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A greater tendency for representation mediated learning in a ketamine mouse model of schizophrenia

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Abstract

Representation mediated learning is a behavioral paradigm that could be used to potentially capture psychotic symptoms including hallucinations and delusions in schizophrenia. In studies of mediated learning, representations of prior experience can enter into current associations. Using a ketamine model of schizophrenia, we investigated whether mice exposed to ketamine during late adolescence subsequently showed an increased tendency to use a representation of a prior gustatory experience to form associations in learning. Mice were given prior experience of an odor and a taste presented together. The odor was subsequently presented alone with gastrointestinal illness induced by a lithium chloride injection. A consumption test was then given to assess whether the taste, despite its absence during conditioning, entered into an association with the induced illness. Such learning would be mediated via a representation of the taste activated by the odor. Our results showed that control mice displayed no aversion to the taste following the procedures just described, but mice that had been treated developmentally with ketamine exhibited a significant taste aversion, suggesting a greater propensity for mediated learning. Complementary to that finding, ketamine-exposed mice also showed a greater susceptibility to mediated extinction. Chronic treatment with the antipsychotic drug, risperidone, in ketamine-exposed mice attenuated mediated learning, a finding that may be related to its known efficacy in reducing the positive symptoms of schizophrenia. These data provide a setting with potential relevance to preclinical research on schizophrenia, to study the neural mechanisms underlying a propensity for aberrant associations and assessment of therapeutics.

Keywords

amphetamine; cognition; dopamine; psychosis; taste aversion

Hallucinations and delusions are defining features of psychosis in which the proclivity for distorted sensory perception and faulty maladaptive beliefs can be debilitating to everyday functioning. The current understanding of these key features is still evolving, with new efforts to conceptualize psychosis in testable frameworks to guide research on how cognitive and neural systems construct false concepts (delusions) and percepts (hallucinations; e.g., Corlett et al., 2016; Fletcher and Frith, 2009; Krystal et al., 2017; Powers et al., 2017).

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Psychosis, however, is exceedingly difficult to model in laboratory animals, hindering efforts to both characterize its underlying neural mechanisms and identify targets for effective drug therapy.

One intriguing recent attempt on that front demonstrated the utility of an associative learning paradigm to probe the capacity to distinguish internally generated representations from external reality using an animal model of schizophrenia (McDannald et al., 2011). The behavioral paradigm is grounded in representation-mediated learning under which a prior experience can enter into current associations (Holland, 1981; Holland and Wheeler, 2008), an account that is consistent with how hallucinations and delusions have been conceptualized in cognitive terms (Adams et al., 2013; Feeney et al., 2017; Powers et al., 2017; McDannald and Schoenbaum, 2009). Using conditioned taste aversion in one variant of the procedures for mediated learning (Wheeler et al., 2013), animals were exposed to a taste-odor compound to endow the odor with the ability to activate a representation of the associated taste. The odor was subsequently paired with gastrointestinal illness in the absence of the taste. A subsequent test with the taste in the absence of the odor was used to assess the presence of a conditioned taste aversion, in which an odor-activated representation of the taste entered into an association with illness. By such mediation, consumption of the taste itself would be reduced relative to consumption of a control taste that did not have the opportunity to form a mediated association with illness. Under such learning paradigms, animals that were used to model other behavioral symptoms of schizophrenia, such as those with neonatal ventral hippocampus lesions (McDannald et al., 2011) or phospholipase C β 1 knockout (Kim and Koh, 2016) were more susceptible to the use of representations from prior experience to form mediated associations in learning.

