Role of drug-associated environmental stimuli in the development of cross-tolerance to the tachycardic effects of nicotine and alcohol in humans

Papel de los estímulos ambientales asociados a la droga en el desarrollo de tolerancia cruzada a los efectos de taquicardia de la nicotina y el alcohol en humanos

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Abstract

According to the Pavlovian conditioning model, drug tolerance is modulated by drug-associated environmental cues. This study evaluated the contribution of drug-associated cues in the development of cross-tolerance to the tachycardic effects of nicotine from tobacco and alcohol in human subjects. Forty undergraduate students were recruited for this experiment, and each student was randomly assigned to one of two experimental conditions. Twenty students smoked nicotine-containing cigarettes in context A and placebo cigarettes in context B, and twenty students smoked nicotine-containing cigarettes in context B and placebo cigarettes in context A. A cross-tolerance test was carried out by dividing the subjects in each condition into two subgroups (n = 10). Each subgroup consumed alcohol in both contexts (A and B). The results of this experiment showed that cross-tolerance between nicotine and alcohol was exhibited only if the cross-tolerance test was carried out in the same context where tolerance had developed to the nicotine from tobacco. These results support the hypothesis that drug-associated environmental stimuli play a modulatory role in the development of cross-tolerance between nicotine from tobacco and alcohol.

Keywords: Cross-tolerance; classical conditioning; tobacco; nicotine; alcohol; heart rate response.

Resumen

De acuerdo con el modelo de condicionamiento pavloviano, las claves ambientales asociadas a la droga modulan la tolerancia a las drogas. Este estudio evaluó la contribución de las claves asociadas a la droga en el desarrollo de tolerancia cruzada a los efectos taquicárdicos de la nicotina de tabaco y el alcohol en sujetos humanos. En este experimento participaron cuarenta estudiantes universitarios. Cada estudiante fue asignado aleatoriamente a una de dos condiciones experimentales. Veinte estudiantes fumaron cigarros con nicotina en el Contexto A y placebo en el Contexto B y veinte estudiantes fumaron cigarros con nicotina en el Contexto B y placebo en el Contexto A. La prueba de tolerancia cruzada fue llevada a cabo dividiendo a los participantes de cada condición en dos subgrupos (n = 10), cada subgrupo consumió alcohol en cada uno de los contextos (A y B). Los resultados de este experimento muestran que la tolerancia cruzada entre nicotina y alcohol se presentó únicamente cuando la prueba de tolerancia cruzada se realizó en el mismo contexto donde se desarrolló la tolerancia a la nicotina del tabaco. Estos resultados concuerdan con la hipótesis de que los estímulos ambientales asociados a la droga juegan un papel modulador en el desarrollo de la tolerancia cruzada entre la nicotina del tabaco y el alcohol.

Palabras clave: Tolerancia cruzada; condicionamiento clásico; tabaco; nicotina; alcohol; frecuencia cardíaca.

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There is a strong relationship between drinking alcohol or ethanol and smoking nicotine-containing cigarettes. Clinical studies have provided the best evidence of this association, suggesting that it is common to find patients diagnosed with alcohol dependence and diagnosed with tobacco/nicotine dependence (Abburi et al., 2016; Abreu-Villaça, Manhaes, Krahe, Filgueiras & Ribeiro-Carvalho, 2017; Drobes, 2002; Funk, Marinelli & Lê, 2006; Oliver et al., 2013). In fact, it has been estimated that 80-90% of alcoholic individuals often also smoke nicotine-containing cigarettes (Taslim, Soderstrom & Saeed, 2011). Similarly, consumption of alcohol is higher in smokers than in nonsmokers (Abburi et al., 2016; Oliver et al., 2013), and it seems that consumption of both nicotine from tobacco and alcohol can enhance or reinforce the effects of each drug (Chi & De Wit, 2003; Enggasser & Wit, 2001; Oliver et al., 2013). A study with human subjects revealed that the interaction of the pharmacological effects of nicotine and low doses of alcohol plays an important role in the motivation for consuming both substances, and this phenomenon contributes to the development of cross-reinforcement and cross-tolerance, as well as dependence on both drugs (Oliver et al., 2013).

