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Socially mediated alcohol preferences in adolescent rats following interactions with an intoxicated peer

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Abstract

This study focuses on "passive social influences" (alcohol-related information acquired by an organism that interacts with an intoxicated counterpart) that can potentially affect alcohol preference in adolescent rats. Five experiments were conducted to investigate whether repeated social interactions with an intoxicated peer can generate alcohol-related memories that lead an animal to exhibit heightened alcohol olfactory preference patterns. Juvenile experiences with alcohol were operationalized as follows: interactions with an alcohol-intoxicated peer (Experiment 1), with an alcohol-scented cotton surrogate (Experiment 2) or with an anesthetized alcohol-intoxicated partner (Experiments 3–5). Periadolescents were then evaluated in a two-way location olfactory test where they had the opportunity to investigate a hole scented with alcohol odor or vanilla (an odorant naturally preferred by the strain of rats here utilized). Only juveniles that interacted with an alcohol-intoxicated peer were found to exhibit a significant change in alcohol odor preferences when compared to appropriate controls that interacted with a non-intoxicated peer. Alcohol odor exposure alone or interactions with an anesthetized alcohol-intoxicated peer were not sufficient to establish changes in preference for alcohol sensory cues. Results indicate that social interactions with an intoxicated peer determine heightened preference for alcohol cues in periadolescents. The establishment of this preference seems to require behavioral manifestations of the intoxicated counterpart, instead of just being dependent on an olfactory pre-exposure to alcohol cues.

Keywords: Alcohol; Social learning; Demonstrator; Observer; Social interaction; Adolescence; Nose-poking

1. Introduction

In humans, social influences on alcohol-related behaviors and expectations have been consistently reported. These influences have been classified into two main categories (Graham et al., 1991): "active social influences" that imply the explicit offer to drink alcohol and "passive social influences" that refer to a subject's perception of the reinforcement patterns of alcohol on other peers. It has been argued that social influences (e.g. peer norms, siblings' and parental drinking patterns as well as perceived availability of alcohol) are among the strongest predictors of alcohol initiation, consumption patterns and plans of future drinking (Epstein et al., 1999a,b; Wood et al., 2001).

Animal studies appear to provide cost-effective models in the examination of neurobehavioral characteristics underlying the rewarding effects of drugs and how social and environmental stimuli modulate drug seeking and self-administration during adolescence (Spear, 2000). Yet, there are relatively few animal studies that have explicitly analyzed how social factors lead to the initiation of alcohol consumption. This social approach could be essential to understand the mechanisms that explain how the indirect perception of alcohol-related effects in an intoxicated peer can modulate latter responsiveness to the drug in young organisms. According to the human literature, active social influences are significantly associated with alcohol use and alcohol-derived problems, but passive social influences

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constitute the strongest predictors of this phenomena (Wood et al., 2001). Thus, the development of animal models related with passive social influences are particularly important when examining the mechanisms of social-mediated juvenile alcohol acceptance.

Adolescent rats exhibit specific patterns of social interactions that are markedly different from those observed in younger and older subjects. They spend more time interacting with peers when compared with other age groups and reach peak levels in terms of playing behavior. The stimuli derived from social interactions between juveniles aids in the establishment of new behaviors and skills that are essential for independence and lead to social adjustments to the demands of adulthood (van den Berg et al., 1999).

Social interaction in periadolescent rats has been considered as a reinforcer in place preference conditioning (Calcagnetti and Schechter, 1992) and maze-learning (Humphrey and Einon, 1981; Normansell and Panksepp, 1990). Furthermore, in rats as well as in other rodent species, food preferences are established via social interactions (rat: Galef and Whiskin, 2000; Galef et al., 1990, 1994; *Acomys cahirinus*: McFadyen-Ketchum and Porter, 1989; *Meriones unguiculatus*: Valsecchi et al., 1996; *Mus domesticus*: Choleris et al., 1997; *Spermophilus beldingi*: Peacock and Jenkins, 1988). These studies are based on the cultural transmission of food preferences that can either enhance the consumption or counteract an aversion to a given food. These effects are attained as a function of the interaction between an animal that has had recent access to an unusual food (demonstrator) and a naïve animal, which interacts with the former (observer). The observer is then tested to determine whether a specific affective component of the food has been transmitted or not (Galef and Wigmore, 1983; Galef and Whiskin, 2000; Posadas-Andrews and Roper, 1983).

According to Galef and Wigmore (1983) and Galef et al. (1994), the learning process generating changes in diet preference must take place in association with a conspecific even though physical contact is not necessary. Observer rats that are allowed to smell but not to have physical contact with anesthetized demonstrators still express the preference for the food eaten by the demonstrator (Galef and Stein, 1985; Galef and Wigmore, 1983; Galef et al., 1985). Observers do not exhibit changes in food preference when the odor of the diet is presented in a rat-sized cotton ball (Galef and Stein, 1985; Galef and Wigmore, 1983; Galef et al., 1985).

Social factors modulate alcohol self-administration in rats. Housing conditions as operationalized through complete, partial or null social isolation exert significant effects upon alcohol intake (Wolffgramm, 1990). Isolated rats drink more alcohol when compared with partially isolated or group-housed subjects. When focusing on group-housed animals, subordinate rats consume more alcohol than dominant ones (Wolffgramm and Heyne, 1991). Heightened alcohol consumption in isolated or subordinate rats appears to obey to antianxiety effects of alcohol that alleviate the stress originated by specific social conditions (Wolffgramm, 1990; Wolffgramm and Heyne, 1991). Under this perspective, negative rather than appetitive reinforcing effects of alcohol seem responsible in the modulation of alcohol self-administration patterns.

It has been reported that heightened alcohol consumption results from the interaction of infant (Hunt et al., 2000) and periadolescent observers (Hunt et al., 2001) with an intoxicated age peer. It is unknown whether this effect obeys to social transmission of food preferences or if the interaction with the intoxicated peer generates a stressful condition in the observer, which later utilizes the drug in order to mitigate an aversive emotional state. It seems necessary to note that alcohol strongly affects social interactions. Alcohol dosing parameters seem crucial in determining either social facilitation or inhibition (Panksepp et al., 1987; Stewart and Grupp, 1985; Varlinskaya et al., 2001). It is conceivable that the observer's perception of these pharmacologically driven effects can play a role in the emotional content of the memory acquired while interacting with the intoxicated peer.

