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The role of COMT, DAT and DRD2 polymorphisms on brain mechanisms of involuntary attention and cognitive control

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A mi mama

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Summary

Our genetic background plays a key role in the way we face environmental changes and adapt our behavior adequately to the requirements of everyday life. The present research focuses on the role of three genes related to dopamine (DA) transmission on relevant cognitive processes, such as shifting attention when required by the environmental demands or processing of unexpected but potentially relevant events. Prefrontal cortex (PFC) and striatum dopamine activity seem to play different roles in attentional processing and interact to regulate stability and flexible update of contexualt information. Therefore, we studied the effect of genes of known functionality for the regulation of PFC dopamine activity (i.e., Catechol-O-Methyltransferase; COMT), reuptake of dopamine diffused on extrasynaptic striatal space (i.e., Dopamine Transporter; DAT) and the density of D2 dopamine receptors (i.e., dopamine D2 receptors; DRD2).

The participants performed two different versions of an auditory-visual distraction paradigm, in which they were instructed to ignore frequent standard tones and rare novel environmental sounds that preceded the task-relevant targets. In two studies, we manipulated the effect of an emotional context on processing on unexpected novel events, due to the adaptive relevance of novel event during a threat situation in which it may be potentially harmful. In three studies, participants with different alleles or allele combinations of the studied genes performed a cued task-switching

paradigm, in which update of sensory and task-set information could be dissociated. Along the six studies work, behavioral and scalp-recorded electrophysiological measures were employed, such as the analyses of time-domain averaged event-related brain potentials, and time-frequency domain brain oscillatory activity.

Three studies revealed the role of DAT gene on the cognitive control of attention, thus suggesting the relevance of striatal DA in cognitive flexibility. Our results suggest a context-independent processing of sensory changes for task-set reconfiguration in individuals with the DAT 9-repeat allele (9R+) and thus resulting in larger striatal dopamine display. However, these individuals showed an earlier detection of task-relevance of sensory changes. The DAT gene seemed to account for the modulation of novelty processing by an emotional context. Individuals without the 9-repeat allele (9R-) and thus resulting in lesser striatal dopamine display showed increased brain response to novel events in a threatening context but no behavioral correlate. In contrast, 9R+ individuals showed a behavioral enhancement of distraction in a threatening situation but largest brain response to novelty in a neutral situation with no further increase in a negative one.

Two studies revealed the epistatic intercation of COMT and DRD2 genes on attentional processes. Individuals with a COMT Val allele and DRD2 A1 allele (A1+) and COMT Met without DRD2 A1 allele (A1-) are suggested to

display balanced interaction of PFC and striatal dopamine. These groups showed behavioral distraction, while individuals Val A1- and Met A1+ were not distracted by novel sounds in an auditory visual distraction paradigm. However, non-distracted groups happened to process novel events through resetting of 40 Hz oscillatory activity. Moreover, those with pressumed balanced interaction seemed to reconfigure task-set information when required, while those with genetically driven either extreme PFC or striatal dopamine display would reconfigure task-set after every sensory change.

The results of theses studies provide evidence for a relevant role of COMT, DAT1 and DRD2 genes in cognitive processes, which helps to understand cognitive disruption associated to dopamine dysregulation in neurological or psychiatric disorders.

Original studies

I. Garcia-Garcia, M., Yordanova, J., Kolev, V., Domínguez-Borràs, J., Escera, C. (2010) Tuning the brain for novelty detection under emotional threat: The role of increasing gamma-phase synchronization. *NeuroImage 49, 1038-1044.*

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Glossary of abbreviations

ADHD	Attention deficit hyperactivity disorder
ANOVA	Analysis of variance
BOLD	Blood-oxygenation leven-dependant
COMT	Cathecol-O-Methyltransferase
DAT	Dopamine transporter
DNA	Deoxyribonucleic acid
DRD2	Dopamine reseptor D2
EEG	Electroencephalogram
EOG	Electro-oculogram
ERP	Event-related potential
fMRI	Functional magnetic resonance imaging
GBR	Gamma band response
ISI	Inter-stimulus interval
MFN	Middle frontal negativity
NEG	Negative emotional context
NEU	Neutral emotional context
NOV	Novel sound
nP3	Novelty-P3
PD	Parkinson's disease
PCR	Polymerase chain reaction
PFC	Prefrontal cortex

PLF	Phase-locking factor
PS	Phase-synchronization
RT	Response time
SNP	Single nucleotic polymorphism
SOA	Stimulus onset asynchrony
VNTR	Variable number of tandem repeat
WCST	Wisconsin Card Sortting Test
WM	Working Memory

Index

1. Introduction

In our everyday life, we are surrounded by an environment rich of stimuli. Even though an adaptive behavior requires selecting relevant information to focus attention on, surrounding stimuli shall not be completely disregarded, as they might provide crucial information for an adaptive behavior, or even for survival. An adaptive behavior requires flexible adjustment to environmental changes through a balanced interaction between stable maintenance of information for cognitive processing and subsequent avoidance of distracters, and a flexible update of information contained in either attended or "unattended" changes in the sensory stimulation. The control of attention is thus a crucial key for understanding the control of behavior. Moreover, the involuntary shifting of attention towards novel environmental stimuli, and the voluntary cognitive control, although seen as different processes, have been proposed to share a common neural network (Barceló et al., 2006).

1.1 Involuntary attention

The involuntary orienting of attention towards unexpected novel events is an adaptive mechanism, which, however, might come in detriment of ongoing cognitive processes. The occurrence of an unexpected novel or deviant stimulus during task performance induces distraction, as shown by increased response time (RT) to target stimuli after novel as compared to

standard events in auditory-visual “distraction paradigms” (Escera, Alho, Winkler, & Näätänen, 1998; Escera, Yago, Corral, Corbera, & Nunez, 2003; Escera & Corral, 2007) derived from a modification of the so-called “oddball” paradigm, in which a high probability standard stimulus is replaced randomly by a rare or “odd” stimulus.

In the mechanisms controlling for attention, a recent body of evidence suggests a constant interaction between exogenous attentional control by novel environmental events and endogenous cognitive control (Pashler, Johnston, & Ruthruff, 2001). For instance, behavioral and electrophysiological data show a decrease of distraction and reduced novelty processing following enhancement of working memory load of the task during an auditory visual distraction paradigm (SanMiguel, Corral, & Escera, 2008).

Similarly, processing of emotionally negative stimuli interacts with processing of other stimuli either facilitating or competing with them, not only within (Anderson, 2005) but also across sensory modalities (Stanley & Knight, 2004). For instance, stimuli with an affective load have been shown to elicit stronger and faster attention capture than non-emotional stimuli (Hansen & Hansen, 1988). Emotional stimuli capture attention at a very early stage of information processing in the human brain (around 100-200 ms after stimulus onset; Carretie, Hinojosa, Martin-Loeches, Mercado, & Tapia, 2004; Krolak-Salmon, Henaff, Vighetto, Bertrand, & Mauguiere, 2004; or

even earlier, 50-100 ms after stimulus onset; Sugase, Yamane, Ueno, & Kawano, 1999) even when the emotional stimuli appear out of the attentional set, that is, automatically (Carretie, Hinojosa, Mercado, & Tapia, 2005; Vuilleumier, Armony, Driver, & Dolan, 2001). A visually induced negative emotion interacts with the processing of concomitant auditory inputs (Stanley & Knight, 2004). A recent fMRI study has reported that areas involved in novelty processing (bilateral superior temporal gyri) are significantly more activated in a negative emotional context, as compared with a neutral one (Dominguez-Borras et al., 2008), demonstrating a modulation of orienting of attention towards salient stimuli by emotional context, analogous to top-down mechanisms of attentional control. On a similar study, the electrophysiological results revealed an enhancement of novelty processing when novel events were processed during the performance of a task involving emotionally negative pictures (Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Dominguez-Borras, Garcia-Garcia, & Escera, 2008b; Garcia-Garcia, Dominguez-Borras, SanMiguel, & Escera, 2008). Unexpected novel stimuli in an emotionally negative situation, such as a threatening or fear environment, acquire a vital importance, as they may be potentially harmful, so a stronger processing becomes crucial and has an obvious adaptive value.

1.2 Cognitive control

Individuals usually have to pay attention sequentially to many different stimuli in response to the changing environment, requiring a flexible adjustment to new cognitive tasks. This adjustment involves an appropriate reconfiguration of the mental set in order to give an adequate response to the new task.

Task-switching paradigms are used for investigating mechanisms of task-set reconfiguration. In task-switching paradigms, each task requires attention to, and classification of, a different attribute of the stimulus, or retrieval from memory computation of a different property of the stimulus. Performance and brain activity are examined following trials where the task changes as compared to trials when the previous task is repeated, in order to obtain evidences of extra processing demands associated with the task-set reconfiguration (Monsell, 2003). The switch-related extra performance and corresponding brain activity is the switch cost, which reflects cognitive processes associated with the task-set reconfiguration, such as retrieving the currently relevant task-set, initializing stimulus-response mapping and suppressing activation of the previously active task (Rogers & Monsell, 1995). The switch cost is behaviorally reflected by a decline in performance, evidenced by an increase in RT and error rate (Karayanidis, Coltheart, Michie, & Murphy, 2003; Rogers & Monsell, 1995). Event-related brain potential (ERP) task-switching studies in which participants alternated

between two tasks in predictable series have disclosed a particular positivity in anticipation of a switch in a task, and a large late negativity following the stimulus to respond for switch as compared to non-switch trials (Karayanidis et al., 2003). This positive response was related to the extra brain activity required for task-set reconfiguration.

However, in order to better establish the onset of the anticipatory task set reconfiguration process and thus be able to manipulate active reconfiguration and passive dissipation, cued task-switching paradigms are used, in which a cue announce whether the task must be changed or repeated for the following stimulus. Cued-task switching paradigms have also found the parietal differential positivity peaking around 400 ms (Nicholson, Karayanidis, Poboka, Heathcote, & Michie, 2005; Rushworth, Passingham, & Nobre, 2002). However, the RT switch-cost and the switch-related parietal positivity does not necessarily reflect the cost of task set reconfiguration, but they may confound task-switch and cue-switch processing (Logan & Bundesen, 2004). In an attempt to dissociate the effect of a change in sensory or task information, different dual cue conditions have been applied. For instance, Nicholson, Karayanidis, Bumak, Poboka, & Michie, (2006) found an enhancement of a prior negative response after cue repetition as compared to cue switch and a switch-related parietal positivity attributable to the task-set reconfiguration, however non accompanied by behavioral switch costs. Differently, (Brass, Ullsperger, Knoesche, von Cramon, & Phillips, 2005) found a fronto-central negativity peaking around

470 ms after cue onset only when task-set reconfiguration was required, and a positive response to both sensory and task switches; moreover, a tonic negative late potential was observed to be larger for repeat than task-switch trials.

A task-cueing paradigm inspired by the classic test of prefrontal cortex (PFC) impairment, the Wisconsin Card Sorting Test (WCST; Rubinstein, Meyer, & Evans, 2001), was implemented, in order to provide a reliable tool to study the event-related brain potentials (ERP) dynamics underlying the executive control of attention and, in particular, the cognitive operations of shifting, updating, and rehearsal of task-sets in working memory (Barceló, Munoz-Cespedes, Pozo, & Rubia, 2000). Barceló, Perianez, & Knight, (2002) found that auditory cues directing a switch in the mental set to a new task elicited a fronto-posteriorly distributed brain potential which resembled the brain responses to novel distracters delivered in auditory-visual distraction paradigms (Escera et al., 1998; Escera, Alho, Schroger, & Winkler, 2000; Escera & Corral, 2007), suggesting that sensory and task novelty processing might share a common neural network (Barceló, Escera, Corral, & Perianez, 2006).

1.3 Role of dopamine in the control of attention

The implication of the dopamine (DA) system in the control of attention has been widely evidenced in the last decades (Kaplan & Oudeyer, 2007; D. A.

Lewis et al., 2001; S. J. Lewis, Dove, Robbins, Barker, & Owen, 2003; Sawaguchi & Goldman-Rakic, 1994). For instance, studies have reported that DA is crucial in control of attention when it is directed by a cue (Cools, Barker, Sahakian, & Robbins, 2001; Cools, Barker, Sahakian, & Robbins, 2003; Cools, 2008), as well as when it is regulated by rewards (Montague & Berns, 2002; Schultz, 2002; Stefani & Moghaddam, 2006). DA drugs administration to PFC neurons can enhance the activity during the delay period of a working memory task at a certain concentration but inhibit firing at other dosages (Williams & Goldman-Rakic, 1995). Behavioral impairment has been observed subsequent to great increases of PFC DA release following noise stress (Arnsten, 1998) or the administration of dopaminergic agents (Zahrt, Taylor, Mathew, & Arnsten, 1997). Moreover, individuals with low working memory capacity, as measured by a digit span task, showed improvements in PFC functioning tasks with low dosage of DA receptor agonist bromocriptine (Kimberg, D'Esposito, & Farah, 1997) or the DA agonist Methylphenidate (Mehta et al., 2000), whereas high-span individuals were either impaired or showed similar performance as before the administration of the drugs.

There is reciprocal relationship between DA D1 and D2 receptor binding (Akil et al., 2003; Meyer-Lindenberg et al., 2002). While D1 receptor binding in the PFC is thought to promote stability of mental representations by increasing distracter resistance (Durstewitz, Seamans, & Sejnowski, 2000), the stimulation of D2 receptors, which are mainly expressed at human

striatum (Camps, Cortes, Gueye, Probst, & Palacios, 1989), is related to the facilitation of cognitive flexibility by allowing the updating of newly relevant representations (Frank, 2005). The multifaceted role of DA activity in the PFC regulated by the extrasynaptic actions of D1/D2 receptors accounts to the efficient updating of representations in working memory through an inverted-U function: middling levels of prefrontal DA result in optimal performance (i.e., N-back task; Callicott et al., 1999), whereas highest and lowest PFC DA concentrations leads to suboptimal updating of information in working memory (Arnsten, 1998; Cools, Clark, & Robbins, 2004; Williams & Castner, 2006). Therefore, the balance between D1/D2 actions might be involved in stimulus-driven control of attention, such as the processing of environmental novel events (Birkas et al., 2006), or updating the task context. This relationship may be explained by the differential effects of D1 and D2 receptor binding, as the D2 receptor binding in the striatum allows the PFC network to respond to new information by updating its working memory system, whereas the D1 receptor stimulation plays a gating role by controlling the threshold of significance above which it can be admitted to working memory and processed by the PFC network (Weinberger et al., 2001)

A biphasic DA modulation of PFC pyramidal neurons has been proposed with interesting functional implications. D1 receptors have been argued to mediate in order to sharpen the tuning of pyramidal cells and to focus the activity on task-relevant items (Sawaguchi & Goldman-Rakic, 1994), as

supported by performance deficits on PFC function tasks showed by patients with deteriorated PFC. Seamans, Gorelova, Durstewitz, & Yang, (2001) showed a short D2-mediated decrease on pyramidal evoked potential, measured by patch-clamp, preceding a long-lasting D1-mediated increase. This D2-mediated decrease would allow multiple representations to be activated closely in time, so even weak representations could pop into the delay-active state easily (Durstewitz et al., 2000). Conversely, in a state dominated by the D1-mediated enhancement, weakly active representations fails to be maintained, and a limited number of strongly active representations become very stable, thus avoiding interfering inputs and noise (Durstewitz et al., 2000). This is revealing the role of D2 receptor stimulation on cognitive functions, setting adaptive flexibility to process novel stimuli and switching attentional resources.

Moreover, several pharmacological studies on human volunteers supported the role of D2 receptors in the control of attention. For instance, the administration of sulpiride, a selective antagonist at postsynaptic D2-receptors, protected participants again distraction during a delayed-response task, but impaired performance on a set-switching task (Mehta, Manes, Magnolfi, Sahakian, & Robbins, 2004). In an auditory oddball paradigm studying involuntary attention shifting, Kahkonen et al., (2002) found a decrease in the amplitude of an attention-related brain potential after administration of D2 receptor antagonist haloperidol suggesting an

impairment of shifting the attention to the deviant stimuli after pharmacological D2 receptor blockage.

Furthermore, D2 receptors are mainly expressed at human striatum (Camps et al., 1989), a structure known to play a critical role in cognitive flexibility (Cools et al., 2003; Cools et al., 2004; Cools, Ivry, & D'Esposito, 2006; Cools, 2008; Ragozzino, Jih, & Tzavos, 2002). Midbrain DA neurons recorded from Ventral Tegmental Area of rats showed activation during occurrence of motivationally salient stimuli that trigger behavioral transition (Wilson & Bowman, 2006). Accordingly, a neuroimaging study revealed that human striatum reflects the level of saliency associated to a stimulus, providing a signal to reallocate limited resources to important events (Zink, Pagnoni, Chappelow, Martin-Skurski, & Berns, 2006). A significant impairment in the WCST and other task-switching related protocols has been observed in patients with striatum degeneration, like Parkinson's disease (Cools et al., 2001; Cools et al., 2003; Cools et al., 2004; Meiran, Friedman, & Yehene, 2004), or striatal focal lesions (Yehene, Meiran, & Soroker, 2008), attributed to a deficit in the flexible use of abstract task rules (Meiran et al., 2004; Yehene et al., 2008).

Thus, the individual differences on attention shifting, and on involuntary attention may be determined by the possibility of DA signaling on D1, D2 receptors. This is mediated by several genetic functional polymorphisms which are expressed on different sites of the dopaminergic system.

1.4 Genetic polymorphisms for COMT, DAT and DRD2

An important issue in cognitive neurosciences is connecting the general properties of human mind to differences observed among individuals. A major advance in the attempt to bridge the gap between general cognitive processes and individual differences has been the discovery of genes that influence cognitive functions such as attention.

Even though many different genes have been functionally related to attentional processes, the above-mentioned relationship between the dopaminergic system and the control of attention led us to focus on some genes regulating D1 and D2 receptors action. Among all genes discovered to regulate these two DA receptor types, three genetic polymorphisms were selected, as previous studies had suggested a strong relationship with cognitive processes that might influence the exogenous control of attention for novelty processing and task switching.

