Cognitive "Omics": Pattern-based Validation of Potential Drug Targets

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Abstract:

Despite the abundance of cognitive enhancer mechanisms identified in basic research, drugs approved for cognitive disorders are scarce and of limited efficacy. Although the so-called "gold standard" animal assays are well suited to study fundamental learning processes, they fail to predict clinical efficacy against complex and robust cognitive defects. Preclinical validation of potential drug targets requires new approaches with higher translational value. Here I propose a rodent cognitive test system that encompasses several learning paradigms, each modelling a certain human cognitive domain. Cognitive deficits are brought about by several impairing methods and a particular mechanism of action is tested on each defective cognitive function. The outcome is a cognitive efficacy pattern which should then be matched to the cognitive deficit patterns of the clinical disorders. The best fit will highlight the clinical indication with the greatest chance for success.

The "translational gap"

When a molecular entity is shown to be involved in a given cognitive function, a quasi-obligatory conclusion at the end of the paper is highlighting its potential in the therapy of one or the other cognitive disorder. It is a recognized attempt by the authors to connect their work to obvious social benefits and thereby emphasize and enhance the importance of the study. Regretfully, these prophetic statements cannot be taken on face value as "playing a role" in a certain cognitive process may well not mean being a "hot spot" of intervention in defective cognitive functions. These "promising" targets need further validation in order to become suitable subjects of feasible industrial drug development projects.

As an example, take two "gold-standard" animal learning assays: scopolamine-induced amnesia in the passive avoidance paradigm and delay-induced forgetting in the novel object recognition task. A PubMed search run for these two methods up to 2015 resulted in 678 hits for the former and 246 hits for the latter. The abstracts were scanned one by one for effective procognitive mechanisms of action identified in the assays. Solely in these two methods 103 different modes of action were detected (Table 1). If one takes into consideration other versions of these two paradigms and other popular cognitive assays (e.g. Morris water-maze, social recognition/discrimination or fear conditioning) a realistic estimation for the number of *potential* cognitive enhancer mechanisms already identified in animal tests totals in the hundreds.

The large number of potential targets confronts the stark fact that only two types of drugs are in clinical application for dementia and memory impairment: the acetyl-choline-esterase (AchE) inhibitors [1] and an NMDA antagonist, memantine [2]. In some European countries a third class, the so called racetams (piracetam, aniracetam, etc.) with unknown mechanism of action are also in use for mild memory impairments. Unfortunately, the efficacy of currently available medications is, at best, moderate [3,4]; the racetams are even not approved in many countries due to lack of clinical evidence. Furthermore, even the "youngest" drug, memantine was launched more than a decade ago (in 2003),and the AchE inhibitors already came to market in the nineties, while the appearance of racetams dates back to the 1970s [5].

The increasingly tense unmet need has driven enormous R&D activity in the field, and yet, the clinical development of new drugs has faced a 100% **attrition rate** (see Glossary) in the past decade. Detailed statistics are published by Ref. [6] for the period 2002-2012 showing a high, 92% attrition rate already in Phase 2 clinical trials, i.e. in the **proof-of-concept studies**.

The overall success rate among AD drug-candidates – owing to the launch of memantine – was, however, slightly different from zero (0.4%) in this period.

This long standing failure has slowly but surely led to a general devaluation of animal models [7,8] and forced many pharma companies to withdraw from preclinical research and development in CNS disorders [9].

The disappointment has not been confined to the industrial R&D. The European flagship research and innovation program, Horizon 2020 consistently avoids funding of animal research in its health domain (the ban is sometimes explicit); while molecular, IT and clinical methodology are most welcome (http://ec.europa.eu/research/participants/data/ref/h2020/wp/2016_2017/main/h2020-wp1617-health_en.pdf/).

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The aim of this article is to propose a preclinical approach, as a possible way out from the current situation, by which the clinical success rate could be increased. First, factors underlying the translational gap will be briefly analysed together with discussing the attempts made so far to remediate the problem. Then a proposal will be put forward for a rodent cognitive test battery with increased predictive power for the clinical efficacy of putative cognitive enhancers. Finally, some related drug development issues will be discussed and emerging feasibility questions will be raised.

Causes of 'target-indication mismatch'

Animal models thus detected a large number of false positive compounds which later failed in the clinic. Ineffectiveness due to insufficient ADME properties (e.g. poor absorption, metabolic vulnerability, low brain penetration, etc.) has gradually faded away as an attrition-factor in the last 20 years [10] as the pharma industry successfully invested a lot of effort into proprerly designing the physico-chemical parameters critical in shaping the ADME character of the candidate molecules [11] and applying biomarker studies checking the presence/action of the compound on the target. For example, PET studies demonstrated that histamine H_3 receptor antagonists and serotonin 5-HT_6 receptor antagonists occupied their respective receptors to a high degree in humans [12,13] at doses where they produced mild or no effect on cognitive performance in patients [14,15].

Thus, the major factor responsible for the missing efficacy must have been the inappropriate/invalid modes of action of the compounds. This invalidity is the direct result of insufficient and inappropriate target validation work, both in human and in animal studies,

preceding the clinical trials. The former would be even more important than the latter, but it is not the subject of this paper.

