Social Memory in Mice: Disruption with an NMDA Antagonist and Attenuation with Antipsychotic Drugs

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Abstract

Social recognition reflects the ability of one animal to learn and remember the identity of another. Animal models of social learning and memory are pertinent to several different CNS diseases involving disruptions in cognition. Moreover, the increased understanding of the basic biology of memory increases the likelihood of discovery of memory-enhancing treatments in these human diseases. In the present study, we investigated the effects of the non-competitive NMDA antagonist ketamine on social recognition in mice across a broad dose range (5–30mg/kg) and time-course (60 min- 7 days). We also tested the ability of two antipsychotic drugs, haloperidol and olanzapine, to block the ketamine effect. Our results show that mice demonstrate social recognition over a several day period, with loss of recognition between 3–7 days. Ketamine disrupts social memory at doses which do not affect task performance. Chronic oral administration of haloperidol or olanzapine attenuates these ketamine-induced effects on social recognition, tending to normalize the memory behavior. The neural mechanisms of these actions are not known, although medial temporal lobe memory systems have been implicated.

Keywords

hippocampus; learning and memory; haloperidol; olanzapine; ketamine

INTRODUCTION

Social memory is an important component of survival in animal groups, and is based on relational learning of complex stimuli in a social environment [1]. Animals recognize each other on the basis of multimodal sensory characteristics, conjunctively encoded [2–4]. Under laboratory conditions, social learning and memory in animals can be studied by exposing rodents to each other for an initial meeting, then testing their recognition as a function of time. When two rodents are exposed to each other for a specified period of time, re-exposure of the two animals at a subsequent episode is characterized by a shorter investigation time. This foreshortened time is taken to represent the social recognition [2–4]. Social recognition in rodents

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and primates has been shown to be dependent on hippocampal function in that ablations of the hippocampus block social recognition [5–8]. Moreover, persistent social memory (longer than 24 hours) is dependent on changes in cyclic AMP responsive element binding (CREB) and protein synthesis[5;9].

Several memory systems exist in brain partitioned by region; the prefrontal cortex is implicated in working memory, the medial temporal lobe (MTL), in declarative memory, and the basal ganglia, in procedural memory. The MTL brain regions implicated in declarative memory facilitate conscious memory for events and facts [10–12]. The MTL memory circuit encodes information about prior objects and events and reactivates this knowledge to inform present decisions and actions [4;13;14]. Hippocampal subfields play distinct roles in memory function as do regions along the MTL rostrocaudal axis [15–17]. Clinical evidence implicates hippocampal dysfunction in several diseases of cognition, including Alzheimer's dementia [18], depression [19;20], post-traumatic stress disorder (PTSD) [21] and schizophrenia [22]; therefore, animal cognition, especially those behaviors requiring hippocampal mediation, could have broad disease relevance.

Phencyclidine (PCP) is often used in animals to model human psychosis [23], based on its known psychotomimetic properties in humans [24;25]. NMDA-sensitive glutamate receptors are localized in high density within cortex and basal ganglia, where PCP provides a non-competitive blockade at the NMDA ionophore. In MTL, glutamate positively modulates what are thought to be some of the cellular processes underlying recognition, learning and memory through enhancement of long term potentiation (LTP) [26;27]. Among the many actions of PCP on brain chemistry are those we have previously reported, that include dynamic and potent neuronal activation [28], an extended alteration (biphasic in nature) in NMDA receptor density [29–31], and dynamic changes in immediate-early gene expression [32], all of which occur most dramatically in hippocampus. These data suggest that PCP and its congeners, including ketamine, [33] can directly disrupt cerebral neurochemistry and function in multiple brain regions including hippocampus.

Social cognition is an aspect of learning and memory that is particularly impaired in psychotic disorders, including schizophrenia and bipolar disorder [34;35]. The possibility that this disturbance in human social cognition could be mimicked in animals using ketamine and measured using social memory was attractive, given the current focus on cognition in psychotic illnesses [36]. We hypothesized that ketamine, by blocking the NMDA-sensitive glutamate receptor in brain, would impair social cognition in mice through glutamatergic mechanisms [37]. Using the same line of reasoning, we anticipated that antipsychotic treatment might attenuate the ketamine action since they improve cognition from the untreated state in schizophrenia. Therefore, in these experiments, we sought to test the effects of NMDA blockade with ketamine on social learning in mice. We examined the effects of ketamine on social recognition behavior across a dose range and a time-course in mice. Then, we studied the effects of a first (haloperidol) and a second (olanzapine) generation antipsychotic drug on those ketamine-induced disruptions in social learning.

Materials and Methods

1. Animals—Adult (8–12 weeks) and juvenile (4–5 weeks) male C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, Maine). Animals were housed 4–5 per cage and given food and water ad libitum. Lighting followed a 12:12-h light-dark cycle and the experiments were always conducted during the light phase of the cycle. Juvenile mice were used as the stimulus animals to minimize aggression. New sets of animals were used for each experiment; no mouse was tested at more than one evaluation time or drug dose. The experimental protocol for these studies was approved by the Institutional Review Committee

for the University of Maryland at Baltimore, covering the use of animal subjects, in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2. Social Memory Assessment—To assess social memory in mice, we followed the method described by Thor and Holloway [2]. The test is based on the tendency of an adult mouse to investigate an unknown juvenile mouse. Overall, in this paradigm, two mice (one adult and one juvenile) are exposed to each other for a short time (2 min), and the time that the adult explores the juvenile is measured. Then, mice are re-exposed at fixed inter-trial intervals; at the second ('trial') exposure, the duration of their investigation is characteristically reduced. The decrement in investigation time between the first and second exposure is used as an indication of social memory. Animal testing in this experiment was not conducted during the normally active period, because constraints on the housing facility prohibited a reverse light-dark cycle. Our concern that the housing conditions would interfere with the behavioral assay was mitigated by the successful control conditions.

On the day of the first exposure, group housed adult and juvenile mice were brought to the observation room to acclimate to the environment. An adult mouse was then placed into a 29cm × 18cm × 12cm empty plastic cage under dim light and allowed to habituate to the test environment for 15 minutes.

Following the 15 min acclimation period, a juvenile mouse was placed into the cage with the adult mouse for 2 min. The duration of the social investigatory behavior within the two minute period was scored by a trained observer. The social investigatory behavior which was quantified included direct contact or sniffing, following, nosing, grooming, pawing or generally inspecting any body surface of the novel juvenile mouse within 1 cm^2. The re-exposure at a fixed interval between the same adult and juvenile pair was conducted exactly like the first exposure. The interval between the first exposure and the test trial was 10, 30, 60, 120 minutes, 1 day, 3 days, 7 days, 15 days or 30 days. A minimum initial investigation time (we adopted 24 sec. from Kogan et al 2000) was required to qualify for an exposure test. A new set of mice were used for assessing each intertribal interval and each new experiment.

