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Ketamine facilitates extinction of avoidance behavior and enhances synaptic plasticity in a rat model of anxiety vulnerability: Implications for the pathophysiology and treatment of anxiety disorders

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ABSTRACT

Anxiety disorders and posttraumatic stress disorder (PTSD) share a common feature of pathological avoidance behavior. The Wistar Kyoto (WKY) rat has been used as a model of anxiety vulnerability, expressing a behaviorally inhibited temperament, acquiring avoidance behavior more rapidly and displaying extinction-resistant avoidance compared to Sprague Dawley (SD) rats. Subanesthetic levels of ketamine have gained attention as a rapid antidepressant in treatment-resistant depression. While traditional antidepressants are commonly used to treat anxiety disorders and PTSD, the therapeutic utility of ketamine for these disorders is much less understood. The hippocampus is critical for the actions of antidepressants, is a structure implicated in anxiety disorders and PTSD, and is necessary for extinction of avoidance in SD rats. WKY rats have impaired hippocampal long-term potentiation (LTP), suggesting that persistent avoidance in WKY rats may be due to deficient hippocampal synaptic plasticity. In the present study, we hypothesized that ketamine would facilitate extinction of avoidance learning in WKY rats, and do so by enhancing hippocampal synaptic plasticity. As predicted, ketamine facilitated extinction of avoidance behavior in a subset of WKY rats (responders), with effects lasting at least three weeks. Additionally, LTP in these rats was enhanced by ketamine. Ketamine was not effective in facilitating avoidance extinction or in modifying LTP in WKY non-responders. The results suggest that subanesthetic levels of ketamine may be useful for treating anxiety disorders by reducing avoidance behaviors when combined with extinction conditions. Moreover, ketamine may have its long-lasting behavioral effects through enhancing hippocampal synaptic plasticity.

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1. Introduction

The lifetime prevalence for anxiety disorders is a staggering 29% (Bandelow et al., 2012). A key feature of anxiety and anxiety-related disorders such as posttraumatic stress disorder (PTSD) is pathological avoidance. Avoidance behavior can be healthy and help facilitate harm reduction by preventing life-threatening or risky situations. However, dysfunction can result when avoidance is taken to an excessive level (Krypotos et al., 2015). Pathological avoidance predicts the severity of anxiety and extent to which individuals will be resistant to treatment (Foa et al., 2006; Karamustafalioglu et al., 2006).

Antidepressants are considered the first line of pharmacological therapy in treating both anxiety and depression (Bystritsky et al., 2013). Approximately 30% of patients will be refractory to treatment (Bystritsky, 2006; Al-Harbi, 2012), demonstrating a need for alternative treatment options to current antidepressants. Ketamine, a non-competitive NMDA receptor (NMDAR) antagonist, has garnered recent attention for its ability to act as a rapid antidepressant and its efficacy in treatment-resistant individuals when administered at subanesthetic doses in humans and rodents (Zarate et al., 2006; Autry et al., 2011; Gideons et al., 2014; Haile et al., 2014; Choi et al., 2015). In humans, a single intravenous infusion of a subanesthetic dose of ketamine exerts antidepressant actions in as little as 2 h, with a peak response at 24 h, and a persistent response

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in 35% of subjects at one week (Zarate et al., 2006). Interestingly, ketamine's effects are not universal and only approximately 50% of all patients receiving ketamine will have a clinically effective response to ketamine, resulting in the classification of ketamine responders and ketamine non-responders (Haile et al., 2014; Wilkinson et al., 2017; Diazgranados et al., 2010; Moaddel et al., 2015). However, the reason why some individuals respond to ketamine and other do not is still unclear. Repeated dosing can prolong the effectiveness of ketamine in responders to at least two weeks, suggesting that long-term effects of ketamine are possible for a subset of patients (Murrough et al., 2013; Aan Het Rot et al., 2010; Singh et al., 2016). Because its actions differ considerably from traditional antidepressants, understanding the long-term effects of ketamine in anxiety disorders, its mechanisms and how the response to ketamine differs at the neurobiological level in responders and non-responders will be critical for developing novel, efficacious anxiolytics.

Like humans, ketamine has rapid and long-lasting effects in animal models of depression. In mice, a single injection of ketamine shows antidepressant effects as early as 30 min and persists for at least one week whereas traditional antidepressants such as imipramine and fluoxetine show no evidence of a rapid antidepressant effect (Autry et al., 2011). Thus, ketamine has the unique ability to act as a rapid therapeutic compared to traditional antidepressant medications. The antidepressant actions of ketamine may be through glutamatergic receptors. Blocking NMDA receptor currents results in a desuppression of protein translation by inhibiting eukaryotic elongation factor 2 kinase (eEF2k) (Nosyreva et al., 2013), leading to an increase in brain-derived neurotrophic factor (BDNF) and increased insertion of GluA1 and GluA2 subunits to facilitate AMPA receptor currents, both of which are important for synaptic plasticity (Bjorkholm and Monteggia, 2016; Nosyreva et al., 2013). Hippocampal synaptic plasticity is necessary for antidepressant actions (Nosyreva et al., 2013; Kanzari et al., 2017). Moreover, the long-term activation of AMPA receptors may be necessary and sufficient for the antidepressant actions of ketamine (Zanos et al., 2016; Suzuki et al., 2017).

There is currently a dearth of data on the efficacy of subanesthetic ketamine in anxiety disorders and PTSD compared to the literature surrounding treatment-resistant depression. In patients with PTSD, ketamine was more effective than midazolam in reducing overall PTSD symptom severity. Ketamine also reduced avoidance 24 h but not 7 days after a single infusion of ketamine, even after controlling for any effect of depression (Feder et al., 2014). Additionally, ketamine effectively reduced the symptoms of anxiety in patients with social anxiety disorder for at least two weeks (Taylor et al., 2017). However, few preclinical models have sought to address the therapeutic potential of ketamine in reducing the overlapping core clinical symptom of avoidance.

The Wistar-Kyoto (WKY) rat is an animal model of behavioral inhibition (Pare, 1994, 2000) and displays many characteristics observed in anxiety disorders and PTSD. Trait behavioral inhibition is a vulnerability factor for the development of anxiety disorders, as behaviorally inhibited children are more likely to develop anxiety disorders (Kagan et al., 1987). WKY rats acquire lever-press avoidance faster and to a higher degree than Sprague Dawley (SD) rats (Servatius et al., 2008). Avoidant behaviors of WKY rats are also more persistent during extinction training than in SD rats, especially at high shock intensity (Jiao et al., 2011; Cominski et al., 2014). Likewise, humans with anxiety disorders and PTSD have a common feature of abnormal and persistent avoidance with avoidance being one of the symptom criteria for PTSD diagnosis in the DSM-5 (American Psychiatric Association, 2013). Humans with anxiety disorders and PTSD display anatomical and functional differences (Pitman et al., 2012; Cha et al., 2016), and in fact a smaller hippocampus and impairments in hippocampal dependent learning may be a vulnerability factor to develop PTSD (Gilbertson et al., 2002, 2007). Likewise, WKY rats have reduced hippocampal volume and impaired hippocampal function (Cominski et al., 2014; Janke et al., 2015). These features make the WKY rat a suitable and advantageous model for studying the effects of ketamine on avoidance symptoms related to anxiety disorders and PTSD.

