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## Janus effect of the anterior cingulate cortex: Pain and emotion

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1 **Janus Effect of the Anterior Cingulate Cortex: Pain and Emotion**

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## Janus Effect of the Anterior Cingulate Cortex: Pain and Emotion

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### Highlights (3 to 5):

- ACC plays a critical role in emotional and pain processing.
- ACC is essential for the emotional component as well as anxio-depressive consequences of chronic pain.
- Imbalance between glutamatergic and GABAergic transmission in the ACC is one of the key mechanisms for the aversive- and anxiodepressive-consequences of chronic pain.
- Recent technological advances and development of several rodent model of chronic pain strengthen our mechanistic understanding underlying the complex role of the ACC in emotion and pain dyad.

## **Abstract**

Over the past 20 years, clinical and preclinical studies point to the anterior cingulate cortex (ACC) as a site of interest for several neurological and psychiatric conditions. The ACC plays a critical role in emotion, autonomic regulation, pain processing, attention, memory and decision making. An increasing number of studies have demonstrated the involvement of the ACC in the emotional component of pain and its comorbidity with emotional disorders such as anxiety and depression. Thanks to the development of animal models combined with state-of-the-art technologies, we now have a better mechanistic understanding of the functions of the ACC. Hence, the primary aim of this review is to compile the most recent preclinical studies on the role of ACC in the emotional component and consequences of chronic pain. Herein, we thus thoroughly describe the pain-induced electrophysiological, molecular and anatomical alterations in the ACC and in its related circuits. Finally, we discuss the next steps that are needed to strengthen our understanding of the involvement of the ACC in emotional and pain processing.

**Key words (12/12):** Anterior cingulate cortex, ACC, Area 24a, Area 24b, Area 32, pain, chronic pain, aversion, anxiety, depression, circuits, rodent models.

## **Abbreviation list**

ACC: anterior cingulate cortex

AMPA:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BDNF: brain derived neurotrophic factor

BLA: basolateral nucleus of the amygdala

CPEB1: cytoplasmic polyadenylation element binding protein 1

E/I: excitatory/inhibitory

GABA:  $\gamma$ -aminobutyric acid

GAD: generalized anxiety disorder

Glu: glutamatergic

HCN: hyperpolarization-activated cyclic nucleotide-gated

LC: locus coeruleus

mGluR5: metabotropic glutamate receptor 5

MAPK: mitogen-activated protein kinase

MKP-1: MAPK phosphatase 1

MCC: middle cingulate cortex

MDD: major depressive disorder

N: nucleus

NMDA: N-methyl D-aspartate

PAG: periaqueductal gray

p-mTOR: phosphorylated mTOR

rACC: rostral anterior cingulate cortex

VTA: ventral tegmental area

## 1           **Introduction**

2   Major depressive disorder (MDD) and generalized anxiety disorder (GAD) are some of the leading  
3   causes of disability worldwide (World Health Organization, 2009), contributing to the global burden  
4   of diseases (Santomauro et al., 2021; Whiteford et al., 2013). Although chronic stress is considered as  
5   the major risk factor for MDD and GAD, other pathologies can also trigger or worsen **these**  
6   **psychiatric conditions. These comorbidities are in part responsible for treatment failures but also**  
7   **dramatically decrease the patients' quality of life.** Aside from the well-known comorbidity between  
8   depression and anxiety themselves, chronic pain is an important determinant for MDD and GAD  
9   (Means-Christensen et al., 2008), **with a prevalence rate ranging from 30 to 80% of chronic pain**  
10   **patients depending on the pain condition** (Bair et al., 2003; de Heer et al., 2014; Demyttenaere et al.,  
11   2007; Gureje et al., 2008; Von Korff et al., 2005; Williams, 2003). **On the other hand, 50 to 60% of**  
12   **patients diagnosed with MDD report chronic pain (Meints and Edwards, 2018; Williams, 2003),**  
13   **highlighting the important interplay between these pathologies.**

14   One hypothesis for the comorbidity between chronic pain and emotional disorders is based on a shared  
15   neuroanatomical substrate, suggesting that **i)** specific brain regions processing pain are also involved  
16   in emotional processing, and **ii)** alterations induced by chronic pain in these regions may alter  
17   emotional processing, possibly resulting in psychiatric disorders. Indeed, in different pain conditions,  
18   recent human and rodent imaging studies reported morphological and functional alterations in the pain  
19   matrix, including the primary and secondary somatosensory, insular and anterior cingulate cortices  
20   (ACC) (Apkarian et al., 2005), and in the mesolimbic and corticolimbic pathways, well-known for  
21   their implication in emotional processing (Serafini et al., 2020). Among these brain structures, the  
22   ACC is at the interface between sensory, cognitive and emotional processes (Vogt, 2005), acting as a  
23   hub for both pain and emotional processing (Shackman et al., 2011).

### 24   *Insight from Human Studies*

25   **The involvement of the ACC in the emotional component of pain was first suggested by clinical**  
26   **studies reporting that patients with stereotactic cingulotomies describe pain less unpleasant (Foltz and**  
27   **White, 1962). These studies have recently been extended by showing that cingulotomy not only**  
28   **alleviates the distress caused by pain but also the depressive symptoms in chronic pain patients (Deng**  
29   **et al., 2019) and it decreases the functional connectivity within the salience network (Jalon et al.,**  
30   **2023). Functional magnetic resonance imaging (MRI), positron emission tomography (PET), voxel-**  
31   **based morphometry (VBM), diffusion tensor imaging (DTI) also clearly showed how different chronic**  
32   **pain components are correlated with ACC alterations. The pioneer study from Rainville and**  
33   **colleagues (1997) demonstrated, using PET scan, a correlation between ACC activity and the**  
34   **perceived unpleasantness of pain. In addition, VBM analyses showed reduced gray matter volume in**  
35   **the ACC of trigeminal neuralgia patients which correlated with pain intensity, depression and anxiety**  
36   **ratings (Wang et al., 2017). Furthermore, several groups reported positive effect of deep brain**

37 stimulation (see review Russo and Sheth, 2015) and transcranial magnetic stimulation of the ACC  
38 (Best and Pavel, 2017), positioning this brain area as a new potential neuromodulatory target to treat  
39 the affective component of chronic pain. These findings are in favor of a strong and dual role of the  
40 ACC in pain and its emotional comorbidities. Since the correlates of human ACC can be found in both  
41 primates and rodents (van Heukelum et al., 2020), this brain structure became a region of interest in  
42 preclinical studies leading to a better understanding of the role of the ACC in pain-associated  
43 emotional disorders.

#### 44 *Insight from preclinical studies*

45 While clinical investigation (Bair et al., 2003; de Heer et al., 2014; Demyttenaere et al., 2007; Gureje  
46 et al., 2008; Von Korff et al., 2005; Williams, 2003) continues to provide insights about the emotional  
47 component of pain, animal experiments offer a unique opportunity to test cellular and molecular  
48 hypotheses to decipher underlying cerebral mechanisms. Indeed, in the last two decades, the  
49 development of preclinical settings and rodent models of chronic pain (see review Kremer et al., 2021)  
50 combined with invasive or virally-mediated approaches, unavailable in Human research (see reviews  
51 Bravo et al., 2020; Humo et al., 2019), have greatly improved our understanding of the  
52 neurobiological mechanisms implicated in this important clinical issue. Thus, these new approaches at  
53 the interface between translational and reverse-translational research brought novel hypotheses that  
54 pave the way to the development of novel treatment strategies. Accordingly, this narrative review will  
55 outline preclinical studies focusing only on the role of the ACC in the emotional component of chronic  
56 pain, published between 2004 and 2022. To that end, publications were chosen using a combination of  
57 three keywords [(pain) and (aversion or anxiety or depression) and (anterior cingulate cortex or  
58 cingulate or ACC or prefrontal cortex or PFC) (**Fig.1**). As a first step, all studies not conducted in  
59 rodents (mouse or rat) were excluded. Secondly, as neuroanatomical description of the ACC differs in  
60 the literature (Fillinger et al., 2017a, 2017b; van Heukelum et al., 2020), we selected the scientific  
61 papers focusing only on the area **24a**, **24b** and **32**, based on the atlas of Paxinos and Franklin, 2012.  
62 We therefore excluded publications that were not studying these areas, even though they mentioned  
63 the ACC in the manuscript.

#### 64 -----**Fig.1**-----

65 In this review, after a brief description of the neuroanatomy of the ACC, we will highlight the  
66 significant role of this cerebral hub at the interface between pain and its emotional components. It is  
67 worth mentioning that most of the studies cited here focus mainly on neuropathic and inflammatory  
68 pain (see Kremer et al., 2021 for detail), with few articles focusing on visceral or cancer pain (**BOX 1**  
69 for preclinical models cited in this review). Finally, we will discuss the next steps needed for  
70 strengthening our understanding of the role of the ACC in the emotional component and consequences  
71 of pain, with a special focus on sex differences.

## 72 1. Neuroanatomy of the ACC

73 In human and non-human primates, the cingulate cortex belongs to the medial wall of the cerebral  
74 cortex (Palomero-Gallagher et al., 2009; van Heukelum et al., 2020; Vogt et al., 1996). Along the  
75 anteroposterior axis, four subregions including the anterior (ACC), the middle (MCC) and posterior  
76 cingulate cortex as well as the retrosplenial cortices compose this gyrus [Fig.2a, (Vogt, 2005)].  
77 Comparative anatomy based on cytoarchitecture shows several striking differences between primates  
78 and rodents, the most obvious being that rodents do not have a cingulate sulcus and therefore lack  
79 primate sulcal ACC areas 24c, s32 and p32. Additionally, while MCC is organized into an anterior and  
80 a posterior region in primates, rodents present a uniform MCC. Moreover, the supracallosal area 33  
81 spans the full extent of ACC and MCC in primates, while its presence is minor in rats and absent in  
82 mice. Finally, the posterior cingulate cortex, massively developed in humans and on a smaller scale in  
83 non-human primates, is absent in rodents (Vogt and Paxinos, 2014). Yet, despite the absence of the  
84 aforementioned subregions in rodents, the cytoarchitectural organization of the common areas found  
85 in human and non-human primates and rodents present sufficient similarities to share a common  
86 nomenclature. In rodents, the ACC is an agranular cortex lacking a true layer IV composed by the area  
87 24, further divided into 24a (ventral) and 24b (dorsal), and the areas 25, 32 and 33 – the latter being  
88 absent in mice (Fig.2b). These areas contain glutamatergic pyramidal neurons (~80%) in all ACC  
89 layers apart from layer I. The inhibitory GABAergic neurons (~20%), including heterogeneous  
90 subpopulations based on calcium binding proteins [parvalbumin, calbindin or calretinin] and peptides  
91 such as somatostatin (Gabbott et al., 1997), are distinctly distributed in terms of density and  
92 connectome over different layers and subdivisions (Gabbott et al., 1997).

93 Concerning its neuroanatomical connections, the ACC presents a wide connectome, similar across  
94 species, with only a few notable differences illustrated for example by the cortico-spinal projection  
95 located in the areas 32 and 24b in rodents, which is found in the deep layers of the MCC in non-human  
96 primates (for details see Fillinger et al., 2017a, 2017b; van Heukelum et al., 2020). Briefly, in all  
97 species, connections of the areas 24a/24b can be divided in four sub-networks, 1) cortical, 2) thalamic,  
98 which are the denser connections of the ACC and mostly reciprocal, 3) forebrain and hypothalamus  
99 and 4) brainstem centers (Fig.2c-f).

100 -----Fig.2-----

101 In rodents, at the cortical level, beside strong interconnections, areas 24a and 24b are reciprocally  
102 connected with frontal regions, mostly with the rest of the ACC and the orbital cortex, as well as the  
103 sensory and temporal cortices. As an exception, the hippocampal region sends moderate projections to  
104 the ACC but does not receive any afferents from it. In regards to the thalamo-cortico-thalamic  
105 connections, four main groups of nuclei can be highlighted as cingulate privileged interlocutors: the  
106 anterior nuclei - mostly the anteromedial nucleus-, the mediodorsal nucleus, the midline nuclei -



107 primarily the reuniens and rhomboid nuclei-, and finally the intralaminar nuclei with the centromedial,  
108 central lateral, paracentral and parafascicular thalamic nuclei (Fillinger et al., 2017a, 2017b).

109 In the forebrain, the basolateral nucleus of the amygdala (BLA) and the claustrum are densely and  
110 reciprocally connected to the ACC, along with a lesser contribution of the lateral and anterior  
111 hypothalamus. Additionally, the lateral septum, as well as the dorsal, and to a lesser extent the ventral,  
112 striatum receive dense ACC projections. In the brainstem, monoaminergic centers [ventral tegmental  
113 area (VTA), substantia nigra, dorsal and median raphe and the locus coeruleus (LC)] are  
114 bidirectionally connected with the ACC (Fillinger et al., 2017a, 2017b). In addition, the ACC sends  
115 unidirectional projections to the superior colliculus and the pretectal nuclei, as well as to the  
116 periaqueductal gray (PAG), more specifically to the dorsolateral column from 24a and the  
117 lateral/ventrolateral columns from 24b in mice. Finally, ACC neurons have been found to project to  
118 the cervical and thoracic spinal cord in rodents (Chen et al., 2018).

119 **In conclusion, as other higher-order cortical areas, the ACC possesses a wide and conserved**  
120 **connectome (Fig.2) making it a cerebral hub responsible for multiple pain-related, emotional and**  
121 **cognitive processes.**

## 122 **2. Role of the ACC in pain-related aversion**

123 The affective dimension of pain refers primarily to the feeling of unpleasantness and secondarily to the  
124 long-term impact of suffering from pain. Such aspects of pain participate in shaping subsequent pain-  
125 related behaviors and reflect aversive and motivational aspects of pain. Hence, pain-related aversion  
126 leads to behavioral avoidance or extinction of behaviors associated with noxious stimuli.

127 Numerous clinical studies have demonstrated the critical role of the ACC in the affective dimension of  
128 pain in human (see Brotis et al., 2009). In preclinical studies, several paradigms were developed as a  
129 mean to evaluate aversion in rodents [Fig.3, (Kremer et al., 2021)]. The place escape/avoidance  
130 paradigm (Fuchs and McNabb, 2012) - measuring animals escape behavior from an evoked painful  
131 stimulus-, place conditioning paradigms (Tappe-Theodor et al., 2019) - relying on the association of a  
132 context with either painful stimuli in conditioned place aversion or pain-relief via an analgesic drug in  
133 conditioned place preference (Navratilova et al., 2013)-, are the most frequently used tests to evaluate  
134 the aversive component of pain.

135 Lesioning studies were part of the milestones assessing the involvement of the ACC in pain-related  
136 aversion. It was reported that ACC lesion (using electrolytic or excitotoxic procedures) in rats blocks  
137 the avoidance to a context paired with acute inflammatory pain (Gao et al., 2004; Shi et al., 2020; Yi  
138 et al., 2011) and prevent the aversiveness of ongoing pain in both neuropathic (Barthas et al., 2015;  
139 Juarez-Salinas et al., 2019; LaGraize et al., 2004; Li et al., 2022; Qu et al., 2011) and inflammatory  
140 pain models (Johansen et al., 2001). Interestingly, none of these studies reported an effect of the ACC  
141 lesion on mechanical hypersensitivity. Interestingly, none of these **forementioned** studies reported an

142 effect on mechanical hypersensitivity which is probably due to drastic effect of lesioning of a given  
143 structure on its connection with whole network. On the contrary, lesion of the posterior insular cortex  
144 blocks the maintenance of mechanical hypersensitivity (Gao et al., 2004; Shi et al., 2020) without  
145 affecting the aversive component of neuropathic pain (Barthas et al., 2015). Accordingly, the ACC  
146 seems to be essential for the aversive component of pain. The next section will thus outline cellular  
147 and molecular alterations in the ACC as well as its circuits involved in pain-related aversion.

148 -----Fig.3-----

## 149 **2.1 Electrophysiological changes and functional alterations**

150 Recently, available technological advances in animal research have accelerated our understanding of  
151 the functional role of the ACC in pain-related aversion. The use of different electrophysiological  
152 approaches, from *ex vivo* whole cell patch clamp recordings to *in vivo* single unit recordings in  
153 anesthetized or freely moving animals (Cao et al., 2012; Lançon et al., 2021; Sellmeijer et al., 2018;  
154 Zhang et al., 2017, 2021) have shown an increased ACC spontaneous firing activity associated with  
155 the expression of aversive-like behaviors, in different pain models. This shift towards ACC  
156 hyperactivity might be due to an imbalance between excitatory and inhibitory (E/I) neurons within the  
157 ACC. Recent *in vivo* studies, aiming to modulate ACC activity, suggest a primary role of the  
158 pyramidal cells in this imbalance (Bliss et al., 2016; Zhu et al., 2022). Indeed, cell-type specific opto-  
159 or chemogenetic inhibition of ACC glutamatergic neurons alleviates the aversiveness of ongoing pain  
160 without affecting mechanical allodynia in inflammatory and neuropathic pain models (Sellmeijer et  
161 al., 2018; Zhang et al., 2017, 2021). On the contrary, optogenetic activation of ACC pyramidal  
162 neurons in naive animals induces aversion (Tan et al., 2017; Zhang et al., 2017) similar to that of pain  
163 models.

164 On the other hand, according to some reports ACC hyperactivity may be due to a loss of GABAergic  
165 inhibitory control. Using a chemotherapy-induced neuropathic pain model, Juarez-Salinas and  
166 colleagues (2019) showed that the transplantation of inhibitory interneuron progenitor cells into the  
167 ACC was sufficient to restore a GABA-A mediated inhibitory control of host pyramidal cells and  
168 block aversion in the conditioned place aversion paradigm. Local administration of the GABA-A  
169 receptor agonist, muscimol, into the rostral ACC (rACC) also reduces aversion after nerve injury  
170 (LaGraize and Fuchs, 2007; Tang et al., 2005). To further dissect cell types involved in pain-related  
171 aversion, Zhang and colleagues (Zhang et al., 2021) focused on ACC neurons expressing the gamma  
172 isoform of the protein kinase C (PKC $\gamma$ ). They showed that chemogenetic activation of ACC PKC $\gamma$   
173 neurons induces mechanical hypersensitivity and aversion in naive animals, while in neuropathic pain  
174 condition these neurons are hyperexcitable and their inhibition blocks mechanical allodynia and  
175 aversiveness of ongoing pain.

