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Deficits in emotional learning and memory in an animal model of schizophrenia

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Abstract

Alterations in N-methyl-D-aspartate (NMDA) receptor function have been linked to numerous behavioral deficits and neurochemical alterations. Recent investigations have begun to explore the role of NMDA receptor function on principally inhibitory neurons and their role in network function. One of the prevailing models of schizophrenia proposes a reduction in NMDA receptor function on inhibitory interneurons and the resulting disinhibition may give rise to aspects of the disorder. Studies using NMDA receptor antagonists such as PCP and ketamine have induced schizophrenia-like behavioral deficits in animal model systems as well as changes in inhibitory circuits. The current study investigated whether the administration of a subanesthetic dose of ketamine (8 mg/kg subcutaneously), that disrupts sensorimotor gating, also produces impairments in a Pavlovian emotional learning and memory task. We utilized both standard delay and trace cued and contextual fear conditioning (CCF) paradigms to examine if ketamine produces differential effects when the task is more difficult and relies on connectivity between specific brain regions. Rats administered ketamine displayed no significant deficits in cued or contextual fear following the delay conditioning protocol. However, ketamine did produce a significant impairment in the more difficult trace conditioning protocol. Analyses of tissue from the hippocampus and amygdala indicated that the administration of ketamine produced an alteration in GABA receptor protein levels differentially depending on the task. These data indicate that 8 mg/kg of ketamine impairs learning in the more difficult emotional classical conditioning task and may be related to altered signaling in GABAergic systems.

Keywords

Schizophrenia; Animal models; NMDA antagonists; Pavlovian learning

1. Introduction

Schizophrenia is characterized by multiple behavioral disruptions including sensorimotor gating deficits, altered emotional processing, and cognitive impairments [1–3]. The latter include impairments in attention, working memory, abstract reasoning, mental flexibility, planning and judgment, and disruptions in certain aspects of information processing [2,4,5]. Several of the negative symptoms have been examined in both patient populations and animal model systems in an effort to evaluate models of the disorder and identify pathogenic

mechanisms [6–8]. These investigations have identified several possible brain regions and cell types as potentially dysregulated in schizophrenia.

One of the current models of schizophrenia proposes a reduction in glutamate signaling, in particular a hypofunction of the N-methyl-D-aspartate (NMDA) glutamate receptor on principally inhibitory neurons, which induces a loss of coordinated network and/or oscillatory function [9,10]. Specifically, the reduced activity of NMDA receptors within a specific subclass of GABAergic neurons has been proposed to result in a loss of the tonic inhibition these cells provide to regulate large populations of other neurons [11]. The data supporting this model comes from post-mortem examinations of schizophrenia populations indicating a reduction in glutamate tone [12], as well as changes in several GABAergic markers including glutamic acid decarboxylase 67 (GAD67) and parvalbumin (PV) [13–20]. Further, data from human populations have established that drugs that block the NMDA receptor such as PCP and ketamine produce psychotic episodes in normal populations [21–23] and exacerbate psychosis in schizophrenia populations [23–27]. Finally, animal model investigations have been carried out in which the administration of these same NMDA receptor antagonists produces behavioral (sensorimotor gating, learning and memory [28,30]) and neurological changes consistent with schizophrenia [7,29–33,77]. Separate data have also established that administration of NMDA receptor antagonists lead to cortical disinhibition [34] consistent with the above proposal of altered network function.

Collectively, these findings provide support for reduced NMDA receptor function being associated with behavioral and cellular changes, and may be relevant to schizophrenia. The potential link to reduced GABA tone and a loss of coordinated network function represents a compelling mechanism. The above studies also suggest that additional investigations of alterations in NMDA receptor function related to GABAergic neuron function are needed to evaluate the importance of excitatory drive (NMDA) on inhibitory systems. One of the behavioral deficits that has been consistently observed in schizophrenia and may be informative in the evaluation of this approach is emotional learning and memory, which is mediated by several discrete neurological regions and requires the interaction of several systems [35,36].

Emotional processing and emotional learning and memory have been found to be impaired in patients with schizophrenia [37]. Specifically, data indicate a deficit in the impact of emotion on memory [38–42], with some arguing the deficits resemble patients with amygdala damage [37,43–46]. In non-schizophrenic populations, neuroimaging studies demonstrate that amygdala activation is positively correlated with hippocampal activity and subsequent memory while viewing emotional scenes [37–39,47]. Patients with schizophrenia display a reduction in amygdala activation when viewing emotional faces and reduced activation in the hippocampus during episodic memory tasks [37,40,48,49]. These findings not only indicate a change in emotional processing, but may also represent an alteration in connectivity between the amygdala and hippocampus.

