Early social isolation augments alcohol consumption in rats

Heidi M.B. Lesscher[a], Marcia Spoelder[a], Marthe D. Rotte[a], Martijn J. Janssen[a], Peter Hesseling[a], José G. Lozeman-van't Klooster[a], Annemarie M. Baars[a] and Louk J.M.J. Vanderschuren[a,b]

There is a considerable degree of individual vulnerability for alcohol use disorder (AUD) as only a subpopulation of individuals who regularly consume alcohol develop AUD. It is therefore very important to understand the factors and mechanisms that contribute towards the individual risk for AUD. In this respect, social influences, in particular during development, may be relevant for AUD as disruptions in early social experiences are associated with an increased risk for AUD. Social play, the most prominent form of social behaviour shown by young mammals, is rewarding and considered to be important for social, emotional and cognitive development. Recent studies suggest that early social isolation, effectively depriving animals from social play, increases the risk for addictive behaviour. The aim of this study was therefore to explore the long-term consequences of early social isolation on alcohol consumption and motivation for alcohol. To this end, rats were socially isolated from postnatal days 21–42, followed by 4 weeks of social housing, and voluntary alcohol consumption and operant responding for alcohol were determined in adulthood. We observed enhanced levels of alcohol consumption in adulthood in previously isolated rats, whereas operant responding for alcohol was not altered. The impact of early social isolation was independent of the individual variation in alcohol consumption. These data indicate that social isolation, during a developmental period when social play is highly abundant, enhances the propensity to consume alcohol in adulthood. This implies that early social experience may be a protective factor against excessive alcohol use. *Behavioural Pharmacology* **26:673–680**

Behavioural Pharmacology 2015, **26**:673–680

Keywords: addiction, alcohol, alcohol use disorder, rat, social isolation, social play behaviour

[a]Department of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University and [b]Department of Translational Neuroscience, Brain Center Rudolf Magnus, University Medical Center Utrecht, Utrecht, The Netherlands

Correspondence to Heidi M.B. Lesscher, PhD, Department of Animals in Science and Society, Division of Behavioural Neuroscience, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 2, 3584 CM Utrecht, The Netherlands
E-mail: h.m.b.lesscher@uu.nl

Received 17 April 2015 **Accepted as revised** 11 June 2015

Introduction

Alcohol use disorder (AUD) is a chronic relapsing disorder that affects ~76 million individuals worldwide, thus representing a major medical and socioeconomic problem for our society (Rehm *et al.*, 2009; World Health Organization, 2011; American Psychiatric Association, 2013). Importantly, there is a considerable degree of variability in the vulnerability for developing AUD. That is, most individuals consume alcohol in a controlled manner, but a subpopulation of 3–5% of individuals who regularly consume alcohol develop AUD (World Health Organization, 2011). Considering that treatment strategies for addiction are currently limited in number and efficacy (O'Brien, 2008; Koob *et al.*, 2009; Pierce *et al.*, 2012; van den Brink, 2012), understanding the factors and mechanisms that contribute towards the individual variability in the propensity for AUD is essential to prevent and treat this devastating disorder.

Alcohol is often consumed in a social context. In fact, social context and peer norms are important determinants of alcohol consumption (e.g. Perkins, 2002; Homish and Leonard, 2008; Lau-Barraco *et al.*, 2012). Recent animal studies also provide support for social influences on alcohol consumption. For example, voles show greater preference for alcohol when housed in pairs and they adjust their alcohol consumption in the presence of lower alcohol-drinking cagemates (Anacker *et al.*, 2011a, 2011b). Conversely, early social insults have been shown to enhance the propensity to consume alcohol and other drugs of abuse, suggesting that a dysfunctional social context during development comprises a risk factor for AUD (Bonin *et al.*, 2000; Alwan *et al.*, 2011; Stickley *et al.*, 2013; Whelan *et al.*, 2014). Furthermore, social disorders in childhood and adolescence, in particular disruptive behaviour disorders, are associated with a higher incidence of AUD (Compton *et al.*, 2005; Goldstein *et al.*, 2007). There is also ample evidence from animal studies supporting a critical role of social development in addiction sensitivity. For example, peer rearing in non-human primates consistently leads to elevated alcohol consumption in adulthood compared with mother-reared conspecifics (Higley *et al.*, 1991; Fahlke *et al.*, 2000; Huggins *et al.*, 2012). In agreement with these findings, maternal separation in rodents is known to induce persistent increases in alcohol consumption in adulthood

0955-8810 DOI: 10.1097/FBP.0000000000000165

(Roman *et al.*, 2005; Cruz *et al.*, 2008; Nylander and Roman, 2013). Furthermore, postweaning isolation rearing in rodents has repeatedly been shown to result in augmented alcohol consumption and operant responding for alcohol in adulthood (Ellison, 1981; Schenk *et al.*, 1990; Wolffgramm, 1990; Hall *et al.*, 1998; Lodge and Lawrence, 2003; Advani *et al.*, 2007; Deehan *et al.*, 2007; McCool and Chappell, 2009; Sanna *et al.*, 2011; Chappell *et al.*, 2013; Butler *et al.*, 2014).

