Oral alcohol self-administration and maintenance of operant behavior in rats [1]

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Abstract

We evaluated the effects of two alcohol induction procedures on food-reinforced lever-pressing in rats. Nine outbreed Wistar rats were assigned to one of three groups. For all subjects a fixed-ratio (FR)11 food-reinforcement schedule was established. The induction group (IG) was exposed to alcohol at increasing concentrations over the course of 10 days up to a final concentration of 10%. The non-induction group (NG) received only the 10% alcohol dose during the same period. The control group (CG) received only water and never had alcohol. After induction, the responses in two groups (IG, NG) were food-reinforced for 10 days with free access to alcohol, followed by 15 days of food reinforcement when water was made available instead of alcohol. The alternating cycle of access to alcohol and water was repeated twice more to complete the experiment. Results were as follows: Rate of reinforcement per minute was lower, body weight increased, sessions duration increased, reduced food intake decreased, and alcohol consumption increased during induction and when ethanol was available. Results are discussed by comparing the behavioral effects of alcohol and water on operant behavior.

Keywords: alcohol, body weight, eating and drinking intakes, induction, oral self-administration, pressing lever, rats.

Resumen

En este estudio evaluamos los efectos de dos procedimientos de inducción al alcohol sobre presionar una palanca reforzada por comida en ratas. Nueve ratas Wistar fueron asignadas a uno de tres grupos. Para todos los sujetos se estableció un programa de reforzamiento de razón fija (RF)11 con comida. El grupo inducción (IG) fue expuesto al alcohol en concentraciones crecientes en el transcurso de 10 días hasta llegar a una concentración final de 10%. El grupo sin inducción (NG) recibió directamente la dosis de alcohol al 10% en el mismo periodo. El grupo control (CG) recibió solo agua y nunca alcohol. Posterior a la inducción, las respuestas en los grupos IG y NG fueron reforzadas con comida durante 10 días con libre acceso al alcohol, seguidas por 15 días de reforzamiento con comida cuando el agua estuvo disponible en vez del alcohol. El ciclo alternando el acceso al alcohol y al agua se repitió dos veces más para completar el experimento. Los resultados fueron: la tasa de reforzamiento por minuto fue menor, el peso corporal aumentó, la duración de las sesiones incrementó, la reducción de ingesta de comida fue

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menor, durante la inducción el consumo de alcohol aumentó y también cuando el alcohol estuvo disponible. Discutimos los resultados comparando los efectos conductuales del alcohol y agua sobre la conducta operante.

Palabras clave: alcohol, auto-administración oral, consumo de comida y agua, inducción, peso corporal, presión de palanca, ratas.

In recent years, researchers have developed experimental models of oral self-administration of alcohol to study the effects of different variables on alcohol consumption in rats (Linseman, 1987; Tabakoff & Hoffman, 2000). According to Kamenetzky and Mustaca (2005), home cage and operant conditioning models are the two main forms of self-administration of alcohol in animals. The main difference between both models is the behavioral requirement established to have access to alcohol. In two cases the animal controls the temporal pattern of intake and dose. In the case of the box-house procedures, the animal has access only to alcohol or water and alcohol for different periods of time, animals have to drink directly from the bottle containing alcohol. In the models of operant conditioning the behavior of the animal is contingent upon access to alcohol under the requirement of an instrumental response (e.g., pressing a lever). Procedures for oral self-administration of alcohol in rats are considered useful for pre-clinical pharmacological evaluation of new drugs and for studying the etiology of intake abuse and processes of alcohol addiction (Carnicella, Yowell, & Ron, 2011; Spanagel & Hölter, 1999). Cunningham, Filder, and Hill (2000) reviewed some of the most effective models for studying oral self-administration of alcohol under different experimental preparations. In one model designed to study patterns of alcohol consumption, rats are allowed access –limited or unlimited– to alcohol throughout the day in their home cages. Other experimental models have used operant conditioning to study various phenomena, including reward value, consumption patterns, the behavioral effects of stress, aggressive behavior, and the relationship between drug use and behavioral effects, among others (Green & Grahame, 2008; Roberts, Heyser, & Koob, 1999; Weatherly, Bauste, Mcdougall, & Nurnberger, 2006; Wolffgramm, 1990). The model of oral self-administration of alcohol using operant conditioning procedures focuses primarily on the motivation to consume alcohol. The experimenter determines the response required to delivery of the reinforcer (i.e., schedule of reinforcement) such that the rat has to work (e.g., press a lever) to drink a very small amount of alcohol (Meisch & Thompson, 1973; Vacca et al., 2002). Studies on operant procedures have collected data suggesting that alcohol intake is maintained due to reinforcing effects (Meish, 1973). These models typically use intermittent reinforcement schedules because they produce high and stable response rates that make it possible to evaluate how the substance administered acts on response rates.

There are also studies of motivational variables that involve tasks in which animals must work to drink alcohol even when they are not hungry or thirsty (Sinclair, 1974). Gill, Amit, and Smith (1996) have reported that the pattern of alcohol consumption in rats is distributed throughout the day, as is the water consumption pattern, and that alcohol consumption is maintained without altering normal eating patterns. Rats exposed to alcohol as the sole fluid source, drink during short and intermittent periods that do not produce significant blood alcohol levels (Cunningham et al., 2000). Models of oral self-administration in home cages have been used to evaluate animals' preferences between two substances (e.g., alcohol *vs.* sucrose), or changes in consumption patterns under conditions of free access (Green & Grahame, 2008). Three major problems have been reported in relation to oral self-administration of various drugs in animals: a) aversive taste; b) delays in the onset of effects on the central nervous system; and, c) consumption of low amounts of the substance.

Cunningham, Fidler and Hill (2000) argued that induction procedures are a common strategy for initiating alcohol consumption in animals at low concentrations and gradually increasing the concentration. Alcohol induction represents a controlled introduction of alcohol into the diet and consumption patterns of the organism (Martínez & Urzúa, 2013). For these reasons, several methods have been used to induce consumption of a new flavor; for example, using an induction procedure that begins at a low concentration of alcohol and gradually increases concentrations diluted in water (Cunningham et al., 2000; Meisch, 2001; Slaweck & Samson, 1997).