The investigation of emotional learning and memory in animal studies using Pavlovian classical conditioning tasks have been very productive [50,51]. One task in particular, cued and contextual fear conditioning (CCF), in which the training protocol produces an association between an initially neutral tone (conditioned stimulus; CS) and an aversive event such as a mild footshock (unconditioned stimulus; US) works well in rodents. The association can be quantified based on the fear response (freezing) to the CS (cued fear) and serves as a measure of how well the animal learned an association. In addition, animals also learn a quantifiable association between the US and the environment in which the training took place (contextual fear). This approach has yielded important data on learning and memory mechanisms that mediate these associations [50,52–56]. An advantage of this task

is that alterations in the training protocol have been demonstrated to recruit differential involvement of specific neurological regions in order to learn the association. For example, in standard delay CCF procedures, when the CS and US overlap in time and co-terminate, the cued fear component requires amygdala function [50,51,57–60] and the contextual fear is dependent on hippocampal function [50,61–63]. A variation known as trace CCF conditioning modifies this task by inserting a temporal gap between the cessation of the CS and the onset of the US (see [51]). The insertion of an interval between the CS and US has been demonstrated to make the association more difficult, as additional training trials are required for equivalent learning [51,64]. Further, data indicate that not only does the task become more difficult, but lesion studies demonstrate the trace cued fear association is dependent on both the hippocampus and amygdala [51,65–71]. This procedural change presents a novel method by which to examine an animal model of schizophrenia that argues for disrupted network function as being in part responsible for aspects of the disorder.

In an attempt to mimic the processes occurring in schizophrenia as well as examine the role of NMDA receptor function in several domains, investigations have utilized subanesthetic doses of ketamine [32,72,73]. The use of ketamine is based on studies that demonstrate ketamine not only is effective at antagonizing NMDA receptor function [74–76] but it has also been suggested to preferentially bind to NMDA receptors on GABAergic interneurons at lower doses [76]. Additional data are required to establish the preferential affinity for NMDA on GABAergic neurons; however, ketamine has been demonstrated to also reduce inhibitory postsynaptic currents in the cortex [78]. In the below studies, we investigated the effects of ketamine at a subanesthetic dose that disrupts sensorimotor gating consistent with animal models of schizophrenia in an emotional learning and memory task. The dose selected is based on a previous examination [79] in which 8 mg/ml ketamine was the minimal concentration that produced deficits in both sensorimotor gating as well as spatial learning. In order to better characterize any ketamine induced deficits we employed both a delay and trace CCF procedure.

Based on the established literature demonstrating trace cued fear requires an interaction between the amygdala and the hippocampus, we hypothesized that a disruption of network function (as indicated above) would produce deficits in excess to any observed that relies more extensively on individual structures (delay cued fear). Finally, we examined the brains of these animals to determine if changes in GABA-mediated signaling may accompany any behavioral deficits. If ketamine results in an alteration in network function via a reduction in GABA release, we hypothesized that GABA receptor protein levels may be altered. The data indicate that ketamine administration produces a selective impairment in fear conditioning and an equally specific alteration in GABA receptor protein levels. These data represent a novel behavioral model for the examination of NMDA receptor function in sensorimotor gating, learning and memory, and potential interactions with GABAergic systems.

2. Materials and methods

2.1. Subjects

Thirty-six male Sprague-Dawley rats (20 for delay CCF and 16 for trace CCF; $n = 10$ for saline and ketamine groups in delay CCF; $n = 8$ for saline and ketamine groups in trace CCF) from Charles River Laboratories (Hollister, CA, USA) weighing between 250 and 350 g were used in this experiment. The animals were pair-housed in a standard animal facility with a 12–12 hr light–dark cycle, with food and water available ad libitum. All procedures were performed during the light phase and in accordance with the University of Nevada, Las Vegas Animal Care and Use Committee and NIH guidelines for ethical treatment of research subjects.

2.2. Drugs

Ketamine HCl was purchased from Henry–Schein (Indianapolis, IN) and diluted in physiological saline to achieve a concentration of 8 mg/ml. All solutions were administered at a dose of 1 ml/kg of body weight via a subcutaneous (SC) route 30 min prior to behavioral testing

2.3. Apparatus

2.3.1. Fear conditioning chambers—Both the delay and trace fear conditioning training and contextual fear tests were carried out in 10 in. $\times$ 10 in. $\times$ 7.5 in. ($L \times W \times H$) Freeze Monitor chambers (San Diego Instruments, SDI) with a stainless steel grid floor and plexiglas walls. The chambers were connected to a computer with the Freeze Monitor software (SDI) to run the experimental sessions. At the end of each animal's session, the chamber was cleaned with 409 solution (Clorox, Oakland, California). For the cued fear test, an altered context consisting of an opaque plastic enclosure with dimensions of 17 in. $\times$ 5 in. $\times$ 10.5 in. ($L \times W \times H$) was used. A vanilla scent was swiped on an inner wall of the chamber and 1% ethanol was used to clean the chamber between animals to ensure that olfactory cues were distinct from training.

