Discovery and development of novel psychoactive drugs for the treatment of affective and other related brain disorders

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Introduction

Affective disorders are common, debilitating and chronic illnesses of the central nervous system (CNS) [1,2]. Although the cost of research and development of novel drugs is continuously rising globally [3], this growth is particularly high for CNS drugs, nearing 150% in the last decade [4]. At the same time, the 8% approval rate of CNS drugs after successful preclinical trials is one of the lowest (e.g., compared to 20% for cardiovascular drugs) [5]. The total clinical research plus the approval review time for CNS drugs is generally 32 months longer (than for non-CNS drugs), further contributing to their high direct/indirect research costs and low success rate [3]. As a result, large pharmaceutical companies continue to reduce their CNS pipelines and cut research personnel worldwide [3]. Thus, CNS drug research and development are presently facing tremendous obstacles in terms of both global health and market pressure. For affective and other common brain disorders, drug development is further complicated by their poorly understood pathogenesis [6], high clinical heterogeneity [7-9], overlapping genetics and frequent comorbidity [10-14].

As modern translational biomedicine focuses on accelerated integration of fundamental research with clinical data [15,16], animal models are an indispensable tool for CNS drugs discovery [17-22], as will be discussed here in detail. However, its slow pace is caused by the apparent mismatch between the long-term goals of preclinical vs. clinical drug screening: while preclinical screens search for the most effective drugs (based on behavioral and molecular assays), human testing aims to ensure their high safety over therapeutic efficacy [23]. Although drug safety is critically important, this discrepancy is at least partially responsible for the low yields of the CNS drug discovery [5] that, combined with high costs of CNS drug discovery, impedes the innovation in the field [24], shifting focus and efforts from brain to more ‘stable’ and ‘predictable’ non-psychiatric diseases [3]. Thus, ‘clinicizing’ preclinical drug research by enhancing safety screens and using endpoints and biomarkers for drug assessment that are more

clinically valid and translationally relevant, may be beneficial to optimize preclinical drug screening, and, hence, reduce cost and improve yields of CNS drug discovery.

Since stress is commonly associated with affective disorders, especially depression and anxiety [25], animal models are widely used to assess CNS stress effects across taxa [26]. For example, chronic mild unpredictable or variable stress models apply frequent and prolonged (e.g., for 1-8 weeks) stress to evoke affective phenotype both in rodents and fish [27,28]. Typical stressors in such models include disturbing circadian rhythms, food deprivation, electric shock, forced swimming, shaking, exposure to predators, crowding and immobilization [26,28] that induce robust affective-like phenotypes (e.g., anxiety and anhedonia) rescued by antidepressants [27]. Another popular stress paradigm, the chronic social defeat stress, uses prolonged daily aggressive social encounters to induce anxiety- and depression-like phenotypes in rodents [26,29,30] and fish [31]. Finally, multiple behavioral tests have also been developed, based on novelty exposure and/or place preference [26] in both taxa, as they similarly prefer 'protective' black/dark bottom over white/lit open environments when anxious [32].

Among the most important paradigms for affective drugs screening are 'behavioral despair' paradigms that assess animal 'learned helplessness' in dangerous inescapable situations and are selective to conventional antidepressants, but not to anxiolytics like benzodiazepines [33,34]. Recently, this approach has been successfully applied to the zebrafish (*Danio rerio*, presently the second most used animal experimental model in biomedicine [35-37]). These findings show high evolutional conservation of the despair-like phenotype, and its high predictive validity due to its sensitivity to antidepressant (but not anxiolytic) drugs [38].

However, despite the existence of valid and popular affective tests and models, their results often show poor reproducibility between and within laboratories [39,40], with overt individual, strain-, population-, sex- and age variability [39,40]. For example, there are clear sex differences in rodent anxiety [41], social [42], predator- [43] and other stress-related behaviors [44]. Likewise, zebrafish sexes differ in aggression [45], social [31], general activity and anxiety

phenotypes [46]. In addition to heterogeneity of targeted subject populations, other challenges in translational affective neuroscience include the role of environmental and epigenetic factors, inefficient experimental logistics, complex dose-effects of psychoactive compounds, ethical concerns, overall complexity of experimental designs and their multifactorial nature [47,48]. The lack of a sufficient number of clear-cut, translatable and reproducible behavioral and especially molecular biomarkers of individual brain disorders, and the unclear parallels between endpoints studied clinically and preclinically, are also recognized in the field [48].

