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MK-801 induces a low intensity conditioned taste aversion

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ABSTRACT

N-methyl-D-aspartate (NMDA) receptor antagonists are often used to assess the role of NMDA receptors in learning and memory processes. However, few studies have explored the possibility that the antagonists may induce a conditioned aversion when administered following flavor consumption. We report five experiments with rats intended to evaluate the MK-801 capacity to induce conditioned taste aversion. Our findings suggest that: i) MK-801 produces a low-intensity aversion following repeated pairings with saccharin (Experiments 1 and 2); ii) such aversion was not the result of a non-associative process (Experiment 3); and iii) pre-exposure to MK-801 does not interact with conditioned taste aversion induced by lithium chloride (Experiments 4 and 5). These findings suggest that MK-801 induces a low-intensity aversion, although the underlying mechanisms of this aversion may differ from those of a conditioned aversion produced by lithium chloride.

1. Introduction

N-methyl-D-aspartate (NMDA) receptors appear to modulate stimulus processing in learning and memory tasks (see, for a review, Riedel et al., 2003). Several pharmacological compounds have antagonistic properties on NMDA receptor activity, including AP5, AP7, MK-801, PCP, and ketamine. While all these substances function as NMDA antagonists, they can differ in the mechanism through which they block NMDA receptor activity. For instance, AP5 acts on the binding site for glutamate (Collingridge et al., 1983), MK-801 acts by gradually blocking the channel (Coan et al., 1987), PCP and ketamine block the receptors by joining to specific binding sites (Anis et al., 1983), and 7Cl Kyn blocks the binding site for glycine (Bashir et al., 1990).

NMDA receptors are activated by the activity of a glutamate molecule that joins to a specific base in the receptor and opens the calcium (Ca^{2+}) channel. When an antagonist inhibits NMDA receptor activity, the process known as long-term potentiation (LTP) is impeded, which has normally been interpreted as memory interference in learning tasks (e.g. Welzl et al., 1990). For instance, Stringer and Guyenet (1982) found that PCP and ketamine administration, both dissociative drugs, significantly interferes with or blocks LTP in the hippocampus C1 area.

NMDA receptor antagonists can interfere with a wide variety of behavioral tasks, whether injected intravenously or injected in specific structures or regions. The behavioral tasks in which interference has been found include spatial learning in a water maze (Morris, 1989), radial maze (Shapiro and Caramaros, 1990), and T-maze (Handelman et al., 1987), as well as olfactory discrimination tasks (Staubli et al., 1989), passive and active avoidance (Danysz, 1990), visual discrimination tasks (Bevenga and Spaulding, 1988), and taste aversion learning (Aguado et al., 1994; see also Walker and Scully, 1996).

In conditioned taste aversion (CTA) experiments, interference with learning might be confounded with a possible aversive effect of NMDA antagonists. For instance, some studies have found that ketamine (25 mg/kg) can produce CTA after one or several pairings with flavor, although the effect is weak compared to the aversion induced by lithium chloride (LiCl) (Welzl et al., 1990; Aguado et al., 1997). A similar result has been found with other NMDA receptor antagonists, such as PCP (3 mg/kg), MK-801 (0.3 mg/kg), (+)-NANM (10 mg/kg), (−)-NANM (3 mg/kg), and ifenprodil (10 mg/kg), when administered after access to saccharin solution (Jackson and Sanger, 1989). With MK-801, CTA appears after several pairings with flavor and, moreover, the effect is dose-dependent, appearing with a 0.3 mg/kg dose but not a 0.1 mg/kg dose (Jackson and Sanger, 1989).

In order to explore the properties of the aversive effect induced by systemic administration of MK-801, we run five experiments evaluating the capacity of the drug to act as an Unconditioned Stimulus (US) in a CTA procedure. In Experiment 1, we injected two different doses of MK-801 (0.2 mg/kg and 0.3 mg/kg) after access to a flavor (saccharin) and we assessed the subsequent level of flavor aversion. In Experiment 2, we lengthened the exposure time to the flavor compared to Experiment 1, to increase the strength of the aversion induced by MK-801 and to check whether CTA induced by the NMDA antagonist could be as intense as that produced by LiCl, a substance traditionally used as a US in the CTA paradigm. In Experiment 3, we injected the drug immediately after the CS or 6 h after the CS, in order to check any possible non-associative effect affecting fluid consumption. In

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addition we used MK-801 doses of 0.3 mg/kg and 3.0 mg/kg to evaluate whether a higher dose of the drug induces a more intense taste aversion effect. In Experiment 4 we examined whether exposure to MK-801 (0.2 mg/kg and 0.3 mg/kg doses) prior to pairing the drug with saccharin would lead to a reduction in the subsequent conditioned aversion (the US-pre-exposure effect, see for example, Randich and Lolordo, 1979). Finally, in Experiment 5, we examined the degree to which pre-exposure to MK-801 (0.2 mg/kg and 0.3 mg/kg) might affect the intensity of an aversion induced by LiCl, which would suggest that the two substances trigger similar processes.