In this regard, several studies have reported evidence that alcohol and nicotine can interact in several ways. Some studies that have explored the long-term behavioral effects of nicotine and alcohol have revealed that chronic use of one drug induces tolerance to the behavioral and physiological effects of the other drug, which increases the potential for coabuse (Taslim et al., 2011). Another study in which mice were chronically treated with different doses of alcohol found that the animals also developed tolerance to the hypothermic effects of an acute dose of nicotine (Majchrzak & Dilsaver, 1992). In vitro studies have also shown that chronic exposure to alcohol decreased nicotine-induced dopamine (DA) release (Dohrman & Reiter, 2003).

Cross-tolerance occurs when the development of tolerance to one drug produces tolerance to a second drug, and the development of cross-tolerance between nicotine and alcohol could explain the increased use of both drugs and contribute to coabuse. Although it is difficult to assess the development of cross-tolerance between nicotine and alcohol in human subjects because both drugs are commonly used and abused, cross-tolerance between nicotine and alcohol is well documented in animal models. For example, alcohol and nicotine produce hyperthermic and tachycardic effects. Studies with mice have shown that chronic administration of alcohol through a liquid diet, which induced tolerance to various effects of alcohol, also produces cross-tolerance to the hyperthermic and tachycardic effects of nicotine (Collins, Burch, De Fiebre & Marks, 1988).

The neurobiological perspective on the cross-tolerance between nicotine and alcohol has suggested at least four possible mechanisms based on the overlapping sites of action for tobacco and alcohol or the neuronal pathways where both substances exert their rewarding effects, particularly in the mesolimbic DA system. The first suggested mechanism is that both substances can modulate the nicotinic acetylcholine receptor. It is clear that nicotinic acetylcholine receptors are the principal site of action for nicotine (Adams, 2017); however, it has been suggested that ethanol can directly or indirectly interact with these receptors, and perhaps this is because ethanol stabilizes open channel states. Some authors have suggested that ethanol and nicotine could desensitize the nicotinic receptors in the central nervous system (CNS) (Adams, 2017; Collins et al., 1988). The second overlapping mechanism between nicotine and ethanol is their ability to increase the release of neurotransmitters such as DA, serotonin, glutamate, and GABA. A third neurobiological explanation of the interaction between nicotine and ethanol is their ability to sensitize corticotropin-releasing factor systems, a component of the stress system (Abreu-Villaça et al., 2017; Funk et al., 2006). The fourth mechanism in which alcohol and nicotine interact is the activation of the brain reward system. Both drugs increase the activity of the mesocorticolimbic DA system, generating a functional interaction between nicotine and ethanol (Adams, 2017). Finally, it is important to note that there are common genetic factors associated with both substances (De Fiebre & Collins, 1993; Madden, Bucholz, Martin & Heath, 2000). It is clear that cross-tolerance between nicotine and alcohol could have a neurobiological explanation. However, this approach does not explain the previously reported influence of environmental stimuli.

There are several ways in which environmental stimuli can influence people's behavior, for example, in the visual discrimination of an alcoholic beverage or its alcoholic content (Sillero-Rejon, Maynard & Ibañez-Zapata, 2020), or inhibitory control levels may vary in real-world alcohol-related settings, where people are surrounded by visual and auditory stimuli associated with alcohol that may affect their ability to control their consumption (Qureshi et al., 2021). From a different perspective, there have been approaches to the behavioral mechanisms involved in drug tolerance. It has been suggested that a Pavlovian conditioning model of tolerance to drugs, originally proposed by Siegel (1977), could explain the cross-tolerance between nicotine and alcohol. This model suggests that the environmental stimuli associated with the administration of a drug acquire the function of the conditioned stimulus (CS), and the pharmacological stimulation acts as the unconditioned stimulus (US). The CS plays a central role in the development of tolerance since it elicits a conditioned compensatory response (CCR) that

attenuates the unconditional effects of a drug, producing tolerance (González, Navarro, Miguez, Betancourt & Laborda, 2016; Ruiz, Vila & Miranda, 2010; Vila, Ruiz, Trejo & Miranda, 2013). In the absence of the CS, the CCR does not occur, and therefore, there is no reduction in the effects of the drug (González et al., 2016; Ruiz et al., 2010; Siegel, 1979; Siegel, Baptista, Kim, McDonald & Weise-Kelly, 2000; Siegel & Ramos, 2002; Vila et al., 2013). A logical consequence of Siegel's model is that if a second drug is administered in the tolerance test in the presence of the stimuli associated with the first drug, it would cause a CCR that would attenuate the unconditional effects of the second drug, producing cross-tolerance.

