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Perspectives on Zebrafish Models of Hallucinogenic Drugs and Related Psychotropic Compounds

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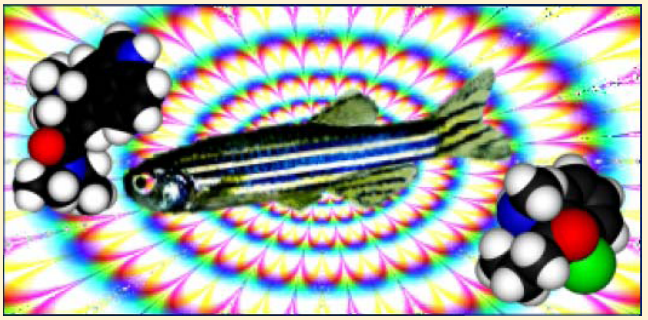
ABSTRACT: Among different classes of psychotropic drugs, hallucinogenic agents exert one of the most prominent effects on human and animal behaviors, markedly altering sensory, motor, affective, and cognitive responses. The growing clinical and preclinical interest in psychedelic, dissociative, and deliriant hallucinogens necessitates novel translational, sensitive, and high-throughput in vivo models and screens. Primate and rodent models have been traditionally used to study cellular mechanisms and neural circuits of hallucinogenic drugs' action. The utility of zebrafish (*Danio rerio*) in neuroscience research is rapidly growing due to their high physiological and genetic homology to humans, ease of genetic manipulation, robust behaviors, and cost effectiveness. Possessing a fully characterized genome, both *adult* and *larval* zebrafish are currently widely used for in vivo screening of various psychotropic compounds, including hallucinogens and related drugs. Recognizing the growing importance of hallucinogens in biological psychiatry, here we discuss hallucinogenic-induced phenotypes in zebrafish and evaluate their potential as efficient preclinical models of drug-induced states in humans.



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Understanding normal and pathological brain functions is an important challenge in biomedical research that requires extensive clinical and preclinical investigation.[1,2] Animal (experimental) models have long been used in translational neuroscience to study brain mechanisms and their effects on behavior.[3,4] Environmental, genetic, and pharmacological manipulations are widely used to probe brain mechanisms and circuits in various animal models.[5,6] Among different classes of psychotropic drugs, hallucinogenic agents (Table 1) occupy a unique niche, underlying the growing interest of clinical and basic scientists in these compounds.[7,8] For example, hallucinogens exert profound effects on human and animal behaviors, sensory perception and processing.[9,10] Thus, increased understanding of the effects of drugs that induce one of the most potent CNS effects, may provide critical insights into normal and pathological brain functioning.[7,11] Furthermore, hallucinogens have very "rich" pharmacology and act via multiple receptors, which remain poorly understood in terms of both their mechanisms and the resultant behavioral responses.[12−14]

In clinical and experimental models, hallucinogens evoke responses that strikingly resemble (and/or are highly relevant to) certain human brain disorders, such as substance abuse,[15,16] psychoses,[17,18] affective disorders,[19,20] and cognitive deficits.[21,22] Thus, the use of hallucinogenic drugs can lead to new sensitive experimental models of brain disorders.[23] Moreover, mounting evidence indicates that hallucinogens may have some clinical value in treating selected brain disorders,[24] including obsessive compulsive disorder,[25,26] post-traumatic stress disorder,[27,28] and depression.[29−31] Therefore, this line of research can lead to widening and innovating the spectrum of potential therapeutic approaches to some serious debilitating pathological conditions (see refs 10, 24, and 32 for discussion).

Evoking strong subjective changes in perception, emotion, and behavior,[9,10] hallucinogens include several classes of psychotropic compounds, such as psychedelic, dissociative, and deliriant agents.[33,34] Typical psychedelic (mind-altering) agents include serotonergic drugs, such as lysergic acid diethylamide (LSD), mescaline, psilocybin, and its biologically active form psilocin. These drugs modulate various serotonin (5-HT) receptors and evoke distortion, depersonalization, somatic symptoms, and sensory hallucinations (Table 1). Albeit not a typical hallucinogen per se, 3,4-methylenedioxy-*N*-methylamphetamine (MDMA) also acts in a similar manner,

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Table 1. Summary of Psychopharmacological Profiles of Major Hallucinogenic Substances in Human and Animal Models

drugs	profile(s)	clinical effects	effects in rodent models	ref
psychedelic hallucinogens				
LSD	nonselective 5HT agonist	perceptual distortions, depersonalization, visual/auditory hallucinations, slowing of time	altered agonistic behaviors, increased exploration	182
mescaline	5H2A/2C agonist	perceptual distortions, depersonalization, agitation, slowing of time	altered agonistic behaviors, hyperlocomotion, anxiety	183
MDMA[a]	monoamine transporter blocker	euphoria, sense of intimacy, antianxiety, empathogenic action	hyperlocomotion, hypersocial behavior	184
ibogaine[b]	5-HT and opioidergic agonist; NMDA antagonist (also possesses some anticholinergic activity)	introspection, dream-like state, oneirophrenic action	decreased drug-induced hypelocomotion	185
dissociative hallucinogens				
PCP	NMDA antagonist	vivid dreams, out-of-body feelings, aggression, anxiety	psychoses-like behavior, hyper-locomotion, motor incoordination, stereotypies, circling, altered pain sensitivity	186
ketamine	NMDA antagonist	dissociation, depersonalization and derealization	antidepressant effect, circling, reduced anxiety, altered pain sensitivity	187
MK-801	NMDA antagonist	long-lasting hallucinations, amnesia	psychoses-like behavior, circling, anxiety behavior	188
salvinorin A[c]	κ-opioid agonist	depersonalization, distortion of reality, hyperactivation, sensation of motion and revisitation of memories	hypolocomotion, altered pain sensitivity	38
deliriants				
atropine	antagonist of M-cholinoreceptors	confusion/disorientation, hallucinations and delusions	hypolocomotion, decreased exploratory action	189
scopolamine	antagonist of M-cholinoreceptors	confusion/disorientation, increased paranoia and agitation	hypolocomotion, memory loss	190

[a]Psychostimulant and mild psychedelic drug. [b]Mixed profile (psychedelic and dissociative), antagonist of NMDA receptor, and 5-HT/opioidergic agonist. [c]Mixed profile (dissociative and psychedelic), opioidergic agonist.

evoking mild psychedelic effects[35,36] associated with the inhibition of monoamine transporters.