Concerning preclinical validation, ample literature deals with the possible causes of the missing predictive power of animal studies. One part of the critiques relates to the **external validity** of the assays, i.e. *what type* of animal paradigms are used; the other part relates to their **internal validity**, i.e. *how* these tests are carried out [16,17,18,19,20,21, 72]. Regarding the latter, several shortcomings in methodology corrupting the reliability, reproducibility and robustness of the results have been identified, such as statistically underpowered study design, lack of randomization and blinding, inappropriate (use of) statistics, publication bias, just to name a few. For remediation of the defects in internal validity several guidelines and recommendations have been set forth [17,19,22,23,24].

The thoroughly analysed internal validity defects, however, do not account for the whole extent of the translational gap. In cognitive enhancer research there are several modes of actions, e.g. muscarinic M_1 agonists, histamine H_3 antagonists, serotonin 5-HT_6 antagonists and nicotinic α7 agonists which have been shown to exert cognitive improving effects on many types of impaired cognitive functions, in several different learning paradigms and with more than a dozen compounds of each type [25,26,27,28]. While a part of the animal studies may be put aside because of methodological deficiencies, the recurrent replication of some findings in certain assays with different compounds, in different labs and with different methodical variants did, indeed, lend the image of reproducibility and validity to the results. This widespread procognitive activity raised non groundless expectations about their clinical potential and all the four underwent extensive clinical investigations [29,30,31,32]. Yet, none of them has managed so far to show up a successful Phase III trial on cognitive symptoms.

Clearly, there should also be problems with the external validity of the models used. Various fashionable cognitive assays have emerged in the literature, like the passive avoidance test in the nineties, or the novel object recognition test in the first decade of this millennium (Figure S1). These assays gain popularity because they are simple, rapid, and involve elementary cognitive functions well suited for studying fundamental learning and memory processes. By producing lots of valuable data on cognition itself, these assays then became a kind of "gold standard" in the field. Indeed, when considering the massive preclinical evidence for the procognitive efficacy of the above mentioned targets, the reproduced findings are in large part coming from these types of assays [26,27,28,33].

In industrial R&D, a consequence has been the acceptance of the – otherwise erroneous – concept that checking the efficacy of potential novel cognitive enhancer drugs in the actual gold standard assay is necessary and at the same time sufficient to provide predictions for clinical effectiveness. Experience shows, however, what is a good model for basic research may not be a good one for target validation [73,74]. First, the elementary cognitive functions which are investigated in these assays do not model the complex cognitive domains affected by the human disease [34,72,75]. Second, learning performance of the animals is usually impaired by relatively mild interventions e.g. a single scopolamine dose or long delay, which cannot, again, model the robust and multiple cognitive deficits characterising a clinical syndrome [73,75].

This dichotomy between assays of basic research on one hand and disease models required for target validation on the other [73,74], is nicely exemplified by the fact that even the terminology was, for a long time, substantially different in animal versus clinical cognitive research. While animal terminology largely classified cognitive functions by the *type of the learning task* (operant vs pavlovian or aversive vs appetitive conditioning, spatial or non-spatial learning, cue- or context-induced response, etc.), human terminology mainly used words describing the *memory-type* under study (**declarative** vs **procedural** or **semantic** vs **episodic** memory, dysexecutive syndrome, **theory of mind**, etc.). This literal translational problem (which clearly reflects fundamentally different approaches), also contributed to the discrepant outcomes in animal learning paradigms and in clinical cognitive trials.

Fortunately, in the past decade there has been a clear move on behalf of animal researchers toward approaching the human terminology and classification. The well-known initiatives like **MATRICS** [35] and **CNTRICS** [36] attempted to map and match the clinical symptoms to animal paradigms. Further on, detailed analyses were carried out to select the most appropriate animal models of several human cognitive domains, e.g. **social cognition** [37], **working memory** [38]; executive control [39], attention [40]. The primary criteria for model selection were cognitive and neurobiological construct validity; the former referring to the ability of the paradigm to specifically measure the targeted cognitive process; the latter meaning the involvement of homologous neural circuits in human subjects and animals [36].

However, potential cognitive enhancer molecules should not simply be tested in models of human cognitive functions, but, instead, in models of *defective* human cognitive functions. Therefore, validity of any animal model essentially and critically depends on the construct of cognitive deficiency. In other words: on how impaired performance is brought about.

