Early social isolation increases persistence of alcohol-seeking behavior in alcohol-related contexts

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Social conditions during rearing are well known to affect adult alcohol consumption, but few experiments have explored the effects of social conditions on behaviors that are related to alcohol dependence, such as the persistence of alcohol seeking. This study compared the effects of isolation (ISO) and interaction (INT) rearing on the persistence of alcohol-seeking behavior. Rats were trained to lever press for a solution of 10% alcohol diluted in water. They were then exposed to a two-component multiple schedule of reinforcement (baseline). Responses in one component were reinforced by a higher rate of alcohol delivery (rich component, variable interval 15 s) and responses in the other component were reinforced by a lower rate of delivery (lean component, variable interval 45 s). The persistence of lever pressing in the presence of each stimulus was then assessed during extinction. The results from baseline showed that response rates in rats in both groups were higher in the rich component than in the lean component, but ISO rats responded significantly more than INT rats in both components. The persistence of responding during extinction in ISO rats in both components was also higher than that in INT rats. The results show that effects of ISO are not restricted to alcohol consumption, but also affect persistence of alcohol-seeking behavior, which may reflect differences in the value of drug-related stimuli. Behavioural Pharmacology 00:000–000

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Behavioural Pharmacology 2016, 00:000–000

Keywords: alcohol seeking, behavioral momentum theory, operant behavior, rat, social isolation

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Received 7 October 2015 Accepted as revised 16 December 2015

Introduction

The persistence of drug-seeking behavior is a central feature of substance abuse. Several attempts have been made to determine the biological and environmental variables that can determine persistent drug-seeking behavior (Mcisch, 2000; Jimenez-Gomez and Shahan, 2007). Laboratory research indicates that contextual stimuli that are paired with drug intake affect the persistence of drug-seeking (Bienkowski et al., 1999; Maccioni et al., 2007). For example, rodent studies that have used various drugs, such as morphine, alcohol, and cocaine, have shown that subjects that are previously trained to self-administer drugs reinstate drug-seeking behavior after long periods of extinction when presented with stimuli that were associated with drug intake, but not when presented with unrelated stimuli (Bienkowski et al., 2000). Notably, drug-seeking behavior in the presence of drug-related stimuli can persist over several sessions without being followed by drug exposure and after long periods of nonconsumption (Weiss et al., 2001).

The persistence of drug-seeking may be explained by behavioral momentum theory (BMT), which provides a framework with which to understand the persistence of operant behavior and its relationship with stimulus contexts (Nevin and Shahan, 2011). In general terms, BMT posits that the persistence of any behavior is the product of Pavlovian relationships between environmental contexts where a behavior occurs and the rate, amount, or quality of the reinforcers that are delivered in that context. Accordingly, a stimulus context that is related to more reinforcer deliveries (i.e. higher magnitudes or a preferred quality) should produce more persistence than stimulus contexts that are related to fewer deliveries (i.e. lower magnitudes or less preferred quality) of reinforcers (Nevin, 1974, 2012). BMT also states that operant persistence in a particular context reflects the value of the reinforcer-related stimulus. Thus, stimuli with higher conditioned value should produce more persistence than stimuli with less conditioned value (Nevin and Grace, 2000; Podlesnik et al., 2013). On the basis of this account, questions on the persistence of drug-seeking may be restated as questions on the determinants of the value of drug-related stimuli.

Some recent studies have provided evidence of the adequacy of BMT for explaining drug-seeking behavior. Jimenez-Gomez and Shahan (2007) assessed the persistence of alcohol seeking in contexts that were associated with differential rates of reinforcement. Rats were trained to self-administer a solution of 10% alcohol on a multiple schedule of reinforcement. One of the components
delivered alcohol according to a random-interval 15 s (RI-15 s) schedule and the other component delivered alcohol according to an RI 45 s schedule. The results showed that response rates were higher during the richer component (RI-15 s). Extinction sessions were then introduced as disruptors. Persistence was higher in the presence of the stimulus that was related to the higher rate of reinforcement. Similar results have been reported with cocaine (Quick and Shahan, 2009), showing that the value of drug-related stimuli is affected by such variables as delivery frequency.

Despite the attempts of BMT to understand the way in which environmental variables affect the value of drug-related stimuli, it is unknown whether environmental variables that are outside the self-administration context can affect the value that is acquired by a drug-related stimulus. Consideration of environmental variables other than the context of self-administration might contribute toward a better understanding of why drug-related stimuli are more likely to produce persistence for some individuals than for others.

