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NEUROPHYSIOLOGICAL ASPECTS

Impairments of Prepulse Inhibition of the Startle Response in Abstinent Alcoholic Male Patients

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Abstract — Aims: Prepulse inhibition (PPI) of the startle reflex, which refers to the ability of innocuous sensory events to reduce the startle reflex, has been described as an operational measure of sensorimotor gating that is reduced in several neuropsychiatric disorders, such as schizophrenia, but experience is lacking in addictions and alcoholism. The aim of this study was to examine the existence of impairments in the startle response and PPI in abstinent alcoholic men. **Methods:** Testing for PPI was conducted on 60 abstinent alcoholic men aged 18–65 years (mean 46.37) who met DSM-IV criteria for alcohol dependence and had been abstinent for more than a month at the time of testing. The comparison group were compared with 37 sex- age- and education-matched controls without alcohol dependence. **Results:** Magnitudes of the startle reflex were lower in patients than in controls. The differences were statistically significant ($P < 0.05$) in trials with prepulses presented 30 and 120 ms before the onset of the startle stimulus. There was also a statistically significant ($P < 0.05$) reduced percentage of PPI when the prepulse was presented 30 ms before the startle stimulus. **Conclusions:** These data suggest that sensory information processing mechanisms could be damaged in abstinent alcoholic patients. The fact that these findings are common to other psychiatric disorders could indicate the existence of a common vulnerability marker and explain the high degree of comorbidity between alcoholism and other mental illnesses.

INTRODUCTION

In recent years, the addictive processes associated with alcohol dependence have been studied using different approaches (Grillon *et al*., 2000). There is a growing interest in neuropsychological and neurophysiological paradigms in the field of addictions, and more specifically in alcoholism (Grillon *et al*., 1997, 2000; Keedwell *et al*., 2001). In fact, several vulnerability markers for the development of alcoholism, such as reduced P300 amplitude (Begleiter *et al*., 1984; Hill *et al*., 1995) and impairments of the startle reflex (Grillon *et al*., 1997) have been already described.

Modulation of the startle reflex, which essentially measures the amplitude of the eye-blink, is a well-replicated phenomenon that has received considerable experimental scrutiny (Donohue *et al*., 2007). Startle reflex is a defensive response to a sudden burst of white noise. It can also be elicited by tactile and visual intense stimuli. The acoustic startle response (ASR) is mediated by a relatively simple neuronal circuit located in the low brainstem (Koch, 1999). It is easily measured in humans by recording its most consistent and persistent component, the amplitude of the eye blink (Landis and Hunt, 1939). The ASR has been used as a measure of behavioural reactivity to external stimuli (Morgan *et al*., 1993). It shows different forms of plasticity including prepulse inhibition (PPI) and habituation. The PPI of the startle reflex refers to the ability of innocuous sensory events presented at an appropriate time (<250 ms) before a startle-eliciting stimulus to inhibit or reduce the startle reflex (Braff *et al*., 1992). It has been described as an operational measure of sensorimotor gating that is reduced in several neuropsychiatric disorders such as schizophrenia (Swerdlow *et al*., 2006) and depression or anxiety disorders (Grillon *et al*., 1997; Mneime *et al*., 2008), but studies are lacking in the area of addictions and alcoholism.

