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Experimental pain models for the evaluation of next-generation analgesics in clinical pharmacology studies

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CHAPTER 1

Introduction: Analgesic drug development: proof-of-mechanism and proof-of-concept in early phase clinical studies

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ABSTRACT

Effective treatment for many pain disorders is still lacking, which is due to the complexity of pain in general and of the underlying pathology of many pain syndromes. This results in most investigational analgesic drugs failing to reach registration; either due to lack of efficacy, or due to the drug's adverse effect profile. To increase the number of analgesics that reach the patient, it is essential to carefully and rationally plan the clinical development program. By including proof-of-mechanism (POM) and/or proof-of-concept (POC) methods in early-phase clinical drug studies, the analgesic drug developer will be better informed about the key characteristics of the studied drug, which will aid in making crucial decisions during the development process. Here, we describe the top 10 currently most developed analgesic drug classes, link them mechanistically to appropriate methods to demonstrate POM and POC in early-phase clinical trials, and include pros and cons of each of the methods described. Lastly, we discuss how each analgesic drug class requires a tailored experimental approach for proper evaluation of POM and POC, and how this can contribute to an efficient and question-based approach in early-phase analgesic drug research.

INTRODUCTION

Pain, while being one of the most common symptoms for which patients seek medical attention, is in terms of available treatment one of the main therapeutic areas in which little progress has been achieved: a mere 59 compounds have been registered for the treatment of pain between 1960 – 2010, with only two-thirds of those being an analgesic. [1] Where major breakthrough discoveries including opioids and acetylsalicylic acid have been discovered decades ago, most first-line therapies currently available still lack either long-term effectiveness (e.g., prolonged use of opioids increases sensitivity to pain (i.e. hyperalgesia (**Figure 1**)), instead of providing pain relief) or have a poor risk-benefit profile (e.g., systemic administration of lidocaine reduces pain but simultaneously induces cardiac arrest). One of the main challenges preventing more analgesics successfully entering the market, is the complexity and multimodality of the underlying pathology of pain. To tackle this, and thus increase the number of analgesics actually reaching the patients, it is needed to understand and evaluate the signal processing dysfunction causing a patient's pain symptoms, rather than developing drugs based on clinical symptoms alone. [2]

Adopting the conventional approach wherein only pharmacokinetics (PK), safety and tolerability are considered main objectives in Phase I/II of the development, leaves essential questions on a drug's actual effects unanswered till late, or even post-approval, which may result in multi-million dollars ill-invested on ineffective drugs, or having severe public health consequences. [3] Instead, by evaluating proof-of-mechanism (POM) and proof-of-concept (POC) early-on in development, the developer is well-informed when making go/no-go decisions. While PK, safety and tolerability assessment unmistakably are important, it should be accompanied by study objectives answering key questions regarding the drug's properties: whether the study drug reaches the target site and if so, if it has its intended pharmacological effect (i.e. POM), or enabling trials with models resembling the (pain) condition(s) the drug is aimed to treat (i.e. POC). [4] Here, we use the term POC for demonstrating analgesic effects, either in patients with pain or in healthy subjects using experimental models to evaluate pain thresholds, as proposed by Campbell et al. [5] We do realize that the term 'POC' is also often used for the first signs of clinical effects in the target population, but believe that in the context of analgesic drug development, it is fair to consider demonstrating effects on

pain thresholds – if the evoked pain test reflects a process involved in the relevant target population with clinical pain – as POC of having analgesic properties. For all biomarkers* that reflect target engagement more 'proximal', i.e. closer to the mechanism of the compound, here we use the term POM, which includes tests of target engagement (binding of the drug to its (receptor-)target), assessed at the body location targeted (e.g., synovial fluid sampled from the knee), and also tests that clearly link to the drugs- pharmacological properties (e.g., pupillometry for μ-opioid receptor (MOR) agonists, see section **Opioids**). [6] Human experimental pain studies are valuable assets to establish POC in early-phase analgesic drug development, and together with POM assessments may provide the drug developer important evidence to help make pivotal decisions on dose selection, which patient (sub)populations to target, and/or evade unnecessary investments in compounds that otherwise were poised to fail later-on. [4,5] In the case of testing first-in-class drugs, it may be that applicable models for both POM and POC are lacking, which may justify not trying to prove mechanism or – in case of analgesics – not demonstrating effects on pain thresholds and directly entering testing in patients, but should never lead to testing neither, and leaving questions unanswered. [3]

Here, we list the top 10 currently most developed analgesic drug classes and link them mechanistically to applicable methods for evaluating POM and POC in early-phase clinical trials, including pros and cons for each method described. We review how experimental studies fit into analgesic drug development, in an effort to contribute to an efficient and question-based approach.

MATERIALS AND METHODS

The Clarivate Analytics Integrity- and Biopharm Insight databases were used as sources to uncover which analgesic drugs are currently in development. [7,8] Both databases aggregate data from various sources including scientific journals, conference papers, statements of regulatory agencies, company websites and clinical trial websites such as clinicaltrials.gov. See **Appendix A** for details on the searches performed. A short term representing the drug class that aligned with the compound's main mechanism

* A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention. [163]

of action was added manually for reporting. If the mechanism of action was not listed, details were searched on the respective manufacturer's website or related business news. If still no details could be obtained, the compound was allocated to the group 'undisclosed'. Listings from both databases were collated and duplicate entries removed.

The top 10 currently most developed drug classes, excluding 'undisclosed', was used for further reporting. Respective methods chosen to evaluate a class's POM and POC have been determined using expert knowledge, with claims supported by literature available in the public domain. An overview of the dosing regimen used in each trial listed is available in **Appendix B**.

RESULTS

Top 10 analgesic compound classes currently in early-phase development

The main mechanism of action was identified for 426 compounds, of the 508 unique entries included in total. **Figure 2**. displays the top 10 most developed analgesic drug classes, which is comprised of 270 (~53% of total enlisted) compounds. Refer to **Appendix C** for the complete list.

The majority of entries identified (n=83) target opioid receptors (**Figure 2**). Most of these opioidergic drugs, as well as those belonging to the non-steroidal anti-inflammatory drug (NSAID)-class (n=47), are therapies or combinations developed using a new drug delivery strategy (e.g., abuse deterrent, prodrug or administration route) rather than classified as a novel drug entity. Voltage-gated sodium channel (Na_V) inhibitors (n=43), the third most developed class, in addition to consisting of marketed drugs with updated drug formulation (e.g., lidocaine patches), includes a substantial amount of novel, selectively targeting drugs (e.g., selective $Na_V1.7$ and $Na_V1.8$ inhibitors). Further within-class details are discussed on a per-class basis in the remainder of this article.

