Functional genomic mechanisms of opioids: a systematic review of animal and human studies

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Abstract

In the past two decades, over-prescription of opioids for pain management has driven a steep increase in opioid use disorder (OUD) and death by overdose, exerting a dramatic toll on western countries. OUD is a chronic relapsing disease associated with a lifetime struggle to control drug consumption, suggesting that opioids trigger long-lasting brain adaptations, notably through functional genomic and epigenomic mechanisms. Current understanding of these processes, however, remain scarce, and have not been previously reviewed systematically. To do so, the goal of the present work was to synthesize current knowledge on genome-wide transcriptomic and epigenetic mechanisms of opioid action, in primate and rodent species. Using a prospectively registered methodology, comprehensive literature searches were completed in PubMed, Embase, and Web of Science. Of the 2705 articles identified, 69 met our inclusion criteria and were considered for qualitative analysis. Focusing on the 5 most studied nervous system structures (nucleus accumbens, frontal cortex, whole striatum, dorsal striatum, spinal cord; 44 articles), we also conducted a quantitative analysis of differentially expressed genes, in an effort to identify a putative core transcriptional signature of opioids. Only one gene, Cdkn1a, was replicated in six studies and, globally, our results unveil surprisingly low consistency across published work, even when considering most recent single-cell approaches. Analysis of putative sources for this poor reproducibility suggest contributions from species, brain structures, duration of opioid exposure, as well as batch effects. To go beyond those limitations, we leveraged threshold-free methods to illustrate how genome-wide comparisons may generate new findings and hypotheses. Finally, we discuss current methodological development in the field, and their implication for future research and, ultimately, better care.

Keywords

Opioid, substance use disorder, genomics, epigenomics, next generation sequencing

Introduction

Opioids are compounds derived from opium that have been used for millennia for their analgesic and euphoric properties [1]. While they remain reference treatment for severe pain, their chronic use also associates with a plethora of adverse effects, including tolerance, physical dependence and, in some individuals, the emergence of an opioid use disorder (OUD). OUD is defined as a problematic pattern of use that leads to clinically significant impairment. This severe and chronic disorder associates with increased mortality, with life expectancy decreased by more than 10 years due to somatic and psychiatric comorbidities. Over the last 2 decades, western countries have faced an increase in death by opioid overdose, due to more frequent prescription for pain management, increasing use of illegal compounds, or misuse of substitution therapies. From a socio-economic point-of-view, OUD imposes a major burden, with an estimated annual cost in the trillion dollar range in the US [2], and increasing use and harm in Europe [3]. This results, overall, in a worsening public health crisis.

OUD results from a variety of biological, psychological and socio-economic factors that show reciprocal interactions [4]. At biological level, it originates from pharmacological effects of opioid drugs, which trigger neurochemical and molecular adaptations within the brain, under modulation by genetic vulnerability and epigenetic regulatory factors. In turn, these effects are thought responsible for behavioral and cognitive dysfunctions that define the disease, including the inability to control drug use despite harmful consequences, or persistent and intense desire for the drug, even after years of abstinence.

Over the last 2 decades, high-throughput approaches have been harnessed to characterize at unprecedented scale, throughout the full genome [5], the molecular underpinnings of opioid effects in brain tissue, across various experimental models and species. In this context, the goal of the present systematic review was to synthesize and discuss current knowledge on functional genomic mechanisms of opioids, defined as changes in gene expression or epigenetic regulation that occur as a function of opioid exposure. To do so, we used a preregistered methodology, and performed an unbiased survey of bibliographic

repositories, focusing on studies that leveraged high-throughput approaches, most notably microarrays and next-generation sequencing. This focus on genome-wide analyses is motivated by the well-known biases associated with candidate gene approaches. It also reflects the conviction that better understanding of heterogeneous behavioral phenotypes such as OUD requires analyzing the full genome.

Based on studies identified through this screening, a qualitative synthesis was first performed to summarize experimental designs and findings. Second, a focused quantitative analysis of bulk tissue transcriptomic studies was conducted, with the goal of defining a core signature of opioids. To our knowledge, previous reviews on opioids and OUD [6,7] were completed more than 15 years ago, and such a systematic synthesis across brain structures and technologies had never been performed. Our results indicate that, although more than 40 genome-wide studies have been published, available evidence for reproducible findings is surprisingly limited. Third, we reviewed most recent work that leveraged cell-type specific and single-cell approaches, or that interrogated epigenetic regulatory mechanisms contributing to opioid plasticity. Finally, we discuss current challenges in the field, as well as avenues for future work.

Methods

Full methodology and bioinformatic code are available as Supplementary Material and File.

Results

The search provided 2705 potentially eligible studies (Fig.1). Once duplicates were removed, a total of 1225 were screened. 1067 articles were excluded because their title or abstract didn't meet eligibility criteria, with an additional 89 articles excluded after full text evaluation. As a result, 69 papers were selected for qualitative synthesis, covering both transcriptomic and epigenomic approaches. Among these, 44 articles that performed transcriptomic analyses in the 5 most frequently investigated regions were selected for more detailed quantitative synthesis: of these, 34 articles investigated a single brain region, 9 covered 2 regions, and

107 only 1 characterized 5 regions [8]. Overall, this resulted in 52 differential expression analyses:
108 18, 11, 11, 6 and 6 in the nucleus accumbens (NAc), frontal cortex, whole striatum, dorsal
109 striatum and spinal cord, respectively.
110
111 **1. Qualitative analysis (n=69 studies)**
112 ***Species and sex ratio.*** Most studies were conducted in rodents (Fig.2A): 30 (43%) in rats,
113 28 (41%) in mice, and 1 in both species. Only a small number investigated humans (n=8, 12%)
114 and macaques (n=2, 3%), reflecting well-known practical limitations. As consequence, current
115 knowledge on conserved or species-specific opioids effects is limited.
116 Regarding sex, epidemiological surveys indicate that OUD prevalence is higher in
117 males than in females [3, 9] (although smaller differences have been reported with DSM-5
118 criteria [10]), with a number of sex-specific risk factors (e.g., ethnicity, income). This clinical
119 situation has long been modeled in rodents, as sex differences in behavioral effects of opioids
120 [11] have been described for analgesia [12], tolerance [13], withdrawal symptoms [14], reward
121 [15] or motivation [16]. In comparison, molecular mechanisms underlying these differences
122 are poorly characterized. The majority of OUD studies reviewed here investigated males only
123 (n=48, 70%; Fig.2B-D), with few analyses conducted in females (n=5, 7%), and only 14 studies
124 (20%) that included both sexes (7 in rodents, 7 in humans). These numbers are consistent
125 with the current landscape in neuroscience research, in which the ratio of articles reporting on
126 males only, as opposed to females only, is around 5 to 1 [17]. We nevertheless note an
127 encouraging trend, as the large majority of studies (8/10) published in 2021 investigated both
128 sexes. Several factors likely underlie this recent evolution, including the recognition that
129 females do not display more experimental variability than males [18], and evidence that
130 pathophysiological mechanisms may differ across sexes, as shown for disorders such as
131 depression [19], schizophrenia [20] or alcohol use disorder [21].
132 Specifically in rodents (Fig.2C), more than three quarters of studies investigated males
133 only (n=47, 80%). Seven articles investigated both sexes (n=7, 12%), using surprisingly low
134 sample sizes (n=1-4 in male/female, and opioid-treated/control, groups; Fig.2D). Among

135 these, 2 studies treated sex as a covariate [22,23], and 3 pooled both sexes for the analysis
136 of opioid effects [24–26], therefore providing no direct description of male/female differences.
137 This is concerning, considering that the 2 remaining studies found little overlap, from 5 to 35%
138 (see [27] and [28]), among morphine-induced changes occurring in each sex. Therefore,
139 available evidence points toward a substantial degree of sex-specificity in opioid-induced
140 transcriptomic plasticity, which will have to be confirmed in future work. Even less is known in
141 human (Fig.2E). Among 8 articles, 7 investigated males and females, while only 1 investigated
142 males only. However, these 7 studies showed a strong bias towards examination of men
143 (Fig.2F), with only one that used equal sample sizes in both sexes [29]. In the later, sex was
144 treated as a covariate, with no direct description of its impact. Altogether, this underscores a
145 significant gap in the opioid literature, and calls for additional work on sex as a biological
146 variable. The hope is that better understanding of divergent pathophysiological routes in males
147 and females may unravel sex-specific therapeutic strategies.

148

149 ***Route and duration of opioid administration.*** Among studies included in qualitative analysis
150 (n=69), the majority used chronic administration (n=55, 80%; see Fig.3A), mostly via self-
151 administration (SA, n=19, 34%, among which 8 corresponded to human studies),) and
152 intraperitoneal injection (ip; n=18, 33%), followed by subcutaneous injection (n=8, 15%). Other
153 procedures were less frequently used: continuous administration (with pellets or mini-pumps;
154 n=5, 9%), intrathecal administration (n=3, 5%), or gavage (n=2, 4%).

155 There is limited information regarding differences among administration procedures.
156 Interestingly, Lefevre et al [30] recently compared 3 conditions: continuous and systemic
157 morphine infusion using osmotic mini-pumps, with ("Interrupted") or without ("Continuous") the
158 induction, twice daily, of a precipitated withdrawal episode (using naloxone, a non-specific
159 opioid antagonist), compared to Controls (saline minipumps). Results indicated 687 and 407
160 differentially expressed genes (DEG) when comparing Interrupted and Control groups in the
161 NAc and dorsal striatum, respectively, with only 1 DEG between Continuous and Control

162 groups. Therefore, rather than opioid exposure per se, it is the repetition of intermittent
163 withdrawal episodes that may be driving the intensity of transcriptomic adaptations affecting
164 the mesolimbic system (whether precipitated, as in this study, or spontaneous, in-between
165 bolus injections). This is consistent with the clinical notion that the alternation of intoxication
166 and withdrawal is critical for the development of OUD.

