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**Papel do Receptor de Peptídeo
Opioide Nociceptina/Orfanina FQ
Através da Regulação dos Canais de
Cálcio Sensíveis à Voltagem em
neuropsicopatologias**

Universidade Federal de Ciências da Saúde
de Porto Alegre

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Orientadora: Dra. Eliane Dallegrave

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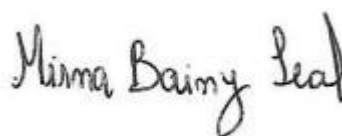
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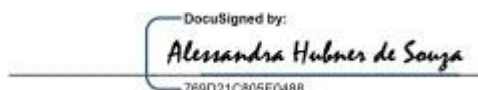
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RESUMO

Os receptores de nociceptina (NOP) são ativados pelo ligante nociceptina/orfanina FQ (N/OFQ). Estes receptores acoplados à proteína G (GPCR) interagem com canais de cálcio sensíveis à voltagem (CCSVs) e canais de potássio retificadores internos acoplados à proteína G (GIRK). Isso causa redução nas correntes de cálcio, desencadeando alterações nos níveis pré-sinápticos, como diminuição na liberação de neurotransmissores, e pós-sinápticos como diminuição da excitabilidade neuronal. Embora tanto o receptor NOP quanto N/OFQ tenham semelhança estrutural e funcional com os outros receptores opioides e seus ligantes endógenos, o receptor NOP não se liga a ligantes opioides clássicos, ao passo que N/OFQ não se liga aos outros receptores opioides, tornando o sistema receptor-ligante NOP-N/OFQ importante em vários aspectos patofisiológicos. Uma vez que CCSVs são importantes reguladores de atividade neuronal e devido aos padrões de expressão generalizados de NOP e CCSVs em múltiplas regiões do cérebro, corno dorsal da medula espinhal e no gânglio da raiz dorsal, eles estão envolvidos em processos centrais. Portanto, esta revisão se concentrará nas descobertas atuais relacionadas ao papel do sistema NOP-N/OFQ e a regulação dos CCSVs em condições fisiopatológicas como ansiedade, respostas ao estresse, depressão e humor, sistema de recompensa, aprendizado, memória, dor e controle motor. Por ser uma revisão narrativa, não é intencionado recapitular a extensa literatura sobre farmacologia do sistema NOP-N/OFQ, para isso, o leitor é encaminhado para várias excelentes análises aprofundadas publicadas recentemente. A ativação de NOP e interação com CCSVs mostra efeitos benéficos no tratamento da ansiedade, respostas ao estresse agudo, e sistema de recompensa, o qual está relacionado ao abuso de alimentos ou de substâncias como drogas (álcool, nicotina, opioides, estimulantes). Por outro lado, agonismo do receptor pode prejudicar funções como humor (depressão, agressividade), aprendizado, memória e controle motor. Ainda, os dados são controversos no que diz respeito à terapêutica da dor. Em suma, pode-se perceber inúmeros pontos sem esclarecimento nas abordagens pré-clínicas dos estudos avaliados aqui, como dados comportamentais sem aprofundamento ou papel dos CCSVs no antagonismo do receptor. Portanto, isto representa um potencial campo de pesquisa em direção a novas terapêuticas com alvo em NOP e CCSVs.

Palavras-chave: Ansiedade. Aprendizado. Comprometimento motor. ORL-1. Receptor opioide. Receptor acoplado à proteína G.

ABSTRACT

Nociceptin receptors (NOP) are activated by the nociceptin/orphanine FQ ligand (N/OFQ). These G protein-coupled receptors (GPCRs) interact with voltage-sensitive calcium channels (VGCCs) and G protein-coupled internal rectifier potassium channels (GIRK). This causes a reduction in calcium currents, triggering changes in presynaptic levels, such as decreased neurotransmitter release, and postsynaptic, such as decreased neuronal excitability. Although both the NOP and N/OFQ receptors have structural and functional similarity to other opioid receptors and their endogenous ligands, the NOP receptor does not bind to classical opioid ligands, whereas N/OFQ does not bind to other opioid receptors, making the NOP-N/OFQ receptor-ligand system important in several pathophysiological aspects. Since VGCCs are important regulators of neuronal activity and because of the widespread expression patterns of NOP and VGCCs in multiple brain regions, spinal cord dorsal horn and dorsal root ganglion, they are involved in central processes. Therefore, this review will focus on current findings related to the role of the NOP-N/OFQ system and the regulation of VGCCs in pathophysiological conditions such as anxiety, stress responses, depression and mood, reward system, learning, memory, pain and motor control. As this is a narrative review, it is not intended to recap the extensive literature on pharmacology of the NOP-N/OFQ system, for which the reader is referred to several excellent in-depth reviews published recently. Activation of NOP and interaction with VGCCs shows beneficial effects in the treatment of anxiety, acute stress responses, and the reward system, which is related to food or substance abuse such as drugs (alcohol, nicotine, opioids, stimulants). On the other hand, receptor agonism can impair functions such as mood (depression, aggression), learning, memory and motor control. Still, the data are controversial with regard to pain therapy. In short, it is possible to perceive numerous points without clarification in the preclinical approaches of the studies evaluated here, such as behavioral data without depth or role of VGCCs in receptor antagonism. Therefore, this represents a potential field of research towards new therapies targeting NOP and VGCCs.

Keywords: Anxiety. Learning. Motor impairment. Opioid receptor. ORL-1. G-protein-coupled receptor.

LISTA DE ABREVIATURAS

ACTH	hormônio adrenocorticotrofina
CCSVs	canais de Ca ²⁺ sensíveis à voltagem
CRH	hormônio corticotrofina
D1	receptores de dopamina tipo 1
D2	receptores de dopamina tipo 2
DA	dopamina
Dacc	córtex cingulado anterior em sua porção dorsal
DOR	receptor de peptídeo opioide δ
DP	doença de Parkinson
Erk1/2	quinases extracelulares reguladas por sinal 1 e 2
GABA	ácido γ -aminobutírico
GIRK	canais de K ⁺ de retificação interna ativados pela proteína G
GPCR	receptores acoplados à proteína G
Gpi	globus pallidus pars interna
GRK	receptor por quinases acoplados à proteína G
HPA	eixo hipotálamo-hipófise-adrenal
HVA	canais ativados por alta voltagem
JNK1-3	quinase c-Jun N-terminal
KOR	receptor de peptídeo opioide κ
LVA	canais ativados por baixa voltagem
MAPK	mitogen-activated protein kinases
MOR	receptor de peptídeo opioide μ
mPFC	córtex pré-frontal medial
N/OFQ	nociceptina/orfanina FQ
NAc	núcleo acumbens
NOP	receptor de peptídeo opioide nociceptina
p38	quinase de estresse
PAG	substância cinzenta periaquedutal
PLC β	fosfolipase C β
ppN/OFQ	prepronociceptina
RVM	medula rostral ventral
SNC	sistema nervoso central

SNc	substância nigra compacta
SNr	substância nigra pars reticulata
TEPT	transtorno de estresse pós-traumático
VTA	área tegmental ventral

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1 INTRODUÇÃO

1.1 RECEPTORES OPIOIDES

O potencial terapêutico de um medicamento se traduz de insights aprofundados desde a descoberta até o desenvolvimento inicial no campo clínico. Os ligantes que ativam o receptor de peptídeo opioide nociceptina (NOP) constituem uma classe de drogas candidatas para aplicações frente à diversas condições, embora em muitas destas a base farmacológica envolvida não esteja bem elucidada. Portanto, é válido destacar os principais mecanismos de ação por trás dos efeitos que este receptor induz em condições estudadas e descritas até o presente momento no intuito de compilar dados e direcionar pesquisas futuras.

Os receptores opioides pertencem à grande superfamília de sete receptores acoplados à proteína G transmembrana (GPCRs), conforme Waldhoer *et al.* (2004). Até o momento, quatro sistemas diferentes de receptores opioides são conhecidos: MOR (μ), DOR (δ), KOR (κ), e de nociceptina (NOP) e seus genes foram caracterizados nos níveis celular, molecular e farmacológico (BODNAR, 2017).

No entanto, existe uma disparidade entre a existência de apenas quatro tipos de receptores opioides e o fato de que muitos ligantes agem promiscuamente nos receptores peptídicos MOP, DOP e KOP, sendo que existem ligantes seletivos para cada receptor (WALDHOER *et al.*, 2004).

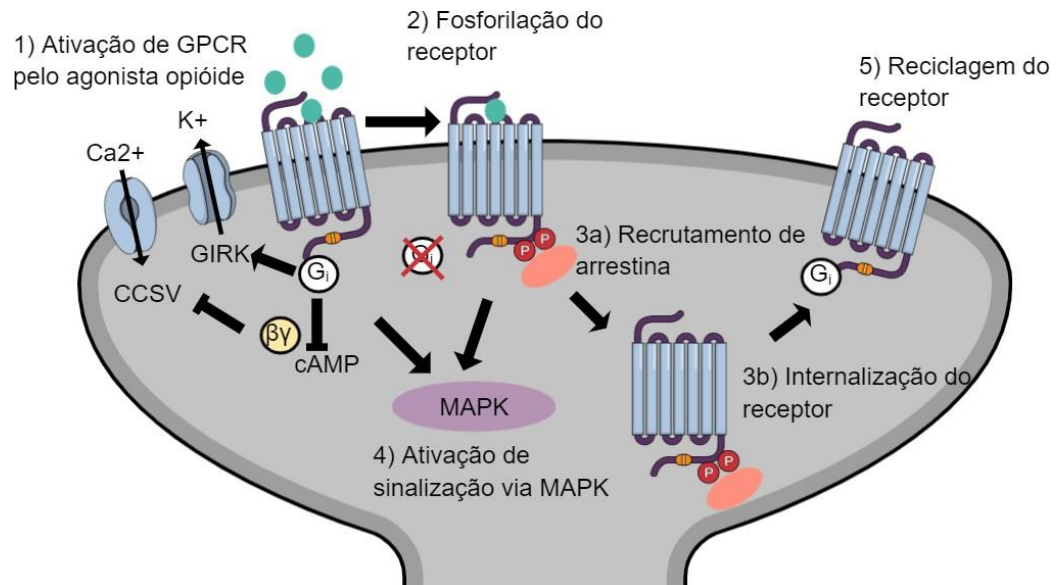
No geral, estes receptores são predominantemente acoplados a proteínas Gi/Go heterotriméricas sensíveis à toxina *pertussis*, segundo Crain e Shen (1990) e após a ativação do receptor, as subunidades α e $\beta\gamma$ da proteína G interagem com vários sistemas efetores celulares, inibindo adenilil ciclases e canais de Ca^{2+} dependentes de voltagem (CCSVs) e estimulando os canais de K^{+} de retificação interna ativados pela proteína G (GIRKs) e fosfolipase $\text{C}\beta$ ($\text{PLC}\beta$) (Figura 1:1-2) (WALDHOER *et al.*, 2004).

Uma das vias de sinalização ativada pela GPCRs acoplada aos receptores opioides é a *mitogen-activated protein kinases* (MAPK), a qual inclui quinases extracelulares reguladas por sinal 1 e 2 (ERK1/2), JNK1-3 e p38 (α , β , γ , δ) quinase de estresse (Figura 1:4) (LEFKOWITZ; SHENOY, 2005; RAMAN *et al.*, 2005).