2.3.2. PPI of acoustic startle—Using acoustic startle chambers, 28 cm $\times$ 28 cm $\times$ 28 cm ($L \times W \times H$), (SDI), acoustic startle and pre-pulse inhibition (PPI) were measured to evaluate sensorimotor gating. The chamber contained a transparent plexiglas tube, 20 cm in length $\times$ 9 cm internal diameter, mounted on an accelerometer sensitive to changes in movement. Data from the accelerometer were recorded to a Cobalt Instruments computer using the Acoustic Startle software package (Startle, SDI).

2.3.3. Tail flick—A circular glass bowl (8 in. in diameter and 3 in. in height) filled with 1800 mL of water heated to 55 °C was placed on a heating plate to maintain water temperature throughout the procedure.

2.4. Procedure

2.4.1. Delay CCF—Subjects were individually taken from the colony room and placed in the conditioning chamber. A training session consisted of an initial 2 min in which the animal was allowed to freely explore the chamber without any presentation of the CS or US. Following the initial 2 min in the chamber, a 2.9 kHz 88 dB (CS) tone was presented for 30 s and a 1 s 0.5 mA footshock (US) was delivered that co-terminated with the CS. Following the offset of the CS and US, animals were allowed to explore the chamber for an additional 2 min before another identical presentation of the CS and US was performed. Following the second CS–US pairing animals remained in the chamber for 2 additional minutes (for a detailed view of the training protocol, see Fig. 1). Freezing was measured by a trained experimenter blind to the experimental groups by making a visual inspection of the animal every 10 s. The criterion for freezing was the absence of movement other than breathing. Data collected for the training session consisted of the proportion time freezing during the initial 2 min in the chamber (pre-training freezing) and the last 2 min in the chamber (post-training freezing).

Cued fear (fear to the CS) was examined 24 h post-training. The animal was placed in the altered context for a total of 11 min. The session consisted of an initial 2 min interval, during which no stimuli were presented in order to evaluate freezing to the novel environment, followed by a 1 min presentation of the same CS used in training. Following the offset of the CS, the animal was allowed to explore for 2 min. These same intervals were repeated until the CS was presented a total of three times, each with the 2-min interval between

presentations. Following the final CS presentation, an additional 2 min were included in order to examine freezing behavior following the last CS. Freezing behavior was recorded throughout the entire session in an identical fashion as in training.

Contextual fear (fear to the training environment) was examined 48 h following training in the original training chamber. Subjects were placed into the original chamber and allowed to explore for a total of 6 min without any presentation of the CS or US. Animals' freezing behavior was recorded in an identical fashion as in training and cued fear. The contextual session was divided into three 2 min blocks in order to evaluate the initial contextual fear as well as any change in freezing across time in the original context without the US presentation.

2.4.2. Trace CCF—For both cued fear and contextual fear testing, all conditions and procedures were identical to the delay CCF procedures outlined above (Section 2.4.1.). The only differences were in the initial training session. In trace conditioning, a 2.5 s interval was inserted between the offset of the CS and the onset of the US (see Fig. 1b). Additionally, a total of four CS–US pairings were presented as previous investigations have demonstrated that the CS–US association is more difficult in the trace protocol [51,65,66,69–71,80]. As in delay CCF, a 2-min interval was present between the CS–US presentations, as well as a final 2-min interval following the last CS–US presentation during training. All other procedures, including evaluation of freezing behavior, were identical to the delay procedure.

2.4.3. Pre-pulse inhibition (PPI)—Animals trained in both the delay and trace CCF procedures were examined for acoustic startle and PPI to assess schizophrenia-like deficits. 24 h following the completion of the fear conditioning tasks, rats were administered the same treatment as in CCF procedures and tested for sensorimotor gating and nociception. Rats were individually placed in the testing chambers and given 5 min to acclimate to the environment with presentation of background noise (65 dB) throughout the entire session. Acoustic startle was tested with 10 ms white noise bursts at 90, 100, 110, and 120 dB. Separate trials contained a pre-pulse of 74, 78, 82, 86, or 90 dB that was presented 100 ms prior to a startle pulse of 120 dB. In order to ensure consistent data, the inter-trial intervals were randomized between 10 and 55 s and the order of trial also randomized. Each session consisted of four counterbalanced presentations of each PPI trial type. For the evaluation of acoustic startle, the first trial at each acoustic startle intensity was removed and an average of the remaining 4 trials was used for calculation of acoustic startle. For PPI, the startle response of each animal to the 120 dB pulse alone was used to calculate percent PPI using the following equation $100 - [(average\ startle\ response\ with\ pre-pulse)/(average\ acoustic\ startle)] \times 100$.