2. Experiment 1

To examine the MK-801 potential to produce CTA, we perform an experiment with four groups. Two groups received the common manipulation employed to induce CTA, with a saccharin solution as the Conditioned Stimulus (CS) and LiCl as a malaise-inducing substance or saline solution as the control substance (groups LiCl and Sal, respectively). For the remaining two groups, access to saccharin was followed by 0.2 mg/kg or 0.3 mg/kg MK-801 administration (groups MK-0.2 and MK-0.3, respectively). If MK-801 produces conditioned aversion, then we should observe a decrease in saccharin consumption across conditioning trials. Based on past findings (e.g. Jackson and Sanger, 1989) we expected that CTA, if induced at all, would be weaker than that induced by LiCl.

2.1. Material and methods

2.1.1. Subjects

The subjects were 32 male Wistar rats, with an average weight of 394 g (ranging from 339 to 467 g). Upon the rats' arrival to the laboratory, they were housed in individual Plexiglas cages (43 x 25 x 15 cm). After a three-week period of habituation to the colony, each animal received a five-minute handling session. Immediately afterwards, a water-deprivation program began in which the animals were given 30 min of access to water per day. The animals were randomly assigned to each condition ($n=8$). The experimental manipulations began seven days after the beginning of the deprivation program. A 12:12 hour light–dark cycle was maintained throughout the entire experiment, and the animals had unlimited access to food. In this and all subsequent experiments the procedures were conducted in agreement with the guidelines established by the Directive 86/609/CEE of the European Community Council, and the Spanish R.D. 223/1988, and all the experiments were approved by the animal care and use committee of Seville University (Spain).

2.1.2. Apparatus

The experimental sessions were carried out in a room different to the colony room. At the beginning of each session the animals were placed in individual transparent Plexiglas cages (30 x 18 x 18 cm). The flavor employed was a 0.04% saccharin solution. Liquids were presented in graduated plastic bottles (150 ml) with a stainless steel sipper tube. The amount of liquid consumed was measured by comparing the pre-session and post-session bottle weights. The saline solution, the LiCl solution (0.4 M, 0.5% of body weight) and the MK-801 solution (0.2 mg/kg or 0.3 mg/kg) were injected intraperitoneally immediately after saccharin access during the conditioning phase.

2.1.3. Procedure

Once the animals had been placed in the experimental cages, they were given access to the saccharin solution for 5 min. Immediately afterwards, the animals received the innocuous, toxin, or pharmacological injection, depending on group membership. This conditioning phase took place over four consecutive days, with one conditioning trial per day. After each experimental session, the animals received 25 additional minutes of water access to preserve the deprivation program initiated at the start of the experiment.

2.2. Results

Fig. 1 shows the average saccharin consumption over conditioning trials for each group. As reflected in the figure, the animals that received the saline solution after exposure to saccharin did not reduce their consumption across conditioning trials. However, consumption decreased sharply for animals in the LiCl group, with maximum conditioning reached after one trial. In groups MK-0.2 and MK-0.3, consumption decreased over conditioning but the decrease was smaller than that observed for LiCl group. This decrease in consumption appeared on the second conditioning trial for MK-0.3 group, and on the third conditioning trial for MK-0.2 group.

These impressions were confirmed by a 4 x 4 mixed ANOVA (Groups x Trials) conducted on mean consumption at conditioning trials. The main factors of Group and Trials were significant, [$F(3,28)=24.9$, $p<0.001$, and $F(3,84)=65.33$, $p<0.001$]. The effect of groups reflects the differences in consumption between the subjects as a result of the different experimental treatments, and the effect of Trials was due to the progressive reduction of consumption across conditioning trials.

The Trials x Group interaction was also significant, $F(3,84)=65.33$, $p<0.001$, and $F(9,84)=17.46$, $p<0.001$, respectively. The two-way interaction was due to the different conditioning rates for each experimental group. The differences between groups (planned comparisons, $p<.05$) have been identified in Fig. 1 by asterisks.

3. Experiment 2

The purpose of Experiment 2 was to examine whether increasing the intensity of the CS (specifically, increasing the exposure time to the CS) would result in a more rapid or intense conditioned aversion produced by MK-801. To this end, we used the same procedure as that of Experiment 1 but increased the time of exposure to the flavor from 5 to 30 min. If the aversion induced by MK-801 is functionally similar to that induced by LiCl, then increasing the exposure time to the CS should produce a more intense aversion.