Several studies have used rodents to model alcohol self-administration, showing that the social environment during early developmental stages can influence alcohol consumption during adulthood (for a review, see Cortés-Patiño & García-Mijares). Adult rats that were isolated during rearing drank more alcohol than rats that were reared in social groups (Ellison, 1987; Rockman et al., 1989; Wolfloggmann and Heyne, 1991; McCool and Chappell, 2009; Chappell et al., 2013) and preferred alcohol over other solutions (Lodge and Lawrence, 2003; Dechan et al., 2007). Furthermore, when allowed access to different alcohol doses, rats that were reared in isolation preferred higher doses (Wolfloggmann and Heyne, 1991, 1995; Hall et al., 1998). Some experiments using operant procedures have suggested that isolation rearing can increase alcohol seeking in addition to consumption. Compared with socially reared rats, isolated rats showed higher breakpoints for alcohol (Dechan et al., 2007; McCool and Chappell, 2009). In addition, when the response requirement for obtaining alcohol was increased gradually on a progressive ratio schedule of reinforcement, isolated rats showed higher breakpoints than socially enriched rats (Dechan et al., 2011). Notwithstanding these findings, it is unknown how isolation affects the persistence of alcohol seeking in the presence of stimuli that signal the availability of alcohol. Exploration of this issue may indicate whether social isolation (ISO) may more generally affect the value of drug-related stimuli.

The aim of the present study was to assess the effects of isolation on the persistence of alcohol seeking in contexts that signaled differential availability of alcohol. One group of rats was reared in ISO (i.e. housed individually) and another group of rats was maintained in group housing during the entire experiment. Alcohol self-administration was measured using an operant procedure of intermittent reinforcement, which enabled the separation of response rate from alcohol consumption. Persistence was measured as the resistance to extinction within a multiple schedule of reinforcement (Nevin, 1974).

**Methods**

**Subjects**

Twenty-four male Wistar rats were obtained at the age of 21 days from the Institute of Biomedical Sciences of the University of São Paulo. All of the rats had free access to water and food during their growth phase (21–60 days). For the rest of the experiment, the rats were maintained at ~ 90% of their free feeding weight using 1 h postsession feeding. The colony room had a 12 h/12 h light/dark cycle (lights on at 08:00 h) with conditions of constant temperature and humidity.

**Environmental conditions**

Immediately after arrival in the laboratory, the rats were assigned randomly to one of two experimental conditions: ISO (n = 12) or social interaction (INT; n = 12). To ensure that all of the subjects experienced their respective rearing conditions during the critical period of neurological development, they were maintained under these conditions for 60 days before the experimental phase began. The rats in the INT condition were housed four per cage in ALESCO mini-isolators (48×34×25 cm). The rats in the ISO condition were housed individually in ALESCO mini-isolators (37×24×24 cm). The bedding material was changed daily for the INT rats and every 3 days for the ISO rats.

**Apparatus**

Eight Med Associates (St Albans, Vermont, USA) operant conditioning chambers (35×25×21 cm) were used for the experiment. Each chamber was contained in a sound-attenuating box with a ventilation fan. The front panel of the chamber was equipped with two response levers located 13 cm apart. Under each lever was a dipper that delivered the solution and above each lever was a white light (1 W). Only the left lever was active during the experiment. Each chamber contained a 28 W houselight at the top of the back panel and a Sonalert (2900±500 Hz, 75–85 dB). Experimental events were controlled in an adjacent room using a Med Associates interface and program.

**Solutions**

Solutions were prepared with 99% ethanol (ET), table sugar, and tap water. ET concentrations were calculated as v/v (ET/tap water) and sucrose (SUC) solutions were calculated as w/v (SUC/tap water). All of the solutions were mixed daily.

**Procedure**

After 60 days of rearing under the experimental conditions, the rats were food deprived and underwent lever-pressing training for a solution of 10% SUC. This SUC solution was...
delivered according to a continuous reinforcement schedule for two consecutive days and then the deliveries were made according to a variable-ratio (VR) schedule of reinforcement to generate higher response rates. The VR schedule was increased gradually from VR5 to VR10 across four sessions. SUC consumption was assessed for 7 days using a VR10 schedule. The same schedule of reinforcement was used during the entire ET self-administration training.

