as predictor of nonresponse to pharmacotherapy in panic disorder with agoraphobia	not examining direct improvement of PD.
2001	Double-blind, controlled, crossover trial of inositol versus fluvoxamine for the treatment of panic disorder	comparision with inositol
2011	The basic neuroscience of emotional experiences in mammals: The case of subcortical FEAR circuitry and implications for clinical anxiety	animal studies
2014	Reduced anterior temporal and hippocampal functional connectivity during face processing discriminates individuals with social anxiety disorder from healthy controls and panic disorder, and increases following treatment	not examining direct improvement of PD.
2009	Effectiveness of current treatment approaches for benzodiazepine discontinuation: a meta-analysis	meta-analysis
2016	AUGMENTATION STRATEGIES FOR TREATMENT-RESISTANT ANXIETY DISORDERS: A SYSTEMATIC REVIEW AND META-ANALYSIS	review
2013	Febrile seizures: recent developments and unanswered questions	Not related to PD
2016	Second-Stage Treatments for Relative Nonresponders to Cognitive Behavioral Therapy (Cbt) for Panic Disorder with or without Agoraphobia-Continued Cbt Versus Ssri: A Randomized Controlled Trial	article including psychological treatment
2013	The effect of escitalopram on metabolic parameters in patients with major depressive disorder, generalised anxiety disorder, and panic disorder: a prospective 6-month follow-up study	not only PD
2008	Efficacy and tolerability of escitalopram in anxiety disorders: A review	review
2007	[Anxiety and depressive disorders in 4,425 long term benzodiazepine users in general practice]	not only PD
2016	Long-Term Pharmacological Treatments of Anxiety Disorders: An Updated Systematic Review	review
2003	Serotonergic modulation of the balance system in panic disorder: an open study	not examining direct improvement of PD.

2004	Modulation of hyperreactivity to 35% CO2 after one week of treatment with paroxetine and reboxetine: a double-blind, randomized study	other class of antidepressants than SSRI
2002	Antipanic drug modulation of 35% CO2 hyperreactivity and short-term treatment outcome	article including tricycles antidepressants
2001	A comparison of citalopram and paroxetine in the treatment of panic disorder: a randomized, single-blind study	comparing two drugs without a placebo
1997	Modification of 35% carbon dioxide hypersensitivity across one week of treatment with clomipramine and fluvoxamine: a double-blind, randomized, placebo-controlled study	article including tricycles antidepressants
2004	Panic disorder: from respiration to the homeostatic brain	review
2011	Antianxiety medications for the treatment of complex agoraphobia: pharmacological interventions for a behavioral condition	article including psychological treatment
2005	Antipanic efficacy of paroxetine and polymorphism within the promoter of the serotonin transporter gene	not examining direct improvement of PD.
2007	Diagnosis and treatment of agoraphobia with panic disorder	review
2016	PREDICTORS OF BENZODIAZEPINES DISCONTINUATION IN AN ACUTE GERIATRIC UNIT	Not only PD
2007	Emerging anxiolytics	not only PD
2017	HEALTHCARE COSTS AMONG PATIENTS WITH LENNOX-GASTAUT SYNDROME TREATED WITH CLOBAZAM	Not only PD
2003	Treatment of depression with comorbid anxiety disorders: differential efficacy of paroxetine versus moclobemide	not only PD patients were evaluated
2010	In a mouse model relevant for post-traumatic stress disorder, selective brain steroidogenic stimulants (SBSS) improve behavioral deficits by normalizing allopregnanolone biosynthesis	animal studies
2004	Neurosteroids and neuroactive drugs in mental disorders	Not related to PD
2012	Distinct Panicogenic Activity of Sodium Lactate and Cholecystokinin Tetrapeptide in Patients with Panic Disorder	not related to PD treatment
2009	Pharmacotherapy of Anxiety Disorders	review

2013	Incremental cost-effectiveness of pharmacotherapy and two brief cognitive-behavioral therapies compared with usual care for panic disorder and noncardiac chest pain	article including psychological treatment
2005	Comorbid anxiety and depression	not only PD
2005	The pharmacotherapy of panic disorder	review
2003	Treatment of panic disorder: focus on paroxetine	review
2007	A double-blind study of the efficacy of venlafaxine extended-release, paroxetine, and placebo in the treatment of panic disorder	evaluating venlafaxine
2000	Sertraline treatment of panic disorder: response in patients at risk for poor outcome	treatment resistant
2003	Combined paroxetine and clonazepam treatment strategies compared to paroxetine monotherapy for panic disorder	combined treatment
2007	A randomized controlled trial of venlafaxine ER and paroxetine in the treatment of outpatients with panic disorder	evaluating venlafaxine
2004	A comparison of venlafaxine XR and paroxetine in the treatment of outpatients with panic disorder	evaluating venlafaxine
2004	A comparison of venlafaxine XR and paroxetine in the treatment of outpatients with panic disorder	meeting abstract
1997	Sertraline treatment of panic disorder: Combined results from two placebo-controlled trials	meeting abstract
1999	Behavioral, neuroendocrine, and cardiovascular response to flumazenil: no evidence for an altered benzodiazepine receptor sensitivity in panic disorder	comment
2007	The effect of repetitive transcranial magnetic stimulation (rTMS) add on serotonin reuptake inhibitors in patients with panic disorder: a randomized, double blind sham controlled study	other biological therapies
2013	Rational Use of Multiple Medications in Medicine and Psychiatry: A Dimensional Perspective	not only PD
2009	A comparison of low-dose risperidone to paroxetine in the treatment of panic attacks: a randomized, single-blind study	evaluating risperidone
2005	Psychiatric comorbidity in migraine: a review	review

2015	Atomoxetine Augmentation in a Case of Treatment Resistant Panic Disorder with Multiple Augments Failure: A Case Report	Case report
2006	New possibilities of treatment for panic attacks in elderly patients: escitalopram versus citalopram	two drugs withou a placebo
2005	Quality-of-life impairment in depressive and anxiety disorders	not only PD
2008	Depersonalization and derealization - Contemporary findings	not related to PD
1999	Use of selective serotonin reuptake inhibitors for the treatment of childhood panic disorder: a pilot study	children
2001	Mirtazapine versus fluoxetine in the treatment of panic disorder	including other class of antidepressant than SS
2004	Alprazolam extended-release in panic disorder	review
1998	Panic disorder: long-term pharmacotherapy and discontinuation	review
2004	Clinical applications of MAO-inhibitors	review
2016	Risk Factors of Opioid/Benzodiazepines-Induced Withdrawal Syndrome in Critically Ill Hispanic Children	Not only PD
2004	Anxiety sensitivity and modulation of the serotonergic system in patients with PD	not examining direct improvement of PD.
2006	Episode of major depression refractory to pharmacotherapy	not related to PD
2017	Engaging clinicians and veterans in efforts to decrease benzodiazepines in posttraumatic stress disorder (PTSD): de-implementing through academic detailing	Not only PD
2004	The development of Clonazepam as a psychotropic: The Massachusetts General Hospital experience	not only PD
2007	Update: New uses for lithium and anticonvulsants	review
2012	Psychoactive "Bath Salts" Intoxication with Methylenedioxypyrovalerone	not related to PD
2008	Critical parameters for D-cycloserine enhancement of cognitive-behaviorial therapy for obsessive-compulsive disorder	not related to PD
2016	Patient outcomes for phenobarbital use with or without benzodiazepines in alcohol withdrawal syndrome: systematic review	Not only PD
2014	Treatment in Nonresponsive Patients With Social Anxiety: Back to the Future With Benzodiazepines	not related to PD