The majority of studies in which PPI has been assessed in the field of alcoholism are experimental animal models and can be grouped into three categories. The first category is prenatal (Potter and Berntson, 1987) and early postnatal (Woolfey *et al*., 2005) alcohol exposure, in which it has been seen that alcohol administration does not affect PPI in rats. The second category is acute or chronic exposure to alcohol, in which different studies have evaluated the magnitude of the startle reflex and PPI as measures of alcohol withdrawal following acute (Wecker and Ison, 1984) and chronic (Pohorecky *et al*., 1976; Gilliam and Collins, 1986; Pahorecky and Roberts, 1991; Rassnick *et al*., 1992; Macey *et al*., 1996; Vandergriff *et al*., 2000; Slawecki and Ehlers, 2005; Slawecki *et al*., 2006) alcohol treatment. Most of the studies in this area have demonstrated that both acute (Brunell and Spear, 2006; Pahorecky *et al*., 1976; Owens *et al*., 2003) and chronic (Rassnick *et al*., 1992) exposure to alcohol reduces the magnitude of the startle response. PPI impairments due to chronic alcohol exposure have also been observed in rats (Rassnick *et al*., 1992), although a recent study did not confirm these results (Slawecki *et al*., 2006). The third category includes studies with rodents selectively bred for alcohol preference and non-preference. These have shown an increase in the magnitude of the startle response (Jones *et al*., 2000; Chester *et al*., 2003, 2004, 2005; Chester and Barrenha, 2007) in alcohol-preferring rodents compared with non-preferring lines. The results for PPI, however, were inconclusive. The study by Jones *et al*. (2000) showed that alcohol-preferring rats exhibited a significant disruption in PPI levels after a low dose of alcohol, but other studies have found no significant differences between alcohol preferring and non-preferring animals in this respect (Sandbak *et al*., 1999; Chester and Barrenha, 2007).

Although the majority of studies in humans have been performed using other startle methodologies such as the affective modulation of the startle response, we focus on studies

involving the PPI paradigm of the startle reflex. Studies assessing startle response and PPI in humans can be grouped in several categories. The first category includes studies that assess how alcohol affects baseline startle and PPI in healthy subjects. These studies demonstrated a global suppression of the startle response (Grillon *et al.*, 1994; Hutchison *et al.*, 1997). However, regarding PPI, results are inconclusive, with one study showing no significant changes in PPI after alcohol intake in healthy subjects (Grillon *et al.*, 1994), and the other concluding that exposure to a low dose of alcohol reduces PPI levels in patients with low baseline PPI and increases PPI levels in patients with high baseline PPI (Hutchison *et al.*, 1997). In a recent study, Hutchinson *et al.* have demonstrated that alcohol decreases both the magnitude of the startle response and PPI in a sample of heavy drinkers. Moreover, they found that actively attending to a prepulse stimulus increases PPI and that actively attending to a startle stimulus increases startle magnitude in this sample, without a significant interaction between alcohol and attention (Hutchison *et al.*, 2003). The second category includes studies that investigate modifications of the startle response and PPI during alcohol withdrawal.

In a study of the modulation of yohimbine and m-chlorophenylpiperazine in recently detoxified alcohol-dependent individuals (Krystal *et al.*, 1997), a relationship between startle amplitude and the number of previous detoxifications was observed. Furthermore, studies of patients experiencing the alcohol withdrawal syndrome have detected an increase in the startle response (Howard and Ford, 1992) and significantly decreased PPI (Keedwell *et al.*, 2001). In the study by Keedwell *et al.*, PPI levels reached their lowest point on the first and third day of abstinence and increased progressively after the first week. These changes were most apparent in patients with a history of delirium tremens.

The last category includes studies of startle response and PPI as vulnerability markers for the development of alcoholism and focus on the offspring of alcoholics. In an initial study, Grillon *et al.* (1997) demonstrated impaired PPI and habituation in children with a family history of alcoholism. In a later study of the effect of alcohol drinking on the startle reflex in non-abusing young male offspring of alcoholics, Grillon *et al.* (2000) found that the startle magnitude was reduced to a lesser degree in the offspring of alcoholics and that PPI was reduced in the offspring of alcohol-dependent individuals compared with a control group of offspring with no parental history of psychiatric disorders. These findings suggest that the startle reactivity and PPI impairments could constitute a vulnerability marker for alcoholism (Grillon *et al.*, 1997, 2000).

While a wide variety of studies have assessed the startle response and PPI in alcoholism, to our knowledge no studies have yet examined whether abstinent alcoholic patients exhibit impairments in PPI compared with healthy controls. Because it has been hypothesized that such impairments may serve as endophenotypes in other psychiatric disorders such as schizophrenia, we believed it would be interesting to assess whether these deficits were present in alcohol-dependent patients.

The aim of the present study was to verify whether abstinent alcoholic patients exhibited a reduced startle response and impairments in PPI compared with healthy controls. Our hypotheses were the following: (1) alcohol-dependent patients will exhibit a lower reactivity of the startle response compare with controls; (2) percentage of the PPI will be reduced among alcoholic patients compared with healthy controls and (3) abstinent alcoholics will show a lower startle habituation compared with controls.