Já a regulação dos receptores opioides é rapidamente controlada por uma cascata de eventos envolvendo a fosforilação do receptor por quinases acoplados à proteína G (GRKs) e subsequente recrutamento de β -arrestina (HASBI *et al.*, 1998; LAW *et al.*, 2000; GUO *et al.*, 2000; PEI *et al.*, 1995).

Assim, a fosforilação por GRK leva ao recrutamento de arrestina, a qual é necessária para internalização, regulação e reciclagem do receptor (Figura 1: 3-5).

Figura 1- Transdução e tráfico do sinal do receptor opioide. Em geral, todos os quatro subtipos de receptores opioides, μ , δ , κ e NOP compartilham essas vias comuns. As setas referem-se às etapas de ativação; as linhas T referem-se ao bloqueio ou inibição da função.



Fonte: Adaptado de Al-Hasani (2011) por Caminski (2022) (não publicado).

Os receptores opioides são implicados em uma série de efeitos comportamentais, incluindo: analgesia, recompensa, depressão, ansiedade e dependência. Portanto, compreender a diversidade de sinalização nos receptores opioides e como a ativação do segundo mensageiro leva à modulação de efeitos pode revelar novos candidatos a medicamentos uma vez que peptídeos opioides e seus receptores são expressos em diversas regiões do sistema nervoso central (SNC) (AL-HASANI et al., 2011).

1.2 N/OFQ: NOCICEPTINA/ORFANINA FQ

Em 1994, foi descoberto um receptor com perfil farmacológico único, agora referido como NOP. Seu neuropeptídeo ligante endógeno, composto por 17 resíduos, foi identificado independentemente por dois grupos em 1995, e denominado nociceptina e orfanina FQ, mas atualmente é referido como N/OFQ. O N/OFQ compartilha homologia de sequência com os outros peptídeos opioides e se origina de uma molécula precursora maior, chamada

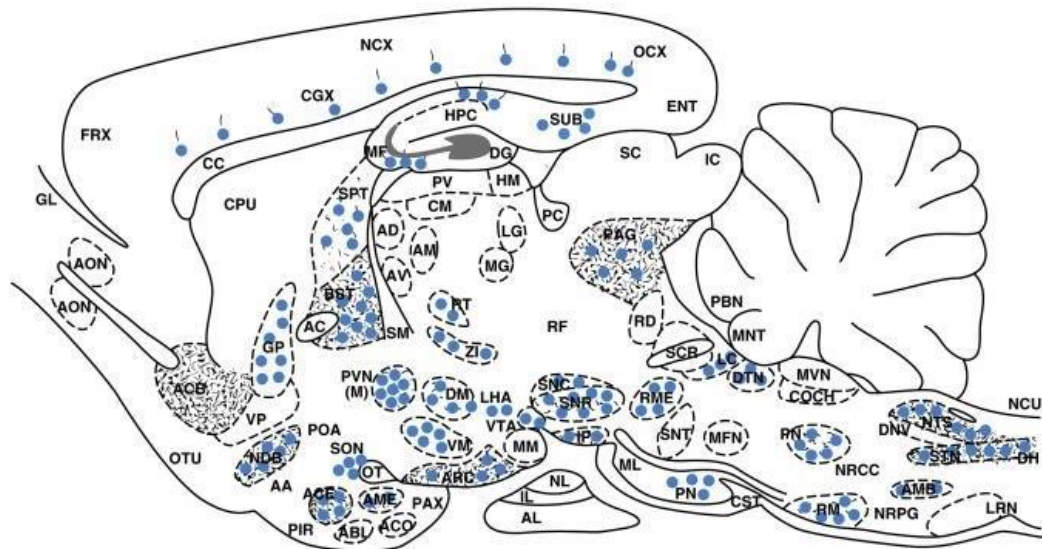
prepronociceptina (ppN/OFQ). A principal diferença entre N/OFQ e ligantes opioides clássicos é a presença de Phe no terminal N, em oposição a Tyr na primeira posição de todos os outros peptídeos opioides endógenos e da maioria de seus análogos sintéticos. A falta de Tyr¹, considerado um importante grupo farmacofórico necessário para a ligação de ligantes aos receptores opioides clássicos, pode ser, pelo menos em parte, responsável pela diferente afinidade do receptor de N/OFQ (WTOREK; JANECKA, 2021).

O NOP está presente em muitas áreas do cérebro e na medula espinhal, conforme Berthele et al., (2003) (Figura 2), mas também em tecidos periféricos e no sistema imunológico, como relatam Mollereau e Mouledous (2000). No sistema nervoso central, a ativação de NOP mostrou inibir a liberação de neurotransmissores como GABA, substância P e noradrenalina (SCHIKLER, MORARI, 2000).

Consistente com sua localização no SNC, a desregulação do sistema NOP-N/OFQ é observada em vários estados patológicos, como dor neuropática e inflamatória, doença de Parkinson, ansiedade e depressão, abuso de drogas (WTOREK; JANECKA, 2021).

No entanto, apesar de haver semelhanças deste receptor com os outros três receptores opioides em termos de estrutura e mecanismos de sinalização, o perfil farmacológico do NOP é bastante singular. A sinalização via NOP medeia eventos intracelulares semelhantes aos provocados por MOR, DOR e KOR, pois todos os receptores opioides acoplam-se às proteínas Gi/Go e inibem a atividade da adenilato ciclase e os CCSVs (TOLL et al., 2016).

Figura 2- Distribuição do peptídeo N/OFQ no SNC de ratos determinado por imunohistoquímica e hibridização *in situ*. Os pericários neuronais são mostrados como círculos sólidos e os terminais das fibras são mostrados como linhas curvas curtas e pontos.



AA, amígdala anterior;
 ABL, núcleo basolateral da amígdala;
 AC, comissura anterior;
 ACB, núcleo accumbens;
 ACE, núcleo central da amígdala;
 ACO, núcleo cortical da amígdala;
 AD, núcleo anterodorsal do tálamo;
 AL, lobo anterior da pituitária;
 AM, núcleo anteromedial do tálamo;
 AMB, núcleo ambíguo;
 AME, núcleo medial da amígdala;
 AON, núcleo olfatório anterior;
 ARC, núcleo arcuado;
 AV, núcleo anteroventral do tálamo;
 BST, núcleo leito da estria terminal;
 CC, corpo caloso;
 CGX, córtex cingulado;
 CM, núcleo centro-medial do tálamo;
 COCH, 14complex nuclear coclear;
 CPU, caudado-putâmen;
 CST, trato corticospinal;
 DH, corno dorsal da medula espinhal;
 DG, giro dentado;
 DM, núcleo dorsomedial do hipotálamo;
 DNV, núcleo dorsal motor do vago;
 DTN, núcleo dorsal tegmentar;
 ENT, córtex entorhinal;
 FN, núcleo fastigial do cerebelo;
 FRX, córtex frontal;
 GL, lâmina glomerular do bulbo olfatório;

GP, globo pálido;
 HM, núcleo medial habenular;
 HPC, hipocampo;
 IC, colículo inferior;
 IL, lobo intermediário da pituitária;
 IP, complexo do núcleo interpeduncular;
 LC, núcleo do loco coruleo;
 LG, núcleo lateral geniculado;
 LHA, área hipotalâmica lateral;
 LRN, núcleo lateral reticular;
 ME, fibras mossy do hipocampo;
 MFN, núcleo motor facial;
 MG, núcleo medial geniculado;
 ML, lemnisco medial;
 MM, núcleo medial mamilar;
 MNT, núcleo mesencefálico do trigêmeo;
 MVN, núcleo medial vestibular;
 NCU, núcleo cuneado;
 NCX, neocórtex;
 NDB, núcleo da banda diagonal;
 NL, lobo neural da pituitária;
 NRG, núcleo reticularis gigantocellularis;
 NRPG, núcleo reticularis paragigantocellularis;
 NTS, núcleo do trato solitário;
 OCX, córtex occipital;
 OT, trato óptico;
 OTU, tubérculo olfatório;
 PAG, área periaqueductal cinza;

PAX, córtex periamidalóide;
 PBN, núcleo parabraquial;
 PC, comissura posterior;
 PIR, córtex piriforme;
 PN, pons;
 POA, área pré-óptica;
 PP, perforant path;
 PV, núcleo periventricular do tálamo;
 PVN(M), núcleo paraventricular (pars magnocellularis);
 PVN(P), núcleo paraventricular (pars parvocellularis);
 RD, núcleo dorsal da raphe;
 RE, núcleo reunions do tálamo;
 RF, formação reticular;
 RM, núcleo magnus da raphe;
 RME, núcleo mediano da raphe;
 SC, colículo superior;
 SCP, pedúnculo cerebelar superior;
 SM, stria medular do tálamo;
 SNC, substância nigra (pars compacta);
 SNR, substância nigra (pars reticulata);
 SNT, núcleo sensorial do trigêmeo;
 SON, núcleo supra-óptico;
 SPT, núcleo septal;
 STN, núcleo espinal do trigêmeo;
 SUB, subiculum;
 VM, núcleo ventromedial do hipotálamo;
 VP, ventral pallidum;
 ZI, zona incerta.

Fonte: Adaptado de Koob et al., (2014).

1.3 CANAIS DE CÁLCIO SENSÍVEIS À VOLTAGEM

Os CCSVs são uma importante fonte de influxo de Ca^{2+} e têm um papel crítico na sinalização em todas as células. O sistema nervoso expressa vários tipos diferentes de CCSVs, cada um com distribuições celulares e subcelulares únicas e funções fisiológicas específicas (BENARROCH, 2010).

Estes canais foram classificados em 2 categorias principais com base em seu limiar de ativação por despolarização, canais ativados por alta voltagem (HVA) e canais ativados por baixa voltagem (LVA) (Tabela 1). Os canais HVA podem ser subdivididos, com base nas características farmacológicas e biofísicas, em tipos L-, N-, P/Q- e R (CATERRALL, 2000; DAI et al., 2009).