2.4.4. Tail flick—In order to ensure any differences observed during the fear conditioning procedure were not tied to changes in nociception (i.e. analgesia), a standard tail-flick nociception test was performed on all animals. Subjects were individually taken from their home cage into a testing room. An experimenter placed the initial 2–3 centimeters of the tip of the tail in 55 °C water and recorded the latency for the animal to flick its tail out of the water. A maximum of 10 s was set as a criterion for withdrawal (flick), however no animal reached this maximum latency.

2.4.5. Tissue collection—Following the completion of the tail flick task, rats were individually euthanized via CO₂ asphyxiation and hippocampi and amygdalae were rapidly dissected out and frozen for SDS-PAGE experiments.

2.4.6. SDS-PAGE-western blotting—Tissue was homogenized using RIPA buffer (20 mM pH 7.5 Tris–HCL, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EGTA, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β -glycerophosphate, 1 mM Na₃VO₄, 1 μ g/ml leupeptin; Cell Signaling, Danvers, MA) with 1 mM DTT, 1 mM PMSF, 20 μ g/ml aprotinin, and 0.1% SDS added. Homogenization was performed using a handheld Polytron (Kinematica Inc., Lucerne, Switzerland) tissue homogenizer. Homogenized tissue was centrifuged for 15 min at 15,000 $\times g$ at 4 °C. The supernatant was removed without disturbing the pellet and a protein concentration assay was performed using the bicinchoninic acid assay (BCA, Pierce, Rockford, IL).

Samples were loaded at a concentration of 20 μ g with a mixture of Laemmli buffer containing 2% SDS (BioRad, Hercules, CA) and water for a total of 10 μ L into 10% or 8% acryl gels (depending on protein of interest). The protein samples were separated via SDS-PAGE according to molecular weight with current being held at a constant 0.04 A for 60 min. Once finished, proteins were then electrotransferred to 0.2 μ m nitrocellulose membranes (GE Osmotics). The membranes were then blocked for 2 h in blocking buffer containing TBST (1 $\times$ Tris-buffered saline with 0.05% Tween-20) with 5% BSA (Rockland Immunochemicals) and 0.01% NaN₃. Membranes were incubated overnight at 4 °C in primary antibody (anti-GABA_{A α 5} polyclonal rabbit, 1:750, Millipore; anti-GABA_{B1} polyclonal rabbit, 1:2000, Cell Signaling; anti-GABA_{B2}, 1:1000; Cell Signaling; anti- β -Actin monoclonal rabbit, 1:10,000, Cell Signaling). Membranes were allowed to return to room temperature the following day and washed with TBST 3 times for 10 min each. Following washes, membranes were incubated in secondary antibody (HRP-conjugated anti rabbit, 1:5000, Vector) for 1.5 h at room temperature. Following 3 additional 10 min washes with TBST the membranes were exposed to Amersham ECL Plus detection system (GE Healthcare Life Sciences) and imaged using a Typhoon 9410 Variable Mode Imager (GE Healthcare Life Sciences). ImageQuant 5.2 software (GE Healthcare Life Sciences) was used to determine protein quantities. The proteins of interest (GABA_{A α 5}, GABA_{B1a}, GABA_{B1b}, and GABA_{B2}) were normalized to β -Actin density. The ketamine group's normalized values were then compared to the average control (saline) normalized values. All SDS-PAGE experiments were run in duplicate to ensure consistent data.

2.4.7. Statistical analyses—CCF training data were analyzed using one-way analysis of variance (ANOVA) between and within groups. Repeated measures ANOVA was used for cued fear (multiple CS) and contextual fear (each 2 min block) data. PPI data were analyzed initially via repeated measures ANOVA across startle and pre-pulse intensities. Following a significant repeated measure ANOVA, a one-way ANOVA was performed for each of the pre-pulse intensities. Mean latencies for tail flick and mean densities for western blotting were analyzed using one-way ANOVA. Tukey post-hocs were performed following a significant ANOVA.