3.1. Material and methods

3.1.1. Subjects

The subjects were 28 male Wistar rats weighing on average 466 g (ranging from 394 to 552 g). Upon the animals' arrival in the colony room, the habituation, handling, deprivation, and the light–dark cycle were the same as described for Experiment 1. The animals were randomly assigned to each condition ($n=7$).

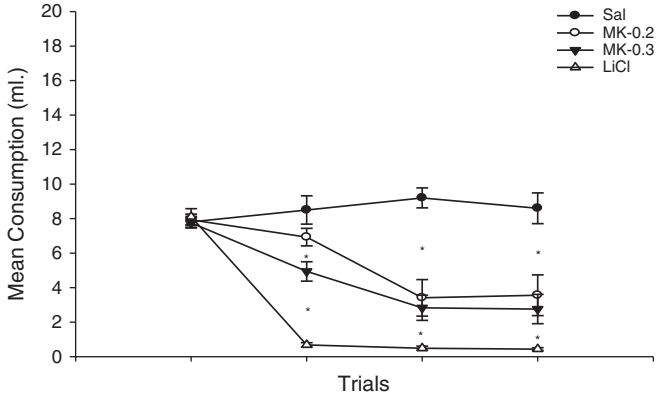

Fig. 1. Mean saccharin consumption (ml) across conditioning trials as a function of groups. Error bars represent SEMs.

3.1.2. Apparatus

As in Experiment 1, the experimental sessions were performed in a room different from the colony room. The cages, solutions, concentrations, and instruments were the same as those used in Experiment 1.

3.1.3. Procedure

Unlike in the previous experiment, the animals had 30 min of access to saccharin over four daily trials. Immediately after each saccharin exposure, the animals received the drug, LiCl or saline solution, depending on group membership.

3.2. Results

Fig. 2 shows saccharin consumption across conditioning trials for the animals having received saline solution, LiCl, and the two doses of MK-801. As can be seen in the figure, LiCl produced a strong conditioned aversion to flavor after the first day of conditioning, reflected in a sharp decrease in saccharin consumption in the animals assigned to this condition. The MK-801 injections (0.2 mg/kg or 0.3 mg/kg) produced a moderate decrease in saccharin consumption that reached its lowest level after the third conditioning trial. In the group receiving saline, saccharin consumption was high and stable throughout the conditioning sessions.

These impressions were confirmed by the statistical analyses. Specifically, a 4 x 4 mixed ANOVA (Trials x Groups) conducted on mean saccharin consumption across conditioning trials revealed a significant main effect of Groups, $F(3,24) = 22.6$, $p<0.001$, due to the differential reduction in consumption between groups. The main effect of Trials was also significant, $F(3,72) = 47.21$, $p<0.001$, due to the general reduction in consumption produced by conditioning. Finally, the interaction was also significant, $F(9,72) = 15.71$, $p<0.001$. An exploration of the interaction using planned comparisons ($p<.05$) revealed different conditioning rates between groups, with lack of conditioning for the SAL group, maximum conditioning for the LiCl Group and an intermediate conditioning for the MK-0.2 and MK-0.3 groups.

The results of this experiment, along with those of Experiment 1, suggest that MK-801 induces a conditioned aversion of medium intensity. This conclusion is based on a comparison between animals receiving MK-801 and those receiving LiCl, with the latter showing a significantly stronger aversion. The effect of MK-801 on saccharin consumption appears to be dependent neither on the dosage nor on the intensity of the flavor, given that the levels of these variables employed in the present experiment produced similar degrees of aversion.

4. Experiment 3

In Experiments 1 and 2 we tested whether intraperitoneal injections of MK-801 (0.2 mg/kg and 0.3 mg/kg) would be effective in inducing CTA when administered after access to a saccharin solution. However, we cannot discard an alternative explanation to the reduction of saccharin consumption observed across conditioning trials, based in some kind of non-associative process (e.g. a reduction in consumption due to generalized illness induced by the drug). To check this possibility, we run an experiment comparing the effect of MK-801 (0.3 mg/kg) injected immediately or 6 h after saccharin consumption. If the reduction in consumption observed in Experiments 1 and 2 in those groups injected with the drug was due to an associative process, this effect would only appear in the no delay group.

4.1. Material and methods

4.1.1. Subjects

The subjects were 14 male Wistar rats weighing on average 337 g (ranging from 293 to 387 g). Upon the animals' arrival to the colony room, the habituation, handling, deprivation, and the light–dark cycle were the same as described for Experiments 1 and 2. The animals were randomly assigned to each condition ($n = 7$).