3 Drug Administration

3.1 Ketamine: Doses of ketamine (10–30 mg/kg, i.p.) were administered to mice 20 minutes prior to their initial social learning exposure. Each ketamine dose group had a saline control (N=10, each dose group). Ketamine was obtained from Fort Dodge Animal Health (Fort Dodge, Iowa) and diluted in sterile saline. The social recognition behavior was tested at 10–120 min and 1–30 days after 10mg ketamine administration. Because ketamine causes a decrease in locomotion behavior at high doses (tested from 5–30 mg/kg, i.p., Table 1), we set 10mg as the highest ketamine dose when administered <u>before</u> testing social learning. In another paradigm, to test the effect of ketamine on only the consolidation phase of learning, we administered ketamine to a set of mice, not previously exposed to the learning environment, immediately <u>after</u> their initial social exposure. Here, we tested doses of up to 30 mg/kg; there was no need to restrict doses because, for consolidation, the social learning was assessed 1–3 days after ketamine when any locomotor effects were gone.

3.2 Antipsychotic drug administration: Three groups of mice were treated with water alone, haloperidol in water (2mg/kg/day) or olanzapine in water (4mg/kg/day), respectively as the only solution available in their home cage water bottle. Drugs were dissolved in drinking water as previously described[38] and administered for 30 days prior to testing. The two test drugs, haloperidol and olanzapine, were separately dissolved in a minimum volume of glacial acetic acid. Each solution was diluted with distilled water and adjusted to PH 5.5 to 6.0, using 10 N NaOH. For the haloperidol solution, the stock was kept at 0.33mg/ml, and diluted to 3.3mg

haloperidol in 100ml solution within 7 days of administration. For the olanzapine solution, the stock was kept at 0.67mg/ml, and diluted in 100ml solution within 7 days of administration.

The dose of drug chosen for each compound was based upon extensive studies done in the rat [32]. The drinking water concentrations of drug known to produce plasma drug concentrations in rat within the human therapeutic range were known; we extrapolated the dosing from the rat to the mouse in order to obtain a similar plasma concentration of each drug in mouse. In order to verify that the concentration extrapolated from rats resulted in appropriate plasma levels in the mouse, we evaluated it. 20 adult mice were given a 2 week treatment with haloperidol (N=10) or olanzapine (N=10), each at two doses, in their drinking water. Plasma was collected by cardiac puncture after 2 weeks and was frozen at −20°C until analysis for drug levels. Plasma drug concentrations were determined using HPLC and mass spectroscopy in the laboratory of Thomas Cooper (Nathan Klein Institute). Plasma levels after 2 weeks at two dose levels of haloperidol and olanzapine are given in Table 2.

When evaluating social memory, each chronically treated animal group (haloperidol at 2 mg/kg/day, olanzapine at 4 mg/kg/day, and water alone) was evenly divided into two groups; one group was injected with saline and the other with ketamine (10 mg/kg) 20 min before social learning testing. The inter-trial interval for evaluation of the antipsychotic drug effect was 3 days. Mice were treated with the antipsychotic drug during the 3 day interval.

4. Data Analysis

Statistical analysis

A two-factor repeated measures analysis using mixed linear models was used to test the interval and trial effect on social recognition with and without ketamine treatment (Figs 1 and 2 respectively). In these models, the factors include the exposure interval (the between group factor), trial (the repeated factor, e.g., initial versus trial), and the interaction between interval and trial. A significant interaction indicates that there are different responses between the groups (exposure intervals or treatments) that are being compared. To test the ketamine effect (Fig 2 and 3), a similar model, but with ketamine dose as the between group factor, was used. The antipsychotic drug effect and antipsychotic drug effect distributed by ketamine and trial effect under different treatments were assessed by a three factor repeated measures model (Fig 4). The alpha=0.05 level of significance was used to test for significant main effects and interactions from the models. Multiple comparisons were made via least squares means contrasts derived from these mixed linear models. For testing the significance of the trial effect in nine exposure interval groups (Fig 1), the Bonferroni–Holm method was used to adjust for multiple testing. For other experiments, p-values were adjusted, using the Bonferroni-Holm approach, for comparisons between three trials, i.e., three trial intervals (Fig 2), three doses (Fig 3), or three treatments (Fig 4). Statistical analysis was performed with SAS 9.1.3 (SAS Institute, Cary, NC).

RESULTS

1. Social Recognition Behavior In Mice

The recognition behavior of an adult mouse toward a previously explored juvenile mouse was assessed by measuring the investigation time during the re-exposure of the adult to the juvenile after varying inter-exposure intervals (Figure 1). The results show that C57BL/6J adult mice show a significant reduction in the duration of investigation of a familiar juvenile mouse. This reduction was significant over inter-exposure intervals (ANOVA, Interval × Trial interaction, $F_{(8, 68)} = 4.88$, $p<0.0001$). This change was observed immediately after an initial exposure (mean change 35.6 sec, SD 19.1) and could still be detected at 3 days (mean change 32.9 sec, SD 28.3). A significant decrease in duration was observed at each inter-trial interval between

10 min-3 days, Bonferroni-Holm adjusted p-values of 0.03 to 0.0009. However, differences were not detected at or beyond 7 days, with these inter-trial intervals having smaller mean changes of 3.1, −4.6, and −3.8 seconds at 7,15, and 30 days, respectively, adjusted p=1.0 within each interval. The reduction in investigation time is taken as an indication of learning which transpires at the initial animal exposure. There was no difference in exploration behavior during the initial exposure across all groups, ANOVA p=0.99. In our hands, as in other laboratories, inhibition of protein synthesis with anisomycin blocked this social memory at inter-trial intervals beyond 24 hours (data not shown).

2. Ketamine –Induced Disruption of Social Recognition

Prior to evaluating ketamine in the social recognition paradigm, the acute effect of increasing doses of ketamine on exploration times in the adult mouse was evaluated to rule out a nonspecific locomotor effect of the drug on exploration. The criterion for a valid initial test was adopted from Kogan [5], namely that the mice show at least 24 seconds of total exploration time during the 2 min exposure. The number of animals who failed to reach this criterion increased with increasing doses of ketamine (Table 1). Because a decrease in exploration time may confound the evaluation of social memory, we set 10 mg/kg of ketamine as the upper limit for testing the drug's ability to disrupt social recognition whenever the drug was administered before the evaluation of social recognition, when the first decrease in exploration time was noted. When ketamine was administered after the evaluation of social recognition (eg, when it was used to evaluate disruption of memory consolidation), higher doses could be evaluated, because social learning was not tested until 1–3 days later. However, all data reported in this paper come only from mouse pairs who met the exploration criterion of 24 sec exploration per 2 min test interval, regardless of ketamine dose.