Methods to evaluate POM and POC per drug class

OPIOIDS Opioids have been widely available for decades and serve as the main therapy of choice for severe pain indications, albeit suffering from a high abuse risk and severe adverse effects (AEs) when

administered at higher doses. Opioids such as morphine achieve pain relief mainly by targeting μ-opioid receptors (MORs), which are abundantly present throughout the human body both peripherally and in the central nervous system, resulting in the wide range of pain indications that opioids can treat. Notable AEs, such as addiction and (fatal) respiratory depression following opioid (over)dosing, however, are also attributable to that same (μ-opioid) receptor. [9] Approximately a third of the opioids listed therefore not only target MORs, but also (ant)agonize the δ- and/or κ-opioid receptors (DOR and KOR, respectively), of which buprenorphine is an example. The KOR, similar to MOR, is abundantly present throughout the body, whereas DOR expression is limited to the brain's basal ganglia and neocortical regions. [10]

METHODS FOR EVALUATING POM

The mechanism of action of opioids, and tests proving those principles, have been well-described over the years. Potency, efficacy and action duration of μ-opioid receptor agonism may be evaluated by assessing miosis using pupillometry, which in addition to confirming POM in humans (i.e. extent of target engagement of MORs), in parallel serves as translational biomarker as MOR-agonism also induces miosis in rabbits and dogs. [6,11] Pupillometry has been used extensively for characterizing the effects of many (experimental) opioid drugs, including fentanyl, naltrexone and buprenorphine, and selectivity of the KOR antagonist LY2456302. [11,12] While easily implementable, it has been debated if opioid-induced miosis is a peripheral effect (as it follows activation of the pupillary sphincter muscle, see e.g., Rollins et al., 2014) rather than affecting the central nervous system (CNS). [6,11] In addition, the method does require specific equipment and analysis methods. The latter also holds true for experimental Functional Magnetic Resonance Imaging (FMRI) studies, in which hemodynamic responses associated with neuronal activation are measured in the brain's pain matrix, following nociceptive stimulation. [13] As such, oral morphine was found to significantly affect brain areas where opioidergic receptors are predominant after heat stimulation using a contact heat evoked potential stimulator. [14]

Classical opioids, as said before, suffer from a high frequency of AEs, which are hypothesized to be absent in the opioids that are currently being developed. Proxies to evaluate the risk-benefit profile early-on in development are therefore also of importance. The most suitable model

depends on the drug's potency, administration route and dose. Applicable methods for MOR-like side effects – or absence thereof – include the dynamic end-tidal forcing technique to study effects on ventilation/respiratory depression, [15] abuse potential of the drug defined as changes on a drug-liking Visual Analogue Scale (VAS), [16] and/or motility of bowel as a measure for opioid-induced constipation by determining gastrointestinal transit times. [17] All mentioned tests directly link to MOR effects and can be tested in sequential fashion with POC tests (see below), but – except for the easily adoptable drug-liking VAS – require specific tools and expertise, limiting their use.

METHODS FOR EVALUATING POC

The cold pressor test, an evoked pain test using cold pain to measure pain thresholds, is primarily used to demonstrate analgesic effects of (MOR-) opioids both in an experimental context [12,18], and clinical context, as the cold pressor test allows for diagnosing fibromyalgia or opioid-induced hyperalgesia (OIH). [19] The model's superiority in detecting analgesia lies in its tonic stimulus, which evokes the opioidergic-linked endogenous central pain inhibiting system [20,21]. See **Table 1** for a detailed description of the method. The effects of potent opioids such as fentanyl have been further characterized by a battery of distinctive nociceptive tests, with changes apart from those noted on the cold pressor test, also reported for heat and electrical pain thresholds; corroborating the broad applicability of opioids as analgesics. [18,22] To assess KOR agonism, visceral pain thresholds induced by a multi-modal esophageal probe may be used, as shown by Arendt-Nielsen et al. and through the suggested role of the KOR in the visceral pain system. [23,24] To note, experimental pain tests require specialized tools and training which limit their applicability. Also, evoked pain thresholds are subject to a relatively high inter-individual variability, which is likely related to the tests' subjective outcome variable (i.e., reporting of when a pain threshold is reached). To counter this, two (or more) period cross-over study designs are often used, which allows comparing treatment effects within a single individual.

For POC studies in clinical pain, i.e., trials assessing the first signal of treatment efficacy in a well-defined patient subpopulation, the dental impaction pain model (including third molar surgery) has been most widely used and found particularly useful for assessing dose ranging and profiling of (novel) opioids, and NSAIDs. [25]

NSAIDS NSAIDs act mainly by targeting the cyclooxygenase (COX) enzymes (COX-1 and -2), that are responsible for inducing fever and inflammatory pain through prostaglandin E_2 (PGE_2) synthesis. [26] Given the side effects induced by classic (non-selective) NSAIDs such as gastrointestinal bleeds attributable to COX-1 inhibition, drug developers turned to selective inhibition of COX-2. While initially praised for their expected efficacy and safety through target specificity, the selective COX-2 inhibitors were later found to induce significant cardiovascular side effects, leading to discontinuation of (the development of) most COX-2 selective inhibitors. [27] Drugs currently being developed and belonging to this class are primarily non-selective COX inhibitors based on marketed NSAIDs, but novel due to their formulation, or by being combined with another drug and developed as a single treatment.

METHODS FOR EVALUATING POM

One of the challenges with COX inhibitors related to proving their pharmacological effects, is the mismatch between drug plasma concentrations and exerted analgesic and/or AEs in inflammatory disease states, which is likely related to the complex pathophysiology of inflammation. [28] Evaluation of (other) biomarkers based on the drug's proposed action mechanism is therefore advised for e.g., calculating dosing regimens. Examples include PGE_2 and thromboxane B2 level determination. For subtype-selective drugs, the IC_{80} of COX-2 (i.e. concentration of drug needed to inhibit COX-2 by 80%) versus effective concentrations at COX-1 can be used for proof-of-specificity. [28] Evaluation of these markers may not be available in a routine laboratory, which then requires assay set-up and additional funds.

METHODS FOR EVALUATING POC

For analgesics intended to treat inflammatory pain, the ultraviolet B (UVB)-induced hyperalgesia model, also referred to as the 'sunburn model', is primarily used as a readout for POC. [13] UVB promotes inflammation through increased production of various cytokines and prostaglandins originating from the affected keratinocytes. [29,30] See **Table 1** for a detailed description of the method. The model has shown robust responses to NSAIDs such as ibuprofen [18,31,32]. Alternatively, freeze injury may be utilized to evoke local hyperalgesia lasting over three days that, in combination with the Von Frey hair filament assessment, has been

shown to be responsive to NSAIDs administered both topically and systemically. [33,34] While yielding robust results with relatively little variability compared to other experimental pain models, application of these models may lead to post-inflammatory hyperpigmentation marks on the study participants' skin, lasting months or even years. [13,35] In addition, studies utilizing the UVB model are hampered by the need for a more homogenous study population, as irradiation levels needed to induce hyperalgesia are only safe in lighter-skinned individuals. [35]

Together with the dental impaction pain model (as mentioned in the section **Opioids – Methods for evaluating POC**), bunionectomy surgery has been used to evaluate an NSAID's efficacy in patients suffering from acute pain, in the POC setting. Others include the joint replacement and soft tissue surgery models, although the former two (dental- and bunionectomy model) yield higher assay sensitivity. [36]

Na_V INHIBITORS Of the human Na_V channels discovered, four ($Na_V1.3$, $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$) have been found to be primarily present on nociceptors. [37] Each of these has unique properties and plays a key role in the generation and/or propagation of action potentials (**Figure 3**). [37,38] As such, $Na_V1.7$ is a key contributor to the initial rising phase of the action potential, but may also amplify subthreshold stimuli, being a low activation channel as $Na_V1.3$ and $Na_V1.9$. $Na_V1.3$ is primarily involved following axotomy and other forms of peripheral nerve injury. [39] Where $Na_V1.3$ and $Na_V1.7$ have fast gating kinetics (i.e., opening and closing of the channel), these properties are for $Na_V1.9$ ultra-slow, also in comparison to $Na_V1.8$. The latter is a high activation threshold channel that acts during the later rising phase to support high frequency firing (i.e., hyperexcitability). [40]

First generation, non-selective Na_V inhibitors have been one of the most widely used class of analgesics in the clinic for decades. Alike opioids, these also suffer from a poor risk-benefit profile, as exemplified by lidocaine to which we alluded in the introduction. [41,42] To this end and following the discovery of $Na_V1.3$, $Na_V1.7$, $Na_V1.8$ and $Na_V1.9$'s contribution to pain signal initiation and propagation, subtype selective Na_V inhibitors are currently being developed to treat acute and neuropathic pain disorders. Most selective inhibitors that came up in our search target either $Na_V1.7$ or $Na_V1.8$. While theoretically a promising target for analgesics, $Na_V1.3$-subtype specific inhibitors are investigated to a limited

extent, likely because $Na_V1.3$ is highly homologous to other sodium channels (up to 85% for $Na_V1.2$). [43] Development of $Na_V1.9$-selective drugs is precluded by the inability to express the channel in heterologous systems, which is needed to study protein structure and function. [44,45]

Currently registered therapies for this drug class include the first-generation anticonvulsants phenytoin and carbamazepine, which are used primarily in the treatment of trigeminal neuralgia and as third-line therapies for other forms of neuropathic pain.