The dissociative hallucinogens, such as ketamine, phencyclidine (PCP), and MK-801 (dizocilpine), act as noncompetitive antagonists of the *N*-methyl-D-aspartic acid (NMDA) receptors, clinically evoking depersonalization, derealization, and dissociation with self and reality (Table 1). Opioidergic agent salvinorin A (acting as a kappa opioid receptor agonist) also possesses strong dissociative (and some psychedelic) hallucinogenic properties in humans,[37,38] and affects behavior in animals.[39−41] Some hallucinogenic agents, such as psychedelic drug ibogaine, have mixed pharmacological profiles, combining antagonism of NMDA receptors with serotonergic and opioidergic agonism (Table 1). Finally, the clinical effect of deliriant hallucinogenic drugs, acting as antimuscarinic cholinergic agents, includes delirium (confusion/agitation and disorganization of behavior), which differs markedly from psychedelic and dissociative states produced by the two other classes of hallucinogens (Table 1). Overall, the resurgence of clinical and preclinical interest in hallucinogenic drug research is encouraging,[24] and may help us understand normal and pathological brain mechanisms modulated by these drugs.

DEVELOPING COMPLEMENTARY NONMAMMALIAN MODELS FOR HALLUCINOGENIC DRUG ACTION

Various primate and rodent models have been used extensively to study mechanisms and neural circuits that underlie neurobehavioral effects of hallucinogenic drugs;[7,42,43] also see ref 14 for review. Typical behavioral responses evoked by hallucinogens in rodent models include serotonin-like behavioral syndrome ("serotonin behavior"), head twitching, behavioral stereotypies, altered startle or prepulse inhibition, and drug discrimination.[14]

Despite their wide use in neuroscience research, mammalian models of hallucinogenic drug action also have their own conceptual and practical limitations.[14] Recently, both *translational* "cross-species" modeling and increasing the spectrum of model organisms have been recognized as key strategies for biological psychiatry research.[44] Reflecting the importance of evolutionarily conserved traits in translational neuroscience,[45] optimal animal models of brain disorders must be *evolutionarily relevant*, that is, targeting common behavioral and physiological phenotypes, neural pathways and circuits in a similar manner across various species.[46] In our view, this approach enables a good focus on ancient, conserved (and, therefore, fundamental and translationally relevant) aspects of brain pathology,[46] including hallucinogenic drug-related phenomena. Collectively, this suggests that developing novel nonmammalian models of hallucinogenic drug action (in addition to the existing mammalian paradigms) is a necessary goal for making further progress in this field.

In line with this, the growing practical need to develop efficient high-throughput in vivo screens for novel psychotropic compounds requires alternative, sensitive, and time/cost-efficient models of psychotropic drug action.[46] Recent evidence indicates that various novel model species, including invertebrates, are emerging as useful tools to target various aspects of hallucinogen-induced CNS phenomena.[47−50] For example, *Drosophila melanogaster* has been successfully used to study visual processing, locomotor activity, and the modulation of gene expression within the brain in response to LSD exposure, suggesting that the fruit fly can serve as a genetically tractable model system to define molecular events leading from hallucinogenic receptor activation to behavior.[48] Among several relatively new model species, zebrafish (*Danio rerio*) offer a unique combination of features, rapidly emerging as high-

Table 2. Summary of Neurobehavioral Alterations Evoked in Zebrafish by Selected Hallucinogenic Agents[a]

		hallucinogenic drugs										
		serotonergic				mixed	glutamatergic			opioid	cholinergic	
phenotypes and domains	test	LSD	mescaline	psilocybin	MDMA	ibogaine[b]	PCP	ketamine	MK-801	salvinorin A	atropine	scopolamine
motor activity												
distance traveled	NTT	0	0	0	0	↑	0	0	↑	↑0[c]		0
velocity	NTT	0	0	0	0	0	0	0	↑	↑		0
anxiety-related behavior												
latency to top	NTT	↓	↓	0	↓	↓	↓	↓				
transitions/time in top	NTT	↑	↑	0	↑	0	0	↑↓	0↑	0↑[c]		0
erratic movements	NTT	0	0	0	↓	↑	↑	0	0			
freezing duration/ bouts	NTT	↑	0	0	↓	↑0	0	0	0			
latency to white	LDB	0		0		↓		0				
transitions/time in white	LDB	0		0		↑		↑0				
social behavior												
shoaling behavior	SBT	↓	↑	↑0[c]	↓	↓	0↓[c]	↓	↓	0*		
social preference	SPT	0		0		0		↑	↓			
cognitive function												
habituation	NTT	↑			↑	↑[d]			↑		0	↓
homebase formation	NTT					↑						
learning	SCT											↓
memory	YMT								↓			↓
neurological phenotypes												
circling behavior	NTT	0[c]	0[c]	0[c]	0[c]	0	↑	↑	↑			
organization of behavior[e]	NTT	↓	↓			↓	↓					
reward												
time in drug-paired sector	CPP									↑		
physiological biomarkers												
whole-brain c-fos expression		↑			↑	0		↑		0[c]		
whole-body cortisol		↑	0	↑		0	↑	↓		0[c]		
ref		66, 126	97, 101	[c]	100, 126	70	101	69, 144	191−194	41, 97	83	72, 73, 154, 191