2001	The effect of selective serotonin reuptake inhibitor treatment of panic disorder on emergency room and laboratory resource utilization	not examining direct improvement of PD.
2001	A randomized effectiveness trial of collaborative care for patients with panic disorder in primary care	not examining direct improvement of PD.
2005	Paroxetine response and tolerability among ethnic minority patients with mood or anxiety disorders: a pooled analysis	not only PD
2003	Personality and symptom sensitivity predictors of alprazolam withdrawal in panic disorder	not examining direct improvement of PD.
2007	Validity and reliability of the Quality of Life, Enjoyment and Satisfaction Questionnaire, Short Form	
2006	GABA(A) receptors as targets for novel anxiolytic drugs	not related to PD
2009	[SSRI discontinuation syndrome: incidence and differences on three groups of patients treated with paroxetine]	not PD related
2008	Selecting among second-step antidepressant medication monotherapies: predictive value of clinical, demographic, or first-step treatment features	not only PD
2003	Imipramine and buspirone in patients with panic disorder who are discontinuing long-term benzodiazepine therapy	article including tricycles antidepressants
2007	Clinical variables which influence the initial therapeutic effect of paroxetine in patients with panic disorder	meeting abstract
2009	Genetic and pharmacokinetic factors affecting the initial pharmacotherapeutic effect of paroxetine in Japanese patients with panic disorder	not examining direct improvement of PD.
2008	Paradoxical aggressive reactions to benzodiazepine use: A review	review
2007	Augmentation of paroxetine with clocapramine in panic disorder	augmentation study
2000	Nonorganic insomnia in panic Disorder: comparative sleep laboratory studies with normal controls and placebo-controlled trials with alprazolam	not examining direct improvement of PD.
2001	Pharmacologic treatment of anxiety disorders in 1989 versus 1996: results from the Harvard/Brown anxiety disorders research program	not only PD patients were evaluated

2004	Escitalopram versus citalopram: the surprising role of the R-enantiomer	Not only PD
2014	Evidence of a suffocation alarm system sensitive to clinically-effective treatments with the panicolytics clonazepam and fluoxetine	not examining direct improvement of PD.
2004	The effects of tianeptine or paroxetine on 35% CO2 provoked panic in panic disorder	evaluating tianeptine effects
2010	CBT, SSRI or both are similarly effective for panic disorder 1-year post-treatment	article including psychological treatment
2006	A randomized, controlled trial of the effectiveness of cognitive-behavioral therapy and sertraline versus a waitlist control group for anxiety disorders in older adults	article including psychological treatment
2006	Tiagabinein anxiety disorders	Not only PD
2005	History repeats itself: Pharmacodynamic trends in the treatment of anxiety disorders	Not only PD
2004	Double-blind, placebo-controlled assessment of combined clonazepam with paroxetine compared with paroxetine monotherapy for generalized social anxiety disorder	combined treatment
2003	Reboxetine and citalopram in panic disorder: a single-blind, cross-over, flexible-dose pilot study	other class of antidepressants than SSRI
1999	[Causes of failure in psychopharmacological treatment of anxiety disorder]	treatment failure
2011	Novel antidepressants and panic disorder: evidence beyond current guidelines	review
1998	Alprazolam reduces response to loud tones in panic disorder but not in posttraumatic stress disorder	artcle evaluating PD and posttraumatic stress
1997	Global measures of outcome in a controlled comparison of pharmacological and psychological treatment of panic disorder and agoraphobia in primary care	article including psychological treatment
2009	An evidence-based review of the clinical use of sertraline in mood and anxiety disorders	review
2007	The speed of onset of action of alprazolam-XR compared to alprazolam-CT in panic disorder	without a placebo

2000	The efficacy of sertraline in panic disorder: combined results from two fixed-dose studies	Patients with subsyndromic levels of depressi
1999	Treatment of panic disorder in older adults: a pilot study comparison of alprazolam, imipramine, and placebo	article including tricycles antidepressants
2002	Efficacy of sertraline in older patients with panic disorder	meeting abstract
2007	Modelling anxiety in humans for drug development	Not only PD
2010	Changes in Cerebral Cortex and Limbic Brain Functions after Short-Term Paroxetine Treatment in Panic Disorder: An [F]FDG-PET Pilot Study	not examining direct improvement of PD.
2009	Open-label support for duloxetine for the treatment of panic disorder	evaluating duloxetine
2009	Next-step strategies for panic disorder refractory to initial pharmacotherapy: a 3-phase randomized clinical trial	treatment resistant
2002	Longitudinal outcome with pharmacotherapy in a naturalistic study of panic disorder	combined therapy
2004	A retrospective data analysis of the impact of the New York triplicate prescription program on benzodiazepine use in Medicaid patients with chronic psychiatric and neurologic disorders	Not only PD
2009	Antidepressant-induced jitteriness/anxiety syndrome: systematic review	review
2015	Pharmacology of cognitive enhancers for exposure-based therapy of fear, anxiety and trauma-related disorders	not only PD
2017	Allostatic load and cognitive outcome after 12 weeks of alprazolam treatment in patients with anxiety disorders	Not only PD
1998	Efficacy studies of alprazolam in panic disorder	review
1999	Adverse reactions of selective serotonin reuptake inhibitors: reports from a spontaneous reporting system	not only PD patients were evaluated
2006	Anxiety states: a review of conceptual and treatment issues	review
2012	Reconsidering benzodiazepines in the treatment of panic disorder	comment
2014	The reappraisal of benzodiazepines in the treatment of anxiety and related disorders	review

2004	Treatment of panic disorder with agoraphobia in an anxiety disorders clinic: factors influencing psychiatrists' treatment choices	article including psychological treatment
2016	Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis	review
2005	The neurobiology of panic disorder: Toward an integrated model	animal studies
2006	Which factors predict placebo response in anxiety disorders and major depression? An analysis of placebo-controlled studies of escitalopram	not PD related
2003	Paroxetine improves somatic symptoms associated with panic disorder and GAD	meeting abstract
2000	Do selective serotonin re-uptake inhibitors enhance the efficacy of very brief cognitive behavioral therapy for panic disorder? A pilot study	article including psychological treatment
2005	Gender differences in clinical presentation and response to sertraline treatment of generalized anxiety disorder	not only PD
2014	CAN WE IMPROVE PSYCHOSOCIAL TREATMENTS FOR CHILD ANXIETY?	children
1999	Behavioral, neuroendocrine, and cardiovascular response to flumazenil: no evidence for an altered benzodiazepine receptor sensitivity in panic disorder	evaluating flumazenil
1998	Effect of flumazenil in lactate-sensitive patients with panic disorder	evaluating flumazenil
2002	GABA(A) receptor-modulating neuroactive steroid composition in patients with panic disorder before and during paroxetine treatment	not examining direct improvement of PD.
2000	Effectiveness of an empirically based treatment for panic disorder delivered in a service clinic setting: 1-year follow-up	article including psychological treatment
2004	Effects of hyperventilation on heart rate and QT variability in panic disorder pre- and post-treatment	not examining direct improvement of PD.
2005	The Role of High-Potency Benzodiazepines in the Treatment of Panic Disorder	review

2008	Long-term outcome of patients with dysthymia and panic disorder: a naturalistic 9-year follow-up study	not examining direct improvement of PD.
2006	Comparison of efficacy and safety profiles among paroxetine, benzodiazepines, and combination therapy in Japanese patients with panic disorder. open trial	meeting abstract
2009	Translocator Protein Ligands as Promising Therapeutic Tools for Anxiety Disorders	not direct evaluating PD symptoms
2011	Structural Requirements to Obtain Highly Potent and Selective 18 kDa Translocator Protein (TSPO) Ligands	not related to PD
2001	Common effects of chronically administered antipanic drugs on brainstem GABA(A) receptor subunit gene expression	not examining direct improvement of PD.
2008	Paroxetine	review
2009	Escitalopram and Venlafaxine for the Prophylaxis of Migraine Headache Without Mood Disorders	not only PD
2006	Managing depressive and anxiety disorders with escitalopram	review
2017	Where's the withdrawal? Retrospective review of benzodiazepine withdrawal	Not only PD
1999	Moclobemide and fluoxetine for panic disorder. International Panic Disorder Study Group	evaluating Moclebemide
2009	Pharmacogenetics of anxiolytic drugs	review
2010	Quality of sleep in escitalopram-treated female patients with panic disorder	not examining direct improvement of PD.
2009	Body temperature in patients with panic disorder treated with escitalopram	not examining direct improvement of PD.
2000	A prospective naturalistic study of 326 panic-agoraphobic patients treated with antidepressants	article including tricycles antidepressants
2012	Antidepressant up-titration: pharmacological and psychological considerations	review
2006	Tryptophan depletion does not modify response to CCK-4 challenge in patients with panic disorder after treatment with citalopram	not examining direct improvement of PD.