4.1.2. Apparatus

The apparatus was the same as described for previous experiments. The saline solution and the MK-801 solution (0.3 mg/kg) were injected intraperitoneally immediately or 6 h after saccharin access during the conditioning phase.

4.1.3. Procedure

As in Experiment 2, the animals had 30 min of access to saccharin over four daily trials. Immediately after each saccharin exposure (for the 0-delay groups) or 6 h after the saccharin consumption (for the 6 h groups), the animals received the MK-801 or the saline solution. For the 6 h groups, the animals returned to their home cages after saccharine consumption and then again to the experimental room to receive the i.p. injection.

4.2. Results

Fig. 3 shows saccharin consumption across conditioning trials for the animals injected immediately after saccharin consumption or 6 h later as a function of MK-801 doses. As can be seen in the figure, a decline in consumption across trials for the MK-0.3/0 min was evident, which did not appear in the MK-0.3/6 h group. This result replicates

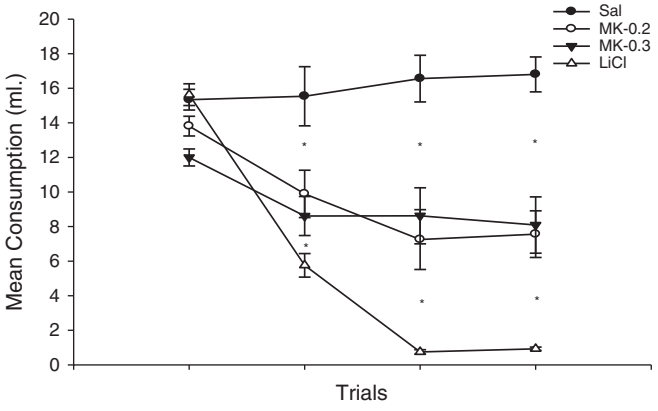

Fig. 2. Mean saccharin consumption (ml) across conditioning trials as a function of groups. Error bars represent SEMs.

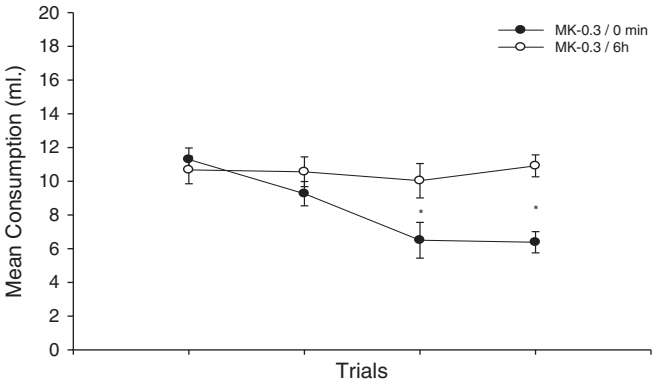

Fig. 3. Mean saccharin consumption (ml) across conditioning trials as a function of groups. Error bars represent SEMs.

the mild taste aversion supported by MK-801 obtained in previous experiments and discards possible non-associative effects as a cause of such an effect.

These impressions were confirmed by the statistical analyses. Specifically, a 4 x 2 mixed ANOVA (Trials x Groups) conducted on mean saccharin consumption across conditioning trials revealed a significant main effect of Trials, $F(3,36) = 7.21$, $p < 0.01$, due to a general decreasing of consumption. The main effect of Group was also significant, $F(1,12) = 6.69$; $p < .05$, due to the reduced consumption showed by the animals in the MK-0.3/0 min (mean = 8.36 ml, SD = 1.37) as compared to the MK-0.3/6 h (mean = 10.54 ml, SD = 1.82).

Finally, the Trials x Group interaction was significant, $F(9,36) = 6.25$, $p < 0.01$. The interaction was examined using planned comparisons ($p < .05$) that showed differences from the third trial between the MK-0.3/0 min and the MK-0.3/6 h, revealing the expected taste aversion in the former group.

5. Experiment 4

Having consistently found medium-intensity CTA with a low MK-801 dose, and having discarded a non-associative explanation of the fluid consumption reduction, in Experiment 4 we explored whether pre-exposure to MK-801 could retard conditioning in the same manner as when animals are pre-exposed to LiCl before it is paired with a flavor (the US pre-exposure effect; for a review, see Randich and Lolordo, 1979). The finding that the decrease in flavor consumption following MK-801 administration involves mechanisms similar to the CTA found when LiCl is used as the US would advance our understanding of the mechanisms involved in the decrease in flavor consumption following repeated administration of MK-801.