METHODS

Patients

Patients were consecutively recruited from the Outpatient Alcohol Programme of the teaching hospital 'Hospital 12 de Octubre' in Madrid, Spain from January 2008 to September 2009. Altogether, 97 men aged between 26 and 65 years (mean 45.44, SD 8.04), were included.

Inclusion criteria were the following: men, aged between 18 and 65 years, who had met DSM-IV criteria for alcohol dependence (APA, 2000), and who were abstinent for alcohol for at least one month. Females were not included because gender differences in the performance of the startle test could constitute a confounding variable. Indeed, it has already been found that women exhibit less startle response compared with men (Kofler *et al.*, 2001), they show less PPI and there are also variations according to the menstrual cycle (Aasen *et al.*, 2005; Kumari *et al.*, 2008).

Patients were excluded if they were under 18 or over 65 years of age, had a systemic or neurological disease which could interfere with coping strategies, an associated neuropsychological deficit, an IQ of under 70, or met criteria for a current major psychiatric disorder such as schizophrenia and other psychotic disorders, affective disorders, obsessive compulsive disorder and anxiety disorders, a hearing or visual impairment which might interfere with the conduct of the experiment, or a score of 15 on the Hospital Anxiety and Depression (HAD) scale (Zigmond and Snaith, 1983) as this indicates a greater likelihood of stress responses. Participants were also excluded if they had consumed alcohol in the last month. Due to the difficulties in recruiting patients with just alcohol dependence, a history of substance use disorders and occasional intake of cannabis were not considered an exclusion criterion. However, patients with a chronic consumption of cannabis or any current consumption of drugs such as cocaine or heroin were excluded, as these drugs have been associated with modifications of the startle response and PPI (Scholes and Martin-Iverson, 2009; Corcoran *et al.*, 2011; Walter *et al.*, 2011). Because most of our patients were undergoing pharmacological treatment at the moment they were tested, specifically with benzodiazepines, anticonvulsant agents, antidepressant, naltrexone or disulfiram, psychopharmacological agents were not considered as an exclusion criterion, with the exception of antipsychotics, as it has been widely described that these agents influence startle responses and PPI (Martinez-Gras *et al.*, 2009; Kishi *et al.*, 2010).

Four patients (4.12%) were excluded because they fulfilled DSM-IV criteria for other psychiatric disorders, three patients (3.09%) were excluded because of cocaine abuse or dependence, five patients (5.15%) were excluded because they had been abstinent for alcohol for <1 month, two patients (2.06%) were excluded because they met DSM-IV criteria for alcohol abuse but not alcohol dependence, and three patients (3.09%) were excluded because they had a positive breath alcohol test before the experiment. In addition, 22

patients (22.68%) were excluded due to artefacts registered during the psychophysiological recordings.

The final sample comprised 60 men fulfilling current DSM-IV criteria for alcohol dependence (American Psychiatric Association, 2000). All the patients had been abstinent for alcohol for at least 1 month. The mean length of education was 15.68 years (SD 2.62); 55% of the sample were married and 10% were unemployed. The clinical and demographic characteristics of the patients are summarized in Table 1.

The final sample ($n = 60$) was compared with a control group ($n = 37$) matched for sex, age and years of education. The study was approved by the local ethics committee and all the patients signed an informed consent form.

Controls

The patients were compared with 37 volunteer healthy male controls (mean age 40.39, SD 8.38), mainly recruited from among health-care professionals working at the hospital. The exclusion criteria for the control group were a systemic, neurological or psychiatric disorder, an IQ of under 70, a hearing or visual impairment which could interfere with the conduct of the test, meeting criteria for a major psychiatric disorder such as schizophrenia and other psychotic disorders, affective disorders, obsessive compulsive disorder and anxiety disorders, a history of psychiatric disease in first-degree relatives (because impairments have been found in the startle response and PPI in subjects with a positive family history of a psychiatric disease, even though they have not developed the disorder themselves (Grillon *et al*., 1997, 2000; Zimmermann *et al*., 2004), current use of psychotropic medication, and a drug abuse/dependence disorder. Twelve of the 49 controls initially assessed could not complete the test because of artefacts registered during physiological recordings.