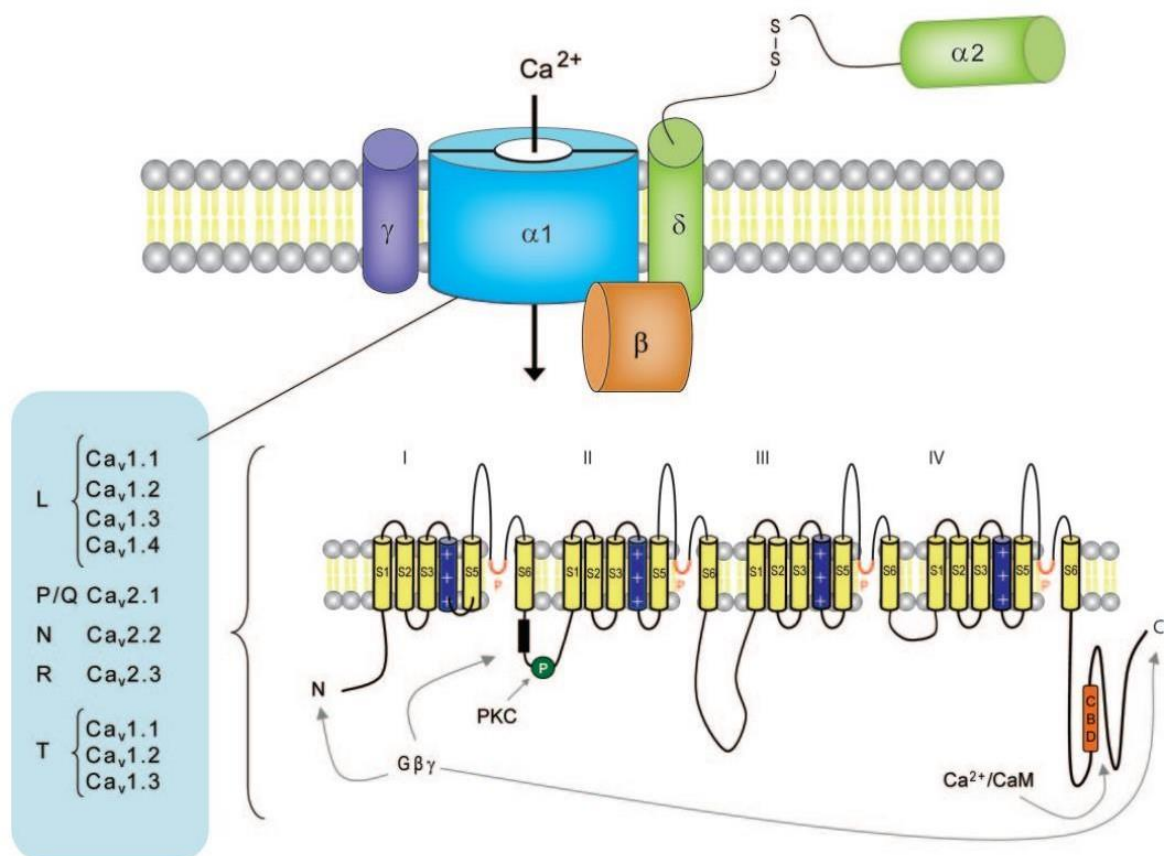
Tabela 1- Principais características dos CCSVs

Tipo fisiológico	Tipo do canal	Subunidade $\alpha 1$	Distribuição	Função
HVA	L	Cav1.1	Músculo esquelético	Excitação-contração
		Cav1.2	Dendritos e corpos celulares no SNC	Plasticidade
		Cav1.3	Cóclea	Transdução sensorial
			Neurônios espinhosos médios do estriado	Disparos em resposta a sinais corticais
			Substância nigra compacta	Atividade marcapasso
		Cav1.4	Retina	Transdução sensorial
	P/Q	Cav2.1	Terminais pré-sinápticos	Liberação de neurotransmissores
			Células de Purkinje	Despolarização
			Tálamo	Disparos tônicos
	N	Cav2.2	Terminais pré-sinápticos	Liberação de neurotransmissores
			Neurônios nociceptivos do DRG	Despolarização
	R	Cav2.3	Terminais pré-sinápticos	Liberação de neurotransmissores
			Hipocampo	Despolarização
LVA	T	Cav3.1-3.3	Dendritos e corpos celulares do tálamo	Atividade marcapasso
			Outros neurônios do SNC	Disparos rítmicos

Fonte: Catterall (2000).

Os canais HVA do Cav1 (tipo L) e as famílias Cav2 (tipos P/Q-, N- e R) são heteromultímeros formados pela associação da subunidade 1α com 3 subunidades auxiliares β , $\alpha 2\delta$ e γ (Figura 3). Já os canais LVA (Cav3, tipo T) parecem ter apenas 1α subunidade, mas isso ainda é controverso. Estudos *in vitro* ou em camundongos *knockout* forneceram informações sobre a função dos diferentes tipos de CCSVs, sendo que os canais do tipo L regulam a excitabilidade neuronal e a expressão gênica; os canais P/Q e N desencadeiam a liberação de neurotransmissores, e os canais do tipo T suportam disparos de explosão rítmica neuronal (Benarroch, 2010).

Figura 3- Estrutura geral de CCSVs. As estruturas das subunidades dos canais são ilustradas como modelos de dobras transmembranares; as hélices α são representadas como cilindros. As regiões ligantes citoplasmáticas da subunidade $\alpha 1$ contêm sítios que são substratos para fosforilação pela proteína quinase C (PKC) e outras quinases, e interação com proteínas reguladoras, como a subunidade $\beta\gamma$ das proteínas de ligação ao nucleotídeo de guanina (G) i/o ($G\beta\gamma$). A região intracelular carboxil (C)-terminal contém um sítio de ligação para calmodulina (CaM), que medeia a inativação desencadeada por Ca^{2+} do canal após despolarização prolongada da membrana.



Fonte: Benarroch (2010).

Evidências de mutantes naturais, camundongos knockout e distúrbios genéticos humanos indicam um papel fundamental de alguns CCSVs em uma ampla variedade de distúrbios neurológicos (BENARROCH, 2010).

Estes canais são importantes reguladores das funções cerebrais, cardíacas e musculares, e sua disfunção pode dar origem a condições fisiopatológicas que variam de distúrbios cardiovasculares a condições neurológicas e psiquiátricas, como epilepsia, dor e autismo. No sistema nervoso, os bloqueadores de CCSVs têm sido usados com sucesso no tratamento de crises de ausência e estão surgindo como potenciais vias terapêuticas para patologias como dor, doença de Parkinson, dependência e ansiedade (ZAMPONI et al., 2016; ANDRADE et al., 2019).

1.4 STRESS, ANSIEDADE E DEPRESSÃO

Os transtornos de ansiedade são marcados pelo medo excessivo (e evitação), muitas vezes em resposta a objetos ou situações específicas e na ausência de perigo real. Acima de tudo, são um problema significativo na clínica, e pesquisas recentes sobre os neurocircuitos dos transtornos de ansiedade tem suas raízes no estudo dos circuitos do medo em modelos animais e no estudo das respostas cerebrais a estímulos emocionais em humanos saudáveis (SHIN; LIBERZON, 2010).

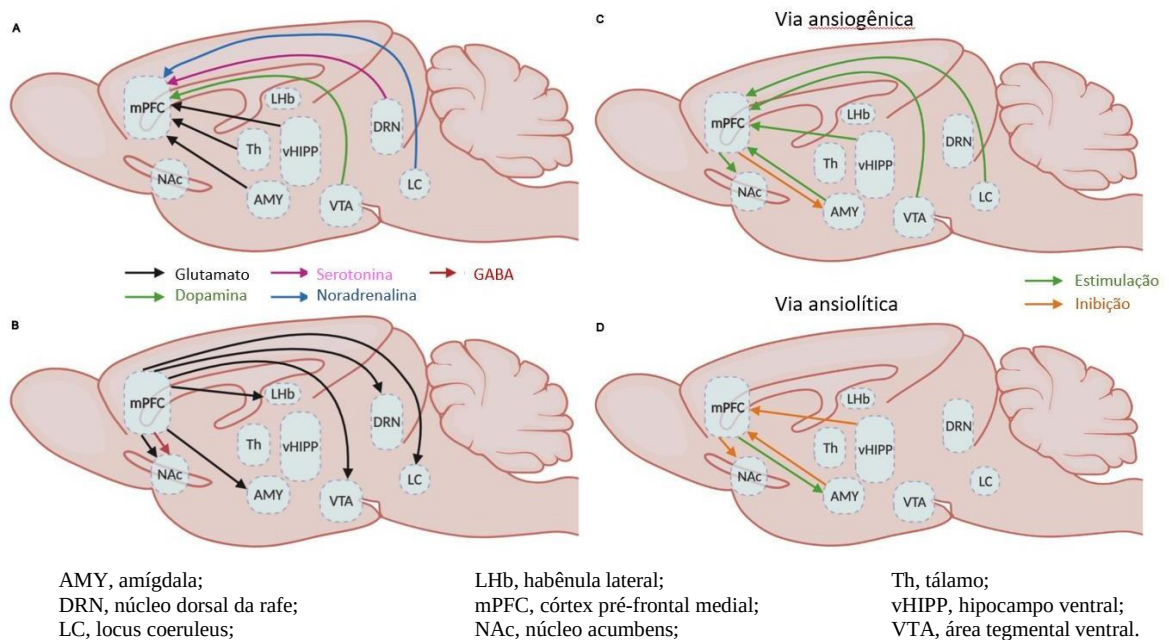
Componentes-chave do circuito do medo, incluindo a amígdala (e seus subnúcleos), núcleo acumbens (incluindo núcleo do leito da estria terminal BNST), hipocampo, hipotálamo ventromedial, substância cinzenta periaquedutal, vários núcleos do tronco cerebral, núcleos talâmicos, córtex insular e alguns núcleos pré-frontais regiões (principalmente córtex infralímbico) foram identificadas nestes estudos (DAVIS, 2006).

Especificamente, o tálamo integra *input* sensorial dos córtices sensoriais primários e envia *output* para a amígdala. A amígdala e o córtex cingulado anterior dorsal (dACC) processam sinais aversivos e enviam saídas para o hipotálamo, gânglios da base e tronco cerebral para produzir comportamentos defensivos. O hipocampo é responsável por codificar informações contextuais associadas à sugestão de ameaça. Dessa forma, o hipocampo tem sido implicado na extinção do medo, desempenhando um papel na regulação negativa da resposta da amígdala em contextos seguros.

O córtex pré-frontal medial (mPFC) fornece controle regulatório da resposta ao medo, recebendo informações do hipocampo e do tálamo e projetando-se para a amígdala para

modular o comportamento do medo com base em informações ambientais complexas, de acordo com Maren (2001), Lee et al., (2013) e Duval et al., (2015) (Figura 4). Esses componentes básicos do circuito do medo estão bem preservados em todas as espécies e provavelmente suportam funções semelhantes em humanos (SHIN; LIBERZON, 2010).

Figura 4- *Inputs e outputs* do córtex pré-frontal medial. Representação esquemática das projeções (A) aferente e (B) eferente com seus respectivos sistemas neurotransmissores, bem como circuitos envolvidos na expressão de (C) respostas comportamentais ansiogênicas ou (D) ansiolíticas.



Fonte: Adaptado de Bittar e Labonté (2021).

Embora o processamento de informações relacionadas à ansiedade envolva uma ampla gama de regiões cerebrais, uma estrutura-chave nessa rede é a amígdala, a qual é percebida com hiperexcitabilidade. A neurotransmissão inibitória desempenha um papel fundamental nos transtornos de ansiedade, como evidenciado pelo efeito ansiolítico da classe benzodiazepina dos agonistas dos receptores do ácido γ -aminobutírico (GABA).

Assim, um interesse substancial se concentrou em entender como os neurônios inibitórios e as sinapses contribuem para os circuitos subjacentes aos comportamentos de ansiedade adaptativos e patológicos. O processamento de informações dentro da amígdala é fortemente dependente do controle inibitório, embora os mecanismos específicos pelos quais os neurônios GABAérgicos da amígdala e as sinapses regulam os comportamentos

relacionados à ansiedade estejam apenas começando a ser descobertos (BABAEV et al., 2018).