176 Different pharmacological and non-pharmacological approaches modulating ACC activity were shown  
177 to be effective to alleviate the aversive component of chronic pain. For instance, a single dose of

178 ketamine induces a prolonged suppression of the ACC pyramidal neuron hyperactivity that is  
179 associated with a reduction of the aversive response to noxious stimuli in rodent pain models.  
180 Strikingly, this anti-aversive effect persists long after the anti-nociceptive effects of ketamine have  
181 ceased (Zhou et al., 2018). Interestingly, Gan and colleagues (2021) reported that repetitive  
182 transcranial direct current stimulation (r-tDCS) of the prefrontal cortex not only blocks aversion, but  
183 also mechanical and thermal nociceptive behaviors in a murine neuropathic pain model. This last  
184 effect could be explained by the fact that the application of repetitive transcranial direct current  
185 stimulation was not restricted to the ACC and might affect other prefrontal areas that are directly  
186 involved in nociception. In a similar manner, high frequency electrical stimulation of the ACC also  
187 rescues pain-related aversion, while low frequency stimulation increases it (Liu et al., 2021). Both  
188 invasive and non-invasive manipulations of ACC seem to be a particular interest to alleviate the pain  
189 related aversion in a translational perspective.

190 Altogether, these findings demonstrate the importance of the ACC I/E activity balance in the  
191 development of pain-related aversion, but also the complexity of the functional alterations induced by  
192 chronic pain in the ACC. Further experiments are required to decipher which mechanism comes first:  
193 an increase in excitatory cell activity followed by a dysregulation of the E/I balance or an alteration of  
194 local inhibitory circuits triggering ACC hyperactivity.

## 195 **2.2 ACC circuits**

196 Besides ACC microcircuits, its macrocircuits are also involved in pain-induced aversion. Considering  
197 ACC afferents, inhibition of the mediodorsal thalamic nucleus projections to the ACC induces  
198 conditioned place preference in animals with nerve injury, while the activation of this pathway or the  
199 inhibition of BLA afferents to the ACC exacerbates aversion (Meda et al., 2019). **Similarly,**  
200 **chemogenetic activation of LC neurons projecting to the ACC exacerbates ongoing pain and produces**  
201 **aversion, in a neuropathic pain model (Hirschberg et al., 2017) without any effect in naive mice.** In  
202 regard to cortico-cortical connections, Singh and colleagues demonstrated that the optogenetic  
203 activation of the primary somatosensory cortex increased the proportion of ACC neurons that respond  
204 to noxious stimuli, suggesting that somatosensory afferents reinforce nociceptive response in the ACC  
205 (Singh et al., 2020). They further observed an enhanced connectivity between these two cortical areas  
206 in chronic pain state and showed that the optogenetic inhibition of this pathway effectively relieved  
207 the aversive component of chronic pain.

208 Concerning ACC efferents, using a chemogenetic approach, Gao and colleagues (2020) showed that  
209 inhibition of CaMKII pyramidal ACC neurons projecting to the nucleus accumbens or the VTA  
210 induced a preference for the compartment associated with the inhibition in neuropathic rats, while  
211 activation of these pathways induced aversive behaviors in non-pathological animals, suggesting this  
212 circuit plays an important role of in pain-related aversion.

213 Overall, many studies demonstrate that the modulation of ACC circuits has no or trivial effects in  
214 naive animals, while the manipulation of these specific circuits in chronic pain conditions either  
215 prevents or exacerbates pain-related aversion, thus strengthening the hypothesis that ACC circuits are  
216 deeply impacted by chronic pain.

### 217 **2.3 Molecular and cellular changes**

218 All the aforementioned alterations are most likely accompanied by important molecular and cellular  
219 changes. In that regard, an increasing number of preclinical studies brought new knowledge about  
220 molecular mechanisms underlying ACC hyperactivity and its role in aversion. For instance, several  
221 groups associated pain related aversion with an increase in brain derived neurotrophic factor (BDNF),  
222 its receptor TrkB, phosphorylated ERK (pERK), phosphorylated mTOR (p-mTOR) and mitogen-  
223 activated protein kinase (MAPK) in the ACC in different pain models (Cao et al., 2014, 2009; Galan-  
224 Arriero et al., 2014; Han et al., 2014; Li et al., 2022; Lu et al., 2016; Thibault et al., 2014; X. Wang et  
225 al., 2017; Zhang et al., 2016, 2018). Manipulation of one of these targets thus impacts aversive-like  
226 behaviors in both naive animals and those suffering from chronic pain (Thibault et al., 2014). For  
227 instance, blocking the ACC TrkB receptors rescues the aversion observed in cancer pain (Li et al.,  
228 2022), while BDNF injection into the ACC is sufficient to induce aversion in naive animals (Thibault  
229 et al., 2014; Zhang et al., 2016). Interestingly, by blocking p-mTOR in the rACC after formalin  
230 injection, Zhang and colleagues (2018) did not only rescue pain-induced aversion, but also prevented  
231 NMDA (N-methyl D-aspartate) receptor 2B upregulation in the rACC, known to occur in pain  
232 conditions (Li et al., 2009), suggesting a complex interaction between different mediators.  
233 Accordingly, in line with the E/I imbalance hypothesis, blocking/silencing ACC NMDA subunits,  
234 with an antagonist or small interfering RNA, in inflammatory, neuropathic and cancer pain rescues  
235 pain-related aversion, but not mechanical hypersensitivity nor acute pain (Feng et al., 2016; Guo et al.,  
236 2015; Lei et al., 2004; Li et al., 2009; Zhou et al., 2018). Similarly, blocking D-serine, an endogenous  
237 ligand for NMDA receptors, either by enzymatic degradation or an antagonist of the glycine site of  
238 NMDA receptors, also attenuates conditioned place aversion produced by inflammatory pain (Ren et  
239 al., 2006), strengthening the role of glutamatergic signaling in pain-related aversion. Galan-Arriero  
240 and colleagues (2014) also showed an increase in ACC metabotropic Glu receptor 5 (mGluR5)  
241 expression in neuropathic rats. Accordingly, preventing this increase by either p38a MAPK inhibition  
242 or by glutamate receptor blockade in the ACC with kynurenic acid is sufficient to prevent aversion  
243 induced by inflammation, while mGluR5 stimulation in naive animals produces conditioned place  
244 aversion (Galan-Arriero et al., 2014; Johansen and Fields, 2004). Kang and colleagues also showed  
245 that inhibiting ACC glutamatergic cells in their inflammatory model induces conditioned place  
246 preference (Kang et al., 2017). Collectively, these results confirm the crucial role played by the  
247 glutamatergic system in pain-related aversion and seem to indicate that chronic pain-induced

248 molecular alterations facilitate the shift towards ACC hyperactivity by increasing the glutamatergic  
249 tone, in various pain models.

250 Alongside the classic excitatory and inhibitory systems, several neuromodulators have been described  
251 as important in pain-related aversion. For instance, in neuropathic rats, bilateral administration of  
252 morphine in the rACC is sufficient to produce conditioned place preference (Gomtsian et al., 2018;  
253 LaGraize et al., 2006; Navratilova et al., 2015), while the microinjection of the opioid antagonist,  
254 naloxone, in the ACC prevented morphine-induced place preference (Navratilova et al., 2015),  
255 suggesting a key role of the opioid system in aversive processes governed by the ACC. Accordingly,  
256 administration of delta opioid receptor agonists in the rACC prevents inflammatory pain-induced place  
257 aversion (Ma et al., 2022), while kappa opioid receptor antagonist blocks the electroacupuncture  
258 rescue of CFA-induced place aversion (Zhang et al., 2011).

259 The role of monoamine signaling such as serotonin (5HT) and dopamine has also been described in  
260 pain-related aversion. Santello and colleagues (2017) demonstrated that injection of a selective 5HT7R  
261 antagonist into the ACC reduces both mechanical hypersensitivity and aversion by enhancing the  
262 function of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels that are  
263 downregulated in ACC layer V neurons. In addition, it was shown that dopamine D1R agonists rescue  
264 the decreased activity of HCN channel in neuropathic mice, which in turn inhibits ACC hyperactivity  
265 (Lançon et al., 2021). Interestingly, chronic systemic L-DOPA or carbidopa treatments not only rescue  
266 ACC HCN channel function but also mechanical and cold hypersensitivity as well as pain-related  
267 aversion. Hence, besides the ACC E/I imbalance, several modulatory systems appear to be at play in  
268 chronic pain conditions, which might be additional targets for new treatment strategies. Interestingly,  
269 these vast alterations in diverse neurotransmission systems can be related to overexpression of ACC  
270 SIP30, one of many soluble N-ethylmaleimide-sensitive factor attachment protein receptors  
271 (SNAREs) which are essential for the regulation of neurotransmitter-containing synaptic vesicles  
272 exocytosis. Indeed, expression of SIP30 is modified by pain conditions and its local silencing reduces  
273 aversion without affecting mechanical hypersensitivity (Han et al., 2014). However, the exact nature  
274 of the neurotransmitters affected by SIP30 silencing has yet to be studied.

275 Finally, it is important to highlight that, as for most of the central nervous system pathologies, neurons  
276 are most likely not the only cells altered by chronic pain. Some studies also point to  
277 neuroinflammation as well as non-neuronal cells as key factors for pain-related aversion. Indeed, an  
278 increase in astrocytic markers, such as GFAP/S100B, and inflammatory cytokines including IL-  
279 1B/TNF- $\alpha$ , have been reported in the ACC in inflammatory rat models (Chen et al., 2012; Lu et al.,  
280 2011) and local injection of an astroglia-specific toxin can prevent pain-induced aversion (Chen et al.,  
281 2012). Moreover, Wang and colleagues (Wang et al., 2020) showed increased immune system activity  
282 indicators in the ACC such as Kynurenine/Tryptophan ratio and indoleamine 2,3-dioxygenase (IDO)

283 enzyme for tryptophan catabolism to kynurenine in a neuropathic pain model and the inhibition of the  
284 latter was shown to reverse pain-related aversion as well as cold and mechanical hypersensitivity.

## 285 **2.4. Conclusions and limitations**

286 Overall, these recent findings (**Table 1**) demonstrate the essential but complex role of the ACC in  
287 pain-related aversion. Chronification of pain seems to trigger electrophysiological and  
288 molecular/cellular changes that lead to a progressive shift towards ACC hyperactivity. This critical  
289 alteration appears to be due to I/E imbalance but also putative contribution of neuromodulatory  
290 systems. However, further studies are necessary to unravel the exact mechanisms leading to ACC  
291 hyperactivity. Optogenetic and chemogenetic studies demonstrated that specific ACC afferents,  
292 mostly involved in sensory (somatosensory cortex, thalamus) and emotional (BLA) processing, and  
293 ACC efferents, notably the VTA or Nucleus Accumbens, participate in pain related aversion.  
294 Interestingly, most of the manipulations of these ACC-related circuits do not induce any effect in  
295 naïve animals, strengthening the view that alterations in these circuits are specific to pain condition,  
296 which underlines the importance of studying pain-induced alteration mechanisms at both  
297 microcircuitry and macrocircuitry levels.

## 298 **3. Role of the ACC in the anxiodepressive-like consequences of chronic pain**

299 In the past two decades, several paradigms addressing the anxiodepressive-like consequences of  
300 chronic pain were developed in rodents [(for detail see Kremer et al., 2021); (**Fig.3 and Box2**)]. In  
301 general, the evaluation of anxiety-like behaviors in rodents relies on the conflict between innate  
302 exploratory behaviors (exploring, feeding, and social behaviour) and innate fear (open lit areas,  
303 foreign objects or an unfamiliar individual). Hence, the most used tests are the elevated plus maze,  
304 open-field, light-dark box, social interaction or burrowing tests (**Fig.3 and Box2**). Regarding  
305 depressive-like behaviors, the currently available paradigms mostly assess despair-like [forced swim  
306 or tail suspension tests], anhedonia-like (sucrose preference test) behaviors or animals' well-being  
307 (splash, nest tests). Although these tests only partially evaluate the anxio-depressive symptoms  
308 observed in human, they present a clinical relevance with face and predictive validity (**Fig.3 and**  
309 **Box2**). For instance, in regard to anxiety, a similar thigmotaxic behavior has been described in human  
310 and rodent and associated with increased anxiety (Walz et al., 2016). In regard to pharmacological  
311 validity, Crawley and colleagues showed that the classical anxiolytic drugs, such as benzodiazepines,  
312 effectively reduce anxiety in the light/dark box tests in rodents (Crawley and Goodwin, 1980) while  
313 Porsolt and colleagues (1977) showed that tricyclic antidepressant reduces immobility time in the  
314 forced swim test. Hence, even though imperfect, these tests have brought and continue to bring  
315 valuable insights about neurobiological mechanisms implicated in psychiatric conditions.

316 One of the first studies demonstrating the involvement of the ACC in the anxiodepressive  
317 consequences of chronic pain came from Seminowicz and colleagues (2009), which showed, in rats, a  
318 correlation between decreased ACC volume and anxiety-like consequences of neuropathic pain using



319 **MRI in a longitudinal fashion.** Lesion studies confirmed these observations by showing that ACC  
320 ablation is sufficient to prevent both anxiety- (Zeng et al., 2018) and depressive-like behaviors  
321 (Barthas et al., 2015) in models of remifentanyl induced hyperalgesia and neuropathic pain  
322 respectively. More specifically, it was shown that optogenetic inhibition of ACC pyramidal neurons is  
323 sufficient to rescue neuropathic pain-induced anxiodepressive-like behaviors in mice (Sellmeijer et al.,  
324 2018). The next sections will discuss the most recent findings on the cellular and molecular alterations  
325 in the ACC as well as in its networks in the anxiodepressive-consequences of chronic pain.

### 326 **3.1 Electrophysiological changes and functional alterations**

327 **Similar to pain related aversion, anxiodepressive-like behaviors in the context of cutaneous**  
328 **mechanical stimulation (Matsumoto et al., 2020), neuropathic pain (Sellmeijer et al., 2018; Zhao et al.,**  
329 **2020), inflammatory pain (Ducret et al., 2022; Zhou et al., 2022) and visceral pain (Brenner et al.,**  
330 **2021; Ren et al., 2022; Zhang et al., 2014) has been associated with increased firing activity and**  
331 **excitability of ACC neurons. In particular, Sellmeijer and colleagues showed that this hyperactivity is**  
332 **absent before the development of depressive-like behavior but persists after the spontaneous recovery**  
333 **of mechanical hypersensitivity. Interestingly, inhibition of pyramidal neurons (Sellmeijer et al., 2018;**  
334 **Zhu et al., 2021) or activation of inhibitory neurons, especially parvalbumin expressing neurons (Ren**  
335 **et al., 2022; Shao et al., 2021a, 2021b), alleviate the anxiodepressive-like behaviors in neuropathic and**  
336 **inflammatory pain, strengthening the causal link between ACC hyperactivity and emotional**  
337 **dysregulation (Sellmeijer et al., 2018). Concurrently, these manipulations reduce pain induced ACC c-**  
338 **Fos levels and hyperexcitability of glutamatergic neurons (Shao et al., 2021a, 2021b). On the contrary,**  
339 **chemogenetic inhibition of ACC GABAergic interneurons in naive animals induces anxiety-like**  
340 **behaviors by enhancing ACC glutamatergic neurons excitability and reducing inhibitory synaptic**  
341 **transmission (Shao et al., 2021b). The next question is how does the information reach to the ACC**  
342 **and how is it then propagate to the efferent structures?**

### 343 **3.2 ACC circuits**

344 Although the literature deciphering the role of ACC circuits in anxiodepressive consequences of  
345 chronic pain is still sparse, Zhu and colleagues (2022) showed that chemogenetic activation of rACC  
346 excitatory neurons projecting to the ventrolateral PAG provokes mechanical hypersensitivity and  
347 anxiety-like behaviors, while the inhibition of this pathway prevents such symptoms in a neuropathic  
348 pain model. Similarly, while chemogenetic activation of the ACC-thalamus pathway induces anxiety-  
349 like behaviors in naive rats, the chemogenetic inhibition of this pathway rescues anxiety-like  
350 behaviors in a CFA rat model (Shen et al., 2021). Concerning ACC afferents, Zhu et al. (2021)  
351 identified two discrete glutamatergic neuronal circuits: one projection from the posterior thalamic  
352 nucleus to primary somatosensory cortex glutamatergic neurons mediating mechanical  
353 hypersensitivity and another one from the parafascicular thalamic nucleus onto both inhibitory and  
354 excitatory ACC neurons respectively and involved in depressive-like behavior induced by chronic

355 pain. The connection between the ACC and the paraventricular nucleus of the hypothalamus also plays  
356 a role in the comorbidity between neuropathic pain and anxiety, as the optogenetic activation of the  
357 paraventricular nucleus neurons projecting to the ACC reduces both mechanical hypersensitivity and  
358 anxiety-like behaviors (Li et al., 2021a). Since the ACC receives a strong projection from the BLA,  
359 Becker and colleagues (2022) studied the role of this pathway in the anxiodepressive-consequences of  
360 neuropathic pain and showed that optogenetic inhibition of the BLA-ACC pathway rescues  
361 depressive-like behavior induced by neuropathic pain while the chronic activation of this pathway in  
362 naive mice mimics depressive-like behaviors. Finally, midbrain projections to the ACC also play a  
363 critical role as chemogenetic inhibition of the ACC inputs from the LC -the principal brain region  
364 secreting noradrenaline- is sufficient to reverse depressive-like behaviors in a rat neuropathic pain  
365 model (Llorca-Torrallba et al., 2022).

366 Hence, as for pain-related aversion, ACC sub-circuits are likely involved in the development of the  
367 anxiodepressive consequences of chronic pain. Indeed, in general, artificial stimulation of various  
368 ACC afferents can trigger anxiodepressive behavior in naïve animals, while inhibition of the same  
369 pathways has beneficial effects in chronic pain models. Nevertheless, further studies are required to  
370 precisely map and understand the role of different circuits in anxiety- and depressive-like  
371 consequences of pain.