In line with the above mechanism, some studies have reported that Pavlovian conditioning processes could be involved in cross-tolerance to several drugs (Cappell, Roach & Poulos, 1981). Although several cross-tolerance experiments have been conducted with laboratory animals (Cappell et al., 1981), it has not yet been demonstrated whether these conditioning processes also regulate cross-tolerance in human subjects using two widely used legal drugs, i.e., nicotine and alcohol (Oliver et al., 2013). The investigation of these processes involved in cross-tolerance could help develop a better treatment for addiction to these drugs. Therefore, in this study, we evaluated the participation of Pavlovian conditioning processes in cross-tolerance to the tachycardic effects of nicotine from tobacco and alcohol in human subjects.

Methods

Participants

The sample was composed of forty undergraduate students from Facultad de Estudios Superiores Iztacala, UNAM (23 men and 17 women), whose average age was 21 years. The inclusion criteria were that they smoked 5 to 7 cigarettes per day and drank alcohol 1 to 2 times per month. Participants with little nicotine and alcohol dependence (4 points maximum in the Fagerström test; 5 points maximum in the AUDIT test) were identified. The participants had an average weight between 60 and 70 kg and a height between 1.60 and 1.70 m. The exclusion criteria were the presence of health problems or taking medically prescribed drugs at the beginning of or during the study. Each subject received an explanation of the experimental protocol, and they were informed of the ethical norms and principles for human research in accordance with the ethical code of the psychologist (Sociedad Mexicana de Psicología, 2009; American Psychological Association, 2010). All students participated voluntarily and gave their informed consent before starting the experiment, being free to abandon the task at any point in the process, though that never happened. They were asked to not use cigarettes for three days before and throughout the experiment. This period of time did not cause withdrawal or any change in the cardiovascular response evaluated, still, allows a stable baseline in all the subjects.

Materials

Heart rate responses of subjects were recorded with a photoplethysmograph (HR / BVP IOIT: Thought Technology LTD, Quebec, Canada). In addition, an AIWA 130 recorder, a reggae music CD and recorded instructions to relax were used.

Drugs and placebo

Two types of drugs were used: nicotine-containing cigarettes (Marlboro, with approximately 0.9 mg of nicotine per cigarette) and alcohol (Absolut vodka, with 40° of pure alcohol). The subjects were instructed to drink alcohol in a vodka mixture (0.65 g/kg) in 100 ml of orange juice. The formula to calculate the amount of alcohol was as follows:

$$\text{Grams of alcohol} = \frac{\text{volume (in c.c.)} \times \text{graduation} \times 0.8}{100}$$

For placebo, cigarettes that did not contain nicotine or any substance that could cause an effect on the cardiovascular system were chosen; Reef LiveTM lettuce cigarettes were used.

Experimental situation

Sessions were carried out in two contexts. Context A and B were created in a room illuminated by two white light lamps, with a table (1.7 m x 0.90 m), a chair and an air extractor that remained in operation across all sessions. In the room, there was an additional table in which the computer and the sound recorder were placed. The differences between the contexts were created by the light intensity and music. Context A included a low intensity light (30 W) and music. Context B was illuminated by 100 W, 100 V lamps and had no music.

Procedure

The subjects were randomly assigned to one of the two experimental conditions, context A or context B, in a counterbalanced manner. Twenty subjects smoked nicotine-containing cigarettes in context A and placebo cigarettes in context B; the remaining 20 subjects smoked nicotine-containing cigarettes in context B and placebo cigarettes in context A. Each subject individually participated in a one-hour session. One of the researchers began the session by informing each subject of the smoking procedure. After clarifying any questions, another researcher recorded the heart rate of the subjects. The subjects were then asked to follow the recorded instructions and relax for 10 minutes, and at the end of the relaxation period, their heart rate was recorded again.