1.4.1 Catechol-O-Methyltransferase Met158/108Val

The DA diffused out of the synaptic cleft and bound to extrasynaptic D1 receptors on PFC is inactivated principally by Catechol-O-Methyltransferase (COMT; Bilder, Volavka, Lachman, & Grace, 2004). A functional polymorphism for the gene synthesizing COMT has been described (rs#116790; Lachman et al., 1996), resulting from a Val to Met substitution at

the 108/158 locus in the peptide sequence. The Val allele substitution increases the efficiency of the enzyme in comparison with the Met allele (Mannisto & Kaakkola, 1999). Thus, Val/Val individuals are expected to have decreased synaptic DA levels in PFC, while Met/Met individuals are expected to have high DA levels. The Val (high activity) allele results in decreased ability to maintain information in working memory (Bilder et al., 2004), but enhanced ability to update its contents with new information (Cools et al., 2004). The Met allele, related to elevated PFC DA levels and increased D1 receptor binding, in turn, is predicted to show better performance of cognitive tasks involving maintenance of information in working memory but lead to decrease cognitive flexibility (Winterer et al., 2004; Egan et al., 2001). Specifically, the Val allele has been associated to poorer performance on the WCST (Egan et al., 2001) and with lower scoring in working memory assessment with the N-back test than Met allele. The Val allele has been suggested to increase risk for schizophrenia, as it enhances DA prefrontal catabolism, and thus, impairs prefrontal cognition and physiology (Egan et al., 2001). In this line of evidence, Abi-Dargham et al., (2002) found increased PFC D1 receptor concentration in schizophrenia patients, and was interpreted as a reflection of diminished dopamine innervation, that had led to an up-regulation of these DA receptors. Schizophrenia patients have been reported to perform poorly on tasks requiring executive functions, such as working memory tasks (Weinberger, Berman, & Zec, 1986). This poorer performance has been related to the excess of DA transmission in subcortical areas of the brain, rich in D2

receptors, and to the deficit of DA transmission at D1 receptors in PFC (Davis, Kahn, Ko, & Davidson, 1991).

1.4.2 Dopamine Transporter DAT 1

The dopamine transporter (DAT) mediates the active reuptake of DA from the synapse and it is the principal regulator of dopaminergic neurotransmission at midbrain. It critically regulates the extent to which DA diffuses in the extracellular space, and thus, the duration of cellular action of DA, especially in the striatum (Sesack, Hawrylak, Matus, Guido, & Levey, 1998). In the cortex, DAT seems to regulate DA volume transmission (Cragg & Rice, 2004), and may play a critical role in regulating cortical signal-to-noise ratio during working memory. Neuronal increased response variability is thought to result from impaired phase resetting of stimulus-induced dynamic changes of rhythmic oscillations generated at the apical dendrites of pyramidal neurons. This PFC noise has been demonstrated to be negatively correlated with working memory performance, and is strongly associated with genetic risk for schizophrenia (Egan et al., 2004). The stimulation of D1 receptors abolishes random firing of pyramidal neurons and increases spike rates in selective frequency bands, such effect accompanied by improved performance across different tasks, such as the oculomotor short-term memory delayed-response task in nonhuman primates (Sawaguchi & Goldman-Rakic, 1994).

A functional variable number of tandem repeat (VNTR) polymorphism was identified in the DAT1 or SLC6A3 gene with repeat copy number ranging from 3 to 11 repetitions, being 9- and 10-repeat (9R and 10R) the most frequent alleles in the population (Vandenbergh et al., 1992). The 10R/10R genotype results in increased DAT expression (Fuke et al., 2001; Heinz et al., 2000; Mill, Asherson, Browes, D'Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005; see however Jacobsen et al., 2000) and putatively, decreased synaptic DA tone in cortico-striatal pathways (Wichmann & DeLong, 1996) modulating the D2 receptors involved in mesocorticolimbic pathways (Neville, Johnstone, & Walton, 2004). Moreover, the 10R allele has been evidenced to be significantly involved in the susceptibility for attention-deficit and hyperactivity disorder (ADHD; Yang et al., 2007).

In a neuroimaging study, Bertolino et al., (2006) compared groups combining COMT and DAT polymorphisms during performance of an N-back working memory task. Although they did not differ in performance, they did in blood-oxygen-level-dependent (BOLD) signal at the working memory (WM) cortical network, being COMT Met and DAT 10-repeat allele individuals who showed more focused response, that is, lesser activation for similar performance. Another fMRI study showed an additive effect of these two genes on brain activation in an N-back task, with subjects homozygous for the Val and the 9-repeat alleles showing the highest activation for the same level of performance (Caldu et al., 2007). Another fMRI study showed a nonadditive epistasis between the DAT1 and the COMT polymorphism in executive

processing (Prata et al., 2009). Because COMT Met allele is related to increased synaptic levels of PFC DA, signal-to-noise ratio of pyramidal glutamatergic neurons would be increased in Met individuals, as compared to Val ones, via stimulation of D1 receptor (Seamans & Yang, 2004). On the other hand, since DAT 10-repeat allele is associated with decreased availability of DA at striatum, and thus reduced D2 receptor signaling, D2-mediated decrease of signal-to-noise ratio of pyramidal neurons would not be expected (Seamans & Yang, 2004). Hence, cortical noise would be suppressed and higher rates of PFC neurons firing would lead to a better performance in working memory tasks (Seamans et al., 2001).

1.4.3 Dopamine D2 receptor DRD2 Taq I A

The D2 DA receptor is located on postsynaptic dopaminergic neurons, and it is centrally involved in reward-mediating mesocorticolimbic pathways (Neville et al., 2004) and mostly expressed at human striatum (Camps et al., 1989). It is also known as a target of antipsychotic drugs that are used to treat neuropsychiatric disorders, such as schizophrenia. Abi-Dargham et al., (2000) showed that that dopamine occupies a greater proportion of striatal D2 receptors in patients with schizophrenia compared with matched control subjects during first episode of illness and subsequent episodes of illness exacerbation.

DRD2 has a functional polymorphism that has been tested in several studies with regard to cognitive functioning. The DRD2 TAQ I A polymorphism is a restriction fragment polymorphism, which is also caused by a mutation in a single nucleotide (rs#126450). For the DRD2 gene, A1 allele carriers show a 30-40% reduction in DRD2 density as compared to A2 homozygous (Ritchie & Noble, 2003). Lower A1 allele-related D2 receptor concentrations would further attenuate the response to striatal dopamine, thus increasing the ratio of PFC pyramidal neurons firing (Seamans & Yang, 2004) and thus improving performance on delay- response tasks (Seamans et al., 2001).

A recent neuroimaging study showed a significant interaction between DAT and DRD2 polymorphisms in PFC and striatum activity during both working memory and encoding of recognition memory (Bertolino et al., 2009). Moreover, Reuter et al., (2005) tested cognitive functions on a sample of individuals combining the different COMT and DRD2 genotypes, finding best performance on STROOP cognitive task on COMT Met/Met carrying DRD2 A1 allele, and worst on individuals carrying COMT Met allele and DRD2 A2A2. An epistasis of COMT and DRD2 genotypes for working memory functioning (Gosso et al., 2008; Stelzel, Basten, Montag, Reuter, & Fiebach, 2009) have beed described, supporting the model claiming that working memory performance needs an optimal level of DA signaling in the PFC, which depend on enzymatic activity controlling DA levels as well as DA receptor sensitivity.

1.5 Electrophysiological markers of cognitive control

During the last decades, several scalp-recorded electrophysiological components have emerged, out of the typical P300 component, as strong markers of attention. In order to establish the role of the above mentioned genes on the control of attention, we focused on three psychophysiological correlates of the control of attention for revealing the role of the genes on the evaluation of novel events and task set reconfiguration, fast detection of task-relevance, and the neural mechanisms associated to the effect of these genes on the cognitive control of attention.

1.5.1 Novelty-P3

Auditory-visual "distraction paradigms" (see reviews in Escera et al., 1998; Escera et al., 2003; Escera & Corral, 2007) are widely used in the study of involuntary attention and novelty processing. They derive from a modification of the so-called "oddball" paradigm, in which a high probability standard stimulus is replaced randomly by a rare or "odd" stimulus. During auditory visual distraction paradigm, scalp-recorded ERPs show a prominent response, the so-called novelty-P3 or P3a, associated with the evaluation of these novel events for subsequent behavioral action (Friedman, Cycowicz, & Gaeta, 2001; Escera & Corral, 2007). The novelty-P3 is an ERP component of the "P300 family", which discloses the early and frontocentral P3a elicited by unexpected stimuli from the later centro-parietal P3b elicited to task-

relevant target stimuli (Polich, 2007). The novelty-P3 component has been described to have two subcomponents, the early and the late ones, clearly disclosed on the basis of their respective latency, scalp distribution and psychological concomitants (Dominguez-Borras, Garcia-Garcia, & Escera et al., 2008b; Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Escera et al., 1998; Escera et al., 2000; Garcia-Garcia et al., 2008; Yago, Escera, Alho, Giard, & Serra-Grabulosa, 2003).

Moreover, the novelty-P3 has been revealed to elucidate the interaction between exogenous attentional control by novel environmental events and endogenous cognitive control (Pashler et al., 2001). For instance, a decrease of behavioral distraction was accompanied by a decrease in the late phase of the novelty-P3 following enhancement of working memory load of the task during an auditory-visual distraction paradigm (SanMiguel et al., 2008). Similarly, an enhancement of the novelty-P3 was observed when novel sounds were processed during the performance of a task involving emotionally negative pictures (Dominguez-Borras, Garcia-Garcia, & Escera et al., 2008b; Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Garcia-Garcia et al., 2008).

Moreover, during a task-cueing paradigm inspired by the classic test of PFC impairment, the WCST (Rubinstein et al., 2001), auditory cues directing a switch in the mental set to a new task elicited a fronto-posteriorly distributed brain potential associated to context updated operations (Barceló et al.,

2002). This potential was seen to resemble the novelty-P3 component to novel distracters delivered in auditory-visual distraction paradigms (Barceló et al., 2002), and furthermore, revealed that sensory and task novelty processing might share a common neural network (Barceló et al., 2006).

1.5.2 Auditory N1wave

According to Näätänen & Picton, (1987), at least three exogenous (i.e., depending upon the physical characteristics of sensory stimulation) and three endogenous (i.e., depending upon subject's factors or informative value of the stimulation) components may be activated simultaneously to produce the auditory N1 wave peaking around 70-100 ms after auditory stimulation. Some of these components are known to be sensitive to attention (Woldorff et al., 1993). A non-specific component of the N1 wave was proposed to be related to the activation following the occurrence of a potentially relevant event, in order to facilitate appropriate sensory and motor responses (Näätänen & Picton, 1987). Moreover, novel auditory events elicited an enhancement of the supratemporal component of the N1 wave in auditory visual distraction paradigms (Alho, Escera, Diaz, Yago, & Serra, 1997; Escera, 1997; Escera et al., 1998) suggesting a role of this component in the exogenous control of attention.

More recent cued task-switching studies have observed an early negative frontocentral response peaking around 100 ms post cue being modulated by

the task relevance of the cue (Barceló, Perianez, & Nyhus, 2007; Brass et al., 2005). The N1 wave seems thus to be an optimal outfit for examining early stages of attentional control.

1.5.3 Gamma activity

Although most previous studies on attention have focused on averaged ERPs, other EEG features such as spectral characteristics and functional connectivity patterns may also provide valuable information. Gamma-band responses (GBRs) are assumed to reflect the association among neurons or neural assemblies that subserve specific information processing (Herrmann, Munk, & Engel, 2004). GBRs are strongly synchronized in the first 100 ms after sensory stimulation and reflect very early stages of stimulus evaluation (Tallon-Baudry & Bertrand, 1999), particularly when the stimuli are presented at very short intervals. GBRs are modulated by attentional processes, as they are enhanced by task-relevant targets relative to irrelevant non-targets (Busch, Herrmann, Muller, Lenz, & Gruber, 2006; Debener, Herrmann, Kranczioch, Gembris, & Engel, 2003; Herrmann et al., 2004; Tiitinen et al., 1993; Yordanova, Kolev, & Demiralp, 1997; Yordanova et al., 2000; Yordanova, Banaschewski, Kolev, Woerner, & Rothenberger, 2001). Specifically, an increase in the phase-synchronization (PS) of GBRs has been associated with the brain mechanisms of increased attention (Fries, Reynolds, Rorie, & Desimone, 2001) and the top-down mechanisms for enhanced sensory information processing (Busch et al., 2006; Debener

et al., 2003; Fries et al., 2001; Herrmann et al., 2004; Yordanova et al., 2001) through the amplification of behaviorally relevant signals in the cortex (Fries et al., 2001; Frund, Busch, Schadow, Korner, & Herrmann, 2007; Womelsdorf, Fries, Mitra, & Desimone, 2006; Yordanova et al., 2001). Moreover, visual stimuli conditioned to emotional pictures elicit stronger low gamma-band PS (around 35 Hz) than non-conditioned ones (Keil et al., 2001; Keil et al., 2007; Keil, Stolarova, Moratti, & Ray, 2007; Stolarova, Keil, & Moratti, 2006), suggesting a neural optimization for rapid processing of emotionally relevant stimuli.

A great number of human and animal studies have thus demonstrated a relevant role of DA neurotransmission in the control of attention by means of different approaches. Yet, the role of COMT, DAT and DRD2, regulators of DA system at PFC and striatum, on the brain mechanisms controlling for involuntary attentional shifting towards novel environmental events and the voluntary cognitive control have not been addressed. Moreover, endophenotypes, measurable components unseen by the unaided eye along the pathway between disease and distal genotype, have emerged as an important concept in the study of complex neuropsychiatric diseases. Endophenotypes represent simpler clues to genetic underpinnings than the disease syndrome itself, promoting the view that psychiatric diagnoses can be decomposed or deconstructed, which can result in more straightforward- and successful-genetic analysis (Gottesman & Gould, 2003). However, to be most useful, endophenotypes for psychiatric disorders must meet certain

criteria, including association with a candidate gene or gene region, heritability that is inferred from relative risk for the disorder in relatives, and disease association parameters. Here, we will try to find EEG endophenotypes in order to fill the gap between gene and behavior.

2. Objectives and hypothesis

2.1 General aim

The general aim of the present PhD thesis work is to reveal some of the factors involved in the inter-individual variability in neural mechanisms of updating contextual information through task-set reconfigutaion and novelty processing. Three DA-related genetic polymophisms were selected due to the wide evidence of the role of the DA system in the control of attention. The polymorphic variations of the DAT are expected to mediate the fast detection of task-relevance, and to play a role in context updating processes of both task-set reconfiguration and novelty processing. Moreover, the interaction of the two polymorphisms for COMT and DRD2, according to the functional relationship of D1 and D2 receptors action, is hypothesized to display different levels of PFC DA activity. Individuals homozygous for Met and lacking the A1 allele (i.e., MetA1-), and those homozygous for Val and presenting the A1 allele (i.e., ValA1+) are expected to display a compensated balance of COMT levels and DRD2 density leading to middling levels of PFC DA. In contrast, individuals homozygous for Met and presenting the A1allele (i.e., MetA1+) would display highest PFC DA activity, while those homozygous for Val and A2 (i.e., ValA1-) would be expected to display suboptimal PFC DA activity (Figure 1). Consequently, the combined effect of COMT and DRD2 genetic variability would be reflected in neural

mechanisms and operations of context updating for task-switch and novelty processing.

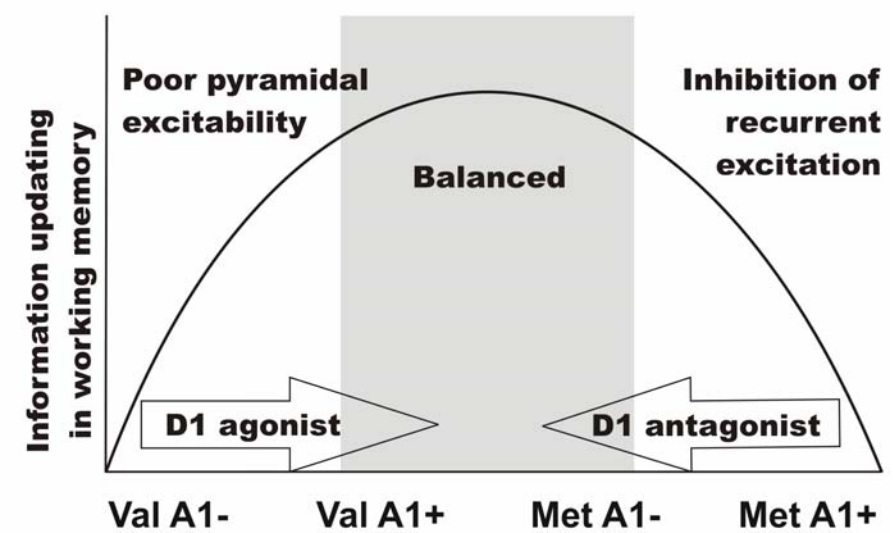

Figure1. *Inverted-U model of PFC DA activity. The y-axis indicates the strength on mnemonic processing, whereas the x-axis indicates the level of PFC DA activity. The four groups of the study combining polymorphisms for the COMT and DRD2 are disposed along the x-axis according to the levels of PFC DA activity reached according to their neurochemical characteristics for D1/D2 receptors binding. The multifaceted role of DA activity account to the efficient manipulation of information in working memory through an inverted-U function, whereby middling levels of prefrontal DA (shadowed) results in optimal performance, whereas highest and lowest PFC DA concentrations leads to suboptimal manipulation of information in WM.*

2.2 Specific aims and hypotheses

Study I

The aim of this study was to explore the mechanisms subserving the interaction between emotional activation and novelty detection by means of

the analysis of the inter-trial phase synchronization elicited by auditory stimuli in an auditory-visual distraction paradigm in which task-irrelevant frequent standard and rare novel sounds were followed by task-relevant visual stimuli of either neutral or negative emotional load. Because gamma band PS plays a role in integrating novelty and emotional information processing in order to guide behavior, we hypothesized that the PS elicited by novel sounds in a negative emotional context should be larger than the one elicited in a neutral one. This study was done in order to examine the neural oscillations at the gamma frequency band reflecting mechanisms of novelty processing and its modulation by endogenous processes, in order to further test these physiological markers in the studies of genetics.

