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LETTER TO THE EDITOR

Probing for Conditioned Hallucinations Through Neural Activation in a Ketamine Mouse Model of Schizophrenia

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Dear Editor,

When two stimuli such as a tone and a visual stimulus are repeatedly presented together, individuals diagnosed with hallucinations tend to report hearing the tone subsequently in response to the visual stimulus alone [1]. Such conditioned hallucinations involved evoked sensory representations as evidenced by increased neural activation in tone-responsive brain regions during the conditioned hallucinations. These and other observations underscore the importance of prior associative experiences in driving hallucinatory perception, and reflect new efforts to conceptualize and understand psychosis from a cognitive perspective.

Based on the cognitive perspective described above, we and others have shown that mice used to model schizophrenia were more susceptible to psychosis-like behavior than normal animals as instantiated by a greater tendency to form associations between stimuli or events that were mediated by prior experience [2–4]. For example, using a well-established ketamine mouse model that is known to recapitulate many of the symptoms seen in schizophrenia [5, 6], we used a representation-mediated learning paradigm to show that, when an odor and a taste are repeatedly paired, ketamine-exposed mice have an increased tendency to use the odor to evoke a putative inner representation of the taste that could enter into an association with illness [3]. Our data in that study also showed that this increased tendency can be blocked by the anti-psychotic dopamine antagonist risperidone, suggesting that the hallucinatory-like perception is likely due to dopamine hyper-function.

In the present study, also using the ketamine model of schizophrenia in which mice were exposed sub-chronically to ketamine during adolescence and tested ketamine-free in young adulthood, we investigated whether an odor activates a representation of a taste based on prior association by examining neural activation in the primary taste-responsive cortical region after odor exposure. Neural activation of the taste-responsive region in this context would provide evidence for an induction of a taste percept at the neural level in the absence of the taste itself, phenomenologically consistent with hallucinations in which a stimulus is perceived without direct external sensory input from the stimulus. To measure neural activation, we used the induction of c-fos, an immediate-early gene induced by neural activity, as a marker to detect the activation of a taste percept in the insular (primary taste) cortex of adult mice previously exposed to either ketamine or vehicle during adolescence. Importantly, prior research has shown that activation of the taste area in the insular cortex resembles the natural neural representation of taste stimuli [7], and stimulation of the taste cortex in the absence of an actual taste can initiate taste percepts to guide behavior [8].

Starting at 35 days of age, mice were exposed to saline or ketamine (16 mg/kg) [3] daily for 2 weeks and then left undisturbed for a week for drug washout before the commencement of odor–taste exposure training. The mice received odor–taste compound exposures on days 1–3 of training, and odor exposure alone (with water to consume) aimed at activating a taste representation on day 4 prior to perfusion (see Supplementary Materials). No differences in

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the mean fluid intake of mice between the vehicle (n = 8) and ketamine (n = 9) groups occurred during training or on day 4 (Fig. 1A). The mean intensity of c-fos mRNA expression in the insular cortex of mice in the ketamine group was significantly stronger than that in the vehicle group [$t(15)$ = 2.2, P = 0.044; Fig. 1B; see Fig. S1 for representative photomicrographs of c-fos mRNA expression in the insular cortex]. Additional analysis of the striatum, which served as a control region, showed no differences between groups (mean c-fos intensity in vehicle, 0.158; with ketamine, 0.150; t < 1, Fig. S2), showing that the increased c-fos expression in the ketamine group did not occur brain-wide. These data, taken together, suggest that a history of ketamine exposure during adolescence increases the tendency for neural activation of an absent stimulus in a brain region known to be responsive to the taste stimulus.

Increased dopamine activation is thought to drive conditioned hallucinations. The excess dopamine activity likely originates in the ventral tegmental area (VTA), while

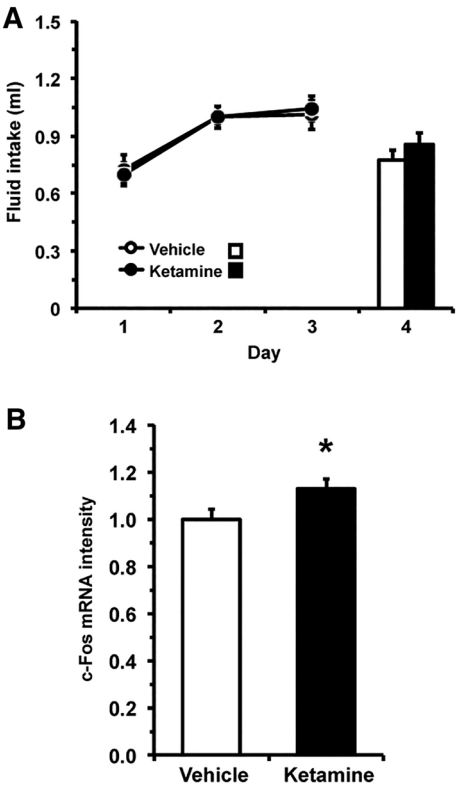

Fig. 1 Fluid intake during training and c-fos expression. **A** Vehicle- and ketamine-exposed mice drank the same amount of sucrose solution on days 1–3 of training in compound exposure with an almond odor. The mice were exposed to the odor with water to drink on day 4 prior to perfusion. **B** Ketamine-exposed mice induced significantly stronger c-fos expression in the insular cortex than in vehicle control mice in response to an odor previously paired with a taste. The expression levels were normalized to the mean of the control group.