In the present studies, a ketamine model of schizophrenia (Koh et al., 2016; Olney et al., 1999; Schobel et al., 2013) was used to extend prior investigations by examining whether mice exposed subchronically to ketamine during late adolescence subsequently showed an increased tendency to use a representation of a prior experience to form mediated associations in learning. Exposure to ketamine, a NMDA receptor antagonist, has been shown to be highly effective in reproducing behavioral features of other positive symptoms of schizophrenia (Corlett et al., 2007; Krystal et al., 1994). In two separate experiments reported here, we used taste aversion procedures to probe mediated learning and mediated extinction in mice that had been treated developmentally with ketamine. Our data show that in both cases, those mice exhibited a greater propensity to form associations between stimuli and events that were not directly related. Importantly, we also found that treatment with the antipsychotic medication, risperidone, blocked this increased susceptibility in the ketamine-exposed mice, a finding that may be related to the known efficacy of dopamine antagonists in reducing positive psychotic symptoms of schizophrenia. Indeed, we established that our ketamine-exposed mice in this study were hyper-responsive to the dopamine agonist, amphetamine, further linking an underlying dysregulation of dopaminergic function to a tendency for making loose associations. Together, these data provide a setting with potential relevance to preclinical research on psychosis to study the neural mechanisms underlying a propensity for forming aberrant associations and for assessment of therapeutic treatment on mediated learning in such models.

Materials and Methods

Subjects

Male C57/BL6 mice (n = 68) were obtained at 30 days old from The Jackson Laboratory (Bar Harbor, Maine). The mice were housed in cohorts of 4 per cage at 25°C and maintained on a 12-hr light/dark cycle. Once ketamine exposure was initiated, all mice were housed individually. Cages were lined with corncob bedding and a nestlet for nest building. Food (Purina autoclave laboratory rodent diet) and water were provided ad libitum unless noted. During training, fluid access was restricted to 15-30 min in the morning, and 1 hr with water in the evening. All procedures in the current investigations were approved by the Institutional Animal Care and Committee in accordance with the National Institutes of Health directive.

Ketamine exposures

Starting at 35 days of age, mice were exposed to saline or ketamine at 16 mg/kg (Koh et al., 2016; Schobel et al., 2013) daily for 2 weeks. Ketamine (VedCo; 100 mg/ml concentration) was diluted to 1.6 mg/ml, and injected subcutaneously at a volume of 10 ml/kg of body weight. Following subchronic exposures, the mice were left undisturbed for 4-7 days for drug washout before the commencement of behavioral training.

Risperidone treatment

A subgroup of the ketamine-exposed mice was treated daily with the antipsychotic agent risperidone (Sigma, Saint Louis, Missouri; n = 10) or vehicle (n = 10) starting on the first day of odor-taste compound training. Risperidone was dosed at 0.18 mg/kg in a vehicle of 0.25% Tween-80 in saline, and injected intraperitoneally in a volume of 10 ml/kg, approximately 1 hr before the start of each training and testing session. The dose selected was within the range that has been shown to be effective in inhibiting dopamine neurons and reversing behavioral deficits induced by NMDA receptor antagonists in rodents (Neill et al., 2016; Skarsfeldt, 1995), and importantly, was below doses that we and others have seen that produced extrapyramidal symptoms including catalepsy (in-house observation; Natesan et al., 2006; Neill et al., 2016).

Odor-taste mediated aversion learning

Control mice (n = 10; mean body weight = 19.3 g), ketamine-exposed mice (n = 20, including 10 injected with vehicle for risperidone; mean body weight = 19.4 g), and ketamine-exposed mice under risperidone therapy (n = 10; mean body weight = 19.7 g) were tested for their tendency to form odor-taste mediated aversion learning. All mice were placed under water restriction and trained to drink daily from a modified graduated pipette made of a 10-ml polystyrene serological pipette with the delivering tip cut off and replaced with a metal drinking spout glued onto the pipette. When filled with fluid, a rubber stopper was inserted at the top end to contain the fluid in the pipette. The filled pipette was carefully positioned at an angle on the metal cage lids, with the drinking spout protruding about 2 cm into the cage. Fluid intake measurement was taken by reading the graduation marks to one

decimal point (0.1 ml) on the pipette before and after drinking, which allowed for accurate and precise measurement.