167 Opioid effects have also been investigated using acute administration (10 studies,
168 14%), corresponding mostly to ip injection (9 articles), with only 1 using subcutaneous
169 injection. While these studies were mainly interested in opioids used as analgesics or
170 anesthetics [31,32], or sought to compare acute effects of various psychoactive drugs [33],
171 available data supports the notion that they may be relevant for the understanding of OUD.
172 Indeed, systematic comparisons across inbred mouse strains have shown that variability in
173 the intensity of opioid physical dependence is strikingly similar when withdrawal is precipitated
174 after acute or chronic injections [34,35]. In other words, there are shared genetic determinants
175 for brain adaptations triggered by acute or chronic exposure. A recent single-cell RNA-
176 sequencing (scRNA-Seq) study [36] provides further evidence that these genetic determinants
177 translate into at least partially similar transcriptional plasticity during acute or chronic opioid
178 exposure (see *3. High cellular resolution approaches*).

179 Another important consideration is that most studies reviewed here (51/69, 73%) used
180 passive modes of administration, which do not model the volitional or compulsive drug-taking
181 aspects of OUD. This reflects the technical difficulty of intravenous SA experiments (IVSA),
182 particularly in the mouse. Interestingly, new paradigms now facilitate such studies, including
183 devices for opioid vapor [37] SA, or the use of optogenetics for light-induced self-stimulation
184 [38]. Few comparisons of passive and active administrations have been conducted. Among
185 these, Tapocik et al [39] compared “yoker” mice (IVSA group) with groups of “yoked-morphine”
186 and “yoked-saline” animals which, each time the yoker self-administered morphine, received
187 passive IV morphine or saline injections. A much higher number of DEG were identified
188 between the yoker and yoked-saline groups (n=1177) than between yoked-morphine and
189 yoked-saline mice (n=244), with only limited overlap between the 2. This suggests that

190 voluntary drug-taking may drive or reflect transcriptional adaptations that are only partially
191 recapitulated by non-contingent administration. Additional studies will be required to deepen
192 this understanding.
193
194 ***Nervous system structures.*** Among articles eligible for qualitative analysis, 18 regions of the
195 nervous system were explored (Fig.2B). Not surprisingly, a large majority focused on the
196 mesocorticolimbic dopaminergic pathway: the NAc (21 articles [8,22,26,28–30,32,40–53]),
197 frontal cortex (13 articles [8,25,27,29,51,54–61]), whole/unspecified striatal complex (11
198 articles [24,33,62–70]), dorsal striatum (7 articles [8,30,48–50,71,72]) and ventral tegmental
199 area (VTA, 5 articles [28,51,73–75]). Other regions included the spinal cord (7 articles [62,76–
200 81]); hippocampus (5 articles [8,63,82–84]); amygdala (3 articles [8,85,86]); locus coeruleus
201 [74, 87], ventral midbrain [42, 88], hypothalamus [89, 90], whole brain with [91] or without [92]
202 cerebellum (1 each); periaqueductal gray matter [93], pituitary gland [90], arcuate nucleus [94],
203 nucleus of the tractus solitarius [31], brainstem [23], cerebellum [84] or dorsal root ganglia [95]
204 (DRG, 1 each). Below, we briefly describe the rationale for studying such diverse structures.
205 The course of OUD involves 3 stages: intoxication, withdrawal/negative affect, and
206 anticipation of next intoxication. These stages reflect gradual adaptation of the brain to drug
207 exposure, and rely on distinct mechanisms. The intoxication stage, mostly driven by acute
208 reward, notably associates with dopamine release, in the striatum and frontal cortex, from
209 neurons located in the VTA. The second stage corresponds to negative reinforcement,
210 whereby drug consumption alleviates physical and negative affective states of withdrawal,
211 involving the locus coeruleus [96], NAc [97], and amygdala [98], among others. Finally, the
212 anticipation stage results from memories of drug-associated cues and contexts
213 (hippocampus), as well as impaired goal-directed behaviors, implicating higher-order
214 structures (e.g. prefrontal and orbital cortices [99]). These relationships are not exclusive, with
215 some structures implicated at multiple stages of the disease (e.g., the striatum, also involved
216 in habit formation through its dorsal part). Remaining regions (spinal cord, DRG, brainstem,
217 periaqueductal gray matter, PAG) were investigated in relation to opioid analgesia, tolerance

218 and hyperalgesia. Finally, few articles were interested in how opioids modulate
219 neuroendocrine systems (hypothalamus, arcuate nucleus, pituitary gland), with implications
220 for their interactions with the stress or reward systems.

221 In addition to these associations, it is also important to consider the distribution of the
222 endogenous opioid system. This system is composed of 3 opioid receptors (mu, delta and
223 kappa; MOR, DOR and KOR), among which the MOR is central and necessary for opioid-
224 induced analgesia, reward, and physical dependence [100]. This receptor, and neurons by
225 which it is expressed, therefore represent pharmacological entry points for brain adaptations
226 to opioids. Comparison of studies reviewed here with MOR distribution in the brain (as
227 assessed using ligand autoradiography [101–106]; Fig.3C) indicates that: i) MOR is highly
228 expressed among structures most frequently studied, as expected; ii) surprisingly, other areas
229 with similarly high expression have received little to no attention (NTS, locus coeruleus, dorsal
230 raphe nucleus, medial habenula, thalamus), calling for more work.

231

232 ***Techniques.*** Thirty-seven and 29 studies used microarrays and RNA-seq, respectively, to
233 analyze the transcriptome (Fig.3D). While the first RNA-seq study was published in 2014[12],
234 this technique has since progressively replaced microarrays, reflecting its well-recognized
235 advantages (higher sensitivity and throughput, potential for gene discovery). For epigenomic
236 profiling, 3 studies recently performed bisulfite sequencing [51, 59, 61], and only 1 used
237 ATACseq (Assay for Transposase-Accessible Chromatin with sequencing) [72]. Considering
238 rapid improvements in throughput and resolution of sequencing methodologies (see below),
239 these trends are expected to strengthen.

240

241 **2. Quantitative analysis of transcriptomics studies**

242 To identify reproducible transcriptomic opioid signatures, we ran quantitative analyses of bulk-
243 tissue results available for the 5 most studied structures (corresponding, after removal of
244 epigenomic studies, to 44 articles; Fig.4A). To do so, gene-level results were retrieved from
245 each study, when available. This yielded only 24 lists of DEG comparing opioid-treated

246 animals and control groups, originating from 17 articles. Among these, 6 articles did not report
247 any p-values, while the remaining 11 used very diverse thresholds on p-values and fold
248 changes (FC; details in Supplementary Table 1). Surprisingly, a small minority (3/44, 6.8%
249 [24,28,29]) provided full results with p-values and FC for all genes. In addition, raw data were
250 identified as available in the public repository GEO in less than half of the cases (8/17, 47%).
251 Overall, this underscores strong heterogeneity in analytical procedures and data reporting
252 which, as discussed elsewhere [107], prevents re-analysis and limits creation of new
253 knowledge. To counter this, efforts have been made to harmonize omics practices [108], with
254 policies enforced by funding agencies [109]. For the present review, a genome-wide meta-
255 analysis combining, for every gene, effect sizes and sample sizes across studies was not
256 possible. We nevertheless: i) Systematically assessed concordance among available lists of
257 DEG; ii) Conducted a threshold-free comparison of the 2 NAc studies for which genome-wide
258 results were available.
259
260 **2.1 Nucleus accumbens (18 studies)**
261 Among 18 articles, only 8 provided lists of DEGs: 4 in mice (in males only [30,32,40,43]), 2 in
262 rats (1 in males only [45], 1 in both sexes [28]), 2 in humans (both sexes [29,47]). The 8 articles
263 provided a total number of 3502 DEG (Fig.4B-E). Among these, the vast majority were
264 identified by 1 study only (n=3017/3502, 86%), while 424, 57 and 4 DEG were identified across
265 2, 3 or 4 studies, respectively. No single genes were common to 5 or more articles. In addition,
266 the best overlap was found among the 2 DEG lists reported by Imperio et al [45] (Fig.3E).
267 Overall, this indicates poor reproducibility in the NAc.
268 To try to define which factors contribute to this heterogeneity, we intersected DEG lists
269 in each species separately (Fig.3C-E). This, however, did not improve concordance: in
270 humans, only 54 genes were common to both studies (Jaccard Index, JI=2.4%, Fig.4C; see
271 Supplementary Table 2 for the full matrix of pair-wise JI across all studies); in mice, 7 genes
272 were identified by at least 2 studies (JI=1%, Fig. 4D); in rats, the JI increased to 15.5%
273 (Fig.4E), mostly driven by high overlap among the 2 aforementioned DEG lists from Imperio

274 et al. In the later work, the authors identified large- (LS) and small-suppressors (SS): in LS
275 rats, greater avoidance of a natural reward (saccharine) that predicted heroin availability was
276 associated, compared to SS rats, with worse outcomes during later heroin IVSA (increased
277 consumption, higher motivation and relapse). Gene expression changes occurring in the LS
278 group (compared to control rats given access to saccharine but not heroin; n=597 genes), and
279 in the SS group (same controls, n=205) showed a JI of 25.5% (with striking concordance in
280 up- and down-regulation). This was much higher than overlaps observed between Imperio et
281 al and the other rat study by Townsend et al, which yielded JI of 1.35% (Imperio-
282 SS/Townsend) and 3.43% (Imperio-LS/Townsend). Overall, this indicates that differences
283 across laboratories, animal facilities or experimental designs lead to poor concordance across
284 studies.
285
286 **2.2 Dorsal striatum (6 studies)**
287 Only 2 dorsal striatum studies provided lists of DEG (Fig.3F). One study used chronic
288 morphine administration interrupted by naloxone injections in male mice [30], as described
289 above, while the other used acute morphine injection in male rats [71]. Reflecting these
290 differences, a very small overlap was found between the 2 (0.62%). Therefore, while the dorsal
291 striatum is thought to gradually gain control over drug-taking behaviors in OUD [110],
292 underlying transcriptomic plasticity is poorly characterized.
293
294 **2.3 Whole striatum (11 studies)**
295 Six of the 11 whole striatum studies provided lists of DEG [24,33,64–67] (Fig.5B): 2
296 investigated chronic heroin [67] or morphine [24] treatment, while 2 compared acute effects of
297 distinct opioids (morphine and heroin, with lists of DEG for each) [33,65], and 2 compared
298 acute and chronic morphine exposure [64,66]. Most DEG were identified by 1 study only
299 (3308/3736, 88.5%), with only 112 genes common to 3 or more studies. Strikingly, consistent
300 with NAc results, strongest overlaps were observed for lists of DEGs coming from the same
301 article, with for example 54 (JI=17%, Korostynski 2013 [33]) and 16 (21%, Piechota 2010 [65])