2005	Acute tryptophan depletion does not modify the response to cck-4 challenge in patients with panic disorder after citalopram treatment	meeting abstract
2017	Imaging drug-drug interaction using positron emission tomography (PET) scans: investigating the impact of diazepam on buprenorphine-induced respiratory depression	Not only PD
2007	The therapeutic potential of escitalopram in the treatment of panic disorder	review
1997	Paroxetine increases heart rate variability in panic disorder	primary outcome heart rate variability
2009	The factors that influence the therapeutic response to paroxetine in patients with panic disorder: longitudinal study	meeting abstract
2016	A pharmacological approach to panic disorder during pregnancy	review
1998	International Study of Expert Judgement on Therapeutic Use of Benzodiazepines and Other Psychotherapeutic Medications: V. Treatment strategies in panic disorder, 1992-1997	review
2000	Do antidepressants selectively suppress spontaneous (unexpected) panic attacks? A replication	article including tricycles antidepressants
2006	Abrupt discontinuation of alprazolam and cognitive style in patients with panic disorder: early effects on mood, performance, and vital signs	not examining direct improvement of PD.
2008	Cognitive style, alprazolam plasma levels, and treatment response in panic disorder	not examining direct improvement of PD.
2006	The relationship between the severity of asthma and comorbidities with anxiety and depressive disorders	not only PD
2001	Smoking and panic disorder	not examining direct improvement of PD.
2004	Antiepileptic drugs in the treatment of anxiety disorders: Role in therapy	review
2010	A randomized trial of cognitive-behavioral therapy or selective serotonin reuptake inhibitor or both combined for panic disorder with or without agoraphobia: treatment results through 1-year follow-up	article including psychological treatment

2008	Is a combined therapy more effective than either CBT or SSRI alone? Results of a multicenter trial on panic disorder with or without agoraphobia	article including psychological treatment
2013	Rate of improvement during and across three treatments for panic disorder with or without agoraphobia: cognitive behavioral therapy, selective serotonin reuptake inhibitor or both combined	article including psychological treatment
2007	A randomized clinical trial of eye movement desensitization and reprocessing (EMDR), fluoxetine, and pill placebo in the treatment of posttraumatic stress disorder: Treatment effects and long-term maintenance	not related to PD
2012	Alprazolam for depression	not related to PD
2014	CO-OCCURRENCE OF ANXIETY AND BIPOLAR DISORDERS: CLINICAL AND THERAPEUTIC OVERVIEW	not related to PD
2004	Clinical pharmacology, clinical efficacy, and behavioral toxicity of alprazolam: A review of the literature	review
2002	Effects of alprazolam on driving ability, memory functioning and psychomotor performance: a randomized, placebo-controlled study	not examining direct improvement of PD.
2017	Association between personality traits and Escitalopram treatment efficacy in panic disorder	not examining direct improvement of PD.
2006	[An additional short-term use of benzodiazepine anxiolytics at the initial stage of the treatment of panic disorders]	article in russian
1997	The effect of citalopram in panic disorder	article including tricycles antidepressants
2003	Paroxetine treatment of mood and anxiety disorders in children and adolescents	children
2014	The potential role of atypical antipsychotics in the treatment of panic disorder	review
2009	Combined psychotherapy plus benzodiazepines for panic disorder	review
2005	An association between 5-HTTLPR and the severity, clinical effect of paroxetine in patients with panic disorder	meeting abstract

2010	Therapeutic response to paroxetine in panic disorder is associated with a C(-1019)G 5-HT1A functional promoter gene polymorphism in a Japanese population	meeting abstract
2007	High plasma concentrations of paroxetine impede clinical response in patients with panic disorder	not examining direct improvement of PD.
2006	An association between paroxetine serum concentrations in panic disorder patients and response to initial treatment	meeting abstract
2007	Controlled study for effectiveness of sport vs. relaxation training in combination with paroxetine vs. Placebo in the treatment of the panic disorder	meeting abstract
2008	Nocturnal urinary cortisol excretion over a randomized controlled trial with paroxetine vs. placebo combined with relaxation training or aerobic exercise in panic disorder	not examining direct improvement of PD.
2002	Naturalistic manner of benzodiazepine use and cognitive behavioral therapy outcome in panic disorder with agoraphobia	article including psychological treatment
2001	Cognitive-behavior therapy for discontinuation of SSRI treatment of panic disorder: a case series	article including psychological treatment
2011	Serum BDNF levels before treatment predict SSRI response in depression	not only PD
1999	Clonazepam treatment of panic disorder in patients with recurrent chest pain and normal coronary arteries	not examining direct improvement of PD.
2002	A randomized, controlled trial of panic disorder treatment initiation in an emergency department chest pain center	not examining direct improvement of PD.
2009	Combining Cognitive Behavioural Therapy and Pharmacotherapy in the Treatment of Anxiety Disorders: True Gains or False Hopes?	not only PD
2000	Fluvoxamine pharmacotherapy of anxiety disorders in later life: preliminary open-trial data	not only PD patients were evaluated
2012	Paroxetine-Associated Hypereosinophilia May Clinically Resemble a Panic Attack	Case report
1999	Effects of paroxetine on heart period variability in patients with panic disorder: a study of holter ECG records	not examining direct improvement of PD.
2000	Effects of nortriptyline and paroxetine on QT variability in patients with panic disorder	article including tricycles antidepressants

2003	Effect of nortriptyline and paroxetine on measures of chaos of heart rate time series in patients with panic disorder	article including tricycles antidepressants
2003	Paroxetine decreases respiratory irregularity of linear and nonlinear measures of respiration in patients with panic disorder	meeting abstract
2004	Paroxetine decreases respiratory irregularity of linear and nonlinear measures of respiration in patients with panic disorder. A preliminary report	not examining direct improvement of PD.
2000	Effects of nortriptyline and paroxetine on QT variability in patients with panic disorder	meeting abstract
2008	Association of 5-HT1A receptor polymorphism with response to SSRI treatment of panic disorder	meeting abstract
2006	Pretreatment attrition and childhood social phobia: Parental concerns about medication	children
2008	Does Acute Treatment with Sedatives/Hypnotics for Anxiety in Depressed Patients Affect Suicide Risk? A Literature Review	review
2008	Platelet serotonin function after paroxetine treatment in patients with panic disorder	meeting abstract
2007	Enhanced reactivity of 5-HT1A receptors in the rat dorsal periaqueductal gray matter after chronic treatment with fluoxetine and sertraline: Evidence from the elevated T-maze	animal studies
2011	Panic disorder and suicidal behavior: a follow-up study of patients treated with cognitive therapy and SSRIs in Hungary	article including psychological treatment
2003	Effects of alprazolam on cholecystokinin-tetrapeptide-induced panic and hypothalamic-pituitary-adrenal-axis activity: a placebo-controlled study	not examining direct improvement of PD.
2003	Xanax XR for panic disorder	review