Expert opinion: New targets, more targets, and more models

Trace amines

Innovating the search for CNS drugs, several novel promising classes of drug targets are worth discussing as illustrative examples of recent successes and challenges. For instance, the trace amines (e.g., β-phenylethyalmine, p-tyramine, tryptamine and p-octopamine) and their Trace Amine Associated Receptors (TAARs) have been recently implicated in various CNS disorders, including schizophrenia, bipolar disorder, depression and drug abuse [49-52]. Although the trace amines have long been known to occur in the brain, their receptors in vertebrates were identified only recently [53,54]. Until recently, only the TAAR1 was thought to be expressed in various brain regions, while other TAARs have been viewed as 'olfactory' receptors, shifting focus of TAAR research to mainly TAAR1-mediated CNS mechanisms [55]. While the *TAAR1* genetic variants are associated with clinical schizophrenia [56], its knockout in mice enhances amphetamine response and impairs prepulse inhibition [57], and the over-expression reduces amphetamine sensitivity [58], implicating TAAR1 in modulating several brain neurotransmitters [55]. TAAR1 agonists also show anxiolytic, antidepressant and antipsychotic activity [55], further supporting this receptor as a putative novel promising therapeutic target. However, the CNS roles of other TAARs have recently become recognized. For example, mouse TAAR5 is expressed in multiple limbic regions and its knockout evokes lower anxiety- and depression-like behavior [59,60], linking TAAR5 to the regulation of

behavior, similarly to TAAR1. In humans, TAAR6 is also expressed in amygdala (with TAAR8), basal ganglia, frontal cortex, substantia nigra and hippocampus [52,53,61], suggesting these TAARs as novel putative targets for CNS drug discovery.

Glutamatergic signalling

Central glutamatergic system is one of the most critical for the regulation of complex behavior. A promising CNS agent, glutamatergic antagonist ketamine, is already in Phase 3 of clinical trials for depression [62]. The fast rise of ketamine use may be partially attributed to its widespread need as an anesthetic clinically, making it a Schedule III drug approved by the US Food and Drug Administration (FDA) for this purpose, unlike psychedelics. Most studies note antidepressant activity of ketamine [62], shown efficacy in multiple reports on treatment-resistance depression [62], with a rapid acute onset (vs. classical antidepressants that need weeks) of therapeutic effects. Ketamine is also beneficial for obsessive-compulsive disorder (OCD) [63-65], post-traumatic stress disorder (PTSD) [66] and addiction [67,68], although its effects usually do not persist for a long time, and require regular administration [65]. In rodents, ketamine exerts similar antidepressant-like effect [69], acting as a glutamatergic N-methyl-D-aspartate (NMDA) receptor antagonist and, possibly, also indirectly modulating other (e.g., gamma-aminobutyric acid/GABA-ergic) systems in various brain circuits [70,71]. In adult zebrafish, acute exposure to ketamine evokes an anxiolytic-like behavior, reduces whole-body cortisol levels [72,73] and dose-dependently modulates (at low doses increasing, and at high does reducing) zebrafish aggression [74]. Collectively, these clinical and preclinical findings support the importance of glutamatergic agents to target evolutionarily conserved signaling pathways for treating various affective brain disorders.

Serotonergic targets

Mounting evidence also suggests classical psychedelic serotonergic hallucinogens and related drugs (e.g., 3,4-methylenedioxymethamphetamine, MDMA) as emerging novel pharmacotherapies for affective disorders, including depression and PTSD [75]. Although

classifying as Schedule 1 drugs for political reasons in the 1960-1980s severely impeded their clinical and preclinical studies, regulatory approval and market introduction [75], they can cause a stable improvement in wellbeing (e.g., by psilocybin treatment) [76], optimism [77], anxiety (e.g., by lysergic acid diethylamide, LSD or psilocybin) [78,79], depression (by ayahuasca, whose primary psychoactive agent is N,N-dimethyltryptamine, DMT) [79-81] and addiction (e.g., by psilocybin) [82,83] clinically. LSD and psilocybin successfully treat clinical anxiety induced by life-threatening diseases [78,84,85], ayahuasca alleviates major depression [80,81], and psilocybin mitigates anhedonia, depression and anxiety in treatment-resistant depressed patients, with effects sustained for months [86,87]. Such prolonged effects of acute psilocybin treatment strikingly differ from the short-term action of acute ketamine, thus making 5-HT_{2A} agonists more promising targets for depression therapy. Likewise, MDMA inhibits monoamine (primarily, serotonin) reuptake and is used in PTSD [88-92] as a 'breakthrough' therapy recognized by the FDA [93]. Paralleling clinical data, 5-HT_{2A} receptor agonists (e.g., 4-iodo-2,5-dimethoxyphenylisopropylamine, DOI) evoke profound neuroplasticity [94], anxiolytic- [95] and antidepressant-like (e.g., repeated LSD) effects in rodent models as well [96] (Fig. 1).