Table 1 shows the design of the experiment. The mice were first exposed to odor-taste compound for 6 days in Phase 1. Two odors (O1 and O2) and two taste solutions (T1 and T2) were used, and the odor-taste combinations were counterbalanced across the control and ketamine groups. The odors were almond (6.7% v/v) and lemon (1.7% v/v) extracts mixed in distilled water, and the taste solutions were sucrose (16% w/v) and maltodextrin (16% w/v). Mice were given O1T1 on odd-numbered days and O2T2 on even-numbered days for 15 min in their home cages in two separate rooms to avoid odor cross-contamination. The odors were presented on saturated filter paper placed around the base of the drinking spout of the modified pipette (approximately 3 cm from the snouts of the mice when drinking). Following compound training, the mice received discrimination training in Phase 2. The mice were given O1 (with water in the pipettes) paired with an injection of lithium chloride (LiCl; 0.15M injected intraperitoneally at 2% body weight) to induce illness on one day, and O2 (with water in the pipettes) paired with saline injection as control on another day. Odor exposure/drinking was limited to 15 min, and the mice were injected within 5 min after the removal of the odor/pipette. The mice were then given a recovery day in which access to water was given in the colony room for 1 hr each in the morning and evening. On the next two days, the mice received taste consumption tests for 30 min with either T1 or T2 in the colony room. Finally, odor tests with either O1 or O2 were given for 30 min on separate days in the training rooms with water in the pipettes.

The mice drank more of maltodextrin in general than sucrose at the concentrations used, likely due to a stronger preference for the former; hence, intake during the test for each taste according to the identity of its compound odor that was paired with LiCl was normalized to the same taste according to its compound odor paired with saline control. A normalized consumption of a 100% thus indicates no aversion relative to control level, and the lower the number relative to that benchmark signifies the stronger the aversion.

Odor-taste mediated aversion extinction

A different set of control ($n = 13$; mean body weight = 20.0 g) and ketamine-exposed ($n = 15$; mean body weight = 18.3 g) mice were tested for their tendency to produce representation-mediated extinction under a taste aversion paradigm. Table 2 showed the experimental design. To simplify the experiment and analysis, we restricted the odor-taste compound training here to one odor (6.7% almond) and one taste (16% sucrose); Phase 1 training lasted 3 days. The presentation of the odor and taste solution to the mice was done in the same way as described in the previous experiment. Over the next 2 days of discrimination training in Phase 2, the mice were given sucrose (without the almond odor) to drink for 15 min in the colony room and then injected intraperitoneally with either saline or LiCl (0.15M at 2% body weight) to induce gastrointestinal illness. Following discrimination training, the mice underwent extinction training in which the almond odor (with water in the pipettes) was presented daily for 15 min over the next 3 days. The mice then received a taste test for 30 min with sucrose on the final day.

Amphetamine-induced locomotor activity

The ketamine-exposed and control mice in the mediated extinction experiment ($n = 28$) were challenged with amphetamine to assess whether our ketamine mouse model recapitulated the dysregulation of dopaminergic function that characterizes schizophrenia. Each mouse was placed in an open field chamber ($42 \text{ cm} \times 42 \text{ cm} \times 30.5 \text{ cm}$) in which locomotion was tracked with the VersaMax animal activity monitoring system (AccuScan Instruments, Columbus, OH). After 20 min of habituation, the mouse was taken out of the chamber and injected intraperitoneally with a small dose of amphetamine (0.5 mg/kg in a volume of 10 ml/kg ; Sigma, Saint Louis, Missouri). The mouse was then returned to the chamber for 40 min of activity monitoring. Total movement time was used as the dependent measure. Note that the data presented here were part of a separate study on the effect of drug treatment on amphetamine-induced locomotor activity; the data analyzed were accrued from control treatment on these mice in which each mouse received intraperitoneal injection of a vehicle solution of 20% Tween-80 in saline at the beginning of baseline assessment.

Results

Representation-mediated learning

Mice received odor-taste compound exposures during Phase 1 of training, and odor exposure alone (with water to consume) during Phase 2 just before conditioning. Table 3 shows the mean fluid intake and standard error of the three groups during both phases; the means for Phase 1 are averaged across trials. An analysis of variance (ANOVA) with repeated measures on the consumption amount in Phase 1 showed no interaction between group and odor-taste compound, $F(2, 37) = 0.09$, $p = .911$, and no effect between O1T1 and O2T2, $F(2, 37) = 0.01$, $p = .974$, but a significant group effect, $F(2, 37) = 10.22$, $p = .001$, likely due to a lower consumption amount by the ketamine-exposed mice under risperidone treatment. Further analysis confirmed that the intake of the risperidone group was significantly lower than those of the control and ketamine groups, $F(1, 18) = 32.65$, $p = .001$ and $F(1, 28) = 10.01$, $p = .004$, respectively; there was no difference in intake between the latter two groups, $F(1, 28) = 2.85$, $p = .102$. Future studies should yoke intake during training to ensure the consumption amount during training among the groups are the same. In Phase 2, the consumption of water in the presence of O1 and O2 was not different among the groups. A repeated measures ANOVA showed no interaction between group and odor, $F(2, 37) = 0.60$, $p = .552$, no difference between O1 and O2, $F(2, 37) = 0.33$, $p = .570$, and no group effect, $F(2, 37) = 1.19$, $p = .310$.

