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Sex-Dependent Attentional Impairments in a Subchronic Ketamine Mouse Model for Schizophrenia

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ABSTRACT

BACKGROUND: The development of more effective treatments for schizophrenia targeting cognitive and negative symptoms has been limited, partly due to a disconnect between rodent models and human illness. Ketamine administration is widely used to model symptoms of schizophrenia in both humans and rodents. In mice, subchronic ketamine treatment reproduces key dopamine and glutamate dysfunction; however, it is unclear how this translates into behavioral changes reflecting positive, negative, and cognitive symptoms.

METHODS: In male and female mice treated with either subchronic ketamine or saline, we assessed spontaneous and amphetamine-induced locomotor activity to measure behaviors relevant to positive symptoms, and used a touchscreen-based progressive ratio task of motivation and the rodent continuous performance test of attention to capture specific negative and cognitive symptoms, respectively. To explore neuronal changes underlying the behavioral effects of subchronic ketamine treatment, we quantified expression of the immediate early gene product, c-Fos, in key corticostriatal regions using immunofluorescence.

RESULTS: We showed that spontaneous locomotor activity was unchanged in male and female subchronic ketamine–treated animals, and amphetamine-induced locomotor response was reduced. Subchronic ketamine treatment did not alter motivation in either male or female mice. In contrast, we identified a sex-specific effect of subchronic ketamine on attentional processing wherein female mice performed worse than control mice due to increased nonselective responding. Finally, we showed that subchronic ketamine treatment increased c-Fos expression in prefrontal cortical and striatal regions, consistent with a mechanism of widespread disinhibition of neuronal activity.

CONCLUSIONS: Our results highlight that the subchronic ketamine mouse model reproduces a subset of behavioral symptoms that are relevant for schizophrenia.

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Schizophrenia is a poorly treated syndrome associated with positive (psychosis involving hallucinations and/or delusions), negative (flat affect, avolition, social withdrawal), and cognitive (deficits in memory, attention, executive function) symptoms (1). Current treatments are only partially effective against positive symptoms and do not meaningfully improve negative or cognitive symptoms, which are associated with poor functional outcomes. Development of more effective medicines has been somewhat limited by a lack of translation between preclinical and clinical efficacy, likely due to animal models that do not reflect key pathophysiology and behavioral tests that do not align with clinical constructs. Therefore, identifying models with relevant pathophysiology by translational measures may be key to improving preclinical predictions of clinical efficacy.

Animal models for psychiatric disorders are limited in that disorders such as schizophrenia are complex, heterogeneous, and found exclusively in humans; nonetheless, they are essential tools for probing key aspects of pathophysiology and provide a critical stepping stone to clinical studies in the development of novel therapeutics. Animal models of schizophrenia are often evaluated for face and construct validity, i.e., how well behavioral dysfunction reflects the positive, negative, and cognitive symptoms seen in schizophrenia and whether these deficits are the result of neurochemical and structural alterations that are also present in the brains of people with schizophrenia. There is increasing evidence that NMDA receptor antagonists (e.g., ketamine, phencyclidine) meet a number of criteria for both face and construct validity (2). In humans, ketamine produces significant increases in positive and negative symptoms assessed by clinical rating scales alongside deficits in episodic memory, semantic memory, working memory, attention, and executive function (3,4). Glutamate dysregulation in schizophrenia has been proposed to occur via NMDA receptor hypofunction on fast-spiking parvalbumin-expressing GABAergic (gamma-aminobutyric acidergic) interneurons in the cortex and hippocampus,

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resulting in disinhibition of glutamate release from pyramidal neurons (5,6). This hypothesis is consistent with the effect of NMDA receptor antagonists, which concurrently decrease activity of GABAergic interneurons while increasing activity of pyramidal neurons (7,8). Downstream effects of NMDA antagonists include disinhibition of midbrain dopaminergic neurons, therefore providing a mechanism for increased striatal presynaptic dopamine function—one of the most robust dopaminergic phenotypes in schizophrenia (9,10). This notable face and construct validity in humans provides a translational link to the behavioral and neurobiological effects of NMDA receptor antagonists in rodents.

In mice, it was recently shown that subchronic ketamine treatment increased presynaptic dopamine function, which was dependent on parvalbumin-expressing GABAergic interneurons in the prefrontal cortex and hippocampus, and activity of midbrain dopaminergic neurons (11). These findings are consistent with both dopamine and glutamate dysfunction in people with schizophrenia and, importantly, were determined by methods consistent with those used clinically. Together, this evidence suggests that the effects of subchronic ketamine treatment in mice may be driven by similar circuit mechanisms that are disrupted in schizophrenia. Behaviors consistent with positive (12,13), cognitive (14–16), and some negative (14,17,18) symptoms have been reported in subchronic ketamine–treated mice; however, significant differences in dosing regimens between studies (ranging from 0.3 mg/kg for 7 days to 100 mg/kg for 10 days) limits the generalizability of such findings. Furthermore, the use of preclinical tests that have little in common with tests used in a clinical setting may provide misleading indications of face validity. Therefore, it is unclear whether the dosing regimen that increases presynaptic dopamine function similarly reflects schizophrenia-relevant behaviors.

Building on the aforementioned construct validity, in this study, we characterized behaviors reflective of positive, negative, and cognitive symptoms in male and female subchronic ketamine–treated mice. To benchmark this model against existing models of positive symptoms, we first assessed spontaneous and amphetamine-induced locomotor activity (LMA). We then used the rodent touchscreen system, which offers a translational platform with better alignment of preclinical and clinical test constructs, to assess attention and motivation, which are key aspects of cognitive and negative symptoms, respectively (19). Finally, we quantified c-Fos expression in key nodes of corticostriatal circuitry to understand the effect of subchronic ketamine treatment on neuronal activity. Our results showed that subchronic ketamine treatment leads to sex-dependent effects on attention while globally affecting behavior relevant to positive symptoms in schizophrenia.