Elimination of pathological avoidance in anxiety disorders and PTSD is the goal of cognitive behavioral therapies (CBT; Olatunji et al., 2010). CBT is based on principles of extinction learning, which is a form of inhibitory learning. Environmental cues signaling safety are especially important in the extinction-resistant avoidance demonstrated by WKY rats (Spiegler et al., 2018). The medial perforant pathway (mPP) is a major input to the hippocampus from the entorhinal cortex (Witter, 2007), and a recent study suggests this pathway is important for the learning of safety signals in an inhibitory avoidance learning paradigm (Micale et al., 2017). We previously found that the WKY rats have impaired synaptic plasticity of the mPP (Cominski et al., 2014) and abnormal use of the safety signal (Spiegler et al., 2018). Moreover, rapid antidepressant actions like those attributed to ketamine occur by the induction of mPP (Kanzari et al., 2017). These findings suggest that impaired LTP in the mPP in WKY rats may be the root cause of pathologically persistent avoidance behavior. Therefore, we sought to determine if ketamine would facilitate extinction of avoidance behavior in an anxiety vulnerable rat strain, the WKY rat. Additionally, because of the relationship between impaired hippocampal function and anxiety disorders/PTSD, we investigated whether improvement in extinction of avoidance is associated with renormalization of hippocampal synaptic plasticity.

2. Methods

2.1. Subjects

A total of 26 male SD rats and 42 male WKY rats were obtained from Envigo (Indianapolis, IN USA) at approximately three months of age. All rats were behaviorally characterized and a subset of these rats (7 SD and 15 WKY) was used for electrophysiology experiments. All rats were individually housed and provided with standard rodent chow and water *ad libitum* in a colony room with a 12:12 light/dark cycle (lights on at 7:00 a.m.). All experiments were performed during the light phase of the light-dark cycle. Prior to beginning any behavioral training, rats were allowed to acclimate to their home cage and environment for two weeks. All procedures followed the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee at the VA New Jersey Health Care System.

2.2. Behavior

All rats were trained on a lever-press avoidance procedure as described previously (Fragale et al., 2016; Cominski et al., 2014). For all avoidance testing procedures, an operant box located within a sound-attenuating chamber was used (Coulbourn Instruments). The operant box consisted of a lever (10.5 cm above the floor), a cue light (20.5 cm above the grid floor), and a speaker on one wall (26 cm above the floor), and a light (14 W) on the opposite wall that remained illuminated during the session (house light). A grid floor was used to deliver scrambled footshocks. The avoidance procedure consisted of three phases: acquisition, extinction, and a single-trial extinction retest session.

Acquisition of avoidance occurred during 12 sessions in which SD and WKY rats were drug-free. Each session was separated by

2–3 days for a total of 3 sessions per week. Each session started with a 60s period during which only the house light was present. This period was followed by 20 trials. Each trial started with the onset of an auditory warning signal (1000 Hz, 74 dB). Sixty seconds after the start of the warning signal, intermittent foot shock (once every 3.5 s, 2.0 mA intensity, 0.5 s duration and 20 shocks maximum) was delivered. A lever press made prior to the start of foot shocks (occurring less than 60 s after the start of the warning signal) prevented shocks from occurring, terminated the warning signal and initiated the intertrial interval; this type of response constituted an avoidance response. If a lever press was made after the start of foot shocks, shocks and warning signal were terminated and the intertrial interval was initiated; this type of response constituted an escape response. If no avoidance or escape response was made, foot shocks and warning signal was terminated after 20 foot shocks and the intertrial interval was initiated; this type of response was considered a failure. The intertrial interval (ITI) was 3 min in duration and signaled by a flashing light throughout the ITI (5 Hz; safety signal).

Prior to the extinction phase, rats of each strain were matched for avoidance performance on session 12 (A12), and then randomized to either saline or ketamine treatment groups for the extinction phase. The extinction phase consisted of six sessions that were the same as sessions during the acquisition phase, except foot shock and the safety signal were omitted. Responses during the first 60 s of the warning signal were classified as “avoidance” responses. Those responses with latencies greater than 60 s were labeled as “escape” responses.

Two weeks following the last day of extinction testing, all rats were tested for persistence of extinction memory using a single-session extinction retest. During the extinction retest, rats were drug-free with their last exposure to drug or saline occurring three weeks prior. For this test, the conditions were the same as during the extinction phase.

2.3. Drugs and treatment

2.3.1. Effect of ketamine on extinction of avoidance

To determine if ketamine facilitated extinction learning, SD and WKY rats were treated with ketamine (5 mg/kg in 0.9% saline; i.p.), a subanesthetic dose shown to have rapid antidepressant actions (Belujon and Grace, 2014). Control rats were given 0.9% saline. Drugs injections were separated by one week, given 24 h prior to the first and fourth extinction sessions.

2.3.2. Effect of ketamine on long-term potentiation

To determine if ketamine facilitated long-term potentiation, behaviorally characterized SD and WKY rats were treated with 5 mg/kg ketamine (i.p.) or 0.9% saline 24 h prior to electrophysiological recording, which was two weeks after the extinction retest session.

2.4. Characterization of ketamine responders and non-responders

Ketamine responders and ketamine non-responders are clearly documented in the clinical literature with responders ranging from 40 to 60% of the total population (Bagot et al., 2017; Haile et al., 2014; Zarate et al., 2006; Cornwell et al., 2012; Henderson, 2016). In order to determine whether ketamine treatment resulted in a bimodal distribution as suggested by the human studies, frequency distributions were constructed for all groups based on performance during the extinction re-test session. To create the frequency distributions, avoidance performance was binned in 10% increments and the number of animals in each bin was recorded. The bimodality coefficient (Pfister et al., 2013) and Hartigan's dip test (Hartigan and Hartigan, 1985) (diptest package for R, v. 0.75–7, 12/5/2016) were calculated for the frequency distribution. Bimodality coefficients greater than 0.555 signify bimodal distributions (Freeman and Dale, 2013). Statistical significance for the Hartigan's dip test indicates the frequency distribution is significantly different from unimodality. As suggested by Pfister et al. (2013), bimodal distributions are best determined by passing both tests because of potential false positive values for each test. The WKY rats treated with ketamine was the only group that passed both tests for bimodality (Table 1). Therefore, those ketamine treated WKY rats with low avoidance were deemed ketamine responders and those with high amounts of avoidance responses were classified as ketamine non-responders using a 50% avoidance threshold. One subject displayed 50% avoidance and was considered a ketamine responder. Analysis of extinction and synaptic plasticity were based on the categorization of ketamine responder and non-responder. Thus, whether or not a ketamine-treated WKY rat would be a responder or a non-responder cannot as yet be determined *a priori*.