302 DEG common to acute morphine and heroin injections, respectively, and 139 DEG (31%,
303 Korostynski 2007 [66]; Fig.5A) common to acute and chronic morphine administration. In
304 sharp contrast, comparisons across, rather than within, studies yielded poor overlaps, even
305 when considering those that used similar drug administration: among chronic morphine
306 studies (Fig.S1A), the JI varied between 0.1% (Coffey 2020 versus Skupio 2017), 1.2%
307 (Korostynski 2007/Skupio 2017) or 2.6% (Korostynski 2007/Skupio 2017), and, among acute
308 morphine studies, from 2.7% to 7.8% (Fig.S1B; Piechota 2010/Skupio 2017, JI=2.7%;
309 Piechota 2010/Korostynski 2007, 3.2%; Piechota 2010/Korostynski 2013, 6.4%; Korostynski
310 2013/Skupio 2017, 6.6%; Korostynski 2013/Korostynski 2007, 7.3%; Korostynski 2007/Skupio
311 2017, 7.8%), with only one comparison standing out among acute heroin studies (30 common
312 DEG, JI=19%, Piechota 2010/Korostinsksy 2013). Importantly, because 5 of these 6 studies
313 were conducted by the same group, it is possible to speculate that this variability may reflect
314 batch effects among experiments conducted in the same facilities but at different time points,
315 rather than variations across different opioids or administration parameters (see also section
316 2.6 below).
317
318 **2.4 Frontal cortex (11 studies)**
319 Seven studies provided DEG lists (Fig.5B): 2 in humans [29, 58], 5 in rats [25,27,55–57]. Data
320 from Odegaard et al [25] were discarded, as they corresponded to transgenerational effects
321 in animals not directly exposed to opioids (administered to dams during pregnancy, or
322 postnatally). For 2 additional studies [55,57], we could not map microarray probes to genes.
323 Among the 4 remaining articles, little overlaps were again found (Kuntz-Melcavage/Mendez,
324 JI=0.5%; Kuntz-Melcavage/Seney, 1.1%; Liu/Mendez, 1.4%; Liu/Seney, 1.6%), with no
325 improvement when conducting comparisons within each species (Liu/Kuntz-Melcavage in
326 rats, JI=0%; Seney/Mendez in humans, 4.1%).
327
328 **2.5 Spinal cord (6 studies)**

329 Among the 6 spinal cord studies, 5 did not provide DEG [76–80], while the 6th one used a
330 homemade microarray[62], for which correspondence between probe IDs and genes was only
331 partially provided. As such, no comparison was possible.
332
333 **2.6 Sources of variability**
334 Considering that pharmacological recruitment of MOR and resulting intra-cellular signaling
335 may trigger a set of common transcriptional adaptations across the 5 regions of interest, we
336 next pooled all lists of DEGs (Fig.5D). Again, the vast majority of DEG (70.1%, 4940/7047)
337 were unique to a single study, while only 21.4% common to 2 studies (1509), 5.9% to 3 studies
338 (418), and 1.7% to 4 or more studies (117; full lists in Supplementary Table 3). Interestingly,
339 Cdkn1a (Cyclin Dependent Kinase Inhibitor 1A) was identified by 11 studies. This gene
340 encodes for p21, a protein involved in the regulation of the cell-cycle and oligodendrocytes
341 [111]. Interestingly, Avey et al [40] found Cdkn1a to be one of the most significantly
342 upregulated genes in response to acute morphine. This upregulation occurred in
343 oligodendrocytes (confirmed by FISH), and was blocked by the opioid antagonist naltrexone,
344 indicating a MOR-dependent mechanism. Therefore, Cdnk1a may contribute to opioid effects
345 across multiple brain sites, possibly through the regulation of the oligodendrocyte lineage. Of
346 note, cocaine, another major drug of abuse, similarly recruits p21, as cocaine-induced
347 behavioral responses were modified in p21 knockout mice [112].
348 We also performed Gene Ontology (GO) analysis using DEG common to at least 3, 4
349 or 5 studies (Supplementary Table 4). No results were significant for the small list of 63 DEG
350 common to 5 or more studies. When considering the 180 DEGs common to at least 4 studies,
351 only 3 unspecific GO terms were enriched: cell periphery, plasma membrane and hormone
352 activity. More consistent results emerged for the 598 DEG common to at least 3 studies, with
353 46 significant GO terms mostly related to synaptic signaling and cell-cell communication, likely
354 reflecting opioid-induced electrophysiological and synaptic plasticity across brain structures.
355 Because weak DEG overlaps were identified within each individual region (even when
356 grouping studies based on species, Fig.4C-E, or treatment chronicity, Fig.S1), we sought to

357 more systematically assess, across the 5 regions, the impact of following factors: species,
358 brain structure, treatment chronicity, drug, and article (Fig.5D). For each factor (e.g., species),
359 pair-wise JI were classified in 2 categories depending on whether they belonged to the same
360 sub-group within that factor (e.g., 2 studies conducted in humans; "intra"), or not (e.g., 1 study
361 in humans compared to another in rats; "inter"). As expected, grouping studies by species
362 (p=6.0E-4) or brain structures (2.3E-4) increased concordance, with higher JI. This is
363 consistent with the notion that, although they remain poorly characterized, these 2 variables
364 significantly contribute to variability in opioid-induced transcriptional plasticity. Treatment
365 chronicity also had a significant, albeit milder, impact (p=0.012). In contrast, the type of opioid
366 used had none (p=0.43), possibly because, despite pharmacokinetic or dynamic differences,
367 all drugs considered act as agonists at the same molecular target, MOR. Importantly,
368 concordance increased most when lists of DEG came from the same publication (p=0.011),
369 despite the fact that only 7 comparisons fell under this category. As already mentioned, this
370 suggests that differences across laboratories explain a substantial part of the lack of
371 reproducibility across studies.

372 Finally, we refined our analysis by considering the directionality (up/down) of DEG,
373 focusing on genes identified by at least 5 studies. Among the 17/24 studies that reported on
374 directionality (Fig.S2), most genes showed discordant results (37/63, 59%): for example,
375 Cables1 was upregulated in Imperio et al and Coffey et al, but downregulated in Seney et al
376 in both the frontal cortex and NAc. In contrast, only 18/63 (29%) DEGs showed concordant
377 directions, with 17 up-regulated genes, and 1 down-regulated. For the remaining 8 genes
378 (13%), directionality was reported by none or only 1 study. Altogether, these results further
379 highlight low reproducibility.

380

381 **2.7 Threshold-free genome-wide comparison**

382 Next, we compared the 2 NAc studies (Seney et al [29], Townsend et al [28]) for which full
383 genome-wide results were available. While both included males and females, only Townsend
384 et al reported sex-specific results. Therefore, we reprocessed Seney et al raw data to identify

385 DEG in each sex separately (see *Supplementary Material*). This slightly increased
386 concordance: while JI were equal to 7.5 and 4% when comparing initial Seney data
387 (male/females pooled) with Townsend data in females or males, respectively (Fig.S3A-B),
388 they increased to 9.5% and 4.5% when considering sex-specific Seney results (Fig.6A-B).
389 These results therefore argue for the importance of accounting for sex when identifying opioid-
390 induced transcriptional changes.

391 We then used RRHO2 (Rank–Rank Hypergeometric Overlap [113]) to conduct
392 genome-wide comparison of the 2 studies. RRHO2 iteratively performs hypergeometric testing
393 for all possible combinations of thresholds applied to each dataset, generating a so-called
394 "threshold-free" analysis. Because this approach takes directionality into account, we also
395 computed, for comparison, selective overlaps among either up- or down-regulated DEG only
396 (using p-value thresholds from each study, and represented as white crosses in RRHO2
397 matrices; Fig.6C-D). Compared to those, much more significant overlaps were identified using
398 RRHO2 when less stringent p-value thresholds were considered within each dataset (best
399 hypergeometric results are shown as black crosses). These increased overlaps, however,
400 came at the expense of the identification of large lists of genes which, although commonly
401 dysregulated in similar direction, individually exhibited milder FC. Of note, these overlaps were
402 detected despite the fact that Seney investigated human post mortem tissue from individuals
403 with OUD, while Townsend focused on rat tissue collected after 24 hours of withdrawal from
404 fentanyl IVSA. In addition, different patterns of convergence were observed in each sex: in
405 females, overlaps were highly significant among both up- and down-regulated genes while, in
406 males, a lower concordance predominantly affected down-regulated genes. This suggests that
407 these rat datasets may differentially capture sex-specific signatures of human OUD. Overall,
408 these analyses demonstrate the utility of reporting full genome-wide results which, in
409 combination with threshold-free approaches, could generate deeper comparisons and
410 meaningful insight, even across species.

411

412 **3. High cellular resolution approaches**

413 Studies reviewed above indicate that opioids induce genome-wide transcriptomic changes,
414 with striking variability across laboratories, experimental designs, and brain regions. The later
415 likely reflects a combination of direct and indirect effects: i) direct pharmacological opioid
416 effects correspond to the recruitment of MOR and downstream intracellular signaling. In case
417 of neurons, this classically results in decreased excitability and activity [114]; ii) then,
418 depending on the chemical identity and connectivity pattern of MOR-expressing cells, their
419 inhibition leads to changes in the release of multiple neuromodulators and transmitters,
420 triggering indirect adaptations [102]. Accordingly, transcriptomic changes described above
421 reflect the sum of these events. This raises 2 important questions: how is each cell type, or
422 even each brain cell, affected by these changes? And what are underlying molecular
423 regulatory principles?