Veale and Myers (1969) developed a gradual method (3%-to-30% v/v) to induce rats to drink larger volumes of alcohol. These authors found that animals exposed to alcohol without an induction procedure, drank smaller amounts compared to animals that were gradually exposed to this liquid. One study of oral self-administration of alcohol (20% v/v) used an induction procedure without adding sugar to show that rats exposed to alcohol (20% v/v) intermittently (3 days per week during induction) consumed significantly more alcohol than those whose responses were reinforced with alcohol (at 10% v/v) induced with sugar. These results confirmed that rats could be trained to respond to alcohol (at 20% v/v) as a reinforcer with no need to use the sweetened procedure (Simms, Bito-Onon, Chatterjee, & Bartlett, 2010). Carnicella et al. (2011) trained Long-Evans rats in an operant procedure using a model of alcohol self-administration (at 20% v/v) that increased the concentration from 2.5% to 60%v/v once the rats had become habituated to drinking alcohol in their home cages. These authors reported an adaptation of the animals to consumption levels and response patterns as the percentage of alcohol was increased until constant levels of blood alcohol were obtained. By increasing the percentage of alcohol, both the frequency of lever-pressing and the delivery of alcohol rose as concentrations were increased gradually from 2.5% to 10% v/v, but that lever-pressing frequency gradually decreased as concentrations rose from 20%-to-60% v/v. It is not yet clear how drinking alcohol without restriction in the rats' home cage affects the reinforcement rate, body weight, food and liquid intake when using reinforcement schedules in food restricted rats.

In behavioral pharmacology, reinforcement schedules have been used as ways of generating substantial changes that can be observed after the administration of some drugs (Laties, 1978). For example, Petry (1997) analyzed the effects on the operant behavior of ip administration of alcohol at different doses (0.0, 0.3, 0.6, and 0.9 g/kg.) in rats. Forced administration of low doses of alcohol resulted in a decrease in response rates under VI´s schedules, but motor performance (measured as locomotor activity, motor coordination and response rate) and sensitivity to reinforcement were not affected and at the highest dose (0.9 g/kg.) there was a decrease in response rate and reinforcement effectiveness (amount of reinforcement needed to maintain the mid-maximal response rate).

It has also been shown that chronic alcohol administration and the development of alcohol tolerance can produce behavioral changes in organisms that cause a loss of reinforcement (i.e., (FR)30). Holloway, King, Michaelis, Harland and Bird (1989) and Holloway and King (1989) using rats as subjects, reported deterioration in the performance of operant behavior (e.g., (FR)30 & DRL) in early phases of chronic intraperitoneal alcohol administration which was significantly improved along of the administration. The authors suggested compensatory learning as a mechanism for the development of alcohol tolerance that has effects on operant behavior. This compensatory mechanism counteracted the disruptive effects of alcohol that caused a reduction in the density of the reinforcement obtained. According to Barret and Stanley (1980) the effects of forced alcohol administration may depend on the response rate, behavioral history, and context where behavior occurs. These authors mentioned that the effects of alcohol on responses under a FR schedule of reinforcement depended on ratio size.

Therefore, it would be interesting to study the relationship between freely-available drinking alcohol in home cage and working for food under an operant procedure in rats. The goal of this study was to examine whether oral self-administration of alcohol in the rats' home cages interferes with continued lever-pressing for food. A second aim was to explore whether an induction procedure generates differences in alcohol consumption and FR performance. The hypotheses were that rats with access to alcohol would show a lower rate of reinforcement on FR schedules compared to control rats offered only water, and that alcohol consumption would be higher in the rats that underwent alcohol induction. These hypotheses were based on an experiment by Martínez and Urzúa (2013) in which they evaluated the effects of three orally self-administered alcohol induction procedures on the maintenance of alcohol consumption, body weight, consumption of food and water in rats. The authors found that the highest level of alcohol consumption occurred during the induction period (12 days) when the alcohol induction consisted of increasing the dose diluted in water (4% - 10% v / v). During this period subjects were required to drink alcohol as the only source of liquid intake. In addition, alcohol consumption remained stable during the phases following alcohol induction and maintained stable body weight and food intake.

Method

Subjects

Nine naïve outbreed female Wistar rats that were 3 months old at the start of experimentation and were obtained from the animal facility of the Institute of Neurosciences at the University of Guadalajara, Mexico -served as subjects. The rats were housed individually and maintained at 80% of *ad libitum* weight. To maintain this weight, if necessary, they received a portion of food at the end of each session. Access to alcohol (10% v/v) or water outside the experimental chambers was unrestricted. The animals were fed with Purina Rodent Laboratory Chow (3% fat, 23% protein, 7% ash, 1% calcium, 6% fiber, 49% of E. L. N, 6% phosphorus and 12% humidity). Room conditions were maintained on a 12-12 hr light/dark cycle (07:00/19:00), and temperature was held constant at 23 ± 2°C. All animal care methods were submitted to, and approved by, the Ethical Committee of the Institute of Neurosciences at the University of Guadalajara # ET042011-100.

Apparatus and Materials

For the experimental sessions, *Lafayette instrument* operant chambers (Model 80003NS) were used. The front wall of each chamber had two non-retractable levers separated by 8 cm and placed at a height of 13 cm from the chamber floor. At the top of each lever was a light (28v/6w). The food dispensers located between the two levers provided pellets (*Bioserv*, dustless precision pellets, rodent grain-base formula, 0.45 mg) according to a schedule. The experimental chambers were connected via an interface to a PC (Lafayette Instrument Abet Models 81401 and 81402). The delivery of reinforcers and data collection were programmed with Abet Lafayette software. A 28v/6w house light served as general illumination. Absolute ethyl alcohol (ethanol 95.5%, *HYCEL*) diluted in water was used in 100 ml bottles (10% v/v).