Figure 1 shows the results of the taste test as indexed by normalized consumption of T1 (with O1 paired with LiCl) relative to T2 (with O2 paired with saline; see Materials & Methods for details on normalization). A benchmark of 100% indicates no mediated taste aversion, and a lower number indicates a stronger aversion. A oneway ANOVA detected group differences among the three groups, $F(2, 37) = 11.74$, $p = .001$. The ketamine-exposed mice showed a significantly stronger aversion than both the control mice, $t(18) = 3.22$, $p = .003$, and the ketamine-exposed mice treated with risperidone, $t(28) = 4.18$, $p = .001$. No difference was found between the mice treated with risperidone and those in the control group, $t(18) = 1.60$, $p = .127$. The mice in those two groups in fact showed no detectable

aversion learning, $t(9) = 1.10$, $p = .302$ and $t(9) = 2.14$, $p = .062$ respectively (one-sample t -tests relative to 100%), while the ketamine-exposed mice showed a significant mediated taste aversion, $t(19) = 4.08$, $p = .001$. A similar analysis of the odor test showed no group differences in water intake in the presence of the two odors, $F(2, 37) = 0.37$, $p = .696$, and none of the groups deviated significantly from 100%. Normalized consumption for the control, ketamine, and ketamine-risperidone groups were 112% (SEM ± 5.3), 111% (SEM ± 10.2), and 124% (SEM ± 13.6), respectively. The conditioned odor therefore formed no direct association with illness, but served to activate the representation of the taste consistent with other reports (Wheeler et al., 2013). Together, these data showed that odor-activated representation of taste has a tendency to enter into an association with illness in mice exposed to ketamine during adolescence, and that risperidone treatment effectively reversed this tendency.

Representation-mediated extinction

Mice were first exposed to odor-taste compound in Phase 1 of training, followed by two days of taste aversion conditioning in Phase 2. The mice were then given repeated non-reinforced exposures to the odor to elicit associatively activated extinction of the taste prior to a final taste test. Table 4 shows the mean intake (and standard error) among the groups during Phase 1 and odor extinction; the means are averaged across trials. The mean sucrose intake during odor-taste compound training was the same between the control and ketamine groups, $F(1, 24) = 0.08$, $p = .774$, and also between the saline and LiCl treatments, $F(1, 24) = 0.28$, $p = .602$. There was however an interaction between the two factors, $F(1, 24) = 6.12$, $p = .021$, primarily due to the differences on the first day of exposure; no such interaction was observed on the last two days of training (data not shown).

Figure 2A shows sucrose intake of the groups on the two days of taste aversion conditioning. Intake on Day 1 prior to conditioning was unsurprisingly the same across the board; there was no group effect, $F(1, 24) = 1.06$, $p = .313$, saline/LiCl treatment effect, $F(1, 24) = 0.05$, $p = .831$, or interaction, $F(1, 24) = 1.28$, $p = .269$. Intake on Day 2 after one day of conditioning was noticeably different. The mice injected with LiCl the day before to induce gastrointestinal illness drank significantly less sucrose than those injected with saline control, $F(1, 24) = 18.50$, $p = .001$, indicating the development of a taste aversion to sucrose in the conditioned group. There was no difference between the control and ketamine groups, $F(1, 24) = 0.07$, $p = .789$, and no interaction between the two factors, $F(1, 24) = 0.11$, $p = .747$. Further analysis confirmed that the control mice that were conditioned with LiCl drank significantly less sucrose than their unconditioned counterparts, $t(11) = 3.54$, $p = .005$. Similarly, the ketamine-exposed mice that were conditioned consumed less than their unconditioned counterparts, $t(13) = 2.70$, $p = .018$. Importantly, the strength of aversion in the control and ketamine groups was identical. The two conditioned groups did not differ from each other, $t(13) = 0.04$, $p = .969$, and likewise for the two unconditioned groups, $t(11) = 0.42$, $p = .680$.