METHODS AND MATERIALS

Animals

Male and female C57BL/6J (n = 24 female, n = 24 male) mice were obtained from the Animal Resource Centre in Perth, Australia. Animals were housed in open-top cages under a reverse 12-hour light/dark cycle (lights off at 8:00 AM). At 8 weeks of age, animals were dosed with ketamine (30 mg/kg intraperitoneal injection in 5 mL/kg dose volume) or saline once daily around midday for 5 consecutive days. Spontaneous LMA and touchscreen behavioral testing was conducted during the active dark phase. For amphetamine-induced LMA only, animals were switched to a normal 12-hour light/dark cycle (lights on at 8:00 AM) and acclimatized for 2 weeks before testing was conducted in the light phase. This was to ensure a greater window (20,21) to detect both increases and decreases in amphetamine-induced LMA and was based on our previous work (22). Food and water were provided ad libitum, except as specified in touchscreen testing. All procedures were conducted under the approval of The Florey Institute of Neuroscience and Mental Health Animal Ethics Committee (21-061-FINMH).

Locomotor Activity

For spontaneous activity, saline- and ketamine-treated mice (n = 12 female, n = 12 male; age 9 weeks) were placed in 27.5 cm × 27.5 cm acrylic glass chambers with 16 infrared beams, and spontaneous activity was recorded for 60 minutes. Ambulatory distance was binned in 5-minute intervals.

For amphetamine-induced activity, saline- and ketamine-treated mice (n = 12 female, n = 12 male, age 24 weeks) were placed in the same acrylic glass chambers, and spontaneous activity was recorded for 30 minutes. All animals were then dosed with dextroamphetamine (2.2 mg/kg in a 5 mL/kg dose volume; intraperitoneal injection) and returned to the testing chambers, where stimulant-induced activity was immediately recorded for an additional 60 minutes. Ambulatory distance was binned in 5-minute intervals.

Touchscreen Apparatus

The touchscreen automated system (Campden Instruments Ltd.) was used as previously described (Supplemental Methods) (20,21).

Behavioral Procedures

Male and female saline- and ketamine-treated animals (age, 9 weeks; saline: n = 12 female, n = 12 male; ketamine: n = 12 female, n = 12 male) were weighed for 3 consecutive days, then food restricted and maintained at 85% to 90% of their free-feeding weights. Animals were given access to a strawberry milk (Nippy's) reward for 2 days immediately prior to behavioral testing to minimize neophobia. The same cohort of animals was used for each task detailed below (Figure 1). Animals were placed back on free feeding between the rodent continuous performance test (rCPT) and progressive ratio (PR) testing to re-establish free-feeding baseline weights; then, prior to commencing PR touchscreen testing, animals were food restricted to 85% to 90% of their updated free-feeding weights.

Rodent Continuous Performance Test

Mice were first habituated to the testing chambers before progressing through four stages of rCPT training (further detail in the Supplemental Methods). In stage 1, animals were required to touch a white square presented in the central window of a 3-hole mask. If a correct touch occurred, the stimulus was removed, and 10 μL of strawberry milk was

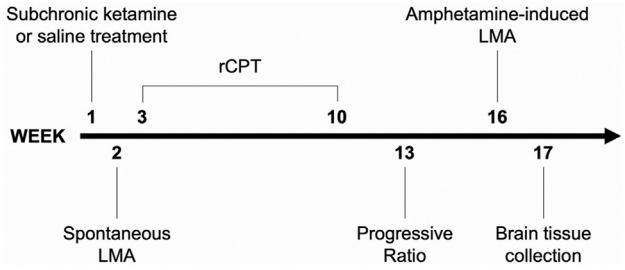

Figure 1. Experimental overview. At 8 weeks of age, male and female mice were dosed with saline or ketamine (30 mg/kg; intraperitoneal injection) once daily for 5 consecutive days (saline: female $n = 12$, male $n = 12$; ketamine: female $n = 12$, male $n = 12$). Spontaneous LMA was assessed 2 days following the last day of ketamine administration. Animals were subsequently food restricted and trained on the rCPT. Free-feeding weights were re-established before animals were food restricted again prior to testing on the progressive ratio task. Animals were placed back on free feeding, and then amphetamine-induced LMA was assessed. Brain tissue was collected following >1 week washout of amphetamine. Animals were housed under reversed light-dark housing conditions and behaviorally tested during the dark phase from experimental week 1 to 13. Subsequently, animals were housed under normal light-dark housing conditions and behaviorally tested during the light phase from experimental week 14 to 17 (acclimatization to lighting conditions occurred during weeks 14–15 with no behavioral testing). LMA, locomotor activity; rCPT, rodent continuous performance test.

dispensed from the reward magazine paired with a light and 1-second tone. Head entry into the reward magazine turned off the light and triggered a 2-second intertrial interval (ITI) before the next trial began with the presentation of a white square. The ITI counter was reset if a touch was made to the central window during this period. If no correct touch was made, the stimulus was removed, and an ITI began before the next trial. Each session ended at the first of either 100 rewards or 60 minutes. The criterion for stage 1 was 100 rewards per session, after which individual animals progressed to stage 2.

In stage 2, the target stimuli (horizontal or vertical black and white lines; counterbalanced across groups) were introduced. The target stimuli remained constant for all subsequent stages. Again, each session ended at the first of 100 rewards or 60 minutes, and criterion was defined as 100 rewards per session, after which individual animals progressed to stage 3.

In stage 3, a single noise nontarget stimulus (snowflake) was introduced and randomly presented on 50% of trials. If the nontarget stimulus was touched, the stimulus was removed, and a correction trial began after the ITI. In a correction trial, the nontarget stimulus was displayed, and trials were repeated until no response was made. The criterion for stage 3 was sensitivity (d′; see Data Analysis section) to target versus nontarget stimuli, which had to be >0.6, and animals had to achieve >80 rewards for two consecutive sessions. One male ketamine, one female saline, and one female ketamine animal did not reach the stage 3 criterion and were therefore excluded from subsequent stages.

In stage 4, the snowflake nontarget stimulus was replaced by four novel stimuli, again with a 50% probability of target stimuli presentation in each trial. In a correction trial, any of the four nontarget stimuli were presented. The criterion for this stage was $d' > 0.6$ and stable group performance; all other parameters remained unchanged.

Following stage 4, animals were tested on a stimulus duration probe in which the stimulus duration was reduced to increase the attentional requirements of the task. Stimuli were presented for 2, 1.5, 1, and 0.5 seconds in a mixed design for three consecutive sessions; all other parameters remained unchanged.