Procedure

Experimental sessions were conducted seven days a week and began approximately at 10:00 am. After recording body weight, the rats were placed in the operant chamber. At the end of each session they were weighed again and returned to their home cages. To start each subject had free access to food and water for five days. On the following day, a regimen of restricted food consumption was imposed to reduce body weight to 80%. Rats were weighed following a session and then fed the difference between their current weight and target weight (80%). If the body weight of some rats at the end of the

experimental session was greater than the required weight, they did not receive extra food, or only a maximum of 6 grams to maintain proper body weight. Thus, all rats received water and a daily food ration to maintain the target weight. All subjects were exposed to a phase of acquisition and maintenance of a basic schedule of fixed-ratio reinforcement (FR)11. This fixed-ratio was chosen on the basis of previous tests with a range of 2-40 responses per reinforcer and 11 responses were considered a reasonable requirement to obtain 90 reinforcers in 30 minutes (Ritz, George, & Meisch, 1989). The baseline was then initiated for 10 days in which subjects were required to obtain 90 reinforcers in a maximum of 30 minutes. After baseline the subjects in the induction group (IG, n = 3) were placed under oral alcohol induction self-administration procedure during 10 days as follows: 2% v/v alcohol for two days with the increased concentration by 2% v/v every two days up to 10% v/v. The non-induction group (NG, n = 3) was exposed directly to alcohol at 10% v/v for the same period as in the induction procedure for the IG. It should be mentioned that subjects had only access to alcohol outside of experimental sessions as the only source of fluid. After the induction procedure, the subjects of both experimental groups were exposed to alcohol access phases (first, second, and third phase with alcohol) lasting 10 days each phase, alternated with three other phases in which they had only access to water (first, second and third phase with water), but with a duration of 15 days each phase of water. During the phases of access to alcohol, the two experimental groups had access to alcohol for approximately 23:30 hours per day, that is, except during the time the subjects were placed in the operant conditioning box where their responses were reinforced with food, had no access to liquids. A third group that only received water all the time and their responses were also reinforced with food throughout the experiment served as control group (CG, n = 3). The other experimental conditions remained the same as those established for the two experimental groups (see Table 1).

Group	Baseline	Induction	1st phase Alcohol	1st phase Water	2nd phase Alcohol	2nd phase Water	3rd phase Alcohol	3rd phase Water
Days	10 days	10 days	10 days	15 days	10 days	15 days	10 days	15 days
Induction group IG	Water	Alcohol 2% -10%	Alcohol 10%	Water	Alcohol 10%	Water	Alcohol 10%	Water
Non induction group NG	Water	Alcohol 10%	Alcohol 10%	Water	Alcohol 10%	Water	Alcohol 10%	Water
Control group CG	Water	Water	Water	Water	Water	Water	Water	Water

Table 1. Experimental design shows baseline, alcohol induction, three phases of alcohol or water for induction group (IG), non-induction group (NG), and control group (CG).

Results

Individual mean data from the last 5 days of baseline and each experimental phase for all 3 groups are summarized in Table 2. The columns represent the data for each subject in each group, while the rows show the five variables measured.

Phase		Alcohol Induction Group (IG)			Alcohol Non-Induction Group (NG)			Control Group (CG)		
		S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
BASE LINE	**BW (g)**	-0.77	-2.45	2	2.88	0.68	2.08	-2.12	-1.22	-3.24
	Food (g)	4	4.4	3.2	3.4	4.4	3.4	3.8	4.2	3.8
	Water (ml)	19.4	37	22.6	26.8	29.2	56.4	30.6	21.6	30.6
	Etoh (g/kg)	-	-	-	-	-	-	-	-	-
	Time (min)	12.51	13.57	11.89	11.35	11.29	11.18	11.27	11.44	11.15
ETOH*	**BW (g)**	4.51	8.13	7.98	6.18	2.32	10.42	-3.44	-2.44	7.82
	Food (g)	0.2	0.2	0.4	0.2	0.2	0.2	2.8	4	3.2
	Water (ml)	-	-	-	-	-	-	33	25.6	31
	Etoh (g/kg)	2.54	2.18	2.14	1.95	1.69	1.58	-	-	-
	Time (min)	16.94	21.7	15.41	16.57	12.82	12.68	16.57	12.82	12.68
ETOH	**BW (g)**	3.03	2.93	10.76	8.32	-0.24	6.38	-2.8	-4.02	2.64
	Food (g)	0	0	0	0	0	0	3	3	3
	Water (ml)	-	-	-	-	-	-	29.2	23.4	31
	Etoh (g/kg)	2.06	2.16	2.46	2.17	2.04	2.25	-	-	-
	Time (min)	17.41	25.07	17.51	19.23	12.67	13.64	19.23	12.67	13.64
Water	**BW (g)**	-0.47	-2.23	-1.44	-1.38	-0.88	-2.98	-2.52	-2.92	-2.42
	Food (g)	3.4	3.4	3.4	3.4	4.2	3.8	3.6	5.6	5
	Water (ml)	27.8	33.8	32.4	24.2	29.6	33.6	27.2	22	27.4
	Etoh (g/kg)	-	-	-	-	-	-	-	-	-
	Time (min)	12.25	15.69	12.78	13.61	10.79	10.19	13.61	10.79	10.19
ETOH	**BW (g)**	3.25	1.49	3.72	-1.2	0.376	1.16	0.56	-0.54	0.04
	Food (g)	0.6	1	0.4	1.8	2	1.4	3	4.4	4.8
	Water (ml)	-	-	-	-	-	-	33.4	22.4	29
	Etoh (g/kg)	4.33	3.69	3.37	3.48	3.29	2.92	-	-	-
	Time (min)	18.12	25.20	25.37	12.18	12.28	12.79	12.18	12.28	12.79
Water	**BW (g)**	1.91	0.87	1.52	-0.3	2.04	2.1	2.08	-0.92	0.22
	Food (g)	3.8	4.4	3.6	4	3.8	3.4	3.6	5.2	4.8
	Water (ml)	30.4	29.4	25	24.8	25.2	37.8	26.8	20	35
	Etoh (g/kg)	-	-	-	-	-	-	-	-	-
	Time (min)	13.06	14.46	15.37	10.22	10.01	10.77	10.22	10.01	10.77
ETOH	**BW (g)**	3.61	1.37	5.08	-1.38	2.18	0.04	1.46	0.66	1.26
	Food (g)	1.6	0.4	0	1	0	0	4.2	4.4	4.4
	Water (ml)	-	-	-	-	-	-	38.2	31.2	40
	Etoh (g/kg)	2.12	2.4	1.8	2.30	2.12	2.08	-	-	-
	Time (min)	17.68	20.38	22.59	11.90	11.77	13.48	11.9	11.77	13.48
Water	**BW (g)**	2.41	-0.49	0.66	0.34	2	1.56	2.06	0.09	1.24
	Food (g)	3.8	4.8	4.8	4	4	4	4	4.2	5
	Water (ml)	28.2	37.6	36.2	30.8	26.6	44.2	41.4	26.2	39.8
	Etoh (g/kg)	-	-	-	-	-	-	-	-	-
	Time (min)	12.56	14.56	10.66	13.49	9.87	10.28	13.49	9.87	10.28

Table 2. Individual mean of the last five days for baseline and each experimental phase for the three body weight groups (BW) measured as grams ±80% of *ad libitum* weight, food intake in grams (Food), water consumption in milliliters (Water), alcohol (Etoh) in g/kg, and session time in minutes. Etoh* indicates the induction phase of alcohol for IG. Horizontal lines (-) mean that neither water nor alcohol was available in that phase.