Study II

This study aimed at exploring the role of the interaction of the COMT and DRD2 genetic polymorphic variations inferred by the D1/D2 receptor action in the processing of environmental novel events by means of behavioral measures and the analysis of the amplitude and inter-trial phase synchronization of early auditory GBRs around 40 Hz (35-45). Behavioral and EEG measures were registered during an auditory-visual distraction paradigm, in which task-irrelevant frequent standard or rare environmental novel sounds were followed by a visual target, which was a digit ranging 1-4 and 6-9. Since WM load modulates stimulus-driven attentional control, the role of COMT/DRD2 genotypes on such modulation was examined as

participants had to classify the target as larger or smaller than 5 in a condition without WM load or as larger or smaller than the one presented in the preceding trial in a condition with WM load. We hypothesized an inverted-U function of genetically-based PFC DA activity and behavioral and the electrophysiological concomitants of processing novel events.

Study III

In this study, we explored the role of the DAT1 genotype in the modulation of novelty processing by a visually induced emotional context. The effect of the 9-repeat allele presence was examined by means of behavioral and electrophysiological brain response to auditory stimuli in an auditory-visual distraction paradigm in which task-irrelevant frequent standard and rare novel sounds were followed by task-relevant visual stimuli of either neutral or negative emotional load. We hypothesized that the presence of the 10R allele (i.e., higher enzyme activity) would lead to decreased reactivity to novel stimuli in a negative emotional context, in comparison with 10R absence,

Study IV

This study aimed at revealing the role of DAT1 genotype on fast detection of task relevance. We here hypothesized a predisposition of the 9R+ group (related to larger D2 binding) for a faster detection of task relevance,

probably through a DA pathway connecting the striatum to the prefrontal cortex. This rapid pathway was measured as an early modulation of the fronto-central N1, brain reponse suggested to be related to task-relevance in task-switching paradigms. In order to test the hypothesis, participants with either high or low striatal DA activity (i.e., 9R+ or 9R- respectively) performed a cued task-switching paradigm in which an acoustic change accompanied by a switch in task set could be dissociated from an acoustic change accompanied by a repetition in the task set.

Study V

In the present study, we explored the role of the DAT1 genotype in the individual differences in cognitive flexibility by examining the influence of the 9-repeat allele in the behavioral and electrophysiological response to a cued task-switching paradigm. We measured the nP3 response, meant to account for operations of context-updating involved in the processing of both sensory and task novelty as reflected by a late fronto-posterior positivity, which is preceded by early fronto-central positivity associated to mechanisms of task rule reactivation. Since reduced striatum DA levels have been shown to impair cognitive flexibility, individuals genotyped 10R/10R are expected to show a more rigid behavior resulting in larger task-switch cost and a less effective gating mechanism for context update than their counter partners, both reflected on the putative endophenotypical nP3 brain response.

Study VI

This study aimed at revealing the role of the interaction between COMT and DRD2 polymorphic variations on operations of updating contextual information. We tested the hypothesis that individuals with a putative optimal balance between PFC DA levels and DRD2 concentrations (i.e., MetA1-ValA1+) would show a more efficient updating of task-set information compared to individuals presenting either the lowest or the highest levels (i.e., ValA1-, MetA1+). As the brain signature of the neural mechanisms of context updating, we measured the EEG-recorded nP3 brain potential for exploring the intrinsic interaction between bottom-up (sensory) and top-down (task) representations, and dissociating the contribution of sensory change and task novelty to context updating. Individuals performed a task-cueing protocol in which the response to a sensory change could be dissociated from update of task-set information.

3. General methods

3.1 Participants

Fifteen subjects (mean age 22.5 ± 3.9 years, range 18-29 years, eight females, all right-handed) participated in the study I. All subjects reported a history with no neurological or psychiatric illness, phobias, or drug consumption, and gave informed consent according to procedures set by the Ethical Committee of the University of Barcelóna. All subjects had normal or corrected to normal vision and were within the normal range of anxiety (assessed by means of the Anxiety Trait and State Scale, STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

Fort the studies II-VI, one hundred and fifty volunteers were interviewed according to an adapted version of the Clinical Interview of the Diagnostic and Statistical Manual (DSM IV-R), for exclusion of individuals with neurological and psychiatric illness, phobias, and drug consumption. DNA was collected with cheek cell swabs for all of them. All participants gave informed consent at each phase of the experimental procedure (interview, buccal cells extraction and EEG recordings) according to the Declaration of Helsinki and the Ethic Committee of the University of Barcelóna. All subjects had normal or corrected-to-normal vision and normal audition.

For studies II and VI, forty subjects (six men, two left-handed, mean age 22 ± 4.2 years, range 18-29 years) were included after exclusion by diagnostic criteria and obtaining the COMT and DRD2 polymorphisms. The participants homozygous for the COMT (Met/Met, Val/Val), and those presenting the most frequent alleles for DRD2 (A1, A2) were selected for an EEG recording session. For the COMT genotype, we selected only homozygous for Val and Met because, as autosomal codominant alleles, heterozygous for the COMT would display middle levels of the enzyme concentration. However, the different frequency in population of the alleles for the DAT and DRD2 did not allow us to focus on homozygotic groups. Participants genotyped as Met/Met were assigned to the MetA1+ group when they presented the A1 allele (A1/A1, or A1/A2) and to the MetA1- group when they were homozygous for the A2 allele for the DRD2 gene. Participants genotyped as Val/Val were assigned to the ValA1+ group when they presented the A1 allele (A1/A1, or A1/A2) and to the ValA1- group when they were homozygous for the A2 allele for the DRD2 gene. In the study II, seven participants were excluded from analyses due to a large amount of artifacts in their EEG recordings. From the remaining 33 individuals, seven composed the MetA1+ group, nine the MetA1- group, seven the ValA1+ group, and ten were included in the ValA1- group. In study VI, five participants were excluded from the analyses due to excessive artifacts in their EEG recordings. From the remaining 35 individuals, six composed the MetA1+ group, nine the MetA1- group, eight the ValA1+ group, and twelve were included in the ValA1- group.

Participants from each of the genetic groups did not differ significantly in age, state or trait anxiety scores (STAI, Spielberger et al., 1983).

For studies III-V, forty individuals (seven men, mean age 22 ± 3.7 years, range 18-29 years) were included after exclusion by diagnostic criteria and obtaining the DAT1 polymorphisms. The participants showing the most frequent genotypes (9R/9R, 9R/10R, 10R/10R; Vandenbergh et al., 1992) were selected for an EEG recording session. Participants genotyped as 10R/10R were assigned to the 9R- group associated with the functional effect of increased DAT expression (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005), and participants genotyped as 9R/10R and 9R/9R were included in the 9R+ group. In study III, twenty individuals composed the 9R+ group and twenty were included in the 9R- group. In study IV, five participants were excluded from the ERP analyses due to a large amount of artifacts in their EEG recordings. From the remaining 35 individuals, eighteen composed the 9R+ group and seventeen subjects were included in the 9R- group. In study V, two participants were excluded from the ERP analyses due to a large amount of artifacts in their EEG recordings. From the remaining 38 individuals, twenty composed the 9R+ group and eighteen subjects were included in the 9R- group.

3.2 DNA isolation and genotyping

DNA was collected with cheek cell swabs and extracted using the Epicentres® BuccalAmp™ DNA Extraction Kit (Epicentre, Madison, WS). Upon isolation of DNA, both single nucleotic polymorphisms (SNP) for the COMT Met108/158Val and DRD2 Taq IA genotyping were performed by real time PCR using fluorescence detection technique by means of the Applied Biosystems TaqMan technology (Applied Biosystems, Foster City, CA, USA). The primers and hybridization probes used were as follows:

• For COMT VAL158MET:

◦ Forward primer: CCCAGCGGATGGTGGAT;

◦ Reverse primer: CAGGCATGCACACCTTGTC;

◦ Probe marked with FAM: TCGCTGGCGTGAAG detecting allele Val108;

◦ Probe marked with VIC: TTCGCTGGCATGAAG detecting allele Met 108.

• For DRD2 TAQ IA, the amplification probe is:

CACAGCCATCCTCAAAGTGCTTGGTC[A/G]AGGCAGGCGCCCAGCTGG ACGTCCA,

where the probe marked with FAM detects allele G (i.e., A2) and the probe marked with VIC detects allele A (i.e., A1).

As for the DAT1 gene, the 40-bp VNTR polymorphisms were obtained for each DNA sample following the procedures described by Sano, Kondoh,

Kakimoto, & Kondo (1993), and modified by amplifying PCR-VNTR using a fluorescently tagged primer. The primers used were as follows:

DAT-F 5' 6-FAM TGTGGTGTAGGGAACGGCCTGAG 3',

DAT-R 5' CTTCCTGGAGGTCACGG-CTCAAGG,

were then followed by a final extension at 72° C for another 10 minutes. Amplification products were analyzed using a capillary electrophoresis on the sequencer ABI Prism® 3730 (Applied Biosystems, Foster City, CA) and through the Fragments Analysis Technique with GeneMapper® Software Version 4.0 (Applied Biosystems, Foster City, CA). The resulting fragments consist of 280 bp for 5 repetitions, 320 for 6, 360 bp for 7, 400 bp for 8, 440 bp for 9, 480 bp for 10, 520 for 11 and 600 bp for 13 repetitions.

3.3 Stimuli and tasks procedures

3.3.1 Auditory-visual distraction paradigm

In studies I-III, two different versions of a well-known auditory visual distraction paradigm (Escera et al., 1998; Escera et al., 2000; Escera, Yago, & Alho, 2001; Escera et al., 2003) were administered. Auditory stimuli were a 700 Hz standard tone (STD) and 100 unique environmental complex novel sounds (NOV), generated as in Escera et al., (1998), chosen amongst the most highly rated by a sample of 30 subjects on a scale of familiarity (Escera et al., 2003). Along the sequence the probability of occurrence of the standard tone was 0.8, and novel sounds occurred with a probability of 0.2,

so that each novel sound was delivered only once within each emotional condition. The duration of all auditory stimuli was 200 ms, delivered binaurally through Sehnheiser® HD202 headphones. All stimuli were presented with the stimulation program Presentation® of Neurobehavioral Systems Inc.

In studies I and III, auditory stimuli preceded in 300 ms the visual stimulus onset. Subjects sat on a comfortable chair and were instructed to press a button of a response pad as fast and accurate as possible whether two pictures appearing simultaneously on the screen were equal or different, while ignoring the sounds. The response buttons (left or right with the same hand) were counterbalanced across participants. Trial duration varied randomly from 1500 ms to 2100 ms (mean length 1800 ± 300 ms). A ten trials practice block was delivered without auditory stimuli before the performance of the task. The visual stimuli were 208 pictures, with either neutral (NEU) or negative (NEG) valence, selected from the International Affective Picture System (IAPS[1]; Lang, Bradley, & Cuthbert, 2005). They included 188 neutral pictures and 120 negative pictures picked among the most highly rated at the Self-Assessment Manikin (SAM; Lang, 1980) for both arousal and valence dimensions. These dimensions were also evaluated by the participants of a similar study (Garcia-Garcia et al., 2008) in order to ensure that they evoked the affective reaction reported by Lang et al., (2005). For the present experiment, each visual stimulus was composed by two pictures of 643x482 pixels of the same emotional valence (duration

on screen 400ms), and a fixation point (a white cross in the center on the screen), subtending a vertical angle of 9º and a horizontal angle of 25º, at 150 cm distance from subject's eyes. The sequence structure was a block design, in which 1000 trials were divided in 66 blocks of 10, 15 or 20 trials of the same valence. All blocks were pseudorandomized in one unique sequence, which could either begin with a higher proportion of neutral pictures that would decrease to a higher proportion of negative pictures, or begin with a higher proportion of negative pictures that would decrease to a higher proportion of neutral pictures. These two different stimulus distributions were counterbalanced across subjects with a Latin square design. Within the sequence, 50% of the pairs of pictures were composed by two identical pictures and 50% by two different pictures.

In study II, the version of auditory-visual distraction task had two experimental conditions: a 1-back working memory condition (WM1) and a 0-back condition with no working memory load (WM0; see SanMiguel et al., submitted). Each trial was formed of a visual target preceded in 300 ms by an auditory stimulus and lasted 1300 ± 300 ms. Participants were instructed to respond to visual stimuli as fast and accurately as possible and to ignore the auditory stimulation. Four blocks of 250 trials were delivered, there being two blocks of each condition. The order of these 4 blocks, as well as the order of the conditions, was counterbalanced across subjects. Visual stimuli were single digits (1-4 and 6-9) presented on a screen for 200 ms in white color against a black background subtending a vertical angle of 1.53^{o} and a

horizontal angle of 2.10 ^{o}. In the WM0 condition, participants had to decide by a button press whether the digit presented was larger or smaller than five. The WM1 condition consisted of a 1-back task in which participants had to decide whether the digit presented was larger or smaller in value than the digit presented in the previous trial. All participants responded with the right and left buttons with the same hand for larger and smaller respectively. Before the experiment, participants performed a five-minute visual-only practice block for each condition, which was repeated until a minimum accuracy of 75 percent was reached.

3.3.2 Task-switching paradigm

A task-cueing protocol inspired by the WCST (Rubinstein et al., 2001) and adapted for measuring ERPs (Barceló, 2003) was administered to participants. Each trial consisted of a tonal cue followed by a target display with four key cards on top of one choice card, all centered on a screen. The target stimulus subtended a visual angle of 4º horizontally and 3.5º vertically, and remained on display until a response was given or up to a maximum of 3000 ms. Subjects were instructed to match the choice card with one of the four key cards following two possible task rules (color or shape). To ensure that all participants could see colors properly, the Test of Ishihara was applied for excluding participants with suspected color blindness. Before target onset, one out of four tonal cues explicitly informed the subject whether to sort the card according to either the ‘color’ (500/1000 Hz) or

'shape' (2000/4000 Hz) rules. Binaural tones were delivered through Sehnheiser® HD202 headphones with a duration of 200 ms, 10 ms rise/fall times and 65 dB SPL. The meaning of the tonal cues was reversed for half of the subjects. All stimuli were presented with the stimulation program Presentation® (Neurobehavioral Systems Inc., Albany, CA). Three trial types were defined in order to dissociate the processing of changes in sensory and task representations. In the *repeat* trials, both the tonal cue and the task were repeated relative to the previous trial. In the *cue-switch* trials, only the cue changed but the task remained the same as in the previous trial. In the *task-switch* trials both cue and task changed. Responses were made using 4 keys on a keyboard, mapped onto the four fingers of the dominant hand, in an array corresponding to the layout of the four key-cards. The far left button designated the key card on the far left of the display, the far right button designated the key card on the far right, and so on. All three trial types were randomly presented with the same overall probability along the 200 trials of the experimental block, as well as during the 50 practice trials. The cues related to each criterion were employed five times during the instruction period of the practice block, and three more times during the instructions of the experimental block, in order to ensure that each participant had correctly learnt the cue-task association. Whenever the hit rate of the practice block was lower than 75%, an additional practice block was administered to ensure full assimilation of the correct cue-task association prior to the run of experimental block. All the task sets declared in the instructions consisted of four-feature-stimulus to four-forced-response mappings. 'Task set' denotes

here, in a broad sense, a set of rules that govern the mapping between sensory inputs and motor responses (Braver, Reynolds, & Donaldson, 2003). The cue-target interval randomly varied between 650 ±150 ms, thus minimizing the effects of a constant preparation interval (Rogers & Monsell, 1995), and the target remained on the screen until a response was given (up to a maximal of 3000 ms). Response-cue intervals also varied randomly around 1100 ±100 ms within the trial block.

3.4 Data acquisition

EEG activity was recorded (ANT Software b.v., Enschede, The Netherlands) during task performance from 64 scalp electrodes following the extended 10/10 convention in an electrically and acoustically shielded room. The experimenters recorded EEG through a double-blind system, by which the experimenter never knew the genotype of the participant. Horizontal and vertical electro-oculographic (EOG) recordings were obtained with electrodes placed at the outer cantus of the right eye and above the right eye. The common reference electrode was placed on the tip of the nose, and the ground was located at the chest. The EEG was amplified and digitized at a sampling rate of 512 Hz. Impedances were kept below 10 kΩ during the whole recording session, which lasted about 20 minutes.

3.5 Data processing and analysis

3.5.1 Behavior

In studies I-III, the first five trials of each block, as well as those trials following a trial containing a novel sound were excluded from the analyses. A correct button press within 100-1200 ms after visual stimulus onset was regarded as a hit, and the mean RT was computed for hit trials only. Hit rate and RT were compared by means of three-factor repeated-measures ANOVA including the between subject factors Novelty (standard, novel), and experimental condition (neutral and negative emotional contexts for studies I and III, and conditions with and without working memory load in study II) and the between-subjects variables of the polymorphisms for the COMT (Met and Val) and the DRD2 (A1+ and A1-) in study II, and for the DAT (9R+ and 9R-) in study III. Pair-wise *post hoc* comparisons were performed to paired out interactions.

In studies IV-VI, any correct button press within 200-3000 ms after target onset was regarded as a hit, and the mean RT was computed for hit trials only. Hit rate and mean RT were submitted to a two-way mixed ANOVA with one repeated-measures factor (Trial type: repeat, cue-switch, task-switch), and one between-subject factor Group for the DAT genotype (9R+ and 9R-) in studies IV and V and the between-subjects variables of the polymorphisms for the COMT (Met and Val) and the DRD2 (A1+ and A1-) in study VI . Pair-wise post hoc comparisons were performed to examine any significant difference between conditions.