Until now, the impact of early social interactions with an intoxicated peer on subsequent alcohol responsiveness has been evaluated using forced or voluntary intake tests (Hunt et al., 2000, 2001). In the present study, socially mediated changes in alcohol responsiveness were evaluated in periadolescent rats through the use of an olfactory preference test. The test procedure was selected to explicitly avoid positive or negative reinforcing properties of the drug during the evaluation phase. In other words, we intended to address whether social interactions with an alcohol-intoxicated peer generates specific seeking behavior of the drug without the intervention of alcohol's pharmacological properties; a strategy that should contribute clarifying the affective value of the memory that is originally established.

As will be described in detail, the first experiment in this study clearly indicated that heightened predisposition to investigate alcohol odor cues are observed in juveniles following brief interactions with a mildly intoxicated partner. Two additional experiments assessed if these odor preferences are also established through mere pre-exposure to the drug's sensory cues (Experiment 2) or through exposure to an intoxicated conspecific whose behavioral repertoire was absent due to anesthesia (Experiments 3–5). These additional experiments provide information relevant to the extent that alcohol-seeking behavior is dependent upon experience with the drug's sensory cues and/or upon ethological cues derived from the intoxicated demonstrator.

2. Experiment 1

Adolescent rats were allowed to freely interact with either an alcohol-intoxicated or a non-intoxicated peer. Intoxicated demonstrators were subjected to the effects of a

subnarcoleptic alcohol dose (1.5 g/kg), which has previously been shown to exacerbate alcohol consumption in periadolescent observers (Hunt et al., 2001). In the present study, observers were evaluated in a two-way olfactory locational test where animals could investigate alternative sections of the apparatus scented with either alcohol or vanilla odors. Preliminary work indicated that juvenile rats systematically spend more time smelling vanilla relative to alcohol. The explicit intention of this experiment was to address whether social interactions with an alcohol-intoxicated peer change this unlearned preference pattern.

2.1. Methods

2.1.1. Subjects

Twenty-four pairs of Wistar-derived periadolescent male rats resulting from 12 different litters were employed. The animals were born and reared in the vivarium at the Instituto Ferreyra. Rats were housed in standard maternity opaque cages filled with pine shavings and maintained on a 14:10-h light/dark schedule (lights on at 0700 h) and controlled temperature conditions (22–24 °C). All subjects had ad libitum access to both rat chow (Cargill, Córdoba-Argentina) and tap water delivered by automatic dispenser valves. The day of birth was considered as postnatal day 0 (PD 0). One day after delivery (PD1), litters were culled to eight pups (four males and four females, whenever possible) and they were weaned on PD 21. After winning, all littermates remain together until the beginning of the experiment. In accordance with the preceding literature (Spear, 2000; Spear and Brake, 1983), periadolescence in the rat takes place between 28 and 42 days of age. Taking this into account as well as maturational parameters corresponding to the above-mentioned strain of rats, all animals employed in the present study were 30 days of age at the beginning of the experiment. From PD 28 throughout the course of the experiment, animals of similar weight were pair-housed in maternity cages. Those pairs were always comprised by periadolescents derived from different litters. Each animal was randomly assigned to either the observer or demonstrator condition. All maintenance and experimental procedures were in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council, 1996).

2.1.2. Procedures

At PD 28, animals were pair-housed and the selected observers were assigned to one of two conditions defined by the pharmacological treatment of the demonstrator (alcohol-intoxicated or alcohol-free). Twelve observers composed each particular group. Only four males were employed from each litter and were distributed into groups as follows: one intoxicated demonstrator, one alcohol-free demonstrator, and two observers (one of them interacted with an intoxicated demonstrator and the other with an alcohol-free one). The experiment was defined by two phases, a training phase and an evaluation phase. The training phase was conducted during 4 consecutive days (days 1, 2, 3 and 4 corresponding to PDs 30, 31, 32 and 33, respectively). Animals were evaluated 24 h after conducting the last training session (day 5 or PD 34).

During each training day, animals composing the observer–demonstrator dyad were socially isolated during 60 min. During this time period, observers remained in their home cages while demonstrators were placed in individual holding cages. Thirty minutes later, demonstrator juveniles corresponding to the alcohol-intoxicated group were subjected to an intragastric (i.g.) administration of a 1.5 g/kg alcohol dose. This dose was achieved by administering 0.015 ml/g of body weight of a 12.6% v/v alcohol solution (vehicle: tap water at room temperature). Alcohol-free demonstrators received a similar volume of tap water. Following the administration procedures, all demonstrators remained in the holding chambers during a 30-min period. They were then returned to the corresponding home cage where both partners were allowed to interact during 30 min. Following this interaction, demonstrators were removed again for 4 h to ensure complete clearance of alcohol. After this time interval, they were again placed with the corresponding social partner until the following training session. Training sessions, comprising observer–demonstrator interactions, were conducted between 1000 and 1130 h.

Twenty-four hours after the last social interaction trial (day 5; PD 34), observers were evaluated in a two-way odor location test. The evaluation intended to assess alcohol odor preference through nose-poking behavior elicited by alcohol odor or by a novel odor (vanilla). The evaluation lasted for 5 min and was videotaped in order to subsequently analyze observer's responsiveness to each odorant. The apparatus utilized in the test session consisted in a black Plexiglas rectangular chamber (50×25×25 cm), which had two holes (diameter: 4 cm). These holes were positioned on the smaller opposite walls of the apparatus. The center of each hole was 4.5 cm above the floor and equidistant from the adjacent sides. A transparent Plexiglas cup (total liquid volume capacity: 16 ml; diameter: 3.4 cm) was positioned on the external side of each hole containing either an alcohol-scented (1.5 ml of 190-proff alcohol, Porta Hnos., Córdoba, Argentina) or a vanilla-scented (1.5 ml of a 0.15% v/v vanilla solution; Montreal, Córdoba, Argentina) cotton ball. The position of the odorants was counterbalanced within each particular treatment group. The rim of each cup was in direct contact with the inferior border of each hole and nose-poking allowed the animals to investigate the corresponding odorants. These cups were removable and were covered with wire mesh that precluded direct contact of the rats' body with the scented cottons. The testing chamber was positioned inside a transparent ducted acrylic hood (volume capacity: 1 m^3) equipped with an air extractor fan. All testing sessions were conducted using dim illumination conditions. The only source of illumination

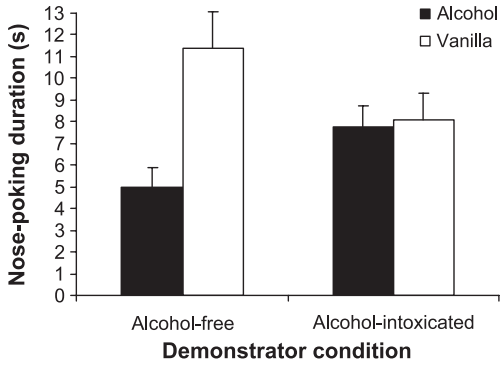

Fig. 1. Overall nose-poking duration (seconds) in the hole scented with alcohol or vanilla odor during the two-way olfactory test. In this experiment (Experiment 1), observers previously interacted with an alcohol-intoxicated or an alcohol-free active demonstrator during training (Demonstrator condition). Vertical lines represent standard errors of the mean (S.E.M.).

was a 40-W electric bulb positioned close to the floor of the room and 1 m away from the hood. Each test session began by placing the animal in the center of the chamber.