Alcohol consumption

The upper part of Figure 1 (A) shows the individual alcohol intake (g/kg) of the IG group that underwent the alcohol induction procedure. All three subjects showed the highest increase (around 2 g/kg) at the end of induction period when alcohol was made available at 10% v/v. In the first phase, alcohol intake was stable (about 2 g/kg) for all three subjects. In the second phase of access to alcohol for the three subjects, intake was higher than before (range: 3-4 g/kg), and S1 reached almost 6 g/kg around the middle of this phase. In the course of the last phase, alcohol consumption by all subjects decreased progressively to approximately 2 g/kg in the final four days of the phase. Unlike IG –and despite some inconsistency– during the first four days of access to alcohol (10% v/v) the NG subjects without the alcohol induction procedure of IG consumed around 2 g/kg (Figure 1B). On the fifth day, there was stability, which continued into the first phase (range: 1.3-2.6 g/kg). In the second phase, consumption by all subjects increased, with S1 and S2 showing the highest consumption (4.5 and 4.9 g/kg, respectively). In the last phase, alcohol consumption was variable for the first four days, but from day five it stabilized at approximately 2 g/kg until the end of the phase.

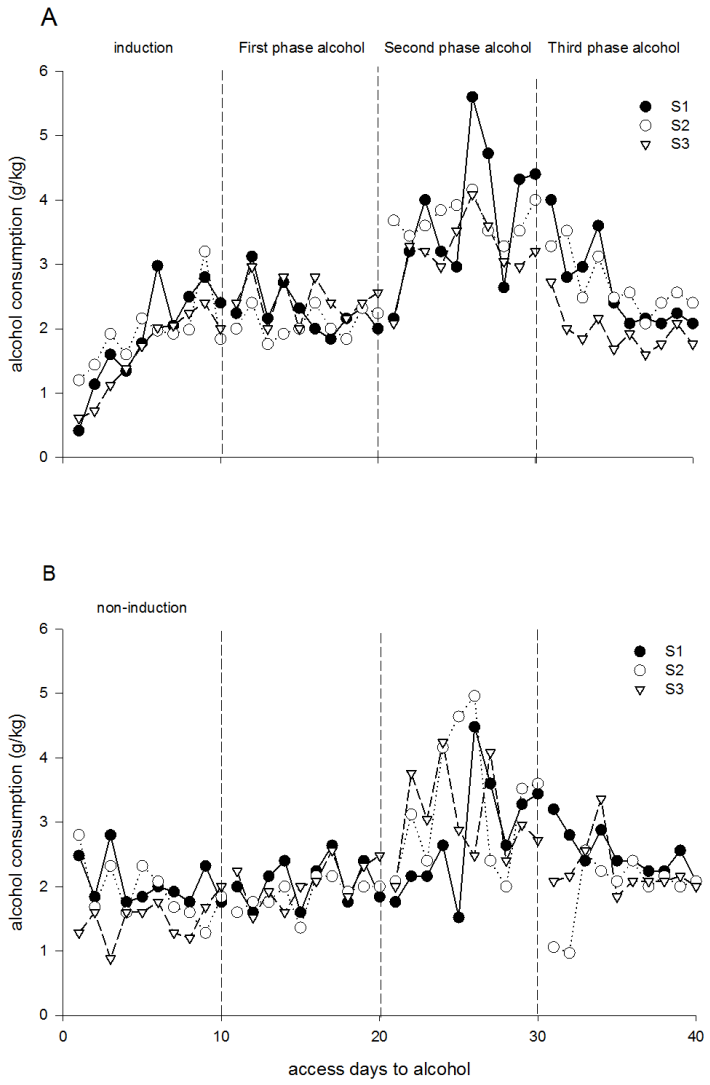

Figure 1. Individual alcohol intake (g/kg) data from the group (IG) that received the alcohol induction procedure (A) and from the group without induction (B). Dashed lines indicate water periods between access days to alcohol.

Water consumption

The upper panel of Figure 2 (A) shows individual water consumption for the IG group during the periods when the only liquid available was water. During baseline water consumption showed differences between subjects. Subject S2 showed the highest consumption (range: 26-40 ml), while for S1 and S3 this varied between 16 and 30 ml. Consumption was similar, although irregular, for the three subjects in the first phase. During the first eight days, consumption was lower compared to the last 7 days when water consumption increased (range: 14-40 ml). In the second phase, S1 and S2 showed lower consumption (around 16 ml) and subject S3 showed the highest (42 ml). Regarding the last phase, for the first day all three subjects showed low consumption. S1 had the lowest intake (9 ml), but on the following days all subjects increased their consumption to a range of 19 ml for S1 and 42 ml for S2 and S3.