ET self-administration training was performed using a modified SUC fading procedure (Tolliver et al., 1988). In the first part of the procedure (fade-in), the SUC concentration was maintained at 10% while the ET concentration increased. The following solutions were presented (where the percentages represent the concentrations of the solute SUC or ET): 10% SUC/2.5% ET for four sessions, 10% SUC/5% ET for four sessions, and 10% SUC/10% ET for five sessions. In the second part of the procedure (fade-out), the SUC concentration in the solution was decreased while the ET concentration was maintained constant. The following solutions were presented: 7.5% SUC/10% ET for three sessions, 5% SUC/10% ET for four sessions, 2.5% SUC/10% ET for four sessions, and 25% SUC/10% ET for eight sessions. This latter solution (0.25% SUC/10% ET) was used during the remaining sessions of the experiment. All of the sessions in this phase ended after 30 min.

After completing self-administration training, alcohol was delivered according to a VI 15 s schedule. Variable interval values were selected without replacement from a 10-interval list that was constructed according to the progression of Fleshler and Hoffman, (1962). Once responding on the VI 15 s schedule stabilized, a multiple schedule was introduced, with two components that delivered differential alcohol reinforcement rates (Jimenez-Gomez and Shahan, 2007). In the multiple schedule, one of the components was signaled by a steady tone and illumination of the houselight and the other component was signaled by pulsing tone and a blinking houselight (0.5 s on,0.5 s off). Initially, responding in both components was reinforced according to a VI 15 s schedule, but for one of the components, the VI value increased gradually across the sessions until it reached VI 45 s. The final multiple schedule that was used in the baseline phase consisted of a VI 15 s component (rich component; 4 dippers/min) and a VI 45 s component (lean component; 1.33 dippers/min). Stimuli that were associated with the rich and lean components were counterbalanced across subjects.

Baseline sessions began with a 15 min blackout and then the first component was selected with a probability of 5. Components alternated during the session and were separated by a 30 s intercomponent interval, during which all of the stimuli in the box were turned off, and responding had no programmed consequences. The components were 60 s long and the sessions ended when each component had been presented 10 times.

Three extinction sessions were performed after the response rate stabilized on the multiple schedule (i.e. five consecutive sessions with no trend toward increased or decreased responding, as determined by visual inspection between 42 and 78 days across subjects). In the extinction sessions, the components were presented as in the baseline sessions, but responding had no programmed consequences. Compound stimuli were presented and the solution was placed outside the chamber, but the dipper did not lift.

Data analysis

ET consumption (g/kg per session) during self-administration training was examined using two-factor mixed analysis of variance (ANOVA), with rearing as a between-subjects factor and session as a repeated measures factor. Because the main effects of session and interactions that involved the session variable were nonsignificant, the data were grouped across sessions to simplify the analysis.

For baseline, measures of consumption (g/kg per session), reinforcement rate, and response rate were obtained for each component. Response and reinforcer rates were measured as the number of lever presses and dipper presentations, respectively, per minute of time spent in each component during the entire session. Mixed ANOVA was performed to analyze the data in this phase, with component (rich and lean) as a within-subjects factor and rearing as a between-subjects factor.

Resistance to extinction was calculated as the log proportion of response rate during extinction relative to the baseline response rate. To perform this calculation, the response rate for each extinction session was divided by the average response rate during baseline, obtained from the last 5 days before disruption, and then transformed into logarithmic units. Log proportions of baseline are the most used measure of resistance to change because they enable the examination of functional relationships without distortions by floor effects. In addition, logarithms render equal proportional changes in equal differences (Nevin and Grace, 2000). Statistical analyses of resistance to extinction were carried out using mixed ANOVA similar to that carried out for baseline data, but with session of extinction as a second within-subjects variable.

An additional analysis measured persistence as relative resistance to change (Grace and Nevin, 1997). This measure enables determination of the difference in the value of two stimulus contexts, in which a greater difference in persistence between components indicates a greater difference in value between the components. Relative resistance to change was calculated by subtracting resistance to extinction in the lean component (i.e. the log proportion of baseline responding in the lean component) from the resistance to extinction in the rich component (i.e. the log proportion of baseline responding...
in the rich component). A higher value of relative resistance to change indicates a greater difference in persistence between the two components. Lower or zero values indicate no differences in persistence between the components. For all of the analyses, effect sizes were computed using $\eta^2$ and statistical significance was determined using a criterion of $P$ value of less than 0.05.