Acquisition phase. The order of presentation of the nicotine or placebo trials was counterbalanced. This phase involved four trials in a session. When the session consisted of smoking nicotine-containing cigarettes in context A, the trials were conducted according to the following procedure. Three minutes before subjects took the first puff, a change was made in the ambient light intensity of the room (from normal to low intensity), and music was turned on. Each trial consisted of smoking four puffs, with a 20-second interval between puffs. Each puff consisted of sucking the smoke from the cigarette by mouth for two seconds and keeping the smoke in the lungs for two seconds. Five minutes after the last puff, the heart rate response was recorded. During the interval between trials, a change in light intensity was made (from 30 W to 100 W), and the music was turned off. When smoking nicotine-containing cigarettes in context B, the trials were identical in terms of the administration of the drug, but no change in ambient light or music occurred.

Placebo (lettuce cigarettes) was administered under the same environmental conditions, that is, in context A and in context B.

Tolerance test phase. The tolerance test was carried out in both contexts A and B. The order of presentation of the contexts was counterbalanced. Five minutes after the last acquisition trial, the tolerance test was carried out. In both contexts, all subjects were allowed to smoke nicotine-containing cigarettes; the smoking instructions were identical to those described in the previous phase. Five minutes after the last puff, their heart rate response was recorded.

Cross-tolerance test phase. A cross-tolerance test was conducted as follows. A researcher gave instructions for consuming the alcoholic drink in approximately 3 minutes. The subjects drank alcohol in the two different contexts (A and B), and the order of presentation of the context was counterbalanced. Five minutes after consuming the beverage, a researcher recorded their heart rate response.

Reacquisition phase. This phase was identical to the first acquisition phase. This phase was carried out to eliminate the influence that the previous evaluation could have and stabilize the tolerance for the subsequent test.

CCR test phase. Five minutes after the last reacquisition trial, the CCR test was carried out. For the CCR test, all subjects were allowed to smoke lettuce-containing cigarettes; the smoking instructions were identical to the previous phase; each subject consumed placebo (lettuce cigarettes) in each context.

For a summary of the procedure, see Table 1.

Table 1. *Cross-tolerance development.*

Acquisition	Tolerance Test	Cross Tolerance Test	Reacquisition	CCR Test
A: NIC	A: NIC	A: ALC	A: NIC	A: P
B: P	B: NIC	B: ALC	B: P	B: P
A: P	A: NIC	A: ALC	A: P	A: P
B: NIC	B: NIC	B: ALC	B: NIC	B: P

Note. A=Context A, B=Context B, NIC=Nicotine, ALC=Alcohol, P=Placebo.