Single dose pharmacological treatment or increased task difficulty may well be criticized in this respect. Notwithstanding that acute scopolamine can powerfully disrupt cognitive performance in many learning tasks (see the review of Ref. [41]), its effect is often not cognition-specific [75] and can be abolished by a single type of pharmacological action, e.g. by increasing the endogenous acetylcholine level. Acetylcholine-esterase inhibitors, which directly produce this effect, show modest potency in early AD [3], but not in other disorders. If the assumptions hold true that both histamine H_3 and serotonin 5-HT_6 antagonists act, at least in part, via indirectly increasing acetylcholine release as a final common pathway [76,77], then not much better efficacy can be expected from these types of compounds than that shown by the AChE inhibitors; neither in terms of magnitude nor of cognitive domains or patient subgroups. Furthermore, if the broad procognitive activity of the compounds typically manifest against scopolamine-induced impairments, then their effects may result from a simple pharmacological interaction that is independent from the cognitive function being studied. It is unlikely that a single mechanism would equally improve diverse cognitive deficits. Assays relying on increased task difficulty, such as the natural forgetting paradigm in the novel object or social recognition tasks, suffer from the discrepancy that increasing the normal learning/memory performance in healthy animals presumes some mobilizable cognitive reserve, which may not be available in an ill, thereby functionally corrupted brain.

Proposal for a rodent cognitive test battery for target validation

If an animal model is intended to be predictive for the human situation, then it should model as closely as possible the human cognitive task and should conform to the human terminology. Therefore, instead of memory tests whose highest values are simplicity and easy measurability (like passive avoidance or novel object recognition), assays with higher therapeutic relevance are needed. These are usually more complex and often time consuming paradigms. The proposed test battery includes animal assays intended to model the human cognitive domains (Table 2). These domains are selected from the 12 domains specified in the review of Ref. [34] to characterize the cognitive deficit patterns of nine psychiatric disorders (schizophrenia, depression, bipolar disorder, autistic spectrum disorders, attentional deficit-hyperactivity disorder, obsessive-compulsive disorder, panic disorder, posttraumatic stress disorder, generalized anxiety disorder) and two neurodegenerative diseases (Parkinson's disease and Alzheimer's disease). The list can be considered fairly comprehensive and covering the full spectrum of cognitive symptoms; and as such, appropriate and sufficient for

target validation. Verbal memory and language use are obviously dropped out from the list, but the other functions can be studied in animals, too. The animal assays suggested for modelling the human functions (Table 2) were chosen partly on the basis of MATRICS and CNTRICS recommendations (working memory, social cognition, executive function, attention), partly by our own judgement. The test list is primarily of illustrative nature; it should by no means be considered exclusive or complete. Many of its items can be replaced by equivalent alternatives, and some of the domains can be further broken down to subcomponents or more specific assays may be constructed for one or the other domain. For example, for modelling **semantic** and **episodic memory**, two different maze-learning paradigms are suggested in Table 2, based on the theory that these cognitive capabilities evolutionary evolved from **allocentric** and **egocentric navigation**, respectively [42]. However, for episodic memory, there exist several well elaborated and more specific animal assays in the literature [43,44,45,46], which could also be used here. Another example for tailoring the list to the needs (and conviction) of the user: if someone wants to go beyond simple social recognition in the social cognition domain and try to approximate the '**theory of mind**' function, a social cooperation paradigm could be included.

However, switching to animal models better mimicking the human cognitive domains is just the first step toward a predictive model-system. Modelling cognitive *deficits* (i.e. deteriorating cognitive functions) is the main challenge. The cognitive deficiency construct to be established depends on whether we want **disease modifying** or **symptomatic treatment** (see Box 1), as the two impose different requirements on the model [73]. The former approach requires disease models, which – in the ideal case – produce all or most of the cognitive symptoms of the disorder by reproducing the pathological process. The latter approach works with symptom models, which are unrelated to the disease process (e.g. do not require neurodegeneration in the background) and constructed to produce defects in distinct cognitive symptoms. The current proposal focuses on the symptomatic approach but can accommodate the disease modifying one, too.

Defective cognitive performance can be brought about by several means: pharmacological agents, cerebral lesions/activations (implying optogenetic/chemogenetic methods as well), stressors, modulation of gene expression, old age, increasing task difficulty or selecting low performers of the population – all may yield low cognitive outcome amenable for improvement. Nevertheless, in lack of exact knowledge on or deliberately being unrelated to the pathomechanism of the given disease (as in case of symptomatic treatment) no distinct

impairing intervention can be considered as the most "appropriate" or "predictive" (see again the failure of the scopolamine-induced amnesia models in predicting clinical efficacy or the critique on the PCP impairment in Box 1). On the other hand, each type of impaired cognitive state holds utilizable information content thus bears a *certain extent* of relevance to the human cognitive deficit. Therefore, to get a better prediction on the expected human efficacy of a putative enhancer mechanism it should be tested against several impairing methods. By doing so, one can make a virtue of necessity and set up a practically applicable "rule of thumb": the more types of impairing methods against which the studied mechanism is effective the higher the chance it will be effective against the cognitive defects – of otherwise unknown or uncertain origin – in the target disease.

Consequently, multiple types of cognitive impairment is suggested in case of each cognitive function, and a 'cognitive domains x impairing methods' matrix of models as test battery is proposed to be used for clinical prediction (Figure 1, Key Figure).

Although in principle each model could be operated as a separate experiment, – i.e. each testing of a compound could be done in a new cohort of naive animals freshly taught for the task and then impaired in performance, – it is not recommended to follow for several reasons. Comprehensive validation of a target in this way would require an unnecessary large number of animals, take unreasonably long time and bring about adversely high variability in the results. In addition, such testing procedure would have low clinical relevance, too.