Progressive Ratio

Because animals had completed operant training during rCPT, minimal training was required for PR. Animals were first trained at a fixed ratio schedule of 1 (FR1), where they were required to touch a white square that was presented indefinitely in the central window of a 5-hole mask. Once a correct touch was made, the stimulus was removed, and 20 μL of strawberry milk was dispensed from the reward magazine paired with a light and 1-second tone. Animals were required to collect the reward before a new trial was initiated, with a 5-second ITI. The session ended at the first of 30 trials or 60 minutes; criterion was 30 trials per session. Animals were then trained at FR5, where 5 operant responses were required for a single reward. On nonreward responses, the stimulus was removed for 500 ms, and a 10-ms tone was played. Again, the session ended at the first of 30 trials or 60 minutes, and the criterion was 30 trials per session.

Upon completion of FR5, animals were tested on a PR schedule of 4 (PR4) where the number of responses required for a single reward increased linearly by 4 each trial (1, 5, 9, 14, etc.). Animals were tested for 3 consecutive days.

c-Fos Immunofluorescence

Following a 1-week washout after amphetamine-induced LMA assays, c-Fos immunofluorescence was assessed as described in the Supplemental Methods.

Data Analysis

Data recorded from rCPT experiments are hits (responses to target stimuli), misses (nonresponses to target stimuli), mistakes (responses to nontarget stimuli), and correct rejections (nonresponses to nontarget stimuli). From these parameters, hit rate and false alarm rate can be calculated as shown below:

$$\textit{Hit rate}\ (HR) = \frac{\textit{Hits}}{\textit{Hits} + \textit{misses}} \quad (1)$$

$$\textit{False alarm rate}\ (FAR) = \frac{\textit{False alarms}}{\textit{False alarms} + \textit{correct rejections}} \quad (2)$$

To provide further information about selective responding, composite measures of HR and FAR were calculated based on signal detection theory. Sensitivity, d′, refers to perceptual discrimination between the target and nontarget stimuli, where a higher d′ indicates better visual discrimination.

$$d' = z(HR) - z(FAR) \quad (3)$$

Response bias, or c, refers to the animals' willingness to make responses; a high c indicates conservative strategies while a low c indicates liberal responding.

$$c = -\frac{z(HR) + z(FAR)}{2} \quad (4)$$

For PR, the main measure of interest was the animals' breakpoint, which is the number of touches made during the last successfully completed trial.

For c-Fos immunofluorescence, the percentage of c-Fos–positive nuclei was determined by automated quantification using Fiji (https://fiji.sc/) (Supplemental Methods).

All statistical analyses were performed using GraphPad Prism 9 (GraphPad Software; https://www.graphpad.com/). LMA was analyzed by a three-way repeated-measures analysis of variance (ANOVA) (main effects of treatment, sex, and time). rCPT data were analyzed by three-way repeated-measures ANOVA (main effects of treatment, sex, and either session for stage 4 data or stimulus duration for probe data). PR data were analyzed by two-way ANOVA (main effects of treatment and sex). c-Fos data were analyzed by two-way ANOVA (main effects of treatment and sex) with Tukey's multiple comparisons test.

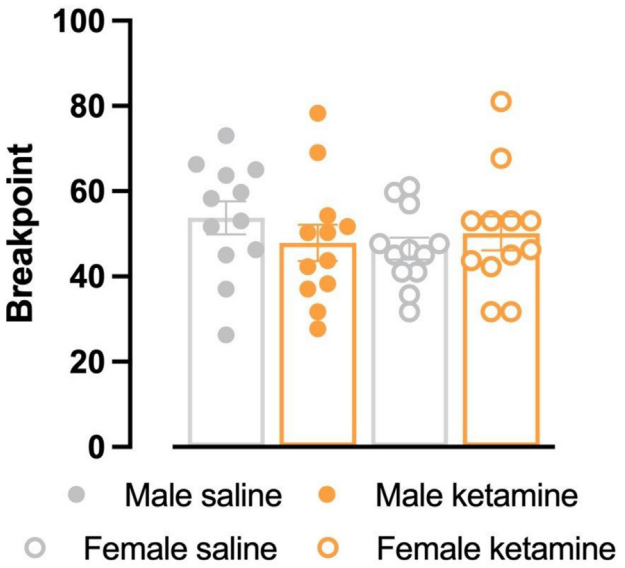

Figure 2. Subchronic ketamine treatment does not alter progressive ratio breakpoint. Two-way analysis of variance, treatment, $F_{1,44} = 0.09755$, $p = .7563$. Individual data points presented with mean ± SEM, $n = 12$.

RESULTS

Motivation Is Unchanged in Subchronic Ketamine–Treated Mice

Avolition is a core negative symptom in schizophrenia and has a direct impact on functional outcome (23). Given that motivational control is associated with striatal dopamine signaling, we investigated whether subchronic ketamine treatment may be a suitable model for motivational deficits in schizophrenia (24). We used a touchscreen-based PR task to assess the willingness to respond for a reward as the response effort requirements increased. Breakpoint, or the number of responses at which the subject stops responding, provides a measure of motivational processing that encompasses the reinforcing properties of the reward and the point at which the effort outweighs the benefit of obtaining that reward. When we assessed responding on the PR task, we found no differences in breakpoint between saline- and ketamine-treated mice (Figure 2, no effect of treatment or sex), indicating that subchronic ketamine did not affect motivational processing.

Subchronic Ketamine Treatment Reduces Amphetamine-Induced Locomotor Response

Assessing the positive symptoms observed in individuals with schizophrenia is challenging in rodents. Increased LMA—in response to novelty, stress, or psychostimulants—has traditionally been used because it reflects changes in striatal dopamine linked to psychosis (25). Therefore, we next investigated the effect of subchronic ketamine treatment on both spontaneous and amphetamine-induced LMA. All animals showed similar levels of spontaneous LMA, habituating as expected to the novel environment over the 60-minute testing period with no significant differences (Figure 3A). We hypothesized that subchronic ketamine treatment would alter the sensitivity to amphetamine-induced LMA due to increased striatal presynaptic dopamine function (11). We found that subchronic ketamine treatment significantly attenuated amphetamine-induced (2.2 mg/kg; intraperitoneal injection) hyperactivity in both male and female mice (Figure 3B). These results suggest that subchronic ketamine treatment might not affect basal extracellular dopamine, but may alter activity-dependent regulation of phasic dopamine release (25,26).