METHODS FOR EVALUATING POM

With Na_V inhibitors primarily acting on action potential firing, the nerve excitability threshold tracking technique may yield detailed information on channel selectivity and amplitude of drug effects on peripheral nerves. [46] This measurement produces information on physiological conditions, the state of ion channels involved in nerve excitation, as well as on the functionality of energy-dependent pumps. It allows for the identification of exposure levels needed for state- and frequency- dependent block of sodium channels. [47] Threshold tracking is generally used to assess motor neuron excitability in e.g., amyotrophic lateral sclerosis patients. It can, however, also be used to assess sensory neuron function, and can be considered POM given it is a distinct readout for the pharmacological effects of Na_V inhibition. [47,48] The non-selective Na_V inhibitors lidocaine, mexiletine and tetrodotoxin have been characterized using this technique. [49–51]

To measure central effects induced by Na_V inhibitors, drug effects can be evaluated using transcranial magnetic stimulation (TMS). TMS may be utilized either by recording TMS-evoked electroencephalographic (EEG) potentials (TEPs), or evoked electromyographic (EMG) responses. See **Table 1** for a detailed description of the method. TEP P180, a late-phase potential controlled by axonal excitability, has shown negative responsiveness (i.e. decreases) to the Na_V inhibitors lamotrigine and carbamazepine, [52,53] whereas motor thresholds as measured by EMG responses were increased following lacosamide and carbamazepine administration. [54] Both threshold tracking and TMS are non-invasive and utilized routinely in experimental studies, but are considered complex both to execute and to analyze generated data. Evaluation of excitability following TMS as POM moreover may may not be applicable for the selective Na_V inhibitors, as they act mostly on ion channels present on peripheral nerves

or dorsal root ganglia (DRG; i.e. compounds targeting $Na_V1.7$ or $Na_V1.8$) (**Figure 3**), while TMS can be used to measure CNS/cortical excitability.

By exposing hyperexcited induced pluripotent stem cell-derived sensory neurons, obtained from patients with inherited erythromelalgia (IEM), to the selective $Na_V1.7$ inhibitor PF-05089771, Cao et al. have presented a method to confirm POM of Na_V inhibitors in a lab-based experiment. It has been proposed that this method may have broader utility than in IEM, e.g., also in other pain conditions of which hyperexcitability is the underlying cause. [55]

METHODS FOR EVALUATING POC

Prior to the reports from the two studies included in this thesis (**Chapter 2** and **3**), no published data were available that reported positive effects of selective Na_V inhibitors on human experimental pain models in a healthy subject population. Preclinical work showed that the selective $Na_V1.8$ inhibitor A-803467 reduced thermal and mechanical hyperalgesia, and attenuated neuropathic pain in multiple preclinical readouts, [56–59] whereas the selective $Na_V1.7$ inhibitor PF-05089771 attenuated sensations of burning pain in patients with diabetic neuropathy. [60] The heat pain test with and without the capsaicin model to induce hyperalgesia and burning sensations may thus be applicable yet noting is was not sensitive to selective Nav1.8 inhibition in two studies (**Chapter 2** and **3**). In another study, the selective $Na_V1.8$ inhibitor VX-150 significantly reduced pain in two studies in patients with acute pain (in patients that underwent bunionectomy surgery, and in patients with knee osteoarthritis (OA)) and small fiber neuropathic pain respectively. [61,62] Stated POC studies in patients therefore are suitable to evaluate (selective) Na_V inhibitors, but we also propose that the cold pressor pain test may establish POC for such compounds considering the above results. The cold pressor pain test namely is sensitive to neuropathic pain treatments as pregabalin and mexiletine, [32], **Chapter 4**] and serves as a readout of TRPM8-mediated cold pain sensations through its interplay with $Na_V1.8$. [63,64] Rationale for this test is discussed in more detail in **Section 1** of this thesis. As an alternative to performing a study with pain models, a POC study in patients with trigeminal neuralgia is proposed based on positive findings of two Na_V inhibitors in this population. Both carbamazepine and more recently selective $Na_V1.7$ inhibitor vixotrigine (BIB074, formerly raxatrigine) proved to be efficacious in this population. [65]

CANNABINOIDS Fueling an ever-growing trend, [66] both the natural *cannabis sativa* L. (cannabis) and cannabis-derived cannabinoids are amongst the currently most developed drugs, with an estimated sale value of 1.9 billion in 2020 in the Unites States alone. [67] Cannabinoids, apart from acting on the cannabinoid-1 and -2 receptor (CB1 and CB2, respectively), [68] may relieve pain by acting on serotonin (5-HT) receptors [69] and transient receptor potential (TRP) channels including the TRPV and -A subtypes. [70] (**Figure 4**) Selective CB2 receptor agonists are of specific interest for drug developers given their observed efficacy in a range of preclinical inflammatory and neuropathic pain models, whilst mitigating psychotropic effects attributed to activation of central CB1 receptors. [71] For cannabinoids, adequate biomarkers largely depend on the receptor targeted, and dose used. Given their action is so distinct, we here define three cannabinoid subgroups for which POM and POC options are discussed: those primarily targeting CB1 and CB2 receptors, those primarily inhibiting fatty acid amide hydrolase (FAAH), and cannabidiol (CBD).

It is important to note that while multiple CB2 agonists and FAAH inhibitors are reported in our search, no recent trials were found on evaluation of these class subtypes in clinical pain. Despite promising preclinical evidence, the CB2 agonist GW842166 was discontinued following its failure to demonstrate meaningful analgesia in patients with acute dental pain, and the FAAH inhibitor PF-04457845 failed to relieve pain in OA patients, although these are reports of almost a decade ago. [72,73] PF-04457845, however, recently was shown to reduce cannabis withdrawal symptoms in men, suggesting that FAAH inhibitors possibly may be better suitable as a treatment for indications other than pain. [74]

METHODS FOR EVALUATING POM

When the drug is a ligand for both CB1 and CB2, e.g., in the case of tetrahydrocannabinol (THC)-formulations or cannabis-based formulations containing THC, motivation and attention-based cognitive tests are most applicable to evaluate POM at low doses, whereas high doses affect blood pressure, heart rate and subjective feeling (e.g., VAS feeling high or evaluation of psychotomimetic feelings). [75] The latter two, effect on heart rate and feeling high, also serve as POM biomarkers for selective CB1 agonists. [76] Lack of observed effects on these proxies may therefore be beneficial for POM of a CB2-selective analgesic. While evidently more costly, radiotracer positron emission tomography (PET) imaging studies, often used

for POM, may provide more detailed information on the availability and (drug) occupancy of CB1 (using e.g., PET tracer [^{18}F]MK-9470) and CB2 (using e.g., [^{11}C]NE4), in the brain. [77,78]