G protein-coupled receptors

The adhesive G protein-coupled receptors (aGPCRs) are a the second largest GPCR family of relatively understudied cell adhesion and signaling proteins [97] whose 33 human orthologs are widely expressed in the brain [98]. The distinctive features of aGPCRs are the large multidomain N-termini and juxtamembrane GPCR Autoproteolysis INducing (GAIN) domain [99,100]. While exact functions and mechanisms of aGPCR remain unclear, some of them have been linked to human and animal CNS pathologies [98,99,101]. For example, Adhesion G Protein-Coupled Receptor B2 (*Adgrb2*) knockout mice display antidepressant-like behavior, enhanced hippocampal cell proliferation, but unaltered locomotor activity and learning [102]. A loss-of-function variant of Adhesion G Protein-Coupled Receptor L3 gene (*ADGRL3*) is associated with human attention deficit and hyperactivity disorder (ADHD) and autism spectrum

disorder [103-107], whereas mice and zebrafish lacking this gene exhibit altered dopaminergic signaling and ADHD-like hyperactivity and impulsivity [108,109]. Adhesion G Protein-Coupled Receptor C3 (*3ADGRC3*) variants are implicated in Tourette's syndrome [110,111] and *Adgrc3* or Adhesion G Protein-Coupled Receptor C2 gene (*Adgrc2*) deletions in mice lead to hydrocephalus and impaired ciliogenesis [112]. Single nucleotide polymorphisms (SNPs) within *ADGRB3* are enriched in schizophrenic patients [113-116], whereas chronically stressed zebrafish markedly increase the *adgrg4b* expression in whole brain samples (log_2 fold change = 8.4), rescued by a selective serotonin reuptake inhibitor (SSRI) fluoxetine (own unpublished data). Collectively, these clinical and preclinical findings implicate aGPCRs in neuropathogenesis and call for further research on their signaling mechanisms in vivo as potential targets for CNS drug discovery, especially related to affective disorders.

Arrestins

Arrestins (mainly β-arrestins expressed in the brain) and ubiquitin-related pathways also warrant attention (Fig. 2). Arrestins are highly homologous proteins that regulate GPCR by supporting their internalization and redirecting signaling to G-protein independent pathways [117-119]. The GPCRs activation by agonist leads to G-proteins activation, followed by second-messenger (cAMP, Ca^{2+}, inositol phosphate) cascades, activating kinases, other proteins and ion channels [120]. However, G protein-coupled-receptor kinases (GRKs) may phosphorylate intracellular domains of GPCRs resulting in the recruitment of β-arrestins to GPCRs followed by receptor desensitization [121]. The GRK/arrestin activity promotes further internalization of GPCRs followed by receptor recycling, degradation or endocytosis [121,122]. GRKs and β-arrestins also bind additional cellular proteins supporting β-arrestins-related (but G-protein independent) pathways activation, including c-Src, ERK1/2, JNK3, p38 MAPK and AKT [123-127].

Recent studies have revealed biased GPCR ligands that activate G-protein and arrestin-related pathways with differential efficiency and target different pathways, thus avoiding

potential undesirable side effects [128]. Importantly, arrestins have also been proposed as potential targets for treating affective pathologies [129]. For example, depressed patients display reduced levels of β-arrestin-1 in mononuclear leukocytes [130,131], whereas antidepressant treatment increases them [131], also similarly elevating their CNS expression in rats [130]. SSRIs, selective serotonin-norepinephrine (SNRIs) and nonselective reuptake inhibitors increase β-arrestin-1 levels in rat cortex and hippocampus following a 10-day treatment, reaching maximal effects on weeks 2 and 3, thus mimicking clinical 'delayed' antidepressants effect [130]. Furthermore, normalization of β-arrestin-1 levels in leukocytes predicted such clinical improvement [131]. In the mouse chronic stress model, both β-arrestin-1 and -2 are down-regulated in the hypothalamus, and fluoxetine treatment recovers their levels [132], whereas β-arrestin-2 knockout mice display reduced fluoxetine responsivity [132].

Importantly, the cellular functions of arrestins depend on ubiquitination that determines the fate of the arrestin-receptor complex, its signaling cascades and the GPCR internalization [121] (Fig. 2). Ubiquitin is a small, highly conserved protein binding to lysine of targeted proteins (ubiquitination) or another ubiquitin, forming polyubiquitin chains on a targeted protein (polyubiquitination) [121,133]. Ubiquitin chains, in turn, guide protein trafficking, endocytosis, degradation and activation of signaling cascades or kinases [121]. For arrestins, ubiquitination occurs after β-arrestin binding and promotes stabilization of receptor complex in the endosome, thus controlling its recycling, endocytosis and scaffolding [121]. Like arrestins, ubiquitin plays an important role in cellular action of antidepressant drugs [134]. For example, while citalopram, imipramine, desipramine and moclobemide all increase mRNA expression of β-arrestin-2 in rat glioma cells, independently on their traditional extracellular signaling effects [134], the β-arrestin-2 protein levels are reduced by antidepressants, due to arrestin ubiquitinylation that promotes its proteasomal degradation [134].