Experimental differences in measuring spontaneous and amphetamine-induced LMA are worth noting. Spontaneous activity was assessed at 9 weeks of age in the dark phase, while amphetamine-induced activity was assessed at 24 weeks of age in the light phase; both age and circadian light/dark cycle can affect spontaneous LMA (27,28). To determine whether there was a potential shift in baseline activity between these experimental conditions, we compared spontaneous LMA from 0 to 30 minutes in both conditions. We found a significant effect of light cycle on LMA (Figure S2) ($F_{1,44} = 7.118$, $p = .0106$), consistent with previous reports (28), but importantly did not see an interaction between subchronic ketamine treatment and light cycle (Figure S2) ($F_{1,44} = 1.446$, $p = .2355$). Together, these findings suggest that the effect of subchronic ketamine treatment on spontaneous versus amphetamine-induced LMA was not driven by differences in light cycle.

Subchronic Ketamine Treatment Impairs Attentional Processing in Female but Not Male Mice

Attention is a core cognitive domain that is impaired in schizophrenia and critically relies on the integrity of prefrontal cortical function (29,30). Therefore, next we wanted to determine whether a disinhibition of cortical microcircuitry by ketamine (5) would translate into attentional deficits in a rodent version of the continuous performance test (CPT), which is commonly used to assess attention in humans (29).

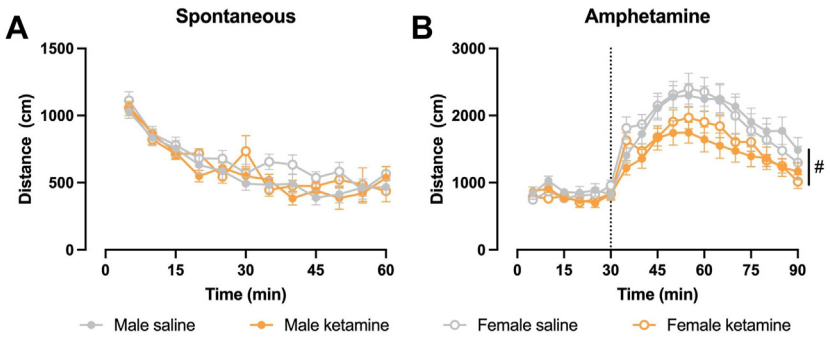

Figure 3. Effect of subchronic ketamine treatment on spontaneous and amphetamine-induced locomotor activity. Subchronic ketamine treatment has **(A)** no effect on spontaneous locomotor activity (three-way repeated-measures analysis of variance, treatment, $F_{1,56}$ = 0.5622, p = .4565), but **(B)** significantly attenuates amphetamine-induced locomotor activity (three-way repeated-measures analysis of variance, treatment, $F_{1,41}$ = 7.210, p = .0104). Main effect of treatment #$p < .05$. All data shown as mean ± SEM, n = 12–15 per group.

All animals successfully completed the earlier rCPT training stages (stages 1–3), and there were no significant differences by treatment or sex (Figure S1). In the last training stage (stage 4), mice were introduced to four novel nontarget stimuli and one target stimulus, thus requiring attentional processing for selective responding. The primary measure of human CPT performance is d′, which reflects sensitivity to accurately detect the target stimulus; therefore, we first interrogated the effect of subchronic ketamine treatment on this measure. While there was no overall effect of subchronic ketamine treatment on d′ in stage 4, we observed a significant interaction between treatment, session, and sex wherein performance of female subchronic ketamine mice plateaued at a lower level compared with female saline mice (Figure 4A and Table 1). To examine this more closely when animals were performing at an established level, we analyzed the average performance during the later sessions of stage 4 (sessions 10–12). Here, we found that female ketamine-treated mice had a significantly lower d′ than female saline-treated mice, while no difference was observed for males (Figure 4B). Once stable performance on stage 4 had been established, mice were tested on probe sessions where the stimulus duration of trials within a session was altered (2 seconds, 1.5 seconds, 1 second, 0.5 second) to measure performance under increased attentional demands. We found that the sex-dependent effect of subchronic ketamine treatment on d′ was exacerbated at lower stimulus durations, where male saline- and ketamine-treated animals converged at 0.5 second, but the difference between female mice was maintained (Figure 4C and Table 1).

The lower d′ observed in female subchronic ketamine–treated mice may reflect an inability to discriminate target from nontarget stimuli, or impaired response inhibition to nontarget stimuli. We therefore analyzed measures of HR (proportion of correct responses to target stimuli) and FAR (proportion of inappropriate responses to nontarget stimuli) that contribute to d′, and c (i.e., response bias), which provides a measure of an animal's strategy to respond in a conservative (high c) or liberal (low c) manner. Ketamine treatment had no effect on response criterion or HR during either stage 4 or the stimulus duration probe (Figure 5A–D; Table 1). However, we found that FARs were altered, indicating that this measure was driving the observed changes in d′ or response sensitivity to accurately detect the target stimulus. During the later sessions of stage 4 training, female subchronic ketamine mice had a higher FAR (making more inappropriate responses to nontarget stimuli) than female saline mice, while no difference