neural activity in the hippocampus plays a key role in controlling dopamine neuron firing in the VTA [9]. Increased hippocampal activity has been noted in patients at risk for schizophrenia during the emergence of psychosis, a condition recapitulated in mice exposed to ketamine during development and in other models of schizophrenia [3, 10–12]. Hippocampal hyperactivity accompanied by increased dopamine in the VTA could therefore comprise a network of circuits that is altered in psychosis to generate a greater cognitive bias toward prior perceptual knowledge over sensory evidence. Here, in a second experiment to elucidate the role of the hippocampus in conditioned hallucinations, we lesioned the dorsal hippocampus of ketamine-exposed mice to assess its effect on the activation of a taste percept at the neural level in the absence of a taste using an odor–taste associative task.

About a week prior to the odor–taste experiment, ketamine-exposed mice with sham (n = 8) or hippocampal lesions (n = 8) were injected with a small dose of amphetamine (1 mg/kg) or vehicle saline (using a within-subject design with counterbalancing for order of exposure), a commonly used behavioral assay to validate dopaminergic perturbation that is central to psychosis. The locomotor activity of sham and lesioned mice during 20 min of baseline (prior to injection) and subsequently after saline or amphetamine administration showed that mice with hippocampal lesions were considerably more active overall than sham controls (Fig. 2), consistent with extensive reports of increased locomotor activity in animals with hippocampal lesions. We first analyzed the effect of the amphetamine challenge in the sham and lesioned groups separately, and then compared the effect size (percentage change in activity) between the groups. In the sham group (Fig. 2A), repeated measures ANOVA during baseline (first 20 min) indicated no interaction or main effect of treatment (saline *versus* amphetamine); a significant main effect of time interval reflected decreased activity during habituation to the arena [$F(1, 7)$ = 51.14, P = 0.001]. After injection, activity in the sham mice was significantly higher in response to amphetamine than saline [$F(1, 7)$ = 9.91, P = 0.016]. No main effect of time or interaction between drug × time was found. In the lesioned group, the same analysis at baseline showed no interaction, main effect of treatment, or main effect of time interval (Fig. 2B). When challenged with amphetamine, the locomotor activity of lesioned mice was dramatically elevated compared to saline injection [$F(1, 7)$ = 13.97, P = 0.001]. No main effect of time or interaction between drug × time was found. Additional analysis showed that, while the locomotor activity of sham-lesioned mice increased in response to amphetamine by an average of 46.5% (compared to saline), the hippocampus-lesioned mice had a markedly larger increase of 124.1% [$t(14)$ = 2.58, P = 0.022]. Taken together, these data