[a]0, no effects; ↑, activated/increased; ↓, reduced/inhibited/impaired; empty cell, not assessed. Tests: NTT, novel tank test; LDB, light-dark box test; SBT, shoaling behavior test; SCT, shuttle chamber test; SPT, social preference test; CPP, conditioned place preference test; YMT, Y-maze test. [b]Pharmacological profile of ibogaine is mixed, and combines serotonergic activity with glutamatergic, opioidergic and cholinergic effects. [c]Own unpublished observations (Kalueff et al., 2011−2012, also see Kyzar et al., 2012a). [d]Inverted habituation profile for ibogaine. [e]Behavioral sequencing assessed in the NTT by the ethograms method.

throughput and sensitive screens to complement existing rodent models of brain disorders.[46]

Importantly, unlike invertebrate models, zebrafish show high physiological and genetic homology to humans, are easy to manipulate genetically, and display robust behavioral phenotypes.[51−58] Possessing a fully characterized genome,[59,60] with functional domains of many key proteins nearly 100% identical to their human homologues,[58,61,62] zebrafish may also be used for in vivo screening of various psychotropic compounds, including hallucinogens.[46,63] Zebrafish behavior has been recently thoroughly described,[64] making neurophenotypic screens based on this model particularly attractive. In addition, zebrafish testing is very cost-efficient, as it may reduce a research budget by 500−1000-fold, compared to rodent testing with similar study designs.[46] Zebrafish are also characterized by well-developed monoaminergic, glutamatergic, opioid, and cholinergic neurotransmitter systems,[54,55,65] all relevant to psychotropic action of hallucinogenic drugs (Table 1). Although fish models will not be able to fully recapitulate all aspects of a complex brain disorder, their attributes make zebrafish aquatic experimental paradigms a valuable tool in psychopharmacological research.[46] Collectively, this renders zebrafish models particularly suitable for drug discovery and in vivo screening of hallucinogens and related compounds (see ref 64 for a comprehensive catalog of hallucinogenic drug-evoked behaviors in zebrafish).

Emphasizing the role of specific receptor systems in the drug-induced phenotypes, recent studies have examined the effects of various hallucinogenic compounds, including LSD, mescaline, MDMA, PCP, MK-801, ketamine, ibogaine, salvinorin A, atropine, and scopolamine in *adult* zebrafish[66−73] (Tables 2 and 3). The recognized strength of adult zebrafish models is their high relevance of adult fish physiology to human brain disorders, well-developed physiological systems, sensitivity to environmental challenges, and a rich spectrum of quantifiable neurobehavioral phenotypes.[46,64,66,74−78]

Table 3. Summary of Studies Investigating Psychopharmacological/Behavioral Profiles of Selected Hallucinogenic Substances in Adult and Larval Zebrafish Models[a]

	larval zebrafish		adult zebrafish	
drugs	effects	ref	effects	ref
LSD			+	66, 126
mescaline			+	97, 101
psilocybin			+0	[b]
MDMA			+	100, 126
ibogaine			+	70
PCP			+	101
ketamine	+	158	+	69, 144
MK-801			+	191−194[b]
salvinorin A			+	41, 97[b]
atropine	+	84, 153		
scopolamine			+	72, 73, 154, 191, 195

[a]+, reported effects; 0, no effects. Note the prevalence of adult fish models and studies, suggesting that larval paradigms may need further attention from zebrafish neuropharmacologists working with hallucinogenic substances. [b]Own unpublished observations (Kalueff et al., 2011−2012, also see Kyzar et al., 2012a).

In addition to adult fish, *larval* zebrafish are also widely used to study brain functions and disorders, becoming indispensable models for genetic research and drug discovery.[79−82] The established strength of *larval* fish models is in their high-throughput nature, ease of genetic manipulations, and well-defined behavioral and neurological phenotypes.[46,64,74] Several studies using larvae (e.g., refs 83 and 84) have already tested hallucinogens, such as a deliriant agent atropine and a dissociative drug ketamine, in zebrafish behavioral models (Tables 2 and 3). Recognizing the growing importance of hallucinogenic drugs as key modulators and potential drug targets in biological psychiatry,[7,24,85] as well as robust behavioral phenotypes of zebrafish highly sensitive to various neuropharmacological manipulations,[46,78,86−88] here we discuss hallucinogenic-induced behaviors in zebrafish, and evaluate their potential as preclinical models of such effects in humans.

MODELING HALLUCINOGENIC DRUG ACTION IN ZEBRAFISH

As already mentioned, hallucinogenic drugs potently affect human perception, emotionality, and cognitive functions.[9,10] Table 2 summarizes current data on responses to major hallucinogenic drugs in zebrafish, revealing both common and unique drug-induced phenotypes in various "key" neurobehavioral domains: locomotor, affective, cognitive, social, and neurological phenotypes. Behavioral experiments to assess these domains in zebrafish typically involve manual observations with computer-aided video-tracking in the novel open arenas (e.g., the novel tank test, NTT or the light-dark box test, LDB), exposure to various cognitive tasks (e.g., the Y-maze test, YMT), as well as assessing individual or group-tested fish (e.g., in the shoaling behavior test, SBT); see refs 89−95 for review of zebrafish behavioral models and testing approaches. Despite the growing utility of zebrafish in neuroscience research, developing aquatic models of hallucinogenic drugs is a challenging task, and may benefit from a detailed analysis of several major neurobehavioral domains.