O transtorno de estresse pós-traumático (TEPT) ocorre após a exposição a eventos traumáticos e é caracterizado por medo e ansiedade avassaladores. Seus modelos de neurocircuitos focam no papel central da amígdala em direcionar a resposta de um indivíduo ao perigo percebido por meio de suas conexões recíprocas com o hipocampo, mPFC e outras áreas corticais associadas a funções cognitivas superiores. Este modelo enfatiza a falta de controle inibitório adequado sobre a amígdala pelo mPFC (especificamente, a porção rostral do córtex cingulado anterior e o hipocampo (KENT; RAUCH, 2004; DAVIU et al., 2019).

Ademais, vê-se diminuição da transmissão serotoninérgica e dopaminérgica e aumento noradrenérgico de acordo com estudos desenvolvidos por Sessa (2017) e Blum et al., (2019). Uma vez que estresse e ansiedade entrelaçaram bases comportamentais e neurais, essas semelhanças são críticas para a compreensão de cada estado, bem como suas interações mútuas.

Os distúrbios no eixo hipotálamo-hipófise-adrenal (HPA) estão envolvidos na patogênese dos transtornos de humor, incluindo ansiedade, TEPT e transtornos depressivos maiores, os quais são apresentados também como comorbidades. Estudos demonstraram a relação entre a resposta do eixo HPA e a vulnerabilidade ao estresse, indicando que o eixo HPA regula o sistema imunológico, a memória do medo e a neurotransmissão (CAI et al., 2022; LIN; LIU, 2022).

A resposta ao estresse inicia no hipotálamo onde neurônios paraventriculares secretam o hormônio corticotrofina (CRH). Este hormônio estimula a liberação do hormônio adrenocorticotrofina (ACTH) pela pituitária, o qual por sua vez estimula a liberação de glucocorticóides pelo córtex da adrenal (por exemplo, cortisol em humanos e corticosterona em animais). Glucocorticóides exercem um feedback negativo no controle do HPA e reduz a resposta noradrenérgica ao stress. O hipocampo e mPFC tem um controle inibitório no efeito do HPA, enquanto amígdala e neurônios aminérgicos estimular o eixo (REGE, 2019).

No TEPT há uma desregulação da sinalização com um feedback negativo sensibilizado do eixo HPA resultando em aumento do CRH e atenuadas respostas a ACTH o qual resulta em reduzida secreção de cortisol (YEHUDA et al., 2015).

Os modelos de depressão atuais recapitularam com sucesso estados de retraimento social, hipofagia e perda de peso, anedonia, alterações circadianas e anormalidades do eixo HPA, embora esses fenótipos sejam frequentemente transitórios e nem todos presentes simultaneamente. A validade de construto mais desafiadora requer um modelo que honre os

fatores etiológicos implicados na depressão, que não são totalmente compreendidos. A maioria dos paradigmas utiliza alguma forma de estresse (de forma física ou psicossocial), dada a conhecida associação entre eventos estressantes independentes da vida e episódios depressivos (KRISHNAN; NESTLER, 2010).

Apesar de décadas de pesquisa, a base neural para a depressão ainda é incompletamente compreendida. Embora muitas regiões do cérebro tenham sido repetidamente implicadas na fisiopatologia da depressão, no entanto, a estimulação de uma variedade de regiões e circuitos cerebrais foi relatada como sendo eficaz na depressão (PANDYA et al., 2012).

Estudos clínicos de indivíduos com transtornos de humor implicaram as redes formadas pelo OMPFC (orbital e PFCm) e áreas anatomicamente relacionadas do estriado, tálamo, córtex temporal e sistema límbico na fisiopatologia da depressão, com base em evidências convergentes de neuroimagem e neuropatologia (PRICE; DEVETS, 2010).

1.5 RECOMPENSA E MEMÓRIA

O sistema de recompensa dirige nosso comportamento para estímulos prazerosos e nos afasta dos dolorosos que exigem mais energia ou esforço. O sistema de recompensa é onde sentimos emoções e as processamos para iniciar ou parar a ação. Aqui, neurônios usam dopamina para se comunicar e sua principal fonte no sistema é produzida na VTA, o qual se projeta para NAc, o centro de recompensa (YAN et al., 2022).

Os neurônios que liberam dopamina são ativados quando esperamos receber uma recompensa. A sinalização pelo neurotransmissor em áreas do cérebro que processam emoções – a amígdala – e regiões envolvidas no planejamento e raciocínio – o PFC – também cria associações emocionais com recompensas (HALBER, 2018).

Para isso, existem duas vias que são particularmente relevantes: a via mesocortical e a via mesolímbica. A via mesocortical projeta-se para múltiplas áreas corticais e é importante para muitos aspectos do processamento de recompensa, incluindo a avaliação hedônica, avaliação comparativa e avaliação de opções. Essa via se projeta principalmente para o PFC, cíngulo e entorrinal em roedores, enquanto a via mesolímbica projeta-se principalmente para o estriado ventral, mas também para outras áreas límbicas como por exemplo a amígdala, tubérculo olfativo e septo (BJORKLUND; DUNNET, 2007).

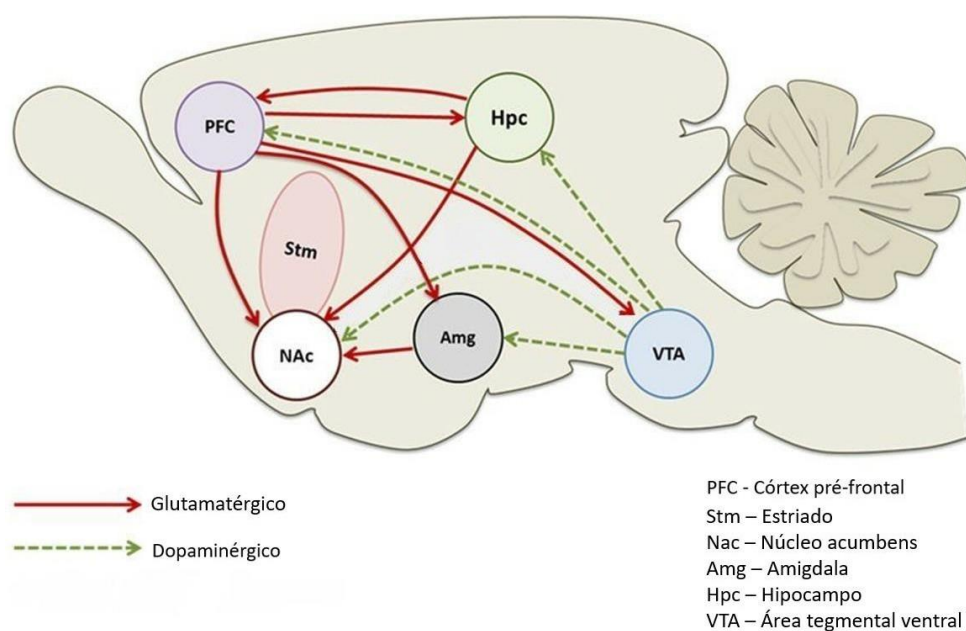
Acredita-se que anormalidades no sistema de recompensa desempenhem um papel em muitos transtornos psiquiátricos, como por exemplo, abuso de substâncias, e aqui podemos

incluir alimentação, conforme Joshi et al., (2022) e o uso de drogas viciantes, que promovem o reforço aumentando a dopamina no sistema mesocorticolímbico, o que altera a transmissão excitatória de glutamato dentro do circuito de recompensa (ALLCHION et al., 2021).

O sistema mesolímbico dopaminérgico é também ativado por todas as principais drogas de abuso e alimentos palatáveis, sendo o estriado ventral uma estrutura chave. Em estudos com animais, a estimulação cerebral necessária para recompensa (limiar de recompensa) é reduzida pela administração aguda destas drogas. Desse modo, se analisa que a ativação no estriado ventral seja importante no estágio de compulsão/intoxicação por recompensa, com envolvimento do estriado dorsal para a formação de hábito que se acredita estar subjacente à progressão para o uso compulsivo (TABER et al., 2012).

Taber et al., (2012) ressalta que a dopamina também melhora as memórias relacionadas à recompensa e fortalece as sinapses no centro de aprendizado e memória do cérebro, o hipocampo. No circuito de recompensa, o hipocampo é responsável por memórias contextuais e pistas associadas, o que sustenta o restabelecimento de comportamentos de busca de recompensa por meio de dicas e gatilhos contextuais. A amígdala (Figura 5) a qual recebe projeções da VTA e envia para o NAc, também cria poderosas memórias de flashes emocionais e provavelmente sustenta a criação de fortes memórias associadas às pistas (KUTLU; GOULD, 2016; MC GAUGH, 2014).

Figura 5- Diagrama esquemático dos principais locais cerebrais do sistema de recompensa e suas conexões dopaminérgicas e glutamatérgicas.



Fonte: Adaptado de Bakhtazad et al., (2020).

1.6 DOR

Quando os neurônios sensoriais da dor, chamados nociceptores, detectam um estímulo potencialmente prejudicial, esses neurônios enviam o sinal como potencial de ação para outros neurônios utilizando os neurotransmissores excitatórios glutamato, que despolariza a célula, e a substância P, que promove inflamação e dor (YAM et al., 2018).

Uma vez que o sinal atinge a base do corno dorsal, uma coluna na medula espinhal que transmite informações sensoriais, ele é então enviado através de uma rede de neurônios chamada trato espinotalâmico que fornece sinais sensoriais da medula espinhal para o córtex somatossensorial que percebe a dor (GOLDBERG, 2013) (Figura 6a).

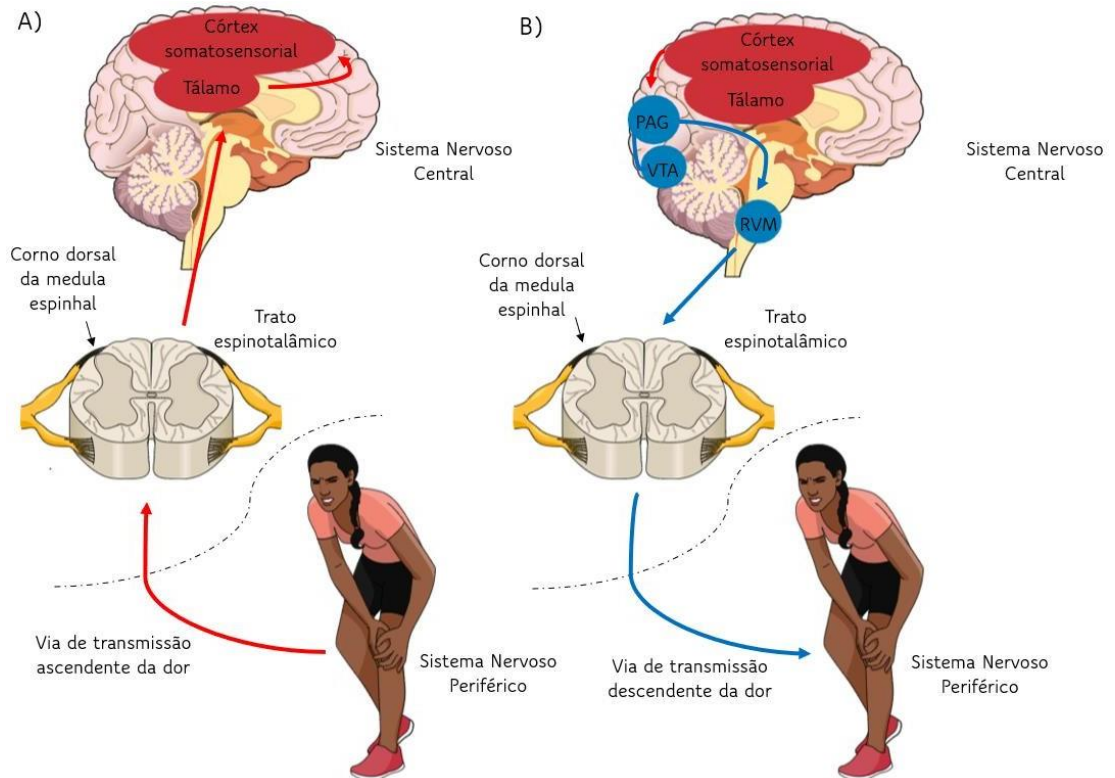
Esse processo, desde a detecção e transmissão do sinal pelo sistema nervoso periférico até a recepção e percepção no sistema nervoso central, é a principal função da via ascendente de transmissão da dor. Em resumo, o caminho ascendente é como sentimos a dor. Uma vez que um sinal de dor da via ascendente atinge o córtex somatossensorial, ele aciona o sistema modulador de dor descendente. O objetivo é permitir que o organismo funcione o suficiente para responder à fonte de dor.