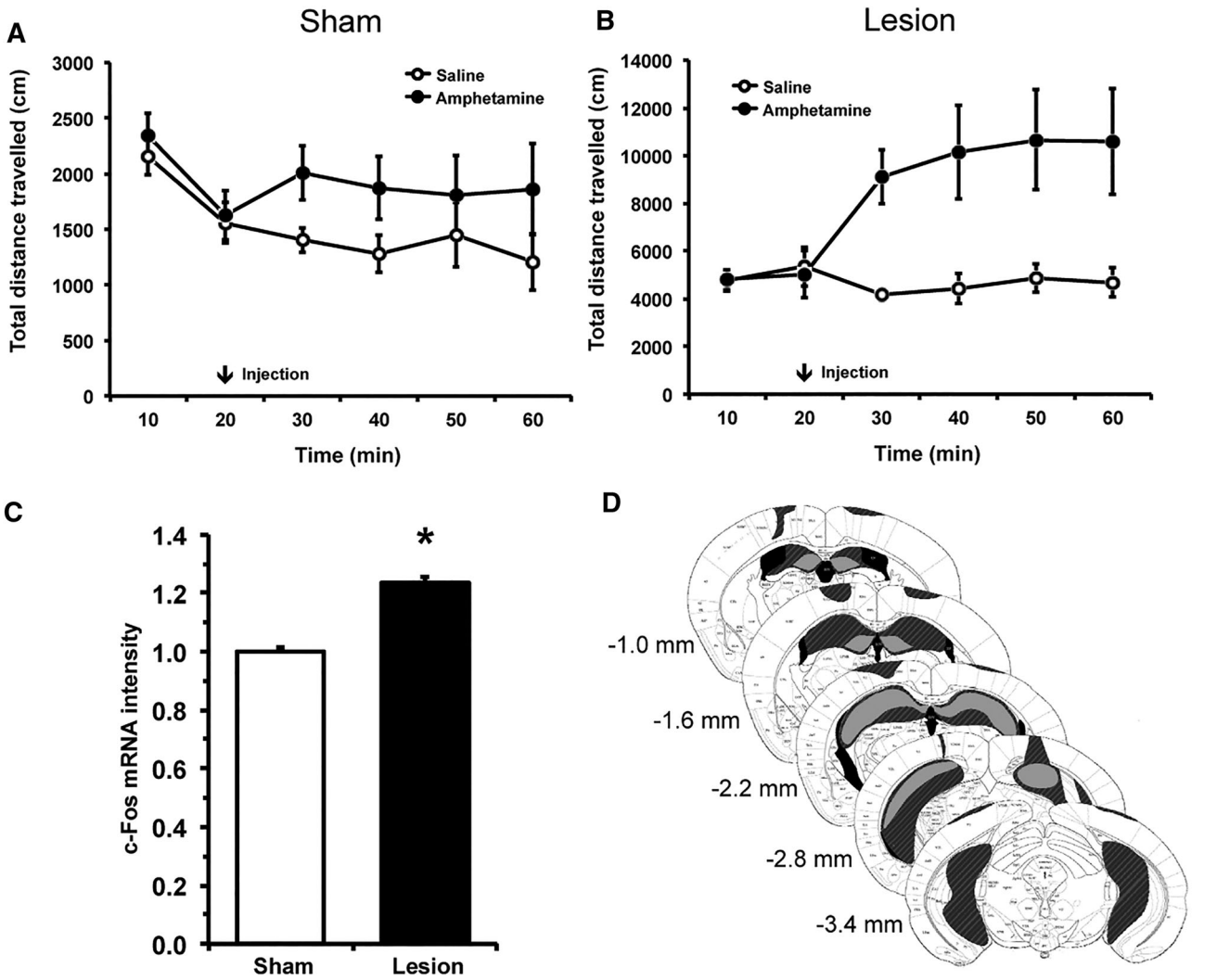

Fig. 2 Hippocampal lesions in ketamine-exposed mice. **A, B** Compared to sham control mice (**A**), ketamine-exposed mice with hippocampal lesions (**B**) showed a markedly larger increase in locomotor activity to amphetamine. **C** Ketamine-exposed mice with hippocampal lesions showed a higher overall expression of c-fos mRNA in the insular cortex than ketamine-exposed mice with sham lesions. **D** Schematics of the largest (hatched dark gray) and smallest (light gray) extent of the hippocampal lesions (coordinates relative to bregma).

showed that ketamine-exposed mice with hippocampal lesions were hyper-responsive to amphetamine, suggesting that damage to the hippocampal formation potentially increases the predisposition to psychosis-like symptoms in this behavioral assay.

The mice with hippocampal and sham lesions were subsequently water-deprived and trained to drink on a limited-access schedule. Training on the odor–taste task commenced thereafter as described above for the first experiment. The mean intensity of c-fos mRNA expression in the insular cortex of the two groups showed that the mice with hippocampal lesions had a stronger neural activation in response to an odor previously paired with a taste than those with sham lesions [$t(14) = 2.32$, $P = 0.036$; Fig. 2C and S3], suggesting that hippocampal lesions in ketamine-exposed mice enhance the associative activation of a taste representation in a primary taste-responsive brain region in the absence of an actual taste. The extent of the hippocampal lesions is illustrated in Fig. 2D.

Using a well-established animal model that recapitulates psychosis-like symptoms, we showed that, in mice exposed to ketamine during adolescence, stronger neural activation was induced in the taste-responsive region of the insular cortex in response to an odor previously associated with a taste than in control mice. The stronger neural activation seen here is consistent with the behavioral findings that ketamine-exposed mice have an increased tendency to evoke an internal representation of a taste that can enter

into an association with illness during conditioning in response to an odor that had gone through odor–taste pairing [3]. Our data are also in line with findings in other animal models of schizophrenia showing an increased tendency in this type of representation-mediated learning [2, 4]. In particular, Fry *et al.* [2] recently showed that transgenic mice with dominant-negative expression of Disrupted-In-Schizophrenia-1 exhibit stronger perceptual processing of an absent taste to an auditory cue previously paired with the taste, and display greater c-fos expression in the insular cortex in response to that cue. In addition, treatment with haloperidol [2] or risperidone [3] effectively reduces the increased tendency for representation-mediated learning in mice used to model schizophrenia, suggesting that dopaminergic hyper-function likely contributes to the heightened tendency.