Table 1

	Bimodality Coefficient	Hartigan's Dip test (p-value)
SD – Saline	.599	.450
SD – Ketamine	.543	.023
WKY – Saline	.510	.023
WKY – Ketamine	.659	.023

2.5. Electrophysiology

Electrophysiology experiments were conducted as described previously (Yoder and Pang, 2005; Cominski et al., 2014). Rats were anesthetized with urethane (1.5 g/kg in 0.9% saline) followed by preparation of the surgical site. A stimulation electrode (125 μm, Teflon coated stainless steel wire) was lowered into the medial perforant pathway (mPP; coordinates from bregma: −8.1 mm AP, 3.1 mm ML, 2.0–3.0 mm ventral from the brain surface for SD rats; −8.1 mm AP, 3.6 mm ML, 2.0–2.8 mm ventral for WKY rats). A recording electrode (75 μm, Teflon coated stainless steel wire) was lowered into the hilar region of the dentate gyrus (−4.0 mm AP, 2.5 mm ML, 2.8–3.2 ventral from the brain surface for SD rats; −4.0 mm AP, 2.8 mm ML, 2.8–3.2 ventral for WKY rats). Electrodes were optimized within the dorsal – ventral range to maximize the evoked field EPSP (fEPSP) and population spike. Baseline evoked responses were generated by stimulating at a rate of 1/15s using constant current stimulation (biphasic pulse, 300 μs duration; AM Systems Isolate Pulse Stimulator, Model 2100, Carlsborg, WA, USA). Evoked responses were amplified 1000 × and bandpass filtered between 0.1 Hz and 5 KHz (AM Systems Differential AC Amplifier, Model 1700, Carlsborg, WA, USA) prior to being visualized, recorded and analyzed (SciWorks software, version 7.2 SP1, DataWave Technologies). After optimizing the evoked response, the electrodes were allowed to settle until evoked responses were stable. Recording started with a 20 min baseline phase followed by a baseline input-output (i/o) curve. For the i/o curve, evoked responses were generated by stimulation intensities ranging from 100 to 1100 μA. Four evoked responses were generated at each intensity. LTP was induced by high frequency stimulation (HFS) using parameters established previously (Cominski et al., 2014). HFS consisted of three sets of four trains. Each train consisted of eight pulses given at a frequency of 400 Hz with an inter-train interval of 10 s. Each set was delivered with an interset interval of 5 min. Stimulus intensity for HFS was determined as a current that evoked half the max population spike and was adjusted for each animal; this intensity was verified both before

and after the baseline i/o curve. To assess whether LTP resulted from HFS, i/o curves were collected at 15 min, 1 h, and 2 h. Mean slope of the initial rise of the fEPSP and amplitude of the population spike were calculated from the 4 evoked responses obtained at each stimulus intensity and the mean values were used to construct the i/o curve for each rat. fEPSP slope and population spike amplitude were normalized to the maximum slope and maximum amplitude at baseline, respectively, and then expressed as a percent change. No baseline group differences were observed prior to normalization for either the fEPSP slope ($F_{(4,19)} = 2.44$, $p = 0.082$) or population spike ($F_{(4,19)} = 0.864$, $p = 0.503$).

2.6. Statistical analyses

All statistical analyses were conducted using SPSS (IBM, version 22). Graphs were produced using MS Office Excel or GraphPad Prism (version 7). For all analyses where sphericity assumptions were violated, a Greenhouse-Geisser correction was used. Corrected p-values are reported only when corrected and uncorrected p-values deviate with respect to significance; otherwise, the uncorrected p-values are reported. When applicable, all post-hoc analyses used Tukey's HSD. Statistical significance was determined as $p < 0.05$. A group (2 or 5) x session (12 or 6) mixed factor ANOVA was used to analyze avoidance behavior. A one-way ANOVA was used to determine differences in avoidance behavior on the extinction retest session. To assess overall differences in population spike LTP and fEPSP LTP, a group (5) x time (4) x intensity (6) omnibus mixed factor ANOVA was used to determine main effects and interactions. To determine if LTP was present within each strain, a time (4) x intensity (6) repeated measures ANOVA was used. Linear regression (GraphPad Prism v. 7) was used to analyze E-S potentiation.

3. Results

3.1. Avoidance behavior

3.1.1. Avoidance acquisition

All rats were untreated during the acquisition phase. SD and WKY rats acquired avoidance over the 12 sessions (main effect of session: $F_{(11,693)} = 117.422$, $p < 0.001$). WKY rats displayed more avoidance behavior than SD rats (main effect of strain: $F_{(1,66)} = 12.922$, $p = 0.001$); however, the rate of avoidance acquisition was similar between the two strains (Session x Strain: $F_{(11, 726)} = 1.755$, $p = 0.091$) (Fig. 1A). These findings are consistent with previous results reporting increased avoidance behavior in WKY rats compared to SD rats (Jiao et al., 2011; Fragale et al., 2016; Servatius et al., 2008).

3.1.2. Avoidance extinction

Upon completion of the acquisition phase, rats in each strain were matched on avoidance performance during the last acquisition session (A12) and randomly assigned to either saline (vehicle) or ketamine treatment conditions. Prior to the first extinction session (E1), rats received an injection of either vehicle or ketamine. Vehicle or ketamine treatment was repeated one week later, 24 h prior to E4. During the extinction phase, shock and safety signal were omitted. Based on avoidance performance on the extinction retest, the ketamine-treated WKY group was the only group that displayed a bimodal distribution (Table 1). Therefore, this group was further categorized as non-responders or responders, resulting in a total of five groups: SD saline, SD ketamine, WKY saline, WKY ketamine responder, and WKY ketamine non-responder. Overall, irrespective of groups the rats decreased avoidance responding during extinction sessions (main effect of session ($F_{(5,315)} = 59.579$, $p < 0.001$; Fig. 1B). However, groups differed in their extinction, as indicated by a significant session x group interaction ($F_{(20,315)} = 2.607$, $p = 0.001$; Fig. 1B) and a main effect of group ($F_{(4,63)} = 10.528$, $p < 0.0001$). Post hoc analysis revealed a significant difference between saline-treated WKY and SD rats, with saline-treated WKY rats avoiding more during extinction ($p < 0.001$). Importantly, WKY ketamine responders had significantly less avoidance responses than saline-treated WKY rats ($p < 0.001$), supporting our hypothesis that ketamine would normalize extinction at least in a subset of WKY rats. Additionally, WKY ketamine responders were not significantly different compared to SD saline rats ($p = 0.99$), suggesting ketamine reduced avoidance to the level of control saline-treated SD rats. In contrast, ketamine WKY non-responders avoided to the same extent as saline-treated WKY rats ($p = 1.0$). Finally, ketamine did not alter extinction of avoidance in SD rats ($p = 0.317$). These findings collectively present striking evidence that ketamine can facilitate extinction learning on an avoidance task in anxiety vulnerable populations. Moreover, the ratio of ketamine responders observed here was similar to clinically observed success in ketamine treated depression (Bagot et al., 2017; Haile et al., 2014; Zarate et al., 2006; Cornwell et al., 2012; Henderson, 2016).