Social recognition was evaluated in the adult mouse after ketamine over inter-trial intervals of 60 min, 1, and 3 days (Fig 2). Social memory at 60 min was not impaired by ketamine, with a mean 25.5 sec, SD=17.5 test-initial investigation time, p=0.03, indicating that ketamine, per se, does not interfere with memory acquisition. Whereas, social memory at more distant times appeared ketamine-sensitive, indicated by a loss of the reduction in exploration time at inter-trial intervals of 1 and 3 days (mean reduction 9.3, SD=16.3, p=0.28 and mean reduction 21.7, SD=30.1, p=0.06, respectively) (Fig 2).

To evaluate the effect of ketamine on memory consolidation only, ketamine was administered <u>after</u> the initial social learning exposure; higher doses could be tested in this paradigm without the drug non-specifically affecting test behavior, since the next social learning exposure was beyond the duration of drug action. When ketamine was administered after initial exposure, memory responses were statistically different between the three dose trials (ANOVA Interval × Trial interaction, $F_{(2,16)}$=6.85, p=0.007), with the saline and 10 mg doses showing 'typical' decreases in investigation times of 24.1 sec (SD=8.3, p=0.0003) and 27.0 sec (SD=6.6, p=0.0003) but the 30 mg ketamine dose showed no significant memory response (Fig 3). No differences were found between experimental groups with regard to initial exploration times in Fig 2 and Fig 3.

3. Chronic Antipsychotic Effect on Social Recognition

Chronic treatment with haloperidol (2mg/kg/day) or olanzapine (4mg/kg/day) was evaluated for its effect on the ketamine-induced disruption in social recognition. To assure adequate antipsychotic doses in the mice, we prospectively determined the oral subacute dose necessary to achieve human therapeutic plasma levels in mice. After one month of oral drug administration, plasma drug levels in mice were assumed to have achieved the human therapeutic antipsychotic dose range for the two different doses of drug tested (as shown in Table 2 for the two week assessment).

After subchronic antipsychotic drug treatment, social recognition occurred in all mouse groups, represented by a significant drop in exploration time at the "trial" compared with the "initial" exposure in both the APD and water groups (p<0.0001 for each of the 6 mouse groups), even though olanzapine, but not haloperidol, modestly but significantly reduced "initial" interaction time (olanzapine "initial" interaction versus the same measure in the water or haloperidol groups, p=0.01). Ketamine administration significantly reduced social memory in the water-treated mice (P=.003), consistent with our other results. In addition, the effect of ketamine on social memory was significantly attenuated in the olanzapine- and haloperidol-treated animals, rendering no difference between the mouse-exploration time after saline or ketamine (p=.67 and p=.64, respectively). That is, we observed a difference in memory between saline and ketamine in the water pretreatment group (p=0.008 adjusted for multiple testing) but not between water and ketamine in either the haloperidol or the olanzapine pretreatment group (p>0.60) (Figure 4). The three way interaction between ketamine, antipsychotic drug, and social memory was significant in this experiment (p=.0124), showing that ketamine has a significantly different effect on memory behavior in the presence of the antipsychotic drugs vs with water only (Figure 4). These results show that a first- or a second-generation antipsychotic drug can block the action of ketamine on the disruption of social learning in mice. Of note, these experiments excluded mice who did not meet the 24 second initial exploration criterion; therefore these results can only be generalized to mice which did meet the initial exploration inclusion criterion.

DISCUSSION

The results of this study confirm that a C57 mouse can effectively form short- and long-term social memories of another mouse, a behavior previously reported in laboratory rats. The adult C57 mouse recognizes a familiar juvenile when tested at 10 – 120 min (short-term) or 1- 3 days (long-term) after a single 2 min interaction, but loses familiarity with the individual beyond 7 days. The familiarity gained by an adult mouse when exposed to an unfamiliar one is reliable and spontaneous, and it occurs within a few minutes after exposure. This long term memory can be disrupted with blockade of protein synthesis using anisomyacin [5]. Our results are consistent with reports of learning and recognition in group-housed rodents from other laboratories [5], which showed that the adult rat forms social memories as quickly as 10 min that last for several days after a single exposure. The advantage of demonstrating this behavior in mice derives from the availability of relevant genetically manipulated mouse colonies and the importance of their behavioral characterization.

Ketamine is a PCP congener and a non-competitive antagonist of the NMDA glutamatergic receptor. It disrupts social recognition behavior in the adult mouse. When ketamine was administered before the initial exposure, it disrupted social recognition without significantly influencing the investigatory activity of the mice, confirming our prediction that a drug blocking NMDA-sensitive glutamate transmission would impair social learning. Ketamine induced a selective diminution of longer-term memory (1–3 days) while minimally disrupting short-term (60 min) memory. This latter observation suggests that ketamine did not disrupt acquisition of information to form the memory, only its subsequent consolidation into a longer-term memory construct. This observation suggests that both non-NMDA mediated plasticity as well as NMDA-mediated plasticity is involved in social learning [39;40]. Whether a part of this effect of ketamine is due to its actions within hippocampus has not yet been tested, but it is our speculation, based on the known actions of ketamine in hippocampus in animals and humans [32;41] and hippocampal involvement in social recognition in rats [5]. Because blocking transmission at the NMDA receptor disrupts social learning in mice, it is plausible to postulate that some of the manifestations of brain diseases associated with social memory, like schizophrenia or autism, could be associated with altered glutamatergic transmission in brain, possibly in the hippocamal formation.

Ketamine was selected as the NMDA antagonist based on its ability to be used in humans as well as in animal models of psychosis [41]. We have already evaluated the ability of MK801 to mimic this ketamine effect on social learning in mice and show that it acts similarly to ketamine, but with greater potency, thus supporting the idea that this disruption of social learning is an NMDA-mediated action. In the data reported here, we show that ketamine when given before the memory acquisition (at 10 mg/kg) was more potent at blocking learning than when given after the acquisition (at 30 mg/kg), even though the ketamine appeared to disrupt memory in both instances. Differential sensitivity to ketamine-induced disruption likely represents a complex interaction between neurocircuitry and neuropharmacology active in the acquisition of a memory versus that involved in memory consolidation. The relative balance of NMDA involvement may be weighted more towards acquisition than consolidation [42;43] or differentially involved in unique forms of memory consolidation (i.e. specific to social recognition) [44].

The two antipsychotics, representative of the first and second generation drugs, alone failed to modify social learning in mice. This suggests that antipsychotic drugs could be devoid of adverse actions on social memory in humans, an observation already suggested in human studies (Green, et al, personal communication). Because these medications have adverse effects on other aspects of memory [45], this sparing of social memory could be of clinical significance. Moreover, given the importance of social memory in overall psychosocial recovery in a psychotic illness like schizophrenia [46], it is important that treatments for the illness do not worsen important domains of function in persons with the illness.