A POM approach for cannabinoid drugs primarily inhibiting FAAH, on the other hand, is through assessment of endocannabinoid levels, with specific focus on anandamide levels as they increase upon FAAH inhibition. [79] FAAH inhibition can be measured using a fluorescence assay. A striking example in which such a POM approach proved to be essential, is the infamous BIA 10–2474 (Bial) trial in which a novel FAAH inhibitor was tested. While safety, tolerability, PK and FAAH inhibition were evaluated, only the former three were used for dose escalation decisions. When the crucial data on the FAAH inhibitory effects had been taken into consideration, it could probably have prevented the death of a healthy volunteer and irreversible brain damage in four other healthy study participants. [80] In the case of CBD, the main non-psychoactive component of cannabis, investigators have difficulty showing POM, as CBD apart from having low affinity for CB1 and CB2, also acts on a plethora of other receptors throughout the body. CBD therefore links to many diseases and (neuro)protective properties. [81] While early-phase studies in the context of CBD and pain are scarce, a possible, yet costly and complex, approach is by evaluating striatal activation during a verbal memory task using FMRI. CBD has been found to augment, and THC attenuate, the striatal activation, therefore this may be utilized for differentiation of CBD from THC-based drugs. [82]

METHODS FOR EVALUATING POC

POC read-outs for CB1/CB2 ligands are complex, as translation between healthy volunteer- and patient studies has been difficult: despite theoretical evidence, THC administered in two distinct experimental pain studies with healthy subjects, induced hyperalgesia rather than analgesia. [83,84] Therefore, testing within a well-chosen patient subpopulation seems more appropriate. For example, pressure pain thresholds, but not spontaneous or electrical pain, assessed in fibromyalgia patients have been found reactive to THC administration, [85] as were pain scores reported by patients with multiple sclerosis. With the latter, it is important to take temporal effects into account when designing such a study, given effects can take weeks to develop. [86]

Genotyping may allow for POC evaluation of a FAAH inhibitory drug: alterations in sensitivity to cold pain are associated with FAAH polymor-

phisms in lower back- and postoperative pain conditions. [87,88] The latter is an expensive approach, and only applicable to a limited patient population. Alternatively, assessing EEG readouts from laser evoked potentials (LEPs) generated on capsaicin-treated skin, may be suitable. Schaffler et al. demonstrated that, in subjects with a confirmed hyperalgesic response to capsaicin, the FAAH inhibitor ASP8477 reduced sensitization, demonstrated by a decrease in LEP N2-P2 peak-to-peak amplitudes compared to placebo. [89]

For CBD, preclinical evidence has established a POC role for the UVB-induced hyperalgesia model (**Table 1**), as CBD reduces keratinocyte-mediated inflammation, and potentially protects keratinocytes against UVB irradiation. [90,91] While there plausibly is a role for experimental pain models in characterizing CBD's analgesic effects, given the beneficial effects from CBD reported by chronic pain patients, [92] there is little to no clinical evidence available in the public domain other than the cited experimental pain study in fibromyalgia patients, where no analgesic effects attributable to CBD could be demonstrated. [85]

NMDA MODULATOR N-methyl-D-aspartate receptor (NMDA) antagonists in a clinical setting have shown robust efficacy in treating (opioid-induced) hyperalgesia, neuropathic pain syndromes and pain following opioid tolerance. [93] Primarily represented by ketamine and methadone, this drug class relieves pain by blocking the excitatory signal at the NMDA receptor, typically induced by binding of glycine and glutamate to their respective receptors. [94] Changes in glutamatergic neurotransmission, however, may also induce notable CNS-side effects, which have led to the recreational abuse of these drugs and failure of many novel NMDA antagonists during development. [95]

METHODS FOR EVALUATING POM

TMS (see **Na_V inhibitors – methods for evaluating POM**) may be used to evaluate effects of NMDA antagonists on motor cortex excitability. Previously, ketamine was found to increase the motor cortex responses, and memantine to significantly affect its plasticity. [96,97] When planning to include TMS for evaluating NMDA receptor modulation, one – apart from the cons mentioned in the previous section – should be aware of a delay in effects, in the case that the to-be-tested drug has prolonged action characteristics. [96]

METHODS FOR EVALUATING POC

NMDA antagonists such as ketamine have been profiled in an experimental setting on a variety of pain paradigms. The (cutaneous) heat pain test and, to a lesser extent, the (cutaneous) electrical pain test most adequately display ketamine's analgesic potential. [18,98] The thermal grill test, during which warm and cold stimuli are applied simultaneously to the skin to evoke a paradoxical pain sensation, is suitable to confirm activation of the glutaminergic- rather than the endogenous opioid system, as ketamine reduced paradoxical pain intensity whereas the opioid-receptor antagonist naloxone did not. [99] Recently, NMDA receptor antagonists have been suggested as potential treatments for central sensitization, [100] which was positively evaluated in an experimental setting using the freeze injury hyperalgesia model (also see. **NSAIDs – Methods for evaluating POC**). [95]

NERVE GROWTH FACTOR (NGF) MODULATOR The interaction of NGF with tropomyosin kinase A (TrkA) – which is highly expressed in the DRG – has been found a key step in the sensitization of nociceptors. [101] Antagonists are therefore expected – and developed – as a treatment for chronic pain with specific focus on inflammatory conditions. [102] Development of this class was temporarily halted by the US Food and Drug Administration following reports that anti-NGF antibodies caused rapid joint destruction in patients with OA. [102] More recent data, however, suggest that lower dose anti-NGF antibodies may have a more favorable risk-benefit profile. [101] Approximately half of the NGF-compounds enlisted are anti-NGF antibodies, the other half TrkA-selective inhibitors.

METHODS FOR EVALUATING POM

For compounds developed to treat localized (inflammatory) pain conditions – such as NGF antibodies to treat knee OA –, distribution to, and availability of the drug in the target tissue is key. Demonstration of this is feasible by performing synovial fluid sampling. [103] TrkA, however, is not highly expressed in blood cells and therefore does not allow for testing of target engagement in blood. Alternatively, in the case where a TrkA inhibitor is studied, skin biopsies can be utilized for studying inhibition of NGF-induced TrkA phosphorylation *ex vivo* . [104]

METHODS FOR EVALUATING POC

TrkA receptors, apart from modulation of various receptors such as TRPV1 through expression in the DRG, are also available on mast cells. NGF, through TrkA, therefore induces a pro-inflammatory response with increase of e.g., histamines, 5-HT and NGF, resulting in a positive feedback loop. [105] The UVB-induced hyperalgesia model (**Table 1**) induces a (local) inflammatory response which, amongst others, results in increased NGF levels, [106] and applicability for POC as previously described. [31] To note, while the capsaicin-induced hyperalgesia model theoretically may also be suitable, given it induces sensitization and local inflammation through TRPV1 and mast cell activation, [107–109] to the best of the authors' knowledge no clinical evidence is publicly available to substantiate the use of this model in the context of POC for this compound class.

5-HT MODULATORS 5-HT mediates pleiotropic behavioral effects including mood and anxiety through a family of 14 different receptor subtypes. Additionally, 5-HT plays a complex part in both hyperalgesic and analgesic states, dependent on the receptor (sub)type targeted and action site, with choice of the descending inhibitory pathways in the CNS, the trigeminal system, or afferent nerve fibers (**Figure 4**). [110] Various 5-HT subtype-selective (5-HT1B,-1D,-1F,-2B) modulators are currently developed, of which approximately half are to treat migraine or other headache syndromes.