3. Results

3.1. Delay CCF

No significant differences in proportion time freezing between the saline-treated and the ketamine-treated animals were observed during the initial training (Fig. 2a; $F_{(1,18)} = 2.477$; $p = 0.133$). A significant increase in freezing was observed within each group between the initial 2 min of the training session (pre CS–US) and the last 2 min of the session (post CS–US; saline, $F_{(1,18)} = 61.793$, $p < 0.01$; ketamine, $F_{(1,18)} = 14.414$, $p < 0.01$). No significant differences were observed in freezing between groups in cued fear testing (CS presentation in altered context) 24 h following training (see Fig. 2c; $F_{(1,18)} = 0.252$; $p = 0.621$). Both groups displayed a significant increase in freezing to the initial presentation of the CS in the

altered context (saline, $F_{(1,18)} = 13.755$, $p < 0.01$; ketamine, $F_{(1,18)} = 7.312$, $p < 0.01$). There were also no significant differences between the ketamine and saline groups in freezing behavior 48 h following training in the original training environment (contextual fear; Fig. 2e; $F_{(1,18)} = 1.858$; $p = 0.190$).

3.2. Trace CCF

Similar to the data from the delay CCF protocol, no significant differences were observed between the treatment groups throughout the training session (Fig. 2b; $F_{(1,14)} = 1.625$; $p = 0.223$). A significant difference within each treatment group was observed in the proportion of freezing between the pre CS-US and the post CS-US (saline, $F_{(1,14)} = 34.413$, $p < 0.01$; ketamine, $F_{(1,14)} = 65.215$, $p < 0.01$). A significant deficit in freezing behavior was observed in the ketamine group during the cued fear testing in the altered context (Fig. 2d; $F_{(1,14)} = 15.545$; $p < 0.01$); indicating that the treatment group did not learn the association as well as the saline group. Before the presentation of the first CS (pre CS1), no significant difference in freezing to the altered context was observed ($F_{(1,14)} = 1.615$; $p = 0.225$). No significant differences in freezing between groups were observed for the contextual fear testing (Fig. 2f; $F_{(1,14)} = 3.849$; $p = 0.070$).

3.3. PPI

Administration of ketamine did not produce any deficits in acoustic startle for animals trained in either the delay (Fig. 3a; $F_{(1,78)} = 0.013$, $p = 0.910$) or trace CCF procedure (Fig. 3b; $F_{(1,62)} = 0.046$, $p = 0.831$). Repeated measures ANOVA of PPI trials revealed a significant effect of treatment in both the delay CCF (Fig. 3c; $F_{(1,78)} = 17.884$, $p < 0.01$) and trace CCF protocol groups (Fig. 3d; $F_{(1,62)} = 18.295$, $p < 0.01$). Following significant repeated measures ANOVA, one-way ANOVA's were performed for each pre-pulse intensity. A significant difference in PPI was observed between the ketamine and saline groups at pre-pulse intensities of 74 dB (delay trained $F_{(1,78)} = 5.045$, $p < 0.05$; trace trained $F_{(1,62)} = 7.623$, $p < 0.01$), 78 dB (delay trained $F_{(1,78)} = 12.077$, $p < 0.01$; trace trained $F_{(1,62)} = 4.676$, $p < 0.05$), 82 dB (delay trained $F_{(1,78)} = 12.634$, $p < 0.01$; trace trained $F_{(1,62)} = 15.240$, $p < 0.01$), and 86 dB (delay trained $F_{(1,78)} = 5.530$, $p < 0.05$; trace trained $F_{(1,62)} = 11.492$, $p < 0.01$). No significant difference was observed between ketamine and saline groups on trials with a 90 dB prepulse (delay Fig. 3c; $F_{(1,78)} = 0.680$; $p = 0.412$; and trace Fig. 3d; $F_{(1,62)} = 3.905$; $p = 0.053$).

3.4. Tail flick

Ketamine and saline-treated animals that were trained in the delay CCF did not exhibit any differences in nociception in the tail flick test (Fig. 4a; $F_{(1,18)} = 2.091$; $p = 0.165$). However, animals that were trained in the trace CCF showed differences in this task with the saline-treated animals demonstrating a longer latency to withdraw their tail (Fig. 4b; $F_{(1,14)} = 6.068$; $p < 0.05$). The significant difference is not consistent with ketamine producing a reduction in nociception (analgesia- longer latency to withdraw) versus saline.