3.1.3. Extinction retest

To determine the extent to which the effect of ketamine on extinction learning was long-lasting, all rats were tested on an extinction retest session. Importantly, the extinction retest session occurred two weeks after E6, and three weeks after the last injection of saline or ketamine. Avoidance performance for the various treatment/strain conditions were significantly different on the extinction retest session ($F_{(4,63)} = 34.588$, $p < 0.001$; Fig. 1C). Post-hoc analyses revealed effects similar to those present at the end of extinction learning (Fig. 1C). Specifically, saline-treated WKY rats avoided significantly more often than saline-treated SD rats ($p < 0.001$). WKY ketamine responders continued to have low levels of avoidance responses as compared to WKY saline rats ($p = < 0.001$), and similar performance to SD saline rats ($p = 0.612$). Conversely, WKY ketamine non-responders were not significantly different than WKY saline rats ($p = 0.071$).

4. Hippocampal synaptic plasticity

4.1. Population spike LTP

To determine if ketamine facilitated extinction learning by enhancing hippocampal function, LTP of mPP to DG pathway was assessed in urethane-anesthetized rats. LTP was generated by high frequency stimulation (HFS), as supported by a significant main effect of time ($F_{(3, 51)} = 12.447$, $p = < 0.001$). Moreover, LTP differed between groups, as the main effect of group ($F_{(4, 17)} = 9.590$, $p = < 0.001$) and the time × group interaction ($F_{(12, 51)} = 2.794$, $p = 0.018$) were significant (Fig. 2). As reported previously (Cominski et al., 2014) LTP was present in saline-treated SD rats (main effect of time; $F_{(3,9)} = 6.516$, $p = 0.012$; Fig. 2A), but not in WKY saline rats ($F_{(3,9)} = 1.279$, $p = 0.339$; Fig. 2C). Whereas ketamine did not facilitate LTP in the SD rats ($F_{(3,6)} = 2.221$, $p = 0.186$; Fig. 2B), ketamine enhanced LTP in the WKY responders ($F_{(3,12)} = 1.279$, $p = 0.021$; Fig. 2D). Similar to the lack of effect of ketamine in avoidance extinction, ketamine was not effective in enhancing LTP in WKY non-responders ($F_{(3,15)} = 1.045$, $p = 0.401$; Fig. 2E). Thus, the results demonstrate that ketamine enhances hippocampal function in the subgroup of WKY rats that responded to ketamine behaviorally.