### 372 **3.3 Molecular and cellular changes**

373 All the electrophysiological alterations at the ACC or within ACC circuits imply important molecular  
374 and cellular changes. For instance, the intracellular MAPK/ERK pathway is altered in a model of  
375 neuropathic pain, which contributes to the anxiodepressive-like consequences of pain (Barthas et al.,  
376 2017; Humo et al., 2020; Xiao et al., 2021). This finding is confirmed by a genome-wide study  
377 reporting that the ACC MAPK signaling pathway was one of the most significantly altered at both  
378 early and late phase of neuropathic pain (Barthas et al., 2017). In another transcriptomic study, Su and  
379 colleagues (2021) showed that most of the genes differentially expressed in the ACC in neuropathic  
380 pain were related to anxiety and depression rather than nociception itself, confirming the crucial role  
381 of the ACC in emotion. In accordance, the expression of MAPK phosphatase 1 (MKP-1), a negative  
382 regulator of this pathway, dephosphorylating downstream proteins such as pERK and phosphorylated  
383 CREB (pCREB), was robustly increased in neuropathic pain (Barthas et al., 2017; Humo et al., 2020;  
384 Wen et al., 2022) and blocking MKP-1 with an antagonist or knocking it down rescued or prevented  
385 anxiodepressive-like consequences (Barthas et al., 2017; Humo et al., 2020). Strikingly, it was also  
386 shown that classical and fast-acting antidepressant drugs such as fluoxetine and ketamine can  
387 normalize ACC MKP-1 levels. Besides MKP-1, increased levels of ACC pERK (Dai et al., 2011; Luo  
388 et al., 2015; Pereira-Silva et al., 2020; Shao et al., 2015; Zhong et al., 2012), decreased levels of  
389 pCREB and BDNF (Bravo et al., 2012; Cong et al., 2021; Imbe and Kimura, 2017; Ishikawa et al.,  
390 2014, 2015; Pan et al., 2018; Yasuda et al., 2014) were reported in animals showing anxiety- and



391 depressive-like behaviors following chronic pain induction. More specifically, in both visceral and  
392 incisional pain, the expression of the ACC pERK increases biphasically (Dai et al., 2011; Zhong et al.,  
393 2012). During the first phase, both allodynia and anxiety-like behaviors are rescued by pERK  
394 inhibition, while in the second phase this inhibition only affects anxiety-like behaviors (Dai et al.,  
395 2011; Zhong et al., 2012). Interestingly, stimulating BDNF synthesis, via 4-methylcatechol intra-  
396 cerebroventricular injection -increases ACC BDNF levels- decreases ACC pERK levels and prevents  
397 depressive-like behaviors in neuropathic pain (Ishikawa et al., 2014). Either knockdown or systemic  
398 injection of a CREB inhibitor rescues mechanical and heat hypersensitivity, anxiety-like behaviors, as  
399 well as CREB, pCREB and BDNF levels in a neuropathic rat model. Different treatment strategies  
400 used for pain and mood disorders, such as imipramine (Ishikawa et al., 2014), neurotrophin (Ishikawa et  
401 al., 2015) or ketamine (Pan et al., 2018), also recruit the MAPK/ERK pathway by altering ACC  
402 pERK, pCREB and BDNF levels. The inhaled anesthetic sevoflurane also impacts ACC pERK levels  
403 in rats and has a positive effect on the nociceptive and anxiety components of inflammatory pain (Luo  
404 et al., 2015). On the contrary, intravenous anesthetics such as propofol and sodium pentobarbital do  
405 not produce the same effect and only block the nociceptive response (Luo et al., 2015). Besides  
406 pharmacological agents, paramedical approaches such as manual acupuncture and electroacupuncture  
407 were shown to alleviate mechanical hypersensitivity and anxiodepressive-like behaviors (Cong et al.,  
408 2021; Shao et al., 2021a, 2015; Shen et al., 2021; Zhu et al., 2022), as well as pERK overexpression  
409 (Shao et al., 2015) and BDNF/CREB downregulation (Cong et al., 2021), respectively.

410 Glutamatergic signaling and its receptors also play a critical role in the pain/anxiodepressive dyad (Du  
411 et al., 2017; Guo et al., 2018; Sun et al., 2016; Wang et al., 2019; Zeng et al., 2018). Indeed, the  
412 expression of ACC AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) and  
413 NMDA receptors were increased in inflammatory (Du et al., 2017; Sun et al., 2016; Wang et al.,  
414 2020), visceral (Ren et al., 2022; Wang et al., 2019) and remifentanil-induced pain (Zeng et al., 2018).  
415 AMPA-R/NMDA-R ratio was reduced in the rACC of an inflammatory-pain model (Ducret et al.,  
416 2022) and blockade of both ACC AMPA-R and NMDA-R -using either CNQX or AP-5- reversed  
417 mechanical hyperalgesia in a chronic pancreatitis model (Ren et al., 2022). Moreover, injection of an  
418 antagonist AMPA-R subunit pGluR1 in the ACC prevented anxiety-like behaviors and remifentanil-  
419 induced hyperalgesia and also decreased ACC c-Fos overexpression (Zeng et al., 2018). Along with  
420 GluR1, several groups also reported dysregulation of other related proteins such as increased levels of  
421 protein kinases PKCzeta and PKMzeta (Du et al., 2017) or CPEB1 (Cytoplasmic Polyadenylation  
422 Element Binding Protein 1) in the ACC (Wang et al., 2019). Interestingly, blocking PKCzeta or  
423 knocking down CPEB1 alleviated pain and anxiety-like behaviors as well as increased GluR1 levels.  
424 On the other hand, it was reported that mGluR5 expression was decreased in a model of inflammatory  
425 pain (Guo et al., 2018) and positive allosteric modulation of mGluR5 alleviated both CFA-induced  
426 anxiety-like behaviors and allodynia. These findings suggest a differential role for AMPAR, NMDAR  
427 and mGluR5 in the emotional dysregulation. Extracellular alterations might trigger or facilitate

428 intracellular changes. Li and colleagues (2021) indeed showed, in neuropathic pain, a downregulation  
429 of LAMB1 -a key component of the extracellular matrix with a dual role in tissues architecture and  
430 cell signaling- that accompanies anxiodepressive-like behaviors. These results suggest a yet  
431 understudied role of the extracellular matrix in chronic pain and its emotional consequences. Indeed,  
432 such extracellular pain-induced modifications might lead to strong alterations in neurotransmission.

433 Besides neurons, glial cells are also involved in the anxiodepressive-consequences of chronic pain.  
434 Indeed, inflammatory (Sun et al., 2016) and neuropathic pain (Wang et al., 2020) induce microgliosis,  
435 astrogliosis and an increased proinflammatory cytokine expression such as TNF- $\alpha$  and IL6 levels in  
436 the ACC. Imaging studies further support alterations in glial cells. Using manganese-enhanced  
437 magnetic resonance imaging, McIlwrath and colleagues showed increased T1 and T2 signals and  
438 bilateral astrogliosis within the ACC (McIlwrath et al., 2020). The implication of glial cells and  
439 cytokines in the anxiodepressive-like consequences of chronic pain was also supported by Wang and  
440 colleagues (2020) showing that chronic IDO1 inhibitors not only alleviate mechanical and thermal  
441 hypersensitivity, but also prevent anxiety-like behaviors by blocking cytokines and microglial  
442 activation.

443 Serotonergic signaling also plays an important role, since injection of the selective 5HT reuptake  
444 inhibitor paroxetine -commonly used as an antidepressant drug- in the ACC rescued anxiodepressive  
445 consequences, but not mechanical hypersensitivity, in animals with neuropathic pain (Matsuzawa-  
446 Yanagida et al., 2008). In addition, Zhou and colleagues (2022) described lower 5HT levels of animals  
447 with inflammatory pain and demonstrated that knockdown of 5HT1A-R in the ACC of naive animals  
448 mimicked anxiety-like behaviors, while 5HT7-R knockdown induced mechanical hypersensitivity,  
449 which points to a dual and differential role for 5HT signaling in chronic-pain induced emotional  
450 disorders. Likewise, chronic cannabidiol treatment was shown to rescue mechanical, heat and cold  
451 hypersensitivity as well as anxiety-like behaviors and increases cannabidiol receptor (CB1-R)  
452 expression in the ACC (Silva-Cardoso et al., 2021). Finally, oxytocin also seems to play a role in the  
453 emotional consequences of neuropathic pain as an acute administration of oxytocin in the ACC  
454 attenuated mechanical hypersensitivity and anxiety-like behaviors. Interestingly, this effect was shown  
455 to act through a reduction E/I ratio in the ACC, with a greater enhancement of inhibitory transmission  
456 due to the reduction of evoked excitatory postsynaptic current amplitude concurrently with  
457 depolarization and increased firing rate of interneurons (Li et al., 2021a).

### 458 **3.4 Conclusions and limitations**

459 **The studies focusing on the role of the ACC in anxiety- (Table 2) and depressive-like (Table 3)**  
460 **consequences of chronic pain revealed multiple common mechanisms with pain related aversion such**  
461 **as E/I imbalance, MAPK/ERK pathway dysfunction (Fig.4). It is thus interesting to further study one**  
462 **hand time-dependent development of these cellular and molecular alterations and on the other hand**  
463 **their implication in the close relation between aversion and mood disorders. The limitations of these**

464 studies base on the fact that sex differences, different type and stage of chronic pain are frequently  
465 neglected. Indeed, almost all studies performed in male, they are mainly use inflammatory and  
466 neuropathic pain models and time-dependent development of pathology is rarely taken into  
467 consideration.

468 -----Fig. 4-----

#### 469 **4. Sex differences, where are we now?**

470 It is important to highlight that the aforementioned studies were mainly performed in male rodents  
471 even though the study of chronic pain and emotional disorders in females is clinically relevant, as  
472 female patients represent 55% of the chronic pain population (Rikard, 2023) and they are twice more  
473 likely to develop anxiety or depression compared to male patients (Gater et al., 1998; Kessler et al.,  
474 1994, 1995; Weissman et al., 1994, 1996). Although sex differences are widely studied in clinical  
475 settings, this is still not the case for preclinical research (Beery and Zucker, 2011; Karp and Reavey,  
476 2019). For example, in the journal PAIN, almost 4 papers out of 5 studied male animals only, while  
477 5% papers studied both sexes without analyzing possible sex differences (Mogil, 2012). The studies  
478 presented in this review were no exception, as only 6 of them were performed either in both sexes (Dai  
479 et al., 2022; Jarrin et al., 2020; Li et al., 2021a, 2021b; Michailidis et al., 2021) or in females only  
480 (Zhong et al., 2012). While most of the studies reproduced the effects observed in male in females,  
481 there are some studies showing striking differences between the two sexes. For instance, Zhong and  
482 colleagues (2012) reported similar results in female compared to male animals (Dai et al., 2011)  
483 concerning the role of pERK in anxiety-like behavior of chronic pain. Similarly, there were no  
484 differences between females and males when looking at LAMB1 expression in the ACC following  
485 spared nerve injury (Li et al., 2021b) or mode of action of oxytocin in the ACC (Li et al., 2021a). In  
486 contrary, a recent pioneer study from Jarrin and colleagues (Jarrin et al., 2020) compared the role of  
487 ACC glutamatergic neurons in pain-related aversion in male and female rats in an inflammatory pain  
488 model and showed that the optogenetic activation of calmodulin-kinase II $\alpha$ -expressing excitatory  
489 neurons decreases formalin-induced nociceptive behaviors during conditioning without affecting  
490 formalin-induced place preference in males while this activation increases nociceptive behavior in  
491 females. However, the optogenetic inhibition of ACC glutamatergic neurons reduces pain-related  
492 aversion in both males and females without affecting the nociceptive behaviors. Similarly, a sex-  
493 dependent mechanism was reported in Michailidis and colleagues' study (2021), as they reported sex  
494 differences in depressive-like behaviors and microglial markers. Indeed, male mice displayed  
495 depressive-like behaviors earlier than female mice in a neuropathic pain mouse model and the authors  
496 observed an increased number of microglial cells in the contralateral ACC of female but not male  
497 mice. In addition, a transcriptomic study showed drastic differences between males and females 2  
498 weeks post neuropathic pain induction (Dai et al., 2022). This latest study further supports the need for  
499 thorough research including both sexes as sex-specific gene expression differences in control animals

500 have already been observed. This could explain the different responses to pain treatment strategies and  
501 emotional consequences in females and males. Even these few examples clearly show robust  
502 differences in the molecular, cellular and system-level mechanisms of pain processing in male and  
503 female.

## 504 **5. Future directions**

505 We believe that advancing our current understanding of chronic pain requires mechanistically explicit,  
506 hypothesis-driven, and clinically relevant models. In this review we highlighted some of the main  
507 findings over the last decades that have contributed to the present knowledge of brain mechanisms of  
508 chronic pain, and how such advances were possible due to a reverse translational research approach.  
509 We argue that this approach is essential in the chronic pain field in order to generate new scientific  
510 hypotheses, probe physiological mechanisms, develop therapeutic strategies and translate findings  
511 back into promising human clinical trials.

512 Even though the studies that we discussed in this present review strongly support the idea that the  
513 ACC is a critical hub and is hyperactive when animals experience pain-related aversion or  
514 anxiodepressive-like behaviours; further research is needed to fully understand how local ACC  
515 alterations occur and what cell types are involved. Most of the studies described the role of excitatory  
516 cells but the exact implication of inhibitory neurons in the ACC E/I imbalance must be studied further.  
517 Indeed, compensatory mechanisms, which could explain such a shift in specific cell type activity,  
518 cannot be excluded. Moreover, our knowledge concerning the role of the ACC extracellular matrix or  
519 non-neuronal cells, such as astrocytes and microglia, is still limited (Kuzumaki et al., 2007; Narita et  
520 al., 2006; Panigada and Gosselin, 2011) and should be further explored.

521 Beside local mechanisms, the pain-induced shift of the ACC E/I balance toward a hyperactive state  
522 likely affects the whole ACC connectome, which likely contributes to the development and  
523 maintenance of pain-induced emotional disorders. Hence, it appears essential to understand how ACC  
524 alterations affect its whole network but also how alterations within brain areas that belong to the ACC  
525 network directly or indirectly affect the ACC as well. Thanks to recent technological advance in brain  
526 imaging and brain activity manipulation, we have started to decipher the role of the ACC-related  
527 connectome in the emotional consequences of chronic pain (Becker et al., 2020; Gao et al., 2020;  
528 Hirschberg et al., 2017; Li et al., 2021a; Llorca-Torralba et al., 2022; Meda et al., 2019; Shen et al.,  
529 2021; Singh et al., 2020; Zhu et al., 2022; Zhu et al., 2021). The most recent breakthrough advances in  
530 multi-site fiber photometry (Sych et al., 2019), as well as functional magnetic resonance imaging  
531 (McIlwraith et al., 2020; Van der Linden et al., 2007), will now enable large scale brain circuits and  
532 will likely bring valuable insight regarding ACC networks. In addition, these techniques provide  
533 necessary tools for longitudinal studies, from pain initiation to the development of pain-induced  
534 emotional disorders, for description of subtle and time-dependent brain dynamic changes, as well as  
535 new potential targets for the development of personalized treatments of emotional disorders in patients

536 suffering from chronic pain. Finally, further studies are needed to better understand the sex-specific  
537 mechanisms implicated in pain related aversion as well as its anxiodepressive-consequences.