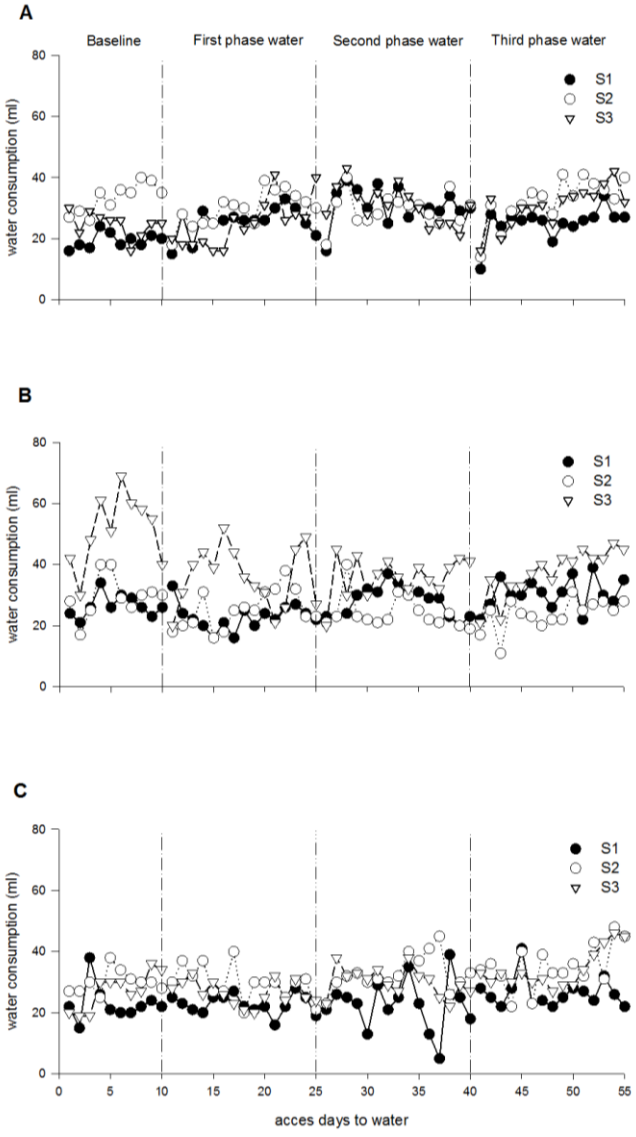

Figure 2. Individual water consumption (ml) data from the group (IG) that received the alcohol induction procedure (A), from the group (NG) without induction (B), and from the group (CG) that received only water during the experiment (C). Except for the control group, dashed lines represent periods when alcohol was available.

The central graph of Figure 2 (B) also shows individual water consumption for the NG group during the days of access to water. In baseline subjects S1 and S2 showed similar consumption (range: 16-40 ml), while the range for subject S3 was 29-69 ml. During the first phase, subjects S1 and S2 maintained

their intake (15-37 ml). Although S3 continued to have the highest consumption, this decreased to a range of 19-51 ml). In the next two phases, the consumption ranges were 19-44 ml (S1 and S2) and 10-46 ml (S3). The lower graph of Figure 2 (C) shows the individual water consumption for the CG group during the days in which water was the only available liquid to IG and NG. During baseline and first phase, the water consumption range for the three subjects in CG ranged from 14-39 ml. In the second phase, water consumption was 4-45 ml, while during the last phase it ranged from 21-48 ml.

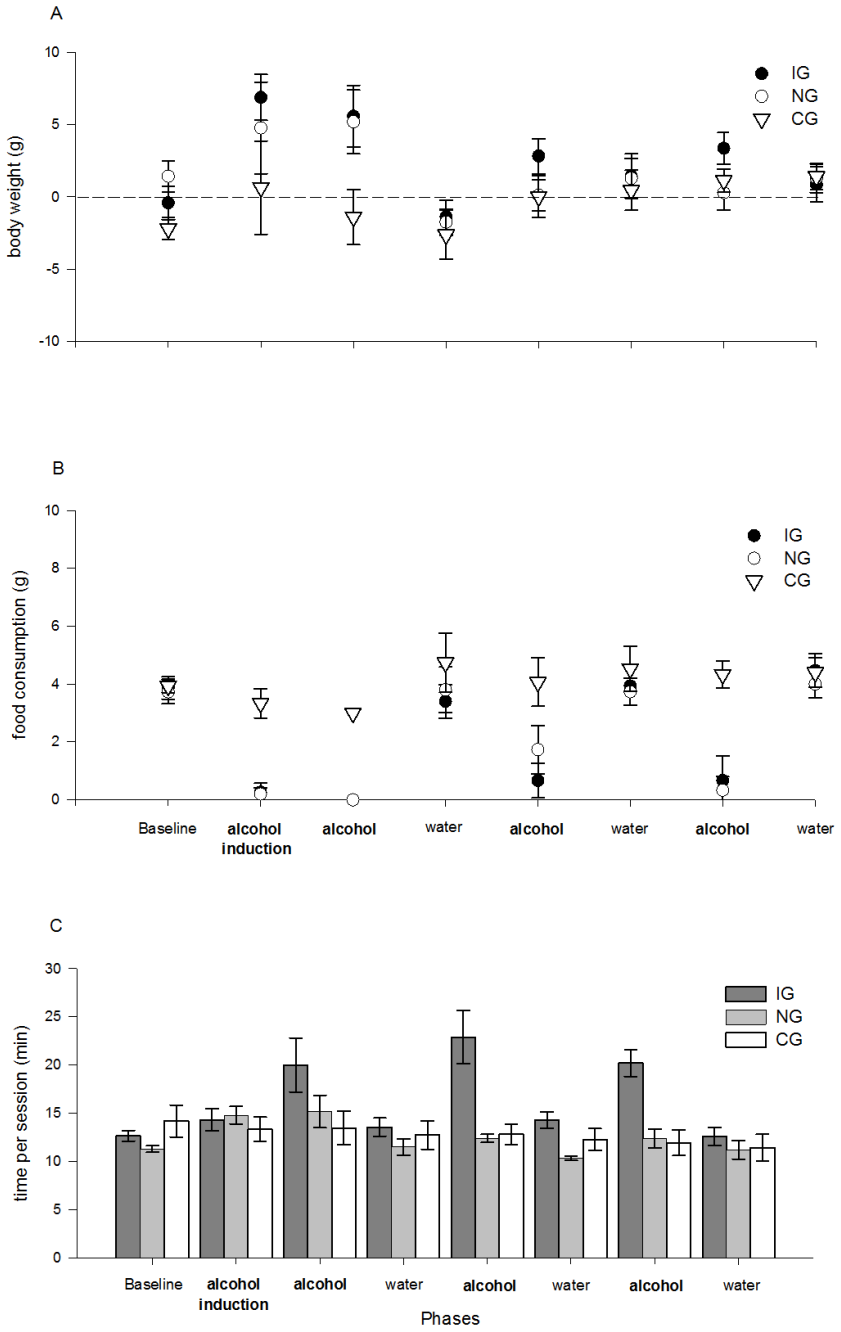

Figure 3. Mean (± 2 SEM) body weight (g) per group for the last five days of each experimental phase (A). Scores of zero represent 80% of body weight; points above zero mean weight gained; while those below zero mean weight loss. Mean (± 2 SEM) food consumption (g) per group for the last five days of each experimental phase (B). Mean (± 2 SEM) reinforcers per minute per group obtained in each phase (C). During induction period: IG = alcohol 2% to 10% v/v; NG = alcohol 10% v/v; CG = water