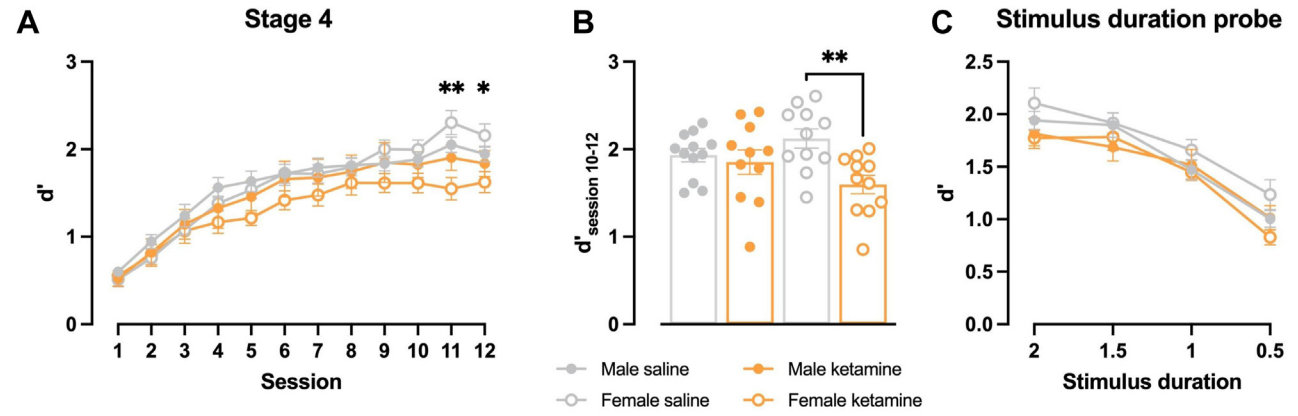

Figure 4. Subchronic ketamine treatment produces selective attentional impairments in female, but not male, mice. Effect of subchronic ketamine treatment on d′ in **(A)** stage 4 (two-way repeated-measures analysis of variance with Tukey's multiple comparisons test, female saline vs. female ketamine, session 11 q = 5.684, df = 18.62; **p = .0039, session 12 q = 4.205, df = 18.47; *p = .0366), **(B)** sessions 10 to 12 of stage 4 (two-way analysis of variance with Tukey's multiple comparisons test, female saline vs. female ketamine q = 4.789, df = 41; **p = .0082) and **(C)** stimulus duration probe (three-way repeated-measures analysis of variance, treatment, $F_{1,41}$ = 3.084, p = .0865). All data shown as mean ± SEM, n = 11–12.

Table 1. Rodent Continuous Performance Test Repeated-Measures Three-Way Analysis of Variance Summary

Factor	Hit Rate	False Alarm Rate	Sensitivity (d′)	Response Bias (c)
Stage 4				
Session	$F_{6.835,277.8} = 50.38$, $p < .0001$[a]	$F_{5.693,231.3} = 62.20$, $p < .0001$[a]	$F_{1,41} = 0.6195$, $p < .0001$[a]	$F_{6.641,269.9} = 6.916$, $p < .0001$[a]
Sex	$F_{1,41} = 0.2783$, $p = .6007$	$F_{1,41} = 0.2170$, $p = .6438$	$F_{1,41} = 0.6195$, $p = .4357$	$F_{1,41} = 0.01287$, $p = .9102$
Treatment	$F_{1,41} = 1.075$, $p = .3059$	$F_{1,41} = 0.9169$, $p = .3439$	$F_{1,41} = 2.828$, $p = .1002$	$F_{1,41} = 0.003779$, $p = .9513$
Session × sex	$F_{11,447} = 0.4095$, $p = .9519$	$F_{11,447} = 1.539$, $p = .1146$	$F_{11,447} = 0.6697$, $p = .7676$	$F_{11,447} = 1.483$, $p = .1346$
Session × treatment	$F_{11,447} = 0.6385$, $p = .7959$	$F_{11,447} = 3.081$, $p = .0005$[a]	$F_{11,447} = 2.274$, $p = .0105$[a]	$F_{11,447} = 1.996$, $p = .0272$[a]
Sex × treatment	$F_{1,41} = 0.05887$, $p = .8095$	$F_{1,41} = 0.7359$, $p = .3960$	$F_{1,41} = 0.6293$, $p = .4322$	$F_{1,41} = 0.4782$, $p = .4932$
Session × sex × treatment	$F_{11,447} = 1.090$, $p = .3678$	$F_{11,447} = 2.043$, $p = .0233$[a]	$F_{11,447} = 2.882$, $p = .0011$[a]	$F_{11,447} = 1.590$, $p = .0985$
Stimulus Duration Probe				
Stimulus duration	$F_{2.306,94.54} = 312.1$, $p < .0001$[a]	$F_{2.764,113.3} = 3.376$, $p = .0239$[a]	$F_{2.916,119.6} = 165.3$, $p < .0001$[a]	$F_{2.362,96.83} = 120.4$, $p < .0001$[a]
Sex	$F_{1,41} = 0.4718$, $p = .4960$	$F_{1,41} = 0.8167$, $p = .3714$	$F_{1,41} = 0.2652$, $p = .6094$	$F_{1,41} = 0.4149$, $p = .5231$
Treatment	$F_{1,41} = 0.04406$, $p = .8348$	$F_{1,41} = 5.736$, $p = .0213$[a]	$F_{1,41} = 3.084$, $p = .0865$	$F_{1,41} = 1.621$, $p = .2101$
Stimulus duration × sex	$F_{3,123} = 0.4760$, $p = .6996$	$F_{3,123} = 0.1386$, $p = .9368$	$F_{3,123} = 0.08577$, $p = .9677$	$F_{3,123} = 0.4298$, $p = .7320$
Stimulus duration × treatment	$F_{3,123} = 0.8451$, $p = .4717$	$F_{3,123} = 0.1803$, $p = .9096$	$F_{3,123} = 1.039$, $p = .3778$	$F_{3,123} = 0.8629$, $p = .4624$
Sex × treatment	$F_{1,41} = 0.3561$, $p = .5540$	$F_{1,41} = 0.6481$, $p = .4254$	$F_{1,41} = 1.063$, $p = .3087$	$F_{1,41} = 0.004827$, $p = .9450$
Stimulus duration × sex × treatment	$F_{3,123} = 1.368$, $p = .2559$	$F_{3,123} = 0.6660$, $p = .5745$	$F_{3,123} = 2.714$, $p = .0478$[a]	$F_{3,123} = 0.2509$, $p = .8606$

[a]$p < .05$.

was found in male mice (Figure 5E, G; Table 1). In the stimulus duration probe, we found a significant effect of treatment on FARs, with both male and female ketamine-treated animals having a higher FAR than saline control animals (Figure 5F; Table 1). Together, these results suggest that female mice displayed greater sensitivity to the effects of subchronic ketamine on attentional processing, specifically impairing response inhibition.