Experiment 4 followed a US-pre-exposure design with two phases (pre-exposure and conditioning) and four groups that differed in terms of the dosage of the drug administered during pre-exposure (saline, 0.2 mg/kg, or 0.3 mg/kg of MK-801) and during conditioning (0.2 mg/kg or 0.3 mg/kg of MK-801). The animals having received saline during pre-exposure were distributed evenly across the two levels of MK-801 dosage at conditioning, while the animals having received MK-801 during pre-exposure received the same dosage at conditioning.

5.1. Material and methods

5.1.1. Subjects

The subjects were 28 male Wistar rats with an average weight of 379 g (ranging from 309 to 455 g). Habituation, deprivation, handling, and the light–dark cycle were the same as those in previous experiments. The animals were randomly assigned to each condition ($n = 7$).

5.1.2. Apparatus

The apparatus and instruments used in this experiment were similar to those described for Experiments 1 and 2.

5.1.3. Procedure

The first three days (pre-exposure) the animals received access to water in the experimental cages for 30 min each day, and immediately afterward received an injection of saline or MK-801 (0.2 mg/kg or 0.3 mg/kg), depending on group membership. On days 4 to 7 (conditioning), the animals were given access to saccharin for 30 min in the experimental cages each day. Immediately afterwards, all animals received an injection of MK-801 (0.2 mg/kg or 0.3 mg/kg, depending on the group), except after the fourth trial, since no further trials were planned and therefore the MK-801 injections were no longer administered.

5.2. Results

Fig. 4 shows water consumption during the pre-exposure phase and saccharin consumption across conditioning trials for each group. As we expected, water consumption was stable throughout the pre-exposure phase and did not differ between the animals receiving saline and those receiving MK-801. During the conditioning phase we only observed a decrease in saccharin consumption across trials in group SAL/MK-0.3, a decrease that did not appear when the drug had been repeatedly pre-exposed prior to conditioning (group MK-0.3/MK-0.3).

A 4 x 3 mixed ANOVA (Trials x Groups) conducted on mean consumption across pre-exposure trials showed neither significant main effects nor interactions (all $p > .10$).

A 4 x 4 mixed ANOVA (Trials x Groups) conducted on mean saccharin consumption across conditioning trials revealed a significant Trials x Groups interaction, $F(9,72) = 5.67$, $p < 0.01$. Planned comparisons ($p < .05$) performed to identify the source of the interaction revealed a significant reduction of saccharin consumption for the Sal/MK-0.3 group as compared to the MK-0.3/MK-0.3 and the MK-0.2/MK-0.2 groups from the second trial on. The reduction in consumption for the Sal/MK-0.2 group as compared to the MK-0.3/MK-0.3 and the MK-0.2/MK-0.2 groups was restricted to the fourth trial. No more comparisons were significant.

Both main effects of Trials and Groups were non-significant, $F(3,24) = 2.88$, $p > 0.05$, and $F(3,72) = 2.52$, $p > 0.05$, respectively.

6. Experiment 5

In Experiment 4 pre-exposure to a MK-801 0.3 mg/kg dose prior to conditioning reduced the degree of aversion induced by this drug, as normally occurs when the US is pre-exposed prior to conditioning with other procedures, including CTA (Domjan and Best, 1980). However, it is possible that the physiological or psychological mechanisms underlying this decrease in consumption, or the decrease in conditioned aversion caused by pre-exposure to the compound, are different to those that underlie the conditioned aversion produced when LiCl is used as the US. Some authors have suggested that the sensory perception of a new flavor or odor could lead to an avoidance response if the odor or flavor is followed by a change in the internal state of the animal (Davis et al., 1986; see also Parker, 2003). In other words, according to this assumption, consumption of a new flavor (or the perception of a smell) followed by a change in the internal state of the animal converts the new flavor (or smell) into a danger signal, giving rise to a conditioned avoidance response. Therefore, as occurs with other drugs, it is possible that the MK-801 injection produces a change in the internal state of the animals and thereby reduces the level of consumption without inducing processes related with nausea.

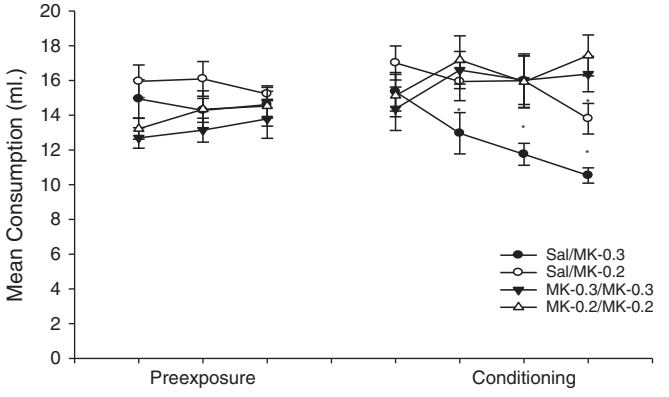

Fig. 4. Mean saccharin consumption (ml) across pre-exposure and conditioning trials as a function of groups. Error bars represent SEMs.