A real-time computer-based program was used to determine the duration and frequency of nose-poking behavior related with each odorant. Time spent in different sections of the apparatus was also recorded. Three sections were established. A middle section corresponding to a 20% of the entire surface of the apparatus and two olfactory sections (equivalent surfaces close to either the vanilla or the alcohol containing cups). Experimenters that recorded nose-poking behaviors or time and frequency of entries into the different sections were blind in regard with the training conditions of the animals. A nose-poke behavior was only recorded whenever the animal introduced the entire head, up to the nape of the neck, through a given lateral hole. Time spent on a given olfactory section of the apparatus was computed whenever the head and the front paws were positioned over such section.

2.2. Results and discussion

Nose-poking duration was analyzed with a two-way mixed ANOVA, which included the condition of the demonstrator as a between factor (alcohol-intoxicated or alcohol-free) and the odorants used in the test (alcohol or vanilla) as a within factor. The ANOVA indicated a significant main effect of odorant as well as a significant interaction between the factors under consideration; $F(1,22)=8.03$, $p<.01$ and $F(1,22)=6.36$, $p<.025$, respectively. Post hoc Fisher's least significant difference tests ($p<.05$) were conducted to further analyze the loci of the significant effects. Periadolescents that interacted with alcohol free demonstrators (control group) spent significantly more time smelling the vanilla odor relative to the alcohol odor, a phenomenon that is in agreement with what was observed in preliminary studies where naïve rats were utilized. This pattern of nose-poking behavior in observers exposed to alcohol-intoxicated peers was clearly different. These juveniles spent similar amounts of time investigating both odorants. Relative to observers that interacted with alcohol-free counterparts, observers trained with alcohol-intoxicated demonstrators exhibited heightened nose-poke duration in the alcohol scented hole and reduced time spent over the vanilla scented cup. These results have been depicted in Fig. 1. A similar pattern of results was obtained when a two-way mixed ANOVA (demonstrator condition×odorant) was utilized to process frequency of nose-poking behavior. The olfactory cue exerted a significant main effect while the interaction between factors also accrued significance, $F(1,22)=4.73$ and $F(1,22)=5.62$, both p's<0.05, respectively. Post hoc tests showed that nose-poke frequencies in control animals were significantly higher in the vanilla-scented hole relative to the alcohol-scented hole. Observers that interacted with an alcohol-intoxicated demonstrator exhibited similar frequencies in terms of investigating the alcohol and the vanilla scents (Fig. 2).

Time spent on the alcohol or vanilla sections as well as the frequency of entries into each section were also analyzed using a two-way mixed ANOVA (demonstrator condition×olfactory cue). This analysis failed to indicate significant effects attributable to the main factors under consideration or the interaction between them. This data has been summarized in Table 1.

In summary, animals that interacted with an alcohol-intoxicated peer were more likely to actively investigate the specific location of the apparatus where alcohol odor was present than were juveniles trained with an alcohol-free counterpart. Prior brief experiences with the intoxicated partner were sufficient to counteract the predisposition of periadolescent animals to prefer the vanilla odorant in relation to alcohol's chemosensory properties. As has been the case when utilizing alcohol intake tests (Hunt and Hallmark, 2001; Hunt et al., 2001), it appears that juvenile experience with alcohol cues derived from social inter-

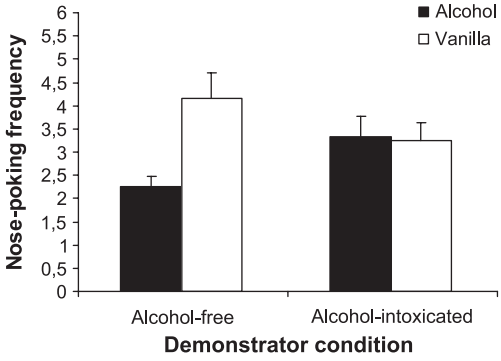

Fig. 2. Nose-poking frequencies showed by observer animals during the two-way olfactory test (alcohol vs. vanilla) in Experiment 1. Demonstrator condition represents whenever the observer interacted with an alcohol-intoxicated or an alcohol-free active demonstrator during training phase. Vertical lines represent standard errors of the mean (S.E.M.).

Table 1
Frequencies and time spent by observers in each section of the apparatus during the evaluation phase

Experiment	Demonstrator condition	Entries into the olfactory section		Time in the olfactory section (s)	
		Alcohol	Vanilla	Alcohol	Vanilla
1	Alcohol-intoxicated (1.5 g/kg)	11.17±0.88	10.33±0.71	131.29±13.05	114.82±10.87
	Alcohol-free	10.50±0.84	10.25±0.69	123.97±12.81	118.80±12.82
2	Alcohol-scented cotton surrogate	08.00±0.70	07.83±0.41	114.09±17.15	124.82±18.85
	Alcohol-free cotton surrogate	08.92±0.73	10.58±0.68	100.90±11.77	136.69±14.34
3	Alcohol-intoxicated (1.5 g/kg+Ket)	10.00±0.46	10.25±0.81	122.70±13.87	112.79±10.53
	Alcohol-free (Ket)	09.92±0.73	11.00±0.82	128.58±12.83	112.79±12.44
5	Alcohol-intoxicated (2.0 g/kg+Ket)	7.44±1.09	8.33±0.76	87.57±10.80	167.11±18.57
	Alcohol-free(Ket)	8.89±0.92	7.89±0.54	100.28±11.51	144.82±11.74

The table shows mean values±standard errors of the mean (S.E.M.) for each behavioral experiment, as a function of the demonstrator condition. "1.5 g/kg" and "2.0 g/kg" refer to the alcohol dose administered to the demonstrator 30 min before social interactions with observer. "Ket" represents the anesthesia administered to the demonstrator (80 mg/kg of ketamine+13.5 mg/kg of xylazine) previous to training sessions.

actions can facilitate alcohol recognition and choice behavior.