To establish a more coherent methodical environment, be suited for the 3R pricinples, and also for mimicking the human clinical circumstances, several cognitive tasks representing different cognitive domains should be taught to the same set of animals, thereby creating a population with "widespread knowledge". This process may take several weeks. These animals are then transformed to a "patient" population by exposing them to a certain impairment method. To increase the human relevance of the induced cognitive deficits, long term interventions should be applied whenever possible, e.g. subchronic pharmacological treatments, stress exposure or lesion/activation. Aging can be considered as a natural way of impairment. Note, that with some impairing methods like constitutive genetic modifications or perinatal treatments the patient population is created "in advance" of teaching and performing the cognitive tasks. Many specific disease models fit into this category. The "patient" population then can subsequently be subjected to one or more improving interventions. Here also, long term treatment is desirable to model the clinical situation. If the applied impairment is reversible, i.e. the memory/learning defects resolve after cessation of

the impairing intervention, further impairment can be sequentially performed in the same cohort of animals for initiating another "drug trial". This is the case, for example, with increasing task difficulty, certain stressors or pharmacological treatments. The outcome of such testing allows not only to judge the efficacy of a certain mechanism of action but the cognitive enhancer pattern may help in selecting the proper target patient population in the clinic (see below).

Patient population selection process

Neurological and psychiatric disorders show diverse patterns of defective cognitive functioning [34], and this pattern-specificity may require compounds with different mode of actions. The traditional way of finding "the right molecule for the right indication" is the one where the target disease is fixed and the appropriate drug is searched for ("marketing-based selection"). Adapting this approach to target validation in the above system would mean that the potential targets are tested in a simplified system containing only those learning/memory paradigms which are relevant for the chosen disease. The smaller set of assays enables higher testing turnover and lower running costs. However, targets potentially effective in other indications may be missed by this approach. Further, and most importantly, finding the valid target, i.e. one which satisfactorily fits the desired activity pattern may take quite a long time.

By contrast, the above described pattern-based validation offers an alternative way of achieving "the right molecule for the right indication" fit. With this approach, the target disease is not fixed in advance, but is rather determined at the end of the validation process. The potential targets are tested in the full system until a mechanism with appealing efficacy and activity pattern is found. Then the disease whose cognitive deficit pattern best matches the cognitive activity pattern of the selected mechanism should be chosen as the target clinical indication ("science-based selection"). Giving a simplistic example: if a certain mechanism of action shows outstanding efficacy in assays measuring attention then it should be tried in ADHD, whereas if it is more active in social cognition paradigms, then autism could be the preferred choice. In this mechanism-based search for indications no promising target is lost and validating a target for an indication may happen within a shorter time. For example, even partial pattern matchings can be utilized if the aim is to relieve certain cognitive symptoms regardless of the disease background. However, establishment of a larger set of models is required which incurs higher running costs and lower testing turnover.

In both cases, a critical methodological factor is how the goodness of pattern-matching is determined/calculated (see Outstanding Questions). Obviously, the better the fit the higher the chance for clinical efficacy, but the exact criteria may be tailored to the needs and expectations of the actual user.

The suggested pattern-based validation has analogous logic to that of the "*omics*" approaches, therefore it may be termed "*cognomics*" (cognitive omics). According to the author's conviction, it will increase the probability of clinical success compared to the predictive power of the so far applied approach which may be best described as "prove efficacy in the gold standard model then run clinical trials in several disorders". However, adopting the cognomics approach will necessitate the changing of the drug discovery paradigm (see Box 2).

Concluding Remarks

Despite their seemingly weak predictive power, animal models can still be utilized in preclinical drug discovery provided they meet external as well as internal validity criteria. The target validating methodology should substantially change by approximating clinical studies regarding patient population, treatment length and outcome measures. For the purpose of predicting clinical efficacy, learning performance of animals should be examined in paradigms really modelling the human cognitive functions. The validity of induced cognitive *deficits* is a critical point either in the disease modifying or the symptomatic treatment approach. A pattern-based validation is suggested to enhance the chance for clinical success. It is worth considering that the clinical target population would be selected on the basis of the merits of the validated targets and not on the basis of *a priori* marketing needs (see Outstanding Questions). Finally, it is essential that the no man's land between basic research and industrial drug discovery be populated by target validating projects (the precompetitive area). The Horizon 2020 bias should be corrected [54], and this type of research should be actively supported in the future.

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Supplemental Information

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Conflict of Interest

The author declares no conflict of interest.

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Table 1. Mechanisms of action found effective in two animal assays: scopolamine-induced amnesia in the passive avoidance paradigm and delay-induced („natural") forgetting in the novel object recognition test. Findings are from a PubMed search, the terms were „passive avoidance AND scopolamine AND [rats OR mice]" and „novel object recognition AND [delay OR retention] AND [rats OR mice]". Bolded are the mechanisms in clinical use. The table shows some peculiarities, such as: i) antagonists as well as agonists of the 5-HT_{1A}, GABA-A, GABA-B, NMDA, opioid receptors were found to be effective; ii) almost all types of selective phosphodiesterase inhibitors showed activity; iii) nearly each serotonin receptor subtype emerged as procognitive target; iv) the enormously high number of herbal cognitive enhancers – a rich source for designing new multitarget drugs.