Motor Activity. In-depth evaluation of locomotor effects of various psychotropic drugs in animal models is a key question in drug screening studies. Analyses of zebrafish locomotor responses shows that some serotonergic (e.g., LSD, mescaline and MDMA) and glutamatergic (e.g., PCP and ketamine) drugs do not significantly alter the "main" zebrafish motor activity indices, such as distance traveled and swimming velocity (Table 2). While glutamatergic agents PCP and ketamine (similarly to the serotonergic agents) did not affect zebrafish locomotion, treatment with MK-801 increased both velocity and distance traveled, showing the hyperactivity that may be attributed to a dose-dependent response, similar to that observed in mice following MK-801 administration.[96] Ibogaine-treated zebrafish exhibited an increase in velocity but unaltered distance traveled, illustrating a mild increase in motor activity. Salvinorin A also elevated motor activity by increasing distance traveled, while its effects on velocity were not reported (also see the lack of effects of various acute doses in Table 2, as assessed in ref 97 and our own unpublished studies). Likewise, a deliriant scopolamine had no effects on distance traveled and velocity in zebrafish (Table 2). At the same time, larval zebrafish locomotor data are mostly lacking for main classes of hallucinogenic drugs, representing an essential knowledge gap to be filled by future studies.

Collectively, our analysis shows that serotonergic, glutamatergic, opioidergic, and anticholinergic drugs either do not affect, or only mildly alter, zebrafish motor activity (Table 2). This suggests that despite being under the influence of hallucinogenic agents (as assessed by other overt behavioral effects of these drugs), motor activity remains mainly intact in zebrafish models following exposure to different classes of hallucinogenic drugs. This observation may be particularly interesting because it is in line with the well-known Hollister's criteria of hallucinogenic drugs as mind-altering agents without major effects on locomotion.[98] Thus, zebrafish appear to show a similar pattern of responsivity to locomotor action of hallucinogens, suggesting the utility of these fish as a relevant *translational* model of drug action.

Anxiety-Related Behavior. Affective action of various hallucinogenic drugs has long been reported in clinical and preclinical literature.[14,23,36,99] Robust anxiety-related behaviors have also been well-established in zebrafish, complementing other important phenotypes, such as social behavior, cognitive function, and reward-related responses[89−95] (also see ref 64 for a comprehensive catalog of zebrafish anxiety-related behaviors). Analysis of the effects of hallucinogenic drugs on zebrafish anxiety-like behavior was based on several key phenotypes assessed in two common and highly sensitive aquatic models of anxiety, the NTT and LDB tests (Table 2). Overall, zebrafish treated with behaviorally active doses of LSD display predominantly anxiolytic-like responses, including increased time spent in top half of the tank, more transitions to the top, shorter latency to the top, as well as fewer freezing bouts and lower freezing duration. The increased exploration of the top portion of the tank observed and the greatly reduced freezing behaviors suggest LSD-induced anxiolytic-like state. Similarly, mescaline also evoked anxiolytic-like behavior in zebrafish (although not as pronounced as LSD), causing increased transitions to the top, increased time spent in the top, and decreased latency to the top (Table 2). While no changes were observed in erratic movements (similar to the effects of LSD), treatment with mescaline yielded no change in freezing bouts and freezing duration, collectively suggesting that mescaline at doses tested does possesses anxiolytic properties, but not at the same magnitude as does LSD.

Like LSD and mescaline, MDMA also reduced anxiety-like behaviors in zebrafish, increasing time in top and reduced latency to top (Table 2; ref 100). However, transitions to the top were also reduced in a dose-dependent manner, likely due to a global increase in top dwelling, as fish spent more time in top and displayed fewer visits to the bottom part of the tank. Interestingly, while LSD and mescaline produced no change in erratic movements (Table 2), MDMA-treated zebrafish exhibited fewer erratic movements, another behavior consistent with lowered anxiety.[100] As with mescaline, administration of MDMA produced no overt changes in freezing frequency or duration. Overall, albeit showing varying degrees of behavioral effects, the serotonergic hallucinogenic agents tested (Table 2) appear to reduce anxiety in zebrafish without inducing anxiogenic-like or overt psychostimulant effects,[66,100,101] the observation that appears to be consistent with the known profile of these compounds in humans and rodents (Table 1).

Among the glutamatergic hallucinogenic agents assessed in zebrafish, ketamine produced strong anxiolytic-like action, including a marked increase in time spent in the top part of the tank, reduced latency to the top as well as unaltered erratic movement, freezing bouts, and duration (Table 2). Ketamine treatment, however, did reduce transitions to the top (which, together with increased time spent in the top, may be attributed to the tranquilizing effect of the drug). Treatment with PCP did not alter time in top, transitions to the top, freezing bouts and freezing duration, but evoked anxiolytic-like decreased latency to the top (also increasing erratic movements). The effects of MK-801, PCP, and ketamine on anxiety-like behavior seem to be in line with clinical and rodent literature that generally shows anxiolytic-like effects evoked by NMDA antagonism;[102−115] also see our recent data on anxiolytic-like profile in zebrafish evoked by another NMDA antagonist, kynurenic acid.[116] Interestingly, while ibogaine-treated zebrafish demonstrated no significant changes in time in the top, transitions to the top, and freezing duration, the drug elicited an anxiolytic-like phenotype in zebrafish by decreasing latency to the top (also note increased erratic movement and freezing bouts[70]). Since ibogaine possesses "mixed" serotonergic and glutamatergic properties, this observation is generally consistent with behavioral effects in zebrafish exposed to other serotonergic and glutamatergic drugs discussed above (Table 2).

The effect of salvinorin A on anxiety-like behavior merits further in-depth investigation using this aquatic model and a wide range of acute and chronic doses. Interestingly, despite prominent acute effects on humans,[37] our pilot studies with a wide dose range of this compound failed to detect consistent anxiety-related effects (ref 97; Kalueff et al., 2009−2012 unpublished observations), suggesting that in vivo screening using a wider spectrum of tests and paradigms may be needed to address complex psychopharmacology of this hallucinogenic agent. To the best of our knowledge, there was only one published study on the effects of deliriants (scopolamine) in adult zebrafish, showing no effects on time in the top sector (Table 2). Likewise, larval zebrafish anxiety-related data are currently lacking all four main classes of hallucinogenic drugs, representing other knowledge gaps meriting future studies.