Antidopaminergic drugs, especially the second generation compounds, have been shown to block the actions of NMDA antagonists on animal behavior [47;48] and neurochemistry [49–51]. First and second generation antipsychotic drugs antagonize MK801 induced locomotion [47;48;52] and stereotypic behaviors [53;54]. Clozapine and olanzapine but not haloperidol antagonized PCP-induced social withdrawal in nonhuman primates [47]. Arvanov et al. [54] showed that olanzapine and clozapine, but not haloperidol, can facilitate glutamatergic transmission and block PCP effects on NMDA-mediated electrophysiologic responses in the medial PFC of rats [55;56]. Thus, antipsychotic drugs can attenuate some but not all behavioral effects of NMDA receptor antagonists. It is often assumed that the differences between the first and second generation of medications are due to the greater serotonin antagonism of later group of drugs, whereas their common actions are due to dopaminergic blockade.

It is not surprising therefore that the antipsychotic drugs blocked ketamine effects on social behavior. The present data demonstrate that both a first (haloperidol) and second (olanzapine) generation antipsychotic drug can antagonize ketamine-induced disruption on social memory. This observation suggests that the dopamine antagonism of these drugs (ie, their common mechanism) is more important in blocking the ketamine effect than the serotonin antagonism (ie, an action relatively distinctive to olanzapine). Because the literature more often reports that olanzapine is more effective in blocking social and NMDA-mediated behaviors than haloperidol, it is somewhat surprising that we have shown an equivalent action in this paradigm between both drugs. The explanation may lie in a species difference since most studies were done in rats or it may be a function of the task since this is the first report of antipsychotic efficacy in a social recognition task. Recent studies conducted in C57BL/6J mice in our lab (unpublished) show equivalent efficacy for haloperidol, olanzapine and clozapine in ameliorating this ketamine action implicating antipsychotic drug efficacy in improving social recognition and memory. Although the mechanism of action of these antipsychotics on ketamine-induced behaviors is unknown and may be indirect, we propose that it is mediated by dopamine receptor blockade. If the site of ketamine action is in hippocampus, the possibility exists that these two drugs act at D4 dopamine receptors, given the substantial density of the dopamine D4 receptor in the hippocampal formation [57;58]. Alternatively, antidopaminergic

agents could act within striatum to attenuate ketamine effects in distal structures mediated through long tract or distant circuit connections[59;60]. Grace suggests that dopamine in the striatum modulates hippocampal function through long tract projections, shuttering hippocampal influence on the prefrontal cortex [61]. Lisman has shown a dopaminergic influence on the Schaffer Collateral projections to CA1 within the hippocampus most likely mediated by the D5 dopamine receptor [62].

In summary, we have confirmed the existence of social memory in mice using an established behavioral paradigm, the disruption of this memory with ketamine and its restitution with antipsychotic drugs, thus, providing new and pertinent pharmacological characterization of this behavior. Because of the demonstrated role of hippocampus in social memory and the known pharmacologic actions of NMDA blockade in hippocampus, we postulate that this ketamine-induced effect may be mediated by its hippocampal actions. Whether the "corrective" action of antipsychotics, among several different possibilities, is a direct action in hippocampus or an effect mediated by long tract neural systems from striatum is unknown but could be further tested. These data suggest that social learning and memory examined with the described paradigms may prove to be a useful animal behavior in the study of hippocampal function and pharmacology [63] and, potentially, to study mechanisms of social cognition.

Acknowledgments

Reference List

1. Lai WS, Ramiro LL, Yu HA, Johnston RE. Recognition of Familiar Individuals in Golden Hamsters: A New Method and Functional Neuroanatomy. J Neurosci 2005;25(49):11239–11247. [PubMed: 16339019]

2. Thor DH, Holloway WR. Social memory of the male laboratory rat. Journal of Comparative Physiological Psychology 1982;96(6):1000–1006.

3. Bluthe RM, Dantzer R. Role of the vomeronasal system in vasopressinergic modulation of social recognition in rats. Brain Res 1993;604(1–2):205–210. [PubMed: 8457849]

4. Dluzen DE, Kreutzberg JD. 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) disrupts social memory/recognition processes in the male mouse. Brain Res 1993;609(1–2):98–102. [PubMed: 8099527]

5. Kogan JH, Frankland PW, Silva AJ. Long-term memory underlying hippocampus-dependent social recognition in mice. Hippocampus 2000;10(1):47–56. [PubMed: 10706216]

6. Maaswinkel H, Baars AM, Gispen WH, Spruijt BM. Roles of the basolateral amygdala and hippocampus in social recognition in rats. Physiol Behav 1996;60(1):55–63. [PubMed: 8804643]

7. Wimersma Greidanus TB, Maigret C. The role of limbic vasopressin and oxytocin in social recognition. Brain Res 1996;713(1–2):153–159. [PubMed: 8724986]

8. Baker KB, Kim JJ. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Mem 2002;9(2):58–65. [PubMed: 11992016]

9. Richter K, Wolf G, Engelmann M. Social recognition memory requires two stages of protein synthesis in mice. Learning & Memory 2005;12(4):407–413. [PubMed: 16077019]

10. Wagner AD, Schacter DL, Rotte M, Koutstaal W, Maril A, Dale AM, et al. Building Memories: Remembering and Forgetting of Verbal Experiences as Predicted by Brain Activity. Science 1998;281(5380):1188–1191. [PubMed: 9712582]

11. Davachi L, Mitchell J, Wagner AD. Multiple routes to memory: distinct medial temporal lobe processes build item and source memories. Proc Natl Acad Sci U S A 2003;100(4):2157–2162. [PubMed: 12578977]

12. Bayley PJ, Gold JJ, Hopkins RO, Squire LR. The neuroanatomy of remote memory. Neuron 2005;46:799–810. [PubMed: 15924865]

13. Corkin S. Lasting consequences of bilateral medial temporal lobectomy: clinical course and experimental findings in H. M Seminars in Neurology 1984;4:249–259.