6.5. Material suplementar artigo 6

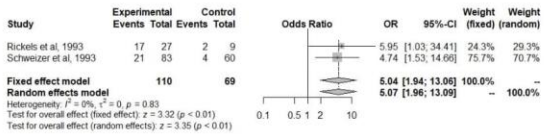
DIFFERENCES BETWEEN PROTOCOL AND REVIEW

The original protocol for this manuscript is registered at PROSPERO (CRD42018104339)

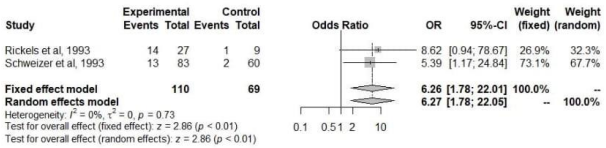
- Peer-reviewers disagreed with our choice of only included short-term studies. We therefore revised our original decision and when we updated our search on January 20th, 2019, we additionally screen for long-term PD studies and tapering investigations. However, since our search terms demonstrated only a few long-term PD studies that did not evaluate the same adverse events, and an investigation without a comparison between SSRIs or BZs with a placebo was an impeditive factor to be included in our statistical analysis, it was unfeasible to summarize long-term PD treatment evidence in a paper. When evaluate tapering in PD, our search strategy, with the included suggested terms, resulted in 3 studies assessing tapering in PD (one comparing tapering between SSRI sertraline and placebo in PD and two comparing tapering between BZs alprazolam and placebo in PD). Since these studies presented different types of adverse events, summarize then in one statistical analysis would be misleading. However, since headache and tremor appeared in both BZs studies, we performed a meta-analysis in R software only with BZs evidence. The results showed that BZs tapering presented a higher risk of causing tremor and headache than placebo in PD patients (OR=6.27, I^2=0, p<0.01, and OR=5.07, I^2=0, p<0.01, respectively). Consider that tapering results were only evaluated in BZs and not in SSRIs, we included the forest plots and the results in supplementary material. However, we understand the importance of evaluating BZs and SSRIs in long-term treatment and tapering. Therefore, to make it possible, we suggest that future research should focus on a more broadly population than just PD patients.

Forest plots BZs tapering:

1. **Forest plot 1. Headache and BZs tapering**



2. Forest plot 2. Tremor and BZs tapering



- Peer-reviewers asked for the analysis of further outcomes. We therefore added craving, dependence, addiction, tolerance, withdrawal, tapering, discontinuation syndrome, impaired driving, driving under the influence, driving while intoxicated, disinhibited behavior, bruising, as other major side-effects that have been discussed in the debate around the BZs and/or SSRIs drugs. Special searches were run on these on January 20th, 2019, search terms are detailed below.

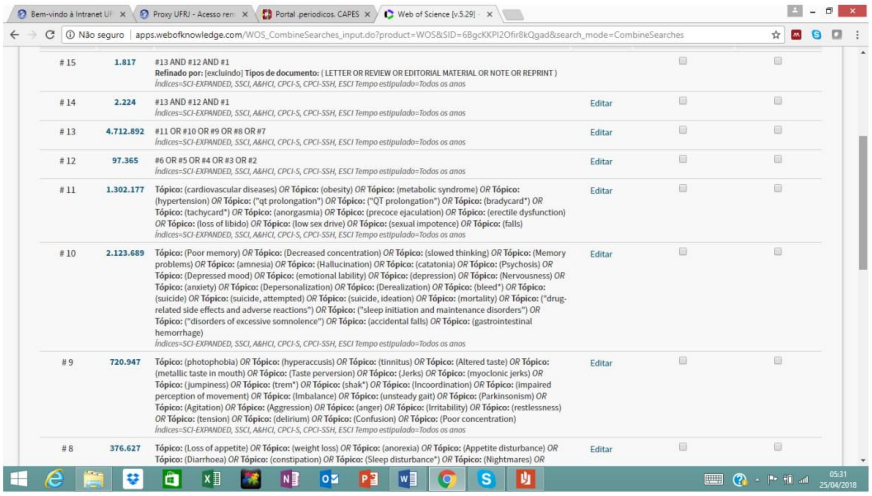
Search Terms:

"panic disorder" OR "panic attacks" AND paroxetine OR sertraline OR fluoxetine OR citalopram OR escitalopram OR fluvoxamine OR alprazolam OR bretazenil OR bromazepam OR chlordiazepoxide OR cinolazepam OR clonazepam OR cloxazolam OR clorazepate OR deloramzeapam OR diazepam OR estazolam OR etizolam OR fludiazepam OR flunitrazepam OR flurazepam OR flutoprazepam OR halazepam OR ketazolam OR loprazolam OR lorazepam OR lormetazepam OR medazepam OR nimatazepam OR nitrazepam OR nodazepam OR oxazepam OR phenazepam OR pinazepam OR prazepam OR premazepam OR quazepam OR temazepam OR tetrazepam OR triazolam OR benzodiazepines OR SSRI OR selective serotonin reuptake inhibitors AND influenza-like symptom* OR flu-like symptom* OR diaphoresis OR sweating OR flushing OR headache OR convulsions OR seizures OR muscle aches OR muscular pains OR myalgia* OR fatigue OR lack of energy OR lethargy OR somnolence OR stiffness OR arthralgias OR palpitations OR skin rash OR itching OR abdominal pain OR abdominal cramps OR nausea OR vomit* OR dry retching OR loss of appetite OR weight loss OR anorexia OR appetite disturbance OR diarrhea OR constipation OR sleep disturbance* OR nightmares or vivid dream* OR ataxia OR dizziness OR lightheadedness OR vertigo OR blurred vision OR difficulty in focusing OR visual disturbance OR sore eyes OR electric shock sensations OR numbness OR fasciculation OR paresthesia OR restless legs OR tingling OR muscle twitches OR hyperosmia OR photophobia OR hyperaccusis OR tinnitus OR altered taste OR metallic taste in mouth OR taste perversion OR jerks OR myoclonic jerks OR jumpiness OR tremors OR shaking OR Incoordination OR impaired perception of movement OR imbalance OR unsteady gait OR parkinsonism OR agitation OR aggression OR anger OR irritability OR restlessness OR tension OR delirium OR confusion OR poor concentration OR poor memory OR decreased concentration OR slowed thinking OR memory problems OR amnesia OR hallucination OR catatonia OR psychosis OR depressed mood OR emotional lability OR depression OR nervousness OR anxiety OR depersonalization OR derealization OR bleed* OR suicide OR suicide, attempted OR suicide, ideation OR mortality OR "drug-related side effects and adverse reactions" OR "sleep initiation and