Social play behaviour is the most prominent form of social behaviour shown by young mammals (Panksepp *et al.*, 1984; Vanderschuren *et al.*, 1997; Pellis and Pellis, 2009). Social play behaviour is rewarding, as shown using place conditioning, operant tasks and T-maze tasks (Mason *et al.*, 1962; Humphreys and Einon, 1981; Calcagnetti and Schechter, 1992; for a review, see Trezza *et al.*, 2011). Importantly, social play behaviour is modulated through neural systems that also mediate the rewarding effects of substances of abuse (Trezza *et al.*, 2010; Siviy and Panksepp, 2011). This suggests a critical role of social play in the development of brain reward circuitry, which may determine an individual's sensitivity to addictive behaviour. Interestingly, acute treatment with alcohol enhances social play behaviour (e.g. Varlinskaya *et al.*, 2001; Varlinskaya and Spear, 2002; Trezza *et al.*, 2009) and the sensitivity to the facilitating effects of acute alcohol on social play behaviour seems to influence the amount of alcohol consumption during adolescence in a sex-dependent manner (Varlinskaya *et al.*, 2015). Postweaning social isolation rearing has been shown to enhance alcohol consumption in rats and mice (Ellison, 1981; Schenk *et al.*, 1990; Wolffgramm, 1990; Hall *et al.*, 1998; Lodge and Lawrence, 2003; Advani *et al.*, 2007; McCool and Chappell, 2009; Sanna *et al.*, 2011; Chappell *et al.*, 2013; Butler *et al.*, 2014). However, these animals were reared in isolation from weaning onward, which leaves the question of whether deprivation of social play behaviour contributed towards the increase in alcohol consumption. In fact, animals that were socially isolated only during the period in development when social play is most abundant [i.e. postnatal day (PND) 21–42, Panksepp, 1981], thus effectively depriving them of social play, show enhanced sensitivity for cocaine self-administration and amphetamine-induced and alcohol-induced conditioned place preference in adulthood (Whitaker *et al.*, 2013; Baarendse *et al.*, 2014). Taken together, these studies suggest that social play behaviour is essential for the adaptive development of brain reward mechanisms, such that deprivation of social play may increase the risk for later addictive behaviour (Trezza *et al.*, 2014). However, the importance of social play behaviour for the sensitivity or resilience to alcohol consumption is unknown. The aim of this study was therefore to explore the long-term consequences of early social isolation on alcohol consumption and motivation for alcohol. Therefore, we socially isolated rats during PND 21–42, and determined alcohol consumption and operant responding for alcohol during adulthood. We hypothesized that early social isolation would lead to enhanced levels of alcohol consumption in adulthood and increased operant responding and motivation for alcohol.

Methods

Subjects

Male Lister Hooded rats (Charles River, Sulzfeld, Germany) arrived in litters of six to eight pups at an age of 14 days with a nursing mother. The rats were housed with free access to food and water under controlled conditions (20 ± 2°C and 50–70% humidity) in a reversed 12 h day/night cycle (lights on, 19.00 h). Experimental procedures were approved by the Animal Ethics Committee of Utrecht University and were conducted in agreement with Dutch laws (Wet op de dierproeven, 1996) and European regulations (Guideline 86/609/EEC).

Early social isolation and alcohol consumption

As in our previous studies (Baarendse *et al.*, 2013, 2014), the rats were weaned and housed either socially in groups of four rats per cage (SOC) or individually (ISO) at 21 days of age. Half of the rats of each litter were assigned to the SOC group and the other half to the ISO group in a semirandomized manner. The rats of the ISO group were resocialized, that is housed together with another previously isolated animal, on day 43. At that time, the animals in the SOC groups were also housed in pairs. After 4 weeks of social housing, all animals were individually housed for subsequent alcohol consumption experiments 2 weeks later. The experiment was conducted in two batches ($n = 24$ and 48).

For alcohol consumption experiments, we adopted the intermittent every-other-day alcohol access model (Wise, 1973; Simms *et al.*, 2008). Two bottles, fitted with stainless steel dual ball bearing drinking spouts, were placed on the home cage; one bottle contained alcohol (20%, v/v) (Klinipath, Duiven, the Netherlands) and the other contained water. The positions of the bottles were switched between sessions to avoid the development of side preference. During 3 consecutive weeks, the rats were allowed 7 h concurrent access to alcohol and water on Monday, Wednesday and Friday, during the dark phase of the day–night cycle. Subsequently, during another 3 consecutive weeks, the rats were allowed 24 h concurrent access to alcohol and water, again on Monday, Wednesday and Friday, starting at the beginning of the dark phase of the day–night cycle. The bottles were weighed before and after each alcohol access period to determine the amount of alcohol and water that the animals consumed. Alcohol intake (g/kg), alcohol preference (percentage alcohol volume relative to the total volume consumed) and total fluid intake (ml/kg) were calculated per rat per session. Subsequently, the alcohol intake, preference and total fluid intake were averaged

across sessions into values representing the average alcohol intake, preference and total fluid intake over the 7 or 24 h alcohol consumption sessions, respectively.

The rats were divided into low, medium and high alcohol-drinking rats on the basis of their average alcohol intake in g/kg; this division was performed within each social housing group (SOC or ISO). The rats were assigned ranking scores (i.e. 1, 2, 3, 4, etc.; corresponding to the number or rats in the group, in this case 1–12 for both the SOC and the ISO group in batch 1 and 1–24 for both the SOC and the ISO group in batch 2) on the basis of their average alcohol intake per week. Then, to calculate a total ranking score, the weekly ranking scores were summed across the 6 weeks of alcohol consumption to select rats with a consistent low or high level of alcohol intake. Rats within the lower, middle and upper 33% of the rank list were designated as low, medium and high alcohol-drinking rats, respectively.

Operant responding for alcohol

After 2 months of alcohol consumption, all rats of the first batch ($n = 12$ for SOC and ISO) and half of the rats of the second batch ($n = 12$ for SOC and ISO) were trained to respond for alcohol in operant conditioning chambers. The other half of the second batch was used for pharmacological studies (not described here). The rats were trained to self-administer alcohol in operant chambers (29.5 cm L, 24 cm W 25 cm H; Med Associates, Georgia, Vermont, USA) that were enclosed in light-attenuating and sound-attenuating cubicles equipped with a ventilation fan. Each chamber was equipped with two 4.8 cm wide retractable levers; the levers were placed 11.7 cm apart and 6 cm from the grid floor. A liquid dipper within a recessed magazine was situated between the levers. A cue light was present above each lever (28 V, 100 mA) and a house light (28 V, 100 mA) was located on the opposite wall. The position of the active and inactive levers was counterbalanced between rats. Pressing the active lever raised the dipper cup containing alcohol (0.1 ml, 20% v/v), illuminated the cue light above the active lever and switched off the house light. Access to alcohol was terminated 10 s after detection of a head entry into the magazine, the cue light was turned off and after a 5 s interval a new trial was started. Pressing the inactive lever was recorded, but had no programmed consequences. To limit alcohol evaporation, the container was filled with a fresh alcohol solution before each session. Experimental events and data recording were controlled using MED-PC for Windows (Med Associates, Georgia, Vermont, USA).