3. Experiment 2

The main goal of this experiment was to assess if juveniles will also change their behavioral repertoire in response to alcohol odor when previously exposed to the smell of the drug in the absence of a social partner. According to prior studies, mere exposure to chemosensory cues of a given diet is not as effective as when the sensory experience occurs within a social context in terms of modulating subsequent choice behavior (Galef, 1989; Heyes and Durlach, 1990). Yet, it is important to note that heightened alcohol acceptance patterns have been observed in preweanling and weanling rats following alcohol odor exposure (Bannoura et al., 1998; Molina et al., 1984). In light of these considerations and as a function of the results obtained in Experiment 1, we examined the effects of mere alcohol olfactory exposure upon juvenile olfactory choice behavior utilizing similar training procedures as those employed in the previous experiment. As will be described, these sensory experiences were conducted while explicitly avoiding the presence of alcohol odor in a social context.

3.1. Methods

3.1.1. Subjects

Twenty-four pairs of Wistar-derived periadolescent male rats were employed. Those animals were extracted from 12 different litters. Genetic and housing conditions of these animals were similar to those reported in Experiment 1. At PD 28, periadolescents were pair-housed, trying to maintain similar body weights between partners. Housing conditions remained unaltered until PD 30 when training began.

3.1.2. Procedures

One animal representative of each pair of subjects was randomly assigned to one of two conditions defined by the nature of the olfactory training procedure (alcohol-free or alcohol-scented cotton roll). Each dyad had animals representative of different litters. During the training phase, these animals were exposed to a cotton roll used as a surrogate demonstrator. The size of the cotton roll was similar to the one of a male periadolescent rat (10 cm long×5 cm diameter) and it was entirely covered with gauze in order to avoid alterations in its original shape. Each roll was only used once during the training phase. Twelve observers were trained with an alcohol-scented roll while 12 additional observers were exposed to an alcohol-free cotton surrogate. With the exception of the use of cotton rolls rather than biological demonstrators, all the procedures employed during the training and evaluation phase replicated those described in Experiment 1.

During each training day (days 1–4; PDs 30–33, respectively) partners were socially isolated during 60 min. During this deprivation procedure, selected observers remained in their home cages while their pair-mates were positioned in individual holding cages. After this isolation period, cotton surrogates containing alcohol (1.5 ml of 190-proof alcohol) or an equivalent volume of water were placed in the cages with the observers. Alcohol or water was carefully injected in the middle internal section of the cotton roll ensuring that liquids were not in direct contact with the gauze cover. Each observer was exposed during 30 min to the corresponding surrogate demonstrator. Four hours after surrogate removal, the biological partners were again reunited in the home cages.

Observer's behavioral responsiveness to alcohol and vanilla scents was evaluated using similar apparatus, procedures and dependent variables as those described in Experiment 1.

3.2. Results and discussion

Data from this experiment (nose-poke duration and frequency) has been depicted in Table 2. From a descriptive perspective, it appeared that, in the case of nose-poking duration, the overall profile of the results was similar to the one obtained in the previous experiment. This general impression was not supported by pertinent inferential

Table 2
Overall durations and frequencies of nose-poking showed by observers during the two-way olfactory test

Experiment	Demonstrator condition	Nose-poking duration (s)		Nose-poking frequency	
		Alcohol	Vanilla	Alcohol	Vanilla
2	Alcohol-scented cotton surrogate	5.54±1.15	7.58±1.87	2.83±0.32	4.08±0.85
	Alcohol-free cotton surrogate	3.47±0.67	8.44±1.58	2.33±0.33	3.92±0.73
3	Alcohol-intoxicated (1.5 g/kg+Ket)	4.47±1.37	6.30±1.67	2.92±0.67	2.67±0.57
	Alcohol-free (Ket)	4.34±1.03	6.22±1.16	2.00±0.25	2.67±0.43
5	Alcohol-intoxicated (2.0 g/kg+Ket)	4.30±0.54	6.92±1.16	3.33±0.17	4.67±0.83
	Alcohol-free(Ket)	3.43±1.09	6.70±0.77	2.33±0.67	4.22±0.62

All scores correspond to mean values±standard errors of the mean (S.E.M.) for behavioral experiments 2, 3 and 5, as a function of the demonstrator condition. "1.5 g/kg" and "2.0 g/kg" are the alcohol doses utilized to administer demonstrator rats 30 min before social interactions with their respective observers partner. "Ket" represents the anesthesia administered to the demonstrator (80 mg/kg of ketamine+13.5 mg/kg of xylazine) previous to training sessions.

analysis. Nose-poking parameters were analyzed through two-way mixed ANOVAs where the olfactory nature of the cotton roll (alcohol-scented or alcohol-free) represented the between factor and the odorants at test (alcohol and vanilla) represented the within factor. The ANOVAs only indicated significant main effects of odorant upon nose-poking duration [$F(1,22)=7.30$, $p<.025$] as well as upon nose-poking frequency [$F(1,22)=7.30$, $p<.025$]. Observers spent significantly more time nose-poking in the hole scented with vanilla relative to the hole scented with alcohol (Table 2). Similar effects were encountered when assessing nose-poking frequency (Table 2). Both dependent variables were unaffected by the cotton roll training condition or by the interaction of the factors under consideration (both $p>.2$).

Time spent over the sections of the test chamber proximal to the lateral holes and frequency of entries into each section were also subjected to two-way mixed ANOVAs (cotton roll condition×odorant at test). These analyses failed to reveal significant effects attributable to the main factors under consideration or the interaction between them. Data corresponding to these dependent variables is shown in Table 1.

Under the present experimental circumstances, exposure to alcohol odor in the absence of a social partner was not sufficient to change the predisposition of periadolescents to investigate this odorant when concurrently presented with a vanilla scent. As observed in preliminary experiments as well as in alcohol-naïve controls employed in Experiment 1, juveniles appear to show a predisposition to prefer vanilla odor rather than alcohol odor. This behavioral pattern was also encountered in rats that were trained in the present experiment with an alcohol-scented cotton roll. Although it appeared that observers exposed to an alcohol-scented cotton were more likely to explore alcohol odor during test than juveniles trained with an unscented cotton, the analysis of the data failed to reveal a significant interaction between training and odorants present in this test. Interestingly, in this study, the olfactory stimulation procedure employed during training and testing was very similar. In both phases, the experimental group was confronted with an equivalent volume of alcohol that served to scent cotton. Hence, the lack of change in alcohol responsiveness across groups in the present experiment strongly suggests that mere pre-exposure to alcohol cues fails to represent the sole factor regulating socially induced alcohol preferences as those reported in Experiment 1.