Overall, our analyses of zebrafish phenotypes (Table 2) reveal strong effects of various hallucinogenic drugs on zebrafish affective-related parameters. These observations may be particularly in line with the well-accepted Hollister's criteria of hallucinogenic drugs as agents strongly affecting mind/thought, perception, and mood/affect.[98] From this point of view, zebrafish appear to show a significant *translational* validity as a model of hallucinogenic drugs' action on affective behavior.

Social Behavior. In both humans and animals, social behaviors are strongly affected by various hallucinogenic drugs.[117−121] For example, social withdrawal is commonly seen in response to hallucinogenic agents in both primate[43] and rodent tests.[122] Since zebrafish are highly social animals, they may be a good model organism to study the effects of various experimental manipulations in the domain of social behavior.[123−126] Thus, an in-depth evaluation of social behaviors, empowered by the newest developments in automated neurophenotyping technology (e.g., monitoring fish shoals),[126] becomes an important direction of research using zebrafish models and tests to study hallucinogenic drugs.

Analysis of zebrafish social behaviors summarized in Table 2 shows that serotonergic agent LSD (while not affecting social preference) markedly decreases shoaling, which may reflect reduced social motivation and anxiety, or altered perception of the environment. For example, although decreased shoaling may parallel the drug's anxiolytic effects described previously, changes in visual perception (e.g., of neighboring zebrafish) may also contribute to reduced shoaling behavior observed, requiring further investigation to examine sensory function in LSD-treated fish. Interestingly, zebrafish observed after acute treatment with mescaline showed increased shoaling, which may be interpreted as increased anxiety. However, given the observed anxiolytic-like effects of this drug in zebrafish in NTT, the increased shoaling suggests that mescaline may, in fact, stimulate social behavior. The effect of mescaline on social preference (e.g., in the three-chamber social preference test, SPT) has not yet been investigated, and may shed light on whether mescaline can promote sociability in zebrafish. An alternative explanation, albeit based on indirect evidence and qualitative observation of zebrafish behavior, may involve "energizing" effects of mescaline (Kalueff et al., 2011−2013, own unpublished observation), which can contribute to higher probability of individual fish running into each other when assessed together in a relatively small and narrow SBT tank. Analyses of the effect of MDMA on shoaling behavior[126] reveal a marked reduction of shoal cohesion, resembling the behavioral effects previously reported for LSD.[66] While the latter effect strikingly differs from well-known pro-social "empathogenic" action of MDMA in humans,[127−129] it resembles the lack of consistent effects of MDMA on social behavior in rodents,[130−135] raising the possibility that the empathogenic action of MDMA may represent a species-specific *human* phenotype, which is difficult to mimic in animal models. For example, given the role of oxytocin on facilitating the pro-social effects of MDMA in several species, including humans,[136−138] differing degrees of the hormone's functional roles among fish[139,140] vs mammals[141−143] may account for some discrepancies.

As shown in Table 2, treatment with PCP evokes no changes in zebrafish shoaling,[101] while its effect on social preference has not yet been tested. Ketamine showed conflicting effects on social behavior, impairing shoaling but increasing social preference.[144] While reduced shoaling may indicate lower sociability, increased social preference suggests that ketamine may affect social behavior or, alternatively, increase motor activity in the preference test. However, MK-801 impairs both social preference and shoaling, implying a reduction in sociability of zebrafish (Table 2), which parallels rodent data

on social withdrawal induced by acute administration of this drug.[145,146]

Zebrafish exposed to behaviorally active doses of ibogaine show effects similar to those produced by LSD, including unaltered social preference but markedly reduced shoaling.[70] To the best of our knowledge, there are no published studies on the effects of salvinorin A and deliriants on adult zebrafish shoaling (Tables 2 and 3), representing an aspect to be addressed further. However, the frequently occurring decrease in zebrafish shoaling produced by both serotonergic (LSD, MDMA, ibogaine) and glutamatergic (ketamine, PCP, MK-801, ibogaine) agents, coupled with unclear effects on social preference, may reflect a global change in social behavior and/or sensory perception of environment and conspecifics, clearly warranting further investigation in zebrafish aquatic tests.

Notably, while social preference tests have long been developed in rodents,[147] shoaling represents a zebrafish-specific behavior that is difficult to adapt to rodent tests. Indeed, adult zebrafish shoaling is not only sensitive to various psychotropic drugs[124,125,148] and can be easily performed in automated high-throughput manner,[126] but is also highly relevant to social "group" behaviors likely to be affected by hallucinogenic drugs in humans (which, like zebrafish, are highly social species). Given the growing importance of *social* context in hallucinogenic drug use and abuse,[129,149] the latter aspect can be of particular interest to assess using zebrafish models. Overall, the prevalence of a disruption of shoaling behaviors in zebrafish exposed to acute doses of hallucinogenic drugs (Table 2) suggests that the social domain is affected by hallucinogenic drugs in zebrafish, similar to other species, including humans. Thus, zebrafish models appear to show some *translational* validity as a potential model of hallucinogenic drugs' action on social behavior.

Cognitive Function. Overall, zebrafish possess excellent cognitive phenotypes, as assessed in various experimental models adapted from rodent cognitive tasks.[14,74,89,150−152] The effects of hallucinogenic drugs on zebrafish cognition have not been extensively investigated, and caution is needed in addressing this aspect, until additional tests are conducted on more specific and/or complex cognitive tasks. However, several studies using hallucinogenic agents have indirectly assessed cognitive phenotypes in this species. For example, LSD and MDMA both reduced intrasession habituation in adult zebrafish, suggesting their altered spatial working memory (Table 2). In line with this, ibogaine reversed normal habituation responses in zebrafish.[70] The anticholinergic agent atropine had no effects on habituation in larval zebrafish,[153] scopolamine impaired startle habituation,[154] and the effects of several glutamatergic agents and salvinorin A have not yet been tested. The latter aspect may be particularly interesting to assess in future studies, given rodent evidence that some agents, such as glutamatergic antagonists, can impair reference and working memory in rats.[155]