Other putative drug targets

Recent studies suggest additional potential pharmacophores underlying therapeutic effects of CNS drugs in the brain. For example, rats exposed to acute high-dose or chronic mild-dose β-phenylethylamine (PEA) show serotonergic syndrome with hallucination-like and hyperactivity behavior [136], whereas acute exposure to Δ9-tetrahydrocannabinol (THC) evokes anxiety-like effects and aberrant locomotor activity in both mice [137] and zebrafish [138]. The bis (7)-cognitin (B7C) is a dimer formed by two tacrine molecules [139], whose multi-target activity includes the inhibition of acetylcholinesterase (AChE), prevention of the aggregation of the β-amyloid (Aβ) protein, regulation of the downstream signaling of the glutamatergic NDMA receptor, inhibition of the nitric oxide synthase (NOS) pathway [140] and a competitive antagonism at the GABA-A receptor [141]. B7C is 150 times more potent and 250 times more selective to inhibit AChE than tacrine, due to the dual interaction with the AChE binding sites [142,143]. In rodents, some tacrine derivatives show positive effects against learning and memory deficits in scopolamine-induced model of amnesia [144], and therefore merit further scrutiny in regard to their other (e.g., affective) putative CNS properties. Neurosteroids are also highly relevant as a potential therapy of CNS disorders [145], modulating both GABA-A and NMDA receptors [146,147]. For example, pregnenolone alleviates depressive episodes in bipolar patients [148], whereas gaboxadol exerts sedative and hypnotic activity [145,149], collectively emphasizing a wide range of signaling pathways relevant to developing novel treatments of CNS disorders.

Where next?

Recognizing the emerging molecular complexity discussed above, and departing from traditional "1 drug – 1 target" approaches that have dominated CNS drug discovery for decades, a promising novel strategy of drug development may therefore be to act via several interacting targets simultaneously ("1 drug – several *coupled* targets"). For example, a ligand that simultaneously impacts both traditional monoaminergic signaling and the associated affiliated mechanism of their regulation (e.g., β-arrestins), may represent a promising 'double-hit'

candidate for such multi-target drug discovery. Furthermore, given high heterogeneity of brain disorders clinically, it is logical to expect that multiple distinct brain and/or molecular systems can be disrupted simultaneously during the disease. As such, polymodal multi-target drugs that simultaneously affect distinct (non-coupled) neurotransmitter signaling processes, are urgently needed, albeit their effectiveness and limitations in the treatment of affective disorders are still unclear [150-152].

Overall, despite multiple known limitations of animal models and the mounting obstacles for innovative CNS drug research, the field must not be swayed by gloomy realities of the current markets. Rather, it should be further promoted, as brain remains the most complex human organ, and its disorders are among the most societally costly public health burdens. Because animal models represent an indispensable tool to assess drug efficiency and safety, further innovation of biological methodology and biomarkers will deepen our understanding of clinical findings, and may contribute to new theories of brain pathogenesis. Thus, the field of CNS drug discovery, especially related to affective disorders, should be reinvigorated, innovated and reintegrated, focusing on new approaches and targets.

The latter strategy also includes embracing novel model organisms. For example, the zebrafish is an excellent model for studying molecular mechanisms of development, also possessing a fully sequenced genome with high (>70%) genetic homology to humans, and multiple practical advantages [153-156]. Zebrafish are particularly useful in CNS modeling since many behaviors correlate with their morphological and physiological features, amenable for the growing number of research technologies [157]. Notably, the cost of research on zebrafish is much lower than on rodents, complemented by the rapid reproduction and high survival rate of embryos, as well as by the simplicity of maintenance and breeding [158]. Zebrafish neuromorphology is well studied and fully described in numerous atlases [159,160]. Their neuroendocrine stress axis is highly homologous to humans [161], and zebrafish possess all major neurotransmitter systems and their signaling cascades as in rodents and humans [162].

Thus, the physiological, anatomical, biochemical and genetic characteristics of zebrafish allow it to be used as successfully as rodent models to study the pathogenesis of affective disorders and their pharmacotherapy [163]. In addition, zebrafish possess transparent embryos and fast development, enabling studying changes in brain morphology and development caused by experimental (e.g., genetic or pharmacological) modulation [164]. The behavioral repertoire of the zebrafish is also thoroughly described and comprehensively catalogued [165], including anxiety-like behavior [166,167], sociability [168], aggressiveness [169,170], cognitive [171] and other phenotypes. While many of these behaviors are evolutionarily conserved across taxa, some behaviors differ from those in mammals. For example, zebrafish have no parental care and develop externally, which precludes monitoring the effect of parental changes on the offspring. Furthermore, maternally deposited cortisol is present during embryogenesis, which may affect larval zebrafish by programming their neuroendocrine development and function [172]. Complementing zebrafish models, other fishes may offer a valuable tool in studying various affective disorders. For instance, the goldfish (*Carassius auratus*) is another popular model species in translational neuroscience research, with well-defined behavioral repertoire [173] and high sensitivity to experimental and pharmacological modulation of CNS responses [174-176].