424 To address the first question, several groups recently used cell-type specific and
425 single-cell methodologies. Coffey et al [115] focused on microglial cells of the whole striatum,
426 using translational ribosome affinity purification (TRAP, to capture their ribosomes and
427 "translating transcriptome" [116,117]). Chronic morphine effects were analyzed with, or
428 without, the induction of naloxone-precipitated withdrawal. Results showed that both chronic
429 morphine and withdrawal triggered numerous gene expression changes, which mostly
430 affected GO terms related to cAMP signaling, regulation of synapses, and the cytoskeleton. A
431 striking anticorrelation was observed between morphine- and withdrawal-induced changes,
432 providing an interesting transcriptomic correlate, at the level of microglia, of the
433 pharmacological antagonism between morphine and naloxone. Surprisingly, to our
434 knowledge, no study has used such approaches to interrogate opioid effects specifically in
435 neuronal cells, let alone MOR-expressing neurons which, arguably, may exhibit particular
436 sensitivity to opioids. This is feasible, as several knock-in mouse lines are now available, in
437 which the Cre enzyme is selectively expressed by MOR-expressing cells [118–120].

438 Achieving even higher resolution, Avey and colleagues applied single-cell RNA-seq
439 (scRNA-Seq, using Drop-seq [121]) to analyze transcriptomic changes occurring, in more than
440 23,000 cells of the mouse NAc, 4 hours after an acute morphine injection (20 mg/kg, ip) [40].

441 As is typical in scRNA-Seq, the sequencing depth achieved yielded expression data for around
442 1,600 genes, corresponding to a reduced representation of the genome. Acute morphine
443 induced gene expression changes that were widespread and, interestingly, most frequent in
444 oligodendrocytes, followed by neurons, astrocytes, and microglia. This therefore uncovers a
445 hierarchy in transcriptional sensitivity to opioids among NAc cell-types, and extend on the
446 previous investigation of microglia[24]. More recently, Reiner et al [36] used single-nucleus
447 RNA-Seq (snRNA-seq, 10x Genomics 3' gene expression assay) to analyze a larger number
448 of nuclei (190,030) and genes (>2000) in the rat NAc, also following acute morphine injection
449 (10 mg/kg, ip). DEG mostly affected neurons and oligodendrocytes, followed by astrocytes
450 and microglia, reminiscent of the former study. Effects of morphine were also analyzed after
451 10 days of IVSA and compared to acute ip effects. Among DEG shared across the 2 conditions
452 (n=431), the vast majority were dysregulated in a similar direction (up/down), suggesting that
453 they may reflect morphine exposure rather than volitional drug-taking (an additional "yoked"
454 group, chronically exposed to morphine, would help substantiate this distinction). Importantly,
455 an even larger number of DEG were specifically dysregulated in only one of the 2 conditions
456 (n=868, with more DEG associated with IVSA), with distinct distributions among NAc cell types
457 (e.g., sub-populations of Dopamine receptor D1- or D2-expressing neurons). By providing the
458 first single-cell description of morphine IVSA, these results reinforce the notion that voluntary
459 drug-intake may recruit molecular mechanisms that are not recapitulated by non-contingent
460 administration.

461 We next directly confronted results from these 2 single-cell studies regarding acute
462 morphine exposure. Both used Seurat [122] and dimensionality reduction (t-SNE [40] or UMAP
463 [36]) to identify clusters, which were then assigned to cell-types using external data. We first
464 compared marker genes reported to define those clusters, when available (Avey: 11/29
465 clusters; Reiner: 11/19 clusters). Low agreement was found, with JI ranging from 0 up to 7.1%
466 (for activated medium spiny neurons, MSNs, in Avey et al and D1R MSN-1 in Reiner et al,
467 Fig.S4A), possibly reflecting the fact that both studies reported low numbers of marker genes
468 (Avey: 39 to 51 marker genes; Reiner: 4 to 12). We then confronted morphine-induced DEG

469 for each cell-type (Fig.6E). Low JI values were found, with maximal overlap (6.4%) obtained
470 when comparing DEG affecting "myelin-forming and mature oligodendrocytes" (MO_all) in
471 Avey et al to "Oligodendrocyte-3" in Reiner et al. In addition, surprisingly, while a group of cell
472 clusters assigned to similar cell-types in the 2 studies showed some similarity in DEG (shown
473 as green squares, Fig.6E), strong concordance was also observed across cell clusters with
474 different identities (e.g. "Astrocyte-2" in Reiner et al compared to MO_all in Avey et al; yellow
475 squares). Also, overlaps among DEG (Fig.6E) did not increase when considering more similar
476 cell-types (Fig.S4B), as would be expected. Therefore, these analyses indicate: i) significant
477 discrepancies across the 2 studies (Fig.S4B), possibly reflecting methodological factors not
478 yet standardized in single-cell approaches, and ii) that, reminiscent of issues raised at bulk
479 tissue level, a more comprehensive reporting of marker genes and differential expression
480 results should be enforced.
481
482 **4. Non-coding and epigenomic mechanisms of opioid plasticity**
483 In parallel to efforts to deepen cellular resolution of transcriptomic analyses, others have
484 sought to decipher underlying non-coding RNA and epigenomic modes of regulation [123].
485 This is motivated by the recognition that, although neurons are post-mitotic cells, they exhibit
486 peculiar plasticity features that may contribute to the encoding of life events, including
487 exposure to drugs of abuse [124].
488 Several non-coding RNA species have been investigated. The first report used
489 custom-made arrays to characterize an unknown number of micro-RNA (miR), and reported
490 bidirectional expression changes affecting 7 of them in rat primary neuronal cultures or the
491 mouse hippocampus, following morphine or heroin exposure [84]. Among these, the miR-339-
492 3p was later shown to target the 3'-UTR and down-regulate MOR in response to opioid
493 treatment [125]. A second study used more recent arrays to interrogate all mouse miR in the
494 NAc, following chronic heroin injections [52]. 111 miR were found to exhibit >25% expression
495 changes (although no statistical significance was reported). This study then focused on miR-
496 218, and showed that its over-expression could inhibit heroin-induced reinforcement (in both

497 conditioned place preference and IVSA), possibly through the targeting of MeCP2 (a protein
498 that binds methylated DNA), suggesting a potential link between opioids, miR and DNA
499 methylation. Other studies explored long non-coding (lncRNA) or circular RNA (circRNA). One
500 group used 2 arrays interrogating 9,000 lncRNA [78] and 15,000 circRNA [81] to characterize
501 chronic morphine effects in the rat spinal cord: 136 lncRNA (84 up, 52 down; nominal p-
502 value<0.05, |log2FC|>1.5) and 2038 circRNA (896 up, 1142 down; nominal p-value<0.05,
503 FC>2) showed expression changes. In parallel, another report used RNA-Sequencing to focus
504 on circRNA in the NAc [53], and found differences for 112 of them when comparing groups of
505 mice receiving chronic morphine injections with or without electroacupuncture (51 up, 61
506 down; nominal p-value<0.05, |log2FC|>1.5). These results remain descriptive, and a large
507 variety of mechanisms have been proposed to explain how non-coding RNA may exert
508 biological functions (including the sequestering of miR by circRNA, or complementary
509 interactions among lncRNA and mRNA). Understanding how these mechanisms are recruited
510 by opioids will require further investigation.

511 Regarding the epigenome, 2 recent studies (1 in the rat [51], 2 in humans [126,127])
512 investigated DNA methylation, a mark increasingly implicated in psychiatric pathophysiology
513 [128]. The rat study used Whole Genome Bisulfite Sequencing (WGBS) to analyze DNA
514 methylation changes during heroin IVSA, in a standard or enriched environment, in 3 brain
515 regions (VTA, NAc and medial prefrontal cortex) [51]. Because of low sequencing depth, DNA
516 methylation levels were averaged for selected gene features across the whole genome
517 (promoters, CG islands). As expected, neither IVSA nor environmental enrichment had any
518 impact on these global metrics, suggesting that the methylome is not massively
519 reprogrammed. It remains possible, however, that opioids may trigger DNA methylation
520 changes at more localized genomic sites, a hypothesis supported by: i) the intuition that large
521 transcriptomic changes associated with opioid exposure or OUD reviewed above are unlikely
522 to occur in the absence of any chromatin plasticity; ii) candidate gene studies which, although
523 outside the scope of the present review, provide evidence for differential DNA methylation at
524 specific loci (the MOR gene in human blood [129–131], and a few loci in the rat brain [132]);

525 iii) and, finally, the 2 aforementioned human studies. Focusing on Broadmann area 9, the first
526 used EPIC arrays to assess DNA methylation in a post-mortem cohort of OUD patients (n=19)
527 and healthy controls (n=11) [127]. Among the >800,000 CG sites investigated (where DNA
528 methylation mostly occurs in the mammalian brain), 11,917 sites located at transcription start
529 sites (TSS) showed nominally significant difference (p-value<0.05), with bidirectional changes.
530 While genomic localization of these probes or full genome-wide results were not described,
531 these results nevertheless support the existence of a form of methylomic plasticity in OUD.

532 Human studies face the difficult task of accounting for confounding factors. Variations
533 in genotype, age, sex, or socio-demographic factors, for example, have all been associated
534 with DNA methylation changes. Also, because epigenetic landscapes determine cellular
535 identity, differences observed in bulk tissue may reflect variations in cellular composition
536 (which may contribute to pathogenesis), rather than transcriptional regulation. The second
537 human study started to address these questions [126]. Using EPIC arrays to analyze bulk
538 tissue, the authors adjusted for socio-demographic characteristics, ancestry and cellular
539 composition. They investigated a large cohort (n=153), focusing on death from acute opioid
540 overdose rather than OUD. Although no CG site survived multiple testing correction, 13
541 passed a relaxed significance threshold (p<1.0E-05), one of which was located in Netrin-1, a
542 gene implicated in KOR activity. At genome-wide level, an association between opioid use
543 and accelerated epigenetic aging was reported, along with enriched differential methylation in
544 GO and KEGG pathways related to substance use. Overall, these studies support the notion
545 that the brain DNA methylome may be modulated by opioid exposure. Additional studies will
546 be necessary to better understand these effects, as well as potential roles for non-canonical,
547 brain-specific forms of DNA methylation (methylation in non-CG context [133],
548 hydroxymethylation at CG sites [134]).