78.

Targets effective in passive avoidance – scopolamine assay		Targets effective in novel object recognition – natural forgetting assay
„-racetams"	1.	***„-racetams"***
5-HT_{1A} agonist	2.	
5-HT_{1A} antagonist	3.	5-HT_{1A} antagonist
5-HT_{1B} antagonist	4.	
	5.	5-HT_{2A} agonist
5$HT2_C$ antagonist	6.	5-HT_{2C} inverse agonist/antagonist
5-HT_3 antagonist	7.	5-HT_3 antagonist
5-HT_4 agonist	8.	5-HT_4 agonist
5-HT_6 antagonist	9.	5-HT_6 antagonist
	10.	5-HT_7 agonist
A_1 (adenosine receptor) antagonist	11.	
	12.	A_2 (adenosine receptor) antagonist (caffeine)
A_3 (adenosine receptor) agonist	13.	
ACE (angiotensin converting enzyme) inhibitor	14.	
ACh (acetylcholine) releaser	15.	
AChE (acetylcholine-esterase) inhibitor	16.	***AChE (acetylcholine-esterase) inhibitor***
adrenerg α_2 antagonist	17.	adrenerg α_2 antagonist
	18.	adrenerg β agonist
agmatine	19.	
AMPA receptor positive modulators	20.	AMPA receptor positive modulators
antioxidants	21.	
	22.	APP^S (secreted amyloid precursor protein)
AT_1 (angiotensin receptor) antagonist	23.	
AT_2 (angiotensin receptor) agonist	24.	
AT_4 (angiotensin receptor) agonist	25.	
AVP (arginine vasopressin)	26.	
BDNF signalling activation	27.	
BZD (benzodiazepine) inverse agonist/antagonist	28.	
Ca^{2+}-channel inhibitor	29.	
	30.	CB1 (cannabinoid receptor) antagonism
CCK (cholecystokinin) agonist	31.	
complement C3a agonist	32.	
COMT (catechol-O-methyltransferase) inhibitor	33.	
cyclooxygenase inhibitor	34.	
D_1 dopamine agonist	35.	D_1 dopamine agonist
D_2 dopamine agonist	36.	D_2 dopamine agonist
D_3 dopamine antagonist	37.	D_3 dopamine antagonist
DARI (dopamine reuptake inhibitor)	38.	
DHEA(S) (dihydroepiandrosteron-sulphate)	39.	
ergot alkaloids	40.	
	41.	erythropoiesis
estrogen	42.	estrogen
GABA uptake inhibitor	43.	

GABA-A agonist / positive modulators	44.	
GABA-A antagonist	45.	GABA-A antagonist
GABA-B agonist	46.	
GABA-B antagonist	47.	
GABAα5 inverse agonist	48.	GABAα5 inverse agonist
gastrin releasing peptide	49.	
	50.	glutamate carboxypeptidase II inhibitor
glutamate derivatives	51.	
GM1 ganglioside	52.	
	53.	GnRH (gonadotropin releasing hormone)
H_1 (histamine receptor) agonist	54.	
H_3 (histamine receptor) antagonist	55.	H_3 (histamine receptor) antagonist
H_4 (histamine receptor) agonist	56.	
	57.	histone deacetylation in the BLA
HDAC (histone deacetylase) inhibitor	58.	
HMG-CoA inhibitor (atorvastatin)	59.	
IL-1α (interleukin)	60.	
IL-6 (interleukin)	61.	
insulin	62.	
K^+-channel inhibitor	63.	K^+-channel inhibitor
M_1 (muscarinic receptor) agonist	64.	
M_2 (muscarinic receptor) antagonist	65.	
MAO (monoamine-oxidase) inhibitor	66.	
melatonin receptor agonist	67.	
	68.	mGluR2/mGluR3 antagonist
NAA (N-acetyl-aspartate)	69.	
NGF (nerve growth factor)	70.	
	71.	nicotinic α4β2 agonist
nicotinic α7 agonist	72.	nicotinic α7 agonist
NMDA antagonist	73.	***NMDA antagonist***
NMDA glycine site agonist	74.	NMDA glycine site agonist
NMDA polyamine site agonist	75.	
	76.	NO (nitric oxide) donor
	77.	NOS (nitric oxide synthase) inhibitor
	78.	neurokinin 3 agonist
NPY (neuropeptide Y) agonist	79.	
	80.	neuropeptide S
	81.	neuropeptide Trefoil factor 3
opioid receptor agonist (morphine)	82.	
opioid receptor antagonist (naloxone)	83.	
opioid κ receptor agonist	84.	
ORL-1 (orphanine receptor) agonist (low dose)	85.	
ORL-1 (orphanine receptor) antagonist	86.	
	87.	PDE-1 (phosphodiesterase) inhibitor
	88.	PDE-2 (phosphodiesterase) inhibitor
	89.	PDE-3 (phosphodiesterase) inhibitor
PDE-4 (phosphodiesterase) inhibitor	90.	PDE-4 (phosphodiesterase) inhibitor
	91.	PDE-5 (phosphodiesterase) inhibitor
PDE-9 (phosphodiesterase) inhibitor	92.	PDE-9 (phosphodiesterase) inhibitor
	93.	PDE-10 (phosphodiesterase) inhibitor
PEP (prolyl-endopeptidase) inhibitor	94.	
PPARγ agonist	95.	
pregnenolon sulphate	96.	
retinoid Am80 (RAR/RXR agonist)	97.	
sigma-1 agonist	98.	
somatostatin	99.	
SRI (serotonin reuptake inhibitor)	100.	SRI (serotonin reuptake inhibitor)
steroid sulphatase inhibitor	101.	
TRH (thyrotropin releasing hormone) agonist	102.	
	103.	vasopressin 1b antagonist
+ ca. 100 types of herbal extracts or derivatives		