538

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1110 -----Table 2-----

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**Box 1. Chronic pain models cited in this review.**

Neuropathic pain	Inflammatory pain	Visceral pain
<i>Bone cancer pain (BCP)</i> : injection of cultured cancer cells into the tibial bone marrow	<i>Chronic facial inflammatory (CFI)</i> : injection of CFA in the vibrissae pad	<i>Acetic acid</i> : intraperitoneal acetic acid injection
<i>Chemotherapy induced</i> : administration of chemotherapeutics	<i>Complete Freund adjuvant (CFA)</i> : hindpaw injection of CFA	<i>Chronic pancreatitis</i> : intraductal administration of trinitrobenzene sulfonic acid
<i>Chronic constriction injury (CCI)</i> : ligation of the sciatic nerve	<i>Formalin</i> : hindpaw injection of formalin	<i>Colitis (DSS)</i> : dextran sulfate sodium in the water
<i>CCI-ION</i> : CCI of the infraorbital nerve	<i>Monoarthritis</i> : injection of CFA in the tibiotarsal joint	<i>Colorectal distension (CRD)</i> : application of pressure inside the colon and rectum with saline
<i>Common peroneal nerve ligation (CPNL)</i> : ligation of the common peroneal nerve		<i>Irritable bowel syndrome (IBS)</i> : zymosan injection
<i>Complete axotomy</i> : removal of a small part of the sciatic and saphenous nerves		
<i>Cuff</i> : compressing the main branch of the sciatic nerve by a polyethylene tube		
<i>Chronic constriction of multiple dorsal root ganglia (CCD)</i> : compression of the DRG of three lumbar nerves		
<i>Partial axotomy</i> : removal of a small part of the sciatic nerve		
<i>Partial sciatic nerve ligation (PSNL)</i> : ligation of half of the sciatic nerve		
<i>Spinal nerve injury (SNI)</i> : ligation of two branches of the sciatic nerve		
<i>Spinal nerve ligation (SNL)</i> : ligation of the spinal nerve of one or more spinal ganglia		
<i>Tibial nerve transection (TNT)</i> : transection of the tibial nerve		
<i>Postsurgical</i> : incision of the skin, deep tissues and muscle of the hindpaw		

NB: this list is non-exhaustive

**Box 2. Behavioral paradigms used to assess aversion, anxiety- and depressive-like behaviors in rodents.**

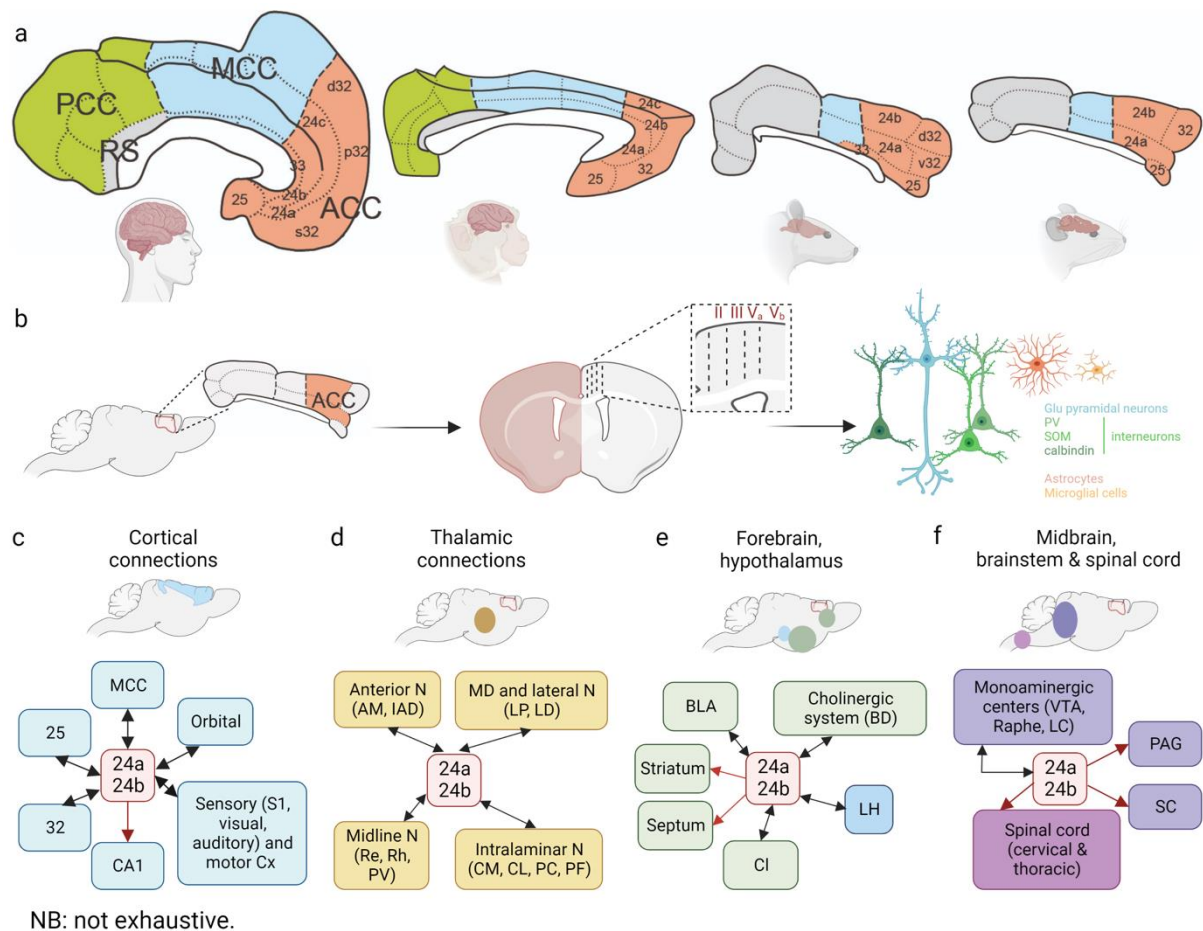
Behavior tested	Test	Principles	Variables	Interpretation	Clinical relevance
<b>Aversive behaviors</b>	<b>Place conditioning (preference/aversion)</b>	The animal is usually injected with an anti-nociceptive or a pro-nociceptive drug and restricted in one compartment during the conditioning, while the other compartment is associated with saline. On the test day, it can access freely both compartments.	Time spent in one specific compartment	Preference/reward or aversion	Interrogate neurobiological processes at play in the integration of stimuli with positive (reward) or negative (punishment) valence
	<b>Place escape/avoidance paradigm</b>	The animal is placed in a dark/light apparatus. It is subjected to a noxious stimulation in the dark "safe" compartment and to a non-noxious stimulation in the light "unsafe" compartment. It is let to explore freely.	Time spent in one specific compartment after a noxious stimulation associated with one of the two compartments	Aversion	
<b>Anxiety-like behaviors</b>	<b>Light/dark box</b>	The animal is placed in an apparatus consisting in a light compartment (>1000 lux) and a dark compartment (<5 lux) and is let to explore freely.	Time spent in the lit or dark compartment	Anxiety	Interrogate neurobiological processes at play in the evaluation of the aversiveness of a given situation
	<b>Open field</b>	The animal is placed in a box of 50x50cm and allowed to move freely within it.	Time spent in different zones (center vs periphery)	Anxiety Exploratory behaviors Locomotion / motor functions	
	<b>Social interaction</b>	The animal is in the middle of a three chambers apparatus. One chamber has an empty cage and the other has a conspecific. The animal is left to explore freely.	Time spent interacting with a conspecific	Sociability Anxiety	Evaluate how a social species reacts to social stimuli and can potentially reveal hypo sociability or social avoidance
	<b>Burrowing test</b>	The animal is placed within a cage filled with sawdust on top of which marbles are placed.	Burrowing behavior (# of item burrowed)	Anxiety	Interrogate neurobiological processes at play when individuals face a potential threat in a familiar environment
<b>Depressive-like behaviors</b>	<b>Forced swim test</b>	The animal is placed within a container filled with water with no possibility to escape.	Time spent mobile vs immobile	Despair Stress coping	Interrogate neurobiological processes at play when individuals face a life-threatening situation
	<b>Tail suspension test</b>	The animal is suspended by its tail with no possibility to escape.	Time spent mobile vs immobile	Despair Stress coping	
	<b>Sucrose preference test</b>	One bottle filled with water and another with a sucrose solution are proposed to the animal.	Water vs sucrose consumption	Anhedonia	Interrogate neurobiological processes at play in the integration when individuals have the choice between a natural reward vs a neutral item
	<b>Splash test</b>	The animal's coat is sprayed with a sucrose solution.	Time spent grooming	Interest in self-centered activities	Interrogate neurobiological processes at play in self-centered behaviors
	<b>Nest test</b>	The animal is placed within a new home cage and provided with nesting material.	Quality of the nest	Interest in self-centered activities	





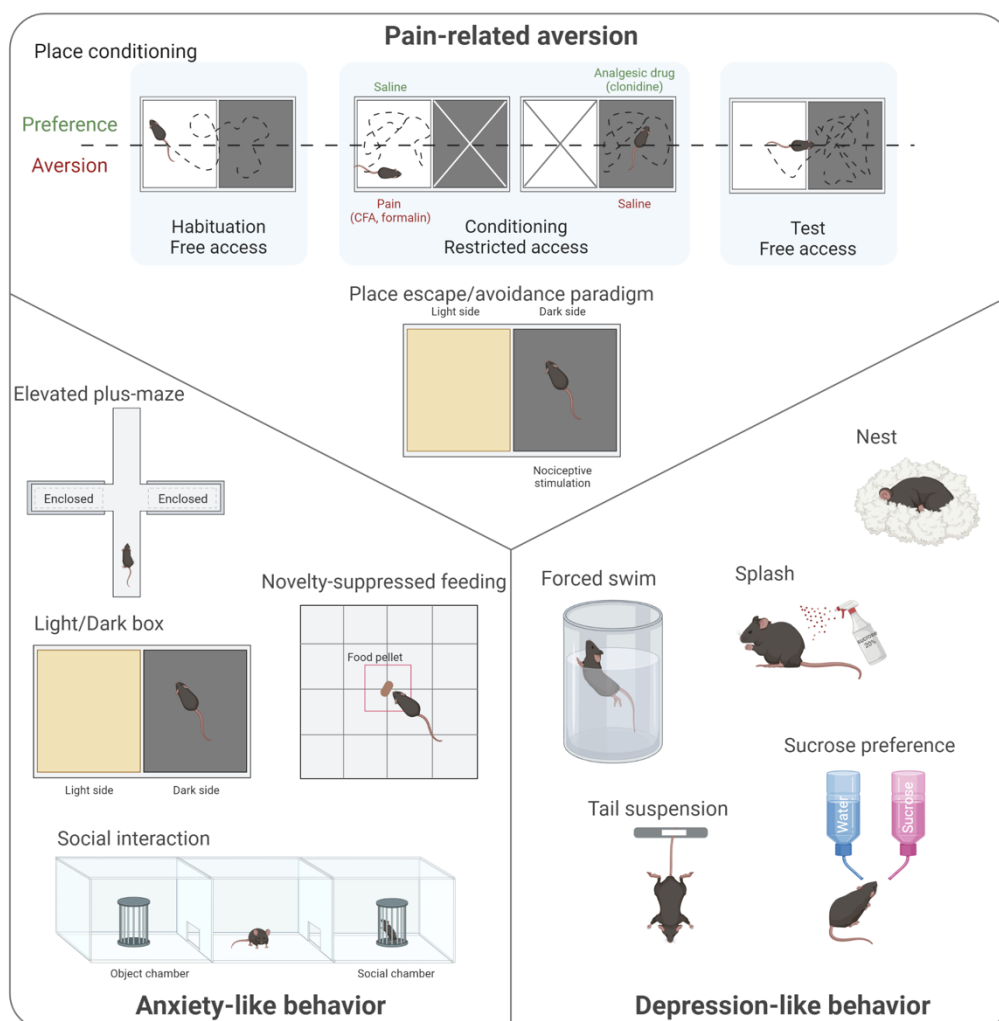
**Fig.1 Search and analysis strategies.**

PubMed was used to search for original studies using a combination of the following terms: [Pain] AND [Aversion OR Anxiety OR Depression] AND [ACC OR Cingulate OR Anterior Cingulate Cortex OR PFC OR Prefrontal Cortex]. Clinical data, reviews and irrelevant publications were excluded. Numbers in light colors (yellow, green and blue) correspond to the total amount of articles for each specific key word combination. Numbers in white correspond to the papers used in this review. Overlapping papers among different topics are indicated in the intersections. *Abbreviations: ACC, anterior cingulate cortex; PFC, prefrontal cortex.*



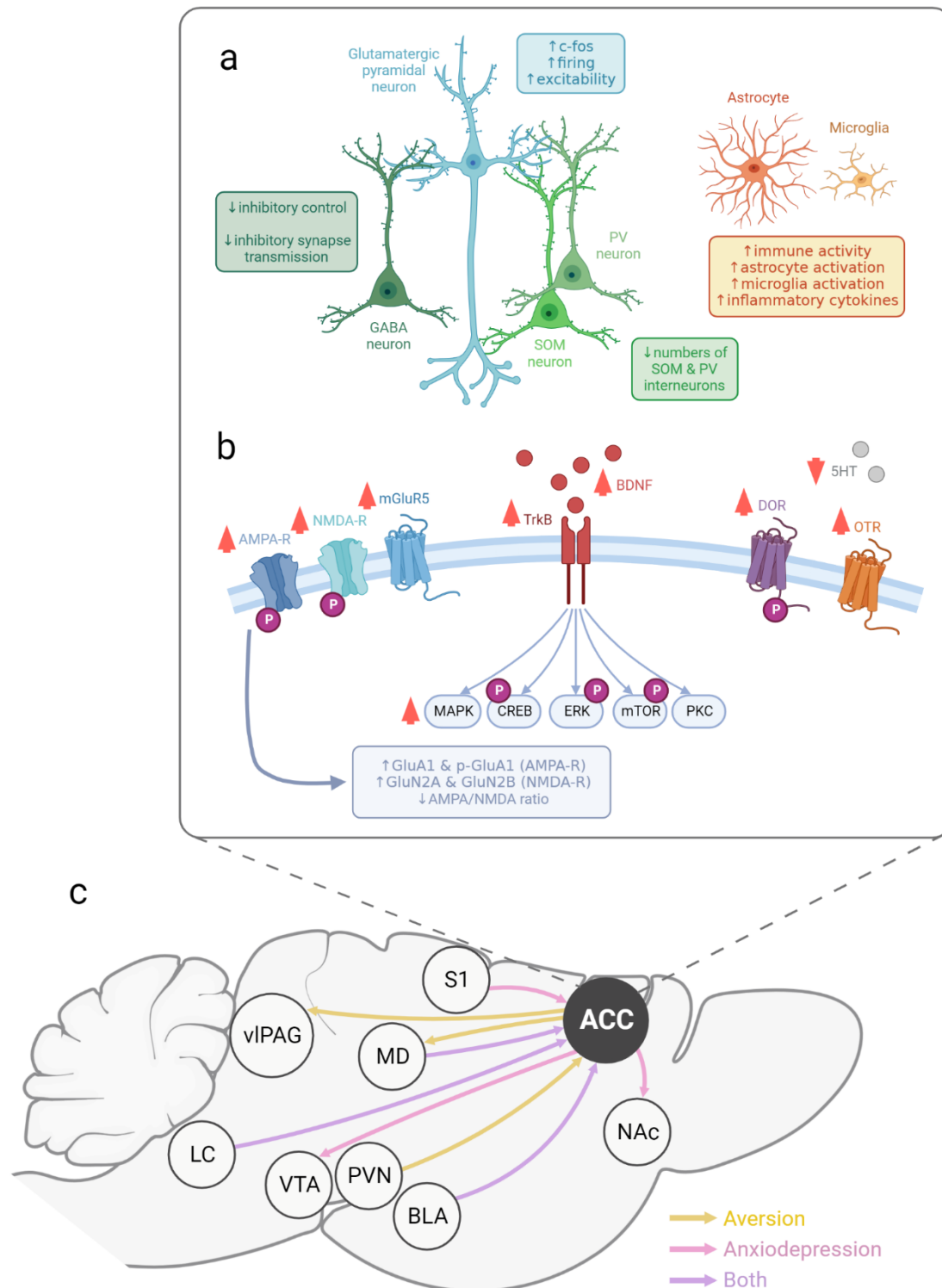
**Fig.2 Neuroanatomical and neurochemical description of the ACC.**

(a) Graphical representation of the cingulate cortex across species, composed by the retrosplenial (RS; grey), posterior (PCC; green), middle (MCC; blue) and anterior cingulate cortex (ACC; orange). (b) Specifically, the rodent ACC is an agranular cortical region comprising the Brodmann areas 24a and 24b, 25 and 32, lacking a true layer IV (middle panel) and composed by multiple different cell types (right panel). Concerning its neurochemical identity, beside the vast majority of glutamatergic neurons (80%), it contains PV, SOM and calbindin GABAergic neurons and non-neuronal cells, such as microglia and astrocytes. (c-f) The areas 24a and 24b of the ACC have a wide connectome that can be divided into 4 sub-categories. The ACC cortical connections (c; light blue) are mostly reciprocal (double arrows), such as its connections with the thalamus (d; gold) and the forebrain (e; light green) and the hypothalamus (e; blue). The ACC has also reciprocal connections with the monoaminergic centers and sends projections to the PAG, SC (violet) and the spinal cord (purple), mostly to the cervical and thoracic levels (f). *Abbreviations: ACC, anterior cingulate cortex; AM, anteromedial nucleus; BD, diagonal band of Broca; BLA, basolateral nucleus of the amygdala; CA1, hippocampal subfield 1; CL, centrolateral N; Cl, claustrum; CM, central medial N; Cx, cortex; Glu, glutamatergic; IAD, interanterodorsal N; LC, locus coeruleus; LD, laterodorsal N; LH, lateral hypothalamus; LP, lateroposterior N; MCC, midcingulate cortex; MD, mediodorsal N; N, nucleus; PAG, periaqueductal gray; PC, paracentral N; PCC, posterior cingulate cortex; PF, parafascicular N; PV, parvalbumin; Re, N reuniens; Rh, rhomboid N; SC, superior colliculus; SOM, somatostatin; VTA, ventral tegmental area.*



**Fig.3 Classical tests used to evaluate pain-related aversion, anxiety- and depressive-like behaviors in rodents.**

Numerous behavioral tests were developed to test pain-related aversion (upper panel), anxiety- (lower left panel) and depressive-like (lower right panel) behaviors in rodents. Most of the pain-related aversion tests are based on the preference that animals experiencing pain develop toward an analgesic drug paired with a specific area (conditioned place preference) or the avoidance of pain-associated environments (conditioned place avoidance and place escape/avoidance paradigm). Tests assessing anxiety-like behaviors rely on the natural conflict between innate behaviors (exploration, feeding, and socialization) and innate fear (open lit areas, foreign objects, unfamiliar individual). Paradigms evaluating depressive-like behavior in rodent assess either self-centered behaviors, such as nest building or grooming behaviors or despair-like behaviors as in the tail suspension or forced swim tests. In regard to anhedonia, the most common test so far is the sucrose preference test that measures the preference for a sweet solution (1 to 5% sucrose solution) compared to water. *Abbreviations: CFA, complete Freund adjuvant.*



**Fig.4 Illustration of the chronic pain-induced molecular and cellular alterations observed in the ACC and its network.**

(a) Chronic pain triggers an E/I imbalance shifting the ACC activity toward hyperactivity. Several studies reported increased c-Fos expression, enhanced activity and excitability in ACC pyramidal cells. On the contrary, a loss of inhibitory control has also been observed in chronic pain models, with a decrease in

transmission at inhibitory synapses. Morphological alterations were also reported, notably a diminution of the number of SOM and PV neurons, in chronic pain conditions. (b) These functional alterations are concomitant with pain-induced molecular modifications including glutamatergic receptor sub-units (AMPA, NMDA, mGluR5) overexpression, MAP/ERK, mTOR and PKC signaling pathway overactivation as well as alterations in opioid, oxytocinergic and serotonergic systems. (c) All these alterations likely affect the whole ACC connectome. Different studies already highlighted the implication of specific ACC pathways in the chronic pain-induced emotional disorders, such as aversion (yellow), anxiety and depression (pink) or both (violet). *Abbreviations: ACC, anterior cingulate cortex; AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain derived neurotrophic factor; BLA, basolateral nucleus of the amygdala; CREB, c-AMP response element-binding protein; DOR, delta opioid receptor; ERK, extracellular signal-regulated kinases; GABA, gamma-aminobutyric acid; Glu, glutamate; LC, locus coeruleus; NAc, nucleus accumbens; LP, lateroposterior nucleus; MAPK, mitogen-activated protein kinase; MD, mediodorsal thalamus; mGluR5, metabotropic glutamate receptor 5; mTOR, mechanistic target of rapamycin; NMDA, N-methyl D-aspartate; OTR, oxytocin receptor; PKC, protein kinase C; PV, parvalbumin; PVN, paraventricular nucleus; R, receptor; SOM, somatostatin; S1, primary somatosensory cortex; TrkB, tropomyosin receptor kinase B; vlPAG, ventrolateral periaqueductal gray; VTA, ventral tegmental area; 5-HT: serotonin.*