549 Beyond covalent modifications of the DNA, epigenomic plasticity also relies on histone
550 modifications and changes in compaction or 3D structure of chromatin. Surprisingly, no data
551 characterizing histone changes in relation to opioid exposure were identified during our
552 systematic screening. Studies available on cocaine [134,135] nevertheless suggest potentially

553 broad adaptations. Interestingly, a recent study compared chromatin accessibility in a cohort
554 of 10 heroin abusers and 10 healthy individuals [72], in the NAc and putamen. ATAC-Seq was
555 used to identify genomic regions where chromatin was "opened" and DNA accessible for
556 tagmentation (i.e., tagging with sequencing adapters by a mutated, hyperactive transposase).
557 The technique was applied to neuronal and non-neuronal cells, isolated using NeuN staining
558 and Fluorescence-Activated Nuclear Sorting (FANS). Heroin use associated with an
559 enrichment of ATAC-Seq peaks in specific gene features (CG islands, promoters, 5'
560 untranslated regions) and annotated regulatory regions (the histone mark H3K4me1, or
561 binding sites for the EZH2 transcription factor), as well as more frequent changes in neurons
562 than in non-neuronal cells (affecting GO terms related to the synapse, among others). Using
563 *in vitro* reporter assays, primary cultures and behavioral rat experiments, the authors then
564 showed that FYN (a member of the Src tyrosine kinase family that exhibited strong heroin-
565 induced changes in chromatin accessibility in human), also significantly contributed to heroin
566 IVSA in the rat. Overall, this work nicely illustrates how epigenetic analyses may uncover
567 chromatin regulatory mechanisms contributing to OUD.
568

569 **Discussion**

570 In the present review, we systematically assessed current knowledge on functional genomic
571 mechanisms of opioids and OUD, and uncovered a surprisingly small overlap in genes for
572 which transcriptomic changes were consistently identified across studies. As a result, a lot
573 remains to be done to move the field forward, and we discuss below most promising
574 perspectives to refine behavioral modelling, improve cellular resolution, and use more diverse
575 sequencing technologies and advanced data integration.

576 While the complexity of OUD is difficult to recapitulate in animal models, their face
577 validity has nevertheless increased over last decades, and it is admitted that addiction-related
578 phenotypes (e.g., uncontrolled consumption, resistance to punishment, increased motivation)
579 can be induced in rodents [136]. We now face the task of deciphering the molecular
580 mechanisms that may account for each of these dimensions, including for example the

581 susceptibility to develop compulsive drug intake. The later susceptibility aspect has been
582 addressed by only one article covered in the present review (Imperio et al [45]), which
583 contrasts with the amount of data available for other psychiatric disorders, in particular
584 depression. Among other examples [137,138], Bagot et al [139] examined transcriptomic
585 adaptations occurring in mice categorized as susceptible or resilient to chronic social defeat.
586 Using network analysis, 2 hub genes (Dkkl1, Neurod2) were identified, whose overexpression
587 was sufficient to potentiate social stress effects, thus unravelling molecular substrates of
588 susceptibility. At epigenetic level, regulatory mechanisms implicating long non-coding RNAs
589 may contribute to mediate such susceptibility [140]. In the addiction field, little is known about
590 molecular substrates of susceptibility. Two reports applied high-throughput methodologies to
591 describe such inter-individual differences in relation to DNA methylation and alcohol oral SA
592 in macaques [141], or to micro-RNA expression in a rat model of cocaine SA [142]. While
593 similar mechanisms are likely recruited in OUD, they have not yet been addressed by open-
594 ended approaches, pointing toward an important goal for future work.

595 Another avenue for improvement is cellular resolution. The brain tissue heterogeneity
596 has long hampered characterization of the role of each cell-type in complex phenotypes. In
597 complement to cell-type specific (TRAP) and single-cell sequencing (sc/snRNA-Seq), which
598 have already been applied to the understanding of OUD (see above), other tools are emerging
599 for molecular profiling of cell or neuronal sub-populations based on 2 factors that determine
600 their functional organization and plasticity : i) connectivity pattern, probed using antero- or
601 retrograde viruses, in combination with TRAP [143] (transcriptomics) or FANS (epigenomics
602 [144]); or ii) spatial context, with methodologies based either on sequencing or microscopy to
603 detect mRNA in space. Among the former, spatial capture followed by transcriptome
604 sequencing (e.g. Slide-seq [145]) enables genome-wide interrogation with 10µm resolution;
605 among the later, single-molecule fluorescence in situ hybridization (smFISH) allows for better
606 spatial resolution (1µm), but requires probe design and target gene selection [146,147]. These
607 spatial transcriptomic technologies have the drawback of interrogating only a fraction of the
608 genome, with a coverage of around a few hundred to a few thousand genes, lower than current

single-cell sequencing [148]. Future work using these techniques, by characterizing molecular effects of opioids in a particular neuronal pathway, or with exquisite spatial resolution, shall deepen the understanding of OUD.

A third aspect relates to gaps in the use of available technologies (ChIP-Seq, Cut&Tag, ATAC-Seq) and their integration in multi-omic models of pathophysiology. Accordingly, several epigenetic layers of regulation have been left unaddressed so far in relation to opioids. This includes chromatin conformation and histone modifications, which have not yet been investigated at genome-wide scale, in contrast with cocaine [72,149]. Because cocaine studies uncovered widespread histone post-translational modifications, it is likely that opioids, notably through hyperdopaminergic activity, may recruit similar plasticity. Along this line, we also speculate that opioid effects on DNA methylation, histone modifications, and other epigenetic substrates (lncRNA, chromatin conformation, etc) may show reciprocal molecular interactions, with possibly locus- and cell-type specific mechanisms that remain unknown. As illustration, we recently conducted a post mortem human study indicating that early-life adversity and depression associate with 2 distinct forms of DNA methylation, in the canonical CG or in the brain-enriched CAC contexts [133]. By combining WGBS with 6 types of ChIP-Seq analysis, the 2 forms of plasticity were found to affect distinct genomic features and sites, and defined by divergent histones modifications and chromatin states. Therefore, combined investigation of multiple epigenetic marks, possibly at the single-cell scale, have the potential to suggest molecular interactions that can then be dissected and manipulated *in vivo*. Importantly, such multi-omic assessment requires implementing dedicated bioinformatic frameworks, which are only emerging in psychiatry [150]. These integrative approaches, by describing how multiple molecular layers of regulation interact, are expected to better capture the chain of events linking disease causes (whether genetic, developmental, environmental, or a combination thereof) to their behavioral manifestation [151].

In conclusion, this is to our knowledge the first systematic review of studies of functional genomic mechanisms of opioids and OUD. Although numerous molecular adaptations have been described across animal models and patient cohorts, replicable

637 findings appear limited. In the future, it is expected that leveraging multi-omic and integrative
638 analyses will pave the way towards the identification of innovative therapeutic strategies, and
639 ultimately contribute to better care.
640
641

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Conflict of Interest

The authors declare no conflicts of interest.

Code availability

All code used in the present review is available as Supplementary File 1.

662 **Figure legends**

663 **Figure 1. PRISMA diagram.** The review protocol was registered prospectively in Prospero
664 (record ID#CRD42022270113), and followed the Preferred Reporting Items for Systematic
665 Review and Meta-Analysis protocol (PRISMA[152]). ***Identification of studies***. Three
666 databases were used for systematic screening: MEDLINE, Embase and Web of Science. The
667 literature search was performed using the following keywords: "microarray" OR "RNA
668 sequencing" OR "bisulfite sequencing" OR "chromatin immunopurification sequencing" OR
669 "single cell RNAsequencing" AND each of the following terms: "opiates", "opioid", "morphine",
670 "fentanyl", "oxycodone", "heroin", "methadone" or "buprenorphine". The initial search provided
671 2705 potentially eligible studies. ***Screening***. Articles were screened based on their title and
672 abstract, and included if they used a high-throughput, genome-wide methodology to assess
673 modifications of gene expression or epigenetic mechanisms as a function of opioid exposure,
674 in the nervous system of primates or rodents (rat, mouse), and were published in English in
675 peer-reviewed journals. Exclusion criteria included tissue other than the nervous system and
676 candidate genes approaches. Once duplicates were removed, a total of 1225 articles were
677 screened, among which 1057 were excluded because their title or abstract didn't meet
678 eligibility criteria. ***Eligibility***. After a more thorough examination of articles' full-text, an
679 additional 89 articles were excluded. As a result, 69 papers were selected for qualitative
680 synthesis. Among these, 44 articles reporting on transcriptomic analyses in the 5 most
681 frequently investigated regions were selected for a more detailed quantitative synthesis. Of
682 these, 37 articles investigated a single brain region, 6 covered 2 regions, and only 1
683 characterized 5 regions [8]. Overall, this resulted in 52 differential expression analyses: 18,
684 11, 11, 6 and 6 in the nucleus accumbens (NAc), frontal cortex, whole striatum, dorsal striatum
685 and spinal cord, respectively.