14. Squire LR, Stark CE, Clark RE. The medial temporal lobe. Annu Rev Neurosci 2004;27:279–306. [PubMed: 15217334]

15. Small SA, Nava AS, Perera GM, DeLaPaz R, Mayeux R, Stern Y. Circuit mechanisms underlying memory encoding and retrieval in the long axis of the hippocampal formation. Nat Neurosci 2001;4 (4):442–449. [PubMed: 11276237]

16. Preston AR, Shohamy D, Tamminga CA, Wagner AD. Hippocampal function, declarative memory, and schizophrenia: anatomic and functional neuroimaging considerations. Curr Neurol Neurosci Rep 2005;5(4):249–256. [PubMed: 15987607]

17. Amaral DG, Witter MP. The three-dimensional organization of the hippocampal formation: a review of anatomical data. Neuroscience 1989;31(3):571–591. [PubMed: 2687721]

18. Zarow C, Vinters HV, Ellis WG, Weiner MW, Mungas D, White L, et al. Correlates of hippocampal neuron number in Alzheimer's disease and ischemic vascular dementia. Ann Neurol 2005;57(6):896–903. [PubMed: 15929035]

19. Campbell S, Macqueen G. The role of the hippocampus in the pathophysiology of major depression. J Psychiatry Neurosci 2004;29(6):417–426. [PubMed: 15644983]

20. Sala M, Perez J, Soloff P, Ucelli di Nemi S, Caverzasi E, Soares JC, et al. Stress and hippocampal abnormalities in psychiatric disorders. European Neuropsychopharmacology 2004;14(5):393–405. [PubMed: 15336301]

21. Bremner JD, Vermetten E. Neuroanatomical Changes Associated with Pharmacotherapy in Posttraumatic Stress Disorder. Ann N Y Acad Sci 2004;1032(1):154–157. [PubMed: 15677402]

22. Medoff DR, Tamminga CA, Lahti AC, Holcomb HH. Abnormal Limbic System Connectivity in Schizophrenia. Arch Gen Psychiatry. 2002Submitted

23. Jentsch JD, Roth RH. The neuropsychopharmacology of phencyclidine: from NMDA receptor hypofunction to the dopamine hypothesis of schizophrenia. Neuropsychopharmacology 1999;20:201–225. [PubMed: 10063482]

24. Pearlson GD. Psychiatric and medical syndromes associated with phencyclidine (PCP) abuse. John Hopkins Med J 1981;148:25–23.

25. Tamminga C. Glutamatergic aspects of schizophrenia. Br J Psychiatry 1999;37(Suppl):12–15.

26. Malenka RC, Nicoll RA. Long-term potentiation--a decade of progress? Science 1999;285(5435): 1870–1874. [PubMed: 10489359]

27. Kandel ER. The Molecular Biology of Memory Storage: A Dialogue Between Genes and Synapses. Science 2001;294(5544):1030–1038. [PubMed: 11691980]

28. Gao XM, Shirakawa O, Du F, Tamminga CA. Delayed regional metabolic actions of phencyclidine. Eur J Pharmacol 1993;241(1):7–15. [PubMed: 8223927]

29. Gao XM, Tamminga CA. MK801 induces late regional increases in NMDA and kainate receptor binding in rat brain. J Neural Transm Gen Sect 1995;101(1–3):105–113. [PubMed: 8695041]

30. Gao XM, Tamminga CA. An increase in NMDA-sensitive [3H]glutamate and [3H]kainate binding in hippocampus 24 hours after PCP. Neurosci Lett 1994;174(2):149–153. [PubMed: 7970171]

31. Gao XM, Tamminga CA. Phencyclidine produces changes in NMDA and kainate receptor binding in rat hippocampus over a 48 hour time course. Synapse 1996;23:274–279. [PubMed: 8855512]

32. Gao XM, Hashimoto T, Tamminga CA. Phencyclidine (PCP) and dizocilpine (MK801) exert time-dependent effects on the expression of immediate early genes in rat brain. Synapse 1998;29:14–28. [PubMed: 9552172]

33. Martin D, Lodge D. Phencyclidine receptors and N-methyl-D-aspartate antagonism: electrophysiologic data correlates with known behaviours. Pharmacol Biochem Behav 1988;31(2): 279–286. [PubMed: 2854262]

34. Kuperberg G, Heckers S. Schizophrenia and cognitive function. Curr Opin Neurobiol 2000;10(2): 205–210. [PubMed: 10753790]

35. Nuechterlein KH, Robbins TW, Einat H. Distinguishing Separable Domains of Cognition in Human and Animal Studies: What Separations Are Optimal for Targeting Interventions? A Summary of Recommendations From Breakout Group 2 at the Measurement and Treatment Research to Improve Cognition in Schizophrenia. New Approaches Conference Schizophr Bull 2005;31(4):870–874.

36. Hyman SE, Fenton WS. MEDICINE: What Are the Right Targets for Psychopharmacology? Science 2003;299(5605):350–351. [PubMed: 12532001]

37. Javitt, DC.; Zukin, SR. Mechanisms of phencyclidine (PCP)-N-Methyl-D-Aspartate (NMDA) receptor interaction: implications for schizophrenia. In: Tamminga, CA.; Schulz, SC., editors. Schizophrenia Research, Vol.1: Advances in Neuropsychiatry and Psychopharmacology. New York, NY: Raven Press; 1991. p. 13-20.

38. Gao XM, Cooper T, Suckow RF, Tamminga CA. Multidose Risperidone Treatment Evaluated in a Rodent Model of Tardive Dyskinesia. Neuropsychopharmacology 2005;31(9):1864–1868. [PubMed: 16319911]

39. Sawtell NB, Huber KM, Roder JC, Bear MF. Induction of NMDA Receptor-Dependent Long-Term Depression in Visual Cortex Does Not Require Metabotropic Glutamate Receptors. J Neurophysiol 1999;82(6):3594–3597. [PubMed: 10601487]

40. Volk LJ, Daly CA, Huber KM. Differential Roles for Group 1 mGluR Subtypes in Induction and Expression of Chemically Induced Hippocampal Long-Term Depression. J Neurophysiol 2006;95 (4):2427–2438. [PubMed: 16421200]

41. Lahti AC, Koffel B, LaPorte D, Tamminga CA. Subanesthetic doses of ketamine stimulate psychosis in schizophrenia. Neuropsychopharmacology 1995;13(1):9–19. [PubMed: 8526975]

42. Lynch G. Memory and the brain: unexpected chemistries and a new pharmacology. Neurobiol Learn Mem 1998;70(1–2):82–100. [PubMed: 9753589]

43. Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. Behav Brain Res 2003;140(1–2):1–47. [PubMed: 12644276]

44. Nadel L, Bohbot V. Consolidation of memory. Hippocampus 2001;11(1):56–60. [PubMed: 11261773]

45. Reilly JL, Harris MS, Keshavan MS, Sweeney JA. Adverse effects of risperidone on spatial working memory in first-episode schizophrenia. Arch Gen Psychiatry 2006;63(11):1189–1197. [PubMed: 17088499]

46. Green MF. What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry 1996;153:321–330. [PubMed: 8610818]

47. Corbett R, Camacho F, Woods AT, Kerman LL, Fishkin RJ, Brooks K, et al. Antipsychotic agents antagonize non-competitive N-methyl-D-aspartate antagonist-induced behaviors. Psychopharmacology 1995;120(1):67–74. [PubMed: 7480537]