Finally, while there is no single animal model that can fully characterize all the range of psychiatric symptoms of a disease [177,178], various behavioral and physiological symptoms of affective disorders can be simulated in both rodents and zebrafish [27,178-181], sensitive to antidepressants that may reverse these symptoms [38,182]. Likewise, despair-like behavior was successfully translated recently from rodents to zebrafish, enabling fast antidepressant screening in this aquatic species [38]. Collectively, this indicates that zebrafish and other fish models may advance the field of affective disorders, also helping to target the evolutionarily conserved ‘core’ mechanisms of affective deficits in vertebrate taxa.

Additional considerations: New methods, tests and screening batteries

Historically, the first examples of targeted development of antidepressant drugs include monoamine oxidase (MAO) inhibitors and SSRIs, currently the most clinically successful groups of antidepressants that also possess anxiolytic properties [183]. How can researchers discover new CNS drugs? Modern drug development concepts are usually based on Rational Drug Design (RDD) and involve identifying a specific target and selecting or modelling de novo the exact ligand structure that would best ensure their binding [184]. For affective disorders, as already mentioned, their therapy is complicated by clinical complexity and heterogeneity [185]. The lack of unequivocal knowledge about the causes of mood disorders, as well as the specific challenges of animal models of mental dysfunction, both reduce the likelihood of a positive outcome, making the chances of a drug being cost-effective to develop very thin for pharmaceutical companies [150,186,187]. Complementing rational drug design methodologies, for psychiatric diseases, phenotypic screening in animal models is also common as an adjunctive approach [161,188,189].

However, the role of bioinformatics and molecular modelling in silico (Table 1) continues to grow in drug design [184,190], especially given cost reduction and shortened duration of drug discovery. Using these methods, the structure of target molecules is recreated by gradual construction of small fragments placed in the active receptor site with minimization of steric factor and maximization of binding energy [191], to build hypothetical structures with a predicted high affinity for the target. The disadvantages of this method are the relatively low reliability of estimating ligand binding affinity and the unclear rules for ligand design that are too generic to accommodate the biological profile of a particular target [192].

For example, the Quantitative Structure-Activity Relationship (QSAR) strategy links the structure of a molecule to its activity [193], either based on the dimensions (e.g., 1-5D) of the descriptors involved in the model, or using the type of biological activity predicted as a dependent variable [193,194]. In addition, QSAR models can also be grouped based on analysis of correlation-linear and non-linear [195], or depending on binding nature of molecule and

receptor [196]. One of QSAR methods, Comparative Molecular Field Analysis (CoMFA) approximates the 3D structure of a ligand with a set of molecular fields separately characterizing its steric, electrostatic, donor-acceptor and other properties, complemented by multiple regression analyses of ligands with known activity [197,198]. The resulting set of fields characterizes the location and properties of substituents in the molecule, which can then be used in virtual screening of compound libraries, acting as analogs of pharmacophores whose activity is determined by the functional groups present [199,200]. Methods determining the similarity of molecules ('fingerprinting') examine certain 'descriptor' properties of a molecule (e.g., the number of H-bond donors or benzene rings) to compare the resulting fingerprint with that of a reference sample, to *predict* their similar molecular activity in vivo [201].

Moreover, the libraries of established 3D structures are used in computer simulation of ligand-protein interaction ('molecular docking'), conformations and mutual affinity [202]. For example, a receptor and its ligand can be modeled as rigid volume figures (hard docking), or afforded conformational flexibility of ligand (semi-flexible docking) and the receptor (flexible docking), with or without solvents [202,203]. Molecular docking has become a powerful approach for discovery and development of novel drugs [204-206]. For example, multiple ligands of adenosine A_{2A} [207,208] and β2-adrenergic receptors [209] have recently been identified using molecular docking. Modern tools also enable complex design of novel molecules based on the key receptor residues, including ligands acting at several targets, as novel dual dopamine D_2 dopamine/serotonin 5-HT^{2A} receptor ligands have been designed as potential schizophrenia treatment [210].

Finally, modern development of genomics and proteomics enables precise identification of targets responsible for pathogenesis of the disease in question [211,212]. Genome-wide association studies (GWAS) also help find associations between the disease, drug responses and their side effects [213]. As such, developing specific pharmacological therapies for individual patients, as part of 'personalized medicine' approach (Table 2), is expected to clarify how

'personality' (individual CNS traits) contributes to pharmacological efficacy in treating affective and other brain disorders.

Conclusion

Although multiple questions remain open in the field (Table 2), the growing prevalence of affective disorders and their major impact on public health necessitate novel psychoactive compounds for their treatment. Here, we call for intensifying preclinical research in the search for novel targets for CNS drugs. However, a balance is also necessary between the strategies to facilitate preclinical research and the importance of integrating such drug discovery with practical needs of clinical medicine. For example, despite differing goals of preclinical vs. clinical drug screening (i.e., the search for most effective vs. safer drugs, respectively) [23], safety considerations are crucial for developing novel therapies. Indeed, potential addictive, pro-psychotic or other side effects of novel drugs (e.g., psychedelic hallucinogens and ketamine) must be carefully evaluated before a novel therapy can reach human patients.