Procedure

All the patients were screened with a portable audiometer (AudioScope 3. Welch Allyn WA®) for hearing disabilities which could interfere with the conduct of the experiment.

Questionnaires completed on initiation of the alcoholism treatment programme at our unit were available for all the patients. After the collection of demographic, social and clinical variables, the patients completed the Structured Clinical Interview for DSM-IV and the Patient Questionnaire (SCID PQ) for the Structured Clinical Interview for DSM-IV Personality Disorders (SCID-P and SCID-II; Firsts *et al*., 1995, 1997) with a psychiatrist. They were also administered the HAD scale to detect severe anxiety and depressive states which could interfere with the results of the study. To assess IQ, the second and third scale of the Cattell Test were used (Cattell and Cattell, 1994).

Subjects were tested with the ASR paradigm in a room run by the Psychiatry Department of our hospital that is specially prepared for this test and is protected against interference from external factors such as environmental noise or non-neutral visual stimulation.

To prevent interference from nicotine consumption-abstinence, smokers were told to smoke during the morning on which the test was to be conducted (to prevent nicotine withdrawal) but to smoke the last cigarette no later than one hour before the test (to prevent acute effects of nicotine on neuropsychological capacities) (Domier *et al*., 2007; Potter and Newhouse, 2008). They were also instructed to abstain from all food and liquids other than water for four hours prior to the experimental session. A breath alcohol test was used to verify alcohol abstinence and a urine drug test was used to check for drug consumption.

Startle response measurement

The startle reflex was elicited and recorded with a commercial computerized human startle response monitoring system (CIBERTEC S.A). Acoustic startle stimuli (pulses and prepulses) were presented binaurally through headphones. Four types of startle stimuli were used: a pulse-alone stimulus of 100 dB white noise presented for 40 ms and three prepulse 30 ms stimuli of 30 dB white noise presented 30, 60, and 120 ms before the pulse. All the stimuli were presented against a continuous background noise of 65 dB. The interstimulus interval was 20 ms ± 2. Previous studies have demonstrated that a 60 dB prepulse preceding a 95 dB pulse reduces the startle response magnitude by 50% (Blumenthal *et al*., 1996).

The eye-blink component of the startle response was measured by recording the electromyographic (EMG) activity of the orbicularis oculi muscle directly beneath the right eye using two miniature silver/silver chloride disk electrodes. The ground electrode was placed on the forehead. Impedance level was kept below 5 kohm. The startle system recorded EMG activity for 250 ms from the onset of the startle stimulus. EMG activity was band-pass filtered (low- and high-pass filters of 5 kHz and 1 Hz, respectively), with a 50-Hz notch

Table 1. Demographic and clinical variables of patients and controls

	Patients ($n = 60$)		Controls ($n = 37$)			
Variables	Mean	SD	Mean	SD	t (97)	P-value
Age (years)	46.37	8.44	40.39	8.38	−3.28	0.01
Age of alcohol use initiation (years)	13.90	3.67	18.89	2.81	7.09	<0.001
Age at which dependence criteria were met	31.62	8.63	0	0	−22.23	<0.001
Time of abstinence (months)	25.65	43.83	0	0	−3.55	<0.001
Previous detoxifications (n)	1.89	2.46	0	0	−4.64	<0.001
Alcohol intake (grams/day)	194.08	100.32	12.43	9.76	−10.96	<0.001
	(n)	%	(n)	%	Chi	P-value
Nicotine dependence	35	(58.3%)	11	(29.7%)	13.39	<0.01
Cannabis dependence	1	(1.7%)	0	(0%)	3.94	NS

filter used to eliminate 50 Hz interference. We used a sampling rate of 1000 Hz and a temporal window for startle measurement of 1 s after the startle. EMG data were stored off-line in the analytical program of the response monitoring system.

Patients were told that brief loud startling sounds would be delivered through the headphones and were asked to keep their eyes open during the test and to avoid moving.