79.

80.

81.

82.

Table 2. Human cognitive domains and their suggested animal models

cognitive domain	animal assay	reference[a]
working memory	delayed non-matching to sample	[38]
semantic memory	Morris water-maze (allocentric navigation)	[47]
episodic memory	multiple T-maze (egocentric navigation)	[47]
visual memory	touchscreen paired associates learning	[48]
attention & information processing	5-choice serial reaction time task prepulse inhibition	[40] [49]
fear extinction	fear conditioning	[50]
social cognition	social recognition / preference	[37]
executive function rule learning decision making response inhibition	 attentional set shifting probabilistic reward learning delayed reinforcement of low rate	 [39] [51] [52]
procedural memory	rotarod learning	[53]

a: the reference papers not only describe the particular assay but also discuss its theoretical background

Box 1

Disease modifying vs symptomatic treatment for cognitive disorders

Disease modifying treatment, which would be the ideal case, relies on our knowledge on the pathomechanism of the disease. The Achilles-heel of any disease modifying approach is the soundness and validity of the underlying hypothesis on the pathomechanism, which can ultimately be checked only in the target patient population.

In Alzheimer disease, such a strong theory has been the amyloid cascade hypothesis [54,56]. Accordingly, transgenic mouse models based on the familial form of the disease and characterized by massive human β-amyloid overproduction formed the key assays in drug testing. However, these animals were much more a model of amyloid intoxication than a model of the disease itself: they lacked tau pathology and the cognitive deficits were discrepant and uncorrelated to the histological changes [57,58,59,60]. The serial failures of the subsequent clinical trials severely punished the overlooking of these caveats of the animal model [61,62] and raised serious doubts about the validity/soundness of the amyloid theory [63,64,65].

In psychiatric disorders the etiological theories are even weaker than in AD as very little is known on the underlying mechanisms of defective cognitive functions. For example, according to the glutamatergic hypothesis of schizophrenia, cortical NMDA glutamate receptor hypofunction plays a central role in the pathomechanism [66]. In harmony with this, subchronic phencyclidine (PCP) treatment induced alterations are widely accepted as a model of glutamatergic dysfunction in schizophrenia [67]. However, a recent review demonstrated that this model is far from being able to recapitulate all the relevant cognitive deficits of the disease [68] pointing out some shortcomings of the theory and/or the model. Even more problematically, subchronic PCP-induced learning/memory impairments were reported to be restored by several atypical antipsychotics [69,70]. These findings are in sharp contrast to the clinical experience where these drugs are not particularly reputed for their memory improving properties. The model thus lacks specificity and may detect false positive compounds.

Symptomatic treatment offers a lower risk – lower benefit alternative. It is based on activating more or less non damaged compensatory cognitive enhancer mechanisms and may feasibly be developed without exact knowledge on the etiology of the disease. The distinct cognitive symptoms (domains) can be modelled and examined separately. On the other hand, a single cognitive enhancer mechanism, be it so potent, cannot compensate for all the complex deficits

of the disorder generated by malfunctions in multiple pathways in the central nervous system. Therefore, the therapeutic effect achievable via the symptomatic approach is predictably less robust as demonstrated e.g. by the acetylcholinesterase inhbitors [3]. However, augmenting the points of symptomatic interventions by combining 2-3 validated targets either via combination therapy or multitarget directed ligands may result in activity on more symptoms and/or higher effect size.

Box 2

Changing the drug discovery paradigm

As the pattern-matching approach implies elevated requirements for a certain mechanism or compound for being deemed "efficacious", the number of real hits will foreseeably be reduced. It may be considered good news on one hand, as the basic translational problem was the high number of false positive hits in the animal literature. On the other hand, because of the scarcity of compounds capable to enter clinical trials and the longer preclinical investigation periods it is also foreseeable that industrial investors and top management may easily become disappointed and decide to refrain from CNS drug development – as it already happened in the near past. Experience shows that the described target validating experimentation does not fit the industrial R&D timeframe and scenery. Therefore, it should be done in the precompetitive area and outside of the conventional industrial settings. Once the target is (deemed to be) valid, drug discovery screening may return to its traditional way, back to the companies' R&D labs, and can be carried out in simple(r) assays with sufficient robustness and capacity. Before entering into developmental phase with the optimized molecule, the selected clinical candidate could be checked again in the target validating paradigms. The study could be considered as a kind of early proof of concept trial and would conform to the 'fail fast' (and cheap…) developmental strategy [71].