4. Experiment 3

According to the preceding experiments, changes in alcohol responsiveness were observed only when periadolescents interacted with an intoxicated counterpart (Experiment 1). Mere pre-exposure to alcohol odor was not sufficient to generate a significant change in how juveniles later investigated this sensory cue (Experiment 2). To further analyze how observers obtain information from a given demonstrator, in the present experiment we examined whether changes in alcohol responsiveness will also occur whenever observer juveniles interact with an alcohol-intoxicated partner that is totally inactive. Specifically, demonstrator rats in this experiment were subjected to similar alcohol treatment as the one employed in Experiment 1 but when placed with the corresponding observer they were under the effects of anesthesia. Prior studies examining social transmission of food preferences have indicated that an unconscious demonstrator emits olfactory signals sufficient to affect observer's subsequent food choices (Galef, 2001; Galef and Wigmore, 1983). According to this information, it should be expected that the presence of a behaviorally passive demonstrator would exert similar effects upon alcohol preferences in observer animals as those detected in Experiment 1. In other words, the presence of alcohol in a social context, independently from the behavior of the intoxicated sibling, should suffice to affect subsequent responsiveness towards alcohol in periadolescent observers.

4.1. Methods

4.1.1. Subjects

Twenty-four pairs of Wistar-derived periadolescent male rats were used. Genetic and housing conditions of these rats replicated those reported for the preceding experiments. At PD 28, rats were pair-housed and randomly assigned to an observer or demonstrator condition. Efforts were made to

keep similar body weights between partners. The day of birth was considered as PD 0 and culling and weaning procedures were the same as the ones used in Experiments 1 and 2. Animals were 30 days of age at the beginning of the experiment.

4.1.2. Procedures

All procedures were similar to those executed in Experiment 1 except for the fact that demonstrators were under the effects of anesthesia during the social interaction training trials (days 1, 2, 3 and 4; PDs 30, 31, 32 and 33, respectively). Alcohol-naïve observers were randomly assigned to one of two groups defined by the nature of the treatment applied to the corresponding demonstrator; i.e. alcohol-intoxicated (n=12) or alcohol-free (n=12) partner. After two days (PD 28 and 29) of pair-housing, training sessions began (PD 30). Both animals were socially isolated for 60 min by removing the demonstrator from the home cage. During this hour, demonstrators in the alcohol-free group were subjected to an i.g. administration of tap water, while those in the alcohol-intoxicated group were administered a 1.5 g/kg alcohol dose. Fifteen minutes later, all demonstrators were subjected to an intraperitoneal injection of an 80 mg/kg dose of ketamine HCl (Vetanarcol, König; Buenos Aires, Argentina) supplemented with 13.5 mg/kg of xylazine HCl (Kensol, König; Buenos Aires, Argentina). Each demonstrator remained in the individual cage for 15 additional minutes following anesthetic procedures. As was the case in Experiment 1, demonstrators were returned to the home cage 30 min following alcohol administration. During each training trial, observers were allowed to interact during 30 consecutive minutes with the corresponding anesthetized demonstrator. Direct observation of the interaction of the observers with the demonstrator indicated that the former animals were highly active in terms of sniffing the mouth and perioral regions and stayed during a considerable amount of time keeping physical contact with the social partner. After this period of time, demonstrators were removed again for 4 h. At this point in time and in accordance with pilot experiments, all demonstrators were completely recovered from the effects of anesthesia. Observers and demonstrators remained together until the next training day. Twenty-four hours after the last social interaction trial (day 5; PD 34), observers were evaluated using similar apparatus, scents, procedures and dependent variables as those employed in the previous experiments.

4.2. Results and discussion

As indicated by a two-way mixed ANOVA (demonstrator condition×odorant), nose-poking duration was significantly higher in the hole scented with vanilla relative to the hole scented with alcohol, $F(1,22)=4.83$, $p<.05$. The interaction between demonstrator condition and odorant failed to significantly affect nose-poking duration. These results have been depicted in Table 2. No significant effects were observed when frequency of nose-poking was the variable under consideration (Table 2).

Time spent in the olfactory sections of the test chamber was analyzed via a two-way mixed ANOVA (demonstrator condition×odorant). This analysis did not show significant main effects of the factors under examination or of the interaction between them. Similar null effects were observed when number of entries into each particular section of the apparatus was analyzed (see Table 1).

In accordance with the control group of Experiment 1 and both groups of Experiment 2, all subjects in the present experiment spent significantly more time investigating the vanilla odor in relation with the alternative olfactory cue (alcohol). This behavioral pattern was not affected by prior interactions with an alcohol-intoxicated counterpart, which was also anesthetized during the training sessions. Frequency of nose-poking, time spent and number of entries in the olfactory sections of the testing chamber were also unaffected by the nature of the training procedure and/or the odorants presented at test. These findings suggest that, under the present experimental circumstances, the presence of a behaviorally passive conspecific treated with alcohol is not sufficient to promote the expression of an alcohol-related memory in juvenile observers as the one observed in Experiment 1. Apparently, changes in alcohol reactivity in periadolescent observers are not observed whenever the behavioral repertoire of demonstrator organisms is completely absent. Yet, the absence of ethological cues provided by demonstrator subjects might not represent the sole factor that impedes social transmission of alcohol olfactory preferences. For example, we ignore if anesthesia alters alcohol's pharmacokinetics, a factor that in turn can imply differences in alcohol direct elimination through expired air or saliva of demonstrator rats. As will be observed, the following experiments pursued the intention of assessing such a possibility.

5. Experiment 4

As indicated, alcohol olfactory preferences were established in observer rats after interacting with an active alcohol-intoxicated demonstrator (Experiment 1) but not with an intoxicated counterpart under the effects of anesthesia (Experiment 3). Alcohol olfactory information is likely to be acquired due to nonmetabolic elimination of alcohol through expired air of the demonstrator animal. In rats, there exists a near perfect positive correlation between blood alcohol levels and those attained in expired air (Hiltunen et al., 1989; Pohorecky and Brick, 1982). Furthermore, a recent study conducted with the same strain of rats as the one utilized in the present study showed that adults treated with ketamine's congener, MK 801, exhibit lower blood alcohol levels (BALs) than when treated with alcohol alone (Manzini et al., 2003). This effect coincides with the results of a preliminary study in which we observed

that BALs in juvenile rats under the effects of ketamine were lower than the ones recorded in unanesthetized rats treated with similar alcohol dose (1.5 g/kg). Based on these observations, in the present experiment, BALs were determined in alcohol-intoxicated periadolescents when these animals were under the effects of ketamine anesthesia or unanesthetized. Two alcohol doses were employed, 1.5 or 2.0 g/kg. The intention was to: (a) explicitly assess BALs when administering a similar alcohol dose as the one used in Experiments 1 and 3; and (b) verify whether a higher alcohol dose (2.0 g/kg) in anesthetized rats would yield comparable alcohol levels relative to the ones encountered in adolescents subjected to the lower alcohol dose (1.5 g/kg) but free from ketamine's effects. The higher alcohol dose was selected in accordance with the above mentioned preliminary studies. In summary, the main goal was to determine whether there are differences in BALs in accordance with the pharmacological treatment of the animals that can be indicative of differential alcohol levels in expired air. If such was the case, the need exists to re-evaluate alcohol olfactory learning derived from an anesthetized peer. The use of a higher alcohol dose to attain similar BALs (and corresponding levels of excretion) as those found in an active alcohol-intoxicated peer should help clarify if appropriate sensory stimulation derived from an inactive partner is sufficient to establish changes in alcohol preferences.