All mice were then given odor extinction training in the absence of the taste on the next 3 days. Table 4 shows the mean intake of water during extinction. No significant differences in intake were observed, $F(1, 24) = 0.14$, $p = .708$ for the main effect of control/ketamine

groups, $F(1, 24) = 3.71$, $p = .066$ for the effect of saline/LiCl treatments, and $F(1, 24) = 2.59$, $p = .121$ for interaction.

A final taste test with sucrose was given to all the mice after the last day of odor extinction training. Figure 2B shows the mean intake of the control and ketamine-exposed mice that previously received saline or LiCl to induce a taste aversion. Overall, an ANOVA shows a significant interaction between control/ketamine groups and saline/LiCl treatments, $F(1, 24) = 12.78$, $p = .002$, suggesting that the odor extinction training had a differential effect on the groups. There was a significant main effect of saline and LiCl treatments, $F(1, 24) = 52.44$, $p = .001$, consistent with the efficacy of LiCl to induce a taste aversion as seen on the second day of conditioning. There was no main effect of control and ketamine groups, $F(1, 24) = 0.63$, $p = .434$. Further analysis showed that mice treated with LiCl in the control and ketamine groups drank significantly less sucrose than their respective counterparts treated with saline, $t(11) = 6.94$, $p = .001$ for control group, and $t(13) = 2.86$, $p = .013$ for ketamine group, indicating that a taste aversion was still present in both groups. Most importantly however, the strength of aversion between the two conditioned groups now differed noticeably; the conditioned mice in the ketamine group showed a significantly weaker taste aversion compared to the conditioned mice in the control group, $t(13) = 3.41$, $p = .005$, suggesting that odor extinction training had a stronger influence on the taste-illness association in the ketamine-exposed mice.

Amphetamine challenge

Mice with a history of ketamine or saline exposures were injected with amphetamine to assess whether the model recapitulated the dysregulation of dopaminergic function that is thought to be a fundamental pathology of schizophrenia. Background analysis prior to amphetamine challenge showed no differences in activity between ketamine-exposed and control mice. In response to amphetamine injection, ketamine-exposed mice were significantly more active than control mice as shown in Figure 3. A repeated measures ANOVA showed a significant main effect of group, $F(1, 26) = 4.52$, $p = .043$, and a main effect of time, $F(3, 78) = 45.80$, $p = .001$, but no interaction between group and time, $F(3, 78) = 0.18$, $p = .907$, as the amphetamine effect subsided similarly over time between the two groups.

Discussion

Mice treated developmentally with ketamine showed a greater tendency to form associations between stimuli and events that were not directly related but mediated by prior experience. In a representation-mediated learning paradigm, ketamine-exposed mice formed a taste aversion in the absence of the taste during conditioning, indicating that an odor-activated internal representation of the taste based on prior experience had entered into an association with illness; control mice by contrast displayed no such mediation in their performance. Similarly, under representation-mediated extinction, ketamine-exposed but not control mice reduced the strength of a taste aversion in response to extinction training with the odor in the absence of the taste. Here, those data indicated that the odor-activated representation of the taste had entered into an association with a safety signal during extinction. In both cases, the

ketamine-exposed mice were more predisposed to the influence of activated representations entering into current associations, consistent with key psychological processes that may contribute to the formation of hallucinations and delusions from a cognitive perspective (Adams et al., 2013; Corlett et al., 2007; Powers et al., 2017). Our data additionally showed that this increased tendency might be due to dopamine dysfunction. The ketamine-exposed mice demonstrated hyper-responsiveness to the dopamine agonist amphetamine, and the ketamine-exposed mice showed a reduced propensity for representation-mediated learning when treated with the dopamine antagonist risperidone.