As soon as the animals had acquired responding, defined as less than a 25% variation in active lever presses over three consecutive sessions under the fixed ratio (FR) 1 schedule of reinforcement, the response requirement was increased to an FR2, then to an FR5 and finally to an FR10 schedule of reinforcement, with the requirement that each rat had to earn at least 10 rewards for two sessions before progressing to FR5 and FR10, respectively. Subsequently, the rats had to earn at least 10 rewards for three sessions during FR10 training before progressing to the progressive ratio (PR) schedules of reinforcement. These requirements were set to ensure that the rats understood the task contingencies and performed at least 100 presses under an FR10 to assess a reliable motivation during PR sessions. Once the rats completed FR10 training, a linear PR schedule of reinforcement was introduced, in which 2 (PR2; i.e. 2, 4, 6, 8, 10, etc.) and subsequently 4 (PR4; i.e. 4, 8, 12, 16, 20, etc.) additional lever presses were required for each subsequent reward. This PR paradigm, rather than the commonly used exponential increase in the response requirement (Richardson and Roberts, 1996), was chosen on the basis of the results of previous studies that showed that (a) alcohol nonpreferring rats have low breakpoints; (b) the required workload should be increased, but before the sedative effects of alcohol begin to interfere with operant performance; and (c) because alcohol is delivered in relatively small volumes (0.1 ml/reinforcement) with a slow absorption rate (Hodos, 1961; Ritz *et al.*, 1994; Rodd *et al.*, 2003). Responding was deemed stable when there was less than 25% variation in reward deliveries over three subsequent sessions. Two rats from the SOC group did not reach stable responding on the PR2 schedule of reinforcement and therefore did not proceed to PR4. The rats were tested for 3 days/week (Monday, Wednesday and Friday), and sessions lasted for 30 min, except for the PR4 schedule of reinforcement, which lasted 60 min. The breakpoint was defined as the maximum number of presses performed in the last, successfully completed ratio in either the 1 h session or when no reward had been obtained in 20 min, whichever came first.

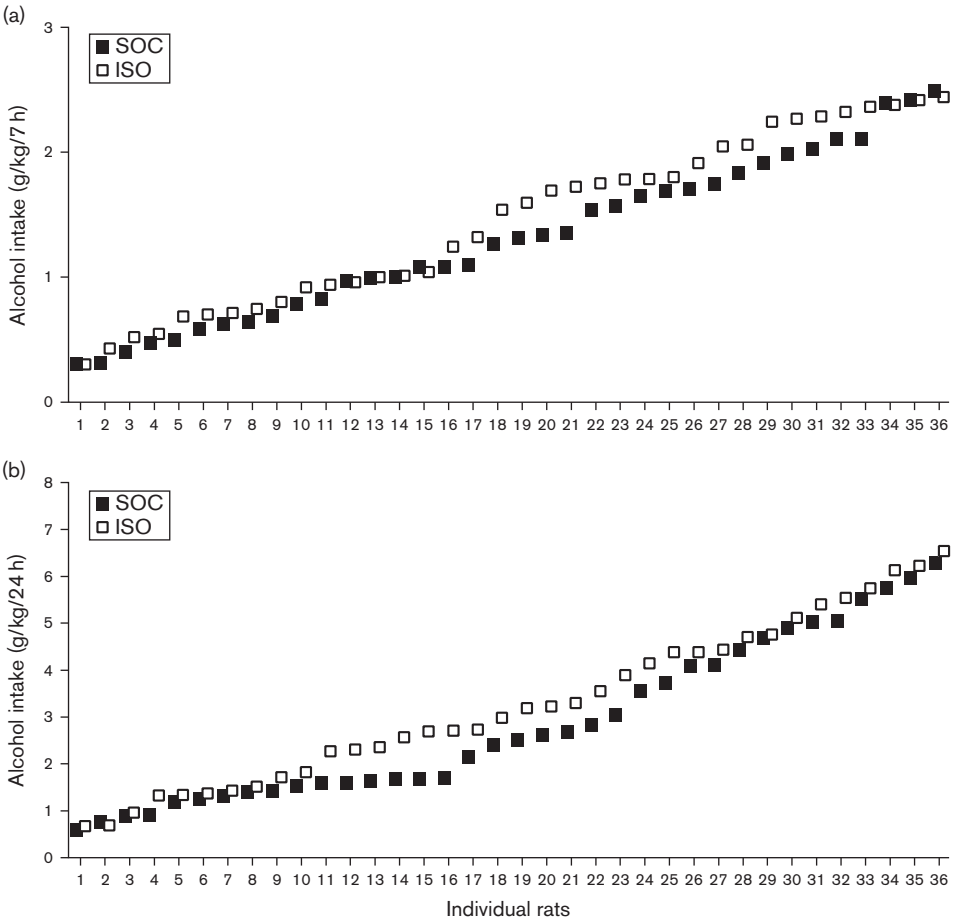
Data analysis

The alcohol consumption data were averaged across the 7 and 24 h sessions, respectively, and analysed by two-way analysis of variance with group (SOC and ISO) and subgroup (low, medium, high) as between-subject factors. For analyses of the operant self-administration data, the numbers of lever presses (FR1) and breakpoints (PR2 and PR4) were averaged over the first three sessions in which the rat acquired the response criteria as described above. These data were also analysed by two-way analysis of variance with group (SOC and ISO) and subgroup (low, medium, high) as between-subject factors. Post-hoc analyses were carried out when appropriate using two-tailed *t*-tests. Differences between pairs of means were considered significant at α less than 0.05. SPSS 22.0 (SPSS Inc., Chicago, Illinois, USA) was used for data analysis. Data are presented as mean ± SEM.

Results

Analysis of the alcohol consumption data showed a large variation in alcohol intake in the population of the rats.