48. Hoffman DC. Typical and atypical neuroleptics antagonize MK-801-induced locomotion and stereotypy in rats. J Neural Transm Gen Sect 1992;89(1–2):1–10. [PubMed: 1358122]

49. Sharp FR, Butman M, Wang S, Koistinaho J, Graham SH, Sagar SM, et al. Haloperidol prevents induction of the hsp70 heat shock gene in neurons injured by phencyclidine (PCP), MK801, and ketamine. J Neurosci Res 1992;33(4):605–616. [PubMed: 1484394]

50. Nakki R, Nickolenko J, Chang J, Sagar SM, Sharp FR. Haloperidol prevents ketamine- and phencyclidine-induced HSP70 protein expression but not microglial activation. Exp Neurol 1996;137 (2):234–241. [PubMed: 8635538]

51. Sharp FR, Jasper P, Hall J, Noble L, Sagar SM. MK-801 and ketamine induce heat shock protein HSP72 in injured neurons in posterior cingulate and retrosplenial cortex. Ann Neurol 1991;30(6): 801–809. [PubMed: 1838680]

52. Terranova P, Chabot C, Barnouin C, Perrault G, Depoortere R, Griebel G, et al. SSR181507, a dopamine D2 receptor antagonist and 5-HT1A receptor agonist, alleviates disturbances of novelty discrimination in a social context in rats, a putative model of selective attention deficit. Psychopharmacology 2005;V181(1):134–144. [PubMed: 15830220]

53. Tiedtke PI, Bischoff C, Schmidt WJ. MK-801-induced stereotypy and its antagonism by neuroleptic drugs. J Neural Transm Gen Sect 1990;81(3):173–182. [PubMed: 1975747]

54. Arvanov VL, Liang X, Schwartz J, Grossman S, Wang RY. Clozapine and haloperidol modulate N-methyl-D-aspartate- and non-N-methyl-D-aspartate receptor-mediated neurotransmission in rat prefrontal cortical neurons in vitro. J Pharmacol Exp Ther 1997;283(1):226–234. [PubMed: 9336328]

55. Svensson TH. Dysfunctional brain dopamine systems induced by psychotomimetic NMDA-receptor antagonists and the effects of antipsychotic drugs. Brain Res Brain Res Rev 2000;31(2–3):320–329. [PubMed: 10719159]

56. Ninan I, Jardemark KE, Wang RY. Differential effects of atypical and typical antipsychotic drugs on N-methyl-D-aspartate and electrically evoked responses in the pyramidal cells of the rat medial prefrontal cortex. 5. Synapse 2003;48:66–79. [PubMed: 12619040]

57. Lahti RA, Roberts RC, Cochrane EV, Primus RJ, Gallager DW, Conley RR, et al. Direct determination of dopamine D4 receptors in normal and schizophrenic postmortem brain tissue: a [3H]NGD-94-1 study. Mol Psychiatry 1998;3(6):528–533. [PubMed: 9857979]

58. Browman KE, Curzon P, Pan JB, Molesky AL, Komater VA, Decker MW, et al. A-412997, a selective dopamine D4 agonist, improves cognitive performance in rats. Pharmacology Biochemistry and Behavior 2005;82(1):148–155.

59. Lisman JE, Grace AA. The Hippocampal-VTA Loop: Controlling the Entry of Information into Long-Term Memory. Neuron 2005;46(5):703–713. [PubMed: 15924857]

60. De Leonibus E, Verheij MMM, Mele A, Cools A. Distinct kinds of novelty processing differentially increase extracellular dopamine in different brain regions. European Journal of Neuroscience 2006;23 (5):1332–1340. [PubMed: 16553794]

61. Charara A, Grace AA. Dopamine receptor subtypes selectively modulate excitatory afferents from the hippocampus and amygdala to rat nucleus accumbens neurons. Neuropsychopharmacology 2003;28(8):1412–1421. [PubMed: 12799620]

62. Otmakhova NA, Lisman JE. Dopamine selectively inhibits the direct cortical pathway to the CA1 hippocampal region. J Neurosci 1999;19(4):1437–1445. [PubMed: 9952420]