However, carrying out all the assays of Table 1 requires a large amount of effort and time and collaboration among labs (see Outstanding Questions). A complete target validation may well be realized within a couple of years. Not a short period, but *a)* it's still much less than the time lost in late clinical phases because of the recurrent trial failures and *b)* it's an investment with high return: a recent analysis [71] pointed out that Phase 2 and 3 success probabilities are the two most important determinants of overall R&D efficiency, and decreasing the corresponding attrition rates by ¼ and 1/3, respectively, may lead to a 1/3 decrease in the

average capitalized cost of a launch. With regard to the high societal needs for novel cognitive therapies, the long history of unsuccessful attempts and the collaborative nature of the job, this kind of target validation activity should obviously be supported by dedicated research funding.

Glossary

allocentric navigation "is characterized by the ability to navigate using distal cues, i.e., cues located outside and at some distance from the organism (e.g., landmarks)." (cited from [47])

attrition rate in clinical development: the ratio between the number of compounds failed in the clinical trials and the number of all tested compounds. Attrition rate can also be calculated for clinical trials instead of compounds in a similar way. While the former demonstrates the net success rate of clinical development, the latter, which results in higher figures, rather reflects the efforts and costs of the development.

CNTRICS initiative: Cognitive Neuroscience Treatment Research to Improve Cognition in Schizophrenia. "… focused on … the identification of cognitive 'constructs' – definable cognitive processes that can be measured at the behavioral level and for which there exist clearly hypothesized and measureable neural-circuit mechanisms. … yielding a scheme of cognitive domains, and within each domain specific constructs considered to be most relevant to the cognitive impairments of schizophrenia. … to develop cognitive neuroscience paradigms for use in humans that could selectively and parametrically measure these constructs at the behavioral level … In a second phase … to further develop homologous assays of the key cognitive constructs within biomarker studies and animal model systems." (cited from [36])

declarative memory: explicit, conscious memory on facts, events and concepts. It can be divided to semantic and episodic memory (see below)

disease modifying treatment: treatment which results in change in the course of the disease process: slowing down, halting or even reversing it. It assumes the pathomechanism of the disease is known to the degree that enables us to directly intervene in the pathological events.

egocentric navigation "is characterized by the ability to find one's way using internal and/or near (proximal) cues. Internal cues include proprioceptive feedback from limb/joint receptors

and stretch receptors in muscles and tendons that provide a sense of speed of motion that, when combined with heading or directional information and signposts about which way to turn, produce a pathway or route to and from different locations." (cited from [47])

episodic memory refers to the memory of our experiences and events happened with us in the past and also to the ability to position ourselves in time and space; e.g. when and where my first date was

executive function: "A purposeful, goal-directed operation such as planning, decision making, problem solving, reasoning, concept formation, self-monitoring or cognitive flexibility (adaptive alternation between different strategies, responses and behaviours)." (cited from [34])

external validity: with regard to animal models of human diseases external validity refers to the "goodness", reliability, precision of inferences which can be drawn from the model onto to the disease. It is usually decomposed to predictive (specificity and sensitivity), face and construct validity (fidelity of the model).

fear extinction: pairing non-aversive contextual or discrete cues (conditioned stimulus) to a fear-provoking aversive (unconditioned) stimulus results in long-lasting fear responses to the formerly neutral stimulus, termed conditioned fear. The acquired fear responses can undergo extinction when the subject recognizes the fear-provoking stimulus is no more coupled to the conditioned stimulus. Fear extinction is an active learning process which is damaged in post-traumatic stress disorder.

internal validity: it reflects those features of a model which enable us to draw solid conclusions on the causal relationships between phenomena studied in the model. Such features are e.g. reliability, reproducibility, robustness, stability, accuracy.

MATRICS initiative: Measurement of Treatment Effects on Cognition in Schizophrenia, initiative of the NIMH with the goal to identify the core cognitive deficits of schizophrenia and to develop a standardized test battery for their measurement (MATRICS Consensus Cognitive Battery). The work continued in the CNTRICS initiative.

procedural memory: implicit, unconscious memory of motor skills, e.g. how to ride a bike

proof of concept trial: a clinical investigation aiming at proving/confirming the scientific hypothesis set on the relationship between drug effect on a given target and disease outcome. Phase 2 trials where the efficacy of a drug is first tested on a smaller number of patients

traditionally belong to this category. Recently, certain biomarker studies carried out on non-patient subjects may also be considered as proof of concept trials.

response inhibition: the ability of the subject to withhold a formerly reinforced or otherwise advantageous "prepotent" response in order to achieve a more favourable goal. Impaired response inhibition is a key component of impulsivity.