We tested this possibility by employing a US-pre-exposure design, similar to that used in the previous experiment, but pre-exposing the animals to MK-801 and conditioning with LiCl. If the decrease in consumption observed in the previous experiments after pairings of saccharin with MK-801 was a consequence of a state of gastric malaise involving the same physiological mechanisms as those that underlie CTA produced by LiCl administration, then pre-exposure to MK-801 should reduce or modify the aversive response induced by LiCl. This interference could take place through several psychological or physiological mechanisms. For instance, (a) MK-801 could trigger an opponent process that reduces the impact of the LiCl US (Solomon and Corbit, 1974), (b) MK-801's association with saccharin flavor could interfere with the subsequent establishment of a LiCl–saccharin association, and (c) the physiological processes triggered by MK-801 could reduce or modify the nausea induced by LiCl. The effect of MK-801 pre-exposure on flavor consumption during a conditioning phase with LiCl would provide information on the mechanisms involved in the reduction of the conditioned response that takes place after repeated administration of MK-801.

In Experiment 5 we used three groups that differed in terms of the concentration of MK-801 injected during preexposure (0.2 mg/kg, 0.3 mg/kg, or saline solution). During the conditioning phase, all animals received access to the saccharin solution followed by an injection of LiCl (0.4 M, 0.5% of body weight).

6.1. Material and methods

6.1.1. Subjects

The subjects were 21 male Wistar rats, weighing 462 g on average (ranging from 370 to 516 g). The animals were randomly assigned to each condition ($n=7$). Handling, deprivation, and the light–dark cycle were identical to those of the previous experiments.

6.1.2. Apparatus

The apparatus and stimuli were the same as those used in the previous experiments.

6.1.3. Procedure

During the pre-exposure phase (days 1–3), the animals received one trial each day in which they had 30 min of access to water followed by an injection of the corresponding dose of MK-801 (0.2 mg/kg or 0.3 mg/kg) or saline solution. In the conditioning stage (days 4–7), the animals received 30 min of access to saccharin followed by an injection of LiCl daily, except in the fourth trial, in which LiCl was not injected because no further trials were programmed.

6.2. Results

Fig. 5 shows the average water consumption (pre-exposure phase) or saccharin consumption (conditioning phase) for each experimental group. As reflected in the figure, pre-exposure did not interfere with conditioning, as all animals showed maximum conditioning after the third trial.

These impressions were confirmed by the statistical analyses. Specifically, a 3 x 3 mixed ANOVA (Trials x Groups) conducted on mean consumption across pre-exposure trials revealed significant main effects of Trials and Groups, $F(2,36)=5.54$, $p<0.01$, and $F(2,18)=4.21$, $p<0.05$, respectively. The effect of Trials was due to a general decreasing of consumption across trials, and the effect of Groups reflects lower level of consumption in the MK-0.2/LiCl groups as compared to the Sal/LiCl group, respectively. The 2-way interaction was non-significant, $F(4,36)=0.31$, $p>0.5$.

In order to analyze differences at the conditioning stage, a 3 x 4 mixed ANOVA (Trials x Groups) was conducted on mean saccharin consumption. The main factor of Group was not significant, $F(2,18)=2.87$, $p>.05$, neither it was the Group x Trial interaction, $F(6,54)=2.1$, $p>.05$. The main effect of Trials was highly significant, $F(3,54)=165.94$, $p<0.01$, due to the strong taste aversion induced by LiCl administration.

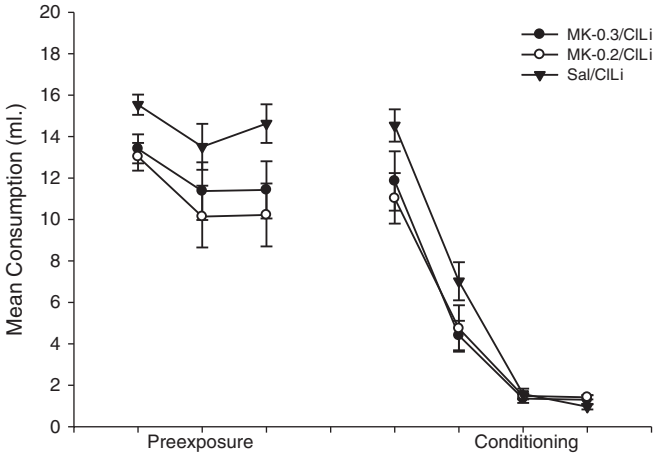

Fig. 5. Mean saccharin consumption (ml) across pre-exposure and conditioning trials as a function of groups. Error bars represent SEMs.