Statistical analysis

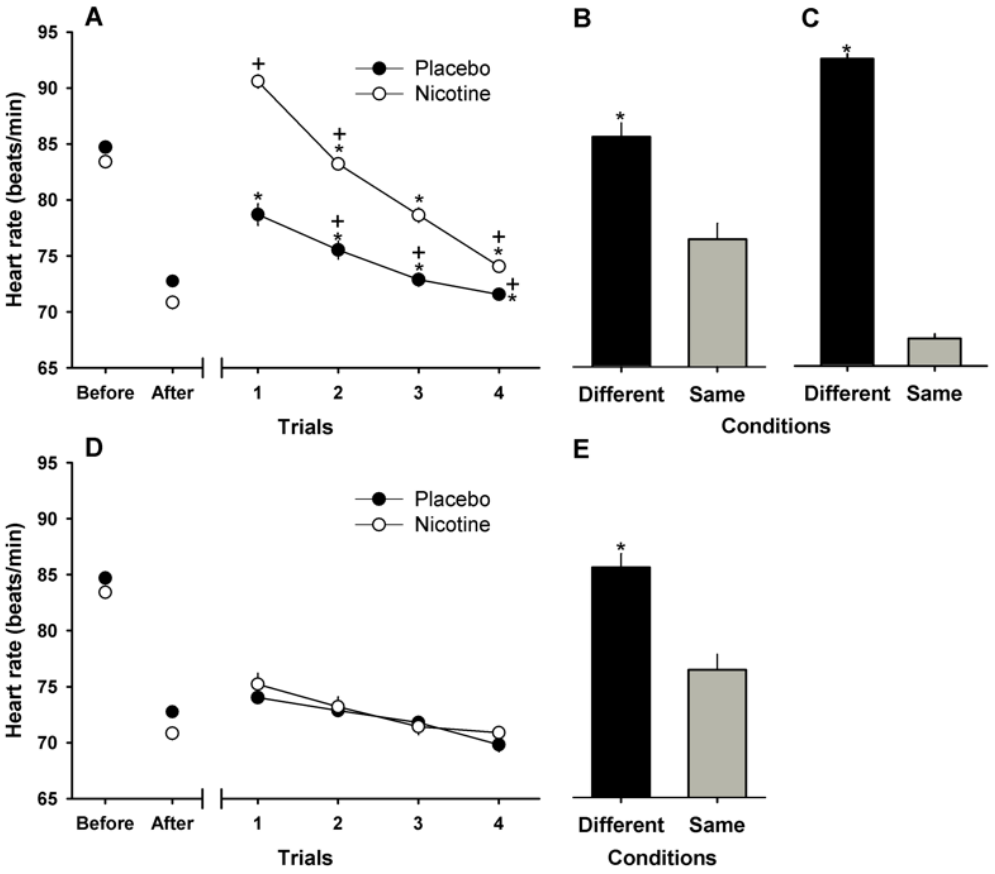
The subject´s heart rate responses were recorded at baseline, during the training, and during the cross-tolerance test. The baseline data were analyzed using two-way ANOVA, with condition (nicotine and placebo conditions) as the first factor and the baseline (before and after relaxation) as the second factor. During the training phase, the data were analyzed using two-way repeated measures ANOVA, with the nicotine and placebo conditions as the first factor and the trial number as the second factor. During the cross-tolerance test, data were analyzed with Student's t-test. Data from the reacquisition phase were analyzed using two-way repeated measures ANOVA, with the nicotine and placebo conditions as the first factor and the trial number as the second factor. For the CCR test, the data were analyzed using Student's t-test. When ANOVAs were significant, multiple comparisons were carried out using Tukey's test. In all tests, the rejection level for type I error was 0.05.

Results

Acquisition phase

The results of the baseline (before and after *relaxation*) are shown on the left side of Figure 1-A. Two-way ANOVA indicated that there were no differences between the nicotine condition (84.8 beats per minute) and placebo condition (83.4 beats per minute) before relaxation. Although there was a significant decrease in heart rate response after relaxation in both conditions, there were no differences between the nicotine (72.8 beats per minute) and placebo (70.9 beats per minute) conditions after relaxation ($F\ [1, 39]=0.357$, $p>0.05$; $F\ [1, 39]=107.601$, $p<0.05$; respectively). Additionally, the test revealed that there were no differences between the after and before conditions based on the nicotine and placebo conditions (i.e., no interaction) ($F\ [1, 39]=1.040$, $p>0.05$). In summary, after relaxation, all subjects started the experiment with a constant heart rate of approximately 70-72 beats per minute.

Different heart rate responses across the four tolerance acquisition trials are shown on the right of Figure 1-A. The data showed that the initial effect of nicotine was to increase the heart rate response. Two-way repeated measures



Note. The results are represented as the mean of the subject's heart rate response ± SEM. A. Development of nicotine tolerance. The first two points represent before and after the relaxation period. It is also shown the development of conditioned tolerance to the tachycardic effects; open circles show the effects after successive smoked nicotine-contained cigarettes, while closed circles show the effect obtained after smoked placebo cigarettes. Asterisk (*) indicates significant differences with respect to the first nicotine trial. The cross (+) indicates significant differences with respect to the first placebo trial. B. Tolerance test. Heart rate of the subjects that smoked nicotine in the Different or the Same context. Asterisk (*) indicates significant differences with respect to the Same context. C. Cross-tolerance test. Results obtained when subjects drank alcohol in the Different and Same context. Asterisk (*) indicates significant differences with respect to the Same context. D. Reacquisition phase. Shows the reacquisition of the tolerance; open circles show the effect of nicotine, while closed circles show the effect obtained after smoked placebo. E. CCR test. Results obtained when subjects smoked placebo in the Different and Same context. Asterisk (*) indicates significant differences with respect to the Same context.

Figure 1. Role of drug-associated cues in the development of cross-tolerance to the tachycardic effects of the nicotine from tobacco and alcohol.