Body weight

Figure 3 (A) shows the mean (± 2 SEM) body weight per group for the last five days of each experimental phase. Scores above zero represent body weight gain, those below zero indicate body weight loss. Scores on the zero line correspond to maintenance of 80% body weight. In baseline the IG subjects remained around 80% of their *ad libitum* body weight (black circles), subjects without induction (NG) were above 80% (white circles), and control subjects (CG) were below 80% of their *ad libitum* figure (white triangles). In the induction to alcohol period, the body weight of the IG subjects increased, reaching a mean of 6.8 g. Subjects in NG had a mean of 4.7 g above *ad libitum* body weight, and subjects in CG, a mean of 0.6 g. In the first phase with alcohol after induction procedure, mean body weight for the IG group was 5.5 g and for the NG group 5.1 g above *ad libitum* body weight. Subjects in the CG group had a mean of -1.3 g below *ad libitum* body weight. During the first phase with water, the body weight for all subjects in all groups declined and remained below 80% of the *ad libitum* figure. The IG group had a mean of -1.3 g, for NG this was -1.7 g, while for CG it was -2.6 g. In the second phase with alcohol, the body weight of all subjects in all three groups increased compared to the previous phase with water and remained above or equal to 80% of their *ad libitum* body weight. For IG, the mean was 2.8 g; for NG, 0.1 g; and for CG, 0.02 g. In the second phase with water, all subjects remained above 80% of *ad libitum* body weight. IG subjects had a mean of 1.4 g; NG rats, 1.3 g; and GC animals, 0.4 g. In the last phase with alcohol, all subjects in the three groups remained above 80% of *ad libitum* body weight. For IG the mean was 3.3 g; for NG, 0.3 g; and for CG, 1.1 g. During the last phase with water, subjects in all three groups remained above 80% of *ad libitum* body weight. For IG the mean was 0.8 g; for NG, 1.3 g; and for CG, 1.4 g.

Food consumption

The middle graph in Figure 3 (B) shows the mean (± 2 SEM) food consumption from the last five days of baseline, induction and each experimental phase for all subjects in the three groups. During baseline all rats consumed about 4 g. In the induction procedure to alcohol, food intake in IG and NG was close to zero g, while in CG it was 3.3 g. In the first phase of alcohol, food consumption in IG and NG continued to be zero g but CG maintained a consumption of around 3 g. In the first phase with water, food intake increased for all subjects. The mean for IG and NG was about 4 g, while for CG it was 5 g. During the second phase with alcohol, food consumption decreased in all the three groups: IG had a mean of 0.6 g; in NG it was 1.6 g; and in CG, 4 g. In the second phase of access to water, IG and NG had a mean food intake of almost 4 g, and subjects in CG consumed 4.5 g. During the last access to alcohol, food intake decreased again in IG and NG, with means of 0.3 and 0.6 g, respectively. For GC the mean was 4.3 g. Finally, food intake by all subjects remained around 4 g in the third phase with water.

Time per session

The bottom graph of Figure 3 (C) shows data (mean ± 2 SEM) for the last 5 days per phase for all subjects in all three groups. During baseline subjects in all three groups showed similar session times. The means were as follows: IG, 12.6 min; NG, 11.2 min; and CG, 14.1 min. In the induction to alcohol the mean respective session times were: IG, 14 min, NG, 15 min, and CG, around 13 min. During the first phase of access to alcohol, session time increased in IG to a mean of 20 min; NG remained constant at around 15 min; and CG increased slightly to 13.4 min. In the first phase with water, session times for all subjects were again similar: IG at a mean of 13.5 min per session; NG at 11.5 min; and CG at 12.7 min. In the second phase with alcohol, IG increased its mean session time to 22.9 min; while the means for NG and CG were 12.4 and 12.8 min, respectively. In the second phase with water, IG decreased its session time to a mean of 14.3 min; NG showed 10.3 min; and GC, 12.29 min. In the last phase with alcohol, IG

again increased its session time, now to a mean of 20.2 min; NG had a mean of 12.3 min; and CG showed a mean of 11.9 min. In the last phase with water, the mean for IG was 12.5 min per session; for NG, 11.2 minutes; and for CG, 11.4 min. The IG group took the longest time to complete the session and the presence of alcohol was more evident in that group compared to the water phases, while NG and CG completed similar times during both phases.

