Pharmacology, Biochemistry and Behavior 90 (2008) 305–311

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Ketamine blocks the formation of a gustatory memory trace in rats ☆

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ARTICLE INFO

Article history:
Received 13 November 2007
Received in revised form 3 March 2008
Accepted 5 March 2008
Available online 12 March 2008

Keywords:
Conditioned taste aversion
Gustatory trace
Ketamine
NMDA receptors

ABSTRACT

N-methyl-D-aspartate (NMDA) receptors appear to play a central role in learning and memory processes, as the administration of antagonistic substances of these receptors hinders learning acquisition by using different behavioral paradigms (e.g., Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. Behavioural Brain Research, 2003;140 (1–2):1–47.). In the specific case of conditioned taste aversion, the administration of ketamine seems to affect the acquisition of conditioning when the drugs are administered before the experimental treatment. In this paper we present three experiments designed to analyze the effect of different ketamine doses (25 mg/kg, 50 mg/kg, 75 mg/kg and 120 mg/kg), administered between exposure to a taste (the conditioned stimulus) and the administration of the unconditioned stimulus, on the acquisition of a taste aversion association. The results reveal that higher ketamine doses (75 mg/kg and 120 mg/kg) have a disruptive effect on conditioned taste aversion by impeding the formation of the gustatory trace.

1. Introduction

Conditioned taste aversion (CTA) is one of the paradigms that has contributed most to the development of theories, models and concepts in learning. CTA is defined as the result of the association of a taste (the Conditioned Stimulus, CS) with inner malaise normally produced by the intra-peritoneal injection of Lithium Chloride (LiCl, the Unconditioned Stimulus, US). After the conditioning stage the animal rejects the consumption of the taste that has been paired with the malaise. This procedure has a series of characteristics that has led some authors to consider it as a specialized kind of learning (Garcia et al., 1989).

CTA has the following series of peculiarities that differentiates it from other preparations of classical conditioning (Klosterhalfen and Klosterhalfen, 1985): (a) it occurs even if the CS and the US are paired in one single trial, (b) it becomes established despite there being prolonged intervals between the CS and the US, (c) the organism shows a preference to pair nausea with the taste but not with other stimuli, (d) the CTA is produced even when the toxic US does not produce visible malaise or a response of avoidance or escape and (e) the CTA appears even when the experimental treatments are administered under anesthetic states induced by the effect of drugs (Garcia and Hankins, 1977). Garcia et al. (1989) proposed that the establishment of an association between taste and malaise seems to be modulated by mechanisms of neural convergence ahead of temporal contiguity between the CS and the US. On a central level, taste aversion learning occurs when the nervous projections that process gustatory and visceral stimuli are activated following a specific temporal sequence.

From an evolutionary point of view, auditory and cutaneous receptors possess very similar functional properties. The efferent fibers of both systems converge towards the thalamus and from there continue rostrally towards the limbic system in the brain cortex. In turn, the taste and visceral receptors share a very similar evolutionary origin. The efferents of these two systems converge towards the nucleus of the solitary tract, located in the brainstem, and from there they proceed together towards the gustatory thalamus, the basolateral amygdala and the gustatory cortex, crossing the parabrachial area (Garcia and Garcia-Robertson, 1984; Braun et al., 1982; Garcia et al., 1985).

There is a high concentration of NMDA receptors in the basolateral amygdala and in the gustatory insular cortex (Monaghan et al., 1983), and both structures play an important role in CTA learning (Yamamoto et al., 1995; Fernández-Ruiz et al., 1993). Therefore, NMDA receptors seem to be involved in conditioned CTA on a molecular level. This relation can be deduced from research using different antagonistic compounds of NMDA that has found the interruption of the CTA. For example, the systemic administration of ketamine (Welzl et al., 1990; Aguado et al., 1994) and MK-801 (Walker and Scully, 1996), as well as the infusion of CPP and AP5 in the insular gustatory cortex (Escobar et al., 1998, 2002), hinder the establishment of the CTA. In addition, blockade of those NMDA receptors located in the basolateral amygdala leads to CTA disruption (Yasoshima et al., 2000).

☆ This research was supported by Spanish MEC SEJ2004-00065 and SEJ2006-1489 grants.