5.1. Methods

5.1.1. Subjects and procedures

Twenty-six Wistar-derived periadolescent male rats were employed. Genetic and housing conditions of these animals replicated those reported for the preceding experiments. Twenty-four hours before sampling BALs, periadolescents were subjected to a surgical procedure aimed at placing a catheter in the right jugular vein. This surgery allowed serial blood sampling during the course of the ethanol toxic process. Animals were anesthetized via ether anesthesia. An incision was then made in the ventral portion of the neck and a catheter (filled with heparin diluted in physiological saline) was inserted into the jugular vein until reaching the cardiac atrium. The catheter was kept in this position by means of a suture procedure to maintain its attachment to the sterno-cleido-mastoid muscle. The free end of the catheter was inserted subcutaneously until it reached the dorsal side of the neck. A small incision was performed in order to express the free end of the cannula and attach it, with one suture stitch, to the skin. To diminish the possibility of post-surgical nociception, a subcutaneous administration of 0.03 mg/kg of buprenorphine HCl (Temgesic, Schering-Plough; Buenos Aires, Argentina) was employed as an analgesic agent.

The following day, rats were randomly assigned to one of three treatments. A first group was only treated with a 1.5 g/kg alcohol dose (n=9). A second group received a 1.5 g/kg alcohol dose and ketamine (n=9) while a third group was subjected to a 2.0 g/kg alcohol dose and ketamine (n=8). As can be observed, the first group of subjects was treated in a similar way as alcohol-treated juveniles employed in Experiment 1. In the remaining groups, experimental procedures were similar to the ones employed in Experiment 3.

Blood samples (100 μl) were collected at each of three alcohol postadministration times (30, 60 and 300 min). Administration and sampling procedures took place between 1000 and 1200 h. Postadministration times were selected on the basis of the experimental procedures described in the preceding experiments where observers were exposed to the intoxicated demonstrator partner 30–60 min after the later animal was intubated with alcohol. BALs at postadministration time 300 min were also recorded since in Experiments 1 and 3 demonstrators were again reunited with the corresponding observers.

Blood samples were subjected to head-space gas chromatography (Hachemberg and Schmidt, 1985; Molina et al., 1992). Samples were placed in microvials (total volume capacity: 700 μl) equipped with a rubber stopper. Each vial was placed on crushed ice to avoid ethanol vaporization. For assessment of BALs, samples were kept in a water bath at 60 °C for 30 min. Gas-tight syringes (Hamilton; Reno, NV, 10 μl) were used to collect the volatile component of the samples and to inject them into the gas chromatograph (Hewlett-Packard, Model 5890; Palo Alto, CA). Column (Carbowax 20 M; 10 m×0.53 mm×1.33 mm film thickness). Oven, injector and detector temperatures were as follows: 60, 150 and 250 °C, respectively. Nitrogen served as the carrier gas (flow rate: 15 ml/min). BALs were computed using linear regression analysis of known standards. Twenty microliters of butanol (52 mg/dl) was added to each blood sample to provide an internal standard control. BALs were expressed as mg of ethanol per dl of body fluid (mg/dl=mg%).

5.2. Results

Complete absence of alcohol in blood was observed in all animals at 300 min postadministration time. Reliable blood alcohol levels were encountered 30 and 60 min following alcohol administration. A two-way mixed ANOVA (treatment×time) was utilized to analyze BALs. This analysis yielded a significant main effect of pharmacological treatment [$F(2,23)=9,70$; $p<.001$] as well as of postadministration time [$F(1,23)=6,61$; $p<.025$]. The variables under consideration failed to significantly interact. Across treatments, BALs were significantly higher at 30 min in comparison with those recorded at 60 min. In turn, ketamine free rats and those exposed to 2.0 g/kg alcohol and ketamine had significantly higher BALs than did anesthetized animals treated solely with the 1.5 g/kg

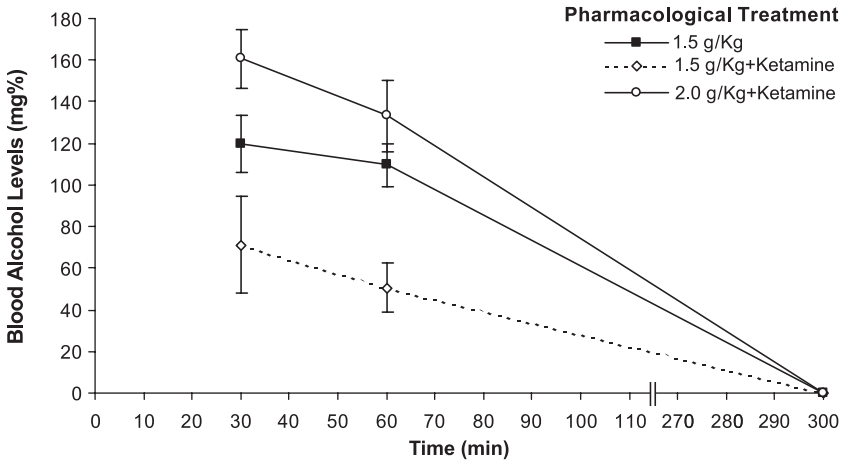

Fig. 3. Blood alcohol levels (mg%) registered at 30 and 60 min. postadministration times during Experiment 4. Periadolescent male rats were under tree different pharmacological treatments resulted from intragastric administrations of: 1.5 g/kg alcohol dose, 1.5 g/kg alcohol dose supplemented with 80 mg/kg of ketamine+13.5 mg/kg of xylazine, and 2.0 g/kg alcohol dose added with 80 mg/kg of ketamine+13.5 mg/kg of xylazine. Vertical lines represent standard errors of the means (S.E.M).

alcohol dose. No other significant differences were observed (Fig. 3).

6. Experiment 5

Based on the results of the previous experiment, it was decided to assess changes in alcohol olfactory responsiveness in subjects that under the effects of ketamine anesthesia show similar BALs as the ones encountered in active juveniles exposed to a 1.5 g/kg alcohol dose. Following this goal, observers had the possibility of interacting with either anesthetized peers administered with a 2.0 g/kg or with alcohol-free animals that also were unconscious due to the effects of ketamine. This experimental strategy intends to maximize the possibility of adequate sensory stimulation provided by the demonstrator animal, which lacks an active behavioral repertoire.