3.5. SDS-PAGE

Trace conditioned animals treated with ketamine displayed a significant increase in GABA_{B1b} receptor subunit protein levels in the amygdala (Fig. 5d; GABA_{B1a}, $F_{(1,18)} = 3.434$, $p = 0.080$; GABA_{B1b}, $F_{(1,18)} = 5.690$, $p < 0.05$) compared to saline-treated controls. No significant differences in hippocampal GABA_{B1} receptor protein levels were found for the trace CCF group (Fig. 5b; GABA_{B1a}, $F_{(1,18)} = 0.364$, $p = 0.554$; GABA_{B1b}, $F_{(1,18)} = 0.143$, $p = 0.710$). Ketamine-treated animals trained in the delay CCF procedure displayed a non-significant increase versus saline controls in GABA_{B1} receptor protein levels in the hippocampus (Fig. 5a, GABA_{B1a}, $F_{(1,18)} = 3.732$, $p = 0.069$; GABA_{B1b}, $F_{(1,18)} = 3.102$, $p =$

0.095) and the amygdala (Fig. 5c, GABA_{B1a}, $F_{(1,18)} = 3.2$, $p = 0.090$; GABA_{B1b}, $F_{(1,18)} = 0.210$, $p = 0.653$).

Ketamine-treated animals from the delay CCF protocol displayed no differences versus saline controls in GABA_{B2} protein levels in the hippocampus (Fig. 6a; $F_{(1,18)} = 1.205$, $p = 0.287$) or the amygdala (Fig. 6c; $F_{(1,18)} = 0.087$, $p = 0.772$). Similarly, animals treated with ketamine in the trace fear conditioning procedure showed no significant differences versus controls in GABA_{B2} protein levels in the hippocampus (Fig. 6b; $F_{(1,18)} = 0.087$, $p = 0.772$) or the amygdala (Fig. 6d; $F_{(1,18)} = 0.407$; $p = 0.531$).

An evaluation of the delay conditioned animals treated with ketamine revealed a significant increase in GABA_{Aα5} receptor protein levels in the hippocampus (Fig. 7a; $F_{(1,18)} = 5.059$, $p < 0.05$) but no significant differences in the amygdala tissue (Fig. 7c; $F_{(1,18)} = 0.392$, $p = 0.539$) compared to saline-treated animals. Animals treated with ketamine in the trace protocol displayed no significant differences in GABA_{Aα5} receptor protein levels in the hippocampus (Fig. 7b; $F_{(1,18)} = 0.121$, $p = 0.732$) or the amygdala (Fig. 7d; $F_{(1,18)} = 0.010$, $p = 0.921$) compared to saline controls.

4. Discussion

In the above studies we have observed a specific emotional learning and memory deficit following the administration of ketamine at a dose that also produces deficits in sensorimotor gating. The administration of ketamine did not produce a global learning impairment but rather a specific deficit in learning the association between the CS and US in only the trace conditioning procedure. Previous investigations have demonstrated that learning the association between the CS and US when a brief interval is inserted between them is more difficult [51,65,66,69–71,80]. The lack of any significant deficits in delay CCF cued fear as well as no significant differences in contextual fear between groups in either the trace or delay conditioning procedure support the conclusion that some aspects of learning and memory were not significantly altered by the administration of ketamine at 8 mg/ml. Previous investigations with alternative NMDA antagonists have produced contextual fear deficits [81,82]. These differences are not surprising as there is evidence that more potent or larger doses of NMDA receptor antagonists produce global deficits in hippocampally-mediated learning [83,84]. Further, findings from several investigations utilizing varying schedules and duration of administration of ketamine have been mixed. For example, administration of 16 mg/kg acutely produced a reduction in contextual freezing [105]; however, the chronic administration of ketamine at 5 mg/kg did not produce contextual fear deficits 24-h after training, but a deficit was observed 17 days following training [106]. In the current study the acute duration and low dose was insufficient to significantly disrupt hippocampal function and produce contextual fear deficits.

The data from the trace fear conditioning may also provide insight into the systems disrupted by the subanesthetic administration of ketamine. Previous research suggests that the neurological regions involved in learning the associations differ for the cued component versus the contextual component [51,59,62,85,50,86–92]. Specifically, the hippocampus has been demonstrated to be necessary for the contextual fear component, as lesions produce impairments in learning an association between the US and the training context for both delay and trace CCF procedures [51,61,62,68]. However, for delay cued fear hippocampal lesions do not disrupt learning the association [50]. It has been demonstrated that for delay cued fear the amygdala is crucial for learning the association, as demonstrated by amygdala lesion studies [57–59,50]. In the above studies, the administration of ketamine did not produce any significant deficits in contextual fear in either protocol and no deficit in delay-cued fear was observed, suggesting somewhat independent hippocampal and amygdala

function were not significantly disrupted by the ketamine. Conversely, the trace cued fear association has been demonstrated to rely on both the hippocampus and the amygdala [65,80]. In order for an animal to learn an association between the CS and US that do not overlap in time, an interaction between the amygdala and hippocampus is necessary to process the temporal gap [51,65–71]. Given the data in the current study are the first to investigate trace cued fear following ketamine, it appears that ketamine only produces a deficit in the component of this task that requires an interaction of the hippocampus and the amygdala. These data suggest that the interaction between these regions was disrupted by the subanesthetic ketamine, which may link to alterations in network function.