METHODS FOR EVALUATING POM

To assess blood-brain-barrier penetrability of the drug, cerebrospinal fluid may be sampled by performing a lumbar puncture. Proper POM studies are lacking for subtype selective 5-HT modulators; however, subclass-related AEs reported so-far may guide POM evaluation. As such, triptans (5-HT1B/1D agonists) are found to induce vasoconstrictive effects and chest tightness. While vasoconstriction can only be assessed *in vitro*, using e.g., isolated arteries obtained from explanted hearts following cardiac transplantation, [111] evaluation of chest tightness is part of the clinical evaluation. The 5-HT1F selective agonist lasmiditan dose-dependently induces dizziness, which can be evaluated using a VAS. [112]

METHODS FOR EVALUATING POC

Receptor hypersensitivity and deficits in the 5-HT descending pain inhibitory pathway following low 5-HT levels have generally been accepted to play a pivotal role in migraine pathophysiology and central sensitization. [113] Using experimental models such as quantification of the conditioned pain modulation (CPM) response, effects of 5-HT selective drugs on central pain systems may be evaluated for POC, although clinical evidence for this approach is still limited and the test itself difficult to execute. [5,114] It is therefore suggested to evaluate pain thresholds in a multimodal test battery that apart from CPM also evaluates heat pain thresholds, as it has been demonstrated that both pain detection and tolerance thresholds are sensitive to 5-HT function following acute tryptophan depletion. [115]

It is noteworthy to mention that, while cilostazol, Calcitonin Gene Related Peptide- and Isosorbide-5-mononitrate-induced headache models do induce migraine-like attacks and are established experimental tests, they fail to respond to the 5-HT agonist sumatriptan in healthy volunteers, which makes these models unsuitable for POC of this specific drug class. [116–118] Rather, the Pituitary Adenylate Cyclase-Activating Polypeptide 38 (PACAP38)-induced headache model can be used, as pretreatment with sumatriptan attenuated headache induced by PACAP38 in a double-blind cross-over setting. [119]

TRPV1 MODULATORS Primarily known for the hot burning sensations caused by capsaicin, the active component of chili peppers, agonists of the TRPV1 channel – abundantly expressed on nociceptive c-fibers (**Figure 4**) – induce hyperalgesia in low concentrations, while overstimulation of that same receptor relieves pain through (temporary) nerve ablation. Antagonism of TRPV1 is of interest for analgesic drug developers as well, following reporting of positive effects in preclinical inflammatory- and cancer pain models. [120] First-generation antagonists, however, induced hyperthermia and impaired noxious heat sensation in many study participants. Development of this class of drugs was therefore initially halted. [121] Apparently it is possible to circumvent this problem, as at least seven later-generation compounds have recently progressed in the clinic without displaying these unwanted effects. [122] TRPV1 modulators have mostly been developed to treat neuropathic pain.

METHODS FOR EVALUATING POM

The relationship between capsaicin, vasodilation and flare is common knowledge for years, and extensively studied as POM. [123] Laser Doppler perfusion imaging, although requiring expensive machinery, is a reliable method to assess macrovascular changes in the skin including capsaicin-induced flare, and is proposed to evaluate mediators of neurogenic inflammation, such as TRPV1 modulators. [124]

METHODS FOR EVALUATING POC

For drugs developed as TRPV1 antagonists, capsaicin-induced hyperalgesia serves as an excellent experimental pain model. In low to medium concentrations (i.e. up to 3%), capsaicin administered either intradermally or topically has been successfully used for decades as a TRPV1-receptor-mediated challenge. [125] Readouts for this challenge include heat- and mechanical pain thresholds, but also effects on capsaicin-induced flare as shown in an early-phase drug study previously. [126] While extensively used, large inter-individual variability is reported for the response to capsaicin. [5] As such, the administration route (intradermal or topical) and test procedure employed (e.g., re-heating of treated area) have been correlated to enhancement of capsaicin-induced sensitization. [127]

More recent advancements in evaluating TRPV1 agonism include assessing changes in nociceptive detection thresholds following intra-epidermal electrical stimulation, which allows for temporally discriminating altered peripheral, versus altered central pain processing mechanisms. [128] The technique, however, is currently still in development and has therefore not yet been applied widely.

Notwithstanding the usefulness of capsaicin to demonstrate POM and POC, translation of positive preclinical data to clinical efficacy has been especially hard for TRPV1 antagonists. No clinically significant effects could be observed for AZD1386 on the Western Ontario and McMaster Universities pain scale, in a POC study in patients with chronic pain from knee OA. [129] Noteworthy, AZD1386 did significantly decrease pain intensity versus placebo in the same study. It has been proposed that by excluding NSAID-sensitive patients – who presumably have an inflammatory component to their pain – study outcomes were negatively influenced, suggesting that patient selection may influence POC study outcomes. [130] In a different study assessing dental pain, AZD1386 elicited significant analgesia, although effects were very short-lived (up to 1 h). [131] Another antagonist,

GRC-6211, while reported to be selective, highly potent and yield good bioavailability across various preclinical models, was discontinued after a clinical trial in OA pain was suspended. For further suggested reading on this topic, see the comprehensive review of e.g., Kort and Kym, 2012. [129]

CALCIUM MODULATORS Calcium channels present on peripheral nerve fibers are responsive to a variety of noxious stimuli. Upon activation, action potential propagation is increased due to an increased calcium influx. Voltage-gated calcium channels at central nerve terminals subsequently propagate pain signals through increased release of glutamate (**Figure 4**). [132] Calcium channel modulators – of which the gabapentioids are by far the most prescribed – inhibit this signal, resulting in their usefulness as treatments for (neuropathic) pain disorders. [133]

METHODS FOR EVALUATING POM

Similar to NMDA antagonists and Na_V inhibitors, the role of calcium channels in membrane excitability makes evaluation of altered excitability following TMS a viable biomarker, although the exact affected parameters vary between the calcium channel inhibitors administered. [134] Previously, it has been shown that intracortical excitability is a Gamma-aminobutyric acid (GABA)-controlled process, involving the interneuronal circuits in the motor cortex. As such, gabapentin prolonged cortical silent periods and the short intracortical inhibition, in addition to reducing intracortical facilitation, whereas in contrast losigamone (a sodium and calcium channel inhibitor without neurotransmitter properties) increased motor thresholds without affecting intracortical excitability, thereby demonstrating specificity of the mentioned cortical excitability parameters for calcium channel modulators. [135–138]

METHODS FOR EVALUATING POC

The analgesic effects of gabapentinoids have been profiled in experimental studies and have demonstrated nociceptive effects in multiple evoked pain tests, including a multimodal test battery. Oral doses of 300 mg pregabalin have shown robust and reproducible effects on pressure- and cold pressor pain thresholds. [32] Results for secondary hyperalgesia to pinprick and allodynia to brushing following topical capsaicin application vary, while noting that the allodynia assessment did produce more robust results. [139]

GABA MODULATORS As the chief inhibitory neurotransmitter, GABA reduces neuronal excitability throughout the central nervous system. Interestingly, many non-selective GABA-ergic compounds including benzodiazepines have evident pharmacological effects, of which analgesia is not one. This may be because significant adverse effects such as sedation precede the drug's antinociceptive effects. [140], As GABA(A) subunits α2 and α3 have been linked to pain relief, while sedation has been attributed to GABA(A) subunit α1, GABA-ergic drug developers seek subtype specificity. Compounds listed in this class either target GABA(A) subunits α2, α3 and/or α5 and aim to treat neuropathic pain, or target GABA(B) for chronic and osteoarthritis pain relief. [141,142]

METHODS FOR EVALUATING POM

For GABA modulators, POM partly depends on demonstrating subtype selectivity, which can be obtained by discriminating the observed pharmacological effects against those observed from non-selective GABA-ergic drugs, including sedation. A selection of neuropsychological and neurophysiological tests may be used to differentiate effects from e.g., α2 and α5-selective-drugs to those from non-selective GABA(A) agonists. A VAS measuring alertness to assess sedation, a test to quantify swaying of the body as proxy for postural imbalance, a VAS measuring 'feeling high' to evaluate a drugs' abuse potential, and effects on saccadic eye movement using a computer-based eye tracking system, have all repeatedly been used to prove GABA(A) selectivity. [143–145] While VAS scales are cheap and easily adoptable, the body sway test and saccadic eye movement tasks do need specific (computer) equipment and trained staff.