6.1. Methods

6.1.1. Subjects and procedures

Eighteen Wistar-derived periadolescent males were utilized. Experimental procedures, with the exception of the alcohol dose, replicated those employed in Experiment 3. During social interaction trials, demonstrators were under the effects of anesthesia exerted by ketamine supplemented with xylazine. Alcohol-naïve observers were randomly assigned to one of two groups defined by the nature of the treatment applied to the corresponding demonstrator; i.e. alcohol-intoxicated (alcohol dose: 2.0 g/kg; n=9) or alcohol-free (n=9) partner. The 2.0 g/kg alcohol dose was achieved by administering 0.015 ml/g of body weight of a 16.8% v/v alcohol solution (vehicle: tap water at room temperature). Alcohol-free demonstrators received a similar volume of tap water. Hence, the volume of administration of the alcohol solution and of water were equivalent to those utilized in the preceding experiments. Following four sessions of social interactions, observers were tested in the same odor location test as the one employed in the previous behavioral experiments.

6.2. Results

A two-way mixed ANOVA (demonstrator condition×odorant) showed that the only factor that significantly affected nose-poking duration was the nature of the olfactory cue at test. Independently from the demonstrator condition, periadolescents spent more time investigating the hole scented with vanilla relative to the hole scented with alcohol [$F(1,16)=18,12$; $p<.001$]. A similar main effect of odorant was also accrued when focusing on nose-poking frequency [$F(1,16)=11.52$; $p<.005$]. These results are depicted in Table 2.

Animals in this experiment were also found to spent significantly less time over the section of the apparatus close to the hole scented with alcohol relative to section proximal to the vanilla scented hole [$F(1,16)=12.05$; $p<.005$]. Demonstrator condition as well as the interaction between this factor and odorant at test failed to reach significant levels. In terms of the number of entries into each particular section, the interaction between the factors under consideration approached significance [$F(1,16)=3.52$; $p=0.08$]. Observers exposed to intoxicated demonstrators that were under the effects of anesthesia showed higher frequencies of entries into the vanilla section relative to the alcohol section. Duration spent on each section and frequency of entries have been summarized in Table 1.

The basal preference for vanilla odor showed by control animals in this experiment is in agreement with the results observed in previous experiments (Experiments 1, 2 and 3). Additionally, as in Experiment 3, interacting with an

alcohol-intoxicated counterpart under the effects of anesthesia was not sufficient to enhance the relative preference for alcohol odor.

In summary, the strategy of increasing the alcohol dose applied to the anesthetized demonstrator as a means of providing adequate sensory stimulation to the observer rat did not result in heightened predisposition to investigate the odorant, as was the case in Experiment 1. In conjunction with the results of Experiment 2 (where rats were only exposed to an alcohol-scented surrogate), the experiments with an anesthetized demonstrator indicate that social learning about alcohol not only requires sensory information about the drug but also ethological cues provided by the intoxicated demonstrator.

7. General discussion

The results of the present study seem to be in agreement with prior research focusing on social transmission of alcohol-related information during periadolescence (Hunt and Hallmark, 2001; Hunt et al., 2001). In the present study, alcohol-related information arising from the interaction with a moderately intoxicated peer was sufficient to modify subsequent responsiveness towards alcohol-related cues. Specifically, heightened predisposition to actively investigate alcohol's olfactory cues was encountered whenever juveniles had brief experiences with a peer subjected to a subnarcoleptic alcohol dose (Experiment 1). Neither mere exposure to alcohol olfactory cues (Experiment 2) nor interactions with an alcohol-intoxicated unconscious demonstrator (Experiments 3–5) were able to promote heightened alcohol odor investigation as observed when juvenile males interacted with an active peer subjected to a moderate state of intoxication.

Social transmission of food preferences has been studied in different species and there is no doubt that it represents a powerful determinant of food choice in young and adult organisms (e.g. Choleris et al., 1997, 1998; Galef, 2001; Galef and Wigmore, 1983; Solomon et al., 2002; Valsecchi et al., 1996). The results related with social transmission of food preferences not necessarily predict what happens in the case of social experiences comprising drugs of abuse such as alcohol. It is impossible to rule out that alcohol, aside from its orosensory components, has pharmacological effects that can substantially modify not only the behavior of the intoxicated rat but also the nature of social interactions. Studies focused on alcohol intake as a resultant of prior social experiences with an intoxicated peer do not seem sufficient to explain the mechanisms that guide a subject to exhibit heightened alcohol intake. Altered social interactions can represent a stressful stimulus leading to self-administration of alcohol, a drug known to exert anxiolytic effects. Animals with high anxiety levels or aversive emotional states show proclivity to self-administer alcohol (Boyd et al., 1989; Pohorecky, 1981; Stewart et al., 1993). The anxiolytic effects of the drug are also encountered when the stressor is operationalized through a variety of social situations (Blanchard et al., 1993a,b; Tornatzky and Miczek, 1995). It was our explicit intention to minimize this pharmacological component of the drug through the use of an olfactory preference test rather than intake assessments in adolescents previously exposed to alcohol in a social context. Under this perspective, the overall pattern of results here reported indicates that sensory experience with alcohol is necessary, but not sufficient, to modulate subsequent alcohol seeking behavior.

It appears critical for the establishment of heightened alcohol seeking behavior that both members of the observer–demonstrator dyad can actively interact. Even when trying to maximize sensory exposure through increases in the alcohol dose applied to an anesthetized demonstrator, we were unable to achieve behaviors indicative of heightened predisposition to accept alcohol odor in the observer (Experiments 4 and 5). The possibility exists that ketamine–xylazine anesthesia can cause respiratory depression when using similar doses as the ones here employed (Komulainen and Olson, 1991; Wixson et al., 1987). A reduction in the breathing rate of the anesthetized demonstrator could imply less alcohol non-metabolic elimination. As a consequence, observers would be less likely to detect alcohol odor in the intoxicated partner. It is also interesting to note that Hunt et al. (2001) have found that a high alcohol dose (3.0 g/kg; probably narcoleptic as described by the authors) completely fails to increase alcohol intake patterns as does a markedly lower dose (1.5 g/kg). Apparently, complete absence of the behavioral repertoire of the intoxicated organism (due to alcohol-induced narcolepsy in Hunt's study, 2001, and anesthesia in the present study) do not permit the establishment of heightened alcohol acceptance patterns in juvenile observers.