3.5.2 Event-related brain potentials

ERPs were averaged offline for each trial type or task condition, for an epoch of 1400 ms including a pre-stimulus baseline of 200 ms. Frequencies above 30 Hz were digitally filtered out from individual EEG epochs prior to ERP averaging. EOG correction was performed via a blind source separation technique with ASA 4.5 of ANT® Software (Enschede, The Netherlands), as described in Belouchrani, Abed-Meraim, Cardoso, & Moulines, (1997). After EOG correction, any epochs containing EEG activity exceeding ± 100 μV peak-to-peak amplitudes were rejected from further analysis.

In the study III, novelty-P3 was isolated in the difference waves obtained by subtracting the STD trial ERPs from those elicited to NOV trials. Novelty-P3 was measured as the mean amplitude in the 200-290 and 290-370 ms latency windows for the early and the late phases, respectively. In the study IV, mean amplitudes of the auditory N1 evoked potential were computed in the latency window from 110 to 140 ms. In the study V, the mean amplitudes of the following ERP components were computed in the specified latency windows: the early fronto-central positivity from 180 to 220 ms, the late fronto-posterior positivity from 300 to 340 ms, the late negative deflection from 420 to 440 ms. Likewise, the slow fronto-parietal negativity was computed in two latency windows, from 600 to 700 ms (SW1), and from 800 to 900 ms (SW2). In the study VI, the mean amplitudes of the fronto-central

positive subcomponent of the nP3 was computed in the latency window from 300 to 340 ms. All these brain responses were measured at channels F3, F4, Fz, C3, C4, Cz, P3, P4 and Pz. ANOVA for repeated measures were performed including factors Frontality (frontal, central, parietal), Laterality (right, midline, left), Context (NEU, NEG) for study III, Trial Type (repeat, cue-switch and task-switch) for studies IV-VI, Phase (early, late; as described in Escera et al. (1998; 2001) for study III, and the between-subject variable Group for the DAT polymorphism (9R+, 9R-) in studies III-V, or COMT (Met and Val) and DRD2 (A1+ and A1-) for studiy VI. Greenhouse-Geisser correction of the degrees of freedom was applied. The *P*-values following correction were reported.

3.5.3 Gamma band responses

Data processing was performed with ASA 4.5.1.0 software (ANT®, ENschede, The Netherlands). For single sweep analysis, time epochs were defined as the time window starting 250 ms before and lasting until 1000 ms after auditory stimulus onset (i.e., 700 ms from visual stimulus onset), with the pre-stimulus period used as a baseline. Automatic ocular correction was performed by applying an independent component analysis (ICA) as in Belouchrani et al., (1997; implemented in the ASA 4.5.1.0 software). In addition, epochs contaminated with ocular movements or muscle artifacts were rejected by automatic artifact rejection procedure if their peak-to-peak amplitude exceeded 150 μV. For analysis of the GBR, the EEG signal was band-pass filtered between 30 and 60 Hz. To equalize the number of sweeps for better

control of possible signal-to-noise ratio differences, all novel sweeps and a similar number of randomly selected standard sweeps were selected from each condition.

To obtain the time-frequency components from the gamma range, epochs averaged for each condition and participant were decomposed by means of a continuous Wavelet transform. Time-frequency transforms were obtained by the application of complex-valued Morlet wavelets, which are Gaussian in both time and frequency domain. Complex Morlet wavelets *w* can be generated in the time domain for different frequencies, *f*, according to the equation:

$$w(t, f) = A\exp(-t^2 / 2\sigma_t^2)\exp(2i\pi ft),$$

where *t* is time, $A = (\sigma_t\sqrt{\pi})^{-1/2}$, σ_t is the wavelet duration, and $i=\sqrt{-1}$.

A ratio of f_0/σ_f = 12 was used, where f_0 is the central frequency and σ_f is the width of the Gaussian shape in the frequency domain. The analyses were performed in the frequency range 30-60 Hz, with a central frequency at 0.75 Hz intervals (40 frequency steps). For different f_0, time and frequency resolutions can be calculated as $2\sigma_t$ and $2\sigma_f$, respectively, where σ_t and σ_f are related by the equation $\sigma_t = 1/(2\pi\sigma_f)$.

PS was calculated by means of the phase-locking of oscillatory activity measured by using the phase-locking factor (PLF) proposed by Tallon-Baudry, Bertrand, Delpuech, & Permier, (1997). This is a measure for phase identity across trials and is bounded between 0 (non-phase-locked signal)

and 1 (phase-locked signal). Statistical thresholds were assessed by means of circular statistics (Rayleigh test) with a significance of p=0.01. Since PLF reached the significant threshold for each sweep and channel, they were submitted to further analyses. A 250 ms baseline (-250 to -50 ms) was used as baseline for the time-frequency information and the mean of this time window was subtracted for the time frequency matrix for each frequency and time point.

Maximal amplitude and PLF values of the GBRs were obtained in the latency windows from 100 to 200 ms, as GBRs are strongly synchronized in the first 100 ms after sensory stimulation and reflect very early stages of stimulus evaluation (Tallon-Baudry & Bertrand, 1999). Because previous studies have referred 40 Hz activity to the increase of attention (Tiitinen et al., 1993; Womelsdorf et al., 2006), analyses were performed for this frequency range around 40 Hz (35-45 Hz).

For analysis of both amplitude and PLF of the GBR, maximal values in the defined latency window were measured for the auditory GBR for 18 of the recorded channels (F3, Fz, F4, FC3, FCz, FC4, C3, Cz, C4, CP3, CPz, CP4, P3, Pz, P4, PO3, POz and PO4) at around 40 Hz frequency range. Repeated measures of four factor ANOVAs were performed including the factors Novelty (standard, novel), emotional contex (neutral and negative) in study I, or working memory load (with or without working memory load) in study II, Region (six levels of frontality corresponding to F, FC, C, CP, P and PO) and

Laterality (three levels for left, midline and right channels), and the between-subjects variables of the polymorphisms for the COMT (Met and Val) and the DRD2 (A1+ and A1-) in the study II. Greenhouse-Geisser correction of the degrees of freedom was applied, with the corrected *P*-values reported. Pair-wise *post hoc* comparisons were performed to paired out interactions.

4. Results

Study I.

Garcia-Garcia, M., Yordanova, J., Kolev, V., Domínguez-Borràs, J., Escera, C. (2010) Tuning the brain for novelty detection under emotional threat: The role of increasing gamma-phase synchronization. *NeuroImage 49, 1038-1044.*

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Tuning the brain for novelty detection under emotional threat: The role of increasing gamma phase-synchronization

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ABSTRACT

Effective orienting of attention towards novel events is crucial for survival, particularly if they occur in a dangerous situation. This is why stimuli with emotional value are more efficient in capturing attention than neutral stimuli, and why the processing of unexpected novel stimuli is enhanced under a negative emotional context. Here we measured the phase-synchronization (PS) of gamma-band responses (GBR) from human EEG scalp-recordings during performance of a visual discrimination task in which task-irrelevant standard and novel sounds were presented in either a neutral or a negative emotional context, in order to elucidate the brain mechanisms by which emotion tunes the processing of novel events. Visual task performance was distracted by novel sounds, and this distraction was enhanced by the negative emotional context. Similarly, gamma PS was enhanced after novel as compared to standard sounds and it was also larger to auditory stimuli in the negative than in the neutral emotional context, reflecting the synchronization of neural networks for increasing of attentional processing. Remarkably, the larger PS increase of GBR after novel sounds in the negative as compared to the neutral emotional context over midline and right frontal regions reveals that a negative emotional context tunes novelty processing by means of the PS of brain activity in the gamma frequency band around 40 Hz in specific neural networks.

Introduction

During a fearful or threatening situation, effective orienting of attention towards unexpected events may provide for rapid awareness of a potential harm and result in adaptive advantage for survival. In humans, stimuli with an emotional load elicit stronger attention capture than non-emotional stimuli (Hansen and Hansen, 1988; Ohman et al., 2001). This is seen at very early stages of information processing (at about 100 ms from stimulus onset; Carretie et al., 2004; Krolak-Salmon et al., 2004; Sugase et al., 1999), and even when the emotional stimuli appear outside the attentional set (Carretie et al., 2005; Pessoa et al., 2002; Vuilleumier et al., 2001). Moreover, visually induced negative emotions interact with the processing of auditory inputs, as shown by startle reflex potentiation while visualizing negative emotional pictures (Bradley et al., 2006; Stanley and Knight, 2004), and by enhanced activity in auditory novelty processing cerebral regions (bilateral superior temporal gyrus) during emotional threat, as shown by fMRI (Dominguez-Borras et al., 2008a, 2008b). Similarly, electrophysiological brain responses associated to auditory novelty processing are enhanced when novel sounds occur in a negative emotional context (Dominguez-Borras et al., 2008a, 2008b; Garcia-Garcia et al., 2008). However, in spite of intensive research (Vuilleumier, 2005) the brain mechanisms by which emotion tunes novelty processing have not yet been elucidated.

In order to explore the mechanisms subserving the interaction between emotional activation and novelty detection, electroencephalographic (EEG) oscillations in the gamma frequency band (30–70 Hz) were analyzed. Gamma-band responses (GBRs) are strongly synchronized in the first 100 ms after sensory stimulation and reflect very early stages of stimulus evaluation (Tallon-Baudry and Bertrand, 1999), particularly when the stimuli are presented at very short intervals. GBRs are modulated by attentional processes, as they are enhanced by task-relevant targets relative to irrelevant non-targets (Busch et al., 2006; Debener et al., 2003; Herrmann et al., 2004; Tiitinen et al., 1993; Yordanova et al., 1997, 2000, 2001). Specifically, an increase in the phase-synchronization (PS) of GBRs has been associated with the brain mechanisms of increased attention (Fries et al., 2001) and the top-down mechanisms for enhanced sensory information processing (Busch et al., 2006; Debener et al., 2003; Fries et al., 2001; Herrmann et al., 2004; Yordanova et al., 2001) through the amplification of behaviorally relevant signals in the cortex (Fries et al., 2001; Frund et al., 2007; Womelsdorf et al., 2006; Yordanova et al., 2001). Moreover, visual stimuli conditioned to emotional pictures elicit stronger low gamma-band PS (around 35 Hz) than non-conditioned ones (Keil et al., 2001, 2007a, 2007b; Stolarova et al., 2006), suggesting a

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neural optimization for rapid processing of emotionally relevant stimuli.

In the present study, we used an auditory-visual distraction paradigm in which task-irrelevant frequent standard and rare novel sounds were followed by task-relevant visual stimuli of either neutral or negative emotional load (Escera et al., 2001, 2003). The PS of human scalp-recorded gamma oscillations in response to auditory standard and novel sounds occurring in either a neutral or a negative emotional context was examined by means of the phase-locking factor (PLF) in early (50–150 ms) and late (150–250 ms) latency windows after stimulus. We hypothesized that if gamma-band PS plays a role in integrating novelty and emotional information processing in order to guide behavior, the PS elicited by novel sounds in a negative emotional context should be larger than the one elicited in a neutral one.

Materials and methods

Participants

Fifteen subjects (mean age 22.5 ± 3.9 years, range 18–29 years, eight females, all right-handed) participated in the present study. All subjects reported a history with no neurological or psychiatric illness, phobias, or drug consumption, and gave informed consent according to procedures set by the Ethical Committee of the University of Barcelona. All subjects had normal or corrected to normal vision and were within the normal range of anxiety (assessed by means of the Anxiety Trait and State Scale (STAI; Spielberger et al., 1983).

Procedure

Participants performed a modified version of a well-characterized auditory-visual distraction paradigm (Dominguez-Borras et al., 2008a, 2008b; Escera et al., 1998, 2001, 2003; Garcia-Garcia et al., 2008). They were instructed to classify visual stimuli presented 300 ms after task-irrelevant auditory stimuli. Each visual stimulus was composed by two pictures of 643 × 482 pixels (50% picture pairs contained two identical pictures and 50% two different ones) of the same emotional valence and a fixation point (a white cross in the center of the screen), presented with a duration of 400 ms and vertical and horizontal angles of 9° and 25°, respectively, at 150 cm from the subject's eyes. A total of 308 pictures (188 of neutral and 120 of negative valence) were selected for the visual stimuli from the International Affective Picture System (IAPS; Lang et al., 2005) according to valence and arousal ratings in Self-Assessment Manikin (SAM; Lang, 1980) and by the participants of a previous study (Garcia-Garcia et al., 2008). Auditory stimuli consisted of a 700 Hz standard tone and 100 unique environmental complex novel sounds, generated as in Escera et al. (1998), all lasting 200 ms and delivered binaurally through Sehnheiser® HD202 headphones at an intensity of 80 dB SPL. Along the sequence, standard tones occurred with a probability of $p = 0.8$ and novel sounds with the complementary $p = 0.2$, so that each novel sound was delivered only once within each emotional condition. A total of 1000 trials were presented in 66 blocks of 10, 15 or 20 trials of the same valence and arousal. All blocks were pseudo randomized in one unique sequence, which could either begin with a higher proportion of neutral pictures ending with a higher proportion of negative pictures, or vice-versa. These two different stimuli distributions were counterbalanced across subjects with a Latin square design. However, the emotional content of the images was task-irrelevant, as the participants were neither informed about their emotional content, nor instructed to pay any attention to it. Subjects were instructed to respond as fast and accurate as possible whether the two pictures were equal or different, while ignoring the sounds. All stimuli were delivered by Presentation® Neurobehavioral System Inc., (Albany, CA). The response buttons (left or right with the same hand) were counterbalanced across participants. Trial duration varied randomly from 1500 ms to 2100 ms (mean length 1800 ± 300 ms). A ten-trial practice block was delivered without auditory stimuli before the experimental session.

EEG data acquisition

Electroencephalographic activity was recorded (EEmagine, ANT Software b.v., Enschede, Netherlands) during task performance from 64 scalp electrodes following the 10/10 convention in an electrically and acoustically shielded room. The horizontal and vertical electrooculograms (EOG) were recorded with electrodes placed on the outer cantus and above the right eye. The common reference electrode was placed on the tip of the nose, and the ground was located on the chest. The EEG was amplified with band limits of 0–138 Hz and digitized with a sampling rate of 512 Hz. Impedances were kept below 15 kΩ during the whole experimental session, which lasted about 35 min, including short resting periods.

Data processing

Data processing was performed with Brain Vision Analyzer software (Brain Products GmbH, Gilching, Germany, version 1.05). For single sweep analysis, time epochs were defined as the time window starting 250 ms before and lasting until 1000 ms after auditory stimulus onset (i.e., 700 ms from visual stimulus onset), with the pre-stimulus period used as a baseline. An automatic ocular correction was performed according to Gratton et al. (1983); as implemented in the Brain Vision Analyzer software. Epochs contaminated with ocular movements or muscle artifacts were rejected by automatic artifact rejection procedure if their peak-to-peak amplitude exceeded 150 μV. For analysis of the GBR, the EEG signal was band-pass filtered between 25 and 70 Hz. To equalize the number of sweeps for better control of possible signal-to-noise ratio differences, a similar number of sweeps were randomly selected from each condition. For each participant and for each condition (standard and novel sounds in both neutral and negative emotional contexts), between 68 and 78 single sweeps were used for analysis.

Data analysis

Time-frequency transforms were obtained by the application of complex-valued Morlet wavelets, which are Gaussian in both time and frequency domain. Complex Morlet wavelets w can be generated in the time domain for different frequencies, f, according to the equation:

$$w(t,f) = A\exp\left(-t^2/2\sigma_t^2\right)\exp(2i\pi ft),$$

where t is time, $A = (\sigma_t\sqrt{\pi})^{-1/2}$, σ_t is the wavelet duration, and $i = \sqrt{-1}$.

A ratio of $f_0/\sigma_f = 12$ was used, where f_0 is the central frequency and σ_f is the width of the Gaussian shape in the frequency domain. The analyses were performed in the frequency range 30–60 Hz, with a central frequency at 0.75 Hz intervals (40 frequency steps). For different f_0, time and frequency resolutions can be calculated as $2\sigma_t$ and $2\sigma_f$, respectively (Tallon-Baudry et al., 1997). σ_t and σ_f are related by the equation $\sigma_t = 1/(2\pi\sigma_f)$.

Phase-locking of oscillatory activity was measured using the PLF (proposed by Tallon-Baudry et al., 1997). PLF is a coefficient ranging between 0 (no phase-locking, random phase-difference between sweeps) and 1 (maximal phase-locking, constant phase-difference). PLF was normalized by subtracting the mean value of the baseline period (250 to 50 ms before auditory stimulus for the auditory potentials) from the PLF for each data point and dividing the difference by the standard deviation of the same baseline (Gruber and

Muller, 2005; Tallon-Baudry and others 1997; Doesburg et al., 2008). The resulting PLF indicates changes from the average baseline PLF expressed in units of standard deviation, ranging between positive and negative values (usually from −3 to +3).

In order to analyze PLF associated with statistically significant synchronization, the maximal PLF value was identified for the respective time window and electrode, and compared with the maximal PLF value detected in the whole epoch from the same set of single sweeps after they were randomly shuffled 200 times thus representing surrogate distributions of PLF values (Lachaux et al., 2000). Statistical significance was concluded if the measured parameters were larger than the respective maximal values in the surrogate PLF distributions. This procedure was conducted for each subject included in the study and the measured PLF values were subjected to further statistical evaluation.

The activity of two wavelet layers reflecting relatively independent time-frequency components was measured. They corresponded to two central frequencies at 35 (30–40 Hz) and 40 Hz (35–45 Hz; Fig. 1A). Firstly, the identification of these gamma-band components was based on visual inspection of the data demonstrating distinctive peaks in synchronization at these frequencies (Fig. 1A). Secondly, this was in accordance with previous literature relating attention and emotion processes to specific 35 and 40 Hz activity (Keil et al., 2001, 2007a, 2007b; Stolarova et al., 2006; Tallon-Baudry and Bertrand, 1999; Womelsdorf et al., 2006). Similarly, after evaluation of the maxima in the grand average (Fig. 1B) the latency windows from 50 to 150 ms and from 150 to 250 ms, were defined for PLF analyzed as early and late GBR, respectively.