63. Storm EE, Tecott LH. Social Circuits: Peptidergic Regulation of Mammalian Social Behavior. Neuron 2005;47(4):483–486. [PubMed: 16102531]
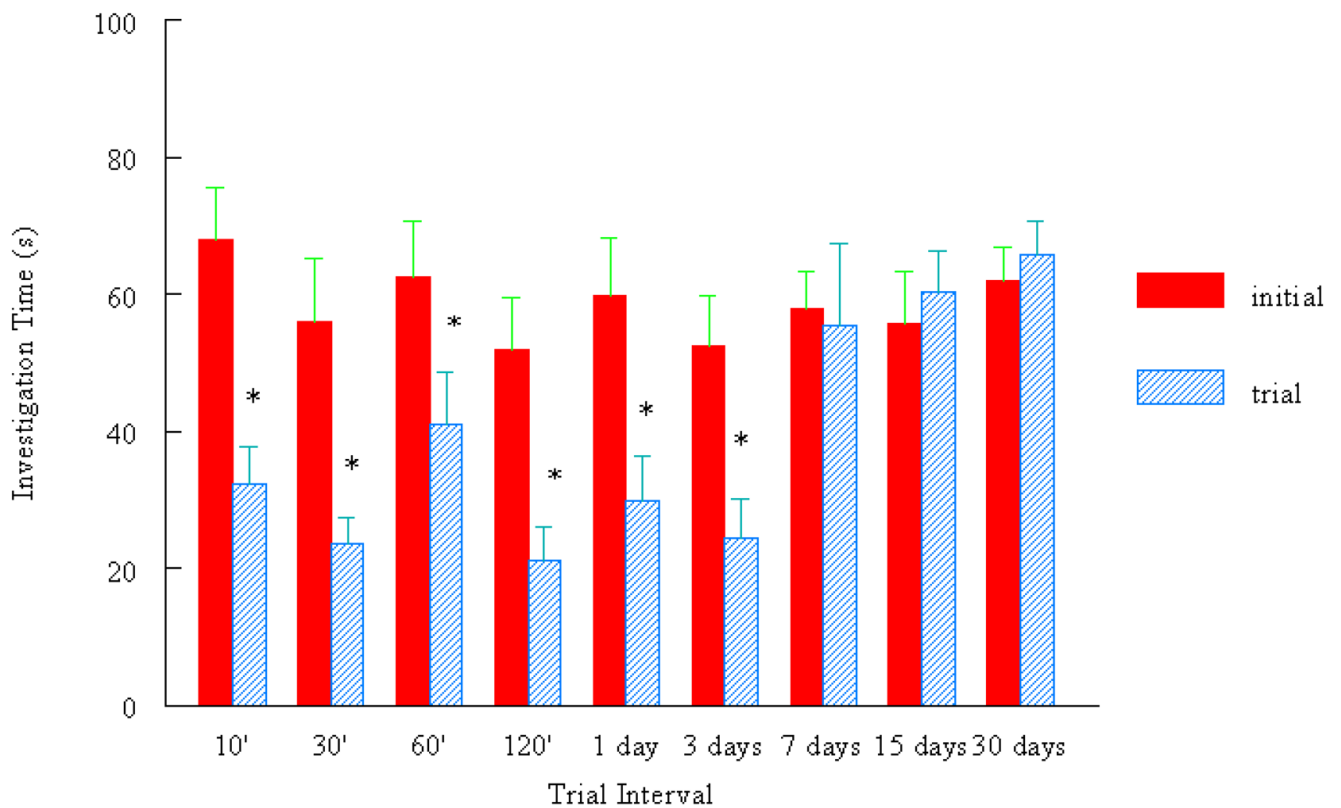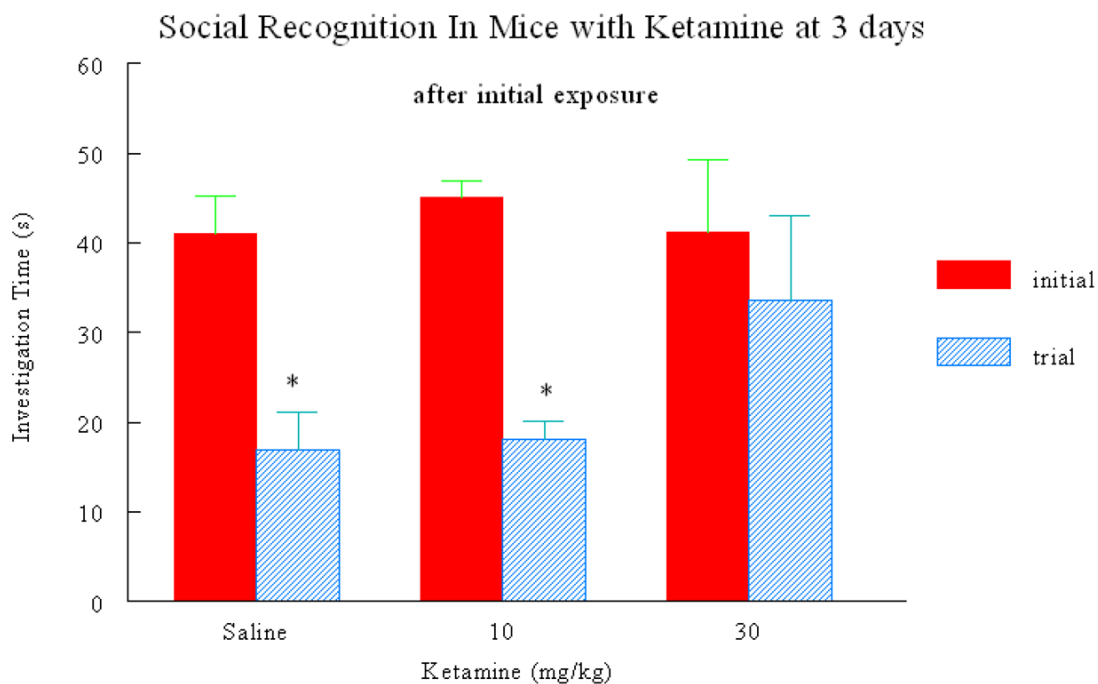
Figure 1.
The time in seconds that an adult mouse investigated a juvenile mouse at an initial meeting
(filled bar) compared with a trial meeting (crossed hatched bar) at inter-trial intervals of 10
minutes to 30 days. New animal pairs were used for each time interval. There was a significant
reduction in the exploration time at the trial meeting between the animals beginning at 10 min
and lasting up to 3 days. This recognition behavior was lost by 7 days.

## Social Recognition In Mice With 10mg/kg of Ketamine
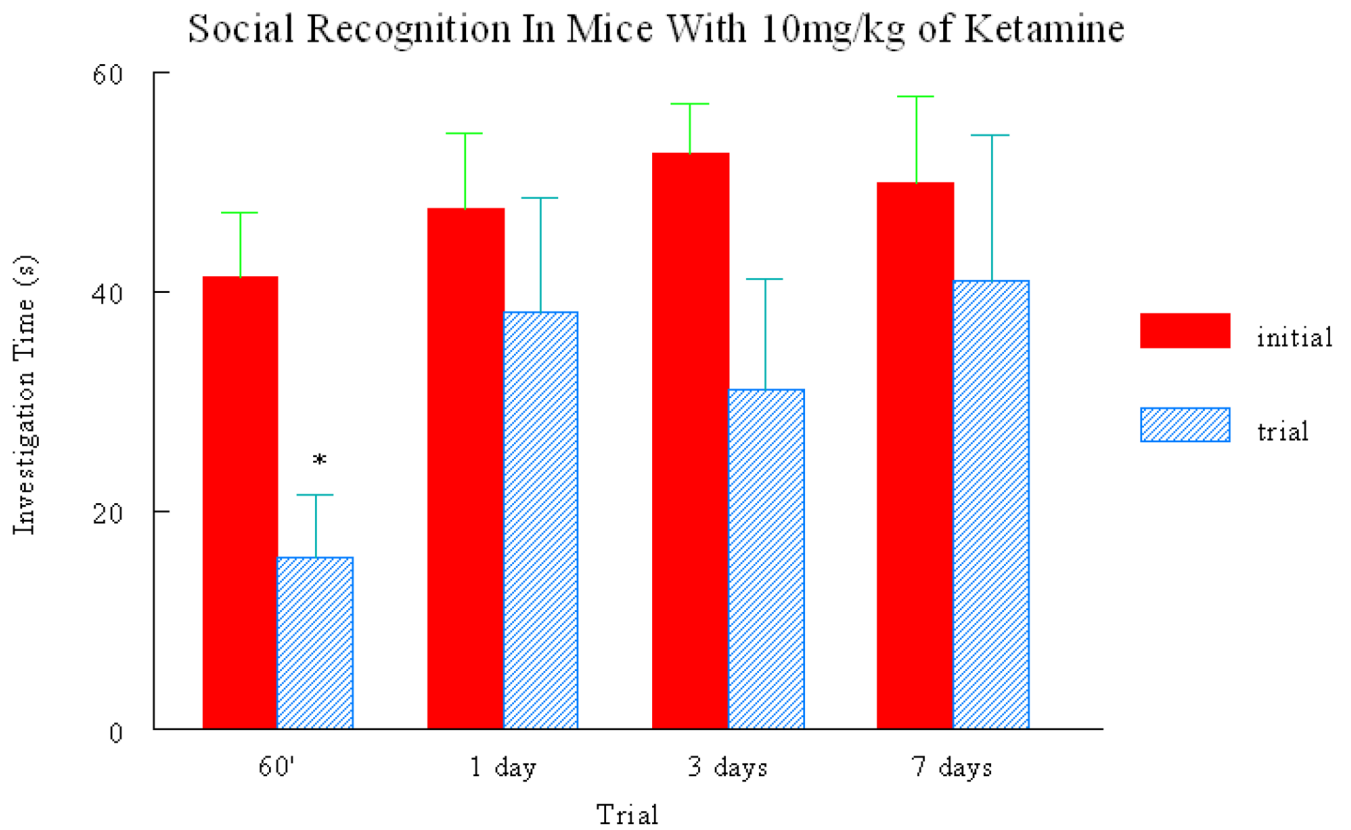
Figure 2.
Ketamine at a dose of 10 mg/kg (administered BEFORE the initial exposure) disrupted the recognition of a juvenile mouse by an adult animal at 1 and 3 day inter-trial intervals (long term), but not after a 60 minute interval (short term). Distinct animal pairs were used for each dose group.