**Table 1. Pain related aversion**

Species	Pain type	Models	Tests	Observation in the ACC in pain related aversion	Reference
Rats	NP, Inf	CCI, formalin	F-CPA	↑ pERK, PKA & pCREB	Cao et al., 2009
Rats	Visceral	CRD	CPA	↑ neuronal firing in response to colorectal distension	Cao et al., 2012
Rats	Inf	Formalin	F-CPA	↑ p38/MAPK & MKK3/6 activation in GABAergic neurons and microglia	Cao et al., 2014
Rats	Inf	CFA	PEAP	↑ GFAP levels	Chen et al., 2012
Rats	NP	SCI	PEAP	↑ mGluR5 & OX42 levels	Galan-Arriero et al., 2014
Mice	NP	SNI	CPP, PEAP	↑ activity	Gan et al., 2021
Rats	NP	CCI	CPP	neurons projecting to the NAc/VTA are hyperexcitable	Gao et al., 2020
Rats	NP	CCI	CPA	↑ ERK, PKA & CREB activation and SIP30 levels	Han et al., 2014
Mice, Rats	NP, Inf	SNI, postsurgical	CPP	↑ ACC pyramidal neurons excitability	Lançon et al., 2021
Rats	Inf	Formalin	F-CPA	↑ cFos+ cells in layers II-VI	Lei et al., 2004
Rats	Inf	Formalin	F-CPA	↑ NR2AR/NR2BR & cFos, ↑ amplitude of NMDA currents	Li et al., 2009
Rats	NP	BCP	CPA, PEAP	↑ BDNF levels	Li et al., 2022
Rats	Inf	Formalin	F-CPA	↑ GFAP/S100B (astrocyte), IL1b/TNFa (inf. cytokines) mRNA & protein	Lu et al., 2011
Rats	Inf	Formalin	F-CPA	↑ p-mTOR & p-p70S6K levels	Lu et al., 2016
Rats	Inf	CFA	CPA	↑ discharge frequencies in the CFA-compartment of CPA	Ma et al., 2022
Mice	NP	CCI	PEAP	↓ HCN channels in apical dendrites of layer V neurons	Santello et al., 2017
Mice	NP	Cuff	CPP	↑ firing, enhancement of excitatory synapse transmission	Sellmeijer et al., 2018
Rats	Inf	CFA	passive avoidance	↑ BDNF, pERK & BDNF+ cells	Thibault et al., 2014
Rats	NP	SNI	CPA	↑ BDNF, TrkB & pERK levels	Wang et al., 2017
Mice, Rats	Inf, NP	Formalin, SNL	CPP	↑ Kyn/Trp ratio, IDO1, GFAP, IL66, pNMDA2BR, pCDK5, pMAP2 & pTau levels and spine density	Wang et al., 2020
Rats	Inf	Formalin	F-CPA	↑ estrogen levels	Xiao et al., 2013
Rats	NP	SNI	CPP	↑ BDNF, TrkB & NR2B levels	Zhang et al., 2016
Rats	Inf	CFA	CPA	↑ firing rate post acute stimulus	Zhang et al., 2017
Rats	Inf	Formalin	CPA	↑ BDNF, p-mTOR, p-p70S6K & NR2B levels	Zhang et al., 2018
Mice	NP	CCI	CPP	↑ excitability, cfos and excitability of PKCy neurons	Zhang et al., 2021
Rats	Inf/NP	CFA, SNI	CPA	layer V/VI hyperactivity	Zhou et al., 2018

**Table 1. (continued)**

Species	Pain type	Models	Tests	Observation in the ACC in pain related aversion after manipulation	Reference
Mice	NP	Cuff	CPP	ACC lesion blocks pain relief (CPP)	Barthas et al., 2015
Rats	NP	BCP	CPA	NR2BR antagonist injection in the rACC rescues PRA	Feng et al., 2016
Rats	Inf	Formalin	F-CPA	ACC lesion reduces the magnitude of PRA	Gao et al., 2004
Rats	NP	SNL	CPP	Morphine injection in the rACC rescues PRA	Gomtsian et al., 2018
Rats	NP	SNL	CPA	Silencing of NR2BR in the rACC rescues PRA	Guo et al., 2015
Rats	NP	TNT	CPP	LC-ACC chemogenetic activation induces PRA	Hirschberg et al., 2017
Rats ♀	Inf	Formalin	F-CPA	Optogenetic inhibition of ACC Glu+ neurons reduces PRA	Jarrin et al., 2020
Rats	Inf	Formalin	F-CPA	Blockade of Glu-R in the rACC prevents PRA	Johansen & Fields, 2004
Rats	Inf	Formalin	F-CPA	rACC lesion rescues F-CPA	Johansen et al., 2001
Mice	NP	SNI, Chemotherapy	CPP	rACC lesion rescues F-CPA	Juarez-Salinas et al., 2019
Mice	Inf	CFA	CPP	Glu+ cells inhibition creates CPP, PV+ cells activation alleviates mechanical hypersensitivity	Kang et al., 2017
Rats	NP	SNL	PEAP	GABAA & GABAB agonist injection in the rACC attenuate PEAP	LaGraize and Fuchs, 2007
Rats	NP	SNL	PEAP	ACC lesion prevents PRA	LaGraize et al., 2004
Rats	NP	SNL	PEAP	Morphine injection in the ACC rescues PRA	LaGraize et al., 2006
Rats	Acute pain	Acute pain	CPA	ACC high frequency stimulation rescues PRA, while low frequency increases PRA	Liu et al., 2021
Mice	NP	SNI, chemotherapy	CPP	Optogenetic inhibition of MD-ACC pathway induces CPP, BLA-ACC activation elicits CPP	Meda et al., 2019
Rats	NP	Postsurgical, SNL	CPP	Injection of morphine in the rACC produces CPP	Navratilova et al., 2015
Rats	NP	Partial/complete axotomy, SNL	CPP	rACC lesion blocks pain relief (CPP)	Qu et al., 2011
Rats	Inf	Formalin	F-CPA	rACC degradation of d-serine & blockade of NMDA receptor attenuates F-CPA	Ren et al., 2006
Rats	Inf	CFA	CFA-CPA	ACC lesion improves PRA	Shi et al., 2020
Rats	Inf, NP	CFA, SNI	CPA, CPP	S1-ACC inhibition induces CPP	Singh et al., 2020
Mice	NP, Inf	SNI, CFA	CPA	ACC optogenetic stimulation leads to CPA	Tan et al., 2017
Mice, Rats	Acute pain	Acute pain	CPP	GABAA receptor agonist in the ACC reduces unpleasant pain memories	Tang et al., 2005
Rats	Inf	Formalin	F-CPA	GABAA blockade rescues PRA	Wang et al., 2005
Rats	Inf	Formalin	F-CPA	ACC lesion rescues PRA	Yi et al., 2011
Rats	Inf	CFA	CPA	rACC KOR antagonist injection blocks electroacupuncture rescue of CPA	Zhang et al., 2011

*Abbreviations: ACC, anterior cingulate cortex; BCP, bone cancer pain; BDNF, brain derived neurotrophic factor; BLA, basolateral nucleus of the amygdala; CCI, chronic constriction injury; CDK5, cyclin-dependant kinase 5; CFA, complete Freund adjuvant; CPA, conditioned place aversion; CPP, conditioned place preference; CRD, colorectal distension; CREB, cAMP response element-binding protein; ERK, extracellular signal-regulated kinase; GABA,  $\gamma$ -aminobutyric acid; GFAP, glial fibrillary acidic protein; Glu, glutamate; HCN, hyperpolarization-activated cyclic nucleotide-gated; IDO1, indoleamine 2,3-dioxygenase 1; IL, interleukin; Inf, inflammatory; F-CPA, formalin-induced CPA; KOR, kappa opioid receptor; Kyn, kynurenine; LC, locus coeruleus; MAPK, mitogen-activated protein kinase; MAP2, microtubule-associated protein 2; MD, mediodorsal thalamus; MKK, ???; mGluR5, metabotropic Glu receptor 5; mRNA, messenger ribosomal nucleic acid; mTOR, mammalian target of rapamycin; NAc, nucleus accumbens; NMDA, N-methyl D-aspartate; NP, neuropathic; NR2A, NMDA subunit NR2A; NR2B, NMDA subunit NR2B; OX, ??; MKK, ???; PK, protein kinase; PRA, pain-related aversion; PV, parvalbumin; R, receptor; rACC, rostral ACC; SIP30, SNAP25 interacting protein of 30; SCI, spinal cord injury; SNI, spinal nerve injury; SNL, spinal nerve ligation; S1, somatosensory cortex 1; TNF $\alpha$ , tumor necrosis factor alpha; TNT, tibial nerve transection; TrkB, tropomyosin receptor kinase B; Trp, tryptophan; VTA, ventral tegmental area; ♀, study realized in both males and females, if not specified, only males were used.*



**Table 2. Anxiety-like consequences of chronic pain**

Species	Pain type	Models	Tests	Observation in the ACC in pain-induced anxiety	Reference
Mice	NP	Cuff	NSF	↑ MKP1, pCREB, cFos and active epigenetic regulations on mkp1 gene	Barthas et al., 2017
Mice	Visceral	Colitis	OFT	↑ cfos levels	Brenner et al., 2021
Rats	NP, Inf	Postsurgical	EPM, OFT	↑ pERK1/2 levels biphasically in time	Dai et al., 2011
Rats	Inf	CFA	OFT, EZM	↑ PKCzeta, PKMzeta and GluR1 levels	Du et al., 2017
Rats	Inf	CFI	EPM, LD	↑ fos in layers II-III/V, ↓AMPA/NMDA ratio	Ducret et al., 2022
Mice	Inf	CFA	OFT	↓ number and function of mGluR5	Guo et al., 2018
Mice ♀	Inf, NP	SNI, CFA	EPM, OFT	↑ ECM genes expression, ↓LAMB1 gene expression	Li et al., 2021b
Mice ♀	NP	CPNL	EPM, OFT	↑ oxytocin receptor expression	Li et al., 2021a
Rats	Inf	Formalin	OFT	↑ pERK levels	Luo et al., 2015
Rats	Acute pain	vF	EPM, OFT	↑ firing in response to cutaneous mechanical stimulation	Mastumoto et al., 2020
Rats	NP	CCI-ION	EPM, LD	↑ T1 and T2 signal (edema and astrogliosis resp.)	Mcllwraith et al., 2020
Mice	NP	SNL	EPM, LD	↑ pDOR immunoreactivity, ↓ DOR function	Narita et al., 2006
Rats	Visceral	chronic pancreatitis	EPM, OFT	↑ fos+ cells in layers II/III & V/VI, vGluT1, membrane GluR1 & pGluR1 (AMPA), membrane NR2B & pNR2B (NMDAR)	Ren et al., 2022
Mice	NP	Cuff	LD, NSF	↑ firing rates, enhancement of excitatory synapse transmission	Sellmeijer et al., 2018
Rats	NP	SNI	EPM, OFT	↓ volume, correlated to mechanical hypersensitivity	Seminowicz et al., 2009
Rats	NP	SNL	EZM	↑ pERK1/2+ cells and pERK levels in layers II-VI	Shao et al., 2015
Rats	Inf	CFA	OFT, EZM, NSF	↓ PV and SOM interneurons	Shao et al., 2021a
Rats	Inf	CFA	OFT, EZM, NSF, marble	↑ fos levels and excitability of pyramidal cells, ↓ PV+ cells in layer V and ↓ inhibitory synaptic transmission	Shao et al., 2021b
Mice	Inf	CFA	EPM, OFT	↑ astrocytes & microglia activation and GluN2A/GluN2B, GluA1, CaMKII-a, pP38 & pJNK MAPK & TNFa/IL6 levels	Sun et al., 2016
Mice	Visceral	IBS	EPM, OFT	↑ GluA1, pGluA1, synaptophysin, PSD95 & CPEB1 levels	Wang et al., 2019
Rats	Inf, NP	Formalin, SNL	OFT	↑ Kyn/Trp ratio,IDO1, GFAP, IL66, pNMDA2BR, pCDK5, pMAP2 & pTau levels and spine density	Wang et al., 2020
Rats	NP	SNI	EPM, OFT, NSF	↑ fos, PSD95, neuroligin2, CREB, pCREB, CaMKIIa levels	Wen et al., 2022
Rats	OIH	Remifentanil	EPM, OFT	↑ pGluR1 and cfos levels, ↓ Arc levels	Zeng et al., 2018
Mice	Visceral	IBS	EPM, OFT, LD	↑ fos expression in layers II-III	Zhang et al., 2014
Rats	NP	CCD	EPM, OFT	↑ pyramidal neurons excitability, ↓ function of BKCa channels	Zhao et al., 2020
Mice ♀	Visceral	Acetic acid	EPM, OFT	↑ pERK1/2+ cells	Zhong et al., 2012
Mice	Inf	CFA	EPM, OFT	↑ fos , ↓5HT levels	Zhou et al., 2022

**Table 2. (continued)**

Species	Pain type	Models	Tests	Observation in the ACC in pain-induced anxiety after manipulation	Reference
Mice	NP	Cuff	NSF, marble	ACC lesion prevents ALB	Barthas et al., 2015
Rats	NP	TNT	OFT	LC-ACC chemogenetic activation induces ALB	Hirschberg et al., 2017
Mice, Rats	NP	SNL	EPM, LD	Paroxetine (SSRI) injection into the ACC rescues ALB	Matsuzawa-Yanagida et al., 2008
Rats	Inf	CFA	OFT	Chemogenetic inhibition of rACC-thalamus rescues ALB	Shen et al., 2021
Rats	NP	CCI	OFT	Subchronic CBD treatment increases CB1 & TRPV1 receptors in the ACC	Silva-Cardoso et al., 2021
Mice	NP	SNI	EPM, OFT	Chemogenetic inhibition of rACC(Glu)-vIPAG pathway rescues mechanical hypersensitivity & ALB	Zhu et al., 2022

*Abbreviations: ACC, anterior cingulate cortex; ALB, anxiety-like behavior; AMPA,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Arc, activity-regulated cytoskeleton-associated protein; BKCa, large-conductance voltage- and Ca<sup>2+</sup>-activated potassium channel; CaMKII $\alpha$ , calcium calmodulin-dependent protein kinase II  $\alpha$ ; CBD, cannabidiol; CB1, cannabinoid receptor type 1; CCD, chronic constriction of multiple dorsal root ganglia; CCI, chronic constriction injury; CCI-ION, CCI of the infraorbital nerve; CDK5, cyclin-dependent kinase 5; CFA, complete Freund adjuvant; CFI, chronic facial inflammatory; CPEB1, Cytoplasmic Polyadenylation Element Binding Protein 1; CPNL, common peroneal nerve ligation; CREB, cAMP response element-binding protein; DOR, delta opioid receptor; ECM, extracellular matrix; EPM, elevated plus maze; ERK, extracellular kinase-regulated kinase; EZM, elevated zero maze; GFAP, glial fibrillary acidic protein; Glu, glutamate; IBS, irritable bowel syndrome; IDO, indoleamine 2,3-dioxygenase 1; IL, interleukin; Inf, inflammatory; JNK, ??; Kyn, kynurenine; LAMB1, laminin subunit beta 1; LC, locus coeruleus; LD, light-dark; MAPK, mitogen-activated protein kinase; mGluR5, metabotropic Glu receptor 5; MKP1, MAPK phosphatase 1; NMDA, N-methyl D-aspartate ; NR2B, NMDA subunit NR2B; NSF, novelty suppressed feeding; NP, neuropathic; OIH, opioid-induced hyperalgesia; OFT, open field test; p, phosphorylated; PK, protein kinase; pMAP2, ??; PSD95, postsynaptic density protein 95 ; PV, parvalbumin; R, receptor; rACC, rostral ACC; SNI, spinal nerve injury; SNL, spinal nerve ligation; SOM, somatostatin; SSRI, selective serotonin reuptake inhibitor; TNF $\alpha$ , tumor necrosis factor alpha; TNT, tibial nerve transection; Trp, tryptophan; TRPV1, transient receptor potential vanilloide 1; vF, von Frey; vGluT1, vesicular Glu transporter 1; vIPAG, ventrolateral periaqueductal gray; ♀, study realized in both males and females; ♀, study realized in females only, if not specified, only males were used.*