Statistical analysis

Only auditory stimuli preceded and followed by visual stimuli of the same emotional valence were included in both behavioral and PLF analyses. The first five trials of each block and the first ten trials of the sequence, as well as trials following a trial containing a novel sound were also excluded from the analysis. A correct button press within 100–1200 ms after visual stimulus onset was regarded as a hit, and the mean RT was computed for hit trials only. Hit rate and RT were compared by means of two-factor repeated-measures ANOVA including the factors Novelty (standard and novel), and Emotional Context (separately for sounds both preceded and followed by neutral pictures and those both preceded and followed by negative pictures). Pair-wise *post hoc* comparisons were performed to paired out interactions.

For analysis of PLF, mean values were measured for the auditory GBR (early and late auditory GBR) for 18 of the recorded channels (F3, Fz, F4, FC3, FCz, FC4, C3, Cz, C4, CP3, CPz, CP4, P3, Pz, P4, PO3, POz and PO4) at 35 and 40 Hz frequency sub-ranges. Repeated measures four factor ANOVAs were performed for the two gamma-band frequency sub-ranges previously defined, including the factors Novelty (standard and novel), Emotional Context (neutral and negative as defined above), Region (six levels of frontality corresponding to F, FC, C, CP, P and PO) and Laterality (three levels for left, midline and right channels). Greenhouse–Geisser correction of the degrees of freedom was applied, with the corrected *p*-values reported. Pair-wise *post hoc* comparisons were performed to paired out interactions.

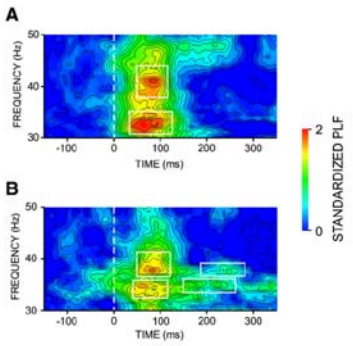

Fig. 1. Time-frequency plots of phase-locking of gamma-band activity. (A) Time-frequency plot of grand average phase-locking factor (PLF) across four stimulus conditions at FCz. The synchronization of early gamma responses (50–150 ms) in frequency-specific gamma sub-ranges (30–40 Hz and 35–45 Hz) is indicated. (B) Time-frequency plot of grand average PLF only for novel stimuli at FCz in order to illustrate that synchronization of gamma responses is observed in the two indicated time windows after auditory stimulus (50–150 ms and 150–250 ms).

Results

Performance

As shown in Fig. 2, an overall hit rate above 91% was attained, which decreased in trials containing novel sounds (Novelty × Context, $F_{1,14} = 4.73$, $p = 0.047$), but only in the condition with pictures of neutral emotional load ($F_{1,14} = 8.19$, $p = 0.013$). Novel sounds distracted visual task performance, as indicated by a delay in the response time (RT) to visual stimuli following novel sounds ($F_{1,14} = 32.67$, $p < 0.001$). The RT was slower in the negative emotional context ($F_{1,14} = 7.51$, $p = 0.016$) as well. Remarkably, the RT increase after novel sounds was larger in the negative (34.5 ± 4.7 ms) than in the neutral emotional context (12 ± 4.5 ms) as supported by the significant interaction Novelty × Context ($F_{1,14} = 26.51$, $p < 0.001$).

Gamma-band synchronization

Relevant synchronization of auditory GBRs was detected for lower and higher gamma frequency sub-bands centered at 35 Hz and 40 Hz, for which the PLF was accordingly quantified. Within the two sub-bands, the PLF was more pronounced over midline-anterior locations for both the standard and novel auditory stimuli and in the two contexts (neutral and negative) (Region, $F_{5,70} = 4.08$, $p = 0.049$, Laterality, $F_{2,28} = 5.16$, $p = 0.015$).

Processing of auditory novelty resulted in a significant increase in the gamma-band PLF at its 35-Hz and 40-Hz frequency sub-ranges. As can be seen in Fig. 3A, the 35-Hz GBR showed an overall stronger PLF

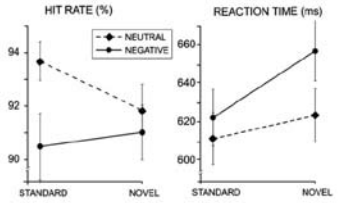

Fig. 2. Hit rate and RT for all four conditions. RT is larger and hit rate is lower in novel trials compared to standard ones, and in the negative context condition compared to the neutral one. Notice that the RT increase caused by novel sounds was larger in the negative than in the neutral emotional context.

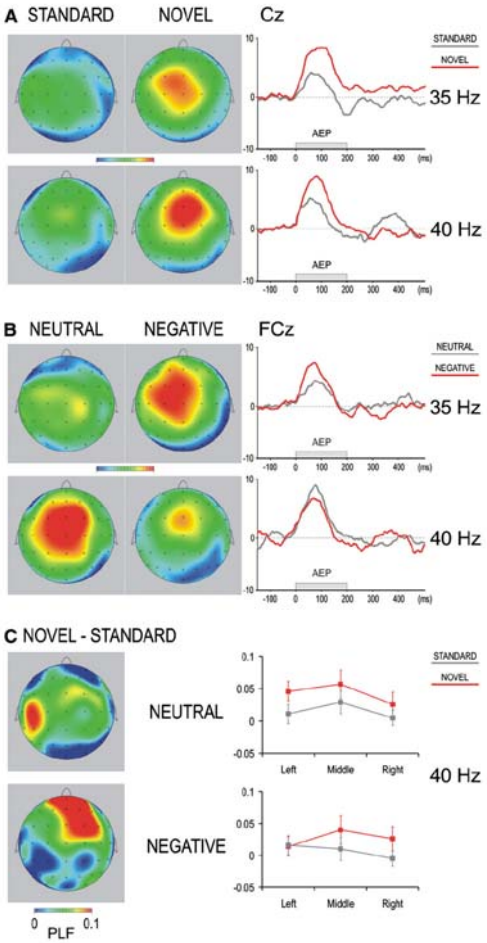

Fig. 3. Scalp distribution of the PLF at 35 and 40 Hz gamma frequency sub-bands. (A) Scalp distribution of PLF at 35 and 40 Hz for the novel and standard sounds in the latency window from 50 to 150 ms. Novel auditory stimuli showed a stronger PLF than standard ones at both 35 and 40 Hz. Waveforms show larger PLF for novel than for standard sounds. (B) Scalp distribution of PLF at 35 and 40 Hz for the neutral and negative emotional contexts. The negative context elicited a frequency-specific stronger PS than the neutral one at 35 Hz over fronto-central regions. Waveforms show larger PLF in the negative than in the neutral emotional context at 35 Hz. (C) Scalp distribution of the subtraction of PLF for novel minus standard sounds in both neutral and negative emotional contexts at 40 Hz. Auditory novelty elicited a larger PLF enhancement in negative than in neutral emotional context over midline and right frontal regions.

within 150–250 ms after novel than after the standard auditory stimuli ($F_{1,15} = 10.92$, $p = 0.005$). For the 40-Hz GBR, a novelty-related increase in PLF was significant in the early latency window, from 50 to 150 ms ($F_{1,14} = 5.04$, $p = 0.041$).

Processing of emotional pictures elicited increased PLF of the lower-frequency portion of the GBR at 35 Hz to the two types of auditory stimuli. Fig. 3B shows that the 35-Hz GBR PLF at fronto-central locations was significantly enhanced in the negative emotional context as compared to the neutral one. This was evidenced by the significant Region × Context interaction ($F_{5,70} = 5.25$, $p = 0.008$), and subsequent *post hoc* comparison showing that while no Region effect on gamma PLF was observed in the neutral context ($F_{5,70} = 1.55$, $p = 0.342$), a significant effect became evident in the negative one ($F_{5,70} = 5.32$, $p = 0.014$).

The most striking finding was that for the 40-Hz gamma activity, the effect of the emotional context interacted with the PLF-increasing effect of novelty over specific scalp regions, as supported by the Novelty × Context × Laterality interaction ($F_{2,28} = 3.91$, $p = 0.041$). As shown in Fig. 3C, novel sounds resulted in stronger PLF of GBR over midline locations ($F_{1,14} = 5.65$, $p = 0.032$) and tended to be stronger over the right-hemisphere ($F_{1,14} = 3.57$, $p = 0.080$) than in the left hemisphere ($F_{1,14} = 0.030$, $p = 0.865$) in the negative emotional context, while no such effects were observed for the neutral emotional context.

Discussion

The present study aimed at elucidating the brain mechanisms subserving the interaction between emotion and novelty processing. Behavioral results supported previous findings revealing slower RT to visual stimuli following novel than standard sounds (i.e., distracted ongoing task performance; Dominguez-Borras et al., 2008a, 2008b; Escera et al., 2001, 2003; Garcia-Garcia et al., 2008), larger RT in the negative than in the neutral emotional context (Dominguez-Borras et al., 2008a, 2008b; Garcia-Garcia et al., 2008), and most importantly, an enhancement of the distraction effect in the negative as compared to the neutral emotional context (Dominguez-Borras et al., 2008a, 2008b; Garcia-Garcia et al., 2008). These behavioral effects were paralleled by a gamma-band PLF enhancement after novel as compared to standard sounds, and PLF to both standard and novel sounds was larger in the negative as compared to the neutral emotional context as well. Remarkably, the behavioral interaction seen as increased distractibility under a negative emotional context was paralleled in the electrophysiology by the finding in a region-specific way of larger PLF increase after novel sounds occurring in the negative emotional context in comparison to that in the neutral one. It should be mentioned that although recent studies evidenced that scalp-recorded induced gamma activity may reflect miniature saccade dynamics (Yuval-Greenberg et al., 2008), gamma-band PS may not be associated with such a source of generation. Therefore, the effect of PLF observed in the present study cannot be attributed to the differential miniature saccade dynamics elicited in the different experimental conditions.

Novelty processing

Novel sounds yielded longer RT and elicited larger PLF of gamma brain activity than standard ones. This PLF increase after novel sounds had a clear mid-central scalp distribution for oscillations around 35 Hz, and a more anterior distribution at around 40 Hz. In the auditory modality, any simple tone elicits a GBR (Debener et al., 2003; Herrmann and Mecklinger, 2000; Yordanova et al., 1997, 2000, 2001), which is larger for attended than unattended stimuli, as shown by scalp-recorded data in humans (Herrmann and Mecklinger, 2000; Herrmann and Knight, 2001; Tiitinen et al., 1993), and by single-cell recordings in monkeys (Womelsdorf et al., 2006). Subsequent studies in humans have confirmed that attention increases the PS of auditory gamma responses (see however Debener et al., 2003). The current results confirm that the PS of auditory gamma oscillations reflect an enhancement of attentional resources re-allocated for processing the auditory distracter, as supported by the behavioral results. According to certain studies, GBRs may be sensory in origin and appears to be generated only by bottom-up binding (Karakas and Basar, 1998). However, since the synchronizing effect was not limited to the auditory stimulus duration (Fig. 3A) and lasted until the visual target appeared, the current gamma-band PS enhancement following novel auditory stimuli may reflect rather a greater re-allocation of attentional resources (Tiitinen et al., 1993; Yago et al., 2003; Yordanova et al., 1997, 2000, 2001) than the more complex physical characteristics of the novel sounds in relation to the standard ones (Karakas and Basar, 1998).

Emotional context

On the other hand, low GBRs showed an early enhancement of PLF at anterior locations to all auditory stimuli in the negative context as compared to the neutral one (Fig. 3B), irrespective of whether the auditory stimuli were standard or novel. This PS enhancement is indicative of an effective modulatory effect of the emotional context on the processing of auditory stimuli which are not emotionally loaded per se. Since auditory stimuli consistently predicted the occurrence of visual targets either emotionally negative or neutral, it could be argued that an association mechanism may have transferred the emotional value of the pictures to the auditory stimuli (Keil et al., 2001, 2007a, 2007b; Stolarova et al., 2006). Alternatively, the frontally distributed PS of brain gamma oscillations may reflect a reorganization of the neural responses (Fries et al., 2001) at the level of frontal attention-related areas, in order to optimize the attention capture by stimuli without an a priori emotional valence but occurring in a context of emotional relevance at a very early stage of auditory processing. Since previous studies have shown a similar gamma-band PS increase locked to visual stimuli conditioned to emotional pictures with an occipital scalp distribution (Keil et al., 2001, 2007a, 2007b; Stolarova et al., 2006), the current results support a specific role of low gamma activity (30–40 Hz) for emotionally triggered functional activation states (Stolarova et al., 2006) irrespective of sensory modality, whose scalp distribution varies according to the nature of the stimuli. The synchronizing effect of both the auditory novelty and negative emotional context on 35-Hz oscillations suggest that low-frequency gamma activity reflects a stronger attentional processing irrespective of the underlying processing leading to this attentional enhancement (Herrmann et al., 2004), although this PS of the gamma oscillations (Herrmann et al., 2004; Womelsdorf et al., 2006) is thought to take place in distinct neural networks, according to the nature of the triggering process (Voss and Paller, 2009).

Tuning of novelty processing by the emotional context

The observation of a distinct regional distribution of synchronized gamma networks contributes to the understanding of the brain mechanisms underlying the interaction between multiple synchronizing sources reflecting the modulation of attentional processes by the emotional context. This modulation of novelty processing by the emotional context was revealed by larger novelty-related gamma-band PLF increase in the negative emotional context over midline and right scalp regions, as compared to the increase elicited by novel sounds in the neutral emotional context. Several previous studies have demonstrated that novelty processing is specifically associated with the activation of superior temporal and right frontal cortices (Opitz et al., 1999; Strobel et al., 2008) as a general orienting response towards any auditory novel stimulus. Since a negative emotional context enhances the orienting of attention towards novel stimuli

(Dominguez-Borras et al., 2008a, 2008b; Garcia-Garcia et al., 2008), as shown behaviorally by larger distraction in a negative than in a neutral emotional context, we suggest that the interaction of the different synchronizing sources subserving the effect of the emotional context on novelty processing, might have recruited a distinct neural network whose PS at 40 Hz was only reflected at midline and right frontal scalp regions (Fig. 3C). While the modulation of novelty processing by a negative emotional context has been previously reported in evoked potentials at later latencies (around 300 ms Dominguez-Borras et al., 2008a, 2008b; Garcia-Garcia et al., 2008), the present analysis of gamma-band activity reveals that differences in emotional context can strongly modify the orienting of attention to external stimuli at very early stages (circa 100 ms) of stimulus processing. Moreover, as it can be observed in Fig. 3C, novelty-related increase in gamma PS is reflected over the left temporo-parietal regions in the neutral condition. This scalp distribution is consistent with the notion of semantic analysis of the novel sound contents, as semantic processing has been correlated with activity from the lower gamma frequency sub-bands (Pulvermuller et al., 1999). In contrast, enhanced emotional arousal seemed to inhibit meaning extraction, favoring thus the contribution of the orienting component of the novelty response in the right frontal regions.

Conclusion

With the present study we aimed at tapping the brain mechanisms supporting enhanced orienting of attention to novel auditory events under emotional threat. We found that auditory involuntary attention was reflected by an increase of gamma-band PS, and that the emotional context enhanced gamma-band PS to auditory stimuli which were not emotionally loaded per se, each with a distinct regional distribution of synchronized gamma networks. More important, gamma-band PS of 40 Hz oscillations reflected the brain mechanisms by which emotion tunes novelty processing, presumably by synchronizing the activity of the GBR related to novelty processing and emotion in different specific neural networks. The observation of the distinct regional distribution of the gamma neural oscillations contributes to the understanding of the brain mechanisms underlying the interaction between multiple synchronizing sources reflecting the modulation of attentional processes by the emotional context. In summary, the present study has revealed that a negative emotional context tunes novelty processing by means of the PS of brain activity in the gamma frequency band around 40 Hz in specific neural networks.

Acknowledgments

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Study II.

Garcia-Garcia, M., Escera, C., SanMiguel, I., Clemente, I.C. COMT and DRD2 genes account for resetting of gamma neural oscillations to novel sounds. *In preparation*

COMT and DRD2 genes account for resetting of the gamma neural oscillations to novel sounds

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Abstract

A biphasic model of the expression of the dopamine transmitter has related D1 receptor binding to stability and D2 receptor binding to flexibility of mental representations. Here, we examined the role of two genes regulating D1 and D2 dopaminergic receptor stimulation (i.e., Catechol-O-Methyltransferase, COMT, and D2 dopamine receptor, DRD2) on the stimulus-driven control of attention. Participants were distributed in groups according to the combination of the polymorphic variations for the COMT and DRD2 genes, and performed a well-known auditory visual distraction protocol for studying stimulus-driven control of attention. COMT Met individuals, related to higher PFC DA concentrations, combined with a high DRD2 density (i.e., without DRD2 A1 allele) and COMT Val participants (low PFC DA availability) combined with low DRD2 density (i.e., with DRD2 A1 allele) showed a delay in response time to the task following appearance of a novel event. In contrast, individuals with other combination of polymorphisms shifted correctly the attention toward novel events without a delay in response time to the task, through a, enhancement of phase-coherence of neural oscillation evoked by the event at 40 Hz. These results provide strong evidence for the role the dopaminergic system, and specially the COMT and DRD2, in the mechanisms of stimulus-driven control of attention.

