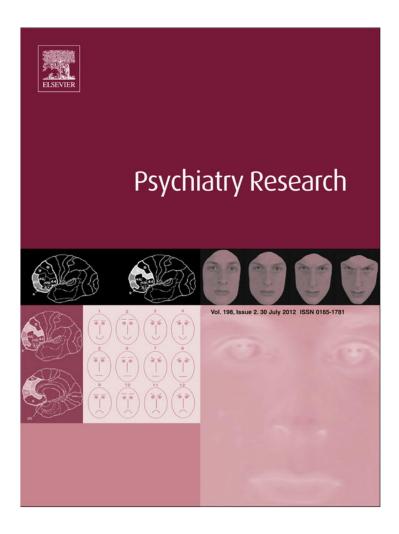
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Investigation of impulsivity in a sample of treatment-seeking pathological gamblers: A multidimensional perspective

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ABSTRACT

Numerous studies have shown that problem gambling is characterised by lack of impulse control. However, they have often been conducted without considering the multifaceted nature of impulsivity and related psychological mechanisms. The current study aims to disentangle which impulsivity facets are altered in pathological gambling. Twenty treatment-seeking pathological gamblers (PGs) and 20 matched control participants completed a self-reported questionnaire measuring the various facets of impulsive behaviours (UPPS Impulsive Behaviour Scale), as well as two laboratory tasks assessing inhibitory control (the go-stop task) and tolerance for delayed rewards (single key impulsivity paradigm). Compared with matched controls, PGs exhibited higher urgency, lower premeditation, impairment in prepotent inhibition, and lower tolerance towards delayed rewards. Nevertheless, complementary profile analyses showed that impulsivity-related deficits found in PGs are highly heterogeneous, and that some PGs are neither impulsive in the impulsivity facets assessed nor impaired in the cognitive mechanisms measured. These findings underscore (1) the necessity to disentangle the construct of impulsivity into lower-order components and (2) that further studies should take into account, in addition to impulsivity-related mechanisms, other psychological factors potentially involved in pathological gambling.

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1. Introduction

When included in the third edition of the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-III*) (American Psychiatric Association, 1980), pathological gambling was defined as an impulse control disorder. Pathological gambling involves an inability to resist overwhelming and irrational gambling-related drives, resulting in adverse consequences in daily life (Raylu and Oei, 2002). The nosography of pathological gambling has been debated, mainly because of its high comorbidity with substance use disorders (Petry et al., 2005). Nowadays, pathological gambling is increasingly considered as part of an addictive spectrum sharing the same underlying risk factors as those of substance addictions (Goodman, 2008). As a consequence, a growing number of studies are being conducted to determine whether established risk factors for

substance addictions are also involved in the aetiology of pathological gambling. Both the early conceptualization of pathological gambling as an impulse control disorder and its affiliation with a broader addictive disorder spectrum have contributed to the emergence of studies investigating the role of impulsivity in the aetiology, maintenance, and relapse of pathological gambling (Blaszczynski and Nower, 2002; Goudriaan et al., 2004; Nower and Blaszczynski, 2006). The great majority of these studies found that pathological gamblers (PGs) have higher levels of impulsivity than control participants (Blaszczynski et al., 1997; Steel and Blaszczynski, 1998; Kim and Grant, 2001; Petry, 2001b; Potenza et al., 2003; Fuentes et al., 2006; Forbush et al., 2008). Impulsivity was also highlighted as a predictor of the severity of pathological gambling symptoms (Moore and Ohtsuka, 1997; Vitaro et al., 1999; Lightsey and Hulsey, 2002; Krueger et al., 2005; Slutske et al., 2005; Mackillop et al., 2006). Crucially, impulsivity in PGs is related to a poor prognosis, as reflected by higher dropout ratios (Leblond et al., 2003) and a global lower likelihood of success in psychological treatments (MacCallum et al., 2007). Some studies, however, found no difference in impulsivity traits between PGs and matched control participants (Langewisch and Frisch, 1998; Lejoyeux et al., 1998).