686

687 **Figure 2. Characteristics of studies included: Species & Sex. (A)** Species repartition in
688 studies identified during systematic search. **(B)** Distribution of sex and year of publication
689 across studies. **(C-D)** Sex distribution specifically in rodent and human studies. **(E-F)** Sample

690 sizes in rodent and human studies comparing opioid-exposed animals to controls (rodent,
691 panel E), or individuals with a diagnosis of opioid use disorder (OUD) to healthy controls.
692
693 **Figure 3. Characteristics of studies included: Injection parameters, Brain structure &**
694 **Techniques.** Among the 69 studies considered during qualitative analysis, a large panel of
695 injection parameters, nervous system structures and techniques were represented. **(A)**
696 Injection parameters used for opioid administration. NB: "mixed" refers to a single study that
697 used a combination of chronic oral administration (through bottles) and acute ip injections of
698 morphine[64]. **(B)** Number of articles investigating each nervous system structure. **(C)** Side-
699 by-side comparison of the number of articles investigating each structure with the protein
700 expression profile of the molecular target of opioids, the mu opioid receptor (MOR; as
701 assessed using [^{3}H]-DAMGO autoradiography, data in fmol/mg of tissue, adapted from[100,
702 103–105]). **(D)** Distribution of high-throughput methodologies and year of publication across
703 studies. *Abbreviations:* FCx, Frontal cortex; NAc, Nucleus accumbens; dStr, Dorsal Striatum;
704 Hc, Hippocapus; HT, Hypothalamus; Th, Thalamus; Amy, Amygdala; VTA, Ventral Tegmental
705 Area; MHb, Medial Habenula; LC, Locus Coeruleus; DRN, Dorsal Raphe Nucleus.
706
707 **Figure 4. Data availability for eligible studies and quantitative analysis in the nucleus**
708 **accumbens. (A)** The graph depicts the number of: i) differential expression analysis results
709 considered for quantitative analysis (with the corresponding number of articles between
710 brackets); ii) cases when lists of differentially expressed genes (DEG) were available; iii) cases
711 when full genome-wide results were available (with both fold changes, FC, and p-values). **(B)**
712 Number of DEG identified in studies that investigated the nucleus accumbens. **(C-E)** Venn
713 diagram of DEG identified in each species (human, mice and rat). **(F)** Venn diagram of DEG
714 in the 2 studies that investigated the dorsal striatum.
715
716 **Figure 5. Quantitative analyses in the dorsal striatum, whole striatum and frontal cortex.**
717 **(A)** Number of differentially expressed genes (DEG) identified in studies that investigated the

718 striatum (see Fig.S1 for separate analyses of studies that used acute or chronic opioid
719 administration). **(B)** Number of DEG in studies of the frontal cortex. **(C)** Number of genes
720 identified as differentially expressed (DE) across different studies. **(D)** Accros the 5 brain
721 regions considered during quantitative analysis, we assessed the impact of 5 factors (species,
722 brain structure, treatment chronicity, drug, and article) on consistency among studies, using
723 pairwise Jaccard indexes. For each factor (e.g., species), pair-wise JI were classified in 2
724 categories describing whether they belonged to the same sub-group (e.g., 2 studies conducted
725 in humans; "intra"), or not (e.g., 1 study in humans compared to another in rats; "inter"), within
726 that factor. NB: for the drug factor, studies in human were not considered as they cannot be
727 assigned to specific opioids.
728
729 **Figure 6. (A-D) Threshold-free comparison of 2 bulk tissue RNA-sequencing studies**
730 **(Seney et al and Townsend et al), and cell-type specific comparison of 2 single-cell**
731 **(Avey et al) and single-nucleus (Reiner et al) RNA-sequencing studies. (A-B)** Venn
732 diagram of differentially expressed genes (DEG) identified across the 2 bulk tissue RNA-
733 Sequencing studies by Seney et al and Townsend et al (DEG defined by arbitrary significance
734 thresholds in each study: p-value < 0.05 for both) for women/female rats (A) and men/male
735 rats (B). **(C-D)** Genome-wide, threshold-free comparison of the same 2 studies using Rank-
736 Rank Hypergeometric Overlap (RRHO2) for women/female (C) rats and men/male rats (D). In
737 RRHO2, briefly, genes are ranked using p-values (signed according to directionality), from
738 upregulated (Seney: left, Townsend: bottom) to down-regulated genes (Seney: right,
739 Townsend: top). Overlaps along the 2 distributions are then iteratively assessed using
740 hypergeometric testing. High p-values in the bottom-left and top-right quadrants indicates
741 concordance, whereas high p-values in the other 2 quadrants (bottom-right and top-left)
742 indicate discordance between the 2 datasets. The white crosses indicate the location of the
743 overlap corresponding to Venn diagrams depicted in panels A-B (split in subgroups of up- or
744 down-regulated genes), while black crosses correspond to most significant overlaps identified

745 using RRHO2. **(E)** Heatmap of pairwise Jaccard indexes for DEG identified across each of the

746 26 and 27 cell-types defined by Avey et al and Reiner et al, respectively.

747

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1183

Figure 1

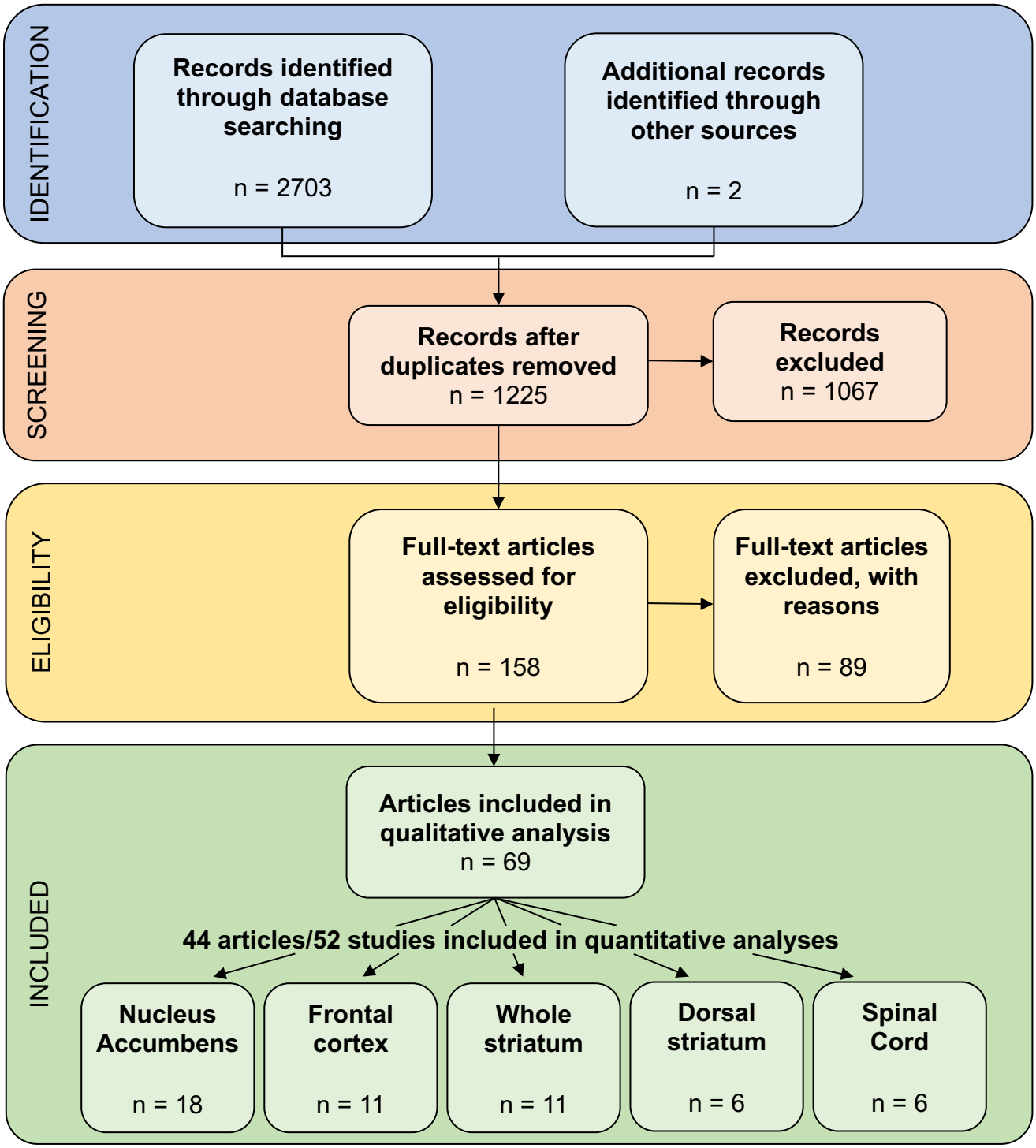

IDENTIFICATION
Records identified through database searching
n = 2703
Additional records identified through other sources
n = 2
SCREENING
Records after duplicates removed
n = 1225
Records excluded
n = 1067
ELIGIBILITY
Full-text articles assessed for eligibility
n = 158
Full-text articles excluded, with reasons
n = 89
INCLUDED
Articles included in qualitative analysis
n = 69
44 articles/52 studies included in quantitative analyses
Nucleus Accumbens
n = 18
Frontal cortex
n = 11
Whole striatum
n = 11
Dorsal striatum
n = 6
Spinal Cord
n = 6

Figure 2

A
SPECIES
Rats (n=30, 43%)
Mice (n=28, 41%)
Human (n=8, 12%)
Monkey (n=2, 3%)
Mice + rats (n=1, 1%)
B
25
20
15
10
5
0
2001-2002
2003-2004
2005-2006
2007-2008
2009-2010
2011-2012
2013-2014
2015-2016
2017-2018
2019-2020
2021
Males (n=48, 70%)
Both (n=14, 20%)
Females (n=5, 7%)
Not specified (n=2, 3%)
C
RODENT studies
Males (n=47, 80%)
Both (n=7, 12%)
Females (n=3, 5%)
Not specified (n=2, 3%)
E
HUMAN studies
Men (n=1, 12.5 %)
Men & Women
(n=7, 87.5 %)
D
Controls
Opiod treated
Vassoler, 2017
Coffey, 2020
Odegaard, 2020
Odegaard, 2021
Townsend, 2021
Borelli, 2021
Liu, 2021
F
Controls
OUD
Albertson, 2006
Sillivan, 2013
Egervari (ATACseq), 2020
Seney, 2021
Mendez, 2021
Liu (RNAseq), 2021
Liu (bisulfite seq), 2021
Shu (bisulfite seq), 2021