Introduction

An adaptive cognitive adjustment to the richness of stimuli around us requires an accurate control of attention by maintaining relevant mental representations active without completely missing potentially relevant stimuli outside the focus of attention. The dopamine (DA) system has been widely demonstrated in the last decades to regulate the control of attention (Kaplan & Oudeyer, 2007; D. A. Lewis et al., 2001; S. J. Lewis, Dove, Robbins, Barker, & Owen, 2003; Sawaguchi & Goldman-Rakic, 1994), even when stimulus-driven (Cools, Barker, Sahakian, & Robbins, 2001; Cools, Barker, Sahakian, & Robbins, 2003; Cools, 2008). A reciprocal relationship between DA D1 and D2 receptor binding (Akil et al., 2003; Meyer-Lindenberg et al., 2002) might shape the relationship between stability and flexibility of mental rapresentations. While D1 receptor binding in prefrontal cortex (PFC) is thought to promote stability of representations by increasing distracter resistance (Durstewitz, Seamans, & Sejnowski, 2000), DA D2 receptor (DRD2) binding is related to the facilitation of the updating of new mental representations (Frank, 2005). The multifaceted role of DA activity in PFC regulated by the extrasynaptic actions of D1/D2 receptors shapes the updating of mental representations through an inverted-U function, whereby middling levels of prefrontal DA result in more effective updating of information in working memory (i.e., N-back task; Callicott et al., 1999), whereas highest and lowest PFC DA concentrations lead to worse updating of information (Arnsten, 1998; Cools, Clark, & Robbins, 2004; Williams &

Castner, 2006). However, the role of this biphasic model of DA function between of D1/D2 actions on stimulus-driven control of attention, such as the processing of environmental novel events (Birkas et al., 2006) has not yet been determined.

Here, we examined the epistatic interaction of two polymorphisms regulating D1 and D2 receptor binding on the stimulus-driven control of attention. For the regulation of the D1 receptor binding in PFC, the Met158/108Val single nucleotide polymorphism of gene of the cathecol-O-methyltransferase (COMT) (rs#116790; Lachman et al., 1996) was examined, as COMT is an enzyme which inactivates the DA diffused out of the synaptic cleft in the PFC (Bilder, Volavka, Lachman, & Grace, 2004). A Val to Met substitution is thought to increase the efficiency of the enzyme (Mannisto & Kaakkola, 1999), so Val homozygous individuals (Val groups) are thus expected to have decreased synaptic PFC DA levels for D1 receptor binding as compared Met homozygous individuals (Met groups) and subsequent decreased stability of representations (Bilder et al., 2004; Winterer et al., 2004). As for D2 regulation, DRD2/ANKK1-Taq-Ia gene restriction fragment length polymorphism for the DRD2 (rs#126450) was examined because A1 allele carriers (A1+ groups) show a 30-40% reduction in DRD2 density as compared to A2 homozygous (Ritchie & Noble, 2003).

According to the reciprocal relationship of D1/D2 receptors action (Akil et al., 2003; Meyer-Lindenberg et al., 2002), four groups were formed:

homozygous COMT Met individuals lacking the DRD2 A1 allele (MetA1-) and homozygous COMT Val individuals presenting the DRD2 A1 allele (ValA1+) are expected to display a compensated balance of COMT levels and DRD2 density, and thus of D1/D2 stimulation, leading to the middling levels of PFC DA activity. In contrast, homozygous COMT Met individuals presenting the DRD2 A1 allele (i.e., MetA1+) will display the highest PFC DA activity, while homozygous COMT Val individuals without the DRD2 A1 allele (ValA1-) would display lowest PFC DA activity. Moreover, the combination of the COMT and DRD2 genes has revealed a beneficial effect of high DRD2 density (i.e., A1-) on Met individuals (i.e., high PFC DA activity; MetA1-) and low DRD2 density (i.e., A1+) on Val individuals (i.e., low PFC DA activity; ValA1+) during manipulation of contents in working memory (Gosso et al., 2008; Stelzel, Basten, Montag, Reuter, & Fiebach, 2009). Therefore, this beneficial effect might result in advantageous performance during distraction in a condition requiring more attentional resources, since working memory load interacts with the exogenous capture of attention by novel events (Lavie & De Fockert, 2005; SanMiguel, Corral, & Escera, 2008).

Participants of the four groups formed according to the combination of COMT/DRD2 genotypes performed an auditory-visual distraction paradigm (Escera, Alho, Winkler, & Näätänen, 1998; Escera, Alho, Schroger, & Winkler, 2000; Escera & Corral, 2007; SanMiguel et al., 2008), in which a task-irrelevant frequent standard or rare environmental novel sounds were followed by a digit ranging 1-4 and 6-9. In a condition without working

memory load (WM0), participants had to classify the target as larger or smaller than 5. In another condition involving a working memory load (WM1), the task was to decide whether the present digit was larger or smaller than the one presented in the preceding trial. Electroencephalographic (EEG) oscillatory activity in the gamma frequency band at 40 Hz was measured to examine control of attention, as it is enhanced for attended as compared to unattended stimuli (Tiitinen et al., 1993; Debener, Herrmann, Kranczioch, Gembris, & Engel, 2003; Yordanova, Kolev, & Demiralp, 1997; Yordanova et al., 2000; Yordanova, Banaschewski, Kolev, Woerner, & Rothenberger, 2001); (Busch, Herrmann, Muller, Lenz, & Gruber, 2006; Herrmann, Munk, & Engel, 2004), even during stimulus-driven attentional control (Garcia-Garcia, Yordanova, Kolev, Dominguez-Borras, & Escera, 2010) and can be detected at very early stages of attentional control (i.e., circa 100 ms post stimulus; (Tallon-Baudry & Bertrand, 1999). Specifically, an increase in the phase-coherence of GBRs has been associated with the brain mechanisms of increased attention (Fries, Reynolds, Rorie, & Desimone, 2001) and the top-down mechanisms for enhanced sensory information processing (Busch et al., 2006; Debener et al., 2003; Fries et al., 2001; Herrmann et al., 2004; Yordanova et al., 2001) through the amplification of behaviorally relevant signals in the cerebral cortex (Fries et al., 2001; Yordanova et al., 2001).

Results

Performance

The participants responded with a mean performance accuracy over 80%, which was reduced in the WM1 relative to the WM 0condition ($F_{1,29}$=60.62, p<0.001), and in trials following a novel sound as compared to those following a standard sound ($F_{1,29}$=104.88, p<0.001; Figure 1a). Larger accuracy decrease was observed after novel trials in the WM1 than in the WM0 condition ($F_{1,29}$=12.89, p=0.001). No interaction between the WM manipulation and the polymorphic groups emerged as statistically significant.

RTs were longer in the WM1 than in the WM0 condition ($F_{1,29}$=10.45, p=0.003) and were delayed in novel relative to standard trials, indicating behavioral distraction ($F_{1,29}$=9.36, p=0.005; Figure 2b). Interestingly, a main interaction between the effect of Novelty and both groups was observed (Novelty X COMT X DRD2: $F_{1,29}$=13.815, p=0.001), indicating that ValA1- and MetA1+ groups showed no distraction, that is, displayed similar RT in standard and novel trials, whereas the ValA1+ and MetA1- groups were distracted by novel relative to standard sounds (ValA1+: $F_{1,6}$=9.45, p=0.022; MetA1-: $F_{1,8}$=13.20, p=0.007; Figures 1b,2a).

Gamma Band Responses

Amplitude of oscillatory activity

Maximal values of oscillatory evoked activity around 40 Hz between 100-200 ms were larger in novel than standard trials (F1,33=64.9, p<0.001). As shown in figure 2a,b, all four groups showed this increase in evoked activity in novel compared to standard trials (ValA1-: $F_{1,12}$=28.8, p<0.001; ValA1+: $F_{1,6}$=15.3, p=0.008; MetA1-: $F_{1,9}$=12.7, p=0.006; MetA1+: $F_{1,6}$=29.0, p=0.002). No effect of WM condition was found for the evoked activity.

Phase-locking factor of oscillatory activity

Maximal values for the PLF of scalp-recorded brain activity around 40 Hz between 100-200 ms were larger in novel than standard trials ($F_{1,29}$=7.62, p=0.010). Remarkably, a significant Novelty x COMT x DRD2 interaction ($F_{1,29}$=7.62, p=0.039) revealed larger PLF values in novel than standard trials in ValA1- ($F_{1,9}$=14.03, p=0.005) and MetA1+ groups ($F_{1,6}$=6.95, p=0.039), but similar PLF values for standard and novel trials in the ValA1+ and MetA1- groups (Figure 2a,b,c). No effect of WM condition was found for the PLF.

Discussion

The current study explored the epistatic interaction of COMT and DRD2 genes regulating DA D1/D2 receptors action on stimulus–driven control of attention. ValA1+ and MetA1- individuals, related to a balance between PFC DA availability and DRD2 density, were behaviorally distracted by auditory novel events (i.e., experienced a delay in RT after the occurrence of novel events in comparison to standard sounds) whereas individuals with a different combination of alleles for COMT and DRD2, and presumably an unbalanced PFC DA, showed similar RT in novel and standard trials (i.e., displayed no behavioral distraction). The amplitude of neural oscillations at 40 Hz evoked to the auditory stimuli increased in novel as compared to standard trials in all groups similarly. However, mechanisms leading to this amplitude increase differed according to the genetically-based DA activity: while sound-locked oscillations showed similar phase-coherence for both auditory stimuli in ValA1+ and MetA1- groups with COMT/DRD2 balance, the phase-coherence of 40 Hz neural oscillations increased in novel as compared to standard trials for ValA1- and MetA1+ individuals.

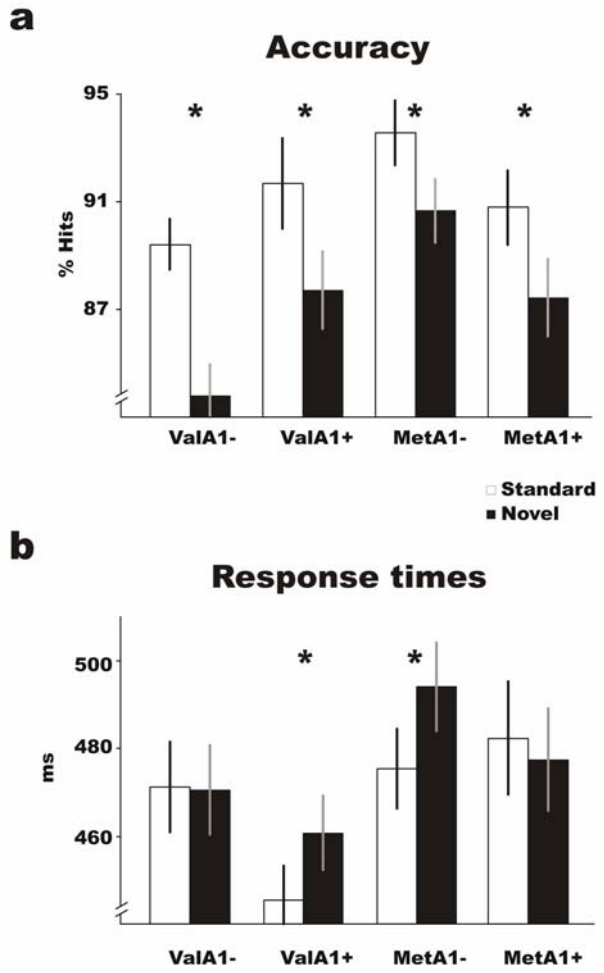

Figure 1. *Task performance for all four groups.* ***a****) Accuracy for all four groups for standard and novel trials. Significant novelty effects are indicated with an asterisk above the bars. Accuracy was lower in trials with novel sounds in comparison to those with standard sounds in all four groups. Notice the inverted-U shape of accuracy in the two trial types for the four groups arranged in the panel according to their levels of PFC DA activity.* ***b****) Response times for the four groups in standard and novel trials. Significant novelty effects are indicated with an asterisk above the bars (*, p<0.03). Notice that two groups display similar RT (i.e., ValA1- and MetA1+) after standard and after novel sounds, whereas two groups (i.e., ValA1+ and MetA1-) show larger RT following novel as compared to standard sounds (i.e., reflecting distraction by the novel sound).*

The genes regulating for D1/D2 receptors action played a critical role in the occurrence of the distraction effect. The present results reflect that the

distraction effect elicited by unexpected events during task performance (Escera et al., 1998; Escera et al., 2000; Escera & Corral, 2007) follows an inverted-U model, where individuals with middling levels of PFC DA activity shift the attention towards novel stimuli by experiencing a disruption in the ongoing task (i.e., delay in RT). Because DRD2 binding leads to the update of mental representation (Frank, 2005), the combined effect of COMT activity and DRD2 density might reflect the interaction between a gating mechanism allowing the updating functions needed for activation of new mental representations and the maintenance of representations promoted by PFC DA concentrations (Arnsten, 1998; Cools et al., 2004; Cools, 2008). Therefore, a balanced interaction between stability and flexibility of mental representations allow ValA1+ and MetA1- individuals to reallocate attention into novel events and the reorient the attention back to the current activity.

However, involuntary attention capture is an ecologically critical mechanism for detecting potentially important stimuli in the environment. Therefore, because none of the groups present any clinical disorder, all groups are expected to detect correctly these salient signals even if they do not experience behavioral distraction caused by such stimuli. In fact, larger amplitudes of oscillatory activity at 40 Hz in novel relative to standard trials in all groups suggest increased attention directed to novel events as compared to standard ones (Busch et al., 2006; Debener et al., 2003; Herrmann et al., 2004; Tiitinen et al., 1993; Yordanova et al., 1997; Yordanova et al., 2000; Yordanova et al., 2001).

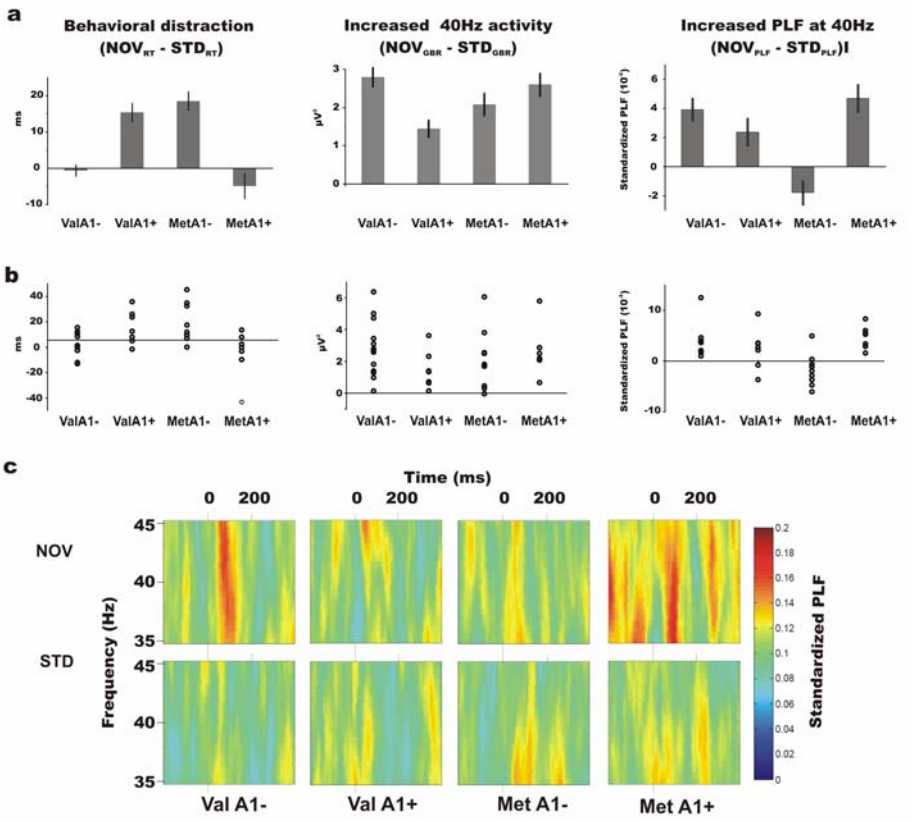

Figure 2. *RT, amplitude and PLF of 40 Hz oscillatory activity for novel relative to standard trials.* ***a****) The plot on the left panel shows the magnitude of the behavioral distraction, i.e., RT in novel trials minus RT in standards trials; notice that only the MetA1- and ValA1+ groups had larger RT for novel as compared to standard trials, i.e., were behaviorally distracted by the novel sounds. The plot in the middle panel shows the increase in amplitude of neural oscillations at 40 Hz locked to novels sounds compared to that locked to standard sounds at the Cz electrode. Notice that amplitudes were larger after novel compared to standard sounds in all four groups. The plot in the right panel the show the increase of the PLF of neural oscillations at 40 Hz locked to novels sounds relative to that locked to standard sounds at the Cz channel. Notice that PLF was larger after novel compared to standard sounds in only in the MetA1+ and ValA1- groups.* ***b****) Scatter plots of individual values for RT (left plot), amplitude of the evoked 40 Hz oscillatory activity (middle plot), and PLF of the 40 Hz oscillatory activity (right plot).* ***c****) Plots of standardized PLF values in novel and standard trials for all 4 groups at the Cz channels for frequencies from 35 to 45 Hz. ValA1- and MetA1+ groups showed enhanced PLF in novel compared to standard sounds around 100 ms post-stimulus, whereas ValA1+ and MetA1- groups show similar PLF values to both stimulus types.*

Nevertheless, while EEG amplitudes reflect rather sustained activation patterns of large cortical patches, the phase of brain oscillatory activity has been related to high temporal preciseness of particular neural firing patterns

(Buzsaki & Draguhn, 2004). Therefore, the increased phase-coherence for novel relative to standard sounds experienced by the ValA1- and MetA1+ groups suggests a critical regulation of phase resetting of neural firing (Klimesch, Sauseng, & Hanslmayr, 2007) during stimulus-driven control of attention by D1/D2 receptors action. This enhancement of phase-coherence in ValA1- and MetA1+ groups, related to distant ratios of D1 and D2 DA receptors binding, probably reflects a resetting mechanism for appraisal of novel events. Moreover, phase-resetting of neural oscillatory activity seemed to set a mechanism to avoid behavioral distraction while evaluating potentially relevant events.