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Despite having established a clear relationship between pathological gambling and high impulsivity levels, these studies bring about limited comprehension of the psychological mechanisms involved, as they have been too often conducted without considering the multifaceted nature of impulsivity. Indeed, it is now established that impulsivity encompasses a combination of multiple and separable psychological dimensions (Evenden, 1999; Enticott and Ogloff, 2006). Whiteside and Lynam (2001) clarified the multidimensionality of impulsivity by subdividing it into four dimensions, which are related but also independent. These four dimensions are defined as follows: urgency, the tendency to act rashly when experiencing negative affect (e.g., individuals with high urgency often engage in behaviours they later regret when they are upset or in a bad mood, whereas individuals with low urgency tend to keep calm in these situations); premeditation, the tendency to take into account the consequences of an act before engaging in that act (e.g., individuals with low premeditation generally do not consider all of the advantages and inconveniences before acting, in contrast to individuals with high premeditation who are more careful and purposeful); perseverance, the capacity to remain focused on a boring and/or difficult task (e.g., individuals with low perseverance often procrastinate and give up tasks, in contrast to individuals with high perseverance who generally finish what they start); and sensation seeking, the tendency to enjoy and pursue new and exciting activities (e.g., individuals with high sensation seeking often engage in potentially risky activities such as extreme sports, in contrast to individuals with low sensation seeking who do not like these types of activities). Notably, a growing number of studies have highlighted specific links between these impulsivity facets and various dimensions of problematic behaviours and/or psychopathological states (Miller et al., 2003; Billieux et al., 2007, 2008; Smith et al., 2007; Verdejo-García et al., 2007). With regard to problem gambling, a few studies conducted on gamblers from the community (non-clinical participants) have shown that adverse consequences resulting from gambling (e.g., financial problems, chasing behaviours) are predicted by high urgency and low premeditation, whereas high sensation seeking predicts only gambling frequency (Smith et al., 2007; Cyders and Smith, 2008). The last impulsivity facet, namely, lack of perseverance, has not yet been shown to be related to problem gambling.

The urgency and lack of premeditation components of impulsivity, which seem to play a role in problem gambling, have been related to specific cognitive mechanisms (Bechara and Van der Linden, 2005). First, recent data suggested that urgency is at least partly underlain by poor prepotent response inhibition (Gay et al., 2008; Billieux et al., 2010). In these studies, inhibition capacities were assessed with laboratory tasks measuring the ability to refrain or interrupt a motor response that was automatised beforehand (go/no-go or stop-signal paradigms) (Verbruggen and Logan, 2008). Crucially, a growing number of studies found prepotent inhibition to be impaired in PGs (Goudriaan et al., 2005, 2006; Fuentes et al., 2006; Kertzman et al., 2008; Roca et al., 2008), and poor inhibition was highlighted as a predictor of relapse in pathological gambling (Goudriaan et al., 2008). Despite almost all published studies found inhibition impairment in PGs, some PGs have also been shown to have inhibition capacities that were comparable to (and sometimes even better than) those of matched controls (Carlton and Manowitz, 1992).

Second, lack of premeditation has been shown to be related to difficulty in balancing immediate benefits with future ones (Lynam and Miller, 2004). More precisely, low premeditators were shown to make less advantageous choices than high premeditators in a "delay discounting" procedure in which they had to choose between a small amount of fake money that was immediately available or a much higher amount of fake money that was delayed. Of note, some studies conducted with delay discounting tasks found that PGs are characterised by similar short-term-based choices (Petry, 2001a; Alessi and Petry, 2003). Although the urgency and lack of premeditation facets of impulsivity rely on specific cognitive processes, they also significantly correlate with each other (Whiteside and Lynam, 2001; Van der Linden et al.,

2006), suggesting that they are not totally independent and may be at least partly underlain by shared psychological mechanisms.

The aims of the current study were twofold. First, we wanted to replicate previous findings regarding urgency and lack of premeditation in a sample of PGs rather than in community participants. Second, we sought to determine at the individual level whether heterogeneous "impulsivity profiles" could be highlighted among PGs. Indeed, from a multidimensional view of impulsivity, it can be supposed that the altered impulsivity components (and related psychological mechanisms) possibly diverge from one PG to another. Moreover, some studies have shown that PGs are not necessarily impulsive (Lejoyeux et al., 1998) or characterised by poor response inhibition (Carlton and Manowitz, 1992), implying that at least part of the PGs included in the study may not be impaired with regard to the measures used in the study. To investigate these topics, we conducted a study in which a group of treatment-seeking PGs (n = 20) and a matched group of control participants (n=20) who do not gamble were compared for (1) impulsivity facets, (2) inhibitory control, and (3) delayed reward tolerance. We found it important to use both self-reported measures and laboratory tasks. Indeed, these two types of measures cannot be considered as isomorphic. More precisely, self-reported questionnaires generally assess broader constructs (the various items of the same dimension often refer to a wide range of situations) and are influenced by a certain bias (e.g., social desirability, lack of insight), whereas laboratory tasks sometimes lack ecological validity (the generalisation from the context of the laboratory to real-life situations).