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doi:10.1016/j.pbb.2008.03.002

For example, Welzl et al. (1990) showed that a subanesthetic dose of ketamine (25 mg/kg), injected before the saccharin–LiCl compound, reduced the level of conditioned aversion to this taste. As no attenuation in learning was produced when the drug was injected between the CS and the US, these authors suggested that the interference was produced with the gustatory trace but not with association. Aguado et al. (1994) found that the administration of ketamine (25 mg/kg), before taste presentation was only effective in reducing learning for the first trial, because after several ketamine–sucrose–LiCl pairings, the animals reached a level of conditioning equivalent to the control group (that received an injection of saline solution before the pairing between taste and toxic substance). Having observed that this dose of ketamine did not produce an effect of interference with phenomena such as habituation of neophobia or latent inhibition, Aguado et al. (1994) considered that the effect of ketamine on learning could be the result of a reduction in the salience of taste, while it would be unlikely for the effect on the acquisition of aversion to be the result of interference with the gustatory memory trace or with the association.

On the other hand, ketamine seems to have an effect on CTA depending on the dose, as an intra-peritoneal injection (25 mg/kg) 30 min before the acquisition phase interrupts learning, while doses of 6 and 12 mg/kg have proved ineffective (Welzl et al., 1990). In contrast, when ketamine (25 mg/kg) is administered between the CS and the US it is not effective in interrupting learning, and neither does it produce interference in the expression of the CTA when it is administered immediately before the test trial (Welzl et al., 1990). However, it is possible that an increase in the dose, when the drug is administered between the taste and the LiCl, might produce some type of interference with learning. Our first experiment was designed to test this possibility. In Experiment 1 different doses of ketamine were injected (25 mg/kg, 50 mg/kg, 75 mg/kg, and 120 mg/kg) between the CS and the US to analyze whether any of the doses interrupted taste aversion learning. In the second experiment we analyzed whether the effect of ketamine on learning is the result of interference with the gustatory trace or with the taste–malaise association. To do this, in experiments 2A and 2B, we introduced a delay between the CS and the US of 30 min and injected high doses of ketamine (75 mg/kg and 120 mg/kg respectively) immediately after the CS, 15 min after the CS or immediately before the US, to analyze whether the interruption of the CTA when the drug is injected between the CS and the US is an effect of limited interference with the formation of the gustatory trace or whether its influence extends to the US and to the association.

In Experiment 1 we ought to observe some level of interference with the expression of the CTA if the NMDA receptors modulate the taste processing or are involved in the formation of the taste–malaise association. On the other hand, if the learning deficit is the result of interference with the gustatory trace, but not with association, in Experiments 2A and 2B the drug ought to be effective for reducing the expression of the CTA when administered immediately after the CS, but not 15 or 30 min after it. In contrast, if the results of the second experiment show that ketamine is effective in producing interference regardless of when it is injected, then it would be possible to state that the blocking of the NMDA receptors either limits the system's capacity to respond to the CS–US contingency, or reduces the motivational or affective impact of the US.

2. Experiment 1

If the injection of ketamine in the interval between the presentation of the CS and the US impedes the establishment of the CTA, it might suggest that the NMDA receptors are participating in the formation of a new gustatory memory trace. To test this hypothesis we used five groups defined by the injected drug dose (saline solution, or ketamine: 25 mg/kg, 50 mg/kg, 75 mg/kg or 120 mg/kg). In each group the animals were exposed to a solution of sucrose diluted in water, followed by the administration of a dose of ketamine and finally, the intra-peritoneal injection of LiCl.

2.1. Method

2.1.1. Subjects

The subjects were 40 adult naïve male *Wistar* rats (mean weight 319 g, range 275–384 g). Subjects were housed in Plexiglas cages (43×25×15 cm) located in a vivarium. A water deprivation schedule (30 min access to water daily) was initiated seven days before the start of the experimental treatment and was maintained throughout the entire duration of the experiment. Standard rat food was continuously available.

2.1.2. Apparatus

All treatments were conducted in Plexiglas cages (30×18×18 cm) located in an experimental room which was separate from the colony room. All liquid rations were provided at room temperature in 150 ml graduated plastic bottles, fitted with stainless steel spouts. Bottles were attached to the front of each cage during liquid presentations. The amount of liquid intake was indexed by the difference between bottle weight before and after liquid presentation. The conditioning and test flavor was a 0.5% sucrose solution. The unconditioned stimulus was a 190 mg/kg i.p. injection of 0.4-M LiCl. Ketamine (25 mg/kg, 50 mg/kg, 75 mg/kg or 120 mg/kg) was also i.p. injected.

2.2. Procedure

2.2.1. Baseline

Water consumption in home cages was registered for the three days before the experimental treatment. In order to minimize differences in baseline drinking, the animals were allocated to groups ($n=8$) depending on the amount of water consumed.

2.2.2. Conditioning

Each rat was placed in its experimental cage and received access to the sucrose solution for 10 min. Then, each animal was injected with the correspondent ketamine dose or the saline solution. The US (LiCl) was administered immediately after the drug injection. The animals were allowed to drink water for an additional 20 min period in their home cages after the experimental treatment.