Notably, ibogaine produced a dramatic increase in homebase formation, raising the possibility of altered spatial memory and/or awareness in zebrafish (note, however, the lack of published data on homebase formation produced by other common hallucinogenic agents; Table 2). Furthermore, as already mentioned, data are scarce or lacking on the effects of major hallucinogenic drugs in more specialized memory or learning tests in adult or larval zebrafish (Table 2), meriting future studies. Likewise, zebrafish evidence is lacking on other critical cognitive tests highly relevant to hallucinogenic drug action, such as prepulse inhibition of startle, time perception tasks and impulsivity/response inhibition (see ref 14 for a detailed review of rodent cognitive paradigms in hallucinogenic research). Given robust startle responses reported in both adult and larval zebrafish,[153,156] the possibility of modeling such responses in specific novel zebrafish cognitive tasks becomes particularly promising. Taken together, data on zebrafish exposed to acute doses of hallucinogenic drugs (Table 2) suggest that cognitive domain is not most strongly impaired by hallucinogens in zebrafish, which is in line with Hollister's criteria of hallucinogenic drugs as agents potently altering mind, perception, and affect, but not human intelligence or memory.[98]

Neurological and Reward-Related Phenotypes. In addition to motor, affective, social, and cognitive behaviors, zebrafish neurological and reward-related phenotypes are highly relevant to hallucinogenic drug action and merit further scrutiny. For example, while serotonergic drugs do not generally evoke *circling* behavior in adult zebrafish, glutamatergic NMDA antagonists ketamine, MK-801, and PCP all induce overt circling in both zebrafish and rodent models (see refs 71, 101, and 157 for details). Although ibogaine was not active on zebrafish circling,[70] salvinorin A was reported to produce circling-like behaviors,[41] suggesting that opiodiergic mechanisms may also be involved in hallucinogenic-induced circling behavior in zebrafish models. There are almost no published studies on the effects of the main classes of hallucinogenic drugs on circling behavior in larval zebrafish, necessitating future testing. The only study screening ketamine effects in larval zebrafish,[158] however, did report aberrant circling-like (looplike) swimming, further paralleling adult fish findings discussed above. Given the similarity of observed drug-induced "circling" behavior across various species (see ref 70 for discussion), zebrafish appear to mimic specific neurological phenotypes evoked by selected hallucinogenic drugs in various nonfish studies.

While some potent hallucinogenic drugs (e.g., LSD, MDMA) have relatively low addictive potential, others (e.g., ketamine, salvinorin A) are frequently abused, justifying the inclusion of most hallucinogenic agents in the control substance lists worldwide.[24] Regardless of their abuse potential, virtually all hallucinogenic drugs evoke reward-like action in both human and animal subjects.[9,159] Recently, the reward properties of salvinorin A have been reported in zebrafish,[41] generally paralleling clinical and rodent data on this drug of abuse.[37,160,161] However, there are no currently available data on the reward effects of any of other classes of hallucinogenic drugs in either adult or larval zebrafish, representing the critical area for future extensive investigation. Similarly, zebrafish data are lacking on other critical reward-related tests relevant to hallucinogenic action, such as drug discrimination and fixed-ratio reinforcement (see ref 14 for detailed review of rodent literature). Therefore, given the well-established zebrafish reward neural pathways,[77,162] the possibility of developing novel aquatic models to target such phenotypes (and their modulation by hallucinogens) becomes timely.

Physiological Biomarkers. In addition to behavioral responses to hallucinogens in zebrafish models, it is critical to link the observed drug-induced phenotypes with other well-validated physiological biomarkers, such as brain *c-fos* and whole-body cortisol,[46,66,70] relevant to the effects of hallucinogenic drugs. As shown in Table 2, selected hallucinogens drugs affect both *c-fos* and cortisol levels in adult zebrafish. Notably,

altered *c-fos* brain expression and cortisol/corticosterone levels have been reported in various mammalian models following hallucinogenic drugs, including LSD,[163,164] MDMA,[165,166] ketamine,[167,168] and PCP.[113,169] Zebrafish display a well-developed neuroendocrine system[74,170] and show high sensitivity of their CNS proto-oncogene expression to various experimental manipulations.[87,144,171] From this point of view, the sensitivity of zebrafish physiological biomarkers to hallucinogenic drugs (Table 2) supports the utility of such aquatic models to parallel behavioral phenotypes with physiological responses.

CRITICAL SYNTHESIS: THE LESSONS FROM ZEBRAFISH MODELS

Like any experimental model, zebrafish have both strengths and limitations, some of which have already been discussed, and others will be addressed further. What "big" lessons can be learned from zebrafish models of hallucinogenic drug action? First, zebrafish appear to be highly sensitive to hallucinogenic drugs (Table 2), demonstrating striking parallels with humans in relative behavioral efficacy of various hallucinogens (see refs 66−73 for details). Taken together, this suggests a high predictive validity of zebrafish models for this area of research. Second, zebrafish show behavioral and physiological responses that parallel rodent and human findings in important, clinically relevant neurobehavioral domains (such as locomotor, affective, cognitive, social and reward; Tables 2 and 3), thereby suggesting high construct and face validity for hallucinogenic drug research. Third, there are methodological and conceptual problems with using animal models to study complex drug-evoked diseases in humans. For example, there is a growing research need in using traditional (e.g., rodent) experimental models to address these challenges. However, given the complexity of zebrafish behavior, aquatic models discussed here seem to provide valuable exploratory insights into complex behavioral and physiological phenotypes underlying hallucinogenic drug action. We also recognize the limitations on time and efficacy imposed by the complex and multifaceted nature of zebrafish paradigms. At the same time, as advanced video-analysis software and detailed zebrafish ethograms become available, research laboratories are now able to rapidly obtain reliable behavioral data, supplemented with video capture and video tracking, and followed by a multistep assays of physiological biomarkers.[63] Several other potential problems exist. For example, the dissection between hallucinogenic behaviors per se and additional domains (likely to be affected by these drugs, e.g., anxiety, activity, and cognition) requires a thorough investigation using more specific behavioral tests. Thus, a proper selection of zebrafish behavioral end points, as well as applying additional tools (e.g., movement pattern analyses) and pharmacological agents (e.g., anxiolytics or antipsychotics), may help such dissection. For example, several antipsychotics showed attenuation of drug-evoked responses in zebrafish,[172−175] suggesting their utility for antipsychotic drug discovery and modeling hallucinogenic-induced psychoses. Furthermore, as polymodal dose-dependent responses and nonspecific sedation are commonly seen for various drugs of abuse, testing a wide spectrum of doses may be needed to fully explore complex drug-evoked behaviors in zebrafish.