2. Methods

2.1. Participants

Two groups participated in the study: a group of PGs (n=20) and a group of control participants (n=20). Demographic data are presented in Table 1.

The PGs were recruited from outpatients of a gambling addiction treatment centre in the psychiatric service of the Sainte-Marguerite Hospital in Marseille, France. They were tested on their arrival in the centre and were thus free from any psychological treatment or psychoactive drugs. Each PG was diagnosed according to DSM-IV criteria and completed the French version of the South Oaks Gambling Screen (SOGS) (Lejoyeux, 1999). The SOGS is a 16-item questionnaire based on the symptoms of pathological gambling in the third edition of the DSM. All PGs have a SOGS > 5, which is a common cut-off used to diagnose pathological gambling (Lesieur and Blume, 1987). Exclusion criteria were substance use

Table 1Means, standard deviations, and group differences.

	• •					
	PGs	CPs				
	n=20	n = 20	Test statistic			
Demographics						
Age (years)	44.10 (11.55)	38.30 (12.27)	t(38) = 1.54			
Gender (M/F)	17/3	17/3				
National Adult Reading Test	34.80 (3.91)	35.65 (1.79)	t(38) = -0.88			
C115						
Gambling	0.50 (4.40)					
South Oaks Gambling Screen	9.50 (1.40)					
Maximum expenditure (in €)	4077.50					
	(10928.00)					
Number of game activities	4.5 (2.52)					
Impulsivity						
UPPS-urgency	34.90 (5.22)	29.65(6.70)	$t(38) = 2.76^*$			
UPPS-lack of premeditation	25.75 (5.95)	21.70 (3.06)	t(38) = 2.70 t(38) = 2.71*			
	. ,	` ,	• •			
UPPS-lack of perseverance	21.30 (6.85)	19.75 (3.34)	t(38) = 0.91			
UPPS-sensation seeking	30.60 (6.44)	30.80 (6.71)	t(38) = -0.10			
Inhibitory functions						
Go-stop (SSRT)	288.23 (86.30)	205.44 (62.36)	$t(38) = 3.12^*$			
SKIP-delay	39.89 (52.58)	102.03 (110.72)	$t(38) = -2.97^*$			
ordi delay	33.03 (32.30)	102.03 (110.72)	1(30) = 2.37			

*Comparisons significant at P<0.05, corrected for multiple comparisons using the false discovery rate procedure. PGs: pathological gamblers; CPs: control participants; M: male; F: female; UPPS: UPPS Impulsive Behaviour Scale; Go-stop (SSRT): stop signal reaction time in the go-stop impulsivity paradigm; SKIP-delay: mean delay between two responses in the single key impulsivity paradigm.

disorder (except tobacco dependence) and any reported history of neurological disorders. Comorbidity screening, not used as an exclusion criterion, was assessed with the mini DSM-IV-TR (Robins et al., 1997) and the French Fagerström test for nicotine dependence (Etter et al., 1999). The comorbidities identified were as follows: tobacco dependence (50%), depressive disorder (50%), general anxiety disorder (40%), panic disorder (25%), agoraphobia (15%), and obsessive-compulsive disorder (10%). The main forms of gambling practised were lotteries (90%), slot machines (85%), scratchcards (75%), sport betting (55%), table games in the casino (e.g., roulette, blackjack) (45%), online casino games (45%), and poker (in the casino) (25%).

The control participants were recruited in the community. They were not paid for their participation. Control participants were matched with the PGs for sex, age, and socio-educational level (see Table 1). Socio-educational level was assessed with the French version of the national adult reading test (NART) (Mackinnon and Mulligan, 2005). Exclusion criteria were past or present gambling practices or a history of psychiatric or neurological disorders. Four control participants (20%) reported that they smoke cigarettes.