While being under severe market and societal pressure, the affective drug research has many ways for improvement and innovation, that may result in paradigm shifts and shed a light on affective pathogenesis. Several novel drug targets and novel compounds, emerging as promising for affective disorders, include trace amine receptors, neurotransmitter signaling (Fig. 1) and aGPCRs, as well as their activity modulation by arrestins and ubiquitin, especially since the drugs selectively targeting G-protein dependent or independent pathways (Fig. 2) may reduce adverse effects, and since they mediate receptors internalization and sensitivity. Finally, the field rapidly develops new paradigms and methods, including computer modeling and the use of novel model organisms (e.g., zebrafish), to better target CNS phenotypes in question (Fig. 3 and 4) and more fully translate them into clinical data. Together, these innovative strategies will empower the development and improve efficiency of CNS drug research for affective disorders.

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CRediT authorship contribution statement

Konstantin A. Demin (Investigation) (Writing - original draft) (Writing - review and editing), Maria V. Seredinskaya (Investigation) (Writing - original draft) (Writing - review and editing), Nataliya A. Krotova (Investigation) (Writing - original draft) (Writing - review and editing), Nikita P. Ilyin (Investigation) (Writing - original draft), Alexander S. Taranov (Investigation) (Writing - original draft), David S. Galstyan (Writing - review and editing), Ksenia A. Nikolaeva (Writing - original draft) (Writing - review and editing), Natalia A. Levchenko (Investigation) (Writing - original draft), Tatiana O. Kolesnikova (Writing - original draft) (Writing - review and editing), Murilo S. de Abreu (Investigation) (Writing - original draft) (Writing - review and editing) (Visualization), Tamara G. Amstislavskaya (Investigation) (Writing - review and editing), Tatyana Strekalova (Writing - review and editing) and Allan V. Kalueff (Conceptualization) (Funding acquisition) (Project administration) (Resources) (Supervision) (Validation) (Writing - review and editing)

Figure 1. Common effects of serotonergic $5HT_{2A}$-active psychedelics, associated with affective disorders treatment, reducing anxiety and depression symptoms (based on [214,215]).

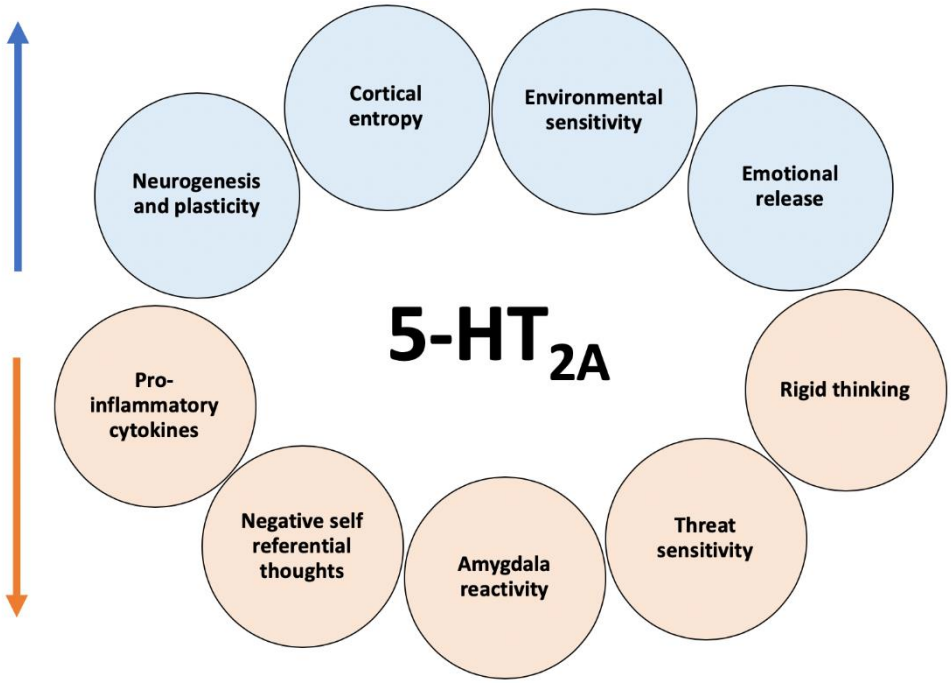

Figure 2. Schematic diagram showing the GPCRs G-protein dependent and independent signaling cascades and their modulation with the β-arrestin and ubiquitin (U) mechanisms. Briefly, agonist binding to extracellular or transmembrane sites triggers common G-protein signaling cascades. However, if this signaling is terminated with GPCRs intracellular phosphorylation by G-protein coupled kinases (GRK), the GPCRs bind β-arrestin, resulting in desensitization of G-dependent pathway by blocking the G-protein binding. Further events include internalization of receptor and/or G-independent signaling pathways activation. Future fate of receptor complex depends on specific ubiquitin sequence binding to β-arrestin. a – agonist, R – GPCR, p – phosphorylated sites. Adapted from [121,216].