As vias de controle descendentes se originam nas regiões do mesencéfalo e do tronco cerebral e se projetam para o corno dorsal da medula espinhal. Eles representam um mecanismo através do qual o sinal de dor transmitido pode ser facilitado – isso aumenta a dor que estamos sentindo, ou inibido – reduzindo assim a dor (SAADE et al., 2015).

Inicialmente, a substância cinzenta periaquedutal (PAG), recebe informações de dor através do trato espinomesencefálico e processa as informações nociceptivas e as retransmite para a medula rostral ventral (RVM). Esses neurônios no RVM então enviam um sinal pela medula espinhal e ativam o sistema opiáceo endógeno para suprimir a dor (LAU et al., 2020).

No estado naive, os interneurônios GABAérgicos na PAG disparam tonicamente, produzindo assim uma liberação constante de GABA e inibição dos neurônios de saída do PAG. Em geral, os mecanismos opioides bloqueiam a liberação do neurotransmissor GABA, suprimindo a inibição, aumentando a saída dos neurônios PAG que se projetam para a medula rostral ventromedial (RVM) (LUEPTOW et al., 2018) (Figura 6b).

Figura 6 - Vias de transmissão da dor. Via ascendente (a) e descendente (b). PAG: substância cinzenta periaquedutal; VTA: área tegmental ventral; RVM: medula rostral ventromedial.



Fonte: Yam (2018) adaptado por Caminski (2022).

Uma vez que os receptores opioides estão localizados no: cérebro (as maiores densidades encontradas no tálamo, no PAG e no RVM); medula espinhal (alta densidade na região do corno dorsal), eles podem regular a dor em vários níveis por reduzir a liberação de neurotransmissores. Esse efeito ocorre pela redução do disparo de potenciais de ação no neurônio de segunda ordem (visto que o trato corticoespinhal começa no córtex cerebral e termina na medula espinhal), bloqueando a transmissão de sinais de dor (Reddi et al., 2013).

1.7 CONTROLE MOTOR

As projeções do mesencéfalo (particularmente substância nigra compacta - SNc) liberam dopamina (DA) no nível do corpo estriado com efeitos mediados por duas famílias diferentes de receptores DA ligados à proteína G localizadas em neurônios estriatais de projeção espinhosa. As projeções estriadas para locais a jusante (e projeções corticais para o corpo estriado) foram entendidas como operando por meio das chamadas vias diretas e

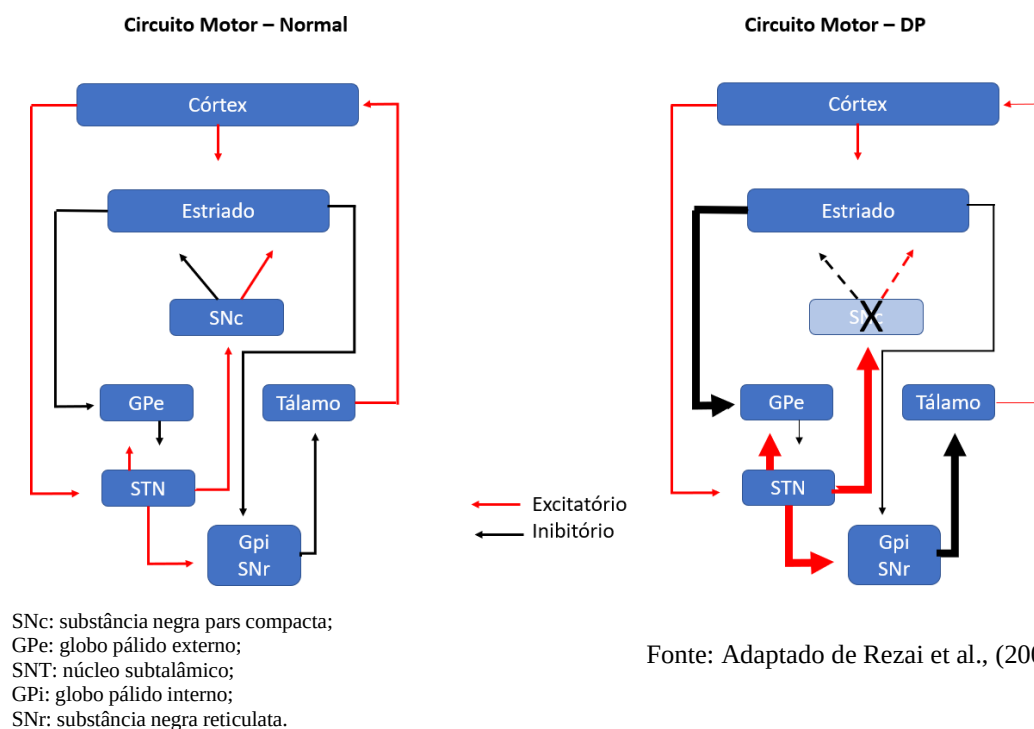
indiretas. De forma evolutivamente conservada Grillner et al., (2016) relata que a via direta inibe, enquanto a via indireta desinibe os *globus pallidus pars interna* e a substância negra pars reticulata (GPe/SNr), que juntos são os principais núcleos de saída inibitória dos gânglios da base.

A saída de GPi/SNr tem efeitos distintos nos núcleos talâmicos para os quais se projetam, com consequente inibição (via indireta) ou desinibição (via direta) do impulso talamocortical (DELONG, 1990).

Além disso, no estado normal, conforme Oswa et al., (2013) a DA facilita a atividade da via direta e inibe a atividade da via indireta. As projeções inibitórias do sistema GABAérgico predominam nessas vias. Além das projeções excitatórias glutamatérgicas do córtex para os gânglios da base e o retorno das projeções talamocorticais excitatórias, as únicas projeções dentro das estruturas cerebrais subcorticais profundas que são excitatórias são as projeções glutamatérgicas que o STN ao GPi/SNr (Figura 7).

A dopamina é um dos neurotransmissores mais poderosos influenciando este circuito e pode ter tanto um papel excitatório ou inibitório nos neurônios estriados, dependendo no subtipo de receptor de dopamina: os receptores D1 estão associados com efeito excitatório, enquanto os receptores D2 são inibitórios (REZAI et al., 2008).

Figura 7- Circuito neural córtico-estriado-palido-tálamo-cortical em estado normal e na doença de Parkinson (DP). O tamanho/intensidade das setas representam o aumento/diminuição da atividade de um aferente cortical específico da sinapse.



Fonte: Adaptado de Rezai et al., (2008).

O principal achado patológico na doença de Parkinson (DP) é a degeneração de neurônios dopaminérgicos na SNc ventrolateral, segundo estudo elaborado por Fearnley e Lees (1991). A deficiência estriatal de DA fornece uma justificativa para o uso do precursor de DA levodopa, bem como de agonistas de DA na doença. Como foi estudado em modelos animais (perda neuronal seletiva de SNc após exposição a 6-hidroxidopamina – 6OHDA - em ratos ou 1-metil-4-fenil-1,2,3,6-tetrahidropiridina em primatas - MPTP), a depleção de DA resulta em alterações consistentemente observadas, onde a via indireta entre o corpo estriado e os núcleos talâmicos torna-se hiperativa no contexto da degeneração dos neurônios dopaminérgicos (MIYAWAKI, 2022).

Aqui, essa hiperatividade leva a redução da ativação do córtex motor, que está envolvido no controle de movimentos voluntários finos e no aprendizado de novas habilidades motoras (HOSP et al., 2011).

O córtex motor integra entradas do córtex pré-motor e conduz saídas excitatórias para a medula espinhal e os gânglios da base por meio de neurônios piramidais glutamatérgicos. Assim, a regulação indireta da DA na função motora através da modulação da atividade dos gânglios da base tem sido amplamente descrita (DEJEAN et al., 2012).

Com base no exposto acima, é evidente que a interação entre NOP e CCSVs pode contribuir como potencial alvo terapêutico no tratamento das condições relacionadas a ansiedade, estresse, recompensa, dor, controle motor, memória e depressão. Sendo assim, apresenta-se então, uma revisão objetivando sumarizar a literatura para fornecer uma compreensão abrangente do atual estado de arte no que tange a interação supracitada. A apresentação é justificada pelo fato que, esta ampla visão pode contribuir para o desenvolvimento da teoria e fornecer futuro direcionamento de pesquisas pré-clínicas com aplicabilidade direta à prática clínica.

2 OBJETIVOS

2.1 Objetivo Geral

Apresentar o papel do sistema NOP-N/OFQ e a regulação dos CCSVs em condições fisiopatológicas baseadas em regiões de co-localização destes.

2.2 Objetivos Específicos

- Descrever o papel do agonismo do NOP e os CCSVs envolvidos na ansiedade, resposta ao estresse, depressão e humor, sistema de recompensa, aprendizado, memória, dor e controle motor;
- Relacionar os subtipos de CCSVs que estão envolvidos em cada condição descrita anteriormente;
- Compilar dados sobre a interação NOP-CCSVs em condições ainda não bem elucidadas pela literatura para guiar a necessidade de futuras abordagens.

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REVIEW

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Regulation of N-type calcium channels by nociceptin receptors and its possible role in neurological disorders

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Abstract

Activation of nociceptin opioid peptide receptors (NOP, a.k.a. opioid-like receptor-1, ORL-1) by the ligand nociceptin/orphanin FQ, leads to G protein-dependent regulation of Cav2.2 (N-type) voltage-gated calcium channels (VGCCs). This typically causes a reduction in calcium currents, triggering changes in presynaptic calcium levels and thus neurotransmission. Because of the widespread expression patterns of NOP and VGCCs across multiple brain regions, the dorsal horn of the spinal cord, and the dorsal root ganglia, this results in the alteration of numerous neurophysiological features. Here we review the regulation of N-type calcium channels by the NOP-nociceptin system in the context of neurological conditions such as anxiety, addiction, and pain.