The methodology used in the startle session was consistent with previous studies (Braff *et al.*, 1992; Martínez-Gras *et al.*, 2009). The session began with a 5-min acclimatization period to reduce initial reactivity and familiarize the participants with the test. The four kinds of startle stimuli previously described were presented in a pseudo-random order such that patients would be unable to anticipate the next trial. The experiment consisted of three blocks: (a) 5 pulse-alone trials; (b) 32 pulse-alone and prepulse-pulse trials with a 30, 60 and 120 ms prepulse-to-pulse interval and (c) 5 pulse-alone trials. A total of 42 trials were conducted in each experiment, and the inter-trial interval averaged 15 s (range: 10–25 s). It lasted over 15 min.

Startle variables

The startle variables considered for our study were: (a) startle responsivity, defined as the amplitude of the startle response to pulse-alone trials (in digital units); (b) PPI, computed as the percentage decrement in startle amplitude in the presence vs the absence of the prepulse and calculated as the difference of the average startle response magnitude in pulse-alone trials minus the magnitude of the average startle response in prepulse trials divided by the magnitude in the pulse-alone trials [% PPI = (pulse – prepulse)/pulse × 100] and (c) startle habituation, measured as the decrease in the amplitude of the startle response throughout the session and calculated as the difference of the average startle response magnitude of pulse-alone trials between the first and last block.

Statistical analysis

The statistical analysis was performed using descriptive statistics for demographic variables and the independent-samples *t*-test for analysing the startle response paradigms. In the descriptive statistics, qualitative variables were described as absolute frequencies and relative percentages for each category, whereas quantitative variables were calculated using means and SDs. To analyse startle response paradigms, the independent variable was the group to which the participants belonged (abstinent alcoholics or controls) and the dependent variables were the startle response magnitude in pulse-alone and prepulse-pulse trials, PPI and startle habituation. We also used Pearson's correlation coefficient to assess correlation between baseline startle and PPI (30, 60 and 120 prepulse trials). Repeated measures analysis of variance (ANOVA) were used to explore the differences in PPI functioning between controls and patients with prepulse types (30, 60, 120 prepulse trials) as the within-subject variable, and the two different groups (patients and controls) as the between-subjects factor. We used the variable of age as covariable, in order to control the potential effect of this parameter on PPI functioning. Data were processed with the statistical computer program SPSS (version 11.5).

RESULTS

Prepulse inhibition

Percentages of PPI exhibited by patients were lower than those exhibited by controls at 30, 60 and 120 ms but the differences were only statistically significant ($P < 0.05$) at 30 ms, although there was a trend towards significance at 120 ms ($P < 0.1$) (Fig. 1). We did not find a statistically significant correlation between PPI and previous number of detoxifications (PPI 30 ms: $R = 0.19$; PPI 60 ms: $R = 0.07$; PPI 120 ms: $R = 0.8$), length of abstinence (PPI 30 ms: $R = –0.11$; PPI 60 ms: $R = –0.1$; PPI 120 ms: $R = –0.31$) or lifetime duration of alcohol use (PPI 30 ms: $R = 0.11$; PPI 60 ms: $R = 0.14$; PPI 120 ms: $R = 0.19$). Regarding PPI, ANOVA only demonstrated a significant effect of group × intervals interactions at 30 ms ($F_{(1,83)} = 6.146$, $P = 0.015$). Bonferroni *post hoc* analysis determined that at 30 ms, controls showed higher percentages of inhibition than patients ($P = 0.015$). However, we did not find statistically significant differences within the other PPI measures (Table 2).

Magnitude of the startle response

The magnitude of the startle response in abstinent alcoholic patients compared with controls was reduced throughout the three blocks in both the pulse-alone and prepulse-pulse trials. These parameters showed a trend towards significance ($P < 0.1$) or reached statistical significance ($P < 0.05$) in the second block. On analysing the sample according to the