Figure 3

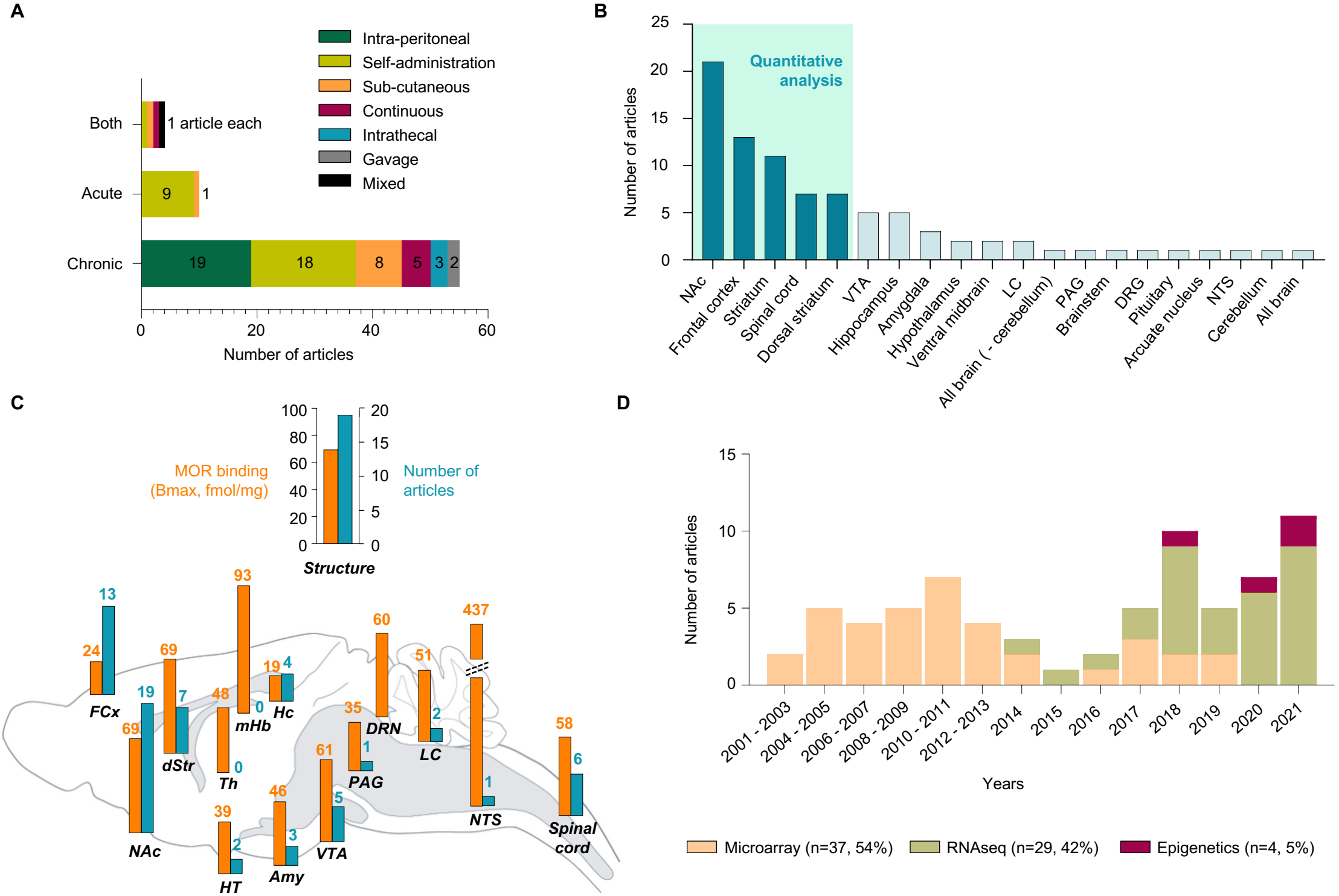

A
Intra-peritoneal
Self-administration
Sub-cutaneous
Continuous
Intrathecal
Gavage
Mixed
Both
1 article each
Acute
9
1
Chronic
19
18
8
5
3
2
0
20
40
60
Number of articles
B
Quantitative analysis
Number of articles
25
20
15
10
5
0
NAc
Frontal cortex
Striatum
Spinal cord
Dorsal striatum
VTA
Hippocampus
Amygdala
Hypothalamus
Ventral midbrain
LC
All brain (- cerebellum)
PAG
Brainstem
DRG
Pituitary
Arcuate nucleus
NTS
Cerebellum
All brain
C
MOR binding (Bmax, fmol/mg)
Number of articles
Structure
FCx
24
13
NAc
69
19
dStr
69
7
HT
39
2
Th
48
0
mHb
93
0
Hc
19
4
Amy
46
3
VTA
61
5
DRN
60
PAG
35
1
LC
51
2
NTS
437
1
Spinal cord
58
6
D
Number of articles
Years
2001 - 2003
2004 - 2005
2006 - 2007
2008 - 2009
2010 - 2011
2012 - 2013
2014
2015
2016
2017
2018
2019
2020
2021
Microarray (n=37, 54%)
RNAseq (n=29, 42%)
Epigenetics (n=4, 5%)

Figure 4

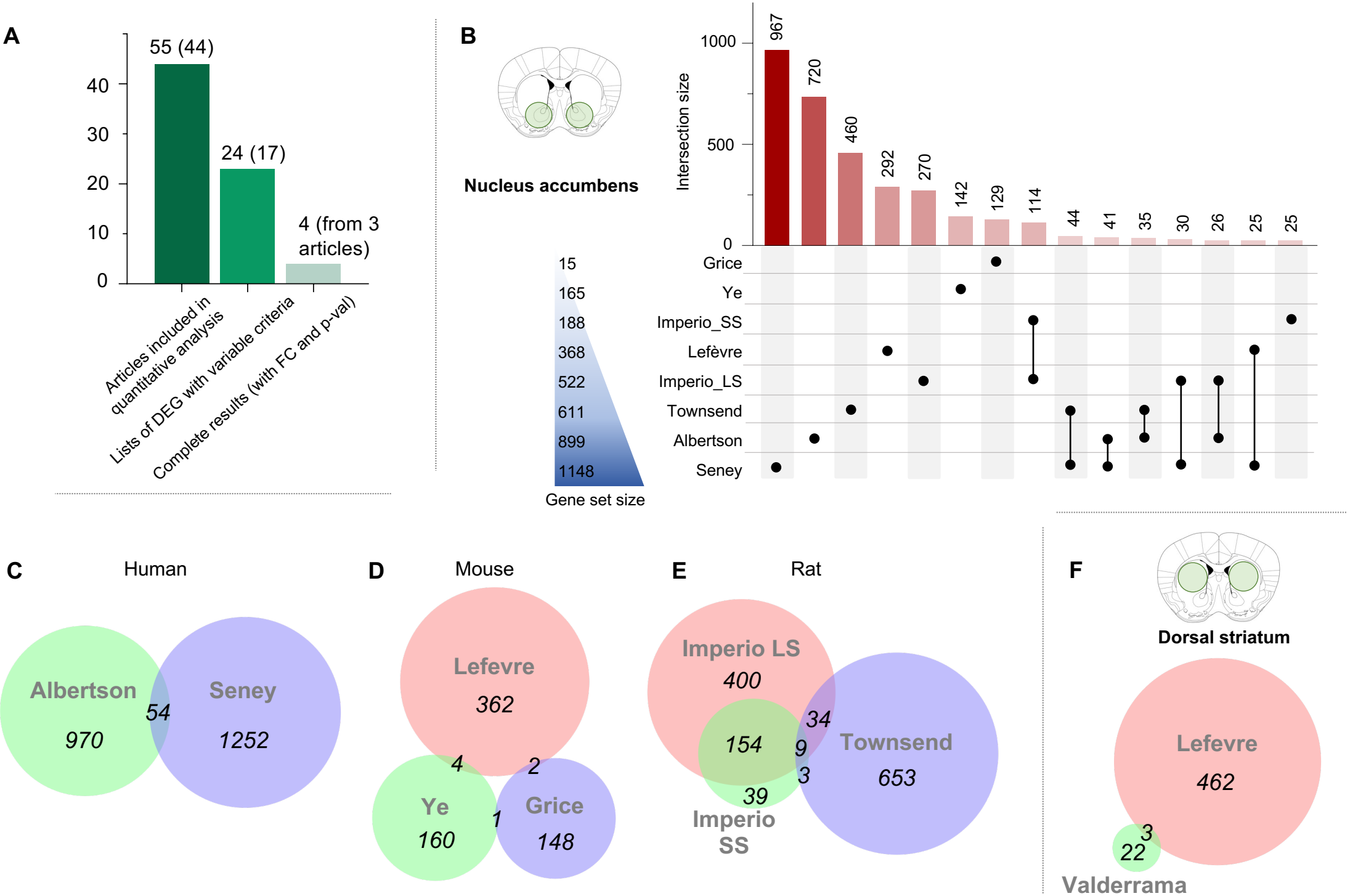

A
55 (44)
24 (17)
4 (from 3 articles)
0
10
20
30
40
Articles included in quantitative analysis
Lists of DEG with variable criteria
Complete results (with FC and p-val)
B
Nucleus accumbens
Intersection size
0
500
1000
967
720
460
292
270
142
129
114
44
41
35
30
26
25
25
Grice
Ye
Imperio_SS
Lefèvre
Imperio_LS
Townsend
Albertson
Seney
15
165
188
368
522
611
899
1148
Gene set size
C
Human
Albertson
970
54
Seney
1252
D
Mouse
Lefevre
362
4
2
Ye
160
1
Grice
148
E
Rat
Imperio LS
400
154
34
9
3
39
Imperio SS
Townsend
653
F
Dorsal striatum
Lefevre
462
3
22
Valderrama