2.2. Instruments

2.2.1. UPPS Impulsivity Scale (UPPS)

The French version of the UPPS (Van der Linden et al., 2006), translated from Whiteside and Lynam (2001), consists of 45 items that evaluate the four different facets of impulsivity, labelled urgency (12 items, e.g., "When I feel bad, I will often do things I later regret in order to make myself feel better now"); (lack of) premeditation (11 items, e.g., "I am a cautious person"); (lack of) perseverance (10 items, e.g., "I concentrate easily"); and sensation seeking (12 items, e.g., "I will try anything once"). All items are scored on a Likert scale from 1 = "I agree strongly" to 4 = "I disagree strongly", with higher scores reflecting higher impulsivity on the respective facet. A validation study found the high internal consistency of the French version and its factorial structure to be similar to the original English UPPS (Van der Linden et al., 2006).

2.2.2. Go-stop impulsivity paradigm (go-stop)

The go-stop (Dougherty et al., 2005) was used to assess the ability to inhibit prepotent response. In this task, two blocks of 160 trials were presented in which a cue stimulus (a black number composed of five digits) was followed (after a 1-second blank screen) by a target stimulus (either the same or a different black number composed of five digits). Each stimulus was presented for 500 ms. Participants were instructed to press a button as quickly as possible if the target stimulus matched the cue stimulus (i.e., if the number was identical). Sometimes, the target matching number changed from black to red, indicating a "stop-signal" where the participant was instructed to withhold responding. The combination of go and stop signals resulted in three trial types: (1) "no-stop trials", in which the cue and target stimuli were identical (25% of the trials); (2) "no-matching trials", in which the cue and the target stimuli were non-identical (50% of the trials); and (3) "stop-trials", in which the stop signal was presented after the apparition of a matching target stimulus (25% of the trials). Stop signals were presented at predetermined intervals before the subject's expected response (50, 150, 250, 350 ms) (Dougherty et al., 2005). The dependent variable that reflects the latency of the inhibitory process is the stop-signal reaction time (SSRT) (Logan, 1994), with higher SSRTs reflecting lower levels of inhibition. SSRTs were calculated separately for each interval and then averaged.

2.2.3. Single key impulsivity paradigm (SKIP)

The SKIP (Dougherty et al., 2005) was developed to assess tolerance for delayed reward. This task lasts 20 min (four blocks of 5 min), during which time the participant is free to respond (by clicking a button) as frequently as desired for reward. The participant collects a point each time he or she presses the response button. However, the magnitude of the reward is related to the length of the delay between consecutive responses. The longer the participant waits between consecutive responses, the more points that will be earned. The size of the reward varies linearly and is directly proportional to the length of delay between two consecutive responses. A point counter at the top of the screen displays the total points won during the session, and another counter at the bottom of the screen indicates the number of points earned in the most recent response. The tolerance for delayed reward was measured as the average delay between the participant's responses (Dougherty et al., 2005).

2.3. Procedure

The study protocol was approved by the human subject committee of the Sainte-Marguerite University Hospital. All participants were over 18 years old and provided informed consent. At initial intake, a detailed clinical history was obtained by means of a semi-structured face-to-face interview. Experienced psychologists and psychiatrists collected socio-demographic and clinical data. Participants were then individually tested with the above-mentioned measures during a single session before starting treatment at the gambling addiction centre.

2.4. Data analysis strategy

Descriptive statistics (means and standard deviations) were computed for all variables. Internal reliabilities (Cronbach's alpha) were computed for the UPPS. Two-tailed Student's *t*-tests for independent samples were computed to compare PGs and control participants for the following variables: age, vocabulary skills (NART), impulsivity facets

(UPPS), SSRT on the go-stop task, and mean delay between responses in the SKIP. The t-tests were considered statistically significant at P < 0.05, corrected for multiple comparisons by using the Benjamini and Hochberg's false discovery rate procedure (Benjamini and Hochberg, 1995). We then considered the proportion of PGs presenting abnormal scores compared with the control group on the various measures. To this end, we fixed a deviance criterion at a threshold of 1.65 S.D. of the mean of the control group. In a normal distribution, this corresponds to the fifth percentile, which is a common threshold to highlight deviance from the mean. This analysis allows the consideration of individual profiles for each PG included in the study.

3. Results

3.1. Preliminary and descriptive analyses

Data for the stop-signal task are missing for one control participant because of technical problems. No other missing data were identified. The SSRT on the go-stop task and the mean delays on the SKIP were transformed by using natural logarithm to decrease the skewness of their distribution. Means and standard deviations for demographic and psychological variables, as well as for gambling-related information for the PG group (SOGS scores, number of different gambling activities practised, maximum expenditures in a single gambling session), are reported in Table 1. The reliability coefficients (Cronbach's alpha) were high for the various impulsivity facets assessed, namely, urgency ($\alpha = 0.84$), lack of premeditation ($\alpha = 0.81$), lack of perseverance ($\alpha = 0.83$), and sensation seeking ($\alpha = 0.79$).