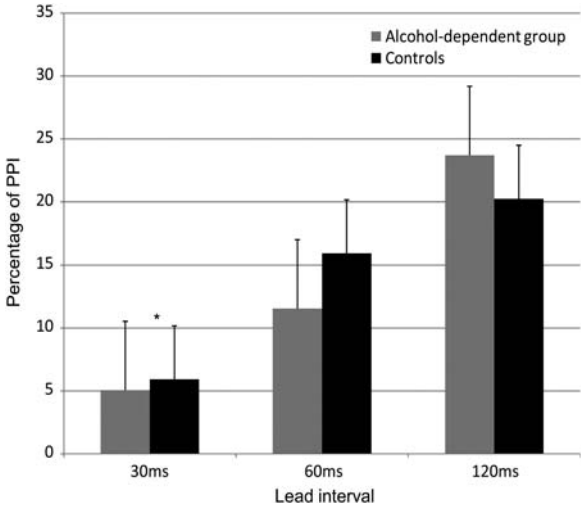

Fig. 1. Percentage of PPI for the 30, 60 and 120 ms prepulse-pulse stimuli in patients compared with controls.

Table 2. Mean (SD) PPI percentage to the 30, 60 and 120 prepulse-to-pulse trials

	Subjects		Controls	
Prepulse-to-pulse interval	Mean	SD	Mean	SD
30 ms	11.21	0.54	19.66	17.03
60 ms	17.63	24.76	20.54	25.36
120 ms	15.59	33.04	26.56	32.82

Table 3. Mean magnitude of the startle response

	Patients ($n = 60$)		Controls ($n = 37$)			
	Mean	SD	Mean	SD	t (97)	P-value
Block 1						
Pulse-alone	0.36	0.45	0.44	0.54	0.80	NS
Block 2						
Pulse-alone	0.24	0.25	0.39	0.57	1.75	NS
30 ms prepulse-pulse	0.19	0.21	0.35	0.56	1.95	<0.05
60 ms prepulse-pulse	0.18	0.22	0.32	0.56	1.67	NS
120 ms prepulse-pulse	0.16	0.19	0.32	0.56	2.08	<0.05
Block 3						
Pulse-alone	0.22	0.31	0.35	0.57	1.39	NS

presence of a history of major depression ($n = 24$) or not ($n = 46$), we found that those with a history of depression had a milder startle response, although the differences were not significant ($P = 0.14$) (Table 3).

Habituation

Habituation was reduced in abstinent alcoholic patients (mean 0.14, SD 0.29) compared with controls (mean 0.09, SD 0.19), but the differences were not significant ($P = 0.43$).

Correlations

PPI scores did not correlate with baseline startle (PPI 30 ms: $R = 0.04$; PPI 60 ms: $R = 0.13$; PPI 120 ms: $R = 0.22$).

DISCUSSION

To the best of our knowledge, this is the first study to assess PPI variables in abstinent alcohol-dependent men. The most relevant findings were that these patients exhibited a reduction in PPI and startle response magnitude compared with controls.

Our results for PPI percentages are in agreement with those reported by studies conducted in the offspring of alcoholics (Grillon *et al*., 1997, 2000) and heavy drinkers (Hutchison *et al*., 2003), and suggest that impairments in PPI might constitute a vulnerability marker for the development of alcoholism. However, caution should be exercised when interpreting these results as they could be due to the toxic effect of alcohol on the central nervous system (CNS) (Hutchison *et al*., 1997, 2003).

PPI has been described as an operational measure of sensorimotor gating (Braff *et al*., 1992), which reduces the impact of irrelevant sensorial stimuli. It is also considered an index of a centrally mediated inhibitory mechanism that regulates not only sensory but also motor and cognitive operations (Swerdlow *et al*., 1992). Consequently, these data suggest that sensory information processing mechanisms could be impaired in abstinent alcoholic patients, who, as a result, would possibly have greater difficulty in suppressing or gating irrelevant information. In such a case, these patients would also have greater difficulty in restraining behavioural automatisms involved in relapse as well as higher stress levels, which would mean a greater tendency to drink to relieve symptoms.

The fact that the PPI impairments detected appeared in 30-ms interval prepulse-to-pulse trials could be related to the existence of impaired pre-attentive mechanisms, as it has been reported that prepulses presented at 30 ms before a pulse are implicated in pre-attentive processes, whereas those presented at 120 ms are related to attentive operational measures (Braff *et al*., 1992).