semantic memory refers to the memory of facts, objects, ideas; our lexical knowledge; e.g. how big an apple can be.

social cognition "refers to processes used to monitor and interpret social signals from others, to decipher their state of mind, emotional status and intentions, and select appropriate social behaviour." (cited from [37])

symptomatic treatment: treatment which only modifies the symptoms of a disease (diminishing or abolishing them) without affecting the pathological sequel. Symptomatic treatment is usually based on activating non damaged compensatory cognitive enhancer mechanisms.

theory of mind refers to the ability to make inferences on someone else's mental state (thoughts, emotions or intentions) and prediction of his/her future behaviour based on social signals and the context of the situation.

working memory: in animals, the term refers to short-term storage of information which can subsequently be transferred to long-term memory stores or dropped (forgotten) if it is no more needed. In humans the term covers a more complex process, including also certain computing activities ("working") with the stored items.

Figure legends

Figure 1. A rodent test battery for characterizing potential cognitive enhancer compounds.

The leftmost column lists assays modelling certain cognitive functions (see Table 1). Other columns represents various impairment methods, thus each cell in the table corresponds to a particular cognitive deficit model. Shading and symbols in the table illustrate hypothetical activity patterns as follows: unshaded and shaded cells indicate impaired and unimpaired cognitive performance, respectively, obtained after applying a concrete type of the impairing method in the column header. Each column has a particular impairment pattern representing a certain "disease state". Symbols show the cognitive improving effects of two compounds, Compound Red and Compound Pink, with distinct mechanisms of action; the latter tested in only two "disease states". 0: no effect, x or +: mild effect; xx or ++: moderate effect; xxx or +++: strong effect. The "results" demonstrate that 1. a compound may have different actions on the different cognitive defects (symptoms) in a given "disease state"; 2. it may have different activity profile in different "disease states"; 3. a particular "disease state" (e.g. old age or stress-induced) may be differently affected by different types of compounds; 4. the resulting outcome of testing a particular cognitive enhancer mechanism is a cognitive enhancer pattern

Abbreviations: PAL: paired associates learning; 5-CSRTT: 5-choice serial reaction time task; PPI: prepulse inhibition; DRL: delayed reinforcement of low rate. For further information on the assays see the references of Table 1.

Outstanding questions:

- What degree of pattern similarity would suffice for a go decision?
- How manyand how large positive effects can be considered sufficient?
- Which has more bearing: effects on many cognitive domains or against many impairments?
- Can weaker efficacy be compensated by a more widespread activity profile and vice versa?
- What if the results with different “probes” of the same mechanism (e.g. two different compounds with similar mode of action) do not converge?
- What if the results in different models of the same cogntive domain (e.g. two different episodic memory models) do not converge?
- Where is the optimal place of target validation activity in the drug discovery/development process?
- What is the time frame of validating a single molecular target?
- How many labs should be involved in a target validating collaboration?
- How should methodical coherence be assured among the collaborating labs?
- Who should fund the work?

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The leftmost column lists assays modelling certain cognitive functions (see Table 1). Other columns represents various impairment methods, thus each cell in the table corresponds to a particular cognitive deficit model. Shading and symbols in the table illustrate hypothetical activity patterns as follows: unshaded and shaded cells indicate impaired and unimpaired cognitive performance, respectively, obtained after applying a concrete type of the impairing method in the column header. Each column has a particular impairment pattern representing a certain „disease state". Symbols show the cognitive improving effects of two compounds, Compound Red and Compound Pink, with distinct mechanisms of action; the latter tested in only two „disease states". 0: no effect, x or +: mild effect; xx or ++: moderate effect; xxx or +++: strong effect. The „results" demonstrate that 1. a compound may have different actions on the different cognitive defects (symptoms) in a given „disease state"; 2. it may have different activity profile in different „disease states"; 3. a particular „disease state" (e.g. old age or stress-induced) may be differently affected by different types of compounds; 4. the resulting outcome of testing a particular cognitive enhancer mechanism is a cognitive enhancer pattern.

Abbreviations: PAL: paired associates learning; 5-CSRTT: 5-choice serial reaction time task; PPI: prepulse inhibition; DRL: delayed reinforcement of low rate. For further information on the assays see the references of Table 1.

animal assay	cognitive deficit induced by						
	drug treatment	lesion/ activation	stress	modulation of gene expression	old age	task difficulty	selecgmenting lowthe performersopulation
delayed non-matching to sample	x	0	0|0	0	0|+	x	0
Morris water-maze	xxx				xx|++	xxx	
multiple T-maze					xxx|0	xx	
touchscreen PAL		xx	xx|++			0	x
5-CSRTT	0	0	0|++		0|+	x	x
PPI			x|+		0|0		0
fear conditioning			0|0				0
social recognition						x	
attentional set shifting	0		x|+	0	x|++	0	x
probabilistic reward learning	0		0|0	0	0|0	0	x
DRL			0|0			0	x

rotarod learning	xx				0 | 0	0	0