Recent advances in molecular biology of zebrafish[176−178] enable precise mapping of neural pathways activated in zebrafish during selected behaviors, and similar neurophysiological approaches can be applied to the effects of various hallucinogenic drugs. Furthermore, in several assays, treatment with hallucinogens was also accompanied by parasympathetic responses (e.g., altered heart rate[84]), strongly supporting the utility of zebrafish models to study additional physiological biomarkers of hallucinogenic drug action, paralleling their strong behavioral effects discussed above in detail. Likewise, gene transcriptomic analyses in relation to hallucinogenic drug action (similar to those already successfully applied to other drugs of abuse; e.g., refs 77 and 179) can enable a better dissection of common (vs drug-specific) gene expression profiles evoked by these agents. Application of *receptorome* and other omics-based approaches[46] to zebrafish may further characterize the diverse patterns of hallucinogenic drugs action, as well as drug interactions, pharmacogenetic mechanisms, and toxicities. In line with this, using transgenic larval zebrafish as biosensors for hallucinogenic drugs' behavioral and genomic effects[158] further supports promising applications of zebrafish-based screens discussed here.

FUTURE DIRECTIONS: WHERE NEXT?

Several existing knowledge gaps in zebrafish responses to hallucinogenic drugs have already been discussed above. Along other available experimental models in biomedicine, the use of zebrafish tests and screens becomes an invaluable tool in studying neurobehavioral disorders. Considering multiple research-driven limitations in this field, one of the main challenges is the development of satisfactory animal models of psychiatric disorders with a high construct, predictive, face and population validity.[14,17,44,180,181] This also coincides with another challenge, a global reduction of research budgets worldwide, necessitating in better and lower-cost options for in vivo drug screening. From this *practical* point of view, larval and adult zebrafish models offer excellent opportunities to foster innovative high-throughput drug discovery, yet preserving the benefits of utilizing *vertebrate* model species for such screening. Furthermore, given the ease of genetic manipulation in the species sharing 80−85% homology with humans, the possibility of developing pharmacogenetic zebrafish models for hallucinogenic drug action becomes another promising and feasible direction of research. Finally, although many models can quantify the behavioral responses induced by psychotropic drugs, there is a growing need in molecular biomarkers of hallucinogenic action on various neurotransmitter pathways, including serotonin, dopamine, glutamate, cannabinoid, opioid, and acetylcholine receptors.[14,21] The effects of hallucinogens on these signaling pathways and receptors are only partially understood, and future research is required to establish the precise neuronal signaling mechanism underlying hallucinogenic drug action.[14]

Discussing the advantages of zebrafish neurobehavioral models, we should also note that zebrafish locomotion (unlike rodent paradigms) occurs in both horizontal (X, Z) and vertical (Y) dimensions, involving swimming in a truly three-dimensional (3D) space. Can we capitalize on the rich neurobehavioral data offered by the 3D nature of zebrafish locomotion? Recently, we have introduced a novel automated neurophenotyping methodology based on analyses of 3D reconstructions of swimming paths, and have applied and validated this method for various experimental manipulations.[63] These studies have developed detailed 3D-based approaches to phenotyping of zebrafish motor and anxiety-related behaviors, offering an innovative high-throughput data-dense methodology for automated visualization and quantification of fish