Figure 5

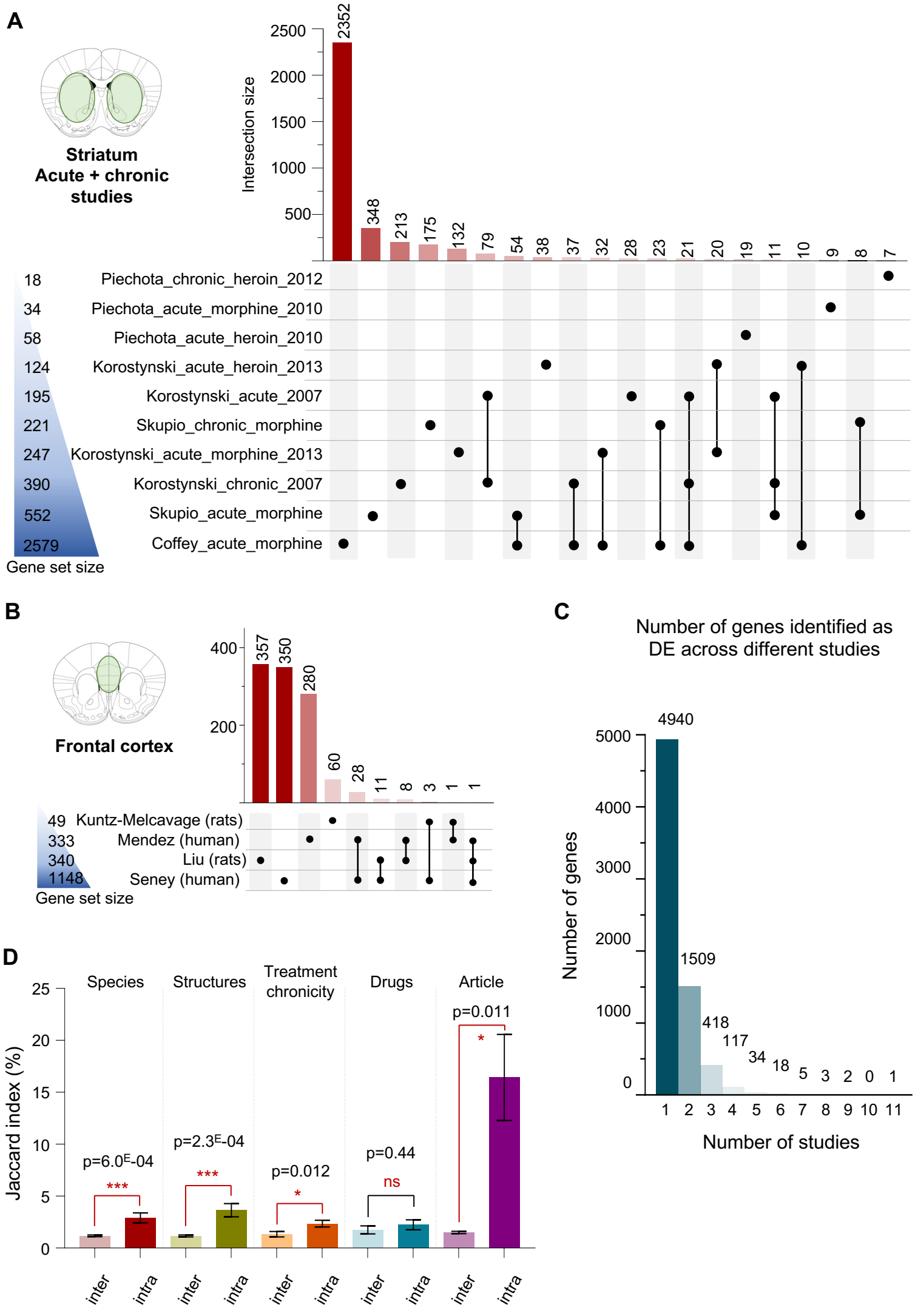
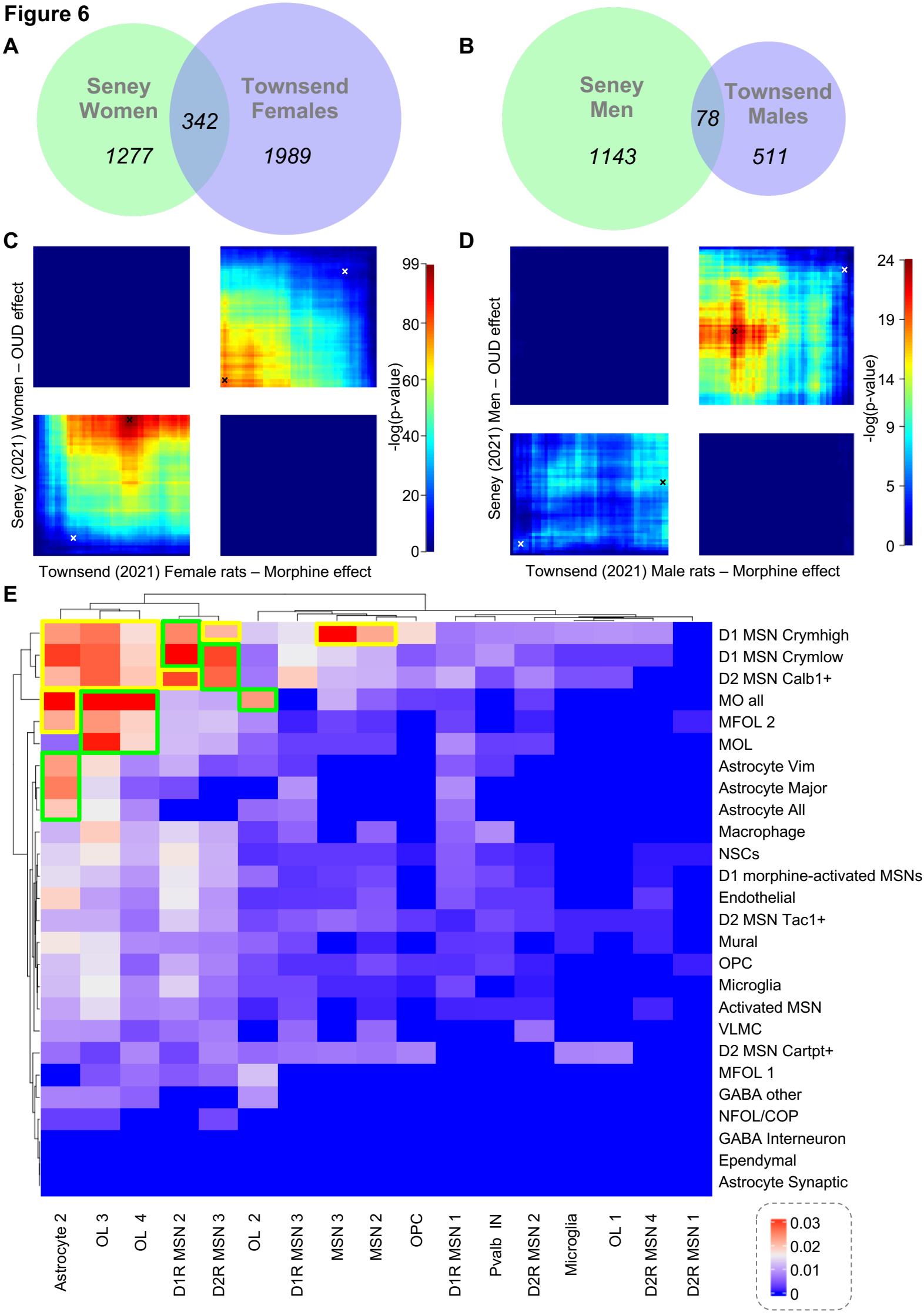

A
Striatum
Acute + chronic studies
Intersection size
2500
2000
1500
1000
500
2352
348
213
175
132
79
54
38
37
32
28
23
21
20
19
11
10
9
8
7
18 Piechota_chronic_heroin_2012
34 Piechota_acute_morphine_2010
58 Piechota_acute_heroin_2010
124 Korostynski_acute_heroin_2013
195 Korostynski_acute_2007
221 Skupio_chronic_morphine
247 Korostynski_acute_morphine_2013
390 Korostynski_chronic_2007
552 Skupio_acute_morphine
2579 Coffey_acute_morphine
Gene set size
B
Frontal cortex
400
200
357
350
280
60
28
11
8
3
1
1
49 Kuntz-Melcavage (rats)
333 Mendez (human)
340 Liu (rats)
1148 Seney (human)
Gene set size
C
Number of genes identified as DE across different studies
Number of genes
5000
4000
3000
2000
1000
0
4940
1509
418
117
34
18
5
3
2
0
1
1 2 3 4 5 6 7 8 9 10 11
Number of studies
D
Jaccard index (%)
25
20
15
10
5
0
Species
Structures
Treatment chronicity
Drugs
Article
p=6.0E-04
***
p=2.3E-04
***
p=0.012
*
p=0.44
ns
p=0.011
*
inter
intra
inter
intra
inter
intra
inter
intra
inter
intra

Figure 6


A
Seney Women
1277
342
Townsend Females
1989
B
Seney Men
1143
78
Townsend Males
511
C
Seney (2021) Women – OUD effect
Townsend (2021) Female rats – Morphine effect
-log(p-value)
99
80
60
40
20
0
D
Seney (2021) Men – OUD effect
Townsend (2021) Male rats – Morphine effect
-log(p-value)
24
19
14
9
5
0
E
D1 MSN Crymhigh
D1 MSN Crymlow
D2 MSN Calb1+
MO all
MFOL 2
MOL
Astrocyte Vim
Astrocyte Major
Astrocyte All
Macrophage
NSCs
D1 morphine-activated MSNs
Endothelial
D2 MSN Tac1+
Mural
OPC
Microglia
Activated MSN
VLMC
D2 MSN Cartpt+
MFOL 1
GABA other
NFOL/COP
GABA Interneuron
Ependymal
Astrocyte Synaptic
Astrocyte 2
OL 3
OL 4
D1R MSN 2
D2R MSN 3
OL 2
D1R MSN 3
MSN 3
MSN 2
OPC
D1R MSN 1
Pvalb IN
D2R MSN 2
Microglia
OL 1
D2R MSN 4
D2R MSN 1
0.03
0.02
0.01
0