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# Effect of the NMDA antagonist MK-801 on latent inhibition of fear conditioning

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ABSTRACT

N-methyl-D-aspartate (NMDA) receptors seem to play a central role in learning and memory processes involved in Latent Inhibition (LI). In fact, MK-801, a non-competitive NMDA receptor antagonist, has proved its effectiveness as a drug for attenuating LI when administered before or after stimulus preexposure and conditioning stages. This paper presents three experiments designed to analyze the effect of MK-801 on LI when the drug is administered before (Experiment 1A) or after (Experiment 1B) preexposure and conditioning stages with a conditioned emotional response procedure. Additionally, we analyze the effect of the drug when it was administered before preexposure, before conditioning or before both phases (Experiment 2). The results show that the effect of the drug varied as a function of the dose (with only the highest dose being effective), the moment of administration (with only the drug administered before the experimental treatments being effective), and the phase of procedure (reducing LI when the drug was administered only at preexposure, and disrupting fear conditioning when administered at conditioning). These differences may be due to several factors ranging from the role played by NMDA receptors in the processing of stimuli of different sensorial modalities to the molecular processes triggered by drug administration.



## 1. Introduction

Latent Inhibition (LI) is generally defined as a retardation in learning that results from previous non-reinforced exposure to the to-be-Conditioned Stimulus (CS). LI has traditionally been considered the result of a failure in the acquisition of the CS–Unconditioned Stimulus (US) association after preexposure due to: i) a reduction in CS associability (e.g., Mackintosh, 1975; Pearce and Hall, 1980), ii) the formation of an association between the preexposed stimulus and the contextual cues (e.g., Wagner, 1981), iii) the conditioning of an inattentional response (e.g., Lubow, 1989), or iv) the reduction of CS novelty (e.g., Schmajuk et al., 1996). More recently, an alternative set of theories has proposed that LI reflects a retrieval failure at the time of testing rather than an associative deficit (Miller et al., 1986; Bouton, 1993). This view implies that a CS–nothing association is formed at preexposure, and an independent CS–US association is established during conditioning. Both associations would compete to obtain behavioral expression at the time of testing, producing a weaker CR (conditioned response) to the CS.

A very influential model of LI, that combines psychological and physiological points of view, is the so-called switching model (Weiner, 1990, 2003; Weiner and Feldon, 1997; Weiner and Arad, 2009) that integrates the evidence available from LI in animal and human experiments. The switching model proposes that the dopaminergic system is the main responsible for producing LI, and its predictive capacity has been demonstrated in the analysis of the effects on LI of some drugs commonly used in the treatment of schizophrenia (e.g., Weiner et al., 1988). Recent developments in this area have led to greater interest in the study of the glutamatergic system (e.g. Rivas-Vazquez and Resnick, 2003), because it is based on a new pharmacological model of LI that complements the dopaminergic model, although the available experimental results are not entirely consistent. Specifically, LI seems to be abolished with aversive conditioning procedures when a NMDA antagonist that impedes normal glutamatergic activity is administered at preexposure stage, regardless whether the drug is administered before or after stimulus exposure (Aguado et al., 1994; Gallo et al., 1998; Traverso et al., 2003; Lewis and Gould, 2004). However, these results may be seen as a consequence of a state dependent learning and the resulting contextual change that it involves (e.g., Siegel, 1988). In fact, when the NMDA antagonist is administered at both preexposure and conditioning stages, the results are apparently contradictory. Some experiments have resulted in intact LI despite drug administration (Weiner and Feldon, 1992; Aguado et al., 1994; Gaisler-Salomon and Weiner, 2003) while other reports have shown complete LI abolition (Turgeon et al., 1998, 2000; Traverso et al., 2003).

On the other hand, fear conditioning is a prominent model of aversive conditioning, that has been frequently used to study LI. In fear conditioning, the effect of MK-801 (a non-competitive NMDA receptor antagonist agent) on LI is far from clear. A summary of the reported effects of MK-801 on LI appears in Table 1. The contradictory results

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could come from the differences between the parameters employed to induce the LI effect, from the different doses injected, and from the administration on the different phases of the procedure. Thus, as can be seen in the table, persistent LI appeared with the lowest doses, from 0.05 mg/kg to 0.15 mg/kg, with one experiment showing intact LI with the 0.05 dose. However, the highest doses, from 0.2 mg/kg to 1.0 mg/kg, resulted in abolition of LI. Therefore, it seems that the amount of MK-801 is critical in determining the effect on LI.

In view of the confusing results described above, we analyzed in the present study the possible differential effect of MK-801 on LI employing a fear conditioning procedure. To this end, we conduct two parallel experiments intended to test the effect of MK-801 (0.1 mg/kg or 0.2 mg/kg) on LI depending on whether the drug is administered before (Experiment 1A) or after (Experiment 1B) pre-exposure and conditioning stages. Experiment 2 was designed to test whether MK-801 (0.2 mg/kg) administration may induce differential effects on LI when drug is administered only before preexposure, only before conditioning, or before both phases.

## 2. Experiments 1A and 1B

These experiments were designed to test whether MK-801 administration before (Experiment 1A) or after (Experiment 1B) preexposure and conditioning disrupts LI, using a procedure of conditioned emotional response (CER). Additionally, we were interested in analyzing possible differential results as a consequence of different drug doses (0.1 mg/kg vs. 0.2 mg/kg).