Subchronic Ketamine Treatment Increases c-Fos Expression in Key Corticostriatal Regions

Attentional and motivational processes have been linked to corticostriatal circuitry involving infralimbic, prelimbic, and anterior cingulate areas of the prefrontal cortex, and dorsolateral, dorsomedial, and ventral functional subdivisions of the striatum (31,32). To investigate whether the behavioral effects of subchronic ketamine treatment would be associated with changes to neuronal activity in these regions, we quantified expression of the immediate early gene c-Fos using immunofluorescence. A significant treatment effect was found for all prefrontal cortical regions (anterior cingulate, prelimbic, and infralimbic), where c-Fos expression was greater in subchronic ketamine–treated animals than in saline control animals (Figure 6A–C and Figure S3). This effect was more pronounced in female mice, with significant differences between female saline- and ketamine-treated animals in prelimbic and anterior cingulate areas (Figure 6A, B). In striatal regions, subchronic ketamine treatment also produced a marked increase in c-Fos in the dorsomedial striatum (Figure 6E) and ventral striatum (Figure 6F), but no significant treatment effect was observed in the dorsolateral striatum (Figure 6D), where c-Fos levels were the lowest. Together, these findings demonstrate that subchronic ketamine treatment produced long-lasting changes (>4 months after treatment) to neuronal activity in regions implicated in attentional and motivational control, consistent with widespread disinhibition of glutamatergic activity (7). Although the animals used in the current study underwent behavioral testing prior to c-Fos quantification, expression was consistent with that reported in other studies with varying subchronic dosing regimens (33,34).

DISCUSSION

Building on the recent finding that subchronic ketamine treatment increases presynaptic dopamine function in mice via schizophrenia-relevant circuitry, we investigated how this might translate into behavioral changes reflecting select positive, negative, and cognitive symptoms of schizophrenia (11). We found that subchronic ketamine treatment had no effect on motivation in a touchscreen PR task (Figure 2). However, we also found that subchronic ketamine treatment reduced amphetamine-induced LMA in both male and female mice in the absence of any effect on spontaneous activity (Figure 3). Furthermore, we saw selective sex-dependent impairments in attention in a touchscreen-based rCPT (Figures 4 and 5). At a cellular level, subchronic ketamine increased c-Fos expression in key corticostriatal regions, highlighting long-lasting neural changes (Figure 6). Broadly, this work highlights the challenges of using animal models to reproduce a complex and heterogeneous human disorder. Despite the widespread application of ketamine treatment, and NMDA receptor antagonists more broadly, in rodents to induce behavioral and neurobiological dysfunction relevant to schizophrenia, we showed here that subchronic ketamine treatment recapitulated only select deficits in mice.

We found that subchronic ketamine impaired attention in female, but not male, mice, an effect that was driven by impaired response selection to nontarget stimuli (Figures 4B

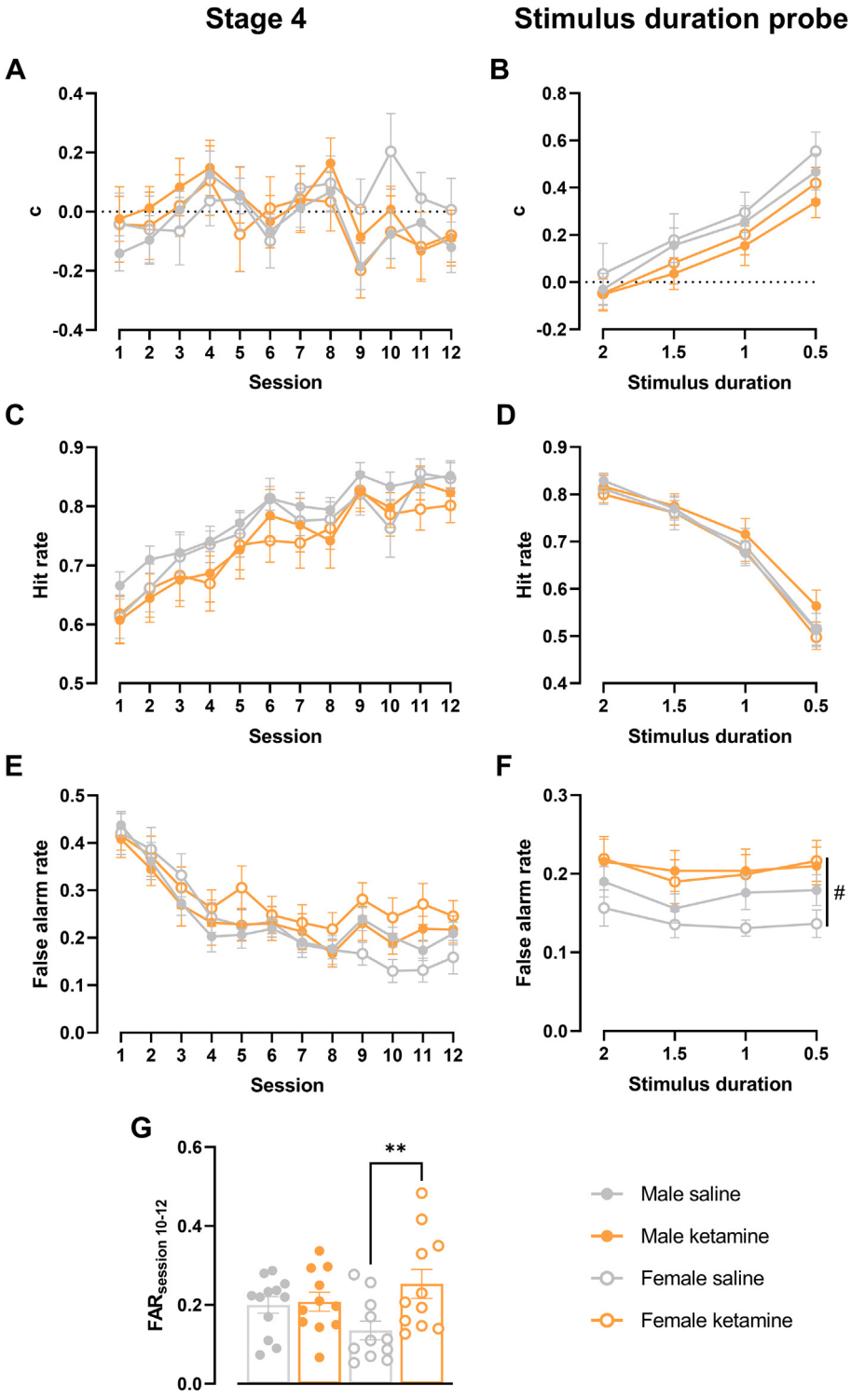

Figure 5. Attentional impairments in female subchronic ketamine–treated mice are driven by increased FAR. Effect of subchronic ketamine treatment on stage 4 **(A)** c, **(C)** hit rate, **(E)** FAR, and **(G)** FAR from sessions 10 to 12; and stimulus duration probe **(B)** c, **(D)** hit rate, and **(F)** FAR. Data analyzed by three-way analysis of variance, treatment #$p < .05$, or two-way analysis of variance with Tukey's multiple comparisons test **$p < .01$. All data shown as mean ± SEM, n = 11–12. FAR, false alarm rate.