2.2.3. Testing

The test trial was conducted 24 h after conditioning and consisted of allowing access to the sucrose solution in the experimental cages for 10 min. There were no injections at this stage. The animals received 20 additional minutes of access to water in the vivarium.

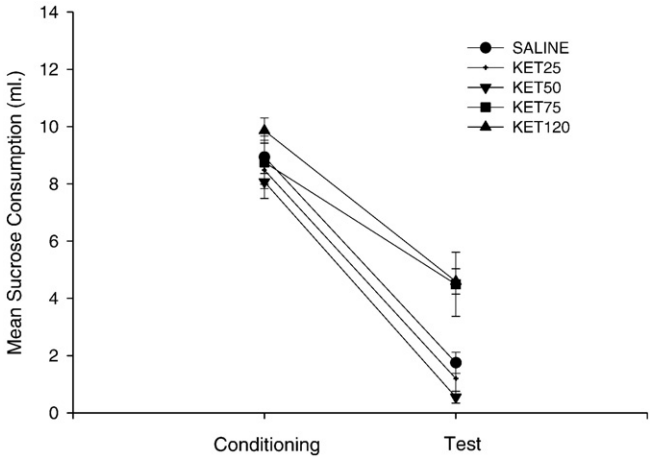

Fig. 1. Mean sucrose consumption (ml) as a function of Groups for conditioning and test trials (see text for procedural details). Error bars represent SEMs.

2.3. Results and discussion

Fig. 1 depicts mean sucrose consumption for conditioning and test trials as a function of Group. As can be seen, a reduction in sucrose consumption was evident in all groups showing conditioned taste aversion conditioning. However, conditioning was less intense for those groups injected with the higher doses of ketamine (75 mg/kg and 120 mg/kg).

This impression was confirmed by a repeated measures 5×2 ANOVA (Groups×Trials) conducted on sucrose consumption. The analysis revealed significant main effects of Trials, $F(1,35)=365.06$; $p<.001$, reflecting a general reduction in consumption from the conditioning to the test trial (the conditioned taste aversion effect), and of Groups, $F(4,35)=6.15$; $p<.01$, due to higher general consumption in the KET75 and KET120 groups compared to the remaining groups. Interestingly, the Trials×Group interaction was significant, $F(4,35)=3.88$; $p=.01$. In order to identify the source of the interaction two ANOVAs with Groups as the main factor were conducted separately on consumption during conditioning and on test days. The ANOVA for the conditioning trial revealed the absence of differences between groups, $p>.35$. However, the ANOVA on the test data revealed significant differences between the groups, $F(4,35)=9.87$; $p<.001$. Post hoc comparisons (Tukey tests, $p<.05$) revealed that KET75 and KET120 consumed more sucrose, i.e., showed less conditioning, than the remaining groups. No other groups were significantly different.

In summary, the experimental results reveal a disruptive effect of the highest ketamine doses (75 mg/kg and 120 mg/kg) on CTA, when the drug is administered between the flavor and the US. This reduction in conditioning was dose dependent because taste aversion was intact when the doses of ketamine injected were lower (25 mg/kg and 50 mg/kg).

3. Experiments 2a and 2b

Experiment 1 demonstrates that the disruptive effect of ketamine on the CTA when the drug is injected between the CS and the US is produced with high doses, which seems to indicate that the NMDA receptors play an important role in the processing of gustatory stimuli. However, we cannot rule out that the injection of the higher doses of ketamine might have had some attenuating effect on the aversive action of the LiCl, which could be at the base of the reduction in conditioning intensity.

To assess this possibility, we performed two experiments where we introduced a time interval of 30 min between the CS and the US; we then administered the ketamine or the saline solution either immediately after the CS, 15 min after or 30 min after the CS (in other words, immediately before the US). In Experiment 2A we used a dose of 75 mg/kg, while a dose of 120 mg/kg was injected in Experiment 2B. In each experiment the groups were formed using a 2×3 (Drug×Delay) factorial design. If the NMDA receptors were involved in the processing of the CS, but did not interfere in the action of the LiCl, we ought to observe a reduction in the conditioned aversion only in the groups that receive the injection of the ketamine immediately after the CS, as the establishment of the gustatory trace would only be impeded in these groups. In contrast, if the drug has any influence on the effect of the LiCl, the greater interference with learning would be observed in all the groups receiving ketamine, regardless of when it was injected, as previous studies have shown that, once administered, the effect of ketamine (30 mg/kg) on NMDA receptors persists for at least 95 min (Lannes et al., 1991).