For demonstrating GABA(A) versus GABA(B) selectivity as an extension of POM, TMS (**Table 1**) experiments may be useful. Using multiple GABA-ergic drugs alprazolam (a classical positive allosteric modulator (PAM) at α1, α2, α3, and α5 subunit-containing GABA(A) receptors), diazepam (classical non-selective benzodiazepine), zolpidem (PAM at α1 GABA(A) subunits only) and baclofen (GABA(B) agonist)), an amplitude increase of the N45 potential has been shown to display GABA(A)-selectivity, whereas a decreased amplitude of the N100 potential showed GABA(B)-selectivity. [146] Oscillatory changes following single-pulse TMS are feasible as readouts also: opposite effects have been demonstrated for GABA(A)- and GABA(B)-ergic compounds on α-band-synchronization measured in the stimulated sensorimotor cortex and lateral frontal cortex. [147]

METHODS FOR EVALUATING POC

While available literature on experimental pain studies with GABA-selective drugs is scarce, the analgesic profile of a partial α2/α3/α5-selective GABA(A) agonist has previously been characterized: pressure-evoked and cold pressor- evoked pain thresholds were inhibited in a similar fashion to 300 mg pregabalin. [148] Another study, testing the benzodiazepines clonazepam and clobazam, reported analgesia in a capsaicin-pressure cuff algometry challenge, further indicating a possible role of pressure pain as a proxy for GABA-ergic analgesic drug effects. [149] It must be noted, however, that clonazepam and clobazam are not regarded as analgesics in general clinical use, their effectiveness in pain being limited to the clinical study setting. [150]

DISCUSSION

In the present article, we have listed the analgesic drug classes that are currently most developed, and have mechanistically linked them to biomarkers suitable for use in early-phase drug studies, to aid in efficient and question-based analgesic drug development. For proper evaluation of POM and POC, each drug class requires a tailored experimental approach. A few methods including TMS, the capsaicin- and UVB-induced hyperalgesia models and the cold pressor evoked pain test were found to be more widely applicable across drug classes.

POM and POC, as we defined these terms in the introduction may not align with how they are commonly used by the scientific community. POC, while here describing experimental models to evaluate pain thresholds in healthy volunteers or patients, is often also regarded as the first signal of clinical efficacy within the relevant target patient population. Although we added the reporting of effectiveness in a healthy population to POC – as it proves the analgesic potential of a compound –, such studies (i.e. positive POC trials performed in healthy volunteers) do not warrant omitting POC studies in an applicable patient population. Evoked pain tests, while preferably mechanistically linked to administered drug and target patient population, only induce pain that is short-lived, neglecting the more chronic, and emotional aspects that coexist in patients experiencing pain. Rather, results from healthy volunteer POM/POC studies allow for an improved and often leaner, therefore more cost-efficient design of successive trials in patients. POM and POC studies therefore serve as a translational step between preclinical experiments and studies in

the relevant patient populations seeking to find the first signal of clinical efficacy. By confirming the active concentration range in a POM or POC study, fewer dose levels need to be evaluated in patients rendering these studies more (cost-)efficient. Moreover, (first-in-human) single or multiple ascending dose (SAD, MAD) studies that are a mandatory part of any drug's development trajectory, often can be enabled with POM and/or POC models. Important information on the drug's characteristics including pharmacokinetic-pharmacodynamic (PK-PD) relationships – especially of interest when evaluating wide dose ranges as during SAD or MAD studies – can then be generated, at little additional cost.

Due to the complexity of pain and its underlying mechanisms, a wide variety of analgesics with ever-increasing specificity are currently being developed. The classes discussed here represent those mostly developed, although it must be noted that compounds within a particular class may still vary substantially, for example due to (sub)type selectivity or route of administration. As such, $Na_V1.7$ contributes differently to analgesia than $Na_V1.8$ does; therefore, arguably other, even more specific biomarkers may be superior in evaluating compounds targeting either channel. The encompassing commentary nonetheless holds true: each technique mentioned does provide a firm handhold for assessing POM and/or POC, and that development of each unique compound needs a tailor-made approach.

While it is thus important to evaluate proxies aligning with the proposed mechanism of action, it is equally important to not narrow the study objectives unnecessarily. By testing multimodally – i.e., in addition to evaluating the desired endpoint, also include models each representing a distinct (pain) pathophysiology to evaluate effects other than expected – an (analgesic) effect profile can be created. [18] Multimodal testing in general does not significantly increase subject burden or study costs, yet provides increased knowledge on the drug's putative mechanism of action, and therefore confidence to make pivotal decisions about the compound's future. [13] This argument, however, only applies to analgesics of which the exact mechanism of action is linked to the suggested test. If there is no scientific rationale behind e.g., a NSAID possibly affecting electrical pain thresholds, it would be futile to add this method to a POC study. Yet even when there is an evident rationale to use a specific test, the relationship between the experimental model and the disease it mimics often is not fully elucidated. E.g., it is rational to use the UVB-induced

inflammatory hyperalgesia model to evaluate a TrkA inhibitor's potential to treat inflammatory pain, although the correlation between the model (increased NGF levels following UVB exposure), and the disease state that it mimics (NGF upregulation in synovial fluid in patients with osteoarthritis) is not fully known. It must therefore be noted that, while POM and POC studies do bridge the gap between preclinical research and studies in patient (sub)populations, positive results generated in such trials do not guarantee a drug's efficacy in a patient population. Two examples are the CB2 agonist GW842166 and the FAAH inhibitor PF-04457845, as discussed in section **Cannabinoids** of this chapter.

Also in a therapeutic area such as pain, research and technology has reached unprecedented levels that allow for meticulous assessment of a compound's (dose-dependent) effects. By incorporating tests such as those mentioned into early-phase trials, success – or failure – of a novel drug may be confirmed rather sooner than later. Moreover, with the change to personalized medicine and target selectivity, drugs developed to treat a multitude of conditions from the start are in decline. Instead, highly selective drugs treating well-chosen patient subpopulations are being developed. POC, but especially POM trials will aid in a crucial aspect related to this change. By incorporating methods that evaluate the drug's mechanism of action accurately, POM studies can confirm target selectivity that may be unachievable using POC experimental models alone. Therefore, results obtained for POM, but also POC – or preferably combined –, can help determine the optimal dose and patient (sub)population to target in the following development phase(s) – and aid in increasing the number of treatments reaching patients.