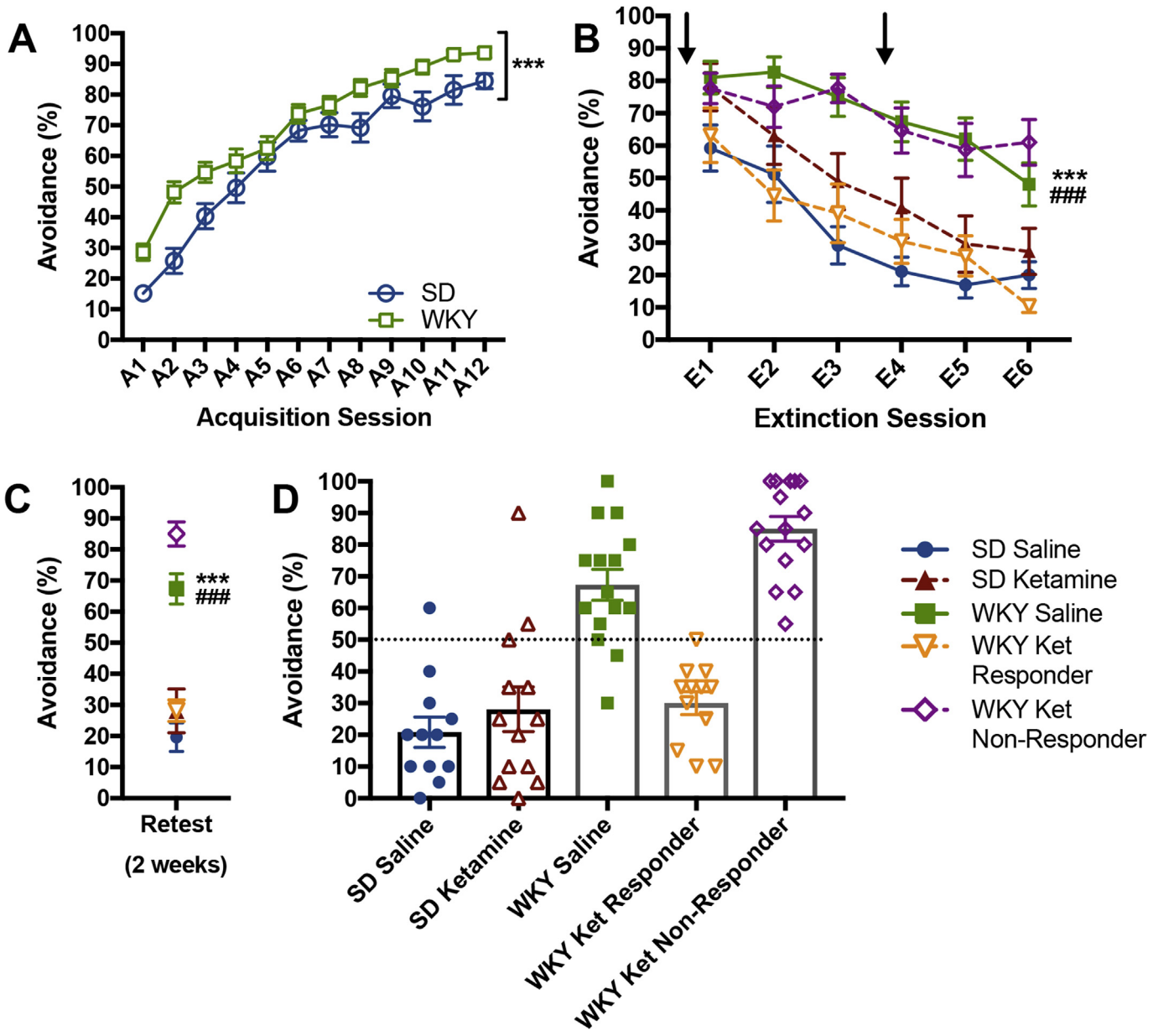

Fig. 1. Ketamine facilitates extinction in WKY rats. Untreated SD and WKY rats were trained on 12 acquisition sessions, during which the anxiety-vulnerable WKY rats acquired avoidance more rapidly than control SD rats (A). (B) During extinction, a subanesthetic dose of ketamine (5 mg/kg) was injected twice: 24 h prior to E1 and 24 h prior to E4 (arrows). Saline-treated SD rats extinguished more rapidly than WKY saline rats. WKY ketamine responders extinguished more rapidly than saline-treated WKY rats, and to the same degree as saline-treated SD rats. Meanwhile, WKY ketamine non-responder and saline-treated WKY groups were not different. (C) Two weeks after E6 (retest session), all rats were tested for extinction recall while drug-free, three weeks after last injection of ketamine. Saline-treated WKY rats and WKY ketamine non-responder rats continued to express high levels of avoidance. In contrast, SD rats and WKY ketamine responders continued to express low levels of avoidance, demonstrating persistent extinction learning without continued ketamine treatment. (D) Individual data for each group on the drug-free retest session from (C) to demonstrate separate clustering of WKY ketamine responders and WKY ketamine non-responders. In (A), ***$p < 0.001$ effect of strain across all days; In (B,C) ***$p < 0.001$ Saline-treated WKY rats vs saline-treated SD rats, ###$p < 0.001$ saline-treated WKY rats vs WKY ketamine responders. See text for complete discussion.

4.2. Field excitatory postsynaptic potential (fEPSP) LTP

In contrast to the population spike, ketamine did not facilitate fEPSP LTP in WKY rats. HFS generated LTP, as supported by a main effect of time ($F_{(3,51)} = 6.8$, $p = 0.003$), and LTP differed between groups, as the main effect of group was also significant ($F_{(4,17)} = 3.358$, $p = 0.034$) (Fig. 3). Follow up analysis revealed that the only group to demonstrate fEPSP LTP was the SD saline group (main effect of time; $F_{(3,9)} = 14.527$, $p = 0.001$; Fig. 3A). The lack of fEPSP LTP in WKY saline rats ($F_{(3,9)} = 1.281$, $p = 0.339$; Fig. 3C) is supported by previous reports that WKY saline rats have impaired fEPSP LTP (Cominski et al., 2014). Ketamine also did not facilitate fEPSP LTP in WKY responders ($F_{(3,12)} = 0.925$, $p = 0.458$), like it did for population spike LTP. Similarly, fEPSP LTP was not observed in WKY ketamine non-responders ($F_{(3,15)} = 1.076$, $p = 0.389$) or ketamine-treated SD rats ($F_{(3,6)} = 0.531$, $p = 0.677$; Fig. 3B–E).

4.3. EPSP-spike potentiation

The finding that ketamine enhanced population spike LTP but not fEPSP LTP in WKY responders suggested that EPSP-spike (E-S) coupling might be altered by ketamine. Previous studies have

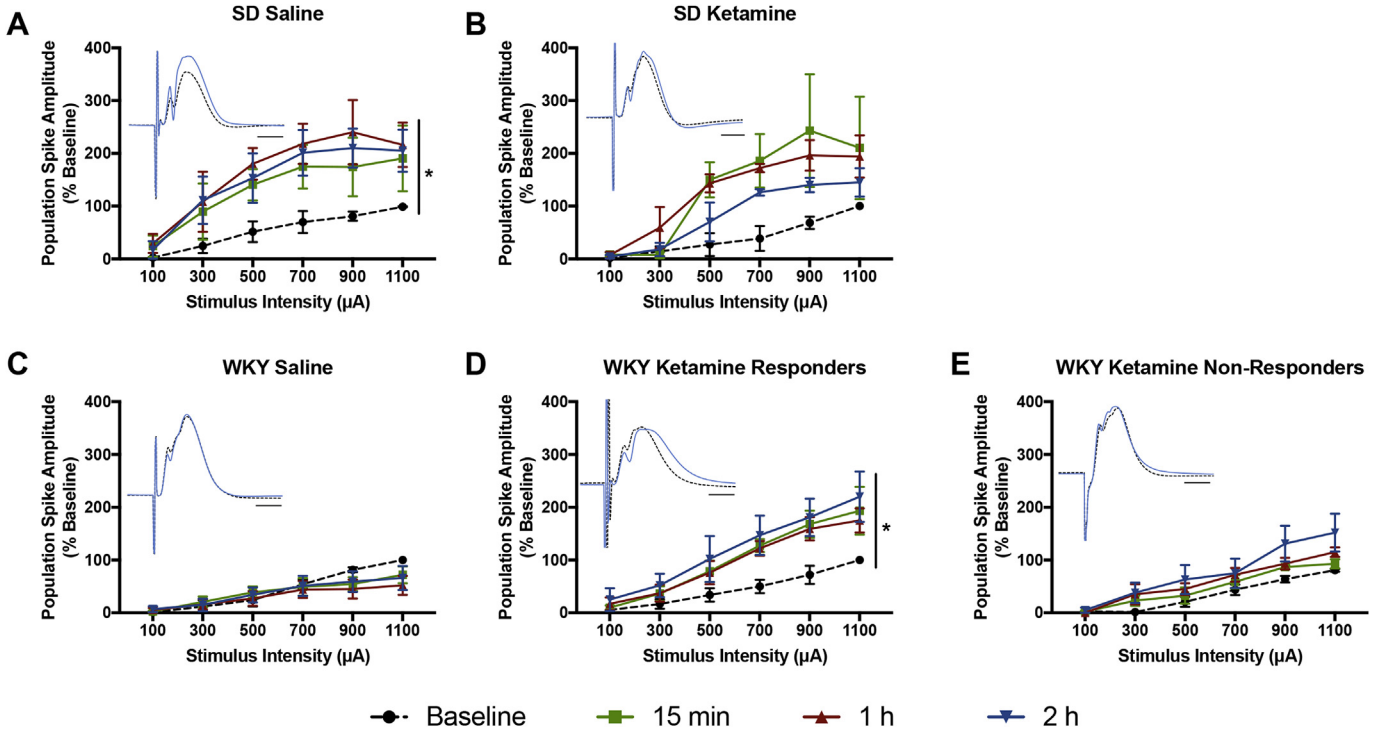

Fig. 2. Ketamine facilitates population spike LTP in the medial perforant path in WKY ketamine responders. LTP of the medial perforant pathway was measured for two hours after HFS. Although saline-treated SD rats (A) had persistent LTP, saline-treated WKY rats did not demonstrate LTP (C). LTP was present in WKY ketamine responder rats (D), but not in WKY ketamine non-responder (E) rats or ketamine-treated SD rats. In (A), $^{**}p < 0.01$ represents main effect of time within group. Details of results are discussed in text. The insets represent representative evoked responses before (black dotted line) and after (blue solid line) high frequency stimulation (HFS) after two hours. Scale bar = 5 ms. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