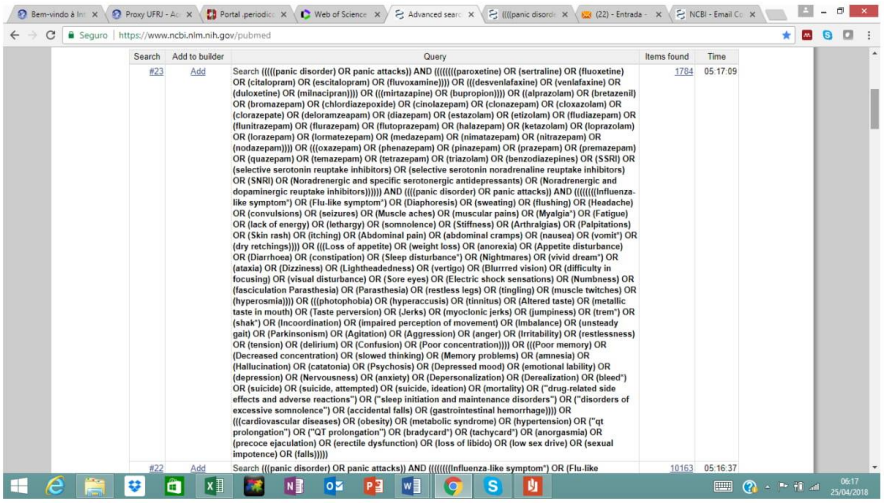
maintenance disorders" OR "disorders of excessive somnolence" OR accidental falls OR gastrointestinal hemorrhage OR cardiovascular diseases OR obesity OR metabolic syndrome OR hypertension OR "QTc prolongation" OR bradycardia OR tachycardia OR anorgasmia OR precocious ejaculation OR erectile dysfunction OR loss of libido OR low sex drive OR sexual impotence OR falls OR bruising OR addiction OR craving OR dependence OR tolerance OR addiction OR withdrawal OR tapering OR “discontinuation syndrome” OR “impaired driving” OR “driving under the influence” OR “driving while intoxicated” OR “disinhibited behavior”. In the search terms, we included items for PD short-term treatment non-regimented by European or North American regulatory agencies with the intention of providing the broadest search possible to avoid missing data. We (LAQ and AEN) manually checked which trials should be included in the systematic review *a posteriori*.

Search results

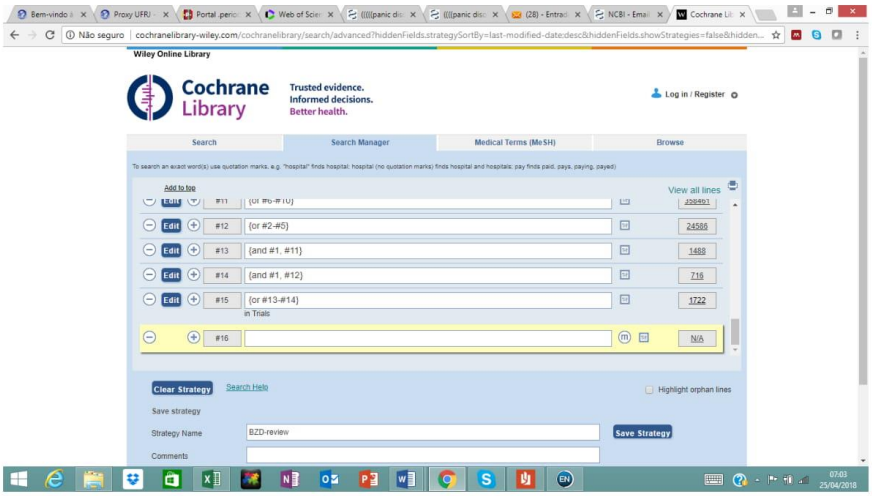
Web of Science Search- 25th april 2018



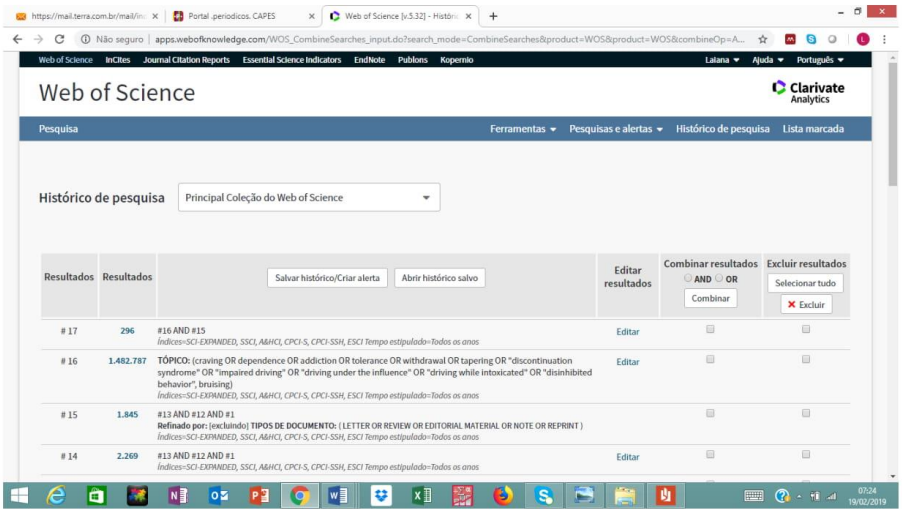
Pubmed Search- 25th april 2018



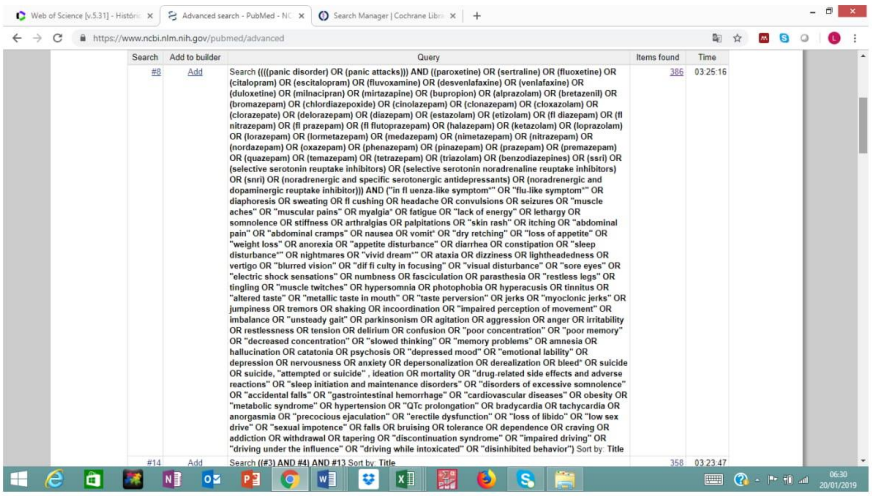
Cochrane Central Register of Controlled Trials Search- 25th april 2018



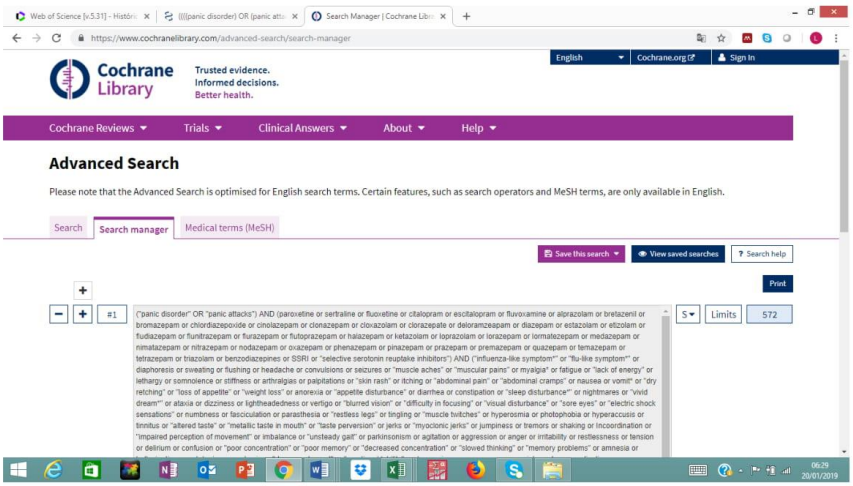
Web of Science Search- 20th January 2019