Neural oscillations in the gamma frequency band are regulated by dopaminergic activity, as they have shown regulation by other DA-related polymorphisms such as for the D4 DA receptor and the dopamine transporter (Demiralp et al., 2007), and by pharmacological DRD2 antagonist haloperidol (Ahveninen et al., 2000). Accordingly, the current results support the role of gamma band oscillatory activity as a putative endophenotype reflecting mechanisms of control of attention regulated by the DA system. Furthermore, altered GBRs to attended stimuli in patients with PFC DA depletion as schizophrenia (Clementz, Blumenfeld, & Cobb, 1997; Gallinat, Winterer, Herrmann, & Senkowski, 2004; Haig et al., 2000) and with poorer D2 stimulation as Attention deficit with hyperactivity disorder (ADHD; Spencer et al., 2007; Yordanova et al., 2001) are a reflection of the attentional deficits present in patients of DA-related disorders. Deficits in the control of attention revealed by larger distraction in patients of schizophrenia

(Cortinas et al., 2008; i.e., treated by blocking D2 receptors, Kapur & Mamo, 2003) might be related to a failure in phase-resetting the neural oscillatory activity to novel sounds, leading thus to a difficulty in shifting attention back to the current task. However, further studies should address the examination of electrophysiological mechanisms of stimulus-driven control of in patients with DA-related disorders.

The genetic base of the diverse patterns of brain and behavioral responses shown during stimulus-driven control of attention in the present study partially fulfill the incognita of the individual variability in cognitive processes. Moreover, the current results establish an inverted-U function of PFC DA activity and behavioral distraction by a novel event, by which middling levels of PFC DA activity lead to a delay in RT to the task while shifting attention towards novel events. However, similar increases of the amplitude of 40 Hz neural oscillation in all groups suggest that all individuals shift attention toward the novel event, as an adaptive process for detecting potentially relevant stimuli. Interestingly, groups with either the lowest or the highest PFC DA activity performed the task with no delay in RT in novel as compared to standard. Presumably, specific patterns of DA neurotransmission set a neural mechanism of phase-resetting of the neural oscillatory activity by which healthy individuals with largest ratios of either D1 or D2 activity would evaluate novel events without a disruption in the ongoing activity. The current results provide strong evidence of epistatic interaction between COMT and DRD2 in the stimulus-driven control of

attention. Furthermore, reported electrophysiological correlates of the control of attention might constitute a reliable endophenotypic marker of the DA system, and could help to isolate in near future dysfunction in the human DA system.

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Authors Contribution

Author contributions: M.G-G., C.E, I.S. and I.C. designed the research; M.G-G. and I.C. performed the research; M.G-G. analyzed the data; and M.G-G., C.E, I.S. and I.C. wrote the paper.

Materials and methods

Participants

Forty subjects (six men, two left-handed, mean age 22 ± 4.2 years, range 18-29 years) participated in the present study. They were recruited from a larger sample of volunteers which were interviewed according to an adapted

version of the Clinical Interview of the Diagnostic and Statistical Manual (DSM IV-R), for exclusion of individuals with neurological and psychiatric illness, phobias, and drug consumption. All participants gave informed consent at each phase of the experimental procedure (interview, buccal cells extraction and EEG recordings) according to the Declaration of Helsinki and the Ethic Committee of the University of Barcelóna. All subjects had normal or corrected-to-normal vision and normal audition. After exclusion by diagnostic criteria and obtaining the COMT and DRD2 polymorphisms, the participants homozygous for the COMT (Met/Met, Val/Val), and those presenting the most frequent alleles for DRD2 (A1, A2) were selected for an EEG recording session. Participants genotyped as Met/Met were assigned to the MetA1+ group when they presented the A1 allele (A1/A1, or A1/A2) and to the MetA1- group when they were homozygous for the A2 allele for the DRD2 gene. Participants genotyped as Val/Val were assigned to the ValA1+ group when they presented the A1 allele (A1/A1, or A1/A2) and to the ValA1- group when they were homozygous for the A2 allele for the DRD2 gene. Seven participants were excluded from analyses due to a large amount of artifacts in their EEG recordings. From the remaining 33 individuals, seven composed the MetA1+ group, nine the MetA1- group, seven the ValA1+ group, and ten were included in the ValA1- group. Participants from each of the genetic groups did not differ significantly in age, state or trait anxiety scores (STAI, Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983)).

DNA isolation and genotyping

DNA was collected with cheek cell swabs and extracted using the Epicentres® BuccalAmp™ DNA Extraction Kit (Epicentre, Madison, WS). Upon isolation of DNA, both single nucleotic polymorphisms (SNP) for the COMt Met108/158Val and DRD2 Taq IA genotyping were performed by real time PCR using fluorescence detection technique by means of the Applied Biosystems TaqMan technology (Applied Biosystems, Foster City, CA, USA).

Stimuli and procedure

Participants performed a modified version of a well-characterized auditory-visual distraction task (Escera et al., 1998; Escera et al., 2000; Escera, Yago, & Alho, 2001; Escera, Corral, & Yago, 2002; Escera, Yago, Corral, Corbera, & Nunez, 2003; Escera & Corral, 2007) with two conditions: a 1-back WM condition (WM1) and a 0-back condition with no WM load (WM0; see SanMiguel et al., submitted). Each trial was lasted 1300 ± 300 ms and formed of a visual target preceded in 300 ms by an auditory stimulus. Participants were instructed to respond to visual stimuli as fast and accurately as possible and to ignore the auditory stimulation. The auditory stimuli were presented through Sehnheiser® HD202 headphones and consisted of a 600 Hz standard tone with an intensity of 90 dB (200 ms duration) in 80 percent of the trials and a unique environmental complex

novel sound in the remaining 20 percent of the trials. Novel sound trials were always preceded by at least one standard tone trial. Novel sounds were 100 unique sounds such as those produced by a drill, hammer, rain, door or telephone ringing, selected from a larger pool (Escera et al., 1998) as the more easily identifiable (Escera et al., 2003), and with similar spectrotemporal features ((Dominguez-Borras et al., 2008). Four blocks of 250 trials were delivered, there being two block of each condition. The order of these four blocks, as well as the order of the conditions was counterbalanced across subjects. Visual stimuli were single digits (1-4 and 6-9) presented on a screen for 200 ms in white colour against a black background subtending a vertical angle of 1.53^{o} and a horizontal angle of 2.10^{o}. In the WM0 condition, participants had to decide by a button press whether the digit presented was larger or smaller than five. The WM1 condition consisted of a 1-back task in which participants had to decide whether the digit presented was larger or smaller in value than the digit presented in the previous trial. All participants responded with the middle and index fingers with the same hand for larger and smaller respectively. Before the experiment, participants performed a five minute practice block for each condition in which the sound was turned off; the practice sessions were repeated until a minimum accuracy of 75 percent was reached.

EEG Data Acquisition

Electroencephalographic activity was recorded (ANT Software b.v., Enschede, The Netherlands) during task performance from 64 scalp electrodes following the extended 10/10 convention in an electrically and acoustically shielded room. Horizontal and vertical electro-oculographic (EOG) recordings were obtained with electrodes placed at the outer cantus of the right eye and above the right eye. The common reference electrode was placed on the tip of the nose, and the ground was located at the chest. The EEG was amplified and digitized at a sampling rate of 512 Hz. Impedances were kept below 10 kΩ during the whole experimental recording session, which lasted about 25 minutes.

Data Processing

Data processing was performed with ASA 4.5.1.0 software (ANT®, ENschede, The Netherlands). For single sweep analysis, time epochs were defined as the time window starting 250 ms before and lasting until 1000 ms after auditory stimulus onset (i.e., 700 ms from visual stimulus onset), with the pre-stimulus period used as a baseline. Automatic ocular correction was performed by applying an independent component analysis (ICA) as in (Belouchrani, Abed-Meraim, Cardoso, & Moulines, 1997); implemented in the ASA 4.5.1.0 software). In addition, epochs contaminated with ocular movements or muscle artifacts were rejected by an automatic artifact rejection procedure if their

peak-to-peak amplitude exceeded 150 μV. For analysis of the GBR, the EEG signal was band-pass filtered between 30 and 60 Hz. To equalize the number of sweeps for better control of possible signal-to-noise ratio differences, all novel sweeps and a similar number of randomly selected standard sweeps were taken from each condition. For each participant an average of 74.9 trials per condition were used for analysis.

Data Analysis

To obtain the time-frequency components from the gamma range, epochs averaged for each condition and participant were decomposed by means of a continuous Wavelet transform. Time-frequency transforms were obtained by the application of complex-valued Morlet wavelets, which are Gaussian in both time and frequency domain. Complex Morlet wavelets *w* can be generated in the time domain for different frequencies, *f*, according to the equation:

$$w(t, f) = A\exp(-t^2 / 2\sigma_t^2)\exp(2i\pi ft),$$

where *t* is time, $A = (\sigma_t \sqrt{\pi})^{-1/2}$, σ_t is the wavelet duration, and $i = \sqrt{-1}$.

A ratio of f_0/σ_f = 12 was used, where f_0 is the central frequency and σ_f is the width of the Gaussian shape in the frequency domain. The analyses were performed in the frequency range 30-60 Hz, with a central frequency at 0.75 Hz intervals (forty frequency steps). For different f_0, time and frequency resolutions can be calculated as $2\sigma_t$ and $2\sigma_f$, respectively. σ_t and σ_f are related by the equation $\sigma_t = 1/(2\pi\sigma_f)$.

PS was calculated by means of the phase-locking of oscillatory activity measured by using the phase-locking factor (PLF) proposed by Tallon-Baudry, Bertrand, Delpuech, & Permier, (1997). This is a measure for phase identity across trials and is bounded between 0 (non-phase-locked signal) and 1 (phase-locked signal). Statistical thresholds were assessed by means of a circular statistics (Rayleigh test) with a significance of p=0.01. Since PLF reached the significant threshold for each sweep and channel, they were submitted to further analyses. A 250 ms baseline (-250 to -50 ms) was used as baseline for the time-frequency information and the mean of this time window was subtracted for the time frequency matrix for each frequency and time point.

Maximal amplitude and PLF values of the GBRs were obtained in the latency windows from 100 to 200 ms, as GBRs are strongly synchronized in the first 100 ms after sensory stimulation and reflect very early stages of stimulus evaluation (Tallon-Baudry & Bertrand, 1999). Because previous studies have referred 40 Hz activity to the increase of attention (Garcia-Garcia et al., 2010; Tiitinen et al., 1993; Womelsdorf, Fries, Mitra, & Desimone, 2006), analyses were performed for this frequency range around 40 Hz (35-45 Hz).

Statistical Analysis

The first five trials of each block, as well as those trials following a trial containing a novel sound were excluded from the analyses. A correct button press within 100-1200 ms after visual stimulus onset was regarded as a hit, and the mean RT was computed for hit trials only. Hit rate and RT were compared by means of three-factor repeated-measures ANOVA including the between subject factors Novelty (standard, novel) and WM load (WM0 and WM1), and the between-subjects variables of the polymorphisms for the COMT (Met and Val) and the DRD2 (A1+ and A1-). Pair-wise *post hoc* comparisons were performed to paired out interactions.

For analysis of both amplitude and PLF of the GBR, maximal values in the defined latency window were measured for the auditory GBR for eighteen of the recorded channels (F3, Fz, F4, FC3, FCz, FC4, C3, Cz, C4, CP3, CPz, CP4, P3, Pz, P4, PO3, POz and PO4) at around 40 Hz frequency range. Repeated measures of four-factor ANOVAs were performed including the factors Novelty (standard, novel), WM (WM0 and WM1), Region (six levels of frontality corresponding to F, FC, C, CP, P and PO) and Laterality (three levels for left, midline and right channels), and the between-subject variables of the polymorphisms for the COMT (Met and Val) and the DRD2 (A1+ and A1-). Greenhouse-Geisser correction of the degrees of freedom was applied, with the corrected *P*-values reported. Pair-wise *post hoc* comparisons were performed to paired out interactions.

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Study III.

III. Garcia-Garcia, M., Clemente, I.C., Domínguez-Borràs, J., Escera, C. Dopamine regulates the modulation of novelty processing by an emotional context: behavioral and electrophysiological evidences. *Submitted*

Dopamine transporter regulates the enhancement of novelty processing by a negative emotional context

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Abstract

The dopaminergic (DA) system has been recently related the emotional modulation of cognitive processes. Moreover, patients with midbrain DA depletion, such as Parkinson's Disease (PD), have shown diminished reactivity during unpleasant events. Here, we examined the role of DA in the enhancement of novelty processing during negative emotion. Forty healthy volunteers were genotyped for the Dopamine Transporter (DAT) gene DAT1 and performed an auditory-visual distraction paradigm in negative and neutral emotional context conditions. 9R- individuals, associated to a lesser striatal DA display, failed to show increased distraction during negative emotion, but experienced an enhancement of the early phase of the novelty-P3 brain response, associated to the evaluation of novel events, in the negative relative to the neutral context. However, 9R+ individuals (associated to larger striatal DA display) showed larger distraction during negative emotion, and larger amplitudes of the novelty-P3, irrespective of the condition. These results suggest a blunted reactivity to novelty during negative emotion in 9R- individuals due to a lesser DA display, and stronger activation of the representation of novel events in the 9R+ group, due to a larger DA availability, thus reaching a ceiling effect in the neutral context condition with no further enhancement during negative emotion. The present results might help to understand the functional implications of dopamine in some neuropsychiatric disorders.

Introduction

The dopamine (DA) neurotransmitter has been revealed to play a relevant role in emotional processing. Animal studies reported higher and inappropriate fear responses (Blackburn & Phillips, 1990) following the administration of a pharmacological antagonist of the DA D2 receptor, while the administration of a D2 agonist blocked fear conditioning and retrieval of emotional memories (Nader & LeDoux, 1999). In humans, the administration of a DA antagonist induced the disruption of emotional stimuli encoding (Mehta, Hinton, Montgomery, Bantick, & Grasby, 2005), suggesting that DA neurotransmission plays a role in human emotional processing. Moreover, a novel molecular imaging technique showed DA release at multiple levels of emotional processing during presentation of emotional stimuli (Badgaiyan, Fischman, & Alpert, 2009).

Importantly, patients with midbrain DA system dysfunction (i.e., Parkinson´s disease, PD; (Lawrence, Goerendt, & Brooks, 2007) showed impairment in the recognition of emotional expressions. Moreover, the potentiated startle reflex found in healthy volunteers while viewing of negative emotional images (Bradley, Codispoti, & Lang, 2006; Stanley & Knight, 2004) is diminished in PD patients (Bowers et al., 2006), reflecting blunted reactivity to highly arousing negative stimuli (Miller, Okun, Marsiske, Fennell, & Bowers, 2009), probably related to the depletion of midbrain DA in these patients. However, whether DA depletion might be involved in the

modulation of environmental novel events processing during an emotional negative situation, as shown by behavioral, neuroimaging (Dominguez-Borras et al., 2008) and electrophysiological data (Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Dominguez-Borras, Garcia-Garcia, & Escera, 2008b; Garcia-Garcia, Dominguez-Borras, SanMiguel, & Escera, 2008; Garcia-Garcia, Yordanova, Kolev, Dominguez-Borras, & Escera, 2010) has not been yet examined.

The dopamine transporter (DAT) is the most important DA regulator at the basal ganglia (Sesack, Hawrylak, Matus, Guido, & Levey, 1998), as it mediates DA active reuptake from the synapse (Lewis et al., 2001) especially at human striatum, where D2 type receptors are mostly expressed (Camps, Cortes, Gueye, Probst, & Palacios, 1989). Therefore, DAT functioning might reflect individual differences in the reactivity during negative emotion in healthy subjects, and thus help to elucidate the neurobiological factors involved in the depressed reactivity to negative stimuli in patients with DA midbrain dysfunction (Miller et al., 2009). We used a functional variable number of tandem repeat (VNTR) polymorphism identified in the DAT1 gene with 9- and 10-repeat (9R and 10R) as the most frequent alleles in the population (Vandenbergh et al., 1992). The 10R/10R genotype results in increased DAT expression (Fuke et al., 2001; Heinz et al., 2000; Mill, Asherson, Browes, D'Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005); see however (Jacobsen et al., 2000) and putatively,

decreased synaptic DA tone in cortico-striatal pathways (Wichmann & DeLong, 1996).

In the present study, we explored the role of the DAT1 genotype on the enhancement of novelty processing by an emotional context. The effect of the 9-repeat allele presence was examined by means of behavioral and electrophysiological brain responses to an auditory-visual distraction paradigm in which task-irrelevant frequent standard and rare novel sounds were preceded and followed by task-relevant visual stimuli of either neutral or negative emotional load. We measured the scalp-recorded event-related potential (ERP) novelty-P3 (nP3) which is associated with the evaluation of these novel events for subsequent behavioral action (Escera, Alho, Winkler, & Näätänen, 1998; Escera & Corral, 2007; Friedman, Cycowicz, & Gaeta, 2001). Previous studies revealed an enhancement of both the behavioral distraction effect and the amplitude of the early phase of the nP3 in a negative relative to the neutral context (Dominguez-Borras, Garcia-Garcia, & Escera et al., 2008b; Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Garcia-Garcia et al., 2008).