**Table 3. Depressive-like consequences of chronic pain**

Species	Pain type	Models	Tests	Observation in the ACC in pain-induced depression	Reference
Mice	NP	Cuff	splash, FST	↑ MKP1, pCREB, cFos and active epigenetic regulations on mcp1 gene	Barthas et al., 2017
Rats	NP	CCI	FST, anhedonia	↑ pERK, ↓ neuronal density	Bravo et al., 2012
Mice	NP	CCI	FST, TST	↓ 5HT, BDNF & CREB levels	Cong et al., 2021
Mice ♀	NP	SNI	splash, FST, TST	Transcriptomic differences stronger in females with neurotransmitter release & ECM impacted	Dai et al., 2022
Mice	NP	Cuff	splash, FST, NSF	↑ MKP1 levels, ↓ pERK levels	Humo et al., 2020
Rats	Inf	FST + CFA	FST	↑ pCREB+ and Egr1+ cells	Imbe & Kimura, 2017
Rats	NP	CCI	FST	↑ pERK1/2+ cells, ↓ BDNF levels	Ishikawa et al., 2014
Rats	NP	CCI	FST	↑ pERK1/2+ cells, ↓ pCREB & BDNF levels	Ishikawa et al., 2015
Mice ♀	Inf, NP	SNI, CFA	TST, SPT	↑ ECM genes expression, ↓LAMB1 gene expression	Li et al., 2021b
Rats	Depression-induced pain	Chronic FST	FST	↑ activity of neurons	Liu et al., 2019
Rats	NP	CCI	FST	LC-rACC projection is activated after pain	Llorca-Torrallba et al., 2022
Mice ♀	NP	SNI	FST, TST, SPT	↑ microglia in females	Michailidis et al., 2021
Rats	NP	SNI	FST, SPT	↓ neuroligin1 & BDNF levels	Pan et al., 2018
Rats	Inf	Monoarthritis	FST	↑ pERK levels	Pereira-Silva et al., 2020
Rats	Visceral	Chronic pancreatitis	FST	↑ fos+ cells in layers II/III & V/VI, vGluT1, membrane GluR1 & pGluR1, membrane NR2B & pNR2B	Ren et al., 2022
Mice	NP	Cuff	splash, FST, NSF	↑ firing rates, enhancement of excitatory synapse transmission	Sellmeijer et al., 2018
Rats	NP	SNI	FST, NSF	↑ fos, PSD95, neuroligin2, CREB, pCREB, CaMKIIa levels	Wen et al., 2022
Mice	NP	Cuff	/	MAPK pathway is altered at 2w & 8w post-surgery	Xiao et al., 2021
Rats	NP	CCI	FST	↑ pERK1/2+ cells, ↓ pCREB+ cells & BDNF levels	Yasuda et al., 2014
Mice	Inf, NP	CFA, SNI	FST, TST, SI	↑ spike number & cFos in Glu+ neurons, ↓excitability of GABA+ neurons	Zhu et al., 2021

Species	Pain type	Models	Tests	Observation in the ACC in pain-induced depression after manipulation	Reference
Mice	NP	Cuff	splash, FST	ACC lesion or optogenetic inhibition of CaMKII+ cells prevent DLB	Barthas et al., 2015 Sellmeijer et al., 2018

*Abbreviations: ACC, anterior cingulate cortex; BDNF, brain-derived neurotrophic factor; CaMKIIa, calcium calmodulin-dependent protein kinase II alpha; CCI, chronic constriction injury; CFA, complete Freund adjuvant; CREB, cAMP response element-binding protein; DLB, depression-like behavior; ECM, extracellular matrix; Egr1, early growth factor 1; ERK, extracellular signal-regulated kinase; FST, forced swim test; GABA,  $\gamma$ -aminobutyric acid; Glu, glutamate; Inf, inflammatory; LAMB1, laminin subunit beta 1; LC, locus coeruleus; MAPK, mitogen-activated protein kinase; MKP1, MAPK phosphatase 1; NMDA, N-methyl D-aspartate; NR2B, NMDA subunit NR2B; NP,*

*neuropathic; NSF, novelty-suppressed feeding; p, phosphorylated; PSD95, postsynaptic density protein 95; R, receptor; rACC, rostral ACC; SI, social interaction; SNI, spinal nerve injury; SPT, sucrose preference test; TST, tail suspension test; vGluT1, vesicular Glu transporter 1; w, week; 5HT, serotonin; ♀, study realized in both males and females, if not specified, only males were used*

## **Janus Effect of the Anterior Cingulate Cortex: Pain and Emotion**

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### **Highlights (3 to 5):**

- ACC plays a critical role in emotional and pain processing.
- ACC is essential for the emotional component as well as anxio-depressive consequences of chronic pain.
- Imbalance between glutamatergic and GABAergic transmission in the ACC is one of the key mechanisms for the aversive- and anxiodepressive-consequences of chronic pain.
- Recent technological advances and development of several rodent model of chronic pain strengthen our mechanistic understanding underlying the complex role of the ACC in emotion and pain dyad.

## **Abstract**

Over the past 20 years, clinical and preclinical studies point to the anterior cingulate cortex (ACC) as a site of interest for several neurological and psychiatric conditions. The ACC plays a critical role in emotion, autonomic regulation, pain processing, attention, memory and decision making. An increasing number of studies have demonstrated the involvement of the ACC in the emotional component of pain and its comorbidity with emotional disorders such as anxiety and depression. Thanks to the development of animal models combined with state-of-the-art technologies, we now have a better mechanistic understanding of the functions of the ACC. Hence, the primary aim of this review is to compile the most recent preclinical studies on the role of ACC in the emotional component and consequences of chronic pain. Herein, we thus thoroughly describe the pain-induced electrophysiological, molecular and anatomical alterations in the ACC and in its related circuits. Finally, we discuss the next steps that are needed to strengthen our understanding of the involvement of the ACC in emotional and pain processing.

**Key words (12/12):** Anterior cingulate cortex, ACC, Area 24a, Area 24b, Area 32, pain, chronic pain, aversion, anxiety, depression, circuits, rodent models.

## **Abbreviation list**

ACC: anterior cingulate cortex

AMPA:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BDNF: brain derived neurotrophic factor

BLA: basolateral nucleus of the amygdala

CPEB1: cytoplasmic polyadenylation element binding protein 1

E/I: excitatory/inhibitory

GABA:  $\gamma$ -aminobutyric acid

GAD: generalized anxiety disorder

Glu: glutamatergic

HCN: hyperpolarization-activated cyclic nucleotide-gated

LC: locus coeruleus

mGluR5: metabotropic glutamate receptor 5

MAPK: mitogen-activated protein kinase

MKP-1: MAPK phosphatase 1

MCC: middle cingulate cortex

MDD: major depressive disorder

N: nucleus

NMDA: N-methyl D-aspartate

PAG: periaqueductal gray

p-mTOR: phosphorylated mTOR

rACC: rostral anterior cingulate cortex

VTA: ventral tegmental area

## 1           **Introduction**

2   Major depressive disorder (MDD) and generalized anxiety disorder (GAD) are some of the leading  
3   causes of disability worldwide (World Health Organization, 2009), contributing to the global burden  
4   of diseases (Santomauro et al., 2021; Whiteford et al., 2013). Although chronic stress is considered as  
5   the major risk factor for MDD and GAD, other pathologies can also trigger or worsen these  
6   psychiatric conditions. These comorbidities are in part responsible for treatment failures but also  
7   dramatically decrease the patients' quality of life. Aside from the well-known comorbidity between  
8   depression and anxiety themselves, chronic pain is an important determinant for MDD and GAD  
9   (Means-Christensen et al., 2008), with a prevalence rate ranging from 30 to 80% of chronic pain  
10   patients depending on the pain condition (Bair et al., 2003; de Heer et al., 2014; Demyttenaere et al.,  
11   2007; Gureje et al., 2008; Von Korff et al., 2005; Williams, 2003). On the other hand, 50 to 60% of  
12   patients diagnosed with MDD report chronic pain (Meints and Edwards, 2018; Williams, 2003),  
13   highlighting the important interplay between these pathologies.

14   One hypothesis for the comorbidity between chronic pain and emotional disorders is based on a shared  
15   neuroanatomical substrate, suggesting that **i**) specific brain regions processing pain are also involved  
16   in emotional processing, and **ii**) alterations induced by chronic pain in these regions may alter  
17   emotional processing, possibly resulting in psychiatric disorders. Indeed, in different pain conditions,  
18   recent human and rodent imaging studies reported morphological and functional alterations in the pain  
19   matrix, including the primary and secondary somatosensory, insular and anterior cingulate cortices  
20   (ACC) (Apkarian et al., 2005), and in the mesolimbic and corticolimbic pathways, well-known for  
21   their implication in emotional processing (Serafini et al., 2020). Among these brain structures, the  
22   ACC is at the interface between sensory, cognitive and emotional processes (Vogt, 2005), acting as a  
23   hub for both pain and emotional processing (Shackman et al., 2011).

### 24   *Insight from Human Studies*

25   The involvement of the ACC in the emotional component of pain was first suggested by clinical  
26   studies reporting that patients with stereotactic cingulotomies describe pain less unpleasant (Foltz and  
27   White, 1962). These studies have recently been extended by showing that cingulotomy not only  
28   alleviates the distress caused by pain but also the depressive symptoms in chronic pain patients (Deng  
29   et al., 2019) and it decreases the functional connectivity within the salience network (Jalon et al.,  
30   2023). Functional magnetic resonance imaging (MRI), positron emission tomography (PET), voxel-  
31   based morphometry (VBM), diffusion tensor imaging (DTI) also clearly showed how different chronic  
32   pain components are correlated with ACC alterations. The pioneer study from Rainville and  
33   colleagues (1997) demonstrated, using PET scan, a correlation between ACC activity and the  
34   perceived unpleasantness of pain. In addition, VBM analyses showed reduced gray matter volume in  
35   the ACC of trigeminal neuralgia patients which correlated with pain intensity, depression and anxiety  
36   ratings (Wang et al., 2017). Furthermore, several groups reported positive effect of deep brain



37 stimulation (see review Russo and Sheth, 2015) and transcranial magnetic stimulation of the ACC  
38 (Best and Pavel, 2017), positioning this brain area as a new potential neuromodulatory target to treat  
39 the affective component of chronic pain. These findings are in favor of a strong and dual role of the  
40 ACC in pain and its emotional comorbidities. Since the correlates of human ACC can be found in both  
41 primates and rodents (van Heukelum et al., 2020), this brain structure became a region of interest in  
42 preclinical studies leading to a better understanding of the role of the ACC in pain-associated  
43 emotional disorders.

#### 44 *Insight from preclinical studies*

45 While clinical investigation (Bair et al., 2003; de Heer et al., 2014; Demyttenaere et al., 2007; Gureje  
46 et al., 2008; Von Korff et al., 2005; Williams, 2003) continues to provide insights about the emotional  
47 component of pain, animal experiments offer a unique opportunity to test cellular and molecular  
48 hypotheses to decipher underlying cerebral mechanisms. Indeed, in the last two decades, the  
49 development of preclinical settings and rodent models of chronic pain (see review Kremer et al., 2021)  
50 combined with invasive or virally-mediated approaches, unavailable in Human research (see reviews  
51 Bravo et al., 2020; Humo et al., 2019), have greatly improved our understanding of the  
52 neurobiological mechanisms implicated in this important clinical issue. Thus, these new approaches at  
53 the interface between translational and reverse-translational research brought novel hypotheses that  
54 pave the way to the development of novel treatment strategies. Accordingly, this narrative review will  
55 outline preclinical studies focusing only on the role of the ACC in the emotional component of chronic  
56 pain, published between 2004 and 2022. To that end, publications were chosen using a combination of  
57 three keywords [(pain) and (aversion or anxiety or depression) and (anterior cingulate cortex or  
58 cingulate or ACC or prefrontal cortex or PFC) (**Fig.1**). As a first step, all studies not conducted in  
59 rodents (mouse or rat) were excluded. Secondly, as neuroanatomical description of the ACC differs in  
60 the literature (Fillinger et al., 2017a, 2017b; van Heukelum et al., 2020), we selected the scientific  
61 papers focusing only on the area **24a**, **24b** and **32**, based on the atlas of Paxinos and Franklin, 2012.  
62 We therefore excluded publications that were not studying these areas, even though they mentioned  
63 the ACC in the manuscript.

#### 64 -----**Fig.1**-----

65 In this review, after a brief description of the neuroanatomy of the ACC, we will highlight the  
66 significant role of this cerebral hub at the interface between pain and its emotional components. It is  
67 worth mentioning that most of the studies cited here focus mainly on neuropathic and inflammatory  
68 pain (see Kremer et al., 2021 for detail), with few articles focusing on visceral or cancer pain (**BOX 1**  
69 for preclinical models cited in this review). Finally, we will discuss the next steps needed for  
70 strengthening our understanding of the role of the ACC in the emotional component and consequences  
71 of pain, with a special focus on sex differences.

72 **1. Neuroanatomy of the ACC**

73 In human and non-human primates, the cingulate cortex belongs to the medial wall of the cerebral  
74 cortex (Palomero-Gallagher et al., 2009; van Heukelum et al., 2020; Vogt et al., 1996). Along the  
75 anteroposterior axis, four subregions including the anterior (ACC), the middle (MCC) and posterior  
76 cingulate cortex as well as the retrosplenial cortices compose this gyrus [Fig.2a, (Vogt, 2005)].  
77 Comparative anatomy based on cytoarchitecture shows several striking differences between primates  
78 and rodents, the most obvious being that rodents do not have a cingulate sulcus and therefore lack  
79 primate sulcal ACC areas 24c, s32 and p32. Additionally, while MCC is organized into an anterior and  
80 a posterior region in primates, rodents present a uniform MCC. Moreover, the supracallosal area 33  
81 spans the full extent of ACC and MCC in primates, while its presence is minor in rats and absent in  
82 mice. Finally, the posterior cingulate cortex, massively developed in humans and on a smaller scale in  
83 non-human primates, is absent in rodents (Vogt and Paxinos, 2014). Yet, despite the absence of the  
84 aforementioned subregions in rodents, the cytoarchitectural organization of the common areas found  
85 in human and non-human primates and rodents present sufficient similarities to share a common  
86 nomenclature. In rodents, the ACC is an agranular cortex lacking a true layer IV composed by the area  
87 24, further divided into 24a (ventral) and 24b (dorsal), and the areas 25, 32 and 33 – the latter being  
88 absent in mice (Fig.2b). These areas contain glutamatergic pyramidal neurons (~80%) in all ACC  
89 layers apart from layer I. The inhibitory GABAergic neurons (~20%), including heterogeneous  
90 subpopulations based on calcium binding proteins [parvalbumin, calbindin or calretinin] and peptides  
91 such as somatostatin (Gabbott et al., 1997), are distinctly distributed in terms of density and  
92 connectome over different layers and subdivisions (Gabbott et al., 1997).

93 Concerning its neuroanatomical connections, the ACC presents a wide connectome, similar across  
94 species, with only a few notable differences illustrated for example by the cortico-spinal projection  
95 located in the areas 32 and 24b in rodents, which is found in the deep layers of the MCC in non-human  
96 primates (for details see Fillinger et al., 2017a, 2017b; van Heukelum et al., 2020). Briefly, in all  
97 species, connections of the areas 24a/24b can be divided in four sub-networks, 1) cortical, 2) thalamic,  
98 which are the denser connections of the ACC and mostly reciprocal, 3) forebrain and hypothalamus  
99 and 4) brainstem centers (Fig.2c-f).

100 -----**Fig.2**-----

101 In rodents, at the cortical level, beside strong interconnections, areas 24a and 24b are reciprocally  
102 connected with frontal regions, mostly with the rest of the ACC and the orbital cortex, as well as the  
103 sensory and temporal cortices. As an exception, the hippocampal region sends moderate projections to  
104 the ACC but does not receive any afferents from it. In regards to the thalamo-cortico-thalamic  
105 connections, four main groups of nuclei can be highlighted as cingulate privileged interlocutors: the  
106 anterior nuclei - mostly the anteromedial nucleus-, the mediodorsal nucleus, the midline nuclei -

107 primarily the reuniens and rhomboid nuclei-, and finally the intralaminar nuclei with the centromedial,  
108 central lateral, paracentral and parafascicular thalamic nuclei (Fillinger et al., 2017a, 2017b).

109 In the forebrain, the basolateral nucleus of the amygdala (BLA) and the claustrum are densely and  
110 reciprocally connected to the ACC, along with a lesser contribution of the lateral and anterior  
111 hypothalamus. Additionally, the lateral septum, as well as the dorsal, and to a lesser extent the ventral,  
112 striatum receive dense ACC projections. In the brainstem, monoaminergic centers [ventral tegmental  
113 area (VTA), substantia nigra, dorsal and median raphe and the locus coeruleus (LC)] are  
114 bidirectionally connected with the ACC (Fillinger et al., 2017a, 2017b). In addition, the ACC sends  
115 unidirectional projections to the superior colliculus and the pretectal nuclei, as well as to the  
116 periaqueductal gray (PAG), more specifically to the dorsolateral column from 24a and the  
117 lateral/ventrolateral columns from 24b in mice. Finally, ACC neurons have been found to project to  
118 the cervical and thoracic spinal cord in rodents (Chen et al., 2018).

119 In conclusion, as other higher-order cortical areas, the ACC possesses a wide and conserved  
120 connectome (Fig.2) making it a cerebral hub responsible for multiple pain-related, emotional and  
121 cognitive processes.

## 122 **2. Role of the ACC in pain-related aversion**

123 The affective dimension of pain refers primarily to the feeling of unpleasantness and secondarily to the  
124 long-term impact of suffering from pain. Such aspects of pain participate in shaping subsequent pain-  
125 related behaviors and reflect aversive and motivational aspects of pain. Hence, pain-related aversion  
126 leads to behavioral avoidance or extinction of behaviors associated with noxious stimuli.

127 Numerous clinical studies have demonstrated the critical role of the ACC in the affective dimension of  
128 pain in human (see Brotis et al., 2009). In preclinical studies, several paradigms were developed as a  
129 mean to evaluate aversion in rodents [Fig.3, (Kremer et al., 2021)]. The place escape/avoidance  
130 paradigm (Fuchs and McNabb, 2012) - measuring animals escape behavior from an evoked painful  
131 stimulus-, place conditioning paradigms (Tappe-Theodor et al., 2019) - relying on the association of a  
132 context with either painful stimuli in conditioned place aversion or pain-relief via an analgesic drug in  
133 conditioned place preference (Navratilova et al., 2013)-, are the most frequently used tests to evaluate  
134 the aversive component of pain.

135 Lesioning studies were part of the milestones assessing the involvement of the ACC in pain-related  
136 aversion. It was reported that ACC lesion (using electrolytic or excitotoxic procedures) in rats blocks  
137 the avoidance to a context paired with acute inflammatory pain (Gao et al., 2004; Shi et al., 2020; Yi  
138 et al., 2011) and prevent the aversiveness of ongoing pain in both neuropathic (Barthas et al., 2015;  
139 Juarez-Salinas et al., 2019; LaGraize et al., 2004; Li et al., 2022; Qu et al., 2011) and inflammatory  
140 pain models (Johansen et al., 2001). Interestingly, none of these studies reported an effect of the ACC  
141 lesion on mechanical hypersensitivity. Interestingly, none of these aforementioned studies reported an

142 effect on mechanical hypersensitivity which is probably due to drastic effect of lesioning of a given  
143 structure on its connection with whole network. On the contrary, lesion of the posterior insular cortex  
144 blocks the maintenance of mechanical hypersensitivity (Gao et al., 2004; Shi et al., 2020) without  
145 affecting the aversive component of neuropathic pain (Barthas et al., 2015). Accordingly, the ACC  
146 seems to be essential for the aversive component of pain. The next section will thus outline cellular  
147 and molecular alterations in the ACC as well as its circuits involved in pain-related aversion.