3.1. Method

3.1.1. Subjects

36 ($n=6$) and 48 ($n=8$) male naïve Wistar rats were used for Experiments 2A and 2B, respectively. The mean weight for all the 90 rats was 385 g (range 310–494). The animals were housed and maintained as described in Experiment 1.

3.2. Apparatus

The same apparatus and stimuli described in Experiment 1 were used in the present experiments, except for a change in the doses of ketamine administered: 75 mg/kg in Experiment 2A and 120 mg/kg in Experiment 2B.

3.3. Procedure

3.3.1. Baseline

The amount of water consumed three days before the start of conditioning stage was computed. As described for Experiment 1, the rats were distributed across the experimental groups considering mean water consumption during these days, in order to minimize differences between groups on conditioning day.

3.3.2. Conditioning

As in Experiment 1, the experimental treatments were conducted in a separate experimental room, not in the colony room. Each animal received access to sucrose for 10 min in the experimental cages. The corresponding doses of saline or ketamine (75 mg/kg in Experiment 2A and 120 mg/kg in Experiment 2B) were administered in the interval between CS and US presentation. In one third of the animals, it was injected immediately after the CS, in the second third 15 min after the CS, and in the last third 30 min after the CS. For all groups LiCl was injected 30 min after the CS.

3.3.3. Test

The test trial was conducted 24 h after the conditioning trial. It consisted of allowing access for 10 min to the sucrose solution in absence of any drug. Fluid consumption was registered as the index of conditioning.

3.4. Results and discussion

3.4.1. Experiment 2a

Fig. 2 shows mean sucrose consumption in the conditioning and test trials as a function of Drug administration time (0, 15 or 30 min after the CS). The upper panel depicts those groups injected with saline and the lower panel those groups injected with 75 mg/kg of ketamine. As the Figure shows, there were no differences between the groups at test stage for the saline condition that reveals similar levels of conditioning. However, conditioning was intact in ket/15 and ket/30 groups, but was clearly reduced in those subjects that received ketamine injection immediately after the CS (ket/0 group).

These impressions were confirmed by a repeated measures 2×2×3 mixed ANOVA (Trials: conditioning vs. test×Drug: saline vs. ketamine×Time of drug: 0 vs. 15 vs. 30 min). The analysis revealed a significant main effect of Trials, $F(1,30)=275.58$, $p<0.001$, due to a general reduction in consumption from conditioning to test trial, that reflects the CTA effect. No more main effects or interactions were significant (all $ps>.15$). In spite of the lack of the predicted 3-way interaction, we conducted some *a priori* comparisons (t tests) based on our hypotheses. A comparison between the ket/0 group and each of the groups that received saline revealed that the animals that received the drug immediately after the CS consumed more sucrose at testing, showing an attenuation of CTA ($p<.05$ for all three comparisons). Interestingly, consumption for the ket/0 group was significantly higher compared with consumption for ket/15 and ket/30 groups, $t(10)=3.78$, $p<.01$ and $t(10)=2.18$, $p=.05$, respectively. There were no significant differences between ket/15 and ket/30, $p>.30$.

3.4.2. Experiment 2b

Fig. 3 depicts mean sucrose consumption at conditioning and test trials as a function of time of drug administration (0, 15 or 30 min after the CS). The upper panel shows those groups injected with the saline