ANOVA indicated that there were differences between the nicotine and placebo conditions ($F [1, 38]=19,204$, $p<0.05$), between trials ($F [5, 90]=36.779$, $p<0.05$) and in the condition-trial interaction ($F [5, 90]=10.541$, $p<0.05$). As shown on the right of Figure 1A, the effect on the heart rate response decreased depending on the number of exposures to the drug. Tukey's test indicated that in the case of the nicotine condition, there was an important decrease ($p<0.05$) from the second trial onwards. On the other hand, although an increase in trial 1 was observed with respect to the baseline in the placebo condition, the mean heart rate response was significantly lower than that observed in trial 1 in the nicotine condition ($p<0.05$).

In the placebo condition, the trials did not present significant differences among themselves, compared to trial 4 of the nicotine condition, or compared to after *relaxation.*

Tolerance test phase

Results from the tolerance test phase showed that when subjects smoked nicotine-containing cigarettes in the context associated with the consumption of the drug, they exhibited a decrease in the heart rate response (bar indicated by "Same" in Figure 1B), whereas when the subjects smoked nicotine-containing cigarettes in the absence of the environmental stimuli associated with the drug, their heart rate response increased (bar indicated by "Different" in Figure 1B). Related Student's t-test confirmed a significant difference between the responses in these two conditions ($t [38] =3.707$, $p< 0.05$).

Cross-tolerance test phase

Results of the cross-tolerance test are shown in Figure 1C. When the subjects drank alcohol in the environment associated with smoking nicotine-containing cigarettes, the subjects exhibited a decrease in the heart rate response (bar indicated by "Same" in Figure 1C), while when the subjects drank alcohol in the absence of environmental stimuli associated with smoking nicotine-containing cigarettes, the heart rate response increased (bar designated as "Different" in Figure 1C). Related Student's t-test indicated a difference between the responses in these two conditions ($t\ [38] = 15.036$, $p<0.05$).

Reacquisition phase

The changes in heart rate responses across the four tolerance reacquisition trials are shown in Figure 1D. The data from this phase showed that the initial effect of smoking nicotine-containing cigarettes did not produce an increase in the heart rate response compared to the baseline response. As can be observed, this effect was maintained over the course of the trials and became less pronounced in comparison with the trials in the placebo condition. A two-way repeated measures ANOVA revealed that there were no significant differences between the groups ($F\ [1, 38] = 2.01$, $p>0.05$). The results also showed that there were no significant differences between the trials ($F\ [5, 70] = 0.248$, $p>0.05$), and there was no condition-trials interaction ($F\ [3, 114] = 0.391$, $p>0.05$).

CCR test phase

The results of the CCR test in the reacquisition phase showed that when subjects smoked lettuce-containing cigarettes in the context associated with the consumption of the drug, they exhibited a decrease in the heart rate response (bar indicated by "Same" in Figure 1E); whereas, when the subjects smoked lettuce-containing cigarettes in the absence of the environmental stimuli associated with the drug, the heart rate response increased (bar indicated by "Different" in Figure 1E). Related Student's t-test confirmed a significant difference between the response in the two conditions ($t\ [19] = 6.688$, $p<0.05$).

Discussion

The purpose of this study was to evaluate the contribution of drug-associated cues in the development of cross-tolerance to the tachycardic effects of nicotine from tobacco and alcohol in human subjects. In addition, the expression of the CCR as a possible mechanism that underlies the cross-tolerance between nicotine and alcohol on the tachycardic effects was evaluated. We found that the development of tolerance to the tachycardic effects of nicotine can be modulated by the environmental signals associated with its consumption. The data also showed cross-tolerance between nicotine and alcohol on heart rate responses, and this effect was only observed if the cross-tolerance test was carried out in the same context in which tolerance to the first drug was developed; when, the cross-tolerance test was carried out in the different context, the cross-tolerance was reversed and an increase in the heart rate response was observed. An additional finding was evidence of a CCR when the subjects consumed placebo in the presence of environmental stimuli associated with nicotine consumption by presenting a decrease in heart rate compared to that in the subjects who consumed placebo in the absence of such stimuli; the results suggest that a CCR could be the mechanism underlying cross-tolerance.