Aims and outline of this thesis

Continuing efforts are made to expand and further improve our knowledge on pain signaling and effective treatment of pain. One is by developing and validating new methods for early-phase clinical drug studies that have improved accuracy or improved resemblance to clinical pathophysiology, and may so improve the evaluation of a drug's mechanism of action and analgesic potential. The other is by actually testing novel compounds that are proposed to have a superior clinical utility, using methods that we believe to be appropriate for evaluating their POM and/or POC. For all types of pain but especially within the field of neuropathic pain, there is

still much to be gained, as illustrated by the large unmet medical need and limitedly efficacious drug treatments that are currently available. [151]

Neuropathic pain is defined by the International Association for the Study of Pain (IASP) as "pain that arises as a direct consequence of a lesion or diseases affecting the somatosensory system". [152] A key contributor to the chronification of neuropathic pain is central sensitization, which may manifest clinically as hyperalgesia (also see **Figure 1**), a symptom non-existent in healthy individuals. Models that can induce hyperalgesia and tools that can reliably assess altered functioning following induction, are sought-after as they may aid in examining the potential of (novel) analgesics as neuropathic pain treatment. Hyperalgesia – in experimental context – may be induced peripherally (i.e., increasing responsiveness to stimuli locally by increasing nociceptor sensitivity at the affected area), or centrally (i.e., increasing responsiveness to stimuli by increasing sensory neuron excitability at the dorsal horn and thalamus; **Figure 4**). [125] Hyperalgesia models that are suitable for use in early-phase drug studies can be an important asset for improved POC of the analgesic drug classes described here in **Chapter 1**. To be 'suitable for use', we applied the general criteria for usability of a biomarker, as described previously:

- The model should induce a clear, consistent response across studies, and across drugs from the same class
- A clear response to therapeutic doses must be observed
- Dose (concentration)-response relationship can be demonstrated (if the study design allows for this)
- There should be a plausible relationship between the model, the pharmacology of the tested drug class and the disease pathophysiology. [153]

As convincing evidence in favor of hyperalgesia models with respect to the above criteria is limited, further research is warranted. The main objectives of this thesis therefore were to evaluate applicable tools for profiling the effects of (novel) analgesics using hyperalgesia models and other established nociceptive tests (**Section I**), and explore other tools that may even better predict an analgesic's effects in healthy volunteers (**Section II**).

Section I

In this first section, we assessed the validity of a panel of nociceptive and hyperalgesia models in context of the assessment of analgesic effects of (novel) NAv inhibitors. We tested a novel and selective NAv1.8 inhibitor, VX-150, in a dedicated POC two-way cross-over study and reported our findings in **Chapter 2**. In **Chapter 3** the safety, tolerability and nociceptive test results of a first-in-human study with NAv1.8-selective inhibitor VX-150 are described. To further study how NAv inhibition modulates nociceptive processing, in **Chapter 4** we tested the two registered, non-selective, NAv inhibitors lacosamide and mexiletine using a nociceptive test battery and UVB-induced hyperalgesia model.

Section II

Next, we evaluated a selection of models on their potential to induce hyperalgesia in healthy subjects. **Chapter 5** describes results from a clinical study in which we studied the suitability of the human endotoxemia to induce inflammatory hyperalgesia. **Chapter 6 and 7** discuss how depriving healthy subjects from sleep induces sex-dependent enhanced pain sensitivity, and report that different readouts may be applicable. In **Chapter 8**, we investigated whether we could improve our existent topical capsaicin (cream) formulation with an updated (ethanolic solution) formulation by testing its potential to induce peripheral and central sensitization.

The main findings of this thesis are summarized and discussed in **Chapter 9**, which also includes general conclusions and recommendations on the use of experimental models in early-phase analgesic drug development.

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Table 1 Key POC methods often used in early-phase analgesic drug development.

Method	Details
TMS	**METHOD** A non-invasive, painless technique to stimulate the brain. Through a coil which is kept on the head of the subject, a magnetic pulse is applied to the brain. This magnetic pulse induces an electric field and electrical currents in the cortex, which in turn, if large enough, depolarize neurons and initiate action potentials. Because the focus of the magnetic field lies just beneath the coil, TMS activates a brain area in the superficial cortical layers of only a few centimeters in diameter. [154,155]
	PRO'S TMS provides the opportunity to assess cortical excitability, which can be regarded as a measure of how easily neurons and cortical networks are activated by the magnetic pulse. [154]
	CON'S Expensive method requiring specific knowledge to be performed and analyzed. Results are subject to operator-variability, and found effects may be difficult to interpret mechanistically.
	CLINICAL TRANSLATION widely used as supportive diagnostic tool for a variety of neurological diseases. [156]
UVB-induced hyperalgesia	**METHOD** First, the minimal dose of UVB needed to induce erythema (MED, in mJ/cm^2) is determined for each subject individually. Subsequently, 18-24h prior to planned test days, 2x or 3x MED is applied on healthy skin, to induce inflammatory hyperalgesia. Readouts include heat pain ratings using a thermode, or mechanical allodynia surrounding affected site using e.g., Von Frey filaments (i.e., secondary allodynia).[13]
	PRO'S Low inter- and intra-subject variability, stable hyperalgesia for 36 h
	CON'S Induction of post-inflammatory hyperpigmentation lasting 6 months (2xMED dose) up to multiple years (3xMED dose). Requires a more homogenous study population as irradiation levels needed to induce hyperalgesia are only safe in lighter-skinned individuals. [35]
	CLINICAL TRANSLATION Agreement between drug effects reported in this model, and trials in patients suffering from burn injury and postoperative pain. [157]
Capsaicin-induced hyperalgesia	**METHOD** Low concentration capsaicin is either topically applied and absorbed for a brief period (e.g., 30 min) or injected intradermally, to induce transient burn-like sensations on and around the treated area/location. The same readouts can be evaluated as mentioned for the UVB-induced hyperalgesia model.
	PRO'S Method that is easy to use, highly customizable and selectively links to TRPV1 agonism. The sensitizing effects after topical application are considered mild with effects approximately lasting a day. Both the topical and intradermal model induce reproducible primary heat sensitizationX. [158] Intradermal injection exerts reproducible effects on the area surrounding the area of application (i.e. secondary area).
	CON'S Subject burden for the intradermal model is high, as the injection induces a near-maximal pain sensation (rating of ~9/10). [159] After topical application, the response on the secondary area is highly variable both between subjects and within the same subject. [127,160]
	CLINICAL TRANSLATION Used as model to induce secondary hyperalgesia. In combination with mechanical stimulation, drug efficacy for this model is associated with effective treatment of trigeminal neuralgia and renal colic. [157] High concentrations are indicated in postherpetic neuralgia through temporary denervation that may last up to months. [13]
Cold pressor pain test	**METHOD** Variations of this test are available, all involving submerging (a part of) the hand or foot in cold water. Cold pressor test methodology often used (Eckhart et al., Jones et al.): A blood pressure cuff is inflated to 20 mmHg below resting-diastolic pressure, after which the ipsilateral hand is submerged in a cold water bath. Pain is rated using a rating scale (e.g., VAS) until pain tolerance or 120s is reached, whichever comes first. Subjects are instructed to remove their hand, the cuff deflates at that same time. [161,162]
	PRO'S broadly applicable, easily adoptable test that may also be used to induce the CPM response.
	CON'S temperature of the water, and ability of equipment to maintain set limit (i.e. by circulating water) results in evident differences between studies, limiting translatability of the model. [13]
	CLINICAL TRANSLATION used for diagnosing fibromyalgia or opioid-induced hyperalgesia [19]

CPM: conditioned pain modulation, MED: minimal erythema dose, TMS: transcranial magnetic stimulation, UVB: ultraviolet B, VAS: visual analogue scale

Figure 1 High-level illustration of definitions of allodynia and hyperalgesia With gradually increasing intensity of a pain stimulus, a normal pain response is expected to increase following a sigmoid curve, as described on the right part of the Figure. Allodynia is defined as perceiving a stimulus as painful where it normally would not be perceived as such, this is defined as allodynia. (blocked area under the left sigmoid curve) E.g., a stroke with a brush or feather that produces a painful sensation. Hyperalgesia is defined as having an increased sensitivity to a painful stimulus, that normally would also perceived as painful (striped area under the left sigmoid curve). E.g., a blow with a hammer that was rated with a pain intensity of 3 out of 10, where the pain typically would be rated as 1 out of 10.