**Results**

**Acquisition (ET fade-in, SUC fade-out)**

Rearing condition did not affect SUC intake during initial training ($P > 0.05$, data not shown). As shown in Fig. 1, no significant differences in ET consumption were found between ISO and INT rats during the ET fade-in procedure when SUC was maintained constant at a concentration of 10%. However, during SUC fade-out, ISO rats drank more alcohol than INT rats. The effect of rearing was significant when the SUC concentration was 2.5 and 0.25% [$F(1,22) = 8.6, P < 0.05, \eta^2 = 0.18$ and 0.28, respectively]. No interaction was found between rearing and session.

**Baseline**

Figure 2a shows the average number of reinforcer deliveries (dipper deliveries) in the last five sessions before extinction. In ISO and INT rats, dipper deliveries were higher in the rich component [dark bars; $F(1,22) = 555.05, P < 0.001, \eta^2 = 0.962$], but ISO rats obtained more alcohol deliveries in both the rich and the lean components [$F(1,22) = 6.4, P < 0.02, \eta^2 = 0.22$]. No interaction was found between these variables.

The analysis of average dipper deliveries in both groups showed that the reinforcer rates were lower than the programmed reinforcer rates (1.33 for the lean component and four for the rich component). The mean reinforcer rates in ISO rats in the lean and rich components were 0.9 and 2.7, respectively, and the mean reinforcer rates in INT rats in the lean and rich components were 0.8 and 2.3, respectively. Nevertheless, the programmed ratio (1 : 3) was maintained. Alcohol consumption (g/kg) was also affected by rearing condition (Fig. 2b). ISO rats consumed significantly more alcohol in both the rich and the lean components [$F(1,22) = 14.06, P < 0.005, \eta^2 = 0.39$]. As expected, both groups consumed more alcohol in the rich component [$F(1,22) = 581.45, P < 0.001, \eta^2 = 0.95$]. In addition, the interaction between factors was significant, reflecting a larger difference in alcohol ingestion between the two components in the ISO group [$F(1,22) = 10.158, P < 0.005, \eta^2 = 0.31$].

Figure 2c shows the responses per minute for the last five sessions that preceded extinction. Both groups emitted higher response rates in the rich component [$F(1,22) = 18.34, P < 0.001, \eta^2 = 0.45$], but the ISO group had a higher overall response rate [$F(1,22) = 6.15, P < 0.05, \eta^2 = 0.21$]. The interaction between factors was not statistically significant.

**Extinction**

Persistence during extinction is shown in Fig. 3. Greater persistence is indicated by a higher log proportion of baseline responding during the extinction sessions. In both groups, the response rates during extinction decreased as the sessions advanced [$F(2,44) = 66.31, P < 0.001, \eta^2 = 0.86$]. In addition, the proportion of baseline responding was higher
in the rich component in both groups \(F(1, 22) = 17.94, P < 0.001, \eta^2 = 0.44\). The interaction between component and session was not significant.

Persistence was affected by rearing conditions (Fig. 3): ISO rats showed higher resistance to extinction than INT rats in both components \(F(1, 22) = 4.69, P < 0.05, \eta^2 = 0.17\).

The difference in persistence between the rich and lean components was not affected by rearing conditions, reflected in comparisons of relative resistance to change between groups \((P > 0.05; \text{Fig. 4})\).

**Discussion**

Social conditions during early stages of development have been shown to affect several behaviors that might increase the vulnerability to drug abuse (Rockman **et al.**, 1988; Wolffgramm and Heyne, 1995; Hall **et al.**, 1998; Araujo **et al.**, 2005). The present study investigated whether social conditions that affect alcohol self-administration can also affect persistence when alcohol is no longer available. Our results indicate that early isolation increased alcohol consumption and responding and also the persistence of alcohol seeking in the presence of drug-related stimuli.