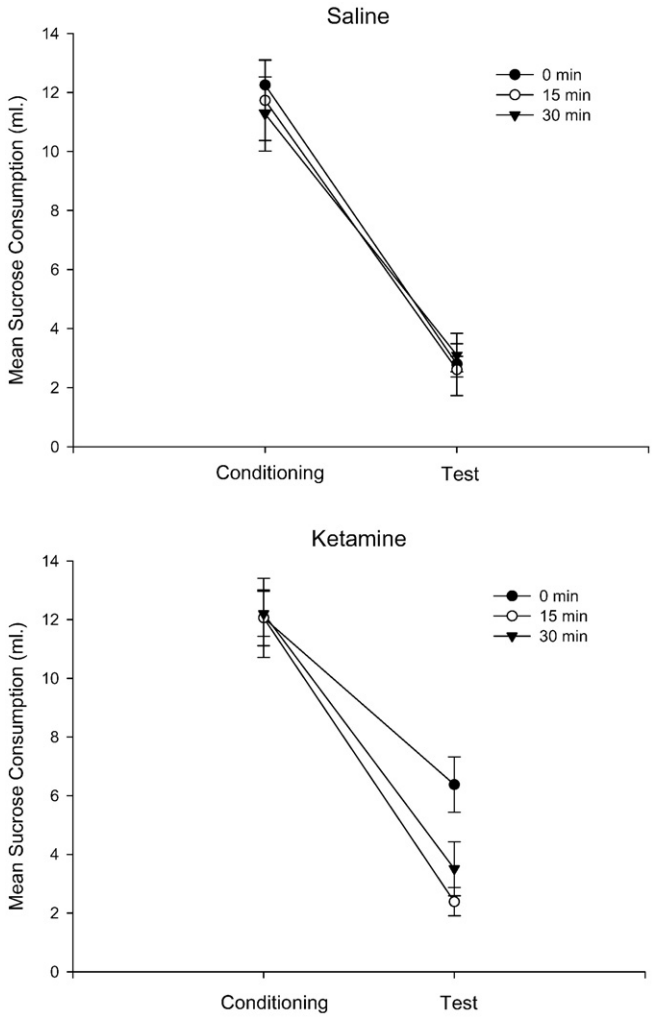

Fig. 2. Mean sucrose consumption (ml) for those groups injected with saline solution (upper panel) or with 75 mg/kg of ketamine (lower panel) as a function of time of drug administration (0, 15 or 30 min after CS presentation) for conditioning and test trials. Error bars represent SEMs.

solution, and the lower panel those injected with 120 mg/kg of ketamine. As can be seen in the section for the saline groups, CTA was evident in all groups, but aversion was higher in the group that received the saline injection immediately after the CS (Sal/0 group). The data for the ketamine groups (Fig. 2, lower panel) seem more complex. Although the absolute differences between the groups were not that great, the within groups variance was minimum. The CTA reduction was apparent only for the group that received the ketamine immediately after the CS (Ket/0 group).

A repeated measures 2×2×3 mixed ANOVA with main factors Trials (conditioning vs. Test), Drug (saline vs. Ketamine), and Time of drug (0 vs. 15 vs. 30 min) revealed significant effects for all main effects. The main effect of Trials, $F(1,42)=3515.19$, $p<.001$, was due to the general CTA that resulted in a strong consumption reduction between conditioning and the test trial. The significant effect of Drug, $F(1,42)=29.53$, $p<.001$, reflects higher general levels of sucrose consumption for those animals that received ketamine (mean=8.90 ml, SD=1.22) compared to the rats that received the saline solution (mean=8.19 ml, SD=1.22). The significant effect of Time of drug, $F(2,42)=16.04$, $p<.001$, reflects more sucrose consumption for those groups that received the injection immediately after the CS (mean=9.06 ml, SD=1.6) as compared to those groups that received the injection 15 or 30 min after the CS (mean=8.31 ml, SD=0.44, and mean=8.25, SD=10.32, respectively).

The interactions Trials×Drug and Trials×Time of drug were statistically significant, $F(1,42)=33.03$, $p<.001$ and $F(2,42)=8.88$, $p=.001$, respectively. The Trials×Drug interaction was due to a general higher conditioning for the saline compared to the ketamine groups. The Trials×Time of drug interaction reflects higher levels of conditioning for the groups injected immediately after CS presentation compared to the groups injected 15 or 30 min after the CS. The 3-way interaction was also significant, $F(2,42)=37.43$, $p<.001$. As Fig. 3 shows, the interaction was due to the reduction in CTA intensity for the group injected with ketamine immediately after the CS (see Fig. 3).

Finally, the Drug×Time of drug interaction was also significant, $F(2,42)=69.39$, $p<.001$, due to a general higher level of consumption shown by those groups injected immediately after the CS compared to those groups injected 15 or 30 min after the CS presentation.

4. General discussion

The results from Experiment 1 show that the administration of ketamine during the period between the CS and the US produces interference with the CTA with doses of 75 mg/kg and 120 mg/kg, while no effect was observed when the injected doses were 25 mg/kg or 50 mg/kg. This last finding is similar to that obtained by Welzl et al. (1990) who used a dose of 25 mg/kg administered between the saccharin and the LiCl and