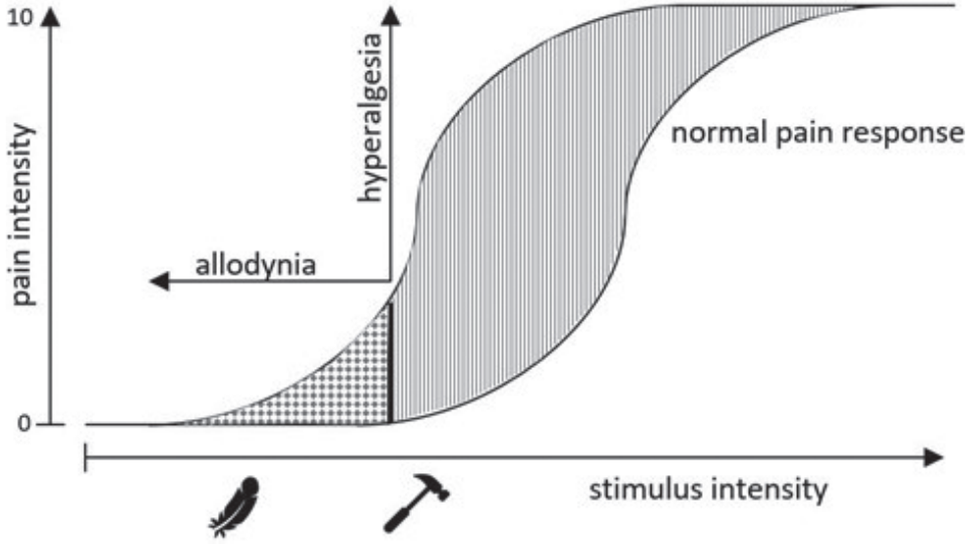
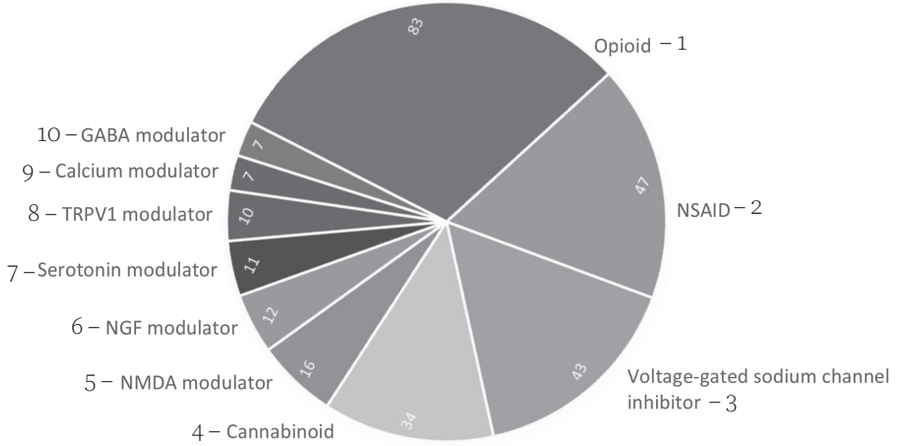


Figure 2 Top 10 analgesic drug classes currently in early phases of drug development (until the therapeutic exploratory phase (phase I/II)) Numbers represent number of unique compounds currently in development, per respective class.



GABA: gamma-aminobutyric acid; NaV: voltage-gated sodium channel; NMDA: N-methyl-D-aspartate receptor; NGF: Nerve Growth Factor; NSAID: non-steroidal anti-inflammatory drug; TRPV1: transient receptor potential cation channel subfamily V member 1.

Figure 3 Illustration of unique role of various Na_V channels in action potential generation. (Adapted from [40]) *(full color version of this illustration on inside of the cover)*

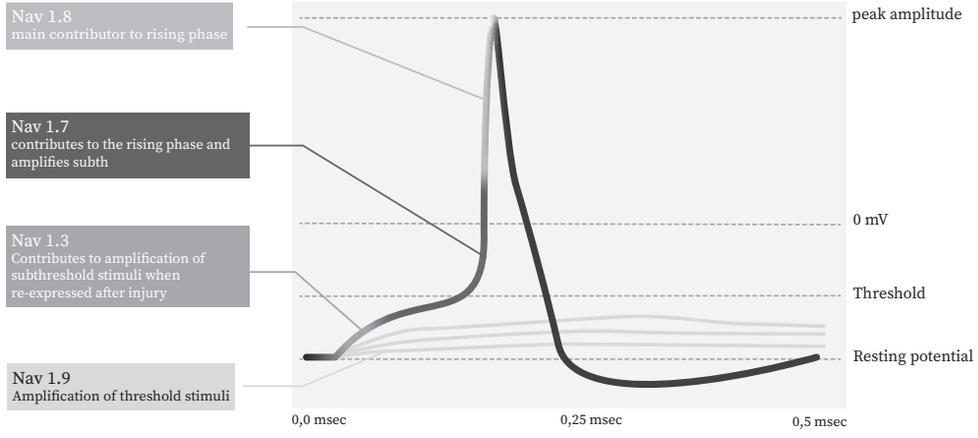
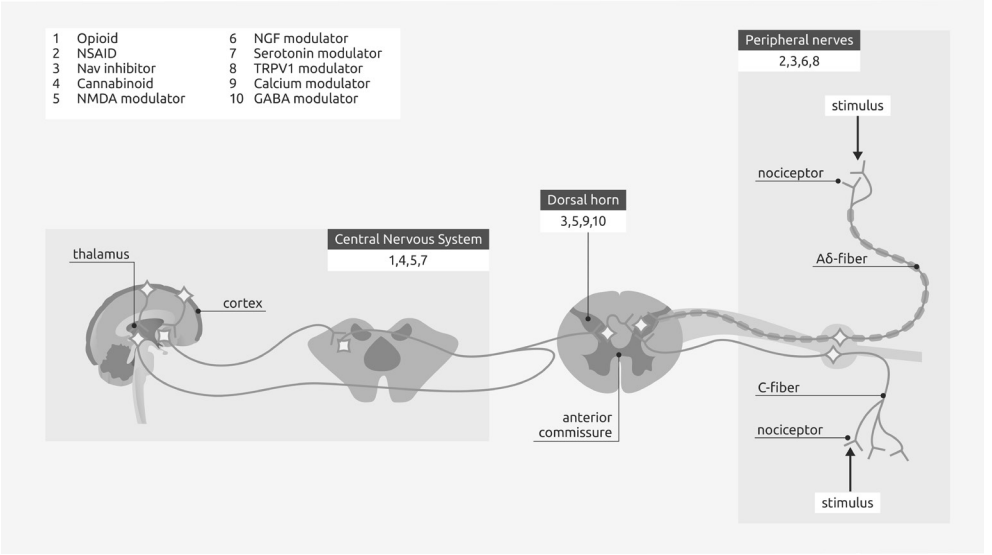


Figure 4 Schematic overview of primary target location per analgesic drug class. Drug classes are described with numbers, legend in top left corner describes which number links to which class. The pain pathway is described as having three distinctive target locations: the central nervous system, dorsal horn, and peripheral nerves. While specific drug classes may target multiple sites to a lesser extent as well, for sake of reasoning only the main target locations are linked to a specific drug class.



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