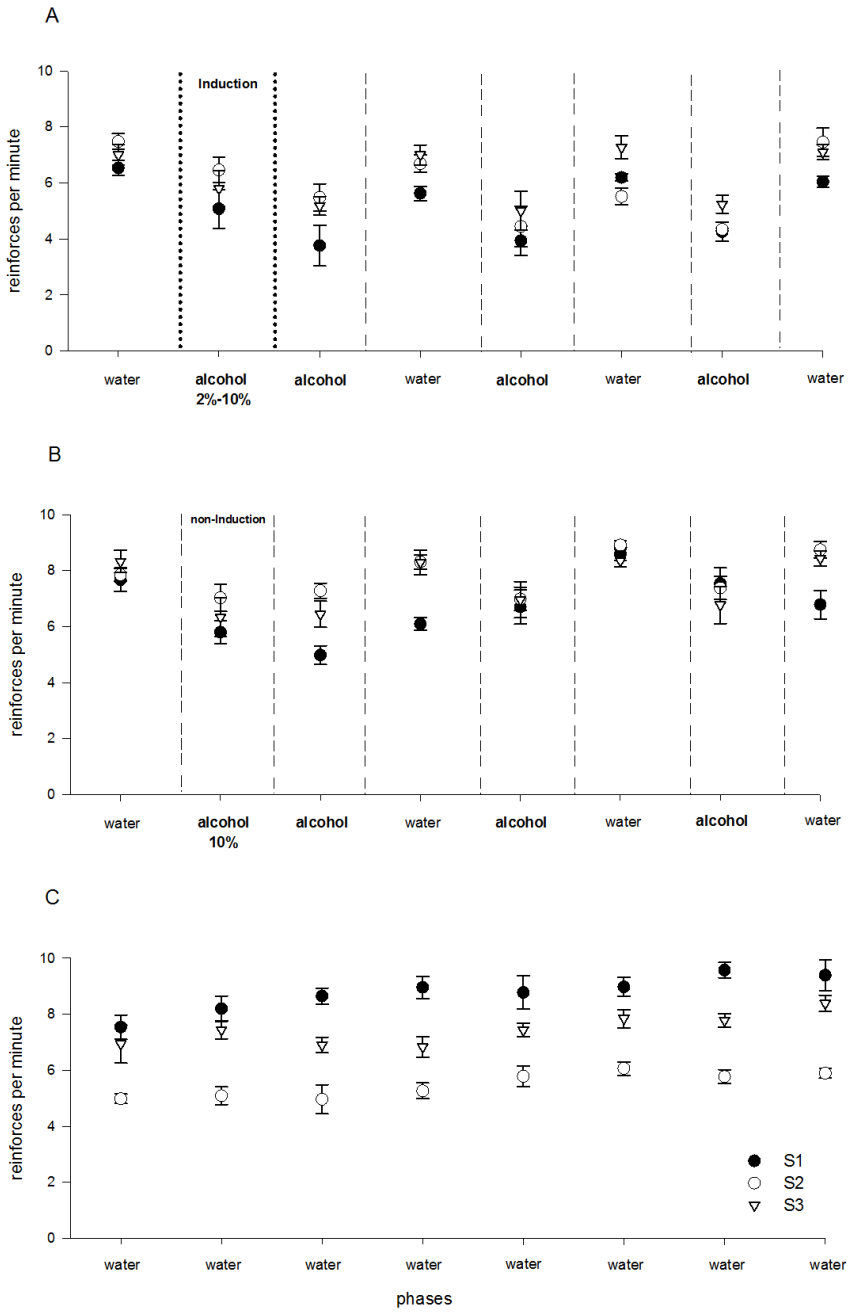

Figure 4. Individual means (± 2 SEM) of reinforcers per minute per phase obtained by each group. The upper graph (A) represents the induction group data, the middle graph (B) the non-induction group data, and the bottom graph (C) the control group data.

Reinforcers per minute

The group IG obtained less reinforcers per minute in the alcohol phases compared to the water phases (Fig. 4A). In the alcohol phases, IG subjects obtained a range of 3-6 reinforcers per minute; while

in the water phases the range was 5-8 per minute. In general, NG obtained more reinforcers per minute in the alcohol and water phases (Fig. 4B), and this group also obtained a higher number of reinforcers per minute during the water phases (range 6-9) compared to the alcohol phases (range 5-8). The control group, which received only water, tended to increase its reinforcers per minute over the course of the sessions. Only one subject (S3) showed a low, but steady, rate of reinforcers per minute.

Discussion

The purpose of the present study was to examine whether oral alcohol self-administration in rats outside of experimental session (period in which the subjects were inside the operant conditioning chamber) interfered with responding maintained by schedules of food reinforcement. A second objective was to explore whether two procedures of self-administration oral alcohol generated differences on alcohol consumption and performance in FR schedules of reinforcement.

Researchers have proposed models of alcohol consumption in animals in order to (1) study the development of chronic alcohol consumption; (2) identify motivational variables related to alcohol consumption through methods that induce effects on consumption by restricting or allowing free access to food (inhibition or facilitation); and, (3) explore compulsive behaviors and relapse, among other phenomena (Kamenetzky & Mustaca, 2005). However, studying other variables is difficult in experimental conditions designed to evaluate the effects of alcohol consumption through voluntary oral self-administration under exposure to operant conditioning using ABAB within-subject designs (Sidman, 1960). Hence, our main objective was to evaluate the effects of free-access to alcohol or water as the sole source of liquid in alternating periods in home cages on performance under a FR food-reinforcement schedule in rats. The parameters evaluated were: Rate of reinforcement, body weight; food intake in the home cages after the experimental sessions; session duration; and alcohol and water consumption.

Body weight

Some researchers have considered it particularly interesting to study the effects of oral alcohol consumption on body weight (e.g., Larue-Achagiotis, Poussard, & Louis-Sylvestre, 1990), but most studies of this kind have been conducted with human subjects (Gruchow, Sobocinski, Barboriak, & Scheller, 1985; Lands, 1995; Yeomans Caton, & Hetherington, 2003). As has been documented, despite the high caloric intake provided by alcohol, human drinkers were not more obese than non-drinkers; indeed, women drinkers had a lower body mass index than female non-drinkers. In male drinkers, findings have shown that body mass index decreased progressively as alcohol consumption increased. It has been suggested that the calories provided by alcohol function as additives in the diets of "light" drinkers, while in those who consume moderate or high amounts of alcohol the calories they ingest may replace other nutrients (Gruchow et al., 1985). In the present study, as expected, due to the restriction of 80% of body weight, food intake by the rats decreased notoriously when they were exposed to alcohol induction and responses reinforced with food, though their body weight increased during induction and the first period of alcohol administration. During these periods, the rats consumed all the food available in the experimental session (90 pellets), but received no additional food in their home cages so as to maintain body weight at around 80%.

For the three subjects in IG, the largest body weight gain occurred during the induction procedure. In the phases with alcohol and water that followed, they did not maintain that weight increase, so their body weight returned to around the 80%. Our results suggest that alcohol consumption in rats under dietary restriction that have to work to obtain food after alcohol induction results in a temporary increase of body weight, since despite the condition of food deprivation their increase in body weight was

notable. During the first period of alcohol administration, the group of NG rats showed a similar pattern of weight gain to that observed in IG; however, the weight gain in the following phases of alcohol and water was not maintained and was closer to 80%. The largest increase in body weight in these subjects was recorded during induction and the first phase of alcohol administration. Some studies involving models of oral self-administration of alcohol have reported that rats have the ability to restrain the weight loss caused by food restriction by consuming the calories provided by alcohol consumption (Rodgers, McClearn, Bennett, Hebert, 1963). One interpretation of these results might suggest that the effect of alcohol consumption on body weight in food-restricted rats that work for food and without alcohol induction was the result of the caloric intake provided by the alcohol, which allowed subjects to maintain their body weight stable despite food restriction.

Our results show intra-group consistencies, but differences between the two experimental groups. In the post-induction subjects, body weight was unsteady, while in the subjects without alcohol induction it showed an increase only during the first phase of access to alcohol before manifesting a tendency to stabilize at around 80% of their initial weight. These results suggest that in rats with oral self-administration of alcohol in home cages, the number of calories provided by alcohol might be the determining variable in the initial increase in body weight.