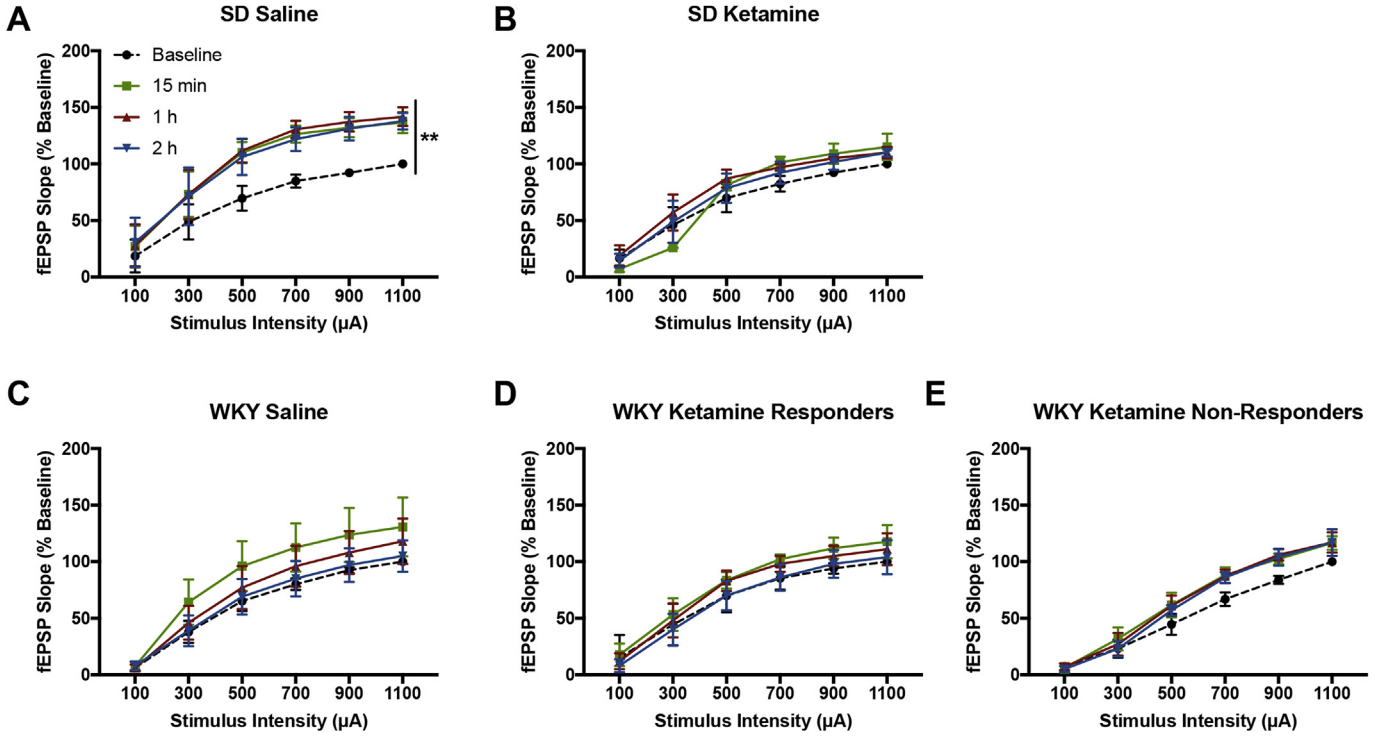

Fig. 3. Field excitatory post synaptic potential (fEPSP) LTP was not affected by ketamine. Although saline-treated SD rats demonstrated LTP following HFS (A), no other group demonstrated potentiation of the fEPSP. In (A), $^{*}p < 0.05$ represents main effect of time within group. Details are discussed in text.

demonstrated that E-S potentiation is different mechanistically than potentiation of synaptic efficiency (fEPSP) (Messaoudi et al., 2002; Bliss and Lomo, 1973). Therefore, E-S potentiation was assessed in SD saline and WKY ketamine responder groups, the two groups demonstrating population spike LTP. For SD saline rats, the E-S plot after HFS was significantly different than the baseline E-S plot ($F_{(1,8)} = 20.88$, $p = 0.002$; Fig. 4A). In particular, the slope of the E-S coupling was increased two hours after HFS, suggesting an increase in excitability of the granule cells. Additionally, values of the E-S plot after HFS are rightward shifted compared to baseline conditions, supporting a potentiation of synaptic efficiency too. WKY ketamine responders also demonstrated an increase in excitability of granule cells following HFS (Fig. 4B). In contrast to the SD saline group, the slope of E-S coupling was not different between the 2 h time after HFS and the baseline condition in WKY ketamine responders ($F_{(1,8)} = 2.419$, $p = 0.159$; Fig. 4B). Instead, the elevation (or alternatively the y-intercepts) was significantly different ($F_{(1,9)} = 71.25$, $p < 0.001$). Noticeably, the E-S plot after HFS is not generally rightward shifted compared to the baseline condition, suggesting that synaptic efficiency was not potentiated similar to LTP in SD saline rats. Therefore, HFS caused enhanced synaptic efficacy and increased excitation of granule cell in SD saline rats, whereas LTP in WKY ketamine responders was only characterized by an increase in granule cell excitability.

5. Discussion

The therapeutic implications of ketamine for anxiety disorders have not been well studied in spite of the significant traction for using ketamine in treatment-resistant depression. Avoidance behavior is a core symptom of anxiety and anxiety-related disorders that predicts resistance to treatment (Foa et al., 2006; Karamustafalioglu et al., 2006). In the present study, administration of a subanesthetic dose of ketamine facilitated extinction of perseverative avoidance behavior in a sub-population of anxiety-vulnerable WKY rats, effectively characterizing a group of responders to ketamine and a group of non-responders. Importantly, ketamine enhanced hippocampal synaptic plasticity in the ketamine responders, but not the non-responders. Therefore, this study is the first to demonstrate that a subanesthetic dose of ketamine can be used to reduce pathological avoidance in an animal model of anxiety disorders and provides insight into a neurobiological mechanism by which this therapeutic action may occur.