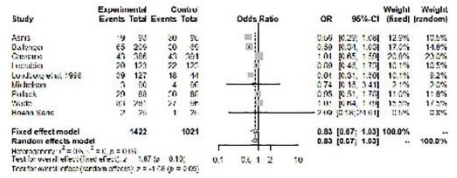
Pubmed Search- 20th January 2019



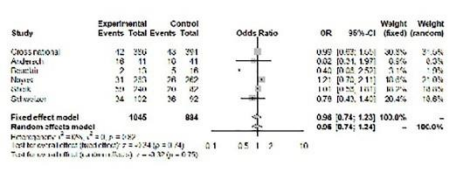
Cochrane Central Register of Controlled Trials Search- 20th January 2019



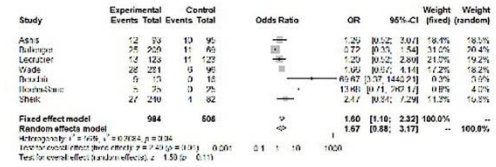
Headache
SSRI X placebo



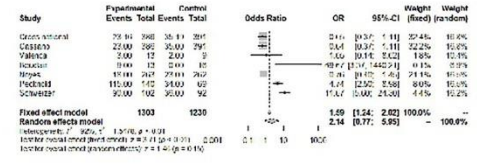
Headache
BZD X placebo



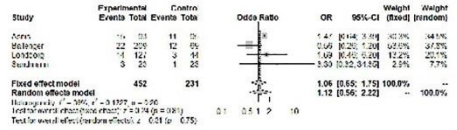
Dizziness
SSRI X placebo



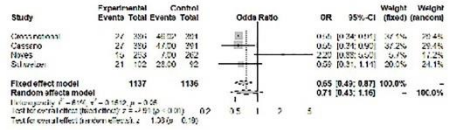
Dizziness
BZD X placebo



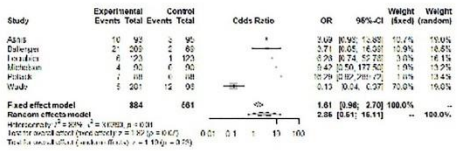
Nervouness
SSRI X placebo



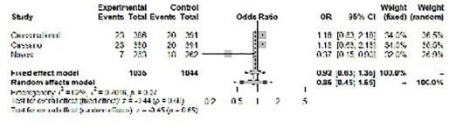
Nervouness
BZD X placebo



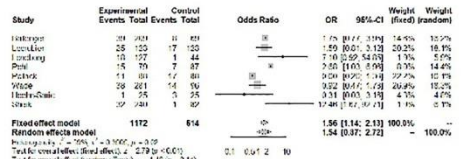
Tremor
SSRI X placebo



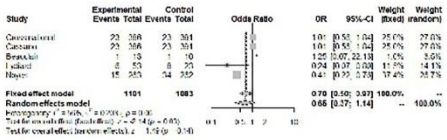
Tremor
BZD X placebo



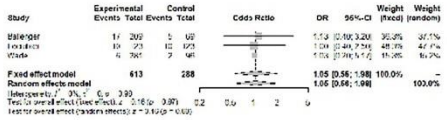
Dry Mouth
SSRI X placebo



Nausea
BZD X placebo



Constipation
SSRI X placebo



PRISMA Harms Checklist

Section/topic (page no)	Item	PRISMA checklist item	PRISMA harms (minimum)	Recommendations for reporting harms in systematic reviews (desirable)	Check if done
Title					Page 1*
Title (3)	1	Identify the report as a systematic review, meta-analysis, or both.	Specifically mention "harms" or other related terms, or the harm of interest in the review.	—	
Abstract					
Structured summary (4)	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	—	Abstracts should report any analysis of harms undertaken in the review, if harms are a primary or secondary outcome.	Page 2
Introduction					
Rationale (5)	3	Describe the rationale for the review in the context of what is already known.	—	It should clearly describe in introduction or in methods section which events are considered harms and provide a clear rationale for the specific harm(s), condition(s), and patient group(s) included in the review.	Page 3 and 4
Objectives (5)	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	—	PICOS format should be specified, although in systematic reviews of harms the selection criteria for P, C, and O may be very broad (same intervention may have been used for heterogeneous indications in a diverse range of patients)	Page 5
Methods					
Protocol and registration (6)	5	Indicate if a review protocol exists, if and where it can be accessed (eg, web address), and, if available, provide registration information including registration number.	—	No specific additional information is required for systematic reviews of harms.	Page 5
Eligibility criteria (6)	6	Specify study characteristics (eg, PICOS, length of follow-up) and report characteristics (eg, years considered, language, publication status) used as criteria for eligibility, giving rationale.	—	Report how handled relevant studies (based on population and intervention) when the outcomes of interest were not reported. Report choices for specific study designs and length of follow-up.	Page 5

Information sources (7)	7	Describe all information sources (eg, databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	—	Report if only searched for published data, or also sought data from unpublished sources, from authors, drug manufacturers and regulatory agencies. If includes unpublished data, provide the source and the process of obtaining it.	Page 5
Search (7)	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	—	If additional searches were used specifically to identify adverse events, authors should present the full search process so it can be replicated.	Supplementary Material 2
Study selection (8)	9	State the process for selecting studies (ie, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	—	If only included studies reporting on adverse events of interest, defined if screening was based on adverse event reporting in title/abstract or full text. If no harms reported in the text, report if any attempt was made to retrieve relevant data from authors.	Page 5 and 6
Data collection process (9)	10	Describe method of data extraction from reports (eg, piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	—	No specific additional information is required for systematic reviews of harms.	Page 6
Data items (9)	11	List and define all variables for which data were sought (eg, PICOS, funding sources) and any assumptions and simplifications made.	—	Report the definition of the harm and seriousness used by each included study (if applicable). Report if multiple events occurred in the same individuals, if this information is available. Consider if the harm may be related to factors associated with participants (eg, age, sex, use of medications) or provider (eg, years of practice, level of training). Specify if information was extracted and how it was used in subsequent results. Specify if extracted details regarding the specific methods used to capture harms (active/passive and timing of adverse event).	Not available
Risk of bias in individual studies (10)	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	—	The risk of bias assessment should be considered separately for outcomes of benefit and harms.	Page 6 and Supplementary material 3

Summary measures (11)	13	State the principal summary measures (eg, risk ratio, difference in means).	—	No specific additional information is required for systematic reviews of harms.	Page 7
Synthesis of results (11)	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (eg, I^2) for each meta-analysis.	Specify how zero events were handled, if relevant.		Page 7
Risk of bias across studies (11)	15	Specify any assessment of risk of bias that may affect the cumulative evidence (eg, publication bias, selective reporting within studies).	—	Present the extent of missing information (studies without harms outcomes), any factors that may account for their absence, and whether these reasons may be related to the results.	Supplementary material 3
Additional analyses (12)	16	Describe methods of additional analyses (eg, sensitivity or subgroup analyses, meta-regression), if done, indicating which were prespecified.	—	Sensitivity analyses may be affected by different definitions, grading, and attribution of adverse events, as adverse events are typically infrequent or reported using heterogeneous classifications. Report the number of participants and studies included in each subgroup.	Page 7
Results					
Study selection (13)	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	—	If a review addresses both efficacy and harms, display a flow diagram specific for each (efficacy and harm).	Figure 1
Study characteristics (14)	18	For each study, present characteristics for which data were extracted (eg, study size, PICOS, follow-up period) and provide the citations.	Define each harm addressed, how it was ascertained (eg, patient report, active search), and over what time period.	Add additional characteristics to: "P" (population) patient risk factors that were considered as possibly affecting the risk of the harm outcome. "I" (intervention) professional expertise/skills if relevant (for example if the intervention is a procedure). "T" (time) timing of all harms assessments and the length of follow-up.	Supplementary material 3
Risk of bias within studies (15)	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	—	Consider the possible sources of biases that could affect the specific harm under consideration within the review. Sample selection, dropouts and measurement of adverse events should be evaluated separately from the outcomes of benefit as described in item 12, above.	Supplementary material 3
Results of individual studies (16)	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	—	Report the actual numbers of adverse events in each study, separately for each intervention.	Supplementary material 3