148 -----**Fig.3**-----

## 149 **2.1 Electrophysiological changes and functional alterations**

150 Recently, available technological advances in animal research have accelerated our understanding of  
151 the functional role of the ACC in pain-related aversion. The use of different electrophysiological  
152 approaches, from *ex vivo* whole cell patch clamp recordings to *in vivo* single unit recordings in  
153 anesthetized or freely moving animals (Cao et al., 2012; Lançon et al., 2021; Sellmeijer et al., 2018;  
154 Zhang et al., 2017, 2021) have shown an increased ACC spontaneous firing activity associated with  
155 the expression of aversive-like behaviors, in different pain models. This shift towards ACC  
156 hyperactivity might be due to an imbalance between excitatory and inhibitory (E/I) neurons within the  
157 ACC. Recent *in vivo* studies, aiming to modulate ACC activity, suggest a primary role of the  
158 pyramidal cells in this imbalance (Bliss et al., 2016; Zhu et al., 2022). Indeed, cell-type specific opto-  
159 or chemogenetic inhibition of ACC glutamatergic neurons alleviates the aversiveness of ongoing pain  
160 without affecting mechanical allodynia in inflammatory and neuropathic pain models (Sellmeijer et  
161 al., 2018; Zhang et al., 2017, 2021). On the contrary, optogenetic activation of ACC pyramidal  
162 neurons in naive animals induces aversion (Tan et al., 2017; Zhang et al., 2017) similar to that of pain  
163 models.

164 On the other hand, according to some reports ACC hyperactivity may be due to a loss of GABAergic  
165 inhibitory control. Using a chemotherapy-induced neuropathic pain model, Juarez-Salinas and  
166 colleagues (2019) showed that the transplantation of inhibitory interneuron progenitor cells into the  
167 ACC was sufficient to restore a GABA-A mediated inhibitory control of host pyramidal cells and  
168 block aversion in the conditioned place aversion paradigm. Local administration of the GABA-A  
169 receptor agonist, muscimol, into the rostral ACC (rACC) also reduces aversion after nerve injury  
170 (LaGraize and Fuchs, 2007; Tang et al., 2005). To further dissect cell types involved in pain-related  
171 aversion, Zhang and colleagues (Zhang et al., 2021) focused on ACC neurons expressing the gamma  
172 isoform of the protein kinase C (PKC $\gamma$ ). They showed that chemogenetic activation of ACC PKC $\gamma$   
173 neurons induces mechanical hypersensitivity and aversion in naive animals, while in neuropathic pain  
174 condition these neurons are hyperexcitable and their inhibition blocks mechanical allodynia and  
175 aversiveness of ongoing pain.

176 Different pharmacological and non-pharmacological approaches modulating ACC activity were shown  
177 to be effective to alleviate the aversive component of chronic pain. For instance, a single dose of

178 ketamine induces a prolonged suppression of the ACC pyramidal neuron hyperactivity that is  
179 associated with a reduction of the aversive response to noxious stimuli in rodent pain models.  
180 Strikingly, this anti-aversive effect persists long after the anti-nociceptive effects of ketamine have  
181 ceased (Zhou et al., 2018). Interestingly, Gan and colleagues (2021) reported that repetitive  
182 transcranial direct current stimulation (r-tDCS) of the prefrontal cortex not only blocks aversion, but  
183 also mechanical and thermal nociceptive behaviors in a murine neuropathic pain model. This last  
184 effect could be explained by the fact that the application of repetitive transcranial direct current  
185 stimulation was not restricted to the ACC and might affect other prefrontal areas that are directly  
186 involved in nociception. In a similar manner, high frequency electrical stimulation of the ACC also  
187 rescues pain-related aversion, while low frequency stimulation increases it (Liu et al., 2021). Both  
188 invasive and non-invasive manipulations of ACC seem to be a particular interest to alleviate the pain  
189 related aversion in a translational perspective.

190 Altogether, these findings demonstrate the importance of the ACC I/E activity balance in the  
191 development of pain-related aversion, but also the complexity of the functional alterations induced by  
192 chronic pain in the ACC. Further experiments are required to decipher which mechanism comes first:  
193 an increase in excitatory cell activity followed by a dysregulation of the E/I balance or an alteration of  
194 local inhibitory circuits triggering ACC hyperactivity.

## 195 **2.2 ACC circuits**

196 Besides ACC microcircuits, its macrocircuits are also involved in pain-induced aversion. Considering  
197 ACC afferents, inhibition of the mediodorsal thalamic nucleus projections to the ACC induces  
198 conditioned place preference in animals with nerve injury, while the activation of this pathway or the  
199 inhibition of BLA afferents to the ACC exacerbates aversion (Meda et al., 2019). Similarly,  
200 chemogenetic activation of LC neurons projecting to the ACC exacerbates ongoing pain and produces  
201 aversion, in a neuropathic pain model (Hirschberg et al., 2017) without any effect in naive mice. In  
202 regard to cortico-cortical connections, Singh and colleagues demonstrated that the optogenetic  
203 activation of the primary somatosensory cortex increased the proportion of ACC neurons that respond  
204 to noxious stimuli, suggesting that somatosensory afferents reinforce nociceptive response in the ACC  
205 (Singh et al., 2020). They further observed an enhanced connectivity between these two cortical areas  
206 in chronic pain state and showed that the optogenetic inhibition of this pathway effectively relieved  
207 the aversive component of chronic pain.

208 Concerning ACC efferents, using a chemogenetic approach, Gao and colleagues (2020) showed that  
209 inhibition of CaMKII pyramidal ACC neurons projecting to the nucleus accumbens or the VTA  
210 induced a preference for the compartment associated with the inhibition in neuropathic rats, while  
211 activation of these pathways induced aversive behaviors in non-pathological animals, suggesting this  
212 circuit plays an important role of in pain-related aversion.

213 Overall, many studies demonstrate that the modulation of ACC circuits has no or trivial effects in  
214 naive animals, while the manipulation of these specific circuits in chronic pain conditions either  
215 prevents or exacerbates pain-related aversion, thus strengthening the hypothesis that ACC circuits are  
216 deeply impacted by chronic pain.

### 217 **2.3 Molecular and cellular changes**

218 All the aforementioned alterations are most likely accompanied by important molecular and cellular  
219 changes. In that regard, an increasing number of preclinical studies brought new knowledge about  
220 molecular mechanisms underlying ACC hyperactivity and its role in aversion. For instance, several  
221 groups associated pain related aversion with an increase in brain derived neurotrophic factor (BDNF),  
222 its receptor TrkB, phosphorylated ERK (pERK), phosphorylated mTOR (p-mTOR) and mitogen-  
223 activated protein kinase (MAPK) in the ACC in different pain models (Cao et al., 2014, 2009; Galan-  
224 Arriero et al., 2014; Han et al., 2014; Li et al., 2022; Lu et al., 2016; Thibault et al., 2014; X. Wang et  
225 al., 2017; Zhang et al., 2016, 2018). Manipulation of one of these targets thus impacts aversive-like  
226 behaviors in both naive animals and those suffering from chronic pain (Thibault et al., 2014). For  
227 instance, blocking the ACC TrkB receptors rescues the aversion observed in cancer pain (Li et al.,  
228 2022), while BDNF injection into the ACC is sufficient to induce aversion in naive animals (Thibault  
229 et al., 2014; Zhang et al., 2016). Interestingly, by blocking p-mTOR in the rACC after formalin  
230 injection, Zhang and colleagues (2018) did not only rescue pain-induced aversion, but also prevented  
231 NMDA (N-methyl D-aspartate) receptor 2B upregulation in the rACC, known to occur in pain  
232 conditions (Li et al., 2009), suggesting a complex interaction between different mediators.  
233 Accordingly, in line with the E/I imbalance hypothesis, blocking/silencing ACC NMDA subunits,  
234 with an antagonist or small interfering RNA, in inflammatory, neuropathic and cancer pain rescues  
235 pain-related aversion, but not mechanical hypersensitivity nor acute pain (Feng et al., 2016; Guo et al.,  
236 2015; Lei et al., 2004; Li et al., 2009; Zhou et al., 2018). Similarly, blocking D-serine, an endogenous  
237 ligand for NMDA receptors, either by enzymatic degradation or an antagonist of the glycine site of  
238 NMDA receptors, also attenuates conditioned place aversion produced by inflammatory pain (Ren et  
239 al., 2006), strengthening the role of glutamatergic signaling in pain-related aversion. Galan-Arriero  
240 and colleagues (2014) also showed an increase in ACC metabotropic Glu receptor 5 (mGluR5)  
241 expression in neuropathic rats. Accordingly, preventing this increase by either p38a MAPK inhibition  
242 or by glutamate receptor blockade in the ACC with kynurenic acid is sufficient to prevent aversion  
243 induced by inflammation, while mGluR5 stimulation in naive animals produces conditioned place  
244 aversion (Galan-Arriero et al., 2014; Johansen and Fields, 2004). Kang and colleagues also showed  
245 that inhibiting ACC glutamatergic cells in their inflammatory model induces conditioned place  
246 preference (Kang et al., 2017). Collectively, these results confirm the crucial role played by the  
247 glutamatergic system in pain-related aversion and seem to indicate that chronic pain-induced

248 molecular alterations facilitate the shift towards ACC hyperactivity by increasing the glutamatergic  
249 tone, in various pain models.

250 Alongside the classic excitatory and inhibitory systems, several neuromodulators have been described  
251 as important in pain-related aversion. For instance, in neuropathic rats, bilateral administration of  
252 morphine in the rACC is sufficient to produce conditioned place preference (Gomtsian et al., 2018;  
253 LaGraize et al., 2006; Navratilova et al., 2015), while the microinjection of the opioid antagonist,  
254 naloxone, in the ACC prevented morphine-induced place preference (Navratilova et al., 2015),  
255 suggesting a key role of the opioid system in aversive processes governed by the ACC. Accordingly,  
256 administration of delta opioid receptor agonists in the rACC prevents inflammatory pain-induced place  
257 aversion (Ma et al., 2022), while kappa opioid receptor antagonist blocks the electroacupuncture  
258 rescue of CFA-induced place aversion (Zhang et al., 2011).

259 The role of monoamine signaling such as serotonin (5HT) and dopamine has also been described in  
260 pain-related aversion. Santello and colleagues (2017) demonstrated that injection of a selective 5HT7R  
261 antagonist into the ACC reduces both mechanical hypersensitivity and aversion by enhancing the  
262 function of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels that are  
263 downregulated in ACC layer V neurons. In addition, it was shown that dopamine D1R agonists rescue  
264 the decreased activity of HCN channel in neuropathic mice, which in turn inhibits ACC hyperactivity  
265 (Lançon et al., 2021). Interestingly, chronic systemic L-DOPA or carbidopa treatments not only rescue  
266 ACC HCN channel function but also mechanical and cold hypersensitivity as well as pain-related  
267 aversion. Hence, besides the ACC E/I imbalance, several modulatory systems appear to be at play in  
268 chronic pain conditions, which might be additional targets for new treatment strategies. Interestingly,  
269 these vast alterations in diverse neurotransmission systems can be related to overexpression of ACC  
270 SIP30, one of many soluble N-ethylmaleimide-sensitive factor attachment protein receptors  
271 (SNAREs) which are essential for the regulation of neurotransmitter-containing synaptic vesicles  
272 exocytosis. Indeed, expression of SIP30 is modified by pain conditions and its local silencing reduces  
273 aversion without affecting mechanical hypersensitivity (Han et al., 2014). However, the exact nature  
274 of the neurotransmitters affected by SIP30 silencing has yet to be studied.

275 Finally, it is important to highlight that, as for most of the central nervous system pathologies, neurons  
276 are most likely not the only cells altered by chronic pain. Some studies also point to  
277 neuroinflammation as well as non-neuronal cells as key factors for pain-related aversion. Indeed, an  
278 increase in astrocytic markers, such as GFAP/S100B, and inflammatory cytokines including IL-  
279 1B/TNF- $\alpha$ , have been reported in the ACC in inflammatory rat models (Chen et al., 2012; Lu et al.,  
280 2011) and local injection of an astroglia-specific toxin can prevent pain-induced aversion (Chen et al.,  
281 2012). Moreover, Wang and colleagues (Wang et al., 2020) showed increased immune system activity  
282 indicators in the ACC such as Kynurenine/Tryptophan ratio and indoleamine 2,3-dioxygenase (IDO)

283 enzyme for tryptophan catabolism to kynurenine in a neuropathic pain model and the inhibition of the  
284 latter was shown to reverse pain-related aversion as well as cold and mechanical hypersensitivity.

#### 285 **2.4. Conclusions and limitations**

286 Overall, these recent findings (**Table 1**) demonstrate the essential but complex role of the ACC in  
287 pain-related aversion. Chronification of pain seems to trigger electrophysiological and  
288 molecular/cellular changes that lead to a progressive shift towards ACC hyperactivity. This critical  
289 alteration appears to be due to I/E imbalance but also putative contribution of neuromodulatory  
290 systems. However, further studies are necessary to unravel the exact mechanisms leading to ACC  
291 hyperactivity. Optogenetic and chemogenetic studies demonstrated that specific ACC afferents,  
292 mostly involved in sensory (somatosensory cortex, thalamus) and emotional (BLA) processing, and  
293 ACC efferents, notably the VTA or Nucleus Accumbens, participate in pain related aversion.  
294 Interestingly, most of the manipulations of these ACC-related circuits do not induce any effect in  
295 naïve animals, strengthening the view that alterations in these circuits are specific to pain condition,  
296 which underlines the importance of studying pain-induced alteration mechanisms at both  
297 microcircuitry and macrocircuitry levels.

#### 298 **3. Role of the ACC in the anxiodepressive-like consequences of chronic pain**

299 In the past two decades, several paradigms addressing the anxiodepressive-like consequences of  
300 chronic pain were developed in rodents [(for detail see Kremer et al., 2021); (**Fig.3 and Box2**)]. In  
301 general, the evaluation of anxiety-like behaviors in rodents relies on the conflict between innate  
302 exploratory behaviors (exploring, feeding, and social behaviour) and innate fear (open lit areas,  
303 foreign objects or an unfamiliar individual). Hence, the most used tests are the elevated plus maze,  
304 open-field, light-dark box, social interaction or burrowing tests (**Fig.3 and Box2**). Regarding  
305 depressive-like behaviors, the currently available paradigms mostly assess despair-like [forced swim  
306 or tail suspension tests], anhedonia-like (sucrose preference test) behaviors or animals' well-being  
307 (splash, nest tests). Although these tests only partially evaluate the anxio-depressive symptoms  
308 observed in human, they present a clinical relevance with face and predictive validity (**Fig.3 and**  
309 **Box2**). For instance, in regard to anxiety, a similar thigmotaxic behavior has been described in human  
310 and rodent and associated with increased anxiety (Walz et al., 2016). In regard to pharmacological  
311 validity, Crawley and colleagues showed that the classical anxiolytic drugs, such as benzodiazepines,  
312 effectively reduce anxiety in the light/dark box tests in rodents (Crawley and Goodwin, 1980) while  
313 Porsolt and colleagues (1977) showed that tricyclic antidepressant reduces immobility time in the  
314 forced swim test. Hence, even though imperfect, these tests have brought and continue to bring  
315 valuable insights about neurobiological mechanisms implicated in psychiatric conditions.

316 One of the first studies demonstrating the involvement of the ACC in the anxiodepressive  
317 consequences of chronic pain came from Seminowicz and colleagues (2009), which showed, in rats, a  
318 correlation between decreased ACC volume and anxiety-like consequences of neuropathic pain using



319 MRI in a longitudinal fashion. Lesion studies confirmed these observations by showing that ACC  
320 ablation is sufficient to prevent both anxiety- (Zeng et al., 2018) and depressive-like behaviors  
321 (Barthas et al., 2015) in models of remifentanyl induced hyperalgesia and neuropathic pain  
322 respectively. More specifically, it was shown that optogenetic inhibition of ACC pyramidal neurons is  
323 sufficient to rescue neuropathic pain-induced anxiodepressive-like behaviors in mice (Sellmeijer et al.,  
324 2018). The next sections will discuss the most recent findings on the cellular and molecular alterations  
325 in the ACC as well as in its networks in the anxiodepressive-consequences of chronic pain.

### 326 **3.1 Electrophysiological changes and functional alterations**

327 Similar to pain related aversion, anxiodepressive-like behaviors in the context of cutaneous  
328 mechanical stimulation (Matsumoto et al., 2020), neuropathic pain (Sellmeijer et al., 2018; Zhao et al.,  
329 2020), inflammatory pain (Ducret et al., 2022; Zhou et al., 2022) and visceral pain (Brenner et al.,  
330 2021; Ren et al., 2022; Zhang et al., 2014) has been associated with increased firing activity and  
331 excitability of ACC neurons. In particular, Sellmeijer and colleagues showed that this hyperactivity is  
332 absent before the development of depressive-like behavior but persists after the spontaneous recovery  
333 of mechanical hypersensitivity. Interestingly, inhibition of pyramidal neurons (Sellmeijer et al., 2018;  
334 Zhu et al., 2021) or activation of inhibitory neurons, especially parvalbumin expressing neurons (Ren  
335 et al., 2022; Shao et al., 2021a, 2021b), alleviate the anxiodepressive-like behaviors in neuropathic and  
336 inflammatory pain, strengthening the causal link between ACC hyperactivity and emotional  
337 dysregulation (Sellmeijer et al., 2018). Concurrently, these manipulations reduce pain induced ACC c-  
338 Fos levels and hyperexcitability of glutamatergic neurons (Shao et al., 2021a, 2021b). On the contrary,  
339 chemogenetic inhibition of ACC GABAergic interneurons in naive animals induces anxiety-like  
340 behaviors by enhancing ACC glutamatergic neurons excitability and reducing inhibitory synaptic  
341 transmission (Shao et al., 2021b). The next question is how does the information reach to the ACC  
342 and how is it then propagate to the efferent structures?