Fig. 1



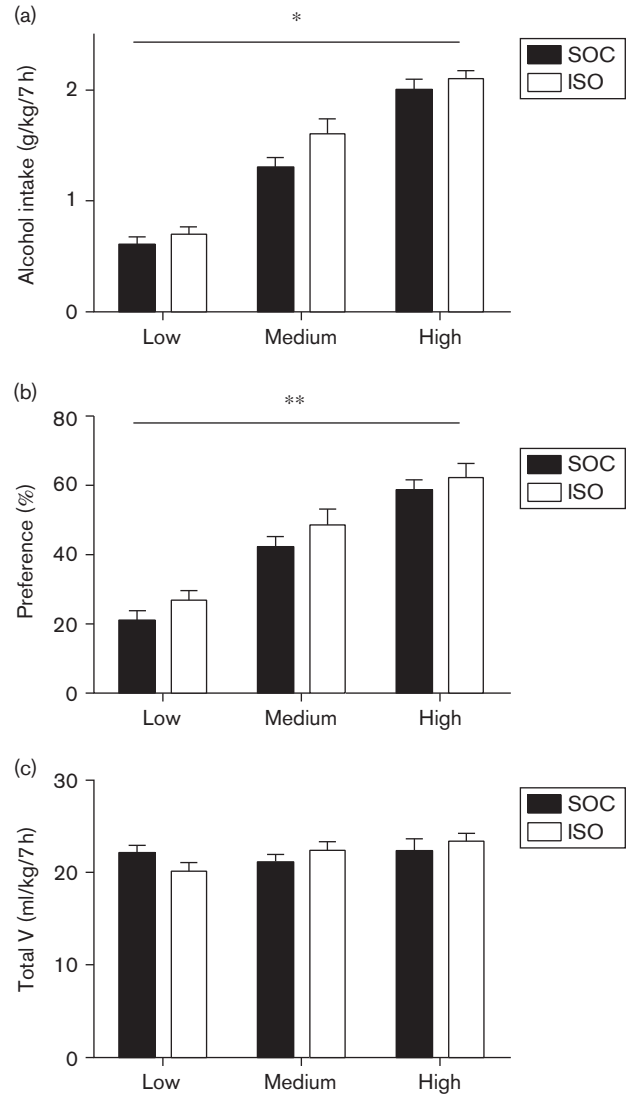
Alcohol consumption for individual isolated rats (ISO) and socially housed control rats (SOC) ($n = 36$). The average levels of alcohol intake (in g/kg) of each individual rat over all 7 h (a) and 24 h (b) access sessions are shown. There is a leftward shift in the distribution curve for the ISO rats compared with the SOC rats.

Inspection of the individual levels of alcohol intake indicated a shift towards higher levels of alcohol intake across the range of alcohol consumption during the 7 and 24 h access sessions for the ISO rats compared with the SOC animals (Fig. 1).

The animals were assigned to subgroups of low, medium and high alcohol-drinking rats. Analysis of the 7 h alcohol consumption data showed that the subgroups (low, medium and high) differed in their level of alcohol intake [$F_{\text{subgroup}(2,71)} = 106.5$, $P < 0.001$]. Moreover, early social isolation increased the level of alcohol intake [$F_{\text{group}(1,71)} = 7.6$, $P < 0.01$], but there was no differential effect of early social isolation in the subgroups of rats [$F_{\text{group} \times \text{subgroup}(2,71)} = 1.1$, NS] (Fig. 2a). The low, medium and high alcohol-drinking rats also differed in their preference for alcohol over water [$F_{\text{subgroup}(2,71)} = 64.2$, $P < 0.001$] and preference for alcohol was significantly higher in the ISO group [$F_{\text{group}(1,71)} = 7.5$, $P < 0.001$], but this effect was not subgroup dependent [$F_{\text{group} \times \text{subgroup}\ (2,71)} = 0.137$, NS] (Fig. 2b). There were no significant differences between the groups or the subgroups in the total volume of liquid consumed by the rats in the 7 h sessions [$F_{\text{subgroup}(2,71)} = 0.90$, NS; $F_{\text{group}(1,71)} = 0.47$, NS; $F_{\text{subgroup} \times \text{group}(2,71)} = 1.3$, NS] (Fig. 2c).

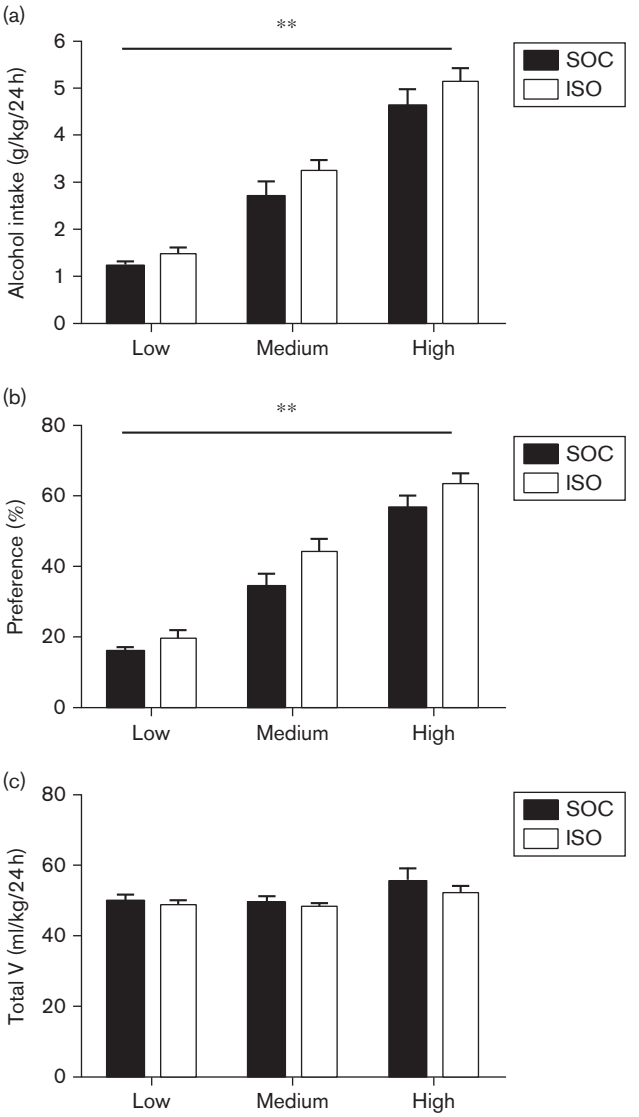
In line with the analysis of the 7 h consumption data, analysis of the 24 h alcohol consumption data confirmed that the amount of alcohol consumed by low, medium and high alcohol-drinking rats was significantly different [$F_{\text{subgroup}(2,71)} = 109.8$, $P < 0.001$]. Moreover, alcohol intake was higher in the ISO rats [$F_{\text{group}(1,71)} = 12.3$, $P < 0.001$], which was independent of the subgroup [$F_{\text{group} \times \text{subgroup}(2,71)} = 1.2$, NS] (Fig. 3a). The low, medium and high alcohol-drinking rats also showed differences in their preference for alcohol over water [$F_{\text{subgroup}(2,71)} = 101.3$, $P < 0.001$], and early social isolation enhanced the preference for alcohol compared with the SOC animals [$F_{\text{group}(1,71)} = 14.6$, $P < 0.001$]. The increase in alcohol preference in the ISO rats was