Synthesis of results (17)	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Describe any assessment of possible causality.	If included data from unpublished sources, report clearly the data source and the impact of these studies to the final systematic review.	Figures 2,3,and 4
Risk of bias across studies (18)	22	Present results of any assessment of risk of bias across studies (see item 15).	—	No specific additional information is required for systematic reviews of harms. See item 15 above.	Figures 2, 3 and 4
Additional analysis (18)	23	Give results of additional analyses, if done (eg, sensitivity or subgroup analyses, meta-regression (see item 16)).	—	No specific additional information is required for systematic reviews of harms.	Page 8
Discussion					
Summary of evidence (18)	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (eg, healthcare providers, users, and policy makers).	—	No specific additional information is required for systematic reviews of harms.	Page 12
Limitations (18)	25	Discuss limitations at study and outcome level (eg, risk of bias), and at review level (eg, incomplete retrieval of identified research, reporting bias).	—	Recognise possible limitations of meta-analysis for rare adverse events (ie, quality and quantity of data), issues noted previously related to collection and reporting.	Page 15 and 16
Conclusions (19)	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	—	State conclusions in coherence with the review findings. When adverse events were not identified we caution against the conclusion that the intervention is "safe," when, in reality, its safety remains unknown.	Page 16 and 17
Funding					
Funding (19)	27	Describe sources of funding for the systematic review and other support (eg, supply of data); role of funders for the systematic review.	—	No specific additional information is required for systematic reviews of harms.	Conflicts of interest statement

*Page numbers refers to the manuscript "Selective serotonin reuptake inhibitors and benzodiazepines in Panic Disorder: a meta-analysis of common side effects in acute treatment".

Table 1. Studies included in the meta-analysis

Authors	N	Age	Primary diagnosis	Duration (weeks)	Comparisons	Response Definition to treatment	AEs assessment	Drop-out rate due to AEs	Overall drop-out rate
Andersch et al.,1991	123	19-63	Panic	8	Alprazolam (6 mg) (N=41) imipramine (150 mg) (N=41) placebo (N=41)	N° of complete remitters according to PAS	Check list	Not reported	Not reported
Cross national collaborative panic study, 1992	1168	34	panic with/without agoraphobia	8	Alprazolam (6 mg) (N=386) imipramine (150 mg) (n=391) placebo (N=391)	N° of patients free of panic attack according to the Panic attack scale	Interview	3.4% alprazolam 3.1% placebo	17.4% Alprazolam 43.7% placebo
Beaudclair et al, 1994	32	34	panic with/ withoutagoraphobia	4	Clonazepam (0.5-5mg) (N=13) Placebo (N=16)	Mean of panic attack	NA	Not reported	Not reported
Ballenger at al, 1998	278	20-65	panic with/with outagoraphobia	10	Paroxetine (10-40 mg) (N= 209) Placebo (N=69)	Mean of panic attacks	NA	5.7% paroxetine 20 mg 11.6% placebo	32.9% paroxetine 20 mg 30.3% placebo
Asnins et al., 2001	188	19-64	panic with/with outagoraphobia	8	Fluvoxamine (50mg) (N=93) Placebo (N=95)	N° of patients free of panic attacks	Interview	10.2% fluvoxamine 17.2% placebo	33.1% fluvoxamine 31.5% placebo
Cassano et al., 1994	1.168	18-65	panic with/with outagoraphobia	8	Alprazolam (1mg) (N= 386) Imipramina (N=391) Placebo (N=391)	NA	Symptoms and Side Effects Checklist	3.4% alprazolam 3.1% placebo	17.4% alprazolam 43.8% placebo
Lecrubier et al, 1997	367	18-70	panic with/with outagoraphobia	12	Paroxetine (10-60 mg) (N=123) Placebo (N=123) Clomipramina (N= 121)	Mean of panic attacks	NA	7.4% paroxetine 6.7% placebo	27.9% paroxetine 35.6% placebo
Londborg et al, 1998	178	18.9-74.5	panic with/with outagoraphobia	12	Setraline (50-200mg) (N=127) Placebo (N=44)	Mean of panic attacks	NA	20% sertraline 4% placebo	37% sertraline 29% placebo
Michelson et al, 2001	180	36.5	panic	12	Fluoxetina (10-20mg) (N=90) Placebo (N=90)	N° of patients free of panic attacks	NA	5.5% fluoxetine 3.3% placebo	16.5% fluoxetine 11% placebo
Pohl et al, 1998	168	37.5	panic	12	Sertraline (50mg) (N=79) Placebo (N=87)	Mean of panic attacks	NA	9% sertraline 1% placebo	26% sertraline 17% placebo
Pollack et al, 1998	176	37.8 +- 11.6	panic with/with outagoraphobia	10	Setraline (25-200mg) (N=88) Placebo (N=88)	Mean of panic attacks	NA	8% sertraline 3% placebo	19% sertraline 17% placebo

Sandmann et al, 1998	46	31.9 +_6.7	panic with/with outagora phobia	6	Fluvoxamine (50-300mg) (N=23) Placebo (N=23)	N° of patients free of panic attacks	NA	4.3% fluvoxamine 2.1% placebo	13% fluvoxamine 10.8% placebo
Valença et al, 2000	24	18-55	panic with/with outagora phobia	6	Clonazepam (2mg)(N=13) Placebo (N=9)	N° of patients free of panic attacks	NA	Not reported	Not reported
Wade et al, 1997	475	18-65	panic with/with outagora phobia	8	Citalopram (10-60mg) (N=281) Placebo (N=96)	N° of patients free of panic attacks	NA	5% citalopram 20 mg 7% placebo	21% citalopram 20 mg 26% placebo
Hoehn-Saric et al, 1993	50	38.0 +- 9.6	panic	8	Flovoxamine (50-300mg) (N=25) Placebo (N=25)	Mean of panic attacks	NA	33.3% fluvoxamine 28.5% placebo	24% fluvoxamine 28% placebo
Lydiard et al, 1992	94	36.3 +-8.1	panic with/with outagora phobia	6	Alprazolam (2-6mg) (N=53) Placebo (N=26)	N° of patients free of panic attacks	NA	19.3% alprazolam 6 mg 3% placebo	28.4% alprazolam 6 mg 45.5% placebo
Noyes et al., 1988	525	37.1 +- 10.2	panic with/with outagora phobia	10	Alprazolam (1-10mg) (N=263) Placebo (N=262)	NA	Symptoms and Side Effects Inventory	4% alprazolam 2% placebo	16% alprazolam 50% placebo
Pecknold et al, 1994	209	18-65	panic with/with outagora phobia	6	Alprazolam CT ou XR (1 - 10mg) (N=70/70) Placebo (N=69)	N° of patients free of panic attacks	NA	0% XR alprazolam 1.4% placebo	14.3% XR alprazolam 17.4% placebo
Schweizer et al, 1993	194	35	panic with/with outagora phobia	8	Alprazolam (1-10mg) (N=102) Placebo (N=92)	Mean of panic attacks	NA	Not reported	Not reported
Sheik et al, 2000	322	39.61	panic with/with outagora phobia	12	Setraline (50-200mg) (N=240) Placebo (N=82)	Mean of panic attacks	NA	19% sertraline 5% placebo	Not reported
Stahl et al, 2003	366	18-80	panic with/with outagora phobia	10	Escitalopram (10-20mg) (N=128) Citalopram (10-20mg) (N=119) Placebo (N=119)	N° of subjects free of panic at post-treatment	NA	6.3% escitalopram 8.4% citalopram 7.6% placebo	Not reported