### 343 **3.2 ACC circuits**

344 Although the literature deciphering the role of ACC circuits in anxiodepressive consequences of  
345 chronic pain is still sparse, Zhu and colleagues (2022) showed that chemogenetic activation of rACC  
346 excitatory neurons projecting to the ventrolateral PAG provokes mechanical hypersensitivity and  
347 anxiety-like behaviors, while the inhibition of this pathway prevents such symptoms in a neuropathic  
348 pain model. Similarly, while chemogenetic activation of the ACC-thalamus pathway induces anxiety-  
349 like behaviors in naive rats, the chemogenetic inhibition of this pathway rescues anxiety-like  
350 behaviors in a CFA rat model (Shen et al., 2021). Concerning ACC afferents, Zhu et al. (2021)  
351 identified two discrete glutamatergic neuronal circuits: one projection from the posterior thalamic  
352 nucleus to primary somatosensory cortex glutamatergic neurons mediating mechanical  
353 hypersensitivity and another one from the parafascicular thalamic nucleus onto both inhibitory and  
354 excitatory ACC neurons respectively and involved in depressive-like behavior induced by chronic

355 pain. The connection between the ACC and the paraventricular nucleus of the hypothalamus also plays  
356 a role in the comorbidity between neuropathic pain and anxiety, as the optogenetic activation of the  
357 paraventricular nucleus neurons projecting to the ACC reduces both mechanical hypersensitivity and  
358 anxiety-like behaviors (Li et al., 2021a). Since the ACC receives a strong projection from the BLA,  
359 Becker and colleagues (2022) studied the role of this pathway in the anxiodepressive-consequences of  
360 neuropathic pain and showed that optogenetic inhibition of the BLA-ACC pathway rescues  
361 depressive-like behavior induced by neuropathic pain while the chronic activation of this pathway in  
362 naive mice mimics depressive-like behaviors. Finally, midbrain projections to the ACC also play a  
363 critical role as chemogenetic inhibition of the ACC inputs from the LC -the principal brain region  
364 secreting noradrenaline- is sufficient to reverse depressive-like behaviors in a rat neuropathic pain  
365 model (Llorca-Torralba et al., 2022).

366 Hence, as for pain-related aversion, ACC sub-circuits are likely involved in the development of the  
367 anxiodepressive consequences of chronic pain. Indeed, in general, artificial stimulation of various  
368 ACC afferents can trigger anxiodepressive behavior in naïve animals, while inhibition of the same  
369 pathways has beneficial effects in chronic pain models. Nevertheless, further studies are required to  
370 precisely map and understand the role of different circuits in anxiety- and depressive-like  
371 consequences of pain.

### 372 **3.3 Molecular and cellular changes**

373 All the electrophysiological alterations at the ACC or within ACC circuits imply important molecular  
374 and cellular changes. For instance, the intracellular MAPK/ERK pathway is altered in a model of  
375 neuropathic pain, which contributes to the anxiodepressive-like consequences of pain (Barthas et al.,  
376 2017; Humo et al., 2020; Xiao et al., 2021). This finding is confirmed by a genome-wide study  
377 reporting that the ACC MAPK signaling pathway was one of the most significantly altered at both  
378 early and late phase of neuropathic pain (Barthas et al., 2017). In another transcriptomic study, Su and  
379 colleagues (2021) showed that most of the genes differentially expressed in the ACC in neuropathic  
380 pain were related to anxiety and depression rather than nociception itself, confirming the crucial role  
381 of the ACC in emotion. In accordance, the expression of MAPK phosphatase 1 (MKP-1), a negative  
382 regulator of this pathway, dephosphorylating downstream proteins such as pERK and phosphorylated  
383 CREB (pCREB), was robustly increased in neuropathic pain (Barthas et al., 2017; Humo et al., 2020;  
384 Wen et al., 2022) and blocking MKP-1 with an antagonist or knocking it down rescued or prevented  
385 anxiodepressive-like consequences (Barthas et al., 2017; Humo et al., 2020). Strikingly, it was also  
386 shown that classical and fast-acting antidepressant drugs such as fluoxetine and ketamine can  
387 normalize ACC MKP-1 levels. Besides MKP-1, increased levels of ACC pERK (Dai et al., 2011; Luo  
388 et al., 2015; Pereira-Silva et al., 2020; Shao et al., 2015; Zhong et al., 2012), decreased levels of  
389 pCREB and BDNF (Bravo et al., 2012; Cong et al., 2021; Imbe and Kimura, 2017; Ishikawa et al.,  
390 2014, 2015; Pan et al., 2018; Yasuda et al., 2014) were reported in animals showing anxiety- and

391 depressive-like behaviors following chronic pain induction. More specifically, in both visceral and  
392 incisional pain, the expression of the ACC pERK increases biphasically (Dai et al., 2011; Zhong et al.,  
393 2012). During the first phase, both allodynia and anxiety-like behaviors are rescued by pERK  
394 inhibition, while in the second phase this inhibition only affects anxiety-like behaviors (Dai et al.,  
395 2011; Zhong et al., 2012). Interestingly, stimulating BDNF synthesis, via 4-methylcatechol intra-  
396 cerebroventricular injection -increases ACC BDNF levels- decreases ACC pERK levels and prevents  
397 depressive-like behaviors in neuropathic pain (Ishikawa et al., 2014). Either knockdown or systemic  
398 injection of a CREB inhibitor rescues mechanical and heat hypersensitivity, anxiety-like behaviors, as  
399 well as CREB, pCREB and BDNF levels in a neuropathic rat model. Different treatment strategies  
400 used for pain and mood disorders, such as imipramine (Ishikawa et al., 2014), neurotrophin (Ishikawa et  
401 al., 2015) or ketamine (Pan et al., 2018), also recruit the MAPK/ERK pathway by altering ACC  
402 pERK, pCREB and BDNF levels. The inhaled anesthetic sevoflurane also impacts ACC pERK levels  
403 in rats and has a positive effect on the nociceptive and anxiety components of inflammatory pain (Luo  
404 et al., 2015). On the contrary, intravenous anesthetics such as propofol and sodium pentobarbital do  
405 not produce the same effect and only block the nociceptive response (Luo et al., 2015). Besides  
406 pharmacological agents, paramedical approaches such as manual acupuncture and electroacupuncture  
407 were shown to alleviate mechanical hypersensitivity and anxiodepressive-like behaviors (Cong et al.,  
408 2021; Shao et al., 2021a, 2015; Shen et al., 2021; Zhu et al., 2022), as well as pERK overexpression  
409 (Shao et al., 2015) and BDNF/CREB downregulation (Cong et al., 2021), respectively.

410 Glutamatergic signaling and its receptors also play a critical role in the pain/anxiodepressive dyad (Du  
411 et al., 2017; Guo et al., 2018; Sun et al., 2016; Wang et al., 2019; Zeng et al., 2018). Indeed, the  
412 expression of ACC AMPA ( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) and  
413 NMDA receptors were increased in inflammatory (Du et al., 2017; Sun et al., 2016; Wang et al.,  
414 2020), visceral (Ren et al., 2022; Wang et al., 2019) and remifentanil-induced pain (Zeng et al., 2018).  
415 AMPA-R/NMDA-R ratio was reduced in the rACC of an inflammatory-pain model (Ducret et al.,  
416 2022) and blockade of both ACC AMPA-R and NMDA-R -using either CNQX or AP-5- reversed  
417 mechanical hyperalgesia in a chronic pancreatitis model (Ren et al., 2022). Moreover, injection of an  
418 antagonist AMPA-R subunit pGluR1 in the ACC prevented anxiety-like behaviors and remifentanil-  
419 induced hyperalgesia and also decreased ACC c-Fos overexpression (Zeng et al., 2018). Along with  
420 GluR1, several groups also reported dysregulation of other related proteins such as increased levels of  
421 protein kinases PKCzeta and PKMzeta (Du et al., 2017) or CPEB1 (Cytoplasmic Polyadenylation  
422 Element Binding Protein 1) in the ACC (Wang et al., 2019). Interestingly, blocking PKCzeta or  
423 knocking down CPEB1 alleviated pain and anxiety-like behaviors as well as increased GluR1 levels.  
424 On the other hand, it was reported that mGluR5 expression was decreased in a model of inflammatory  
425 pain (Guo et al., 2018) and positive allosteric modulation of mGluR5 alleviated both CFA-induced  
426 anxiety-like behaviors and allodynia. These findings suggest a differential role for AMPAR, NMDAR  
427 and mGluR5 in the emotional dysregulation. Extracellular alterations might trigger or facilitate

428 intracellular changes. Li and colleagues (2021) indeed showed, in neuropathic pain, a downregulation  
429 of LAMB1 -a key component of the extracellular matrix with a dual role in tissues architecture and  
430 cell signaling- that accompanies anxiodepressive-like behaviors. These results suggest a yet  
431 understudied role of the extracellular matrix in chronic pain and its emotional consequences. Indeed,  
432 such extracellular pain-induced modifications might lead to strong alterations in neurotransmission.

433 Besides neurons, glial cells are also involved in the anxiodepressive-consequences of chronic pain.  
434 Indeed, inflammatory (Sun et al., 2016) and neuropathic pain (Wang et al., 2020) induce microgliosis,  
435 astrogliosis and an increased proinflammatory cytokine expression such as TNF- $\alpha$  and IL6 levels in  
436 the ACC. Imaging studies further support alterations in glial cells. Using manganese-enhanced  
437 magnetic resonance imaging, McIlwrath and colleagues showed increased T1 and T2 signals and  
438 bilateral astrogliosis within the ACC (McIlwrath et al., 2020). The implication of glial cells and  
439 cytokines in the anxiodepressive-like consequences of chronic pain was also supported by Wang and  
440 colleagues (2020) showing that chronic IDO1 inhibitors not only alleviate mechanical and thermal  
441 hypersensitivity, but also prevent anxiety-like behaviors by blocking cytokines and microglial  
442 activation.

443 Serotonergic signaling also plays an important role, since injection of the selective 5HT reuptake  
444 inhibitor paroxetine -commonly used as an antidepressant drug- in the ACC rescued anxiodepressive  
445 consequences, but not mechanical hypersensitivity, in animals with neuropathic pain (Matsuzawa-  
446 Yanagida et al., 2008). In addition, Zhou and colleagues (2022) described lower 5HT levels of animals  
447 with inflammatory pain and demonstrated that knockdown of 5HT1A-R in the ACC of naive animals  
448 mimicked anxiety-like behaviors, while 5HT7-R knockdown induced mechanical hypersensitivity,  
449 which points to a dual and differential role for 5HT signaling in chronic-pain induced emotional  
450 disorders. Likewise, chronic cannabidiol treatment was shown to rescue mechanical, heat and cold  
451 hypersensitivity as well as anxiety-like behaviors and increases cannabidiol receptor (CB1-R)  
452 expression in the ACC (Silva-Cardoso et al., 2021). Finally, oxytocin also seems to play a role in the  
453 emotional consequences of neuropathic pain as an acute administration of oxytocin in the ACC  
454 attenuated mechanical hypersensitivity and anxiety-like behaviors. Interestingly, this effect was shown  
455 to act through a reduction E/I ratio in the ACC, with a greater enhancement of inhibitory transmission  
456 due to the reduction of evoked excitatory postsynaptic current amplitude concurrently with  
457 depolarization and increased firing rate of interneurons (Li et al., 2021a).

### 458 **3.4 Conclusions and limitations**

459 The studies focusing on the role of the ACC in anxiety- (Table 2) and depressive-like (Table 3)  
460 consequences of chronic pain revealed multiple common mechanisms with pain related aversion such  
461 as E/I imbalance, MAPK/ERK pathway dysfunction (Fig.4). It is thus interesting to further study one  
462 hand time-dependent development of these cellular and molecular alterations and on the other hand  
463 their implication in the close relation between aversion and mood disorders. The limitations of these

464 studies base on the fact that sex differences, different type and stage of chronic pain are frequently  
465 neglected. Indeed, almost all studies performed in male, they are mainly use inflammatory and  
466 neuropathic pain models and time-dependent development of pathology is rarely taken into  
467 consideration.

468 -----Fig. 4-----

#### 469 **4. Sex differences, where are we now?**

470 It is important to highlight that the aforementioned studies were mainly performed in male rodents  
471 even though the study of chronic pain and emotional disorders in females is clinically relevant, as  
472 female patients represent 55% of the chronic pain population (Rikard, 2023) and they are twice more  
473 likely to develop anxiety or depression compared to male patients (Gater et al., 1998; Kessler et al.,  
474 1994, 1995; Weissman et al., 1994, 1996). Although sex differences are widely studied in clinical  
475 settings, this is still not the case for preclinical research (Beery and Zucker, 2011; Karp and Reavey,  
476 2019). For example, in the journal PAIN, almost 4 papers out of 5 studied male animals only, while  
477 5% papers studied both sexes without analyzing possible sex differences (Mogil, 2012). The studies  
478 presented in this review were no exception, as only 6 of them were performed either in both sexes (Dai  
479 et al., 2022; Jarrin et al., 2020; Li et al., 2021a, 2021b; Michailidis et al., 2021) or in females only  
480 (Zhong et al., 2012). While most of the studies reproduced the effects observed in male in females,  
481 there are some studies showing striking differences between the two sexes. For instance, Zhong and  
482 colleagues (2012) reported similar results in female compared to male animals (Dai et al., 2011)  
483 concerning the role of pERK in anxiety-like behavior of chronic pain. Similarly, there were no  
484 differences between females and males when looking at LAMB1 expression in the ACC following  
485 spared nerve injury (Li et al., 2021b) or mode of action of oxytocin in the ACC (Li et al., 2021a). In  
486 contrary, a recent pioneer study from Jarrin and colleagues (Jarrin et al., 2020) compared the role of  
487 ACC glutamatergic neurons in pain-related aversion in male and female rats in an inflammatory pain  
488 model and showed that the optogenetic activation of calmodulin-kinase II $\alpha$ -expressing excitatory  
489 neurons decreases formalin-induced nociceptive behaviors during conditioning without affecting  
490 formalin-induced place preference in males while this activation increases nociceptive behavior in  
491 females. However, the optogenetic inhibition of ACC glutamatergic neurons reduces pain-related  
492 aversion in both males and females without affecting the nociceptive behaviors. Similarly, a sex-  
493 dependent mechanism was reported in Michailidis and colleagues' study (2021), as they reported sex  
494 differences in depressive-like behaviors and microglial markers. Indeed, male mice displayed  
495 depressive-like behaviors earlier than female mice in a neuropathic pain mouse model and the authors  
496 observed an increased number of microglial cells in the contralateral ACC of female but not male  
497 mice. In addition, a transcriptomic study showed drastic differences between males and females 2  
498 weeks post neuropathic pain induction (Dai et al., 2022). This latest study further supports the need for  
499 thorough research including both sexes as sex-specific gene expression differences in control animals

500 have already been observed. This could explain the different responses to pain treatment strategies and  
501 emotional consequences in females and males. Even these few examples clearly show robust  
502 differences in the molecular, cellular and system-level mechanisms of pain processing in male and  
503 female.

## 504 **5. Future directions**

505 We believe that advancing our current understanding of chronic pain requires mechanistically explicit,  
506 hypothesis-driven, and clinically relevant models. In this review we highlighted some of the main  
507 findings over the last decades that have contributed to the present knowledge of brain mechanisms of  
508 chronic pain, and how such advances were possible due to a reverse translational research approach.  
509 We argue that this approach is essential in the chronic pain field in order to generate new scientific  
510 hypotheses, probe physiological mechanisms, develop therapeutic strategies and translate findings  
511 back into promising human clinical trials.

512 Even though the studies that we discussed in this present review strongly support the idea that the  
513 ACC is a critical hub and is hyperactive when animals experience pain-related aversion or  
514 anxiodepressive-like behaviours; further research is needed to fully understand how local ACC  
515 alterations occur and what cell types are involved. Most of the studies described the role of excitatory  
516 cells but the exact implication of inhibitory neurons in the ACC E/I imbalance must be studied further.  
517 Indeed, compensatory mechanisms, which could explain such a shift in specific cell type activity,  
518 cannot be excluded. Moreover, our knowledge concerning the role of the ACC extracellular matrix or  
519 non-neuronal cells, such as astrocytes and microglia, is still limited (Kuzumaki et al., 2007; Narita et  
520 al., 2006; Panigada and Gosselin, 2011) and should be further explored.

521 Beside local mechanisms, the pain-induced shift of the ACC E/I balance toward a hyperactive state  
522 likely affects the whole ACC connectome, which likely contributes to the development and  
523 maintenance of pain-induced emotional disorders. Hence, it appears essential to understand how ACC  
524 alterations affect its whole network but also how alterations within brain areas that belong to the ACC  
525 network directly or indirectly affect the ACC as well. Thanks to recent technological advance in brain  
526 imaging and brain activity manipulation, we have started to decipher the role of the ACC-related  
527 connectome in the emotional consequences of chronic pain (Becker et al., 2020; Gao et al., 2020;  
528 Hirschberg et al., 2017; Li et al., 2021a; Llorca-Torralba et al., 2022; Meda et al., 2019; Shen et al.,  
529 2021; Singh et al., 2020; Zhu et al., 2022; Zhu et al., 2021). The most recent breakthrough advances in  
530 multi-site fiber photometry (Sych et al., 2019), as well as functional magnetic resonance imaging  
531 (McIlwraith et al., 2020; Van der Linden et al., 2007), will now enable large scale brain circuits and  
532 will likely bring valuable insight regarding ACC networks. In addition, these techniques provide  
533 necessary tools for longitudinal studies, from pain initiation to the development of pain-induced  
534 emotional disorders, for description of subtle and time-dependent brain dynamic changes, as well as  
535 new potential targets for the development of personalized treatments of emotional disorders in patients

536 suffering from chronic pain. Finally, further studies are needed to better understand the sex-specific  
537 mechanisms implicated in pain related aversion as well as its anxiodepressive-consequences.

538

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1109 -----Table 1-----

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1111 -----Table 3-----