The present findings are in line with those reported by McDannald et al. (2011) in which rats with neonatal ventral hippocampal lesions, another neurodevelopmental model of schizophrenia, showed an increased likelihood to form a food aversion under representation-mediated learning conditions when the cue predicting the food was paired with gastrointestinal illness. Note that the animals in both studies were not generally more sensitive to developing a taste aversion than control animals as indicated by normal learning when the taste or food was paired directly with illness (see Fig 2A for Day 2 in our study and Fig 1 in McDannald et al., 2011). Our findings from the extinction study further support the concept of augmented mediation of learning in this model; ketamine-exposed mice in fact exhibited a weaker taste aversion than control mice as a result of non-reinforced presentations of a cue in extinction that activated the representation of the taste. It is notable that the animals in our control groups that had not been exposed to ketamine and those in McDannald et al. (2011) showed no evidence for representation-mediated learning, which is not always the case (e.g., Holland, 1981; Wheeler et al., 2013). Procedural differences including the amount of cue training have been shown to affect the content of cue-activated representations necessary to support mediated learning (Holland, 1998; 2005). Although our experimental procedures did not optimize the occurrence of mediated learning in normal control mice, it is clear that the ketamine-exposed mice were more susceptible than control mice trained and tested under the same experimental parameters in our studies, as observed in other models (e.g., McDannald et al., 2011).

Ketamine is used extensively in preclinical and clinical research to engender symptoms reminiscent of those in schizophrenia including psychosis (e.g., Lahti et al., 1995; Moghaddam and Krystal, 2012; Powers et al., 2015; Schobel et al., 2013). The psychomimetic effect of ketamine has been ascribed to its action on glutamate and dopamine neurotransmissions (Kapur and Seeman, 2001). Our data here and elsewhere with amphetamine stimulation (Koh et al., 2016; 2017) support a key role for dopamine; exposure to ketamine developmentally produced behavioral responses that are consistent with augmented dopamine activation. We thus hypothesized that the augmented dopamine function might also contribute to the increased propensity for representation-mediated learning. Indeed, chronic treatment with the antipsychotic risperidone, which acts as an antagonist for dopamine (and serotonin) receptors (Love and Nelson, 2000), attenuated mediated learning in the ketamine-exposed mice. We would expect the same impact of risperidone treatment on mediated extinction in ketamine-exposed mice.

Regarding the neural basis of mediated learning in the ketamine model, increased dopamine activation such as that occurring in the ventral tegmental area in animal models of

schizophrenia has also been tied to neural hyperactivity in the hippocampus, an area known to be necessary for representation mediated learning (Iordanova et al., 2011; Wheeler et al., 2013). Hippocampal output via the subiculum controls dopamine neuron firing in the VTA (Floresco et al., 2001), and excess dopamine activity in the VTA was reduced by targeting hippocampal hyperactivity with intracranial administration of a GABA agonist into the hippocampus (Gill et al., 2011). Pertinent to our model, hippocampal hyperactivity is also observed in mice exposed to ketamine developmentally, which recapitulates an increased hippocampal metabolic state during the emergence of psychosis in patients at risk for schizophrenia (Schobel et al.; 2009; 2013). Taken together, hippocampal hyperactivity accompanied by increased dopamine in the VTA could comprise a network of circuits that is altered in psychosis to generate a greater cognitive bias toward mediated learning. Targeting hippocampal overactivity could therefore present a target for the treatment of psychosis by attenuating dopamine-associated positive symptoms via downstream control of VTA dopaminergic function and concurrently treat other cognitive impairment for which antipsychotics lack efficacy (see Koh et al., 2017).

With respect to the translational relevance of our current findings, recent studies with human subjects have highlighted the strong influence of prior experiences in the formation of hallucinations and delusions. For instance, patients with psychosis trained repeatedly to associate a tone at threshold with a visual stimulus had a greater propensity than those without psychosis to report the presence of the tone upon the presentation of the visual stimulus alone, and those judgments included a higher decision confidence in their conditioned hallucinations (Powers et al., 2017). Strikingly, functional neuroimaging showed brain activity in tone-responsive regions of these patients during conditioned hallucinations, suggesting the activation of an auditory percept, in the absence of a tone, at the neural level. Computational modeling of these data further pointed to a stronger bias in those with conditioned hallucinations favoring prior perceptual knowledge over sensory evidence (see also Teufel et al., 2015). The behavioral data in our studies are consistent with these observations; the ketamine-exposed mice showed a greater influence of their prior odor-taste experience such that the presentation of the odor alone activated a representation of the taste that could enter into associations with other events. Analogous to the other findings in neuroimaging, our lab reported strong activation of neural ensembles in the gustatory cortex, a taste-responsive region, by an odor cue previously paired with a taste (Saddoris et al., 2009), suggesting a neural basis for associatively activated representations in the absence of actual current experience with the stimulus. Overall, these findings support the view that representation-mediated learning could be utilized as a valuable tool in animal research to probe cognitive processes that contribute to the maladaptive occurrence of hallucinations and delusions in psychiatric illness.