Fig. 2



Average alcohol intake (a), alcohol preference (b) and total fluid consumption (c) for all 7 h alcohol consumption sessions. The average data for low, medium and high subgroups of socially housed (SOC) and social play-deprived (ISO) rats ($n = 12$) are shown. Early social isolation increased alcohol consumption and alcohol preference in 7 h sessions without affecting the total fluid intake (*$P < 0.01$, **$P < 0.001$; main effect of early social isolation). Total V, total volume.

Fig. 3



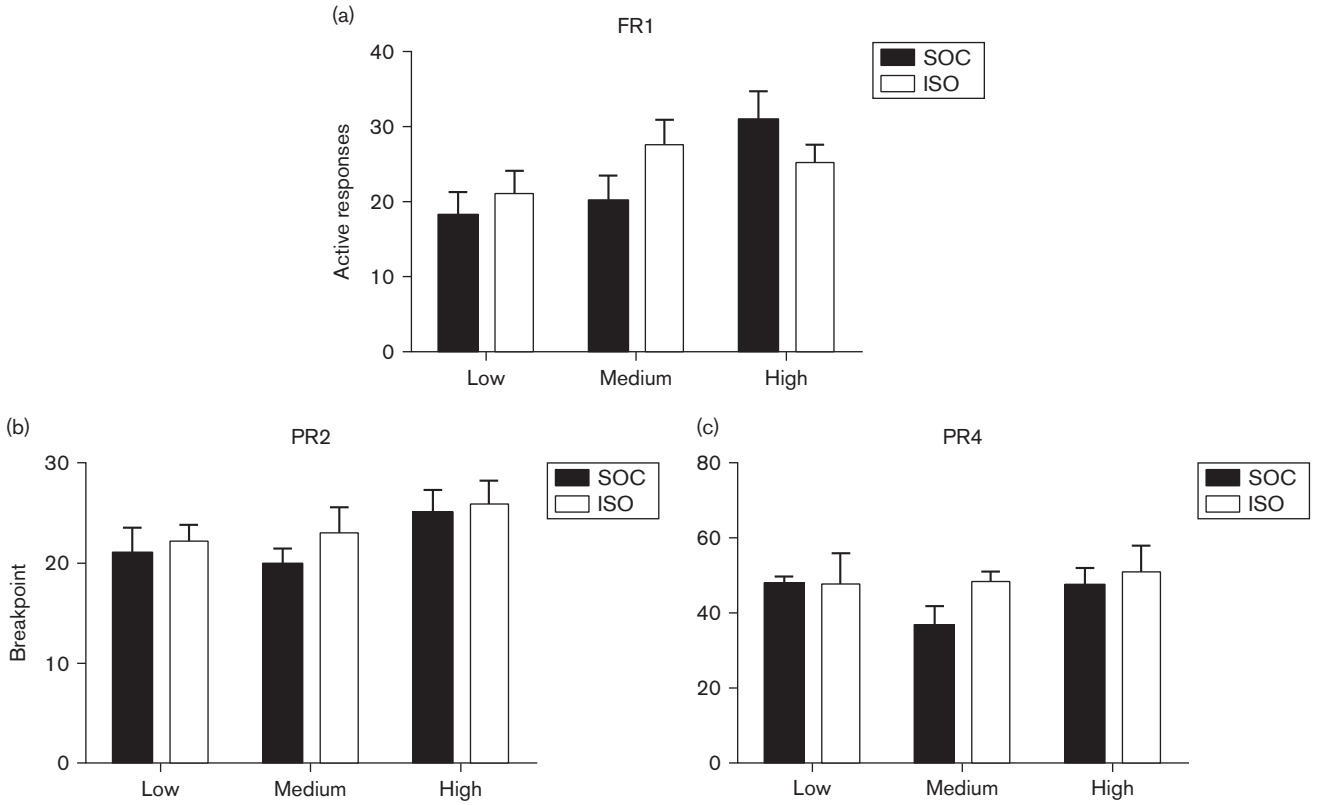
Average alcohol intake (a), alcohol preference (b) and total fluid consumption (c) for all 24 h alcohol consumption sessions. The average data for low, medium and high subgroups of socially housed (SOC) and social play-deprived (ISO) rats ($n = 12$) are shown. Early social isolation enhanced alcohol consumption and alcohol preference without altering the total fluid intake in 24 h sessions (**$P < 0.001$; main effect of early social isolation). Total V, total volume.

independent of the subgroup [$F_{\text{subgroup} \times \text{group}(2,71)} = 1.5$, NS] (Fig. 3b). The subgroups consumed equal total volumes of liquid in the 24 h sessions [$F_{\text{subgroup}(2,71)} = 2.6$, NS] and there was no significant effect of early social isolation on the total volume consumed [$F_{\text{group}(1,71)} = 0.1$, NS], nor was there a significant interaction between the two factors on the total fluid intake [$F_{\text{subgroup} \times \text{group}(2,71)} = 0.08$, NS] (Fig. 3c).

Subsequent to the home-cage alcohol consumption, the rats were trained to respond for alcohol. Analysis of the FR1 self-administration data indicated no significant effect of early social isolation [$F_{\text{group}(1,48)} = 0.32$, NS]. The reinforcing effects of alcohol were dependent on the subgroup, as apparent from a trend [$F_{\text{subgroup}(2,48)} = 3.0$, $P = 0.063$], but there was no differential effect of early social isolation in the subgroups of rats [$F_{\text{group} \times \text{subgroup}(2,48)} = 1.9$, NS] (Fig. 4a).