Our findings demonstrate that ketamine facilitates extinction of avoidance behavior in anxiety-vulnerable rats. Ketamine treatment resulted in two distinct populations of WKY rats, non-responders (56%) and responders (44%). The categorization of ketamine responders and ketamine non-responders is not unique to this study and, in fact, has been reported previously in humans and animals in similar proportions (Bagot et al., 2017; Haile et al., 2014; Zarate et al., 2006; Cornwell et al., 2012; Henderson, 2016). In humans, factors that may make an individual more likely to be a responder compared to a non-responder following ketamine treatment have been studied using patients with treatment-resistant depression. A person is more likely to be a ketamine responder if he or she has a first degree relative with an alcohol use disorder, higher body mass index (Niciu et al., 2014), and low levels of plasma D-Serine (Moaddel et al., 2015). Additionally, there is mixed evidence in humans suggesting that an increase in BDNF following ketamine treatment is associated with significantly fewer symptoms in ketamine responders compared to ketamine non-responders (Haile et al., 2014; Machado-Vieira et al., 2009). Although there is still more to be learned regarding factors driving the response to ketamine, emerging research in this area will be useful for understanding and developing effective therapies for treatment-resistant neuropsychiatric disorders, as well as for gaining insight into etiology of various related diseases.

Ketamine itself has a half-life of a few hours, yet it is capable of producing long-term effects (Zanos et al., 2016; Bjorkholm and Monteggia, 2016). One mechanism for ketamine's long-lasting effects may be its metabolism into (2S,6S; 2R, 6R)-hydroxynorketamine (HNK). HNK is required for the antidepressant response to ketamine and increases GluA1, GluA2, and BDNF protein in synaptoneurosomes of the hippocampus, but not prefrontal cortex 24 h after a single injection (Zanos et al., 2016). This delayed increase in AMPA receptor conductance and BDNF expression

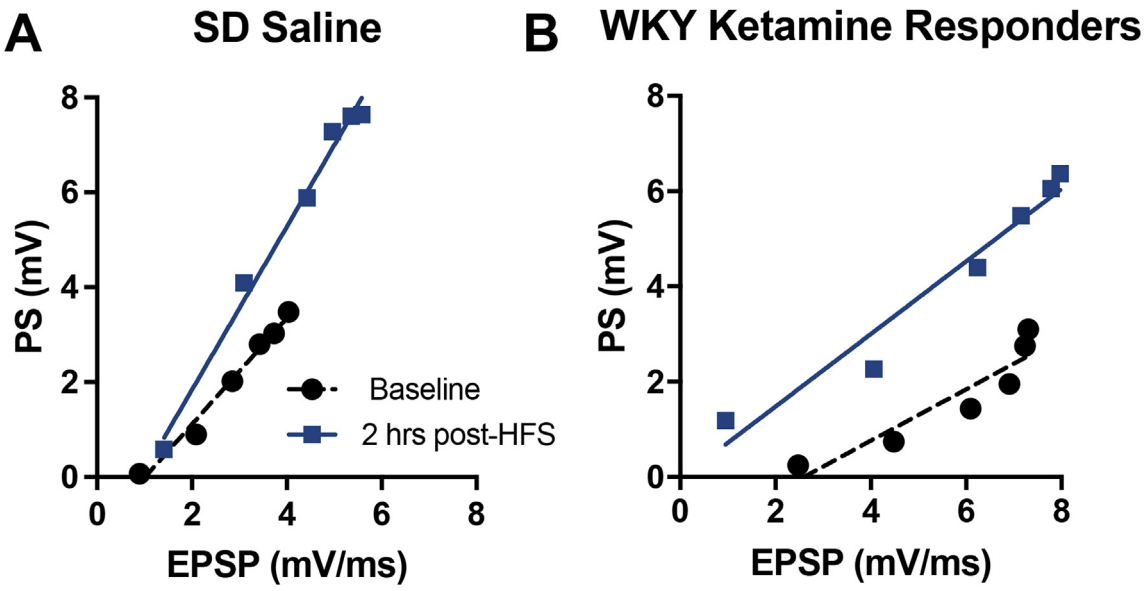

Fig. 4. Both saline-treated SD rats and WKY ketamine responders demonstrated EPSP-population spike (E–S) potentiation two hours after high frequency stimulation (HFS). In saline-treated SD rats, LTP consisted of enhanced synaptic efficacy (EPSP responses after HFS was increased relative to baseline responses) and increased excitability of the granule cells (E-S potentiation), as demonstrated by larger population spike after HFS that could not be accounted for by changes in the fEPSP (A). WKY ketamine responder rats only demonstrated increased excitability in granule cells following HFS, indicated by a significant change in elevation of the E-S plot after HFS (B).

following ketamine or HNK treatment may be essential for boosting synaptic plasticity or the potential for synaptic plasticity related to learning in areas such as the hippocampus.

Mechanisms of extinction have been widely studied in numerous behavioral models, resulting in an extensive amount of data on the extinction of drugs of abuse and conditioned fear. In general, the hippocampus is critical for extinction, and the prefrontal cortex and amygdala have also been implicated in extinction depending on the nature of the task (Fiorenza et al., 2012; Fragale et al., 2016). In the current study focused on the hippocampal dysfunction, ketamine facilitated LTP in WKY responders, but not in non-responders. Additionally, WKY ketamine responders demonstrated enhanced LTP similar to that of control SD rats. These findings suggest that the behavioral response to ketamine may be dependent on changes in synaptic plasticity within the hippocampus, specifically the medial entorhinal cortex to dentate gyrus pathway through the medial perforant pathway. Alternatively, the enhanced hippocampal LTP after ketamine treatment in WKY rats may be reflective of a more global enhancement of synaptic plasticity that may be driving the therapeutic behavioral effect. In our avoidance task, damage to either prefrontal cortex or hippocampus in Sprague Dawley rats was sufficient to recapitulate the extinction-resistant phenotype observed in WKY rats, suggesting larger networks may be important for extinction of active lever-press avoidance (Cominski et al., 2014; Fragale et al., 2016). Supporting the importance of the prefrontal cortex and hippocampus in pathological avoidance in WKY rats is the finding that synaptic plasticity in these two areas is impaired in WKY rats as compared to SD rats (Fragale et al., 2016; Cominski et al., 2014). Future studies will be necessary to determine the anatomical and molecular pathways by which ketamine facilitate avoidance extinction in anxiety vulnerability.