Abbreviations: AE, adverse events; NA, not available; PAS, Panic attack scale

Table 2. Quality score

Study	Random sequence generation	Allocation concealment	Blinding of participants	Blinding of outcome assessment	Incomplete outcome data	selective reporting	other bias
Andersch et al, 19991	? *	?	LR **	?	LR	LR	LR
Beauclair et al, 1994	?	LR	LR	LR	LR	LR	LR
Lydiard et al, 1991	?	?	LR	LR	LR	LR	LR
Noyes et al, 1988	?	LR	?	?	LR	?	LR
Pecknold et al, 1994	?	?	LR	?	LR	?	LR
Sheikh et al, 2000	?	?	LR	?	LR	?	LR
Lecrubier et al, 1997	?	LR	LR	LR	LR	LR	LR
Ballenger et al, 1998	?	?	LR	LR	LR	?	LR
Asnis et al, 2001	?	LR	LR	LR	LR	LR	LR
Londborg et al, 1998	LR	LR	LR	LR	LR	?	LR
Michelson et al, 2001	?	?	LR	?	LR	LR	LR
Pohl et al, 1998	?	LR	LR	LR	LR	LR	LR
Pollack et al, 1998	LR	LR	LR	LR	LR	LR	LR
Sandmann et al, 1998	?	?	LR	LR	?	LR	LR
Valença et al, 2000	?	?	LR	?	LR	LR	LR
Wade et al, 1997	?	?	LR	LR	?	LR	LR
Cross national collaborative panic study, 1992	?	LR	LR	LR	LR	?	LR
Hoehn-Saric et al, 1993	?	?	LR	?	LR	?	LR
Stahl et al, 2003	LR	LR	LR	LR	LR	LR	LR
Cassano et al, 1994	?	LR	LR	LR	LR	?	LR
Schweizer et al, 1993	?	LR	LR	LR	LR	LR	LR

* ?, not available.
**LR, low risk.

Table 3. Adverse events

Study	Cross national, 1991				Asnis et al, 2001				Ballenger et al, 1998				Cassano et al, 1984				Lecrubier et al, 1997			
Event	BZD treated group	N patients group	Placebo treated group	N control group	SSRI treated group	N patients group	placebo	N control group	SSRI treated group	N patients group	Placebo treated group	N control group	BZD treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patients group	Placebo treated group	N control group
diaphoresis	16	386	27,37	381													27	123	15	123
headache	42,46	386	43	381	18,6	93	20,9	95	65	209	30	69	43	386	43	381	20	123	22	123
fatigue	50,18	386	35,13	381	20,46	93	10,45	95	29	209	4	69	52	386	35	381	14	123	5	123
somnolence					22,32	93	6,44	95	53	209	12	69	112	386	43	381	9	123	7	123
nausea	23,16	386	23,46	381	40	93	31	95	56	209	18	69	23	386	23	381	24	123	18	123
xerostomia	42,46	386	27,37	381					39	209	6	69	43	386	27	381	25	123	17	123
Diarrhoea									31	209	3	69					13	123	5	123
constipation	46,32	386	19,55	381					17	209	5	69	46	386	20	381	10	123	10	123
Dizziness	23,16	386	35,19	381	12	93	10	95	25	209	11	69	23	386	35	381	13	123	11	123
nervouness	27	386	46,92	381	14,88	93	10,45	95	22	209	12	69	27	386	47	381				
Poor memory	54	386	19,55	381									54	386	19	381				
sexual dysfunction									14	209	0	69					16	123	1	123
insomnia	15,44	386	27,37	381	23	93	10,45	95	51	209	14	69	15	386	31	381	16	123	8	123
sedation	111,34	386	43	381																
tachycardia	11,58	386	23,46	381									12	386	23	381				
tremor	23,16	386	19,55	381	10,23	93	2,85	95	21	209	2	69	23	386	20	381	6	123	1	123

Study	Londborg et al, 1998				Michelson et al, 2001				Pohl et al, 1998				Pollack et al, 1998				Sandmann et al, 1998			
Event	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group
diaphoresis																				
headache	39,37	127	17,6	44	3	90	4	90					29	88	30	88				
fatigue	16	127	1,76	44									17	88	21	88				
somnolence	14	127	3	44									12	88	9	88	2	23	1	23
nausea	41,91	127	8,8	44	7	90	5	90	26	79	15	87	29	88	20	88	9	23	6	23
xerostomia	17,78	127	1	44					15	79	7	87	11	88	17	88				
Diarrhoea	19	127	4	44					19	79	10	87	24	88	9	88	1	23	3	23
constipation																				
Dizziness																				
nervouness	14	127	3	44													3	23	1	23
Poor memory																				
sexual dysfunction	24,13	127	0	44					17	79	0	87	4	88	1	88				
insomnia	27,94	127	7	44	3	90	2	90					30	88	19	88	3	23	1	23
sedation																				
tachycardia																				
tremor					4	90	0	90					7	88	0	88				

Table 4. Adverse events

Study	Stahl et al, 2013				Valenca et al, 2000				Wade et al, 1997				Andersch et al, 1991				Beauclair et al, 1994				Hoehn-Saric et al, 1993			
Event	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	BZD treated group	N patient group	Placebo treated group	N control group
diaphoresis									41	281	7	96	9,43	41	11	41								
headache									83	281	27	96	15,58	41	17,86	41	2	13	5	16	2	25	1	25
fatigue																								
somnolence					7	13	0	9																
nausea									79	281	17	96					1	13	1	16				
xerostomia									38	281	14	96	14	41	17	41					1	25	3	25
Diarrhoea																								
constipation									6	281	2	96	10	41	3	41								
Dizziness					3	13	2	9	23	281	6	96					9	13	0	16	5	25	0	25
nervousness																								
Poor memory	25,83	128	6	119	2	13	1	9									3	13	0	16				
sexual dysfunction					1	13	0	9	14	281	0	96					3	13	0	16				
insomnia									24	281	7	96												
sedation													34,85	41	17,48	41								
tachycardia	3	128	12	119																				
tremor									5	281	12	96												

Study	Lydiard et al, 1992				Noyes et al, 1988				Sheikh et al, 2000				Pecknold et al, 1994				Schweizer et al, 1993			
Event	BZD treated group	N patient group	Placebo treated group	N control group	BZD treated group	N patient group	Placebo treated group	N control group	SSRI treated group	N patient group	Placebo treated group	N control group	alprazolam CT	N patient group1	Placebo treated group	N control group	BZD treatment	N patient group	Placebo treatment	N placebo group
diaphoresis					5	263	15	262												
headache					31	263	26	262	58	240	20	82					34	102	36	92
fatigue	19	53	6	23	42	263	29	262	27	240	6,6	82	57	140	20,7	69				
somnolence									34	240	3	82								
nausea	6	53	8	23	15	263	34	262	66	240	13	82								
xerostomia	12	53	2	23	31	263	20	262	32	240	1	82	42	140	16	69				
Diarrhoea					21	263	23	262	38	240	4	82								
constipation	12	53	4	23	31	263	13	262												
Dizziness					18	263	23	262	27	240	4	82	115	140	33,81	69	30	102	36	92
nervousness					15	263	7	262									21	102	28	92
Poor memory	12	53	7	23	26	263	18	262					60	140	19	69				
sexual dysfunction	9	53	1	23	39	263	10	262	27	240	0	82								
insomnia	4	53	11	23	30	263	26	262	21	240	10	82								
sedation	24	53	6	23	89	263	10	262												
tachycardia	7	53	9	23	5	263	13	262					20	140	20,7	69				
tremor					7	263	18	262												