7. General discussion

The results of Experiments 1 and 2 suggest that MK-801 produces an aversive effect of medium intensity, and Experiment 3 discarded a possible non-associative effect supporting the observed reduction of the flavored consumption. This finding matches with the results of other studies showing that ketamine (Welzl et al., 1990; Aguado et al., 1997), PCP, and MK-801 (Jackson and Sanger, 1989), act as low-intensity USs. This aversive effect appears to differ from that produced by LiCl and appears to be weaker, becoming stable after the second or third conditioning trial and never completely eliminating consumption. However, these results are in contrast with those of Aguado et al. (1994), who found that ketamine produced an aversive effect that reached asymptote after several pairings of sucrose/ketamine. Nevertheless, other studies show that ketamine may act as a low-intensity US (Welzl et al., 1990; Aguado et al., 1997).

In Experiment 4 we observed retarded conditioning after repeated pairings of saccharin with MK-801 when MK-801 had been pre-exposed during a previous phase. Thus, pre-exposure to MK-801 effectively reduced subsequent conditioning. The US-pre-exposure effect traditionally has been interpreted as resulting from a context–US association that forms during pre-exposure interfering with the formation of a CS–US association during conditioning (e.g. Wagner, 1981).

The results of Experiment 5 show that repeated injections of MK-801 (0.2 mg/kg, or 0.3 mg/kg) prior to pairing the flavor with LiCl, a US commonly used in CTA experiments, did not interfere with the flavor–LiCl association (see also Aguado et al., 1997). This finding supports the hypothesis that different physiological mechanisms take place when LiCl and MK-801 are injected during pre-exposure and conditioning. MK-801 is effective in retarding conditioning when it is injected in both phases (pre-exposure and conditioning) but it has no impact on conditioning when LiCl is administered during the conditioning phase.

However, the results of Experiment 5 may have alternative explanations. For instance, perhaps the association between the context and the malaise induced by MK-801 was too weak to interfere with the formation of an association between saccharin and the malaise induced by LiCl. If MK-801 activates physiological mechanisms different than those activated by LiCl, it is possible that no cross-effects of

MK-801 pre-exposure on LiCl–saccharin pairings would be observed. Therefore, the activation of some type of nausea during US pre-exposure might be necessary for the strength of conditioning in the next phase to be dampened.

In summary, the lack of a significant effect of MK-801 preexposure on conditioning observed in Experiment 5 does not allow us to contemplate the use of the NMDA antagonist at the doses administered in our experiments as an unconditioned stimulus in taste aversion procedures. However, the results from the remaining experiments indicate that the use of MK-801 to evaluate memory or learning processes in taste aversion experiments could add a source of confusion to the results, namely the mild aversion learning induced by the drug. As the described experiments only tested the toxic effect of a very narrow MK-801 dose (0.2–0.3 mg/kg), our conclusions should be restricted to the effects of a low concentration of MK-801, although such dose range was selected because it covers the effects of the most usual doses employed in learning and memory experiments.

Overall, the results show that MK-801 may act as a medium-intensity US. However, the mechanisms underlying the effects of injecting NMDA antagonists (intraperitoneally, in the present case) are not well understood. The literature contains few studies specifically examining the capacity of these drugs to induce CTA. In some cases, a retardation of habituation to neophobia has been confounded with aversive conditioning. For instance, Jackson and Sanger (1989) found that MK-801 (0.3 mg/kg) produced a retardation of habituation to a novel flavor (saccharin), but given that consumption did not decrease across trials, this phenomenon may not be easily labeled as CTA.

Welzl et al. (1990) found that administering ketamine as the US, following the presentation of a saccharin solution, reduced saccharin consumption, although the decrease in consumption was small compared to that induced by LiCl. Some authors have proposed that ketamine's interfering effect on CTA could result from its US-like properties, whereby presenting ketamine prior to the conditioning phase could retard or alter asymptotic learning (Aguado et al., 1994). However, Aguado et al. (1997) found that pre-exposure to ketamine is not effective in retarding the learning of a flavor–LiCl association, a result that is consistent with the results of our Experiment 5.