The behavioral results described above are consistent with those of existing studies demonstrating the influence of the context-specificity of tolerance using different procedures with rats and using different drugs including nicotine (Field & Duka, 2001; McDermut & Haaga, 1998; Mucha, Pauli & Angrilli, 1998; Naqvi & Bechara, 2006) and alcohol (Duncan, Alici & Woodward, 2000; Le, Poulos & Cappell, 1979; White, Roberts & Best, 2002).

With regard to the modulatory role of the environmental context on cross-tolerance, the present results are the first evidence that shows the contribution of the drug-associated cues in the development of cross-tolerance between the effects of nicotine from tobacco and the effects of alcohol in human subjects and suggest that the tolerance that develops to a particular drug effect in a specific context may contribute to the expression of tolerance to the effects of a second drug that has not been previously used by the subjects if the second drug is consumed in the same environment where the first drug was consumed. Therefore, it can be suggested that the Pavlovian conditioning processes that contributed to the expression of the cross-tolerance between nicotine and alcohol corresponded to the effects reported in previous studies carried out using different procedures with animals and could be used as evidence regarding the knowledge of the phenomena that contribute to the development and maintenance of drug addiction since the development of cross-tolerance can be an important component in the progression or increase in consumption and the development of dependence on drugs of abuse (Cappell et al., 1981; Carmona-Perera, Sumarroca-Hernández, Santolaria-Rossell, Pérez-García & Reyes del Paso, 2019; Collins et al., 1988; De Fiebre & Collins, 1993; Oliver et al., 2013).

An initial explanation of the results of our investigation involves the study of the mechanisms that underlie the development of nicotine and alcohol addiction and dependence, which have been widely evaluated (Little, 2000). The neurobiological mechanisms constitute a first set of factors that has provided an explanation of not only these phenomena but also the cross-tolerance between

nicotine and alcohol. In this case, the explanation has focused on the indirect actions that these drugs have on the reward system. Nicotine increases DA concentrations in the nucleus accumbens (nAcc) by three different mechanisms (De Kloet, Mansvelder & De Vries, 2015): activation of ventral tegmental area (VTA) neurons through nicotinic receptors with α4, α6, α7 and β2 subunits; activation of DAergic neurons by cholinergic activation from the pedunculopontine nucleus; and inhibition of GABAergic interneurons in the VTA by desensitization of nicotinic receptors with β2 subunits. On the other hand, alcohol also increases DA concentrations and acts on GABAA receptors on the GABAergic interneurons in the VTA. GABAA activation produces a decrease in the release of GABA in the VTA and consequently increases DA release in the nAcc (Leggio, Kenna & Swift, 2008). Additionally, it is known that alcohol and nicotine can produce differential effects on different behavioral and physiological variables that could be the same depending on the dose of drug consumed, and it has even been suggested that both drugs share at least one genetic component that could produce a predisposition to the use or abuse of these drugs (Funk et al., 2006). Based on the similarity in the mechanisms, it has been proposed that nicotine pharmacological stimulation of specific sites generates plastic changes in neurons. Since these changes do not disappear and are the same site of action of the second drug, when alcohol is consumed for the first time, the phenomenon of tolerance occurs, even in the absence of previous experience. This pharmacological explanation seems to explain the cross-tolerance phenomenon; however, it does not explain the environmental specificity of the cross-tolerance. That is, it does not explain why cross-tolerance occurred only in the context where tolerance had developed and did not occur when the context was different. Thus, cross-tolerance can be studied as a pharmacological phenomenon or as a learning phenomenon; however, pharmacological theories are silent in terms of the role of environmental stimuli associated with the administration of a drug in the development of cross-tolerance or with respect to the incentive value of a drug. Therefore, understanding how the development of tolerance to a drug can modulate tolerance to a second drug requires an appreciation of both learning principles and pharmacological principles.

Another factor that could contribute to the explanation of the development of cross-tolerance is the role of environmental stimuli associated with the administration of the drug. The drug tolerance conditioning model proposed by Siegel (1977) predicts that the development of tolerance is influenced by environmental variables, particularly the history of association of environmental stimuli with the administration of a drug. Thus, the development of tolerance occurs because the conditioned stimuli cause a CR that is opposite to the effects of the drug, and this antagonistic CR attenuates the unconditional effects of the drug (Dafters & Anderson, 1982; Duncan et al., 2000; Newlin, 1986; Siegel, 1977, 1979).