Differences in alcohol consumption in rats that were reared in ISO conditions (ISO group) and group INT conditions (INT group) were observed early in the acquisition of alcohol drinking, and these effects lasted for the entire study. During the fading procedure, initial SUC consumption was not different between groups. However, as the alcohol concentration increased, alcohol consumption in ISO rats was higher compared with INT rats. This difference was significant at the lower SUC concentrations, in which INT rats showed a marked decrease in alcohol consumption compared with ISO rats. During baseline, in which alcohol was delivered in two different contexts (rich and lean), ISO rats continued to show a higher response rate for alcohol, irrespective of the component. Increased drinking during the acquisition phase and higher levels of responding during baseline support the hypothesis that the reward value of alcohol is increased by isolation. It is noteworthy that our results are similar to previous studies that utilized operant models, in which rats that were reared in isolation showed higher response rates for alcohol under continuous (McCool and Chappell, 2009) and intermittent (Deehan **et al.**, 2007, 2011) schedules of reinforcement.

The analysis of resistance to extinction showed that ISO rats were more persistent in alcohol-related contexts than INT rats. Recently, Whitaker **et al.** (2013) reported similar results using a different experimental paradigm. These authors found that the rate of learning about an alcohol-related stimulus in a conditioned place preference paradigm was faster in ISO-reared rats than in group-reared rats. Isolated rats also showed more persistent alcohol-seeking behavior during the extinction of conditioned place preference compared with group-reared rats. These results suggest that the persistence of alcohol seeking is affected by isolation rearing.

The persistence of alcohol-seeking behavior was higher in the rich context than in the lean context in both the ISO and the INT groups. These results indicate that relative
Persistence was unaffected by the rearing conditions. In contrast to these results, the data suggest that the relative persistence of alcohol seeking is affected by pharmacological treatments that decrease alcohol consumption. For example, using a similar procedure, Jimenez-Gomez and Shahan (2007) assessed the effects of the opioid receptor antagonist naltrexone, which has been shown to decrease alcohol consumption, on the persistence of alcohol seeking behavior in contexts that were associated with different alcohol-delivery rates. Their results showed that before naltrexone injections, the response rates were higher in the richer component. After naltrexone treatment, however, the response rates were similar in both the rich and the lean components. Therefore, naltrexone appeared to affect the relative resistance to extinction.

The differences between our results and those obtained in previous studies that used naltrexone suggest that although both social factors and the opioid system are involved in the determination of alcohol consumption (Gustafsson et al., 2007; Palm et al., 2013; Palm and Nylander, 2014), the mechanisms by which ISO and naltrexone affect persistence of seeking behavior might be different. Recent data suggest that changes in synaptic plasticity in dopaminergic areas might be the mechanism by which early isolation affects alcohol seeking. The dopaminergic system that originates in the ventral tegmental area plays an important role in learning about which environmental stimuli lead to reward (Schultz, 2010, 2013). Increases in synaptic plasticity in the ventral tegmental area system, resulting from repeated exposure to drugs, are responsible for the formation of enduring memories about drug-related stimuli (Hyman et al., 2006; Everitt et al., 2008). Whitaker et al. (2013) showed that isolation during adolescence had an effect that was similar to that of repeated drug exposure, in that isolation enhanced the long-term potentiation of N-methyl-D-aspartate receptor-mediated glutamatergic transmission in dopaminergic neurons. Such neurophysiological changes may explain the effects of isolation on learning about reinforcer-related stimuli that have been reported in previous studies of drugs (Zakharova et al., 2009; Kennedy et al., 2012) and SUC (Harmer and Phillips, 1998; van Den Berg et al., 1999). Nevertheless, more studies are needed to explore the relationship between the physiological and behavioral effects of early isolation.

**Fig. 3**

Resistance to change during extinction sessions. Log proportions of baseline responses per minute are presented for ISO and INT groups. Filled lines represent responding in the rich component and dotted lines represent responding in the lean component. INT, interaction; ISO, isolation.

**Fig. 4**

Relative persistence for rich and lean components during extinction. Data were analyzed after subtracting the log proportion of the baseline responding in the lean component (\(B_2/B_0\)) from the log proportion of baseline responding in the rich component (\(B_1/B_0\)). INT, interaction; ISO, isolation.
to better understand the way in which social conditions affect processes that are linked to drug addiction. Further studies could also identify the mechanisms that are involved in the increase in alcohol seeking that is produced by early isolation.

In conclusion, the present results confirm previous findings that suggested that early ISO increases alcohol consumption and responding for alcohol. In addition, the present study supported the hypothesis that ISO increases persistence in the presence of alcohol-related stimuli. If, as proposed by BMT, persistence is a measure of the conditioned value of a stimulus, then we could argue that the value of alcohol-related stimuli is increased by early isolation.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References


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