Finally, the rats were tested under PR2 and PR4 schedules of reinforcement to determine whether early social isolation affects the motivation to respond for alcohol (Fig. 4b and 4c). As a requirement to progress to PR

Fig. 4



Operant responding for alcohol under different schedules of reinforcement: (a) FR1 ($n = 7$–9), (b) PR2 schedule and (c) PR4 ($n = 5$–9). Early social isolation did not affect alcohol reinforcement or the motivation for alcohol self-administration. FR, fixed ratio; ISO, social play-deprived rats; PR, progressive ratio; SOC, socially housed rats.

schedules of reinforcement, each rat had to earn at least 10 rewards for two to three sessions before progressing from FR2 to FR5 to FR10 and ultimately to PR2 schedules of reinforcement. Of the SOC rats, 96% fulfilled these requirements, as opposed to only 80% of the ISO rats. Analysis of the PR data indicated no significant effect of early social isolation under either PR schedules [$F_{\text{group}(1,41)} = 0.69$, NS for PR2 and $F_{\text{group}(1,39)} = 1.3$, NS for PR4]. In addition, the motivation for alcohol was not significantly different between the low, medium and high alcohol-drinking rats [$F_{\text{subgroup}(2,41)} = 1.5$, NS for PR2 and $F_{\text{subgroup}(2,39)} = 0.82$, NS for PR4], nor was there a subgroup-dependent effect of early social isolation on PR responding [$F_{\text{group} \times \text{subgroup}(2,41)} = 0.20$, NS for PR2 and $F_{\text{group} \times \text{subgroup}(2,39)} = 0.47$, NS].

Discussion

In this study, we show that social isolation during a period in development during which rats show high levels of social play behaviour, that is PND 21–42, augments alcohol consumption in adulthood. The increase in alcohol consumption was restricted to home-cage drinking as responding for alcohol under FR1 or PR schedules of reinforcement was not altered by early social isolation. Social isolation after weaning has been shown to enhance alcohol consumption in previous studies in rats and mice (Ellison, 1981; Schenk *et al.*, 1990; Wolffgramm, 1990; Hall *et al.*, 1998; Lodge and Lawrence, 2003; Advani *et al.*, 2007; Deehan *et al.*, 2007; McCool and Chappell, 2009; Sanna *et al.*, 2011; Chappell *et al.*, 2013; Butler *et al.*, 2014). Importantly, however, in these studies, the animals were reared in isolation from weaning onwards. As a result, it is not possible to discern whether the increase in alcohol consumption results from social isolation during alcohol consumption or is the consequence of neuroadaptive changes that are induced by social isolation during postweaning development. In support of the latter possibility, the present findings show that social isolation during a restricted time window (i.e. between PND 21 and 42) results in increased alcohol consumption in adulthood. This is in line with recent findings of augmented alcohol-induced conditioned place preference after early social isolation (Whitaker *et al.*, 2013). An important element of this study, and ours, is that the animals were resocialized for at least 5 weeks in-between the social isolation and the alcohol consumption or place conditioning tests. Together, these data identify the period between PND 21 and 42, which is characterized

by an abundance of social play behaviour (Panksepp, 1981), as a sensitive period for social isolation to augment alcohol reward in adulthood. Importantly, the increased sensitivity to alcohol reward (Whitaker *et al.*, 2013; this study) that results from early social isolation extends to other drugs of abuse as amphetamine-induced conditioned place preference and cocaine self-administration are also enhanced following social isolation during PND 21–42 (Whitaker *et al.*, 2013; Baarendse *et al.*, 2014). This suggests that social play experience serves to develop resilience to addictive behaviours in adulthood.

The isolation window between PND 21 and 42, followed by resocialization, was chosen to selectively prevent the animals from reaping the benefits of social play experience during postweaning development, without depriving them completely from social contact during development into adulthood. However, using this approach, it is not possible to specifically attribute the consequences of social isolation to the lack of social play. Previous studies indicate that the lack of social play is an important determinant of the consequences of social isolation during early postweaning development (Einon *et al.*, 1978). Thus, Juarez and Vazquez-Cortes (2003) showed that social isolation during PND 25–35 enhanced alcohol consumption, but not when rats were intermittently exposed to a social partner during the isolation period. Moreover, Whitaker *et al.* (2013) reported that the augmented alcohol-induced and cocaine-induced conditioned place preference after social isolation between PND 21 and 42 was not observed in animals isolated from PND 21–28 (with the possibility for substantial social play experience after PND 28) or from PND 42–63 (that had social play experience before a period of social isolation of similar length). Taken together, these findings suggest that it is indeed the experience gained during social play behaviour that serves to properly develop brain mechanisms that are important in reward processes (Whitaker *et al.*, 2013) or cognitive control over behaviour (Baarendse *et al.*, 2013) that protects against addictive behaviour in adulthood.

It is important to mention that the effects of early social isolation were independent of the individual variability in alcohol consumption. That is, they were present in low, medium as well as high alcohol-drinking rats. This implies that this social insult, that is the lack of social play behaviour during postweaning development, sums with other factors that determine individual levels of alcohol consumption in adulthood. Interestingly, studies by Ellison (1987) have reported a large degree of individual variation in the propensity to consume alcohol within colonies of rats, which correlated with several aspects of social behaviour, such as grooming, dominance, chasing and aggression, supporting the notion that social behaviour is an important factor contributing towards an individual's propensity for alcohol consumption. Interestingly, however, the effect of early social isolation on alcohol consumption and alcohol preference was restricted to the situation in which the animals had free access to alcohol in their home cages. The lack of an effect on operant responding for alcohol suggests that early social isolation impacts on the consummatory, perhaps hedonic aspects of alcohol reward, rather than the appetitive and incentive motivational properties of alcohol assessed in operant settings. Alternatively, during the operant sessions, animals could only earn relatively small amounts of alcohol, which may have obscured the effect of early social isolation, that is, socially vulnerable individuals are particularly at risk for enhanced alcohol consumption when large amounts of the substance are available.