3.2. Comparisons between groups

We computed *t*-tests corrected for multiple comparisons between groups, as reported in Table 1. Concerning impulsivity facets, PGs were found to have higher levels of urgency (UPPS) and lower levels of premeditation (UPPS). Nevertheless, no significant difference took place between groups concerning the perseverance and sensation-seeking facets of impulsivity (UPPS). With regard to the go-stop task, PGs were characterised by a lower capacity than control participants for prepotent response inhibition. For the SKIP, PGs demonstrated a lower ability than control participants to delay a reward.

3.3. Individual profile analyses

Further analyses were then performed to identify, at the individual level, which impulsivity facets and inhibitory functions were deviant in PGs in comparison with the data obtained from control participants. These analyses revealed that the impairments (and their associations) were highly heterogeneous among PGs. More precisely, the proportions of PGs characterised by deviant scores were as follows: UPPS-urgency, 15%; UPPS-lack of premeditation, 45%; UPPS-lack of perseverance, 30%; UPPS-sensation seeking, 5%; go-stop inhibition task, 40%; and SKIP inhibition task, 30%. When considering the number of deviant scores on both UPPS facets and inhibition tasks for each PG, the proportions were as follows: none, 30% of the PGs; one, 20%; two, 20%; three, 20%; four, 5%; and five, 5%. The individual profiles obtained for all PGs included in the study are reported in Table 2.

4. Discussion

In the current study, we investigated impulsivity facets and related psychological mechanisms in a sample of treatment-seeking PGs and a matched group of control participants. Compared with controls, PGs exhibited high urgency and low premeditation, and were found to be impaired in two tasks assessing inhibitory control and preference for immediate rewards. Nonetheless, complementary profile analyses showed that impulsivity-related impairments found in PGs are highly heterogeneous.

The current study primarily confirms, in a clinical sample of PGs, previous findings obtained for a community sample of gamblers

Table 2 Specific deficits among the pathological gamblers.

Participant	Urgency	Lack of premeditation	Lack of perseverance	Sensation seeking	Go-stop task	SKIP
PG1						
PG2					X	
PG3						
PG4						
PG5						
PG6		X	X			
PG7						
PG8	X	X	X			
PG9					X	
PG10	X	X	X	X	X	
PG11		X	X			
PG12					X	
PG13		X	X			
PG14	X					X
PG15						
PG16		X			X	X
PG17		X			X	X
PG18		X			X	X
PG19		X	X		X	X
PG20						X

X indicates an abnormal score, based on a deviance criterion at a threshold of 1.65 S.D. of the mean of the control group; PG1-PG20: the 20 pathological gamblers included in the study; SKIP: single key impulsivity paradigm.

(Smith et al., 2007; Cyders and Smith, 2008), namely, the fact that urgency and lack of premeditation are involved in problem gambling. It is now acknowledged that problematic gambling behaviours frequently take place to relieve negative affect (e.g., dysphoria, boredom) in the short term (Jacobs, 1986; Shead and Hodgins, 2009), which engenders negative outcomes (e.g., financial problems, relational conflicts). From this perspective, it can be supposed that gamblers with high urgency are more likely to gamble to regulate affective states through the relief of negative emotions. In our view, this type of maladaptive coping strategy can be considered to result from disadvantageous decision making in emotional contexts, underlain by an inability to make choices while taking into account the balance between immediate and future consequences. In support of this assumption, recent data have shown high urgency to be associated with disadvantageous choices in a laboratory task assessing the ability to make advantageous decisions in emotional contexts (Xiao et al., 2009; Billieux et al., 2010). We also found PGs to have low premeditation, suggesting that they demonstrate a tendency to act without forethought in general, that is, not necessarily in emotional contexts. Thus, it is possible that low premeditators are likely to have poor planning abilities because of inadequate deliberative processes (e.g., they do not take into account all relevant information when making a decision) (Evans, 2003). For example, starting to gamble to improve a financial situation can be considered to result from a non-adapted deliberative process.