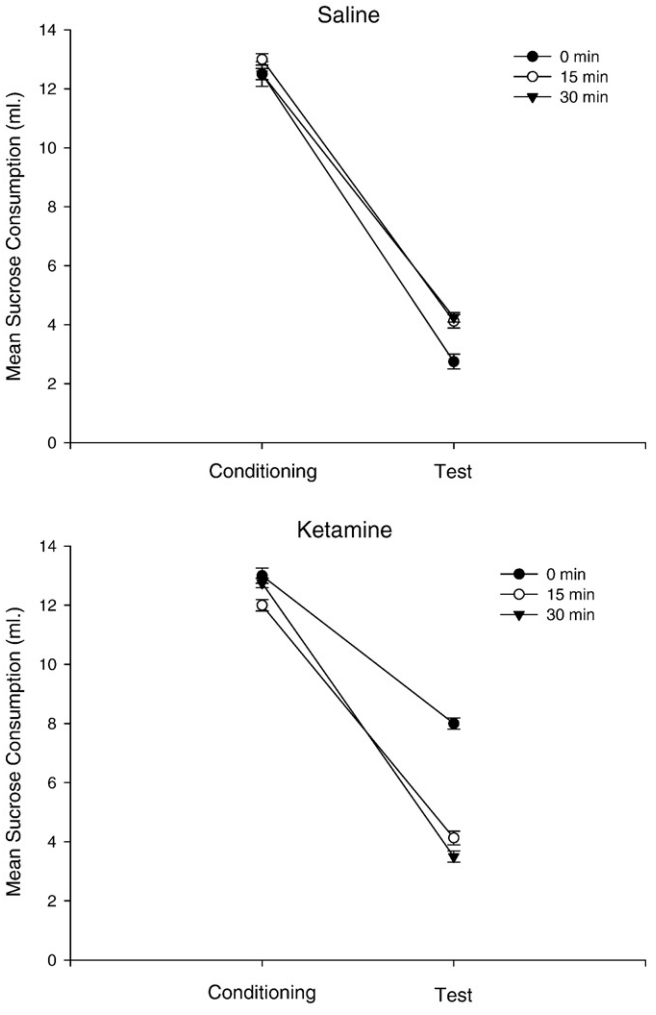

Fig. 3. Mean sucrose consumption (ml) for those groups injected with saline solution (upper panel) or with 120 mg/kg of ketamine (lower panel) as a function of time of drug administration (0, 15 or 30 min after CS presentation) for conditioning and test trials. Error bars represent SEMs.

found no effect on the CTA. These results confirm that high doses of ketamine have a disruptive effect on learning expression.

In Experiments 2A and 2B, the injection of 75 mg/kg and 120 mg/kg of ketamine, respectively, immediately after exposure to the CS, produced a reduction in CTA, while the injection of the same doses after 15 or 30 min did not interfere with learning. This result allows us to conclude that the disruptive effect of ketamine on CTA was not determined by a possible anesthetic or analgesic action of the drug on the aversive effect of the LiCl, neither does it seem to interfere directly with the establishment of the association between the CS and the US. Instead ketamine seems to act indirectly on conditioning by impeding the establishment of the gustatory memory trace that would be associated with the malaise induced by the US in the absence of the drug.

The overall reduction in saccharin consumption observed for all groups at testing indicates that some degree of conditioned taste aversion was evident in spite of the drug administration. Therefore, we can conclude that the drug administration was effective in reducing taste aversion for those groups in the ketamine condition, but did not completely impede the association between the flavor and the US (for similar results see Aguado et al., 1994).

In short, the results obtained in these experiments show that NMDA receptors play an important role in the formation of the gustatory memory trace. Specifically, it appears that NMDA receptors modulate the processing of the gustatory stimulus in the central nervous system. This hypothesis was originally proposed by Welzl et al. (1990), as the administration of ketamine (25 mg/kg) produced interference in the CTA when administered before taste presentation, but was ineffective for interrupting learning when the intra-peritoneal injection was given between the CS and the US. Aguado et al. (1994) refuted this hypothesis as the systemic administration of the same ketamine dose did not produce effects on the habituation of neophobia, or on latent inhibition when the drug was injected before the preexposure and conditioning stages. According to these authors, although their results seem to indicate that the NMDA receptors are not directly involved in the formation of the gustatory memory trace, they do not rule out the possibility that the ketamine is acting on mechanisms related to learning and memory. It would therefore be possible for the NMDA receptors to be only partially blocked, and this blocking might be compensated for by stimuli intensity, or by prolonged training. Alternatively, learning might depend on other neurophysiological mechanisms that could compensate the induced inactivity of the NMDA receptors. However, other studies have obtained results that contradict Aguado et al.'s findings (1994). On this point, it is notable that the administration of MK-801 produces an interruption of habituation to neophobia when administered systemically (Jackson and Sanger, 1989) and this effect depends on the dose injected (it appears with a dose of 0.3 mg/kg but not with 0.1 mg/kg and 0.03 mg/kg). Specifically, when the highest dose was administered the amount of fluid consumed did not change across trials, but with the lowest doses (0.1, and 0.03 mg/kg) the amount of saccharin consumed increased across trials, revealing the interference with habituation of neophobia for the highest dose. On the other hand, intracraneal administration of AP5 on insular gustatory cortex did not affect habituation of neophobia (Gutierrez et al., 2003), suggesting the lack of involvement of NMDA receptors in the transition from a novel to a familiar flavor (Bermúdez-Rattoni, 2004). However, MK-801 infused in the same location blocked habituation of neophobia (Figueroa-Guzman et al., 2006), indicating a differential effect on flavor processing of the NMDA antagonist type employed (e.g., competitive vs. Non-competitive). Some experiments have analyzed the effect of AP5 infusion in the insular gustatory cortex on CTA, showing an abolition of conditioning when the drug was administered before CS and US presentation (e.g., Escobar et al., 2002; Ferreira et al., 2002). However, the results were less clear when the drug was injected between CS and US presentations, because in some cases this procedure led to learning disruption (Rosemblum et al., 1997), while in others the aversion remained intact (Ferreira et al., 2002). Such differences are probably related to the different AP5 concentrations administered in the experiments. On the other hand, latent inhibition was interrupted when MK-801 was injected intra-peritoneally before (Turgeon et al., 2000) or after (Traverso et al., 2003) the preexposure and conditioning stages. In turn, ketamine has been effective for eliminating latent inhibition when injected before (Aguado et al., 1994) or after the preexposure stage (Gallo et al., 1998). These results support the hypothesis that NMDA receptors could be involved in the modulation of the gustatory trace. To end with, ketamine administration (50 mg/kg and 100 mg/kg) impedes recognition of a familiar flavor in fetal rats (Mickley et al., 2000).