Furthermore, explicit comparison of overall nose-poking duration (time spent nose-poking on alcohol and vanilla scented holes) across behavioral experiments seems to indicate that the absence of ethological cues markedly diminishes subsequent olfactory exploration of observer rats during the test [overall means±S.E.M.: Experiment 1 (active demonstrator), 16.11±1.27 s; Experiment 2 (surrogate demonstrator), 12.52±1.45 s; Experiment 3 (1.5 g/kg alcohol plus anesthesia), 10.66±1.64 s and Experiment 5 (2.0 g/kg alcohol plus anesthesia), 10.67±1.08 s]. It seems prudent to take into account that rats avoid chemosensory cues previously associated with a poisoned partner. Poisoning is frequently operationalized through the use of unconditioned stimuli capable of generating conditioned chemosensory aversions (e.g. lithium chloride: Coombes et al., 1980; Revusky et al., 1982). Ketamine can serve as an effective unconditioned stimuli that mediates conditioned chemosensory aversions. It has also been reported that mere exposure to a poisoned partner is sufficient to generate rejection of novel substances in the observer rat. This effect

seems to respond to generalized neophobia as a function of prior interactions with the poisoned peer. This nonassociative learning effect has been detected when testing conditions are defined by voluntary selection of substances characterized by novel chemosensory attributes (Hishimura, 2000). Hence, it seems prudent to accept the possibility that overall decrements in nose-poking behavior observed in Experiments 3 and 5 can obey to observer's generalized neophobia towards the odorants available in the two-way location test. Despite this explanation, other factors (e.g. seasonal changes in spontaneous or elicited activity related with the fact that the experiments were conducted sequentially) could also help to explain changes in olfactory reactivity across studies.

Short- and long-term alcohol odor preferences have been observed in preweanling rats after only being acutely or chronically exposed to the scent of the drug. This has not been the case for the adolescents here employed (Experiment 2). Changes in alcohol preference in observers were only found after interactions with demonstrators treated with a 1.5 g/kg alcohol dose. This dose falls within the range of alcohol doses that do not inhibit social activity (Varlinskaya et al., 2001). Social interactions during adolescence reach peak levels in comparison with younger and older age groups (Spear, 2000) and are known to be rewarding under different experimental conditions (Burgdorf and Panksepp, 2001; Calcagnetti and Schechter, 1992). The preceding observations in conjunction with the pattern of results here reported suggest that juveniles encode alcohol sensory information associated with ethological cues provided by peers. In other words, an alcohol-intoxicated demonstrator does not merely act as a passive carrier of alcohol-related cues but rather can represent, in terms of associative learning, a reinforcer capable of establishing the hedonic content of the acquired memory.

Among others, ethological cues that could be emitted by demonstrators and sensed by observers are changes in ultrasound vocalizations and/or changes in the behavioral repertoire of the dyad produced by the drug. Adult rats do not appear to be sensitive to alcohol doses equivalent to 0.6 and 1.2 g/kg in terms of modifying ultrasonic vocalizations when exposed to an inactive same-sex conspecific (Blanchard et al., 1993b). Even when utilizing high alcohol doses (4.0 g/kg), periadolescents (35 days of age) during the phase of alcohol hangover do not show altered patterns of ultrasound emissions (Brasser and Spear, 2002). This result seems to rule out the possibility that, following social training trials as those here utilized, observers are stimulated with altered vocalizations of demonstrators experiencing recovery from alcohol's postabsorptive effects. In terms of the behavioral repertoire of adolescents exposed to an intoxicated peer, the subnarcoleptic dose here employed does not seem to strongly impact upon different social behaviors of Sprague–Dawley juveniles (Varlinskaya et al., 2001). Yet, it should be noted that adolescent Wistar-derived rats reared in our colony exhibit heightened locomotion during the initial phase of the toxic process (Duarte, 2002) and proclivity to establish more social contacts than those recorded when using a sober demonstrator (Fernández-Vidal, Spear and Molina, unpublished data). These effects have been encountered when using the same alcohol dose (1.5 g/kg) as the one here employed.

The prolific work of Galef et al. (1988) has indicated that carbon disulfide (CS_2), normally present in the rat's breath, participates in socially induced food preferences. The association between a novel substance and this volatile sulfur-containing semiochemical, as a mechanism that explains alcohol preferences as those here observed, seems unlikely in light of the null effects observed when employing anesthetized demonstrators (Experiments 3 and 5). To our knowledge, there is no available data suggesting that ketamine–xylazine affects CS_2 excretion in rats or that these agents produce salient olfactory cues that could mask or change the perception of alcohol odor eliminated by an intoxicated rat. Obviously, further studies are required to establish the relevance of possible chemosensory signals and/or behavioral changes in demonstrator rats that can give rise to social-mediated alcohol memories.

The effects of social interactions comprising alcohol upon ethanol ingestion in periadolescent rats have been assessed with tests conducted immediately after termination of a training session (Hunt et al., 2001). Recently, there have been indications that distributed trials can promote long-term retention of infantile experiences with an intoxicated peer that modulate voluntary ethanol ingestion 24 h or 6 days following such training trials (Hunt and Hallmark, 2001). Our results also indicate that periadolescents are capable of expressing alcohol odor-related memories 24 h following distributed training sessions (Experiment 1).

Abundant experimental as well as epidemiological research has indicated that early experiences with alcohol exert profound effects on later responsiveness to the drug (Baer et al., 1998, 2003; Bachmanov et al., 2003; Chotro and Arias, 2003; Dominguez et al., 1998; López and Molina, 1999; Spear and Molina, 2001; Yates et al., 1998). There are numerous reports that show how early memories about alcohol arise through specific social interactions and are capable of modulating short- and long-term alcohol seeking and consumption behaviors. For example, infantile experiences with an intoxicated dam within the nursing context generate specific memories concerning the sensory nature of the drug and predispose the organism to self-administer this pharmacological agent (Molina et al., 2000; Hunt et al., 1993; Pepino et al., 1998, 1999). According to the present results and those derived from Hunt and colleagues' work (Hunt et al., 2000, 2001), infants and adolescents exposed to intoxicated conspecifics acquire specific information that regulates subsequent alcohol acceptance and preference patterns. Early onset of alcohol use is one of the strongest predictors of later alcohol dependence (Grant, 1998). Hence, it is certainly critical to develop animal models to better understand mechanisms

that lead a young organism to initiate alcohol intake. Under this perspective, this study, as well as recent investigations (Hunt et al., 2000, 2001) focused on socio-environmental influences on initial reactivity to alcohol, might help explain particular circumstances that can determine alcohol seeking and consumption behaviors and/or reactivate previously acquired memories concerning this drug.

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