Figure 3.
Ketamine was administered at a dose of 10 mg/kg or 30 mg/kg (AFTER the initial exposure) and was evaluated at 3 days. Social recognition was disrupted with 30 mg/kg ketamine but not at 10mg/kg, showing that ketamine could disrupt the consolidation of the social recognition memory but only at higher dose levels.
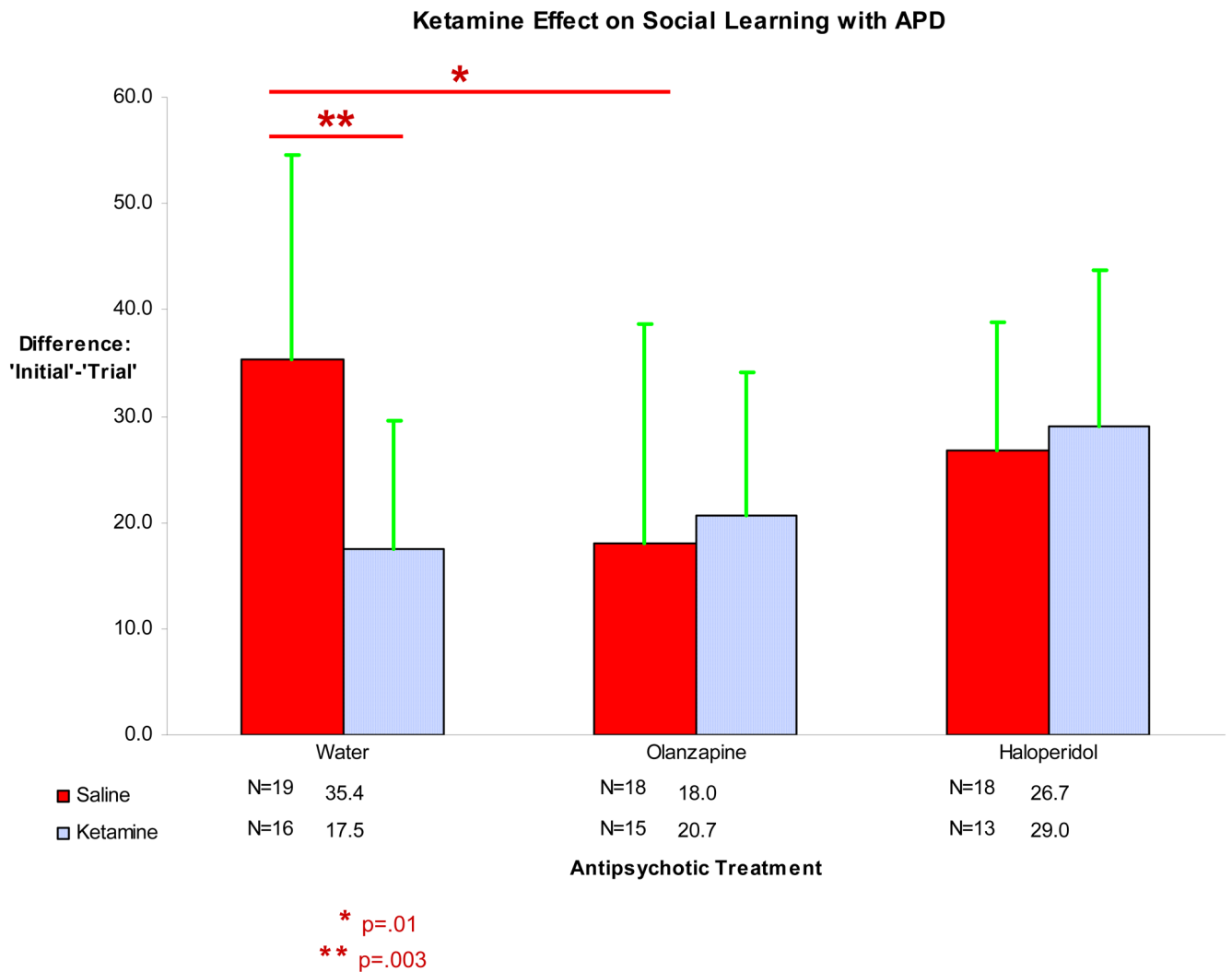
Figure 4.
Ketamine or saline were administered just before the "initial" exposure between two animals making up three different groups, those that were treated with water only, haloperidol (2mg/kg/day) or olanzapine (4mg/kg/day) for 30 days. At the "trial" exposure, social recognition was present in the water/saline condition but reduced by water/ketamine; while subchronic treatment with olanzapine but not haloperidol reduced the initial responding, both antipsychotic drugs (haloperidol/ketamine and olanzapine/ketamine) blocked the disruptive action of ketamine on social learning.

Table 1

Dose Effects of Ketamine on Initial Investigation Time

Treatment	Dose (mg/kg)	Investigation time (second)	Qualifying Mice (%)
Saline	0.1ml/10g	54.4±13.7	75
ketamine	5	45.2±6.4	75
Ketamine	10	47.6±18.3	75
Ketamine	20	45.8±16.7	50
Ketamine	30	44.0±28.2	25

The Investigation time is reported only for the qualifying mice, the percentage of which are noted in the last column. These data were used to identify the highest dose of ketamine that failed to decrease the percentage of investigation time compared with saline as 10 mg/kg.

Table 2

Plasma Drug Concentrations in C57 BL6J Mice

Drug (mg/kg/day)	Drug Plasma Level (ng/ml) Mean±SD	Human Therapeutic Plasma Range (ng/ml)
Haloperidol (2)	7.94±2.1	4–16
Haloperidol (5)	12.5±3.7	4–16
Olanzapine (4)	31.75±16.1	10–30
Olanzapine (8)	37.5±10.6	10–30