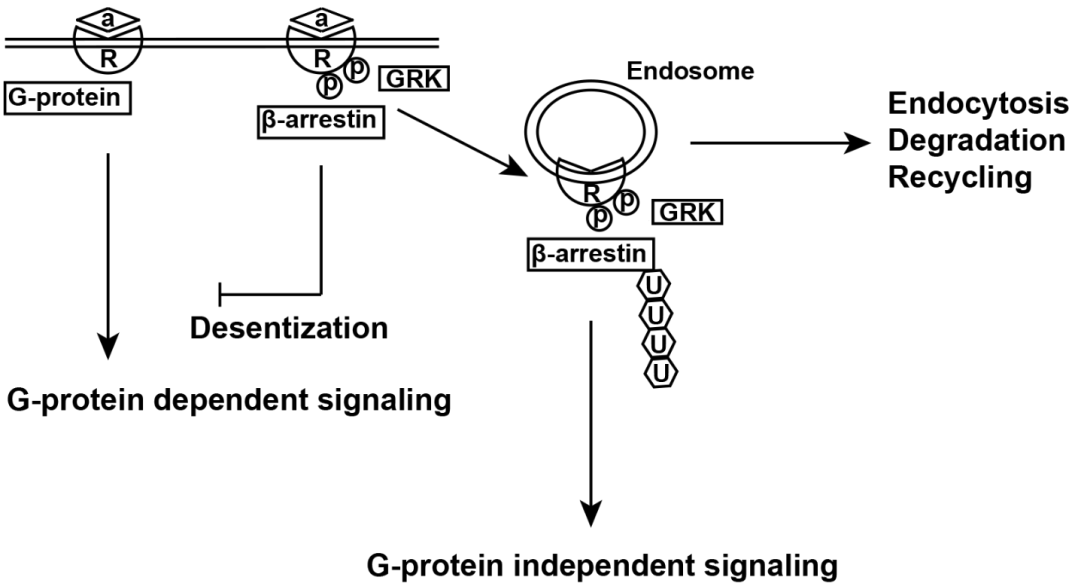

Figure 3. Complementing various stages of preclinical drug development with computer modeling methods

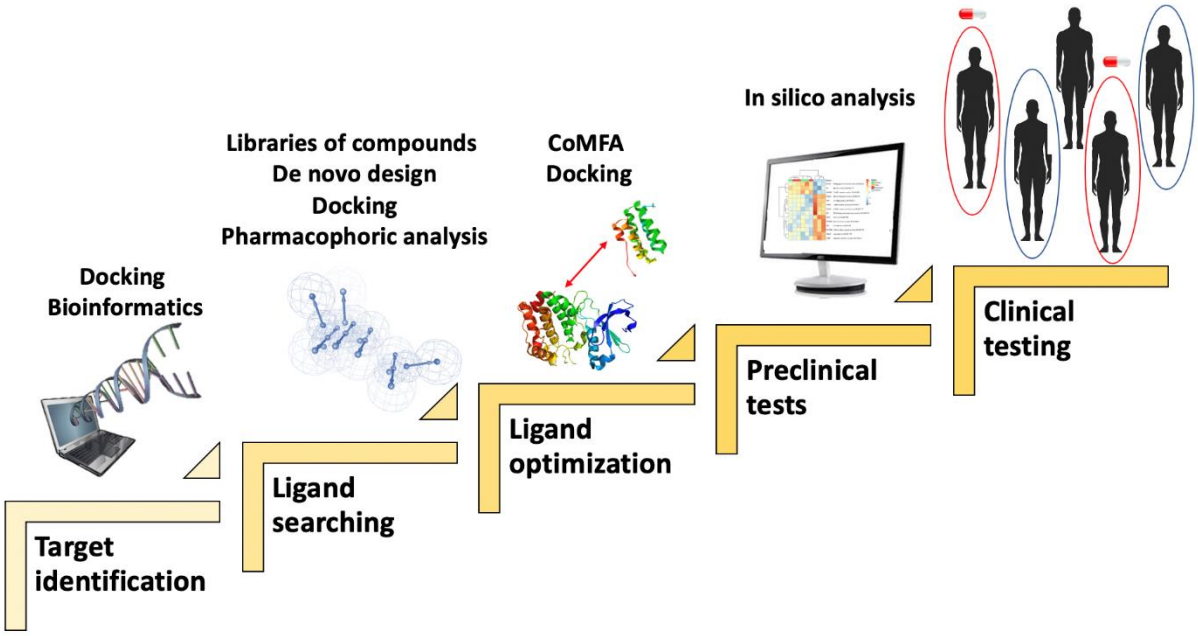

Figure 4. The use of preclinical models and different model organisms in translational CNS drug discovery research. As the global prevalence of affective disorders has increased in the last three decades[2], preclinical models using both rodents and zebrafish, become a valuable tool for screening novel pharmacological and genetic therapeutic targets