Food intake

Epstein and Leddy (2006) have suggested that the reinforcing value of food is affected by various factors, such as restrictions or palatability, among others. According to our results, food served as a reinforcer to press the lever for all subjects. In most sessions, the rats received 90 reinforcers and food consumption outside the experimental sessions was always dependent on the body weight obtained at the end of each one. The rats received food in their home cages in accordance with the percentage of body weight (+/- 80% of initial body weight). This condition was maintained in order to keep subjects within the percentage of body weight stipulated, and to ensure adequate deprivation so that they would press the lever for food. For this reason, both the IG and NG subjects showed decreases in food intake during the alcohol phases. Although they consumed alcohol outside the experimental sessions, all subjects had to press the lever to obtain food, thus confirming that the pellets had a reinforcing effect on that response.

Water and alcohol consumption

Animal studies have reported that subjects under dietary restriction show an increased motivation to drink alcohol at low percentages (e.g., beer alcohol at 2.7% v/v) due to the nutritious and palatable characteristics of these beverages (McGregor, Saharov, Hunt, & Topple, 1999). Our results, however, differed from initial expectations. In the IG subjects, alcohol consumption was high during induction, but only at higher concentrations (8%, 10% v/v), while at low concentrations (2%, 4% and 6% v/v) it was at the same level as water consumption during baseline. After induction, alcohol intake remained high during the first period of alcohol administration, while in the second phase it increased. In the last phase of alcohol, the pattern of alcohol consumption returned to the levels of the first phase. It has been documented that rats exposed to food restriction, but with free access to alcohol at doses of 8%, 16% or 32%, consumed large quantities at all concentrations (Stiglick & Woodworth, 1984). It has also been reported that rats which consume alcohol under conditions of food restriction continue to drink it when they are allowed to recover their body weight, though at considerably lower levels (Linseman, 1987).

For subjects without alcohol induction, alcohol consumption increased only during the second alcohol phase. In the first and third phases of alcohol, consumption did not differ among them, showing in general a consumption pattern similar to IG. These data allow us to confirm, once again, that the

energy intake of alcohol was an important factor in maintaining self-administration in food-restricted rats during the phases in which they had free access to alcohol as the only source of liquid.

Duration of sessions and reinforcers per minute

Session duration after induction to alcohol showed increases in the phases in which alcohol was the only liquid available. Subjects that received no alcohol induction showed a steady pattern during the alcohol phases, although session time was clearly lower when compared to IG. In the alcohol phases, IG decreased its rate of reinforcers per minute. During the induction period this was slower, but the rate of reinforcement was not maintained in the remaining alcohol phases and, therefore, was a temporary effect. When the alcohol concentration was 10% v/v, the reinforcement rate per minute remained constant as with the non-induction group upon its first exposure to alcohol. This decrease in the reinforcement rate per minute was confirmed in NG. The reinforcement schedule (FR)11 required the subjects to make some effort to gain the reinforcer. Clearly, when the rats drank alcohol it took them longer to accomplish 90 reinforcers within 30 minutes, which was the limit on session length. These data suggest that one effect of drinking alcohol is slow performance (Williams & Woods, 2000). The consistent performance of the control subjects that drank only water would support the idea that drinking alcohol could reduce the procuration of reinforcers over time.

Alcohol induction

Another aim of our study was to analyze the effects of using a progressive induction procedure to drink alcohol. Few studies have examined the behavioral adaptation process of operant conditioning models to facilitate the gradual increase in alcohol intake from low-to-high concentrations (Carnicella et al., 2011). The results of our study show a process of behavioral adaptation by the subjects that underwent alcohol induction compared to those that were not exposed to this procedure. The rats that were exposed to alcohol induction showed lower consumption at concentrations of 2%-6% v/v compared to concentrations of 8% and 10% v/v. Also, in the following phases with alcohol at 10%v/v they drank large amounts compared to the phases of access to water and to the non-induction group.

Based on the results of our experiment, alcohol-induced rats (IG) showed greater body weight gain due to increased alcohol consumption when compared to subjects with no previous alcohol progressive induction (NG) and (CG). The largest increases in body weight were observed precisely during the induction procedure. Subjects without alcohol progressive induction showed body weight gain in the first phase of alcohol self-administration, but in the following alcohol phases showed similar body weight to the water self-administration phases. Food intake in both experimental groups decreased during periods of access to alcohol because during these phases body weight increased, so the amount of food in the home cages was reduced to maintain body weight at around 80%. Alcohol consumption was higher for subjects with alcohol induction (IG), with larger increases in high concentrations of alcohol (8% and 10%) compared to lower concentrations (2%, 4% and 6%). Subjects without alcohol induction (NG) showed lower alcohol consumption than those in IG (see Table 1). Session duration for the subjects from IG increased in phases of alcohol self-administration; while in subjects without alcohol induction increases in time per session were not as clear.

It has been pointed out the importance of seeking to narrow basic research with clinical problems, so that experimental approaches to the phenomenon of alcohol intake can provide relevant information for the treatment of alcoholism (Marín, Jurado-Barba, Martínez-Grass, Ponce, & Rubio, 2014; Pérez Nieto, 2014). The experimental animal models have been shown to be a relevant way to investigate variables that could be effective for the treatment of human clinical problems (Gómez & Martínez, 2013).

This study was designed to provide experimental evidence of the effects of oral self-administration of alcohol outside the experimental session on the performance of rats exposed to a food reinforcement schedule, and the effectiveness of an induction procedure in propitiating high alcohol consumption and maintaining a lever-pressing behavior to obtain food. Our results suggest that the differential effects of alcohol consumption on responding (i.e., duration of sessions and reinforcers per minute) were determined by previous exposure to an alcohol induction procedure. This study focused on determining the effects of alcohol oral self-administration on subjects under conditions of food restriction. Saving the differences, using these experimental conditions, we attempt to simulate human alcohol consumption outside work and its effects on their behavior in terms of the time spent working. Based on our results, it would be interesting to determine the effects of oral self-administration of alcohol on free access in subjects without food restriction, a condition which would imply that oral self-administration of alcohol is not motivated by caloric need but by other variables that it would be useful to explore.

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