We also found a decrease in the magnitude of the startle response in abstinent alcoholic patients. Previous studies have reported highly heterogeneous results in this respect for both animal models and humans. Whereas some studies have reported a decrease in ASR during alcohol withdrawal in animal models (Gilliam and Collins, 1986; Chester *et al*., 2005; Slawecki and Ehlers, 2005; Slawecki *et al*., 2006; Chester and Barrenha, 2007), others have reported the contrary (Pahorecky *et al*., 1976; Pahorecky and Roberts, 1991; Macey *et al*., 1992; Rassnick *et al*., 1992; Vandergriff *et al*., 2000). In studies of humans, Grillon *et al*. (1997) found no differences between the offspring of alcoholic patients and controls with respect to the magnitude of the startle response. However, Hutchison *et al*. (2003) demonstrated that alcohol decreased the magnitude of the startle response in a sample of heavy drinkers (Hutchison *et al*., 2003).

The fact that, unlike authors who have studied the offspring of alcoholic patients, but according to authors who have studied heavy drinkers, we found a decrease in the magnitude of the startle response could be because our patients already had impaired measures and a high risk of developing alcohol dependence (they were all alcohol dependent) or because they developed impairments due to repeated alcohol consumption.

A reduced magnitude of the startle response could be, in this way, due to a characteristic of alcohol-dependent patients or because of anhedonia and depressive symptoms that alcoholic patients often exhibit. Alcohol-dependent patients frequently state that their daily affective life is not very stimulating. A recent study of affective ratings and startle modulation reported low baseline startle in individuals with depressive symptoms and anhedonia (Mneime *et al*., 2008). This association has been reported to be highly heritable (Anokhin *et al*., 2003, 2007), suggesting that baseline startle may be a good candidate as an endophenotypic marker of vulnerability to psychiatric disorders.

In our study, individuals with a psychiatric history of depression exhibited lower startle reactivity than those without such a history, although the differences did not reach statistical significance.

Finally, we found reduced habituation in abstinent alcoholic patients compared with controls, although the differences were not significant. These results are in line with findings by Grillon *et al*. (1997), who reported that children with a family history of alcoholism exhibited a reduced startle habituation compared with controls. This lower habituation shown by our patients could reflect impaired inhibitory mechanisms, as discussed above.

In summary, the aim of this study was to investigate the existence of impairments in PPI and ASR amplitude in abstinent alcoholic patients to explore in subsequent studies whether or not such impairments might constitute a vulnerability marker for the development of alcoholism, as has previously been described. We have found that alcohol-dependent individuals are characterized by a poor inhibitory control, as reflected by

a reduced PPI, and a poor response to threaten stimulus, as reflected by a lower magnitude of the startle response. Our results should be interpreted cautiously because reduced PPI has also been reported in several psychiatric disorders. Indeed, the fact that reduced PPI levels are found in other psychiatric disorders could indicate the existence of a common vulnerability marker and possibly explain the high degree of comorbidity between alcoholism and other mental illnesses.

Several limitations of this study should be noted. Firstly, as already mentioned, it is difficult to determine whether the impairments in PPI and ASR amplitude found in abstinent alcoholic patients preceded alcohol consumption, which would support a vulnerability marker for the development of alcoholism as previously reported (Grillon *et al*., 1997, 2000), or were indeed due to chronic alcohol intake, which would exert a toxic effect on the CNS. Secondly, this was an exploratory study involving just a small sample. The exclusion criteria used were also very broad, leading to considerable heterogeneity in parameters such as age, abstinence length, time since onset of alcohol dependence or history of grams of alcohol intake. Finally, several confounding variables, such as nicotine dependence and a history of other substance use disorders, might have interfered with the results reported.

Further studies are necessary in this area to shed light on the use of these paradigms as specific vulnerability markers for the development of alcoholism or as an endophenotype shared with other psychiatric disorders. It would be also useful to study in depth whether these paradigms could be used as markers of the severity of alcohol dependence or of chronic alcohol-related cognitive deficits. To that effect, we intend to conduct new studies with larger and more homogeneous samples and to take into account variables such as age, concomitant intake of drugs, medicaments, abstinence length, time since onset of alcohol dependence and history of alcohol consumption.

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