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Figure 1.

When tested for representation-mediated taste aversion learning, ketamine-exposed mice showed a significantly stronger taste aversion than mice in the control group indicating a greater tendency to form taste-illness association in the absence of taste during conditioning. Chronic treatment with the antipsychotic risperidone in ketamine-exposed mice effectively attenuated mediated learning. A normalized consumption of a 100% indicates no aversion relative to control level, and the lower the number relative to that benchmark signifies the stronger the aversion. Error bars indicate mean $\pm$ SEM.

Figure 2.

Mice were conditioned and tested for representation-mediated extinction. (A) Mice were conditioned to develop a taste aversion over two days. On Day 1 prior to conditioning, no differences in sucrose intake were evident between LiCl and saline conditions across control and ketamine-exposed groups. On Day 2, mice that had received taste-illness pairing the day before drank significantly less sucrose than those that received taste-saline pairing indicating the development of a taste aversion in the conditioned groups. The strength of aversion in the control and ketamine-exposed mice that were conditioned was identical. (B) Mice were tested for mediated extinction to taste aversion after repeated presentation of non-reinforced odor trials. Ketamine-exposed mice that previously received taste-illness pairings showed a significantly weaker taste aversion after odor extinction than their counterparts in the control group. Error bars indicate mean $\pm$ SEM.

Figure 3.

Mice were assessed for hyper-responsiveness to an amphetamine challenge. Ketamine-exposed mice were more active in an open field in response to an amphetamine injection than control mice. Error bars indicate mean $\pm$ SEM.

Table 1

Experiment design for representation mediated learning.

	Phase 1	Phase 2	Taste Test	Odor Test
Mediated Learning	O1T1 / O2T2	O1 → LiCl / O2 → Saline	T1 / T2	O1 / O2

Table 2

Experiment design for representation mediated extinction.

	Phase 1	Phase 2	Extinction	Taste Test
Mediated Extinction	Almond Odor + Sucrose	Sucrose → LiCl Sucrose → Saline	Almond Odor	Sucrose

Table 3

Fluid intake for representation mediated learning.

Group	<u>Phase 1 (tastants in ml $\pm$ SEM)</u>		<u>Phase 2 (water in ml $\pm$ SEM)</u>	
	O1T1	O2T2	O1	O2
Control mice	1.24 ($\pm$ 0.06)	1.23 ($\pm$ 0.06)	0.83 ($\pm$ 0.05)	0.76 ($\pm$ 0.07)
Ketamine mice	1.14 ($\pm$ 0.06)	1.12 ($\pm$ 0.05)	0.71 ($\pm$ 0.04)	0.74 ($\pm$ 0.04)
Ketamine mice on risperidone	0.93 ($\pm$ 0.07)	0.96 ($\pm$ 0.06)	0.73 ($\pm$ 0.05)	0.70 ($\pm$ 0.06)

Table 4

Fluid intake for representation mediated extinction.

Group		Phase 1	Phase 2	Odor Extinction
		(sucrose in ml $\pm$ SEM)		(water in ml $\pm$ SEM)
Control mice	Saline	1.08 ($\pm$ 0.07)	See Figure 2A	0.81 ($\pm$ 0.02)
	LiCl	0.94 ($\pm$ 0.04)		0.74 ($\pm$ 0.02)
Ketamine mice	Saline	0.98 ($\pm$ 0.03)		0.79 ($\pm$ 0.02)
	LiCl	1.07 ($\pm$ 0.04)		0.78 ($\pm$ 0.02)