Ketamine is a non-competitive antagonist; it therefore blocks the channel of the NMDA receptor progressively. Indeed, the kinetics of ketamine could be playing a relevant role in our study. However, there are only a few studies analyzing the effect of ketamine active metabolites on rats' behavior. In fact, some contradictory results appear in the literature: Norketamine, the primary ketamine metabolite, induces effects similar to ketamine; however, a similar dose of the secondary ketamine metabolite, (*Z*)-6-Hydroxynorketamine, did not induce either an anesthetic effect or central nervous system activation (Leung and Baillie, 1986). The exact amount of time that ketamine and other similar drugs take to block the channel is unknown, but the process is relatively slow and depends on whether the ionic channel is open or closed by the agonist's action. Once the channel is open, it is blocked rapidly by the drug, but as the probability of the NMDA receptor channel being open is low, the overall blocking process is slow and gradual (see Huettner and Bean, 1988; MacDonald et al., 1987). Other authors have shown that antagonist effects on NMDA receptors of ketamine, injected intra-peritoneally to a dose of 30 mg/kg, extends from 20 min to an interval ranging from 95 min to 170 min (Lannes et al., 1991). These data suggest that ketamine is effective for reducing or limiting the system's capacity for establishing associative connections and that the attenuation of learning would occur after a time interval ranging from 10 min to 20 min. In our study we observed that as the distance in time between drug administration and sucrose consumption increased, the effectiveness of learning interruption decreased, which seems to indicate that if the NMDA receptors are not completely blocked when the taste–malaise connection is established at a central level, its ability to interfere in the association decreases significantly. In this way, in the study by Welzl et al. (1990), the administration of 25 mg/kg of ketamine could have been ineffective for producing the interruption of the CTA when administered between the CS and the US for two reasons: the dose of the drug may have been insufficient to block the NMDA receptors and, secondly, by injecting ketamine 50 min after the presentation of the CS and 10 min before the US presentation, the NMDA receptors might not have been completely blocked at the moment of establishing the taste–malaise association.

On the other hand, both in our study and in the experiments described by Welzl et al. (1990), it is possible that, during the first period of 15 min after flavor exposure, a gustatory trace was formed that remained unaffected by ketamine administration. Thus, this flavor trace would still be active when the US appears resulting in a normal association. Finally, ketamine (75 and 120 mg/kg) interfered with CTA when the LiCl was injected immediately after flavor consumption (Experiment 1) or 30 min after flavor consumption (Experiments 2A and 2B). Therefore, the concentration of the drug 30 min after its administration in blood plasma with these ketamine doses was intense enough to interfere with the formation of the CS–US association.