and 5G). Previous work investigating attentional impairments produced by ketamine in rodents has predominantly used the 5-choice serial reaction time task (5-CSRTT), the primary measure of which is accuracy (i.e., HR in rCPT) (35). Both acute and subchronic ketamine treatment in male animals has no effect on accuracy in 5-CSRTT (36–39), consistent with the

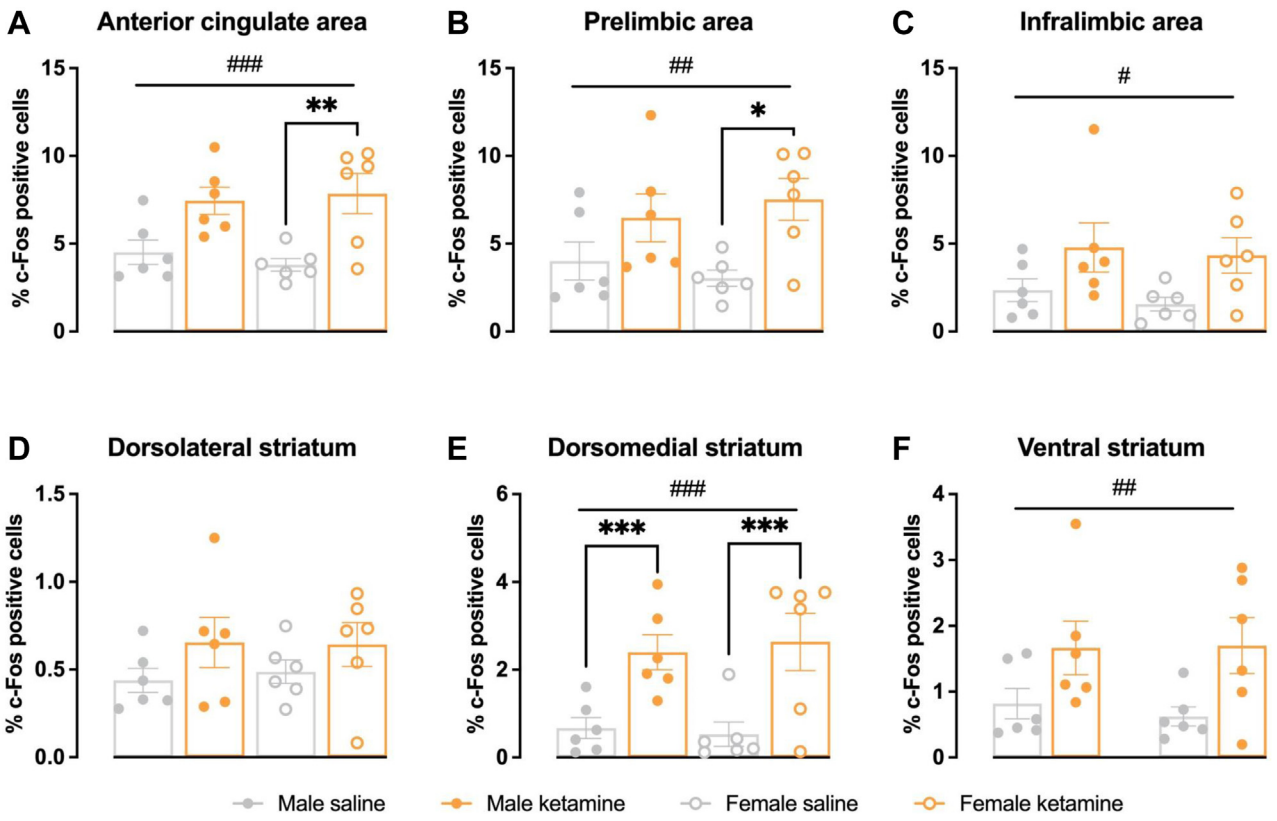

Figure 6. Effect of subchronic ketamine treatment on c-Fos expression in key regions of corticostriatal circuitry. Prefrontal cortical regions **(A)** anterior cingulate area (treatment, $F_{1,20} = 19.33$, $p = .0003$), **(B)** prelimbic area (treatment, $F_{1,20} = 10.30$, $p = .0044$), and **(C)** infralimbic area (treatment, $F_{1,20} = 7.640$, $p = .0120$); **(D)** dorsolateral (treatment, $F_{1,20} = 3.067$, $p = .0952$), **(E)** dorsomedial (treatment, $F_{1,20} = 20.46$, $p = .0002$), and **(F)** ventral (treatment, $F_{1,20} = 8.796$, $p = .0076$) functional subdivisions of the striatum. Data analyzed by two-way analysis of variance with Tukey's multiple comparisons test. Main effect of treatment #$p < .05$, ##$p < .01$, ###$p < .001$. Multiple comparisons *$p < .05$, **$p < .01$, ***$p < .001$. Individual data points presented with mean ± SEM, $n = 6$.

results from the rCPT reported herein (Figure 5C, D). However, the exclusion of female animals in these studies means that any sex-dependent effects of ketamine on 5-CSRTT performance are unclear. With respect to rCPT performance in other rodent models for schizophrenia, female subchronic ketamine–treated mice resemble *Gpr88* knockout and MAM-E17 models, where a lower d′ is driven by an increased FAR [M. Langiu, Ph.D., *et al*., unpublished data, November 2017; (40)]. In contrast, attentional deficits in a 22q11.2 microdeletion model are driven by a lower HR (41). Given that d′ is the primary measure reported in human CPT studies, it is unclear whether there are similar subtypes of attentional deficits in people with schizophrenia and if this has a consequence for treatment response.