Keywords: Anxiety, Learning, ORL-1, Opioid receptor, Pain, G protein-coupled receptor

Structure and physiological roles of N-type calcium channels

Voltage-gated calcium channels (VGCCs) mediate calcium influx upon the membrane depolarization, regulating many physiological processes, such as neurotransmitter release, hormone secretion, muscle contraction, and gene expression [1]. The dysfunction of these channels has been linked to a wide range of pathological conditions including pain, epilepsy, migraine, night blindness, cardiac arrhythmias, and congenital deafness among many others [2, 3], making them important pharmacological targets [4]. VGCCs have been classified based on their electrophysiological and pharmacological profiles. They can be separated into low-voltage activated (LVA) calcium channels, which include the T-type calcium channels (Cav3.1, Cav3.2, and Cav3.3),

or high-voltage activated ones (HVA), including N-type (Cav2.2), R-type (Cav2.3), P/Q-type (Cav2.1), and L-type (Cav1.1, Cav1.2, Cav1.3, and Cav1.4) channels [5, 6]. With the exception of Cav1.1 which is skeletal muscle-specific, these channels are all expressed in the brain, but show varying albeit overlapping distributions and support specific neurophysiological roles [1]. Cav2 channels operate predominantly at presynaptic terminals where they are responsible for fast synaptic transmission [7], whereas Cav1.3 and Cav1.4 channels can both be expressed at ribbon synapses (where they support features such as auditory and visual synaptic transmission [8, 9]; with Cav1.2 and Cav1.3 being expressed as well on cell bodies and dendrites of many types of central nervous system neurons [10]. All calcium channels contain a pore forming Cav α 1 subunit that is obligatory to obtain a functional channel and which are comprised of four transmembrane domains that are connected by cytoplasmic linker regions and flanked by cytoplasmic N- and C-terminal regions [1, 5]. HVA channel Cav α 1 subunits co-assemble with ancillary Cav α 2 δ and Cav β subunits in

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a 1:1:1 stoichiometry [11]. These subunits help promote plasma membrane expression and regulate the biophysical and pharmacological features of the channel [12, 13]. The mammalian genome expresses four different types of Cav β and four types of Cav $\alpha_2\delta$ subunits, with the latter being post-translationally cleaved, relinked via a disulfide bond, and then attached to the outer leaflet of the plasma membrane via a GPI anchor [14, 15]. In contrast, Cav β subunits are cytoplasmic and attach to the Cav α_1 subunit at the linker between transmembrane domains I and II. With additional diversity arising from alternative splicing events, the existence of multiple ancillary subunits gives rise to a wide range of different calcium channel complexes [16, 17]. The cryo-EM structure of Cav2.2 (and other) calcium channels has been solved [18–21] and has provided insights into the structural basis of channels function and pharmacology.

Cav2.2 calcium channels are expressed predominantly at presynaptic nerve terminals where they (along with Cav2.1 channels) are critically involved in the release of neurotransmitters [22, 23]. Their expression is primarily restricted to neurons, with wide distribution in the brain as well as in peripheral sensory afferents. Cav2.2 channels are potently inhibited by peptide toxins isolated from different types of marine fish hunting cone snails such as ω -conotoxin MVIIA (*Conus magus*) and ω -conotoxin GVIA (*Conus geographus*) whose selectivity of action on these channels has helped elucidate key roles of these channels in neurophysiological processes [24, 25]. Intrathecal delivery of such toxins mediates potent analgesic effects [26, 27], consistent with a key role of these channels in neurotransmission between sensory afferents and neurons in the spinal dorsal horn. Case in point, a synthetic version of ω -conotoxin MVIIA (a.k.a. Ziconotide or Prialt) is FDA approved for the treatment of refractory cancer pain [28]. Additional insights into the physiological roles of Cav2.2 channels have been gained from genetic models such as knockout mice lacking these channels, from small organic inhibitors of Cav2.2 channel activity, and from molecules that modulate post-translational modification of these channels. Mice deficient in Cav2.2 show hyposensitivity to different pain modalities [29–31], consistent with the data obtained with pharmacological inhibition. These mice also show increased vigilance [32], hyper-aggression [33], reduced anxiety levels [29], and appear to exhibit a reduction in the symptoms of alcohol withdrawal [34]. The latter is also consistent with the action of small organic Cav2.2 inhibitors [35–37]. A link between Cav2.2 channels and reward-seeking was highlighted by studies that target the interaction of Cav2.2 with collapsin mediator protein 2 (CRMP2) which is involved in regulating Cav2.2 channel expression at the plasma membrane [38, 39]—interfering with

this regulatory pathway alters reinstatement of cocaine seeking [40]. Pharmacological inhibition of Cav2.2 has been shown to have anxiolytic effects [41, 42], and this may be related to an inhibition of GABAergic synaptic transmission in the basolateral amygdala and prefrontal cortex [43]. On the other hand, it was shown that alternative splicing of exon 37 of Cav2.2 enhances responses to aversive stimuli via alteration of glutamatergic signaling [17]. Deletion of Cav2.2 channels either directly, or by depletion of its ancillary Cav β 3 subunit has been linked to impairment of memory [44, 45]. Finally, delivery of the Cav2.2 inhibitor ω -conotoxin GVIA directly into the brain has been shown to cause depression-like behavior in rodents [46], although it should be noted that depression is more commonly associated with altered L-type calcium channel activity. Overall, these findings indicate that Cav2.2 channels mediate important physiological roles and that modulation of the activity of these channels is expected to have consequences that affect behaviors such as anxiety and pain responses.

Modulation of Cav2.2 channels by NOP receptors

Cav2.2 channels are subject to modulation by a wide range of G protein coupled receptors (GPCRs) [47]. This includes the nociceptin opioid receptor (NOP, previously called the opioid receptor-like 1 receptor (ORL-1), which was first described by Mollereau et al. [48] and added as a fourth member of the opioid receptor family of μ -, κ -, and δ -opioid receptors [49, 50]. However, the NOP receptor is not activated by any of the known endogenous opioids that act on classical opioid receptors, but instead by the endogenous agonist nociceptin/orphanin FQ [51, 52]. The crystal structure of the NOP receptor reveals a typical 7 transmembrane helix topology with an additional short helix contained within the proximal C-terminus [53].

Like other G protein-coupled receptors (GPCRs), the activation of NOP by nociceptin triggers the exchange of GDP for GTP on the G α subunit, causing its dissociation from the G $\beta\gamma$ dimer. The dissociated subunits then proceed to act on different signaling pathways and effector systems. The NOP receptor couples primarily to G $\alpha_{i/o}$, inhibiting adenylyl cyclase and thus cyclic adenosine monophosphate (cAMP) production [54]. In addition, activation of NOP receptors activates phospholipase C and MAP kinase activity via coupling to G α_{14} and G α_{16} [55]. The dissociated G $\beta\gamma$ subunits, in addition to regulating various intracellular signaling cascades, activate G protein-coupled inwardly rectifying potassium (GIRK) channels [56], and inhibit VGCCs [47], thus reducing neuronal excitability and neurotransmitter release (Fig. 1A).

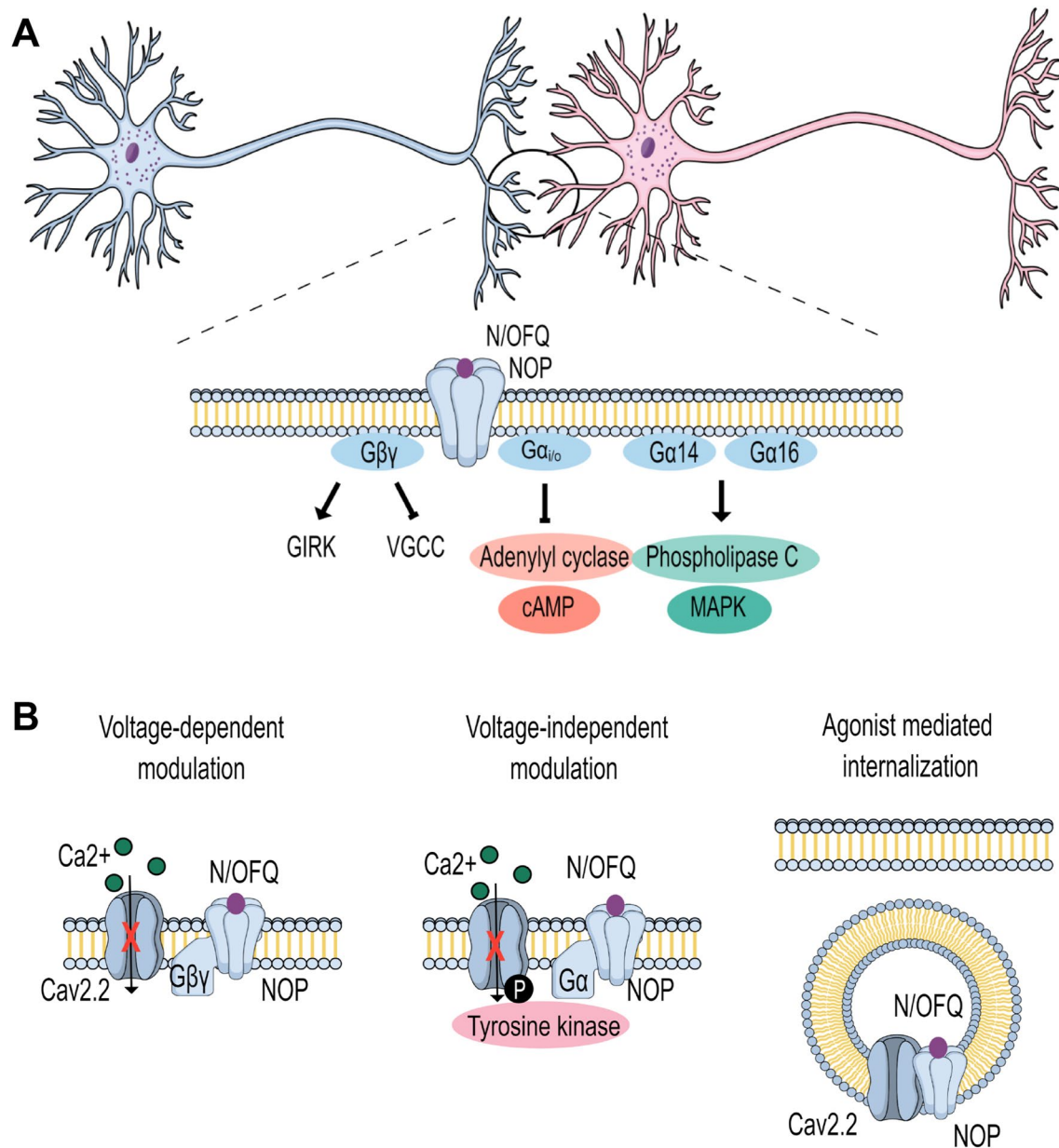


Fig. 1 Key aspects of NOP receptor signalling. **A.** NOP receptors at synaptic terminals. Activation of NOP by N/OFQ triggers several G protein dependent signalling pathways. **B.** Activation of NOP receptors triggers Gβγ mediated voltage-dependent inhibition of Cav2.2 channels, as well as Gα/tyrosine kinase dependent voltage independent modulation. Due to the formation of signalling complexes, prolonged agonist application triggers the co-internalization of NOP receptor Cav2.2 channel complexes

Specifically, Gβγ subunits inhibit members of the Cav2 calcium channel family (Fig. 1B) by directly interacting with the Cavα₁ subunit at its cytoplasmic N-terminal and domain I-II linker regions [57–59], with Cav2.2 channels being inhibited more potently than Cav2.1 [60–62]. Direct Gβγ modulation of Cav2.2 is sensitive to the membrane potential and can be reversed by the application of a depolarizing pre-pulse

or by trains of action potentials—it is therefore referred to as voltage-dependent inhibition [63–65]. The extent of G protein modulation is also dependent on the Cavβ subunit [66–68] and critically dependent on the Gβ isoform [61, 69], thus allowing receptors to mediate a wide range of inhibitory voltage-dependent processes. Many G protein-coupled receptors also cause voltage-independent modulation of channel activity, most likely via

the activation of protein kinases and receptor tyrosine kinases (Fig. 1B). This type of inhibition is insensitive to membrane depolarizations and has also been observed with NOP receptors [70].