While the fear conditioning deficit indicates a subtle learning impairment due to ketamine, the addition of the PPI deficits observed in both the delay-trained or trace-trained groups demonstrate that ketamine induced sensorimotor gating deficits. These findings are consistent with several other reports of sensorimotor gating impairments following NMDA receptor antagonists [1,28] as well as numerous animal models of schizophrenia [93–95]. In addition, the tail flick data clearly demonstrate that the dose of ketamine was subanesthetic and any learning and memory differences were not a result of reduced nociceptive response. The lack of deficit in delay CCF further supports the dose of ketamine was not producing learning impairments via anesthesia. Our interpretation of the above combined data is that the ketamine administration was able to induce deficits consistent with alterations in connectivity and may prove useful to investigations of deficits associated with schizophrenia.

The data collected from the SDS-PAGE experiments may also provide a potential link between the ketamine administration and the deficits in both CCF and PPI. Diminished NMDA receptor function principally on GABAergic interneurons has been reported to result in diminished and/or altered activity of specific interneurons [78], which has been proposed to be involved in schizophrenia. In the above studies, a similar alteration in GABAergic transmission is hypothesized to result from subanesthetic administration of ketamine. Decreased excitation of GABAergic neurons would likely result in diminished GABA release, which we hypothesized may alter GABA receptors (diminished GABA may produce a compensatory increase in protein levels for GABA receptor subunits). The significant increase in the GABA_{B1b} receptor subunit in the trace CCF trained group appears to be consistent with the above hypothesis. The upregulation in the ketamine group that also displayed a deficit in CCF is particularly interesting as the metabotropic GABA_B receptor has been implicated in network and oscillatory function [96,97]. An alteration in GABA_B receptors would likely result in a change to the sustained inhibitory current they provide [17,98] and could potentially be related to a loss of hyperpolarization that mediates oscillatory function. The GABA_{B1} subunit that was altered in the present study has been characterized as the GABA binding site as opposed to the G-protein subunit (GABA_{B2} subunit; [99,100]). These data suggest an upregulation of the binding site for GABA in response to ketamine administration. Further, a limited number of studies have attempted to isolate the GABA_{B1a} and GABA_{B1b} subunits to either postsynaptic or presynaptic localization. Previous literature has indicated that the GABA_{B1a} subunit is principally presynaptic [101,102] and acts as an autoreceptor [99], while GABA_{B1b} receptors have been found postsynaptically to provide sustained inhibitory currents [98,101–103]. Considerably more data are needed to fully characterize the localization of the receptor subunits, however, based on the existing characterization the data from the above study indicate that ketamine resulted in an upregulation of the postsynaptic GABA binding subunit of the GABA_B receptor, perhaps in response to diminished GABA release.

Alterations in GABA_A receptors, in particular the GABA_{Aα5} subunit have been proposed in schizophrenia, as well as indications they mediate several behaviors disrupted in the disorder

[104]. Interestingly, the SDS-PAGE experiments indicated that ketamine induced a significant change in GABA_{Aα5} subunit in the hippocampus of the delay CCF trained animals. While the delay CCF group did not exhibit any significant deficits in cued or contextual learning, the sensorimotor gating deficits do demonstrate a behavioral alteration. The lack of a similar change in the trace-trained group makes the upregulation of GABA_A difficult to associate with the behavioral changes. However, it may be that the reductions in GABA release proposed to result from ketamine forces a shift in GABA receptors. In both the trace and delay CCF ketamine groups an upregulation of a GABA receptor subunit was observed. If the specific mechanisms (receptors) that are altered depend on the degree the hippocampus and amygdala are being utilized (delay vs. trace) the upregulation may differ in recruiting additional GABA_A versus GABA_B. Additional studies are required to further characterize these changes in both GABA_A and GABA_B receptors.