In conclusion, our present data show that disruption of early social play interactions during postweaning development enhances the propensity to consume alcohol in adulthood, identifying early social experience as an important protective factor against excessive alcohol use.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

Advani T, Hensler JG, Koek W (2007). Effect of early rearing conditions on alcohol drinking and 5-HT1A receptor function in C57BL/6J mice. *Int J Neuropsychopharmacol* **10**:595–607.

Alwan H, Viswanathan B, Rousson V, Paccaud F, Bovet P (2011). Association between substance use and psychosocial characteristics among adolescents of the Seychelles. *BMC Pediatr* **11**:85.

American Psychiatric Association (2013). *Diagnostic and statistical manual of mental disorders*, 5th ed. Washington, DC: American Psychiatric Association.

Anacker AM, Loftis JM, Kaur S, Ryabinin AE (2011a). Prairie voles as a novel model of socially facilitated excessive drinking. *Addict Biol* **16**:92–107.

Anacker AM, Loftis JM, Ryabinin AE (2011b). Alcohol intake in prairie voles is influenced by the drinking level of a peer. *Alcohol Clin Exp Res* **35**:1884–1890.

Baarendse PJ, Counotte DS, O'Donnell P, Vanderschuren LJ (2013). Early social experience is critical for the development of cognitive control and dopamine modulation of prefrontal cortex function. *Neuropsychopharmacology* **38**:1485–1494.

Baarendse PJ, Limpens JH, Vanderschuren LJ (2014). Disrupted social development enhances the motivation for cocaine in rats. *Psychopharmacology (Berl)* **231**:1695–1704.

Bonin MF, McCreary DR, Sadava SW (2000). Problem drinking behavior in two community-based samples of adults: influence of gender, coping, loneliness, and depression. *Psychol Addict Behav* **14**:151–161.

Butler TR, Ariwodola OJ, Weiner JL (2014). The impact of social isolation on HPA axis function, anxiety-like behaviors, and ethanol drinking. *Front Integr Neurosci* **7**:102.

Calcagnetti DJ, Schechter MD (1992). Place conditioning reveals the rewarding aspect of social interaction in juvenile rats. *Physiol Behav* **51**:667–672.

Chappell AM, Carter E, McCool BA, Weiner JL (2013). Adolescent rearing conditions influence the relationship between initial anxiety-like behavior and ethanol drinking in male Long Evans rats. *Alcohol Clin Exp Res* **37** (Suppl 1): E394–E403.

Compton WM, Conway KP, Stinson FS, Colliver JD, Grant BF (2005). Prevalence, correlates, and comorbidity of DSM-IV antisocial personality syndromes and alcohol and specific drug use disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. *J Clin Psychiatry* **66**:677–685.

Cruz FC, Quadros IM, Planeta Cda S, Miczek KA (2008). Maternal separation stress in male mice: long-term increases in alcohol intake. *Psychopharmacology (Berl)* **201**:459–468.

Deehan GA Jr, Cain ME, Kiefer SW (2007). Differential rearing conditions alter operant responding for ethanol in outbred rats. *Alcohol Clin Exp Res* **31**:1692–1698.

Einon DF, Morgan MJ, Kibbler CC (1978). Brief periods of socialization and later behavior in the rat. *Dev Psychobiol* **11**:213–225.

Ellison G (1987). Stress and alcohol intake: the socio-pharmacological approach. *Physiol Behav* **40**:387–392.

Ellison GD (1981). A novel animal model of alcohol consumption based on the development of extremes of ethanol preference in colony-housed but not isolated rats. *Behav Neural Biol* **31**:324–330.

Fahlke C, Lorenz JG, Long J, Champoux M, Suomi SJ, Higley JD (2000). Rearing experiences and stress-induced plasma cortisol as early risk factors for excessive alcohol consumption in nonhuman primates. *Alcohol Clin Exp Res* **24**:644–650.

Goldstein RB, Dawson DA, Saha TD, Ruan WJ, Compton WM, Grant BF (2007). Antisocial behavioral syndromes and DSM-IV alcohol use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Alcohol Clin Exp Res* **31**:814–828.

Hall FS, Huang S, Fong GW, Pert A, Linnoila M (1998). Effects of isolation-rearing on voluntary consumption of ethanol, sucrose and saccharin solutions in Fawn Hooded and Wistar rats. *Psychopharmacology (Berl)* **139**:210–216.

Higley JD, Hasert MF, Suomi SJ, Linnoila M (1991). Nonhuman primate model of alcohol abuse: effects of early experience, personality, and stress on alcohol consumption. *Proc Natl Acad Sci USA* **88**:7261–7265.

Hodos W (1961). Progressive ratio as a measure of reward strength. *Science* **134**:943–944.

Homish GG, Leonard KE (2008). The social network and alcohol use. *J Stud Alcohol Drugs* **69**:906–914.

Huggins KN, Mathews TA, Locke JL, Szeliga KT, Friedman DP, Bennett AJ, Jones SR (2012). Effects of early life stress on drinking and serotonin system activity in rhesus macaques: 5-hydroxyindoleacetic acid in cerebrospinal fluid predicts brain tissue levels. *Alcohol* **46**:371–376.

Humphreys AP, Einon DF (1981). Play as a reinforcer for maze-learning in juvenile rats. *Anim Behav* **29**:259–270.

Juarez J, Vazquez-Cortes C (2003). Alcohol intake in social housing and in isolation before puberty and its effects on voluntary alcohol consumption in adulthood. *Dev Psychobiol* **43**:200–207.

Koob GF, Kenneth Lloyd G, Mason BJ (2009). Development of pharmacotherapies for drug addiction: a Rosetta stone approach. *Nat Rev Drug Discov* **8**:500–515.

Lau-Barraco C, Braitman AL, Leonard KE, Padilla M (2012). Drinking buddies and their prospective influence on alcohol outcomes: alcohol expectancies as a mediator. *Psychol Addict Behav* **26**:747–758.

Lodge DJ, Lawrence AJ (2003). The effect of isolation rearing on volitional ethanol consumption and central CCK/dopamine systems in Fawn-Hooded rats. *Behav Brain Res* **141**:113–122.