In our previous work (e.g. Traverso et al., 2003) we have generally observed that MK-801 produces a small decrease in fluid consumption when the drug is administered after access to a flavor in the pre-exposure phase of a typical latent inhibition procedure. This effect appears more clearly in the pre-exposed groups, which are repeatedly exposed to the flavor to be conditioned, than in the non-preexposed groups, who receive water during the pre-exposure phase. For this reason, a possible aversive effect of the drug has consistently muddled the interpretation of the results. However, some findings have contradicted the notion that the aversive effect may explain the decreased consumption observed following the administration of these types of compounds. For instance, Gallo et al. (1998) found that repeated pairings of saccharin and ketamine (50 mg/kg) did not lead to any significant CTA.

Other findings from our laboratory similarly argue against an aversive effect of MK-801. Specifically, administering MK-801 after the flavor (sucrose) and before LiCl administration significantly decreases CTA, at the same dose as that used in the present experiments (0.2 mg/kg), (Traverso, unpublished results). This result appears to rule out the possibility that MK-801 produces an aversive effect that is positively correlated with the toxic effect of LiCl. However, it does not rule out the possibility that MK-801 produces an opponent process that interacts with or reduces the aversive impact of LiCl (see, for example, Solomon and Corbit, 1974). However, the operation of an opponent process appears to be unlikely here, given that administering MK-801 immediately following the conditioning phase has no effect on CTA (see non-pre-exposed groups in Traverso et al., 2003).

Ketamine administration (75 mg/kg or 120 mg/kg) between the CS and US also substantially interrupts CTA (Traverso et al., 2008), although ketamine's effect is highly dose-dependent, with subanesthetic doses (25 mg/kg, see for example Welzl et al., 1990) not interrupting CTA. Using a dosage that does not sufficiently block NMDA receptors at the moment at which stimulus processing takes place should only partially interrupt learning. Thus, Aguado et al. (1994) found that 25 mg/kg of ketamine did not interrupt habituation to neophobia, although it produced a slight decrease in consumption, while the disruptive effect on CTA disappeared after several pairings of ketamine–sucrose–LiCl.

We cannot rule out the possibility that administration of the drug (MK-801) used in our study produces a general decrease in consumption that reflects some type of interaction with mechanisms of a motivational or nutritional nature. For instance, Jahng and Houpt (2001) found that administering MK-801 produced a decrease in water consumption in rats that were not in a state of deprivation, while Burns and Ritter (1997) found no effect on consumption in water-deprived rats. Other studies found increased consumption of sucrose following MK-801 administration (Burns et al., 1998; Treece et al., 1998), leaving us with a mixed overall picture. In our study, we observed high variability in consumption following MK-801 administration, suggesting that differences in fluid consumption could have resulted from interactions of the drug with some poorly-understood metabolic processes. Increasing the number of subjects per group might compensate for this variability to a certain extent.

Finally, and regarding possible sites of action of MK-801, Bermudez-Rattoni (2004) described those structures involved in CTA. The pathway involved in the transmission of visceral information includes structures such as the area postrema, the nucleus of the solitary tract, the parabrachial nucleus, the insular cortex, the thalamus and the amygdala. Different studies have shown that LiCl administration induces an increase of glutamate levels in the amygdala and in the insular cortex (Miranda et al., 2002). In addition, intracranial injections of glutamate into the amygdala produce a strong conditioned aversion when administered just before a low LiCl dose (Miranda, et al., 2002), whereas NMDA antagonists injected between taste consumption and LiCl injection impair conditioning (Yasoshima et al., 2000). Similar results were found when NMDA antagonists were inoculated into insular cortex (Ferreira et al., 2002). Therefore, NMDA receptors appear to be involved in conditioned taste aversion, and also appear to play a relevant role in the transmission of visceral information. However, to the best of our knowledge, there are no data related to the mechanisms or structures involved in the reduction of flavor consumption after NMDA antagonist administration.

8. Conclusions

The findings of our experiments may reflect the existence of two aversive processes of a different nature, depending on the malaise-inducing substance. These results concur with a recent hypothesis that certain pharmacological compounds producing a change in the physiological state of the animal will be treated as potentially dangerous, resulting in the appearance of a conditioned avoidance response (Parker, 2003). This hypothesis assumes that a hierarchical defense system acts in response to toxic compounds (Davis et al., 1986). The first line of defense is modulated by taste and olfactory receptors. The second line is modulated by receptors of the gastric system and reacts to stimuli that induce some type of nausea. The third line of defense corresponds to the vascular system and the central nervous system chemoreceptors that respond to toxic compounds. While LiCl activates all three levels of defense and produces a conditioned aversion response, it is possible that MK-801 only induces a state change, without activating the second and/or third lines of defense, giving rise to a conditioned avoidance response.

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