5. Conclusions

The data from the current study are consistent with numerous investigations of emotional processing and learning and memory impairments in schizophrenia populations. Our findings indicate that the administration of an NMDA receptor antagonist produces a subtle deficit in an emotional learning and memory task only when it was made more difficult and relied on an interaction of amygdala and hippocampal circuits. Our data also indicate an alteration in GABA receptor proteins associated with the administration of ketamine. While additional investigations are needed to characterize the consequences of the alterations in GABA receptor proteins, as well as if the changes correspond to pre or postsynaptic regions, the data provide a linkage of an NMDA antagonist altering GABAergic systems. Secondly, these data add to the literature indicating that subanesthetic administration of NMDA receptor antagonists produce learning and memory impairments consistent with schizophrenia at the same dosage that produces an impairment in sensorimotor gating. Even more compelling is that the learning deficits we observed are only in the portion of the task that is dependent on both the hippocampus and amygdala. These impairments would suggest that the connectivity required for the more difficult task is impaired by the administration of ketamine.

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Fig. 1.

Schematic representation of delay and trace CCF training protocols. In both protocols an initial 2-min interval was utilized to compare groups prior to the first CS–US presentation, as well as after the last CS–US pairing. The presentation duration for the CS and US are identical in both procedures. (a) Delay CCF training, in which animals receive 2 CS–US presentations that temporally overlap and co-terminate. (b) Trace CCF training, a 2.5 s interval is inserted between the cessation of the CS and onset of US. A total of 4 CS–US presentations are included in trace CCF training as this version is more difficult. Figure adapted from [51].

Fig. 2.

Mean proportion time freezing (±SEM) in all sessions for delay CCF (a, c, and e) and trace CCF (b, d, and f). Freezing during the initial 2 min prior to CS–US pairing (Pre CS–US) and the final 2 min (Post CS–US) on training day for (a) delay CCF and (b) trace CCF for ketamine and saline groups. No significant differences were observed between groups. (c and d) Mean proportion time freezing (±SEM) 24 h after training in the altered context for saline or ketamine groups. Freezing during the first 2 min with no CS (Pre CS1) and during each of the three CS presentations (CS1, CS2, CS3). No significant difference was found between treatment groups in delay CCF (c) while a significant deficit in the ketamine treated group trace CCF was observed (d). (e and f) Mean proportion time freezing (±SEM) 48 h after training in the original context following the administration of saline or ketamine. Freezing behavior over 6 min divided into three blocks of 2 min. Both the delay CCF (e) and trace CCF (f) showed no significant differences in freezing between treatment groups. (*) $p < 0.05$ ketamine-treated rats versus saline-treated controls.

Fig. 3.

Acoustic startle and prepulse inhibition for all experimental groups. Administration of saline or ketamine after delay CCF (a) or trace CCF (b) produced no differences in mean startle amplitudes ($\pm$ SEM) between treatment groups ($p > 0.05$). Animals administered ketamine and trained in both the delay CCF (c) and trace CCF (d) procedures exhibited deficits in PPI at all pre-pulse intensities except for 90 dB. (*) $p < 0.05$ for ketamine treated versus saline treated controls.

Fig. 4. Mean latency ($\pm$ SEM) in seconds to withdraw the tail in the tail-flick nociception task following administration of saline or ketamine. Animals trained in delay CCF (a) exhibited equivalent latency to withdraw between treatments while the ketamine group trained in the trace CCF (b) indicated a reduced latency to withdraw the tail. (*) $p < 0.05$ for ketamine versus saline treatment animals.

Fig. 5.

Proportion ($\pm$ SEM) and representative blots of GABA_{B1a} and GABA_{B1b} protein levels normalized to β -Actin from the hippocampus (a) and amygdala (c) of the delay CCF conditioned groups. No significant differences were observed between groups. Proportion ($\pm$ SEM) of GABA_{B1a} and GABA_{B1b} protein levels normalized to β -Actin from the hippocampus (b) and amygdala (d) of the trace CCF conditioned groups. No significant differences were observed between groups in the hippocampus but a significant increase was observed in the ketamine group for GABA_{B1b} in the amygdala. (*) $p < 0.05$ for ketamine versus saline treatment animals.

Fig. 6. Proportion ($\pm$ SEM) and representative blots of GABA_{B2} normalized to β -Actin from the hippocampus (a) and amygdala (c) of the delay CCF conditioned groups and the hippocampus (b) and amygdala (d) from the trace CCF conditioned groups. No significant differences were observed.

Fig. 7.

Proportion ($\pm$ SEM) and representative blots of GABA_Aα5 normalized to β-Actin from the hippocampus (a) and amygdala (c) of the delay CCF conditioned groups and hippocampus (b) and amygdala (d) of the trace CCF groups. A significant increase was found in the ketamine group from the delay protocol. No significant differences were observed in the amygdala. No significant difference in protein levels between ketamine and saline treated animals was detected in trace CCF groups.