Mason WA, Hollis JH, Sharpe LG (1962). Differential responses of chimpanzees to social stimulation. *J Comp Physiol Psychol* **55**:1105–1110.

McCool BA, Chappell AM (2009). Early social isolation in male Long-Evans rats alters both appetitive and consummatory behaviors expressed during operant ethanol self-administration. *Alcohol Clin Exp Res* **33**:273–282.

Nylander I, Roman E (2013). Is the rodent maternal separation model a valid and effective model for studies on the early-life impact on ethanol consumption? *Psychopharmacology (Berl)* **229**:555–569.

O'Brien CP (2008). Review. Evidence-based treatments of addiction. *Philos Trans R Soc Lond B Biol Sci* **363**:3277–3286.

Panksepp J (1981). The ontogeny of play in rats. *Dev Psychobiol* **14**:327–332.

Panksepp J, Siviy S, Normansell L (1984). The psychobiology of play: theoretical and methodological perspectives. *Neurosci Biobehav Rev* **8**:465–492.

Pellis S, Pellis V (2009). *The playful brain: venturing to the limits of neuroscience*. Oxford: Oneworld.

Perkins HW (2002). Social norms and the prevention of alcohol misuse in collegiate contexts. *J Stud Alcohol Suppl* **14**:164–172.

Pierce RC, O'Brien CP, Kenny PJ, Vanderschuren LJ (2012). Rational development of addiction pharmacotherapies: successes, failures, and prospects. *Cold Spring Harb Perspect Med* **2**:a012880.

Rehm J, Mathers C, Popova S, Thavorncharoensap M, Teerawattananon Y, Patra J (2009). Global burden of disease and injury and economic cost attributable to alcohol use and alcohol-use disorders. *Lancet* **373**:2223–2233.

Richardson NR, Roberts DC (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods* **66**:1–11.

Ritz MC, Garcia JM, Protz D, Rael AM, George FR (1994). Ethanol-reinforced behavior in P, NP, HAD and LAD rats: differential genetic regulation of reinforcement and motivation. *Behav Pharmacol* **5** (4–5):521–531.

Rodd ZA, Bell RL, Kuc KA, Murphy JM, Lumeng L, Li TK, McBride WJ (2003). Effects of repeated alcohol deprivations on operant ethanol self-administration by alcohol-preferring (P) rats. *Neuropsychopharmacology* **28**:1614–1621.

Roman E, Gustafsson L, Hyytia P, Nylander I (2005). Short and prolonged periods of maternal separation and voluntary ethanol intake in male and female ethanol-preferring AA and ethanol-avoiding ANA rats. *Alcohol Clin Exp Res* **29**:591–601.

Sanna E, Talani G, Obili N, Mascia MP, Mostallino MC, Secci PP, *et al.* (2011). Voluntary ethanol consumption induced by social isolation reverses the increase of alpha(4)/delta GABA(A) receptor gene expression and function in the hippocampus of C57BL/6J mice. *Front Neurosci* **5**:15.

Schenk S, Gorman K, Amit Z (1990). Age-dependent effects of isolation housing on the self-administration of ethanol in laboratory rats. *Alcohol* **7**:321–326.

Simms JA, Steensland P, Medina B, Abernathy KE, Chandler LJ, Wise R, Bartlett SE (2008). Intermittent access to 20% ethanol induces high ethanol consumption in Long-Evans and Wistar rats. *Alcohol Clin Exp Res* **32**:1816–1823.

Siviy SM, Panksepp J (2011). In search of the neurobiological substrates for social playfulness in mammalian brains. *Neurosci Biobehav Rev* **35**:1821–1830.

Stickley A, Koyanagi A, Roberts B, Richardson E, Abbott P, Tumanov S, McKee M (2013). Loneliness: its correlates and association with health behaviours and outcomes in nine countries of the former Soviet Union. *PLoS One* **8**:e67978.

Trezza V, Baarendse PJ, Vanderschuren LJ (2009). Prosocial effects of nicotine and ethanol in adolescent rats through partially dissociable neurobehavioral mechanisms. *Neuropsychopharmacology* **34**:2560–2573.

Trezza V, Baarendse PJ, Vanderschuren LJ (2010). The pleasures of play: pharmacological insights into social reward mechanisms. *Trends Pharmacol Sci* **31**:463–469.

Trezza V, Campolongo P, Vanderschuren LJ (2011). Evaluating the rewarding nature of social interactions in laboratory animals. *Dev Cogn Neurosci* **1**:444–458.

Trezza V, Baarendse PJ, Vanderschuren LJ (2014). On the interaction between drugs of abuse and adolescent social behavior. *Psychopharmacology (Berl)* **231**:1715–1729.

van den Brink W (2012). Evidence-based pharmacological treatment of substance use disorders and pathological gambling. *Curr Drug Abuse Rev* **5**:3–31.

Vanderschuren LJ, Niesink RJ, Van Ree JM (1997). The neurobiology of social play behavior in rats. *Neurosci Biobehav Rev* **21**:309–326.

Varlinskaya EI, Spear LP (2002). Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol Clin Exp Res* **26**:1502–1511.

Varlinskaya EI, Spear LP, Spear NE (2001). Acute effects of ethanol on behavior of adolescent rats: role of social context. *Alcohol Clin Exp Res* **25**:377–385.

Varlinskaya EI, Truxell EM, Spear LP (2015). Sex differences in sensitivity to the social consequences of acute ethanol and social drinking during adolescence. *Behav Brain Res* **282**:6–13.

Whelan R, Watts R, Orr CA, Althoff RR, Artiges E, Banaschewski T, *et al.* (2014). Neuropsychosocial profiles of current and future adolescent alcohol misusers. *Nature* **512**:185–189.

Whitaker LR, Degoulet M, Morikawa H (2013). Social deprivation enhances VTA synaptic plasticity and drug-induced contextual learning. *Neuron* **77**:335–345.

Wise RA (1973). Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia* **29**:203–210.

Wolffgramm J (1990). Free choice ethanol intake of laboratory rats under different social conditions. *Psychopharmacology (Berl)* **101**:233–239.

World Health Organization (2011). Global status report on alcohol and health. Geneva, Switzerland: World Health Organization.