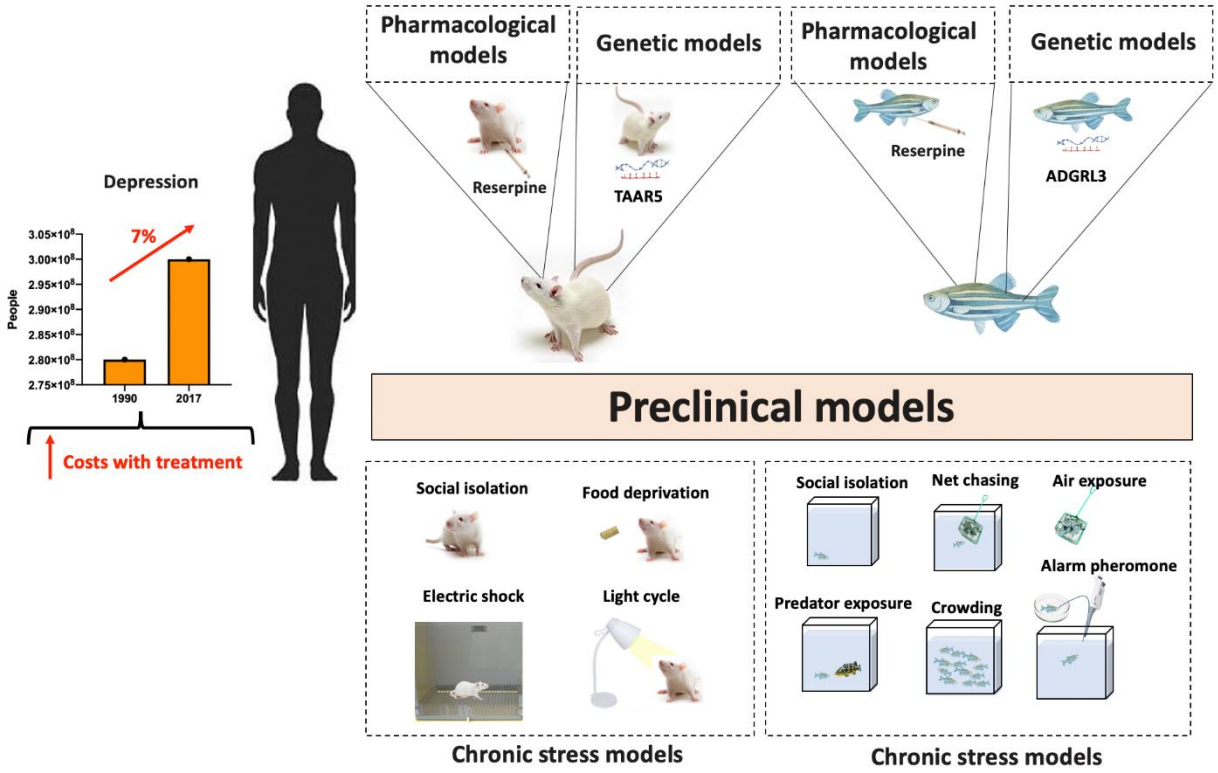

Table 1. Main strategies used in computer modeling of biological activity of substances, depending on the availability of information on the structure of ligands and receptors.

Receptor structure	**Ligand structure**	
	Known	**Unknown**
Known	Docking	De novo design
Unknown	Quantitative Structure-Activity Relationship (QSAR)	Similarity search, screening

Table 2. Selected open questions in the field of affective drug research and development

Questions
General
• How to bring back (and eventually increase) the investments to CNS drugs research both in industry and academia? • What is the core (shared) pathobiological links between comorbid affective disorders that can be targeted by novel drugs? • How to maximize synergistic effects from computer-based, cellular, animal in-vivo models and clinical data? • What is the link between the effects of classical antidepressants, serotonergic psychedelics and ketamine in terms of affective disorder treatment, speed and duration of their effects? • What is the overlap between traditional and novel molecular drug targets involved in affective behavioral spectrum? • How can multi-target drugs be developed, to maximize their therapeutic effects and reduce costs and efforts? • How to optimize drug repurposing to promote innovative CNS drug discovery?
Specific
• What other novel model species may be used to study affective pathogenesis? How to best integrate (and translate) rodent research with studies using novel species? • How to properly assess and model affective phenotypes in other species? Can we assess affective phenotypes in rodents that have no clear behavioral correlates clinically? • How to develop specific pharmacological therapies for individual differences in affective disorders, as part of 'personalized medicine' approach? How does 'personality' (individual traits) contribute to pharmacological efficacy of drugs in affective disorders? • What is the best pharmacological class for the treatment of affective disorders for each sex? • What is the role of epigenetic modulation in pharmacological efficacy for affective disorders? How to develop novel CNS drugs to modulate epigenetic regulation? • Can non-pharmacological approaches (e.g., environmental enrichment) potentiate and synergistically interact with novel drugs developed for affective disorders? • Can early-life experience affect their pharmacotherapeutic efficacy?

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