The results are also consistent with previous data that related pathological gambling to poor inhibitory control (Goudriaan et al., 2005, 2006; Fuentes et al., 2006; Kertzman et al., 2008; Roca et al., 2008) and delay discounting capacities (Petry, 2001a; Alessi and Petry, 2003). Crucially, it has been shown that these specific cognitive mechanisms are involved in adapted decision making (Dunn et al., 2006; Doya, 2008), which is often impaired in PGs (Cavedini et al., 2002; Goudriaan et al., 2005, 2006; Roca et al., 2008). From this perspective, and in accordance with the explanations outlined earlier, we postulated that in pathological gambling, both inhibition impairment and poor ability to postpone reward seeking contribute to short-term-based decision making. Thus, we argue that diminished impulse control and preference for immediate rewards are displayed when a gambler is not able to refrain from gambling (for a variety of reasons, such as regulating affect or seeking stimulation, or for more idiosyncratic purposes such as improving a financial situation) in order to shift from pursuing this behaviour to another one that is not maladaptive in the long term.

Another important finding of the current study is the heterogeneity of the deficits presented by the PGs. Indeed, individual profile analyses demonstrated, on the one hand, that the impairments found are either relatively specific (i.e., restricted to certain components of impulsivity) or are representative of a more global self-control impairment, and, on the other hand, that some PGs included in the study (30%) have no alteration in the various impulsivity facets and related cognitive mechanisms measured. This has important implications, both at the theoretical and at the clinical level. At the theoretical level, our results add supplementary support to the position that pathological gambling is a multifaceted psychopathological state and that PGs should be clustered into distinct subgroups (Blaszczynski and Nower, 2002; Ledgerwood and Petry, 2006; Stewart et al., 2008; Milosevic and Ledgerwood, 2010). Indeed, although a subgroup of PGs presented (heterogeneous) impulsivity-related impairments, alternative pathways to problem gambling have to be envisaged. For example, other subgroups of PGs may incorporate individuals with elevated emotional vulnerability or who are prone to gambling-related cognitive distortions (e.g., Blaszczynski and Nower, 2002). The present results eventually emphasised that the current nosography of pathological gambling as an impulse control disorder is unsatisfactory, as some of the persons with this diagnosis are not impulsive. From a clinical point of view, the diversity of impulsivity-related impairments highlighted herein supports the development of personalised (custom-made) interventions targeting specific psychological mechanisms. For example, a PG presenting a high level of urgency and inhibition impairment, but whose other facets of impulsivity are not altered, could benefit from a psychological intervention designed to improve emotion regulation strategies and to increase inhibition capacities (see Friese et al., 2011, for techniques devoted to improvement of self-control abilities).

No significant difference was found between the sensation seeking level of PGs and that of control participants (and only one PG was characterised by excessive sensation seeking based on individual profile analysis). This observation adds to the debate surrounding the role of sensation seeking in problematic gambling. Indeed, published results on this topic are inconsistent: some emphasised a high level of sensation seeking in PGs, whereas others found no difference in sensation seeking between PGs and matched controls. Of note, a few studies highlighted a lower level of sensation seeking in PGs (see Hammelstein, 2004, for a review about the role of sensation seeking and gambling). In fact, growing evidence supports sensation seeking as relying more on actual gambling (e.g., frequency of gambling, preferences for certain types of games; Bonnaire et al., 2006; Smith et al., 2007) than on problematic gambling. It can thus be supposed that in PGs with high sensation seeking, the main cause of the problems is not the degree to which they look for exciting activities, but rather their inability to prevent excessive gambling as a way to satisfy their search for exciting sensations.

In conclusion, PGs exhibit alterations in specific impulsivity facets (urgency, lack of premeditation) and related cognitive mechanisms (inhibition, delayed reward tolerance), which underscores the necessity of disentangling the construct of impulsivity into lower-order components. Nevertheless, the heterogeneity of impulsivity-related impairments in PGs also implies that further studies should take into account other psychological factors involved in pathological gambling (e.g., cognitive distortions, gambling motives).

Contributors

Joël Billieux participated in the statistical analyses and their interpretations, and wrote the manuscript. Guillaume Lagrange carried out the data acquisition for both the PGs and control participants. Martial Van der Linden contributed to the interpretations of the results, as well as the editing and review of the final manuscript. Marc Adida assisted in the computer implementation of the behavioural tasks and participated in the preliminary statistical treatment of the data. Christophe Lançon contributed to the design of the clinical aspects of

the study as well as assisted in the recruitment of PGs. Régine Jeanningros provided the design of the study and managed the experimental data acquisition.

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