Thus, each experiment comprised a 2×3 factorial designs, with main factors Preexposure (PE vs. NPE) and Drug (Saline vs. 0.1MK vs. 0.2MK). Those subjects in the PE condition received tone-alone presentations, while the NPE subjects spent the same amount of time in the experimental chamber, but without stimulus presentations. As for the Drug factor, one third of the animals received Saline, the second third was injected with 0.1 mg/kg of MK-801, and the last third received 0.2 mg/kg of MK-801. If NMDA receptors play a role in the general processing of stimuli, we would anticipate a disruptive effect of MK-801 on LI with a CER procedure. According to previous results (Gaisler-Salomon and Weiner, 2003) the administration of both doses of MK-801 before the stimulus should result in LI disruption by means of a reduction of conditioning in the NPE groups in Experiment 1A. In Experiment 1B, MK-801 administration after stimulus presentation should be effective to disrupt the LI effect if blocking of NMDA receptors occurs before processing of the auditory stimulus. This possibility seems unlikely, because the auditory stimuli are encoded in a very short time (Maren and Quirk, 2004).

### 2.1. Material and methods

#### 2.1.1. Subjects

84 adult male Wistar rats participated in this experiment (42 in Experiment 1A and 42 in Experiment 1B, n=7). Mean weight was 436 g (range 357–561 g) The animals had previously participated in a flavor preference experiment, but they were naïve to any kind of drugs, and to the procedures and stimuli used in the present experiments. A water deprivation schedule (30 min of water availability daily) was implemented 7 days before initiating the experimental manipulations. Access to food was unrestricted for the entire duration of the experiments.

**Table 1**
Effect of MK-801 on LI with a fear conditioning procedure.

| Experiments | Administration time | Dose | Result |
|---|---|---|---|
| Gaisler-Salomon and Weiner (2003) | Before CS–US | 0.05 mg/kg | LI persistence |
| Lewis and Gould (2004) | Before CS exposure | 1 mg/kg | LI disruption |
| Lipina et al. (2005) | Before CS exposure<br>Before CS–US | 0.15 mg/kg | LI persistence |
| Davis and Gould (2005) | Before CS exposure | 0.5 mg/kg | LI disruption |
| Gould and Lewis (2005) | Before CS exposure | 0.05 mg/kg | Normal LI |
| Gaisler-Salomon et al. (2008) | Before CS–US | 0.05 mg/kg | LI persistence |

#### 2.1.2. Apparatus

Four identical conditioning chambers were used in both experiments. Each box measured 24 cm (length)×29 cm (width)×35 cm (height). The side walls were made of aluminum. The front and back walls, as well as the ceiling, were made of clear acrylic plastic. The floor consisted of steel bars, parallel to the front wall, 0.4 mm in diameter, spaced 1.4 cm from center to center. A hole (3×3 cm) located in the front wall (2 cm above the floor, and 1.5 cm from the right side of the wall) allowed access to an inverted 150-ml graduated bottle fitted with an acrylic spout. During each session, water was delivered through the bottle. Licks were detected by a drinkometer circuit (Letica model 300-70). The to-be-conditioned preexposed stimulus was a 30-s 2.8 kHz at 90 dB tone provided by a generator (Letica model 300-43) wired to identical speakers in each chamber. The US was a 0.5-mA 1-s scrambled footshock provided by a Letica 100-26 shock sources. A personal computer was used for equipment programming and data recording.

#### 2.1.3. Procedure

*2.1.3.1. Baseline.* On days 1 to 5, each animal was placed in an experimental chamber where it remained for 20 min. Each contact with the water-bottle tube was registered as a response, with the total number of responses in each session being computed. At the end of each baseline session, all animals received 10 additional min of free access to water in their home cages.

*2.1.3.2. Preexposure.* This stage started the day following the last baseline session and lasted for two consecutive days. Water consumption was allowed during preexposure sessions in the same way as in the baseline sessions. Each preexposure session for the PE groups consisted of 15 presentations of a 30-s tone with an interstimulus interval of 50 s (+/−30 s). Animals in the NPE condition spent an equivalent period of time in the experimental cages but without tone presentations. Mean duration of each session in this stage was 20 min and 50 s. For those rats in Experiment 1A the correspondent dose of MK-801 (0.1 mg/kg or 0.2 mg/kg) or the saline solution was i.p. injected 20 min before each session. For those rats in Experiment 1B the injection was applied immediately after the session ended.

*2.1.3.3. Conditioning.* Conditioning was conducted on the first day following the last preexposure session. The conditioning procedure, the same for all animals, included two tone-shock pairings with an Inter Trial Interval of 360 s (+/−30 s). Access to water-bottles was allowed during the session. The mean duration of this session was 19 min. After the conditioning session, animals had access to water in their home cages for 30 min. As described for preexposure, drug or saline injection was given before the conditioning session for Experiment 1A and after conditioning session for Experiment 1B.

*2.1.3.4. Repeat baseline and testing.* The next day all animals were given an additional 20 min baseline session without any additional stimuli. A test session was conducted on the following day for all groups. The test session consisted of a single Tone-alone presentation (180 s) that started immediately after lick number 125. Conditioning strength was indexed by computing the time to complete five licks in the presence of the tone CS. The session ended with the tone offset. There was no drug administration at this stage.