Our data for hippocampal lesions in ketamine-exposed mice showed increased behavioral responsiveness to amphetamine above the level of ketamine-exposed animals with sham lesions, indicating significant elevation of dopaminergic function from the lesions beyond the effect of ketamine exposure, and foreshadowing a potential for stronger conditioned hallucinations. Indeed, when the same mice were trained and tested in the odor–taste associative task, those with hippocampal lesions had stronger c-fos activation in response to the odor in the insular cortex than sham control mice. Taken together, these findings indicate that ketamine-exposed mice evoke a stronger hallucination-like percept at the neural level, and hippocampal damage in such animals further heightens these effects.

The activation of a taste percept at the neural level in the absence of an actual taste is similar in principle to a hallucination in which a stimulus is experienced in its absence. Whether hallucinations are triggered by associative stimuli or events is supported by evidence emphasizing the importance of prior associative experiences in eliciting hallucinations [1]. The conceptualization of hallucinations in such a cognitive framework allows for new ways to study and understand the phenomenon of hallucination and its neurobiological underpinnings, including in the laboratory setting with human and animal subjects using Pavlovian conditioning to establish prior associations [1–4]. While human test participants can verbally report the perception of a (hallucinatory) stimulus, behavioral studies with animals require indirect inference of the perception of an absent stimulus through downstream changes in behavior such as with representation-mediated taste aversion learning. In one version of that learning paradigm for example, odor activates a taste representation in the presence of illness to subsequently induce a conditioned taste aversion which serves as the primary measure to infer the occurrence of taste representation as a surrogate for a hallucination-like response. Our study offers another way to probe for hallucination-like perception through neural activation of the absent stimulus in animals (see also the recent study by Fry *et al.* [2]). Converging evidence of enhanced hallucination-like perception from both methods, such as that with the ketamine animal model, would strengthen our confidence in the findings.

Individuals with schizophrenia and ketamine-exposed mice show increased metabolic activity in the hippocampus, and hippocampal activity is known to exert considerable control over dopaminergic activity in the mesolimbic system. For example, intracranial administration of a GABA agonist into the hippocampus has been shown to reduce excess dopamine activity in the VTA and psychosis-like symptoms in an animal model of schizophrenia [9]. Lesions of the hippocampus as in the present study could similarly have the same effect although increased behavioral impairment including psychosis has been noted in schizophrenic patients with hippocampal structural deficits [13]. Our data with ketamine-exposed mice in fact showed that hippocampal lesions increased dopaminergic function coupled with enhanced neural activation in gustatory cortex in the absence of a taste stimulus. One possible explanation is that functional compensation for hippocampal lesions results in upregulation of dopamine release in regions such as the nucleus accumbens, although the exact mechanism of action by which that would occur is not fully delineated. It is worth noting that the dorsal and ventral hippocampus may exert different effects on downstream dopaminergic activity. While inhibition of neural activity in the ventral hippocampus reduces dopaminergic responsiveness [9, 14], the disruption of dorsal hippocampal activity *via* lesions such as in our study appears to increase dopaminergic responses that likely contribute to the stronger activation of a hallucination-like taste percept.

The most common hallucinations experienced by humans are auditory and visual [15, 16]. The use of taste to induce a conditioned taste hallucination in rodents partly takes advantage of the species' highly sensitive taste/gustatory system, as well as being influenced by the precedents set by pioneering studies on representation-mediated taste/food aversion learning [17]. Future studies of conditioned hallucinations in animals are needed to investigate other sensory modalities to assess the generality of these effects. For example, a recent study using a newly-developed single-neuron-resolution optogenetic technique to control neuronal activity patterns in the visual cortex of mice successfully imaged neural ensembles associated with the perception of visual stimuli, and then optogenetically evoked hallucinatory visual percepts of those stimuli using the recorded neural ensembles [18]. Whether prior associative experiences between a visual stimulus and an auditory cue would subsequently render the auditory cue,

either from hearing or *via* optogenetic stimulation of selected neurons in the auditory cortex, with the ability to evoke the same visual percepts, is intriguing. Similarly, investigating whether an extended chain of prior associations or associations established under high stress supports or intensifies conditioned hallucinations in animals used to model psychosis would also be useful to expand the boundaries of our understanding.

Insofar as taste hallucinations in rodents serve as a useful proxy to investigate the experience of hallucination in humans, our findings show that prior associative experience with odor and taste readily endow an odor with the ability to more strongly trigger taste-processing at the neural level in ketamine-exposed mice than in controls, and that hippocampal lesions in the ketamine-treated animals amplify dopamine hyper-responsiveness and further heighten the perceptual processing of an absent stimulus akin to a hallucination.

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Conflict of interest The authors claim that there are no conflicts of interest.

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