In addition to contextual specificity providing an empirical basis for the participation of Pavlovian conditioning in tolerance, the model indicates that the production of a CCR is fundamental in the explanation of tolerance. The CCR can usually be evidenced in subjects who have undergone a process of acquisition and development of tolerance to a drug, and in a subsequent phase, a placebo is administered in the presence of the environmental stimuli associated with the chronic administration of the drug (Newlin, 1986; Ruiz et al., 2010). One example is previous research that we conducted in our laboratory showing that environmental stimuli are an important component in the explanation of tolerance; stimuli that produced the CCR attenuated the unconditional effects of the drug, but the absence of these stimuli did not cause the CCR, and therefore, reduced drug effects were not observed (Ruiz et al., 2010).

In this way, the model could suggest an additional explanation of the cross-tolerance between nicotine and alcohol observed in the present experiment. Environmental stimuli associated with smoking nicotine-containing cigarettes could have produced a CCR that was able to add to the acute effects of alcohol (Cappell et al., 1981; González et al., 2016; Ruiz et al., 2010; Vila et al., 2013). In other words, in the cross-tolerance test, the contextual stimuli caused a decrease in the heart rate response in the subjects. This effect was algebraically added to the increase in the heart rate caused by alcohol consumption. The result that we observed was a decrease in the acute effects of alcohol on the heart rate compared with the effects observed in absence of the contextual stimuli, which allows us to suppose that the CCR also underlies the cross-tolerance observed in this experiment. In this way, our results support the hypothesis proposed by several researchers that contextual cues are an important component in the explanation of the cross-tolerance between nicotine and alcohol, assigning a central role to Pavlovian associative processes (Ruiz et al., 2010; Siegel, 1979, Siegel et al., 2000; Siegel & Ramos, 2002; Vila et al. 2013). Although these results could not be reduced to pharmacological mechanisms, we could state that both mechanisms (pharmacological and conditioning) are complementary.

The clinical significance of our results lies in the pertinence of conditioned cross-tolerance in human nicotine and alcohol abuse based on Siegel's demonstration that the lethality of a drug dose can be greatly influenced by the presence of drug-related stimuli. In a nondrug-related environment, the absence of drug-opposite conditioned responses and thus the absence of conditioned cross-tolerance results in an increase in drug

potency, thereby increasing the potential for overdose. Because of the potentially lethal consequences for the active drug abuser, further research is needed to more comprehensively determine the incidence of conditioned cross-tolerance in the natural environment and the extent to which it is involved in accidental overdose. At the applied level, these findings are particularly relevant for clinicians, considering that treatments for drug-related disorders have not been very effective. The results presented here could help improve therapy in the clinical environment by implementing techniques that have been shown to reduce the influence of environmental stimuli associated with the use of a drug.

Another aim of treatment programs is the prevention of resumption of excessive drug use. Typically, following a period of detoxification, the patient will no longer display withdrawal distress and no longer report craving. Therefore, when patients are released and return to environments where they previously used drugs, they display withdrawal distress, report craving, and relapse. The factors contributing to relapse may be that the capacity of drug-associated stimuli to elicit craving and withdrawal distress has not been reduced during treatment.

These results require future research since a full discussion of the potential mechanisms that explain the development of cross-tolerance to heart rate responses produced by nicotine and alcohol requires incorporating an analysis of the symmetric effects of the two drugs used, so it would be important to evaluate whether cross-tolerance develops when the order of presentation of the drugs is reversed; that is, first administering alcohol for the development of tolerance and then nicotine to observe cross-tolerance. Additionally, it would be important to evaluate whether cross-tolerance can be observed with different effects produced by these drugs.

Conclusions

The results of this investigation showed that the cross-tolerance between nicotine and alcohol was modulated by the environmental stimuli associated with the administration of the first drug and the resulting CCR. Our results have important implications in the study of the mechanisms of addiction and drug dependence. The interaction of conditioning factors, such as the control of environmental stimuli in tolerance and cross-tolerance, could play an important role in drug abuse and dependence.

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Conflict of interest

No conflict of interest to report.

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