Inhibition of Cav2.2 calcium channels by NOP receptors has been described in both neurons and heterologous expression systems [70–77]. In addition to the classical G protein inhibition, the C-terminus of the NOP receptor physically interacts with the Cav2.2 C-terminus, allowing it to control channel density at the plasma membrane through enhanced forward trafficking by possibly promoting ER export, and via channel/receptor co-internalization after prolonged agonist exposure [70, 78, 79] (Fig. 1B). This physical interaction also allows for a tonic inhibition of Cav2.2 channels by G $\beta\gamma$ in the absence of an agonist, presumably due to the receptor's constitutive activity. Another potential avenue by which the NOP can modulate Cav2.2 channels is via heterodimerization with other opioid receptor family members. NOPs form heterodimer complexes with all of the other three opioid receptors, and the prolonged activation of μ -opioid receptors triggers the internalization of Cav2.2 channels, but only in the presence of NOP receptors [80].

A number of different ligands of NOP receptors have been identified and they are not considered to be opioids. As already noted above, the archetypal agonist of the NOP receptor is nociception—a 17-amino acid peptide that is structurally related to dynorphin A [54]. A wide range of synthetic NOP receptor agonists have been described (for review, see [81]) and have been examined in preclinical and clinical studies of anxiety and pain. Conversely, a wide range of NOP receptor antagonists have been developed and tested in animals and human subjects in phase I and II studies for the treatment of cognitive impairment, depression, and addiction [81, 82]. This includes LY2940094 (aka BTRX-246040) which appears to be effective in the treatment of depression and well tolerated [83]. Mixed agonists of NOP and opioid receptors such as AT-121 and cebranopol have also been explored as potential systemically active pain therapeutics including in phase III clinical testing [84, 85]. The NOP receptor is widely distributed in the central and peripheral nervous systems [86]. In the brain, it is expressed in regions that are involved in the processing of pain, learning, memory, food intake, and fear [86–88], consistent with the above pharmacological studies. Given the widespread expression of VGCCs, they are co-expressed with these receptors in many areas of the brain. Below, we will highlight the role of the NOP-N/OFQ system in neuro-physiological processes that are known to rely on Cav2.2 channels.

NOP receptors as modulators of pain

NOP receptors are expressed in the dorsal and ventral horns of the spinal cord which integrate sensory processing [89]. NOP receptor expression is enhanced in the spinal cord in rodent models of persistent inflammatory and neuropathic pain [90–92] and there is an upregulation of plasma concentrations of nociceptin in chronic pain patients [93]. Intrathecal delivery of nociceptin mediates analgesia in rodent models of pain [94–97], as do non-peptide agonists [98–100]. In non-human primates, systemic or intrathecal injection of NOP agonists causes antinociception [101–105]. These analgesic effects are likely mediated by an inhibitory action on presynaptic Cav2.2 channels expressed in sensory afferents (note that NOP receptors are expressed on both A and C fibers [79, 86, 106], along with possible activation of postsynaptic GIRK channels). In addition, NOP receptor activity has been shown to modulate the excitability of spinal interneurons and superficial layer neurons projecting to the brain [107–109].

Mice lacking the NOP receptors show normal nociceptive thresholds, only slightly reduced locomotor activity, and normal immune responses, but compromised hearing ability [110]. These mice however also show alterations in morphine tolerance. Like NOP receptors, activation of μ -opioid receptors by morphine or DAMGO also inhibits Cav2.2 channel activity [111]. While NOP receptors are intrinsically resistant to the μ -opioid specific agonist morphine, they appear to affect morphine tolerance via crosstalk with μ -opioid receptors. It was shown that mice lacking NOP receptors exhibit resistance to morphine tolerance without alterations in the acute effects of morphine [112, 113], whereas mice with morphine tolerance exhibit upregulation of NOP receptors [113]. Along these lines, NOP receptor activation diminishes the development of morphine tolerance in rats and blocks morphine-induced place preference [114, 115], although this concept has been challenged [116]. Other studies have reported an antagonistic effect of NOP receptor activation on opioid-induced analgesia [117, 118]. In addition, increased NOP expression is seen after prolonged morphine exposure [113, 119, 120]. Finally, bi-functional ligands of NOP and μ -opioid receptors help prevent adverse effects associated with μ -opioid receptor activation [121–123]. To what extent this crosstalk is mediated by selective actions via Cav2.2 calcium channels remains to be determined, but the notion that μ -opioid receptors, NOP receptors, and Cav2.2 channels can form signaling complexes [80] hints at the possibility that converging signaling by these two receptors on Cav2.2 as a common target may play a role.

While NOP receptors mediate antinociceptive actions at the spinal level likely by inhibition of spinal excitatory

neurons and synaptic transmission at peripheral afferent terminals [124], they appear to exhibit pronociceptive effects at the supraspinal level, as observed after injection of nociceptin into the brain [51, 52]. It is not entirely clear how these pronociceptive effects occur at the cellular and molecular level. One possibility may be an inhibitory effect on stress-induced analgesia that is known to be modulated by NOP receptors [125–127]. Indeed, there is evidence that NOP receptor activation contributes to the occurrence of allodynia and hyperalgesia in post-traumatic stress disorders (PTSD) [128], with NOP antagonists reversing this type of pain behavior [129]. It has been suggested that at supraspinal sites, the activation of NOP receptors may inhibit neuronal activity via activation of GIRK channels in brain loci that are relevant for nociceptive transmission such as the periaqueductal gray region and dorsal raphe nucleus [124], but exactly how this modulation causes pro-nociceptive effects remains to be worked out.

Role of NOP receptors in anxiety

Anxiety is marked by excessive fear (and avoidance) in response to perceived threatening situations and even in the absence of true danger [130]. NOP receptors are highly expressed in neurons and synaptic terminals of brain areas that are implicated in anxiety and depression [131]. This includes the amygdala, the nucleus accumbens (NAc), the hippocampus, the hypothalamus, the periaqueductal gray, the ventral tegmental area (VTA), and some of the prefrontal regions among others [132–134]. The medial prefrontal cortex (mPFC) exerts inhibitory control over amygdala activity, preventing an improper expression of emotion, but in anxiety disorders, this control becomes imperfect and consequently triggers aberrant amygdala activation through dysfunction of GABAergic and glutamatergic transmission [135–138]. NOP activation produces presynaptic inhibition of VGCCs, and thus the release of glutamate in the lateral amygdala [139, 140]. NOP agonists delivered orally or via intraperitoneal injections have revealed anxiolytic effects in rodents [141–144]. Little is known about the biochemical pathways by which NOP agonists induce anxiolytic effects but they may involve serotonin availability and changes in firing rates in the dorsal raphe nucleus (DRN, which projects to serotonergic axons of the amygdala), and concomitantly, by counteracting anxiogenic CRF actions into the bed nucleus of *stria terminalis* [145]. It should be noted that some studies have, however, reported anxiogenic responses to nociceptin, and the reason for these discordant findings is unclear [146, 147].

Prenatal ethanol exposure (PEE) induces an anxiety-like phenotype in adult and adolescent rats [148], and this is frequently used as an animal model to study anxiety. This

results in the induction of neuronal hyper-excitability by augmenting glutamatergic transmission, which does not appear to be attenuated by GABAergic inhibition in the basolateral amygdala [149, 150]. NOP activation via intracerebroventricular administration of nociceptin reportedly inhibits associated anxiety behaviors [151]. In this model, there is an alteration of dopaminergic neuron morphology [152], along with a reduction of NOP gene expression [153], and an increase in the expression of the precursor of nociceptin (ppN/OFQ) [151]. Although the authors did not find any alterations of the NOP-nociceptin pathway in the amygdala, alterations of upstream inputs may lead to altered amygdala activity, thus perhaps contributing to the anxiety phenotype.

Altogether, anxiolytic-like effects can be observed with the delivery of nociceptin and synthetic agonists into the brain. Cav2.2 channels are also known to play a role in anxiety and are a potential pharmacological target for anxiolytics [41, 154, 155]. Indeed, as noted earlier, inhibition or depletion of Cav2.2 channels has been shown to mediate anxiolytic effects in mice [29, 156], and therefore a putative inhibition of these channels by activation of NOP would be expected to result in similar outcomes. It is however unclear precisely in which brain structures and neuronal subtypes of NOP receptor regulation of Cav2.2 channels may take place in the context of anxiety.

NOP receptors and stress

The fear/anxiety neuro-circuitry overlaps with the neuro-circuitry that regulates stress responses [130]. The amygdala integrates information from cortical and thalamic sensory inputs, to generate fear and anxiety-related behavioral outputs [157], with fear conditioning models demonstrating an increase in synaptic strength in the lateral amygdala (LA) and the PFC [158–160]. PTSD involves an over-reactivity of the HPA axis, decreased inhibitory signaling by GABA, and increased excitatory neurotransmission. This is associated with increased activity in emotion-processing brain regions, such as the limbic system (hippocampus, amygdala, PFC). Overall, a major characteristic of PTSD is impaired fear extinction, following exposure to a traumatic event [161]. These responses against chronic stress lead to altered NOP receptor signaling which may contribute to an increase in corticosterone levels [145]. In PTSD experimental models, corticosterone levels, as well as nociceptin and NOP expression are increased in different brain regions and the cerebrospinal fluid, and consequently, NOP antagonists protect against molecular and behavioral deficits [162, 163]. Much of the underlying mechanisms appear to involve alterations in L-type calcium channel activity [156, 164–170]. However, given that Cav2.2 channels are present at synaptic junctions in the HPA axis, and are

typically inhibited by NOP receptor agonists, it is possible that these channels play a detrimental role in fear extinction during PTSD, in addition to their potential pronociceptive effects that were discussed earlier. On the other hand, it is interesting to note that compounds such as topiramate which block R-type and N-type channels appear to show some promise in treating PTSD [171]. Clearly, more work is needed to elucidate the potential role of Cav2.2 channels in PTSD and its modulation by the NOP receptors.