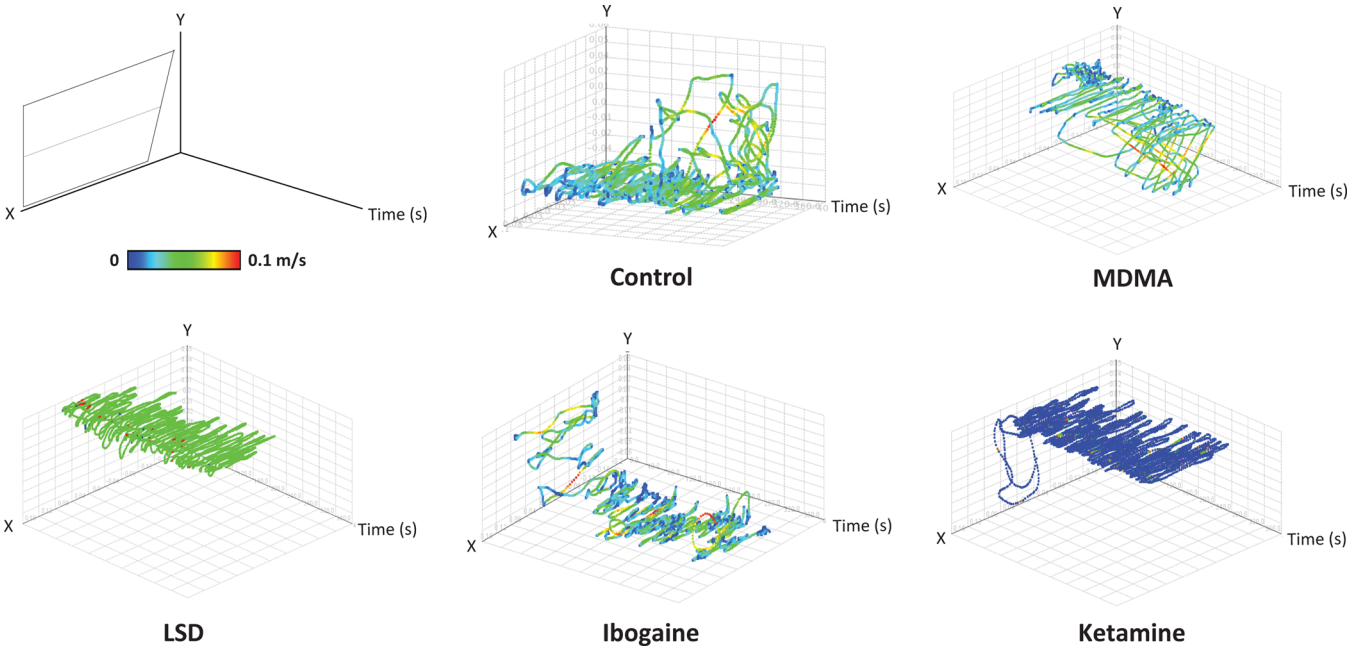

Figure 1. Three-dimensional (3D) spatiotemporal reconstructions (in X, Y, Time coordinates) of adult zebrafish swim paths[63] reveal marked phenotypical differences between representative hallucinogenic drugs, including LSD (250 ug/L), ketamine (20 mg/L), ibogaine (20 mg/L), MDMA (120 mg/L) vs control (drug-free) fish. Following acute 20-min exposure, zebrafish novel tank behavior was video-tracked using EthoVision XT7-8.5 program, and raw tracks were processed, formatted, and visualized in a 3D scatter plot (X, Y, Time) using RapidMiner 5.0 software, according to ref 63. Representative 3D reconstructions were selected on a consensus basis by 2−3 independent highly trained observers, comparing swim paths of all subjects within a cohort, ranking them from 1 to *n* based on similarity to each other (no/low to high activity) and choosing the middle for the illustrations. For a more detailed analysis of 3D reconstructions, the average velocity (m/s) of each fish was reflected by the changes in color (from blue, green, yellow, to red) as the velocity increases.[63] In particular, LSD and ketamine evoked a clear top dwelling, with ketamine exposed fish also exhibiting some decrease in velocity relative to controls. Similarly, MDMA also reduced anxiety-like behaviors in zebrafish, increasing time in top and reduced latency to top. Ibogaine-treated fish demonstrated a mild increase in motor activity, and a reversal of their natural diving response (geotaxis), inducing initial top swimming followed by bottom dwelling. Overall, these 3D traces reveal overt behavioral effects on zebrafish exposed to different hallucinogenic agents, thereby enabling a rapid visualization and interpretation of the observed drug-induced phenotypes (see ref 63 for details).

Table 4. Summary of Major Zebrafish Neurobehavioral Domains That Parallel Targeted Human Brain Disorders Relevant to Hallucinogenic Drug Action[a]

domain	relevant human brain disorder	currently available zebrafish models	ref
motor activity	psychoses, ADHD	drug-induced hyperlocomotion, antipsychotic drug action, genetic models of ADHD, motor retardation	174, 175, 196
mood	depression	chronic unpredictable stress, anhedonic behavior, motor retardation, genetic models of depression, antidepressant action	152, 197[b]
anxiety	anxiety	reduced exploration, increased anxiety-like responses	66, 100, 144
social behavior	social withdrawal	reduced shoaling behavior	123, 126, 198
cognition	cognitive deficits	affected habituation, memory performance, altered homebase formation	191, 192, 199
neurological phenotypes	behavioral stereotypies, OCD	stereotypic circling	144, 194
reward	addiction	conditioned place preference (CPP), drug discrimination, genetic models of reward	77, 200, 201

[a]ADHD, attention deficit/hyperactivity disorder; OCD, obsessive-compulsive disorder. [b]Own unpublished observations (Kyzar et al., 2012b).

swimming activity in both X, Y, Z (spatial) and X, Y, Time (spatiotemporal) coordinates. The use of 3D reconstruction of movement patterns (see Figure 1 for example) to study hallucinogenic drugs[66,70] enables a more precise deconstruction of zebrafish behavior affected by various agents. As rodent models used in neurobehavioral research are mainly based on 2D movement, zebrafish paradigms offer an increased dimensionality of behavioral phenotyping. Therefore, our growing understanding of zebrafish 3D behavior enhances neurobehavioral research using these models,[63] including their application to studying hallucinogenic drug action (see Figure 1 for examples) and targeting several key, translationally relevant domains (Table 4).

CONCLUDING REMARKS

We have previously discussed the unique advantage of zebrafish neurobehavioral research based on the availability of both larval and adult zebrafish models.[46] Mounting evidence, summarized in Tables 3 and 4, indicates that both these models can be sensitive to various hallucinogenic drugs, and may therefore

have the potential to foster further high-throughput drug discovery, neurodevelopmental studies, and substance abuse research using zebrafish.[46,86] In summary, this report outlines the rationale, current successes, and conceptual framework for developing novel efficient models of hallucinogenic drug action in zebrafish. The growing utility of such aquatic models to study hallucinogen-induced phenotypes will foster future experimental studies in this field, markedly enhancing both mechanistically driven *top-down* translational research, as well as *bottom-up* high-throughput screening for novel biomarkers and drug targets.

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N.N., A.M., and A.M.S. have contributed equally to this paper. N.N., A.M., R.A., V.G., D.K., A.M.S., M.K.P., and A.V.K. searched the literature, wrote parts of the manuscript, and participated in conceptual discussion of this study. A.M.S. searched the literature, prepared graphs, wrote, and edited the manuscript. A.V.K. coordinated the study and literature search, conceived the topic of the review, as well as wrote and edited the manuscript.

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Notes

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