There have been mixed reports of sex differences in attention in people with schizophrenia (42–48). Interestingly, we found that attentional impairments produced by subchronic ketamine treatment were greatest in female mice, while no sex-dependent effects were observed on motivation or amphetamine-induced LMA. Female rodents have greater sensitivity to the antidepressant effects of ketamine (49,50); therefore, it stands to reason that at higher doses in a subchronic dosing regimen, they may also be more sensitive to the detrimental effects on cognition, as seen in rCPT (Figures 4 and 5). Indeed, one study found that subchronic ketamine treatment (10 mg/kg; 21 days) produced beneficial antidepressant-like effects in male mice but increased anxiety- and depressive-like behavior in female mice (51). The differential behavioral effects in male versus female rodents are likely related to sex differences in ketamine metabolism and pharmacokinetics. In adolescent and adult rats, the ketamine C_{max} is significantly higher in the medial prefrontal cortex, dorsal striatum, and hippocampus of females than males (52,53). Plasma concentration of ketamine in males versus females is the subject of conflicting reports; however, this may be related to species and strain differences (53–55). Furthermore, ketamine is metabolized predominantly to (2*R*,6*R*;2*S*,6*S*)-HNK in female, but norketamine in male mice (55). Together, these findings highlight varying levels of ketamine and ketamine metabolite exposure in male and female rodents that may underlie the sex-dependent effects of subchronic ketamine treatment on attention.

Dopamine signaling, particularly in the ventral striatum, is heavily implicated in reward- and motivation-related pathways (56). In PR tasks, both D_1 and D_2 antagonists reduce motivation, indexed as a lower breakpoint, while inhibiting dopamine reuptake or increasing dopamine release increases willingness to respond for a reward (57–59). Despite this, we found no link

between the dopaminergic phenotype of subchronic ketamine–treated animals, breakpoint in a PR task of motivation, or c-Fos expression in the ventral striatum. Perhaps the most obvious limitation of the current study is that we were unable to assess presynaptic dopamine function; therefore, it is possible that we did not replicate the dopaminergic phenotype. Nevertheless, there is little evidence in humans for associations between presynaptic dopamine function and behaviors relating to reward and motivation (60–63).

While interview-based clinical rating scales used to assess symptom severity in people with schizophrenia are not feasible in animal models, one component of positive symptoms, namely increased striatal dopamine that is linked to psychosis, is commonly assessed in rodents by measuring increased LMA in response to novelty, stress, or psychostimulants (64). In particular, rodent hyperactivity in a novel environment has typically been attributed to the manifestation of psychomotor agitation in people with schizophrenia (65). While we report that subchronic ketamine treatment did not induce this symptom (Figure 3A), the lack of effect on spontaneous activity may be useful. Hyperactivity can be a confounding factor in rodent models for schizophrenia, where it is unclear if poor performance on a task is specifically due to cognitive dysfunction driven by changes to relevant circuitry, or an inability to appropriately engage in the task due to hyperactivity. Notwithstanding, the differential response of subchronic ketamine– and saline–treated animals to amphetamine points to maladaptive changes in striatal dopamine signaling, consistent with positive symptoms in schizophrenia. Amphetamine acts directly at presynaptic terminals in the striatum to release dopamine via actions at dopamine and vesicular monoamine transporters (66). The smaller locomotor response in subchronic ketamine–treated animals (Figure 3B) may be due to a paradoxical increase in amphetamine sensitivity, given the effects of subchronic ketamine treatment on presynaptic dopamine content (11,67) and an inverted U-shaped dose-response relationship, as has been reported in other models of increased striatal dopamine function (68,69).

The prefrontal cortex has an established role in attention and response control in both humans and rodents (31,70). In particular, lesions of the anterior cingulate cortex and prelimbic area in rodents significantly impair attention in rCPTs (31,71).Therefore, we wondered whether subchronic ketamine treatment might preferentially disrupt neural activation (measured using the activity marker c-Fos) in the prefrontal cortex more than striatal regions given the attentional deficits observed. Interestingly, we found that c-Fos expression was broadly increased following subchronic ketamine treatment in both female and male mice in prefrontal and striatal regions (Figure 6). While these differences were only statistically significant in females, the pattern was consistent in both sexes and regions, likely reflecting the variability in the data rather than a lack of effect. Indeed, in rats it has been shown that the ventromedial (including prelimbic and infralimbic areas) and dorsomedial (including the anterior cingulate) prefrontal cortices have distinct patterns of activation during a task of sustained attention and that silencing of each region during various stages of the task produces differential effects (72). Importantly, we acknowledge that our study provides only a snapshot of neuronal activity at the end of testing on different behavioral tasks and thus does not capture dynamic information either in behaviorally naïve animals or during performance on one task. Despite this, our findings do demonstrate that subchronic ketamine treatment produces long-lasting changes (>4 months after treatment) to neuronal activity in prefrontal and striatal regions, which is relevant for understanding the cognitive changes.

In conclusion, we demonstrated that our particular subchronic ketamine mouse model produced only a subset of behavioral symptoms relevant to those observed in schizophrenia, despite reproducing a key dopaminergic phenotype in people with schizophrenia (11). In particular, female, but not male, subchronic ketamine–treated mice may be an appropriate model of impaired attention, while neither sex reproduced motivational deficits. Animal models are limited but remain necessary tools for probing pathophysiological mechanisms underlying schizophrenia, and identifying how novel treatments may work to improve symptoms in people with schizophrenia. There has been an evolution in the way that we view animal models for psychiatric disorders. We perhaps held unrealistic expectations that a single animal model would recapitulate the spectrum of symptoms observed in schizophrenia. However, it is clear that establishing a toolbox of animal models validated against specific features, which together represent the spectrum of symptoms observed in schizophrenia, is the more realistic and tractable approach. Our findings raise important considerations about how we evaluate the validity of animal models and question what a gold standard model might look like given the enormous heterogeneity in symptoms observed clinically across individuals with schizophrenia. In this light, a manipulation that produces only some behavioral and neurobiological features of schizophrenia is not necessarily a bad model, as long as the limitations are acknowledged and outcomes are not overgeneralized. Therefore, there is a continued need for more comprehensive assessment and detailed reporting of behavioral and neurobiological features in any given animal model. The current work has begun to define the distinct profile of behaviors that are disrupted by subchronic ketamine treatment in mice.

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