NOP receptors as targets for addiction

Dopamine participates in drug-seeking reward behavior by modulating the activity of the mesolimbic pathway [172]. The dopaminergic axons in the ventral tegmental area (VTA) project to downstream regions such as the nucleus accumbens, the nucleus of the stria terminalis, and the lateral hypothalamus [173]. Nociceptin and NOP receptors are densely expressed in the VTA where they regulate dopaminergic transmission [174, 175]. This occurs by disinhibition of GABAergic control over dopaminergic neurons, such that NOP agonists mediate suppression of dopamine release [115, 176, 177]. Genetic deletion or pharmacological inhibition of NOP receptors in rats was shown to inhibit nicotine-motivated behaviors [178]. Along these lines, alcohol reward-seeking behavior was shown to be inhibited upon NOP receptor antagonism or deletion [179–184]. This fits with observations that nociceptin and NOP receptor expression are aberrantly increased in many brain regions of Marchigian Sardinian alcohol-preferring rats, including the central amygdala [185]. However, as with the role of NOP receptors in anxiety, there are also several studies that show that NOP agonists may in fact inhibit reward-seeking behavior related to alcohol and cocaine [186–188]. The reason for this discrepancy is not entirely clear, and a recent review article suggested the possibility of some NOP agonists acting as functional antagonists [163]. This then makes it difficult to predict how NOP receptors modulate the reward circuitry by putative actions on calcium channels.

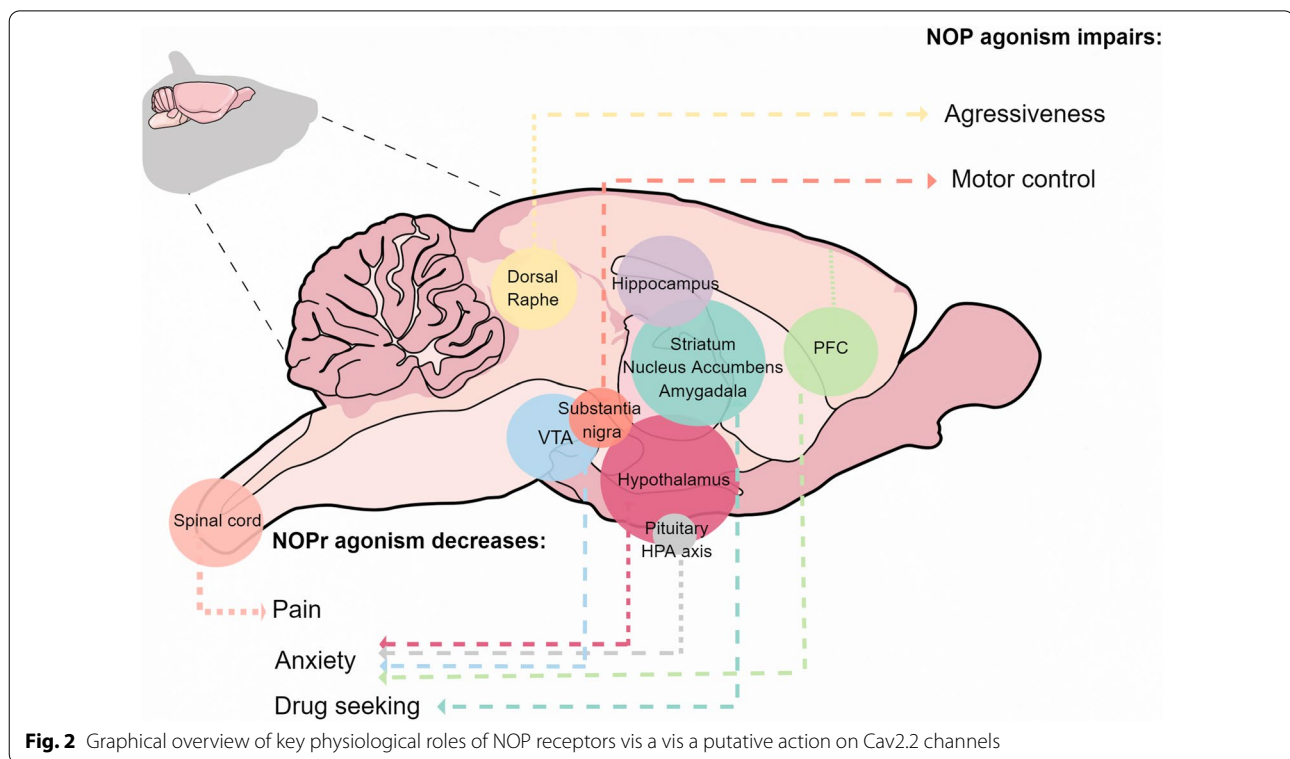
L-type channels have been extensively implicated in reward-seeking behavior and are known to be dysregulated during states of addiction [155, 189–192]. But there is also evidence that N-type calcium channels play a role, as Cav2.2 knockout mice exhibit reduced ethanol consumption [34]. Along these lines, the mixed N-type/T-type calcium channel inhibitor NP078585 reduced alcohol-induced intoxication and reinstatement, but failed to do so in mice lacking Cav2.2. This indicates that its primary target was indeed the N-type channel [35] and altogether suggests that the Cav2.2 channel is a potential target for the treatment of alcoholism [36].

While an inhibitory action of NOP agonists on Cav2.2 channels would be consistent with some of the results discussed above, the fact that NOP antagonists can also attenuate reward-seeking behavior suggests that Cav2.2 channels are not the exclusive effector of NOP receptors in the context of addiction.

Role of NOP receptors in other neuropsychiatric conditions

Khan et al. [193] suggested a potential role of the NOP system in schizophrenia. Some indications that support this concept are derived from experiments involving a resident-intruder test—an animal model of aggression that is thought to reflect certain symptoms of bipolar disorder, schizophrenia, or PTSD. In this model, systemic injection of NOP antagonists had little effect, but the aggressive behavior of mice that were treated with NOP agonists was greatly increased [194]. The observed effects were similar to those seen with those of para-chlorophenylalanine, an inhibitor of serotonin synthesis [194]. An equivalent increase in aggressive behavior was seen in Cav2.2 null mice, or upon microinjection of a Cav2.2 channel inhibitor into the dorsal raphe nucleus of wild-type mice [33]. Hence, it is tempting to speculate that the aggressive behavior observed with NOP receptor agonists may have involved the inhibition of Cav2.2 channels. This might fit with the idea that Cav2.2 null mice also presented with an increase in serotonin levels in the hypothalamus. It is important to note that a recent study has shown that P/Q-type calcium channels may also play a role in aggressive behavior [195]—a Cav2 channel subtype that is also inhibited by NOP receptors.

Modeling bipolar-like behavior through the administration of psychostimulants affects several neurotransmitter systems, and thus it is difficult to attribute precise cellular and molecular mechanisms that underlie the resultant behavioral phenotype [196]. In mouse models of methylphenidate-induced manic-like behavior, the synthetic full NOP agonist Ro 65-6570 attenuated drug-induced hyperlocomotion, but did not affect spontaneous locomotor activity, whereas NOP receptor antagonists had no effect [197]. Interestingly, methylphenidate mediated similar effects in WT and NOP receptor null mice, indicating that the establishment of the hyperlocomotion does not per se involve a dysregulation of the NOP receptor system [197]. It has been shown that N-type and P/Q-type channel inhibitors prevent methylphenidate-induced locomotion [198, 199], potentially by inhibiting dopamine release. This is thus consistent with a mechanism by which NOP receptor activation mediates stimulant-induced hyperlocomotion by inhibition of Cav2.2 channels.



NOP receptor function in movement disorders

Central vestibular neurons are important for processing motion-related multisensory signals and their transformation into motor commands [200]. Participation of NOP receptors and nociceptin in vestibular afferent excitability has been demonstrated via the locomotor performance of rats after traumatic head injury. Such rats exhibit motor deficits and increased levels of nociceptin in the afferent vestibular nuclei, as well as an increase in NOP receptor expression in brain regions that are associated with the vestibular function [201, 202]. The behavioral and functional deficits that occur following a traumatic brain injury also correlate with the elevated NOP expression in brain regions that are associated with vestibular function, including the motor cortex, the dorsal striatum, and the vestibular nuclei [76]. In cultured rat vestibular afferent neurons, nociceptin inhibits HVA calcium currents (principally, those carried by Cav2.2), without modifying LVA channels, and this inhibitory action occurs via voltage-dependent G $\beta\gamma$ modulation [76]. This fits with the idea that blockers of Cav2.2 channels are neuroprotective during traumatic brain injury [203].

In Parkinson's disease, the degeneration of the dopaminergic neurons of the substantia nigra (SN) pars compacta affects the entire basal ganglia network

[204, 205], leading to hyperactivity that is sustained by enhanced glutamatergic inputs [206, 207]. In addition to the basal ganglia, dopamine depletion in the cortical and subcortical motor areas results in the upregulation of the nociceptin-NOP system in the SN, and a down-regulation in the striatum, and this may contribute to associated motor dysfunction [208, 209]. 6-hydroxydopamine (6-OHDA) hemilesioned rats exhibit an elevated expression and release of nociceptin into the SN [210] thereby causing motor deficits. There are conflicting results from pharmacological studies in Parkinson's disease models. On one hand, exogenous nociceptin or synthetic NOP agonists, when administered systemically, attenuated dyskinesias [211, 212]. However, there was a biphasic effect on motor function, where low doses improved Parkinson-like symptoms, and higher doses disrupted motor coordination and global behavior [212]. Conversely, NOP antagonists also produced antiparkinsonian effects [213, 214] by ameliorating akinesia, bradykinesia, and overall gait problems at low doses, but they inhibited motor activity at intermediate concentrations. Interestingly, these different doses differentially regulated glutamatergic and GABAergic signaling in the SN and the ventromedial thalamus [215]. While the precise contributions of VGCCs to this NOP-dependent regulation of excitatory and inhibitory neurons remain to be determined, there is evidence

that 6-OHDA mediates a PKA-dependent upregulation of Cav2.2 channels in the SN [216]. Hence, a putative inhibition of Cav2.2 channels would be consistent with a protective effect of NOP agonists.

Conclusion

The nociceptin-NOP pathway is expressed widely in the peripheral and central nervous system and dysregulated in a number of neurological and neuropsychiatric conditions. A key target of NOP receptor activation are Cav2.2 channels which are typically inhibited upon receptor activation, but whose forward and reverse trafficking is also regulated by NOP receptor association with the channel complex. In the afferent pain pathway, there is a tight correlation between Cav2.2 channel activity and NOP receptor activation in the context of pain signaling (Fig. 2). In conditions such as anxiety, reward-seeking, and motor control the importance of Cav2.2 channels as downstream targets of NOP receptors is more tenuous (Fig. 2). While actions on Cav2.2 channels are consistent with what is known about this VGCC subtype, NOP receptors activate a plethora of downstream signaling cascades that are expected to have complex effects on neurons, glial cells, and neuronal circuits. Future studies involving the activation of these receptors in Cav2.2 null mice will ultimately provide deeper insights into putative linkages.

Abbreviations

NOP: Nociceptin opioid peptide; GABA: Gamma-aminobutyric acid; PKA: Protein kinase A; HVA: High voltage-activated; LVA: Low voltage-activated; VTA: Ventral tegmental area; SN: Substantia nigra; PTSD: Post-traumatic stress syndrome; PFC: Prefrontal cortex; GIRK channel: G protein-coupled inwardly rectifying potassium channel; VGCC: Voltage-gated calcium channel; CRMP2: Collapsin mediator protein 2; SN: Substantia nigra; 6-OHDA: 6-Hydroxydopamine.

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The authors declare no competing interest.

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