Ketamine has anesthetic properties (e.g. Haas and Harper, 1992). In this sense, our results reveal that the injection of anesthetic (120 mg/kg) or subanesthetic doses (75 mg/kg) produces blocking in learning. If in our experiments the cortex was under anesthetic when the taste was processed, there may have been an attenuation of the CTA. For example, Bures and Buresova (1989) established a time lapse of 2 h between the injection of saccharin and the LiCl injection and compared the effect that the administration of pentobarbital (50 mg/kg) produced at different intervals before and after the presentation of the CS. An attenuation of the CTA was observed when the pentobarbital was injected before the

saccharin or 30 min afterwards, but no effect was observed when a time interval of 1 h or more had passed between the taste and the anesthetic. If in our study the ketamine induced a general anesthetic state blocking the formation of the gustatory trace, the application of the drug 15 or 30 min after the presentation of the sucrose ought to have produced an attenuation of the CTA. However, this was not the case. The anesthetic seems to block the initial formation of the memory trace for the taste, but it does not prevent its association with the malaise induced by the LiCl (Buresova and Bures, 1977). In turn, the cortex seems to bear the initial formation of the gustatory memory trace, but the connection of this engram with the information referring to the gastrointestinal malaise may occur without cortical participation (Buresova and Bures, 1973). Therefore, we cannot completely rule out that the reduction in learning of taste aversion is due to the anesthetic state induced by the ketamine, as it is possible that 15 min or 30 min after the presentation of the taste a memory trace has been formed which does not require cortical support and which can establish a functional connection with the aversive stimulus. In contrast, the induction of an anesthetic state through the injection of ketamine immediately after exposure to sucrose would have been effective in blocking the formation of the memory trace. However, having adopted the appearance of ataxia and the loss of righting reflexes as an indication of anesthesia, some authors found that the effective dose for inducing anesthesia in mice and rats is 150 mg/kg (Irufine et al., 1991; Kelland et al., 1993). As our doses were inferior (75 mg/kg and 120 mg/kg) it seems improbable that they would have induced a state of anesthesia capable of limiting or blocking the formation of the initial gustatory memory trace. In addition, other research has found that it is possible to establish learning of taste aversion despite the animals being anesthetized (Roll and Smith, 1972; Rozin and Ree, 1972). This association is established even when the pentobarbital is administered after the presentation of the taste, with the malaise being induced by the effect of LiCl, or gamma radiation (Rabin and Rabin, 1984).

Ketamine can produce a range of effects, amongst which is the ability to produce analgesia (e.g. Subramaniam et al., 2004). Therefore, in our study, a possible alternative is that the analgesic effect of the ketamine might have diminished the effect of the LiCl, reducing the perception of gastrointestinal malaise and ultimately interfering with the establishment of the CS–US association. However, as occurs with the anesthetic effect of ketamine, this does not seem very probable, as a possible interference in learning through an analgesic effect on the US ought to have occurred even when the drug was injected 15 min after the CS or immediately before the US (Experiments 2A and 2B). In addition, Mickley, Remmers-Roeber, Dengler, McMullen, Kenmuir, Girdler, Crouse and Walker (2002) reported that ketamine administration (1 mg/kg, 10 mg/kg and 25 mg/kg) prior to saccharin–LiCl pairings did not affect saccharin consumption at testing. This result was considered as a demonstration of the absence of an effect of the drug on the sensory perception of the aversive properties of LiCl.

The interruption of the CTA is obtained both with ketamine (Welzl et al., 1990; Aguado et al., 1994), and with MK-801 (Walker and Scully, 1996), when both drugs are injected before the stimuli. As our results with ketamine replicate those obtained with MK-801 (Traverso, Ruiz, Camino & De la Casa, unpublished results), demonstrating that disruption of learning of taste aversion also occurs when drugs are injected after the taste, it is possible to eliminate the side effects of both types of compounds as a source of variance. Ketamine produces effects such as anesthesia, analgesia, sensory alterations, hyperlocomotion, ataxia, stereotypy, etc; while MK-801 produces similar sensorimotor effects to ketamine (sideways movements, ataxia, stereotypy, etc.), and although it is not an effective anesthetic (Kelland et al., 1993), it does encourage the anesthetic properties of other compounds (Daniell, 1990). In this sense, both drugs could limit the sensory and perceptual capacity of the animal, significantly reducing the level of learning when the drug is injected before presentation of the Pavlovian treatments. In contrast, the presentation of the pharmacological compounds after presentation of the CS reduces the influence of the sensorimotor effects on the animal's ability to adequately process the stimuli. However, animals under the effect of subanestethic doses of ketamine (1 mg/kg, 10 mg/kg, and 25 mg/kg) can discriminate between different saccharin intensities (0.3% vs 0.6%), (Mickley et al., 2002).

In sum, our results together with those appearing in the literature seem to indicate that ketamine produces interference in learning of taste aversion, without it being possible to attribute this effect to analgesic or anesthetic effects or to interference with the animal's sensory-perceptual capacity. Therefore, we can conclude that this effect seems to be the consequence of interference during the initial stage of the formation of the gustatory trace.

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