Previous studies have demonstrated two forms of LTP generated by HFS (Bliss and Lomo, 1973; Messaoudi et al., 2002). The first is an increase in synaptic strength, and the second effect is an increase in excitability of the postsynaptic neuron (i.e., E-S potentiation). Our results clearly demonstrate that ketamine enhances the latter, but not the former, type of LTP in WKY ketamine responders. This conclusion is further confirmed by an E-S plot (Fig. 4). An increase in neuronal excitability should be expressed as a parallel shift from the baseline E-S plot, such that each EPSP value results in a larger population spike, as seen for WKY ketamine responders (Fig. 4B). An increase in synaptic strength should be observed as post HFS values continuous with the baseline E-S plot but shifted to the right, demonstrating that each stimulus intensity of the i/o curve produces a larger fEPSP (right shift) that leads to a proportionally larger population spike. LTP in the SD saline group demonstrated both a right shift of the fEPSP values (increase in synaptic strength) and a proportionally larger population spike than cannot be accounted for by changes in fEPSP (E-S potentiation) (Fig. 4A).

Since E-S potentiation is dependent on GABAergic transmission (Tomasulo et al., 1991), ketamine may be decreasing the activity of local GABAergic interneurons, resulting in a net excitation of dentate granule cells through disinhibition. Although this mechanism has not been demonstrated in the dentate gyrus following subanesthetic ketamine, support for ketamine- or NMDA-mediated disinhibition has been described in the prefrontal cortex (Moghaddam and Javitt, 2012; Wohleb et al., 2017; Zhang et al., 2008). Increased glutamate signaling in the prefrontal cortex following ketamine treatment is attributed to NMDA antagonist activity selectively targeting fast-spiking GABAergic interneurons in the prefrontal cortex. GABAergic interneurons are particularly vulnerable to NMDA antagonism because they are more depolarized and have more NMDA receptors (Moghaddam and Javitt, 2012; Wohleb et al., 2017). Subanesthetic ketamine treatment may also enhance theta rhythm by a disinhibitory mechanism (Caixeta et al., 2013; Hangya et al., 2009). Therefore, the finding that only LTP of the population spike but not fEPSP was enhanced by subanesthetic ketamine in WKY rats suggests a mechanism involving inhibitory interneurons and disinhibition. Furthermore, evidence that specific regulation of GABA receptors may be responsible for disinhibition offers a unique and novel mechanism for studying and treating extinction-resistant avoidance.

Interestingly, ketamine in SD rats impaired LTP of the fEPSP and had no effect on LTP of the population spike. It is well known that the acute effect of ketamine, an NMDA antagonist, is to block hippocampal LTP. Because ketamine was administered 24 h prior to recording in the current study, longer-lasting metabolites such as HNK (Zanos et al., 2016) could have been responsible for effects on LTP observed at 24 h. Long lasting effects of ketamine have been reported in the nucleus accumbens, where a single subanesthetic dose of ketamine impaired EPSP LTP at 24 h and 7 days after injection in mice (Yao et al., 2017). The impairment of LTP in the nucleus accumbens was not related to changes in basal transmission via AMPA or NMDA receptors. In our study, ketamine blocked LTP of fEPSP in SD rats, as would be expected from Yao et al. (2017). Because WKY rats did not demonstrate LTP of the fEPSP, any impairing actions of ketamine on EPSP LTP would not be observable. In contrast, ketamine actions on LTP of the population spike are likely due to disinhibition of granule cells, supported by the E-S potentiation exhibited by WKY responders (see above). We have previously demonstrated less inhibitory neurons in the basal nucleus of the amygdala in WKY rats compared to SD rats (Jiao et al., 2011). If less inhibitory neurons were also present in the hippocampus of WKY rats, the effects of ketamine to disinhibit hippocampal circuits may be more noticeable because of the reduced inhibitory tone in WKY rats compared to SD rats.

Recent evidence suggests that the effectiveness of ketamine may be prolonged when combined with cognitive-behavioral therapy (CBT) for the treatment of obsessive compulsive disorder (Rodriguez et al., 2016). We observed that two injections, one-week apart, were sufficient to induce long-lasting effects on extinction (at least three weeks), supporting the idea that ketamine and CBT may offer an optimal approach to treatment. Impaired extinction is the result of deficits in inhibitory learning. Extinction learning requires the processing of contextual information and the hippocampus is essential for processing contextual information. As such, the hippocampus has been shown to have a modulatory role in extinction (Maren et al., 2013). The context-dependent nature of extinction suggests that any impairments in the hippocampus may lead to deficits in extinction (Quirk and Mueller, 2008). Individuals with PTSD in addition to those with a diagnosis of social anxiety disorder have smaller hippocampal volumes compared to healthy controls (Irle et al., 2010; Kitayama et al., 2005). Specifically, the dentate gyrus and the CA3 subregions demonstrate the greatest reduction in total volume (Wang et al., 2010). Therefore, ketamine could be facilitating extinction through dentate-gyrus mediated mechanisms. In humans, a smaller DG may contribute to the generalization observed in PTSD (Wang et al., 2010). Similarly, rats with dorsal hippocampal lesions exhibit normal levels of freezing in the fear conditioning context but are impaired in discriminating between similar contexts that are not associated with footshock (Maren and Fanselow, 1997, Frankland et al., 1998, Antoniadis and Mcdonald, 2000). Generalization of context-dependent memories results in an inability to distinguish between a dangerous and neutral context, the result of failed associations of the context with an aversive event (Keiser et al., 2017). Therefore, because WKY rats have reduced activity-dependent BDNF in the dentate gyrus, smaller hippocampi, and impaired dentate gyrus LTP (Janke et al., 2015; Cominski et al., 2014), these findings could collectively

suggest that impaired dentate gyrus plasticity leads to poor pattern discrimination, resulting in a failure to adequately process environmental cues to facilitate extinction of avoidance under normal circumstances.

In summary, the hippocampus is well studied for its role in learning and memory and as a target for traditional antidepressant action. Rapidly mimicking the antidepressant response through non-conventional drugs has utility not only for treatment-resistant depression but bipolar disorder and anxiety disorders alike. Indeed, subanesthetic doses of the NMDA antagonist ketamine provide same-day relief in humans while providing a classical antidepressant action in the hippocampus within 24 h. Here, we provide evidence that ketamine could be used as an adjunct pharmacotherapy with exposure therapy to facilitate extinction in anxiety and anxiety-related disorders. Remarkably, only two ketamine administrations resulted in a long-lasting extinction memory of up to at least three weeks later. Additionally, we provide insight to a potential mechanism by which ketamine could be facilitating extinction learning. Future studies should focus on the role of GABA receptors and inhibitory circuits as mechanisms for the effects of ketamine and to enhance the efficacy of subanesthetic ketamine in non-responders.

Conflicts of interest

The authors do not have any competing interests to declare.

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