Materials and methods

Participants

Forty individuals (seven men, mean age 22 ± 4.2 years, range 18-29 years) participated in the present study. They were recruited from a wider sample of volunteers which were interviewed according to an adapted version of the Clinical Interview of the Diagnostic and Statistical Manual (DSM IV-R), for exclusion of subjects with neurological and psychiatric illness, phobias, and drug consumption. All participants gave informed consent at each phase of the study (interview, buccal cells extraction and EEG recordings) according to the Declaration of Helsinki and the Ethic Committee of the University of Barcelóna. All subjects had normal or corrected-to-normal vision and normal audition. After exclusion by diagnostic criteria and obtaining the DAT1 polymorphisms, the participants showing the most frequent genotypes (9R/9R, 9R/10R, 10R/10R; (Vandenbergh et al., 1992) were selected for an EEG recording session. Participants genotyped as 10R/10R were assigned to the 9R- group associated with the functional effect of increased DAT expression (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005), and participants genotyped as 9R/10R and 9R/9R were included in the 9R+ group. Twenty individuals composed the 9R+ group and twenty were included in the 9R- group. Participants from each of the two genetic groups did not differ significantly in age, gender and state or trait anxiety scores (STAI; (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

DNA isolation and genotyping

A functional variable number of tandem repeat (VNTR) polymorphism was identified in the 3'-untranslated region of the DAT1 gene with repeat copy number ranging from 3-to-11, being 9- and 10-repeat (9R and 10R) the most frequent in population (Vandenbergh et al., 1992). In order to genotype the participants for the DAT1 gene, DNA was first collected with cheek cell swabs and extracted using the Epicentres® BuccalAmp™ DNA Extraction Kit (Epicentre, Madison, WS). Upon isolation of DNA, the 40-bp VNTR polymorphisms for the DAT1 gene (rs#28363170) were obtained for each DNA sample following the procedures described by (Sano, Kondoh, Kakimoto, & Kondo, 1993), and modified by amplifying PCR-VNTR using a fluorescently tagged primer. An initial 4-minutes denaturing at 95° C, 30 cycles of denaturing at 95° C during 30 seconds, annealing at 68° C for 30 second and extension at 72° C for additional 90 seconds performed in the presence of the primers DAT-F 5' 6-FAM TGTGGTGTAGGGAACGGCCTGAG 3' and DAT-R 5' CTTCCTGGAGGTCACGG-CTCAAGG, were then followed by a final extension at 72° C for another 10 minutes. Amplification products were analyzed using a capillary electrophoresis on the sequencer ABI Prism® 3730 (Applied Biosystems, Foster City, CA) and through the Fragments Analysis Technique with GeneMapper® Software Version 4.0 (Applied Biosystems, Foster City, CA).The resulting fragments consisted of 280 bp for

5 repetitions, 320 for 6, 360 bp for 7, 400 bp for 8, 440 bp for 9, 480 bp for 10, 520 for 11 and 600 bp for 13 repetitions.

Stimuli and Task

The emotional visual stimuli were 208 pictures, with either neutral (NEU) or negative (NEG) valence, selected from the International Affective Picture System (IAPS[1]; (Lang, Bradley, & Cuthbert, 2005). A total of 188 neutral pictures and 120 negative pictures were selected among the most highly rated at the Self-Assessment Manikin (SAM; (Lang, 1980) both for arousal and valence dimensions. These dimensions were also evaluated by the subjects of a similar study (Garcia-Garcia et al., 2008) in order to ensure that they evoked the affective reaction reported by(Lang et al., 2005). For the present experiment, each visual stimulus was composed by two pictures of 643x482 pixels characterized by the same emotional valence (duration on screen 400ms), and a fixation point (a white cross in the center on the screen), subtending a vertical angle of 9º and a horizontal angle of 25º, at 150 cm distance from subject's eyes. The sequence structure was a block design, in which a total of 1000 trials were divided in 66 blocks of 10, 15 or 20 trials of the same valence. All blocks were pseudorandomized in one unique sequence, which could either begin with a higher proportion of neutral pictures that would decrease to a higher proportion of negative pictures, or begin with a higher proportion of negative pictures that would decrease to a higher proportion of neutral pictures. These two different

stimulus distributions were counterbalanced across subjects with a Latin square design. Within the sequence, 50% of the pairs of pictures were composed by two identical pictures and 50% by two different pictures.

Auditory stimuli were a 700 Hz standard tone (STD) and 100 unique environmental complex novel sounds (NOV), generated as in (Escera et al., 1998), chosen among the most highly rated by a sample of 30 subjects on a scale of familiarity (Escera, Yago, Corral, Corbera, & Nunez, 2003). Along the sequence, the probability of occurrence of the standard tone was 0.8, and novel sounds occurred with the complementary of 0.2, so that each novel sound was delivered only once within each emotional condition. The duration of all auditory stimuli was 200 ms, delivered binaurally through Sehnheiser® HD202 headphones. All stimuli were presented with the stimulation program Presentation® of Neurobehavioral Systems Inc.

Participants performed a modified version of a well-characterized auditory-visual distraction paradigm (Escera et al., 1998; Escera, Alho, Schroger, & Winkler, 2000; Escera, Yago, & Alho, 2001; Escera et al., 2003; Escera & Corral, 2007). Auditory stimuli preceded in 300 ms the visual stimulus onset. Subjects sat on a comfortable chair and were instructed to press a button of a response pad as fast and accurate as possible whether the two pictures were equal or different, while ignoring the sounds. The response buttons (left or right with the same hand) were counterbalanced across participants. Trial duration varied randomly from 1500 ms to 2100 ms (mean length 1800 ±

300 ms). A ten trial-practice block was delivered without auditory stimuli before the performance of the task.

EEG Data Acquisition

Electroencephalographic activity was recorded (ANT Software b.v. Netherlands) during task performance from 64 scalp electrodes, following the 10/10 convention, in an electrically and acoustically shielded room. The horizontal and vertical electro-oculogram (EOG) was recorded with electrodes placed on the outer cantus of the right eye and above the right eye. The common reference electrode was placed on the tip of the nose, and the ground was located on the chest. The EEG was amplified and digitized at a sampling rate of 512 Hz. Impedances were kept below 10 kΩ during the whole experimental recording session, which lasted about 35 minutes, including short rest periods.

Data processing

ERPs were averaged offline for NOV and STD auditory trials separately, for an epoch of 1400 ms including a pre-stimulus baseline of 200 ms. Only those auditory stimuli that were both preceded and followed by visual stimuli of the same emotional valence were selected for averaging. The first five trials of each block and the first ten trials of the sequence, as well as standard trials following a novel trial were excluded from analysis.

Frequencies above 30 Hz were digitally filtered out from individual EEG epochs prior to ERP averaging. EOG correction was performed via a blind source separation technique with ASA 4.5 of ANT® Software (Enschede, The Netherlands), as described in (Belouchrani, Abed-Meraim, Cardoso, & Moulines, 1997). After EOG correction, all epochs containing EEG activity exceeding ± 100 µV peak-to-peak amplitudes were rejected from further analysis. On average, 82.6% of epochs with STD, 82.9% of epoch with NOV sounds in NEU, as well as 84.7% epochs with STD and 86.6% epochs with NOV in NEG were retained for averaging.

Data analysis

For behavioral analysis, a correct button press within 100-1200 ms after visual stimulus onset was regarded as a hit, and the mean response time (RT) was computed for hit trials only. Hit rate and RT were compared by means of a three-factor repeated-measure ANOVA including the factors Sound (STD, NOV), Context (NEU, NEG), separately for sounds preceded and followed by NEU pictures and those preceded and followed by NEG pictures, and the between-subject variable Group (9R+, 9R-). Finally, pair-wise *post hoc* comparisons were also performed.

Novelty-P3 was isolated in the difference waves obtained by subtracting the STD trial ERPs from those elicited by NOV trials. Novelty-P3 was measured as the mean amplitude at F3, Fz, F4, C3, Cz, C4, P3, Pz and P4 in the 200-

290 and 290-370 ms latency windows for the early and the late phases, respectively. ANOVA for repeated measures were performed including the factor Context (NEU, NEG), Frontality (frontal, central, parietal), Laterality (right, midline, left), Phase (early, late; as described in (Escera et al., 1998; Escera et al., 2001) and the between-subject variable Group (9R+, 9R-). Greenhouse-Geisser correction of the degrees of freedom was applied. The *P*-values following correction are reported below.

Results

Behavioral results

Response times (RT) were larger after NOV than STD trials ($F_{1,38}$=37.4, p<0.001) as well as in NEG compared NEU context ($F_{1,38}$=15.3, p<0.001). Although no group effect was found for the RT, an interesting Novelty x Context X Group interaction ($F_{1,38}$=4.6, p=0.039) revealed that whereas RT increase after NOV was enhanced in NEG as compared to NEU context for 9R+ individuals ($F_{1,19}$=7.5, p=0.013), 9R- displayed similar RT increase after NOV in both NEU and NEG contexts. This can be seen in figure 1A showing the RT for the two groups in all conditions, and much clearer in figure 1B, which illustrates the interaction of the distraction effect between groups and condition.

Novelty-P3

The analysis of the mean amplitudes of the novelty-P3 revealed a remarkable interaction Context x Phase x Frontality x Group ($F_{2,76}$=3.8, p=0.046). This interaction revealed a early phase-specific (Context effect in 9R- group: $F_{1,19}$=5.1, p=0.036) amplitude enhancement in NEG as compared to NEU context which was only observed in the 9R- group (Context x Phase x Frontality: $F_{2,38}$=6.7, p=0.014) in central ($F_{1,9}$=5.4, p=0.031) and parietal locations (F1,19=7.6, p=0.013; Figure 2). In addition, the amplitude of the novelty-P3 appeared to be, overall, substantially larger in the 9R+ than 9R- group ($F_{1,38}$=7.2, p=0.011).

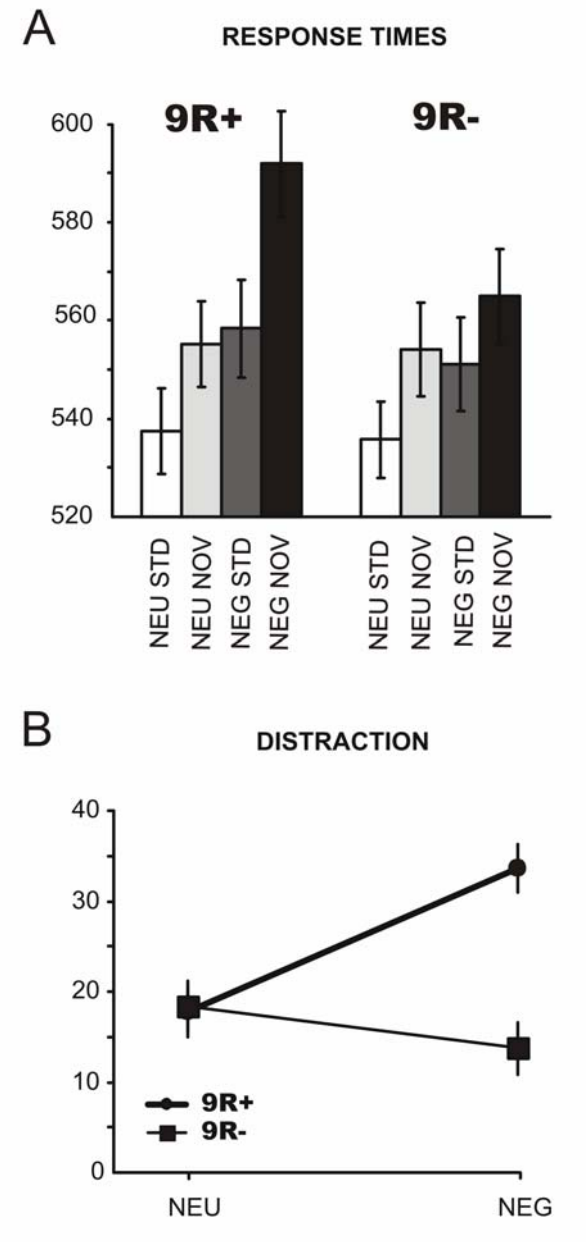

Figure 1. *Response times and distraction. (A) RT of both 9R+ and 9R- groups in all four conditions. RTs were larger after NOV than STD and in NEG than in NEU for both groups. However, notice the larger RT increase in the 9R+ group in NEG NOV compared to the other conditions which is not observed in the 9R- group. (B) Distraction effect (RT after NOV minus RT after STD) in both NEU and NEG emotional context and both for 9R+ and 9R- groups. The distraction effect was similar in NEG and NEU in the 9R- group. Remarkably, distraction increased in NEG as compared to NEU in the 9R+ group.*

Discussion

The present study aimed at revealing the role of the DAT1 genotype on the enhancement of auditory novelty processing during a negative emotional context. Whereas both groups showed behavioral distraction through an enhancement of the RT following NOV sounds relative to STD, only 9R+ individuals (with higher inferred striatal DA display) showed an increase of distraction in a NEG relative to a NEU context. However, 9R+ individuals failed to show any modulation of the nP3 component by the emotional context, whereas the 9R- group showed an amplitude enhancement of the early phase of nP3 in the NEG as compared to NEU condition. Moreover, the mean amplitude of the nP3 component was substantially larger in the 9R+ than in the 9R- group.

During a fearful or threatening situation, effective orienting of attention towards unexpected events may provide for rapid awareness of a potential harm and result in adaptive advantage for survival. An enhancement of novelty processing during emotional context has been shown by behavioral, ERP (Dominguez-Borras, Garcia-Garcia, & Escera et al., 2008b; Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Garcia-Garcia et al., 2008), brain oscillation phase-coherence (Garcia-Garcia et al., 2010) and neuroimaging (Dominguez-Borras et al., 2008) measures. Moreover, individual differences in such enhancement based on gender have been elucidated (Garcia-Garcia et al., 2008). Even though a behavioral effect of

the emotional was revealed in both groups by larger RT in the NEG relative to the NEU, the increase of behavioral distraction in NEG compared to NEU context was only shown by in the 9R+ group but not in 9R-. Individuals presumably with lesser striatal DA display (i.e., 9R-; (Fuke et al., 2001; Heinz et al., 2000; Mill et al., 2002; VanNess et al., 2005)) showed a diminished reactivity to distraction elicited by unexpected novel events than 9R+ individuals, expected to display larger striatal DA. Similarly, blunted reactivity during unpleasant events was elucidated in patients with midbrain DA dysfunction, through reduced emotional modulation of the startle reflex relative to healthy control (Bowers et al., 2006). The current data support the notion of decreased reactivity elicited by negative arousing images following DA depletion (Miller et al., 2009), in agreement with previous pharmachological studies showing impaired recognition of emotionally negative stimuli following D2 receptors blockage (Mehta et al., 2005).

Despite the lack of distraction increase during negative emotion in 9R- individuals, larger amplitudes of the early nP3 were observed in NEG relative to NEU emotional contexts. As revealed by enhanced activity in auditory novelty processing cerebral regions (bilateral superior temporal gyrus) during emotional threat (Dominguez-Borras et al., 2008) and increased phase coherence of neural oscillatory activity associated to auditory novelty in a NEG emotional context (Garcia-Garcia et al., 2010), larger amplitudes of early nP3 in NEG as compared to NEU context (Dominguez-Borras, Garcia-Garcia, & Escera et al., 2008b; Dominguez-

Borras, Garcia-Garcia, & Escera, 2008a; Garcia-Garcia et al., 2008) indicates stronger processing of novel unexpected events during negative emotion; hence this enhancement seems to reflect an effective stronger orienting of attention towards unexpected events during a fearful or threatening, as an adaptive mechanism for rapid awareness of a potential harm.

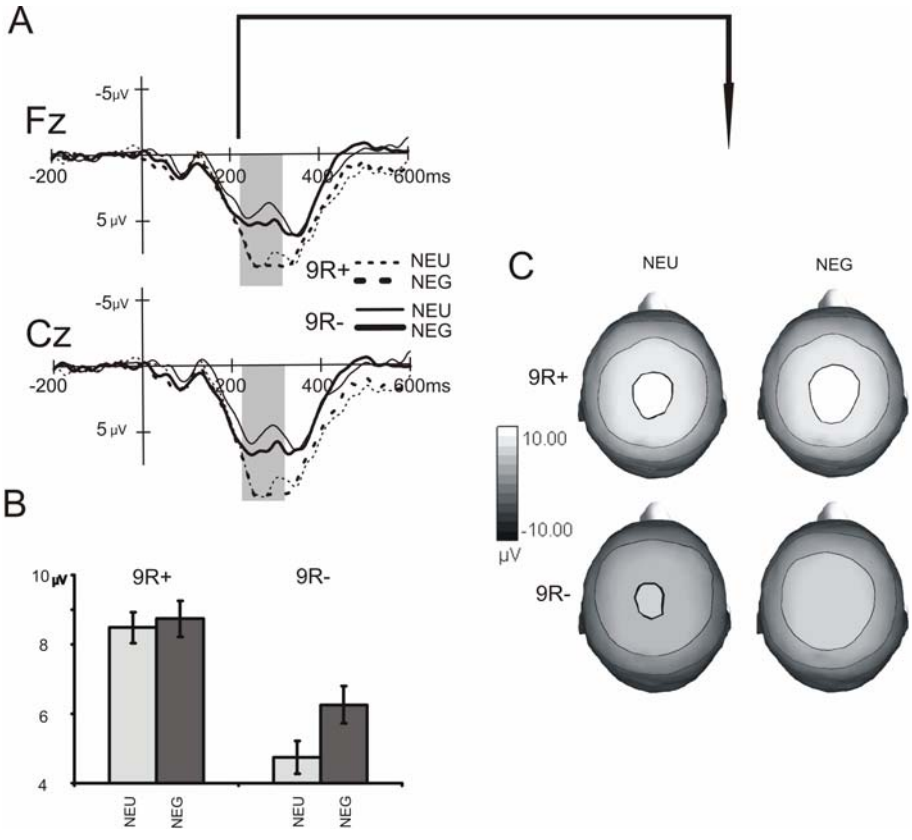

Figure 2. *Brain event-related brain potentials (ERPs). (A) ERPs showing the difference waves obtained by subtracting ERPs after NOV minus ERPs after STD for both NEU and NEG emotional context in both the 9R+ and 9R- groups at the locations Fz, Cz and Pz. The 9R- group showed larger amplitudes of the early nP3 (shadowed time window) in NEG as compared to NEU, while the 9R+ group showed similar early nP3 amplitudes in both emotional contexts. The 9R+ group shows substantially larger amplitudes of nP3 than the 9R- group. (B) Graph*

displaying the mean amplitudes of the early nP3 at Cz location in both 9R+ and 9R- groups and in both NEG and NEU emotional contexts. (C) Scalp distributions of the early phase of the nP3 for the 9R+ and 9R- groups, in both the NEU and NEG context conditions. Larger amplitudes of the early phase of the nP3 are observed in 9R+ relative to 9R- individuals independently of the condition on central distributions; incontrast, 9R- showed larger amplitude on central distribution in the NEG than the NEU emotional context.

Moreover, the 9R+ group showed substantially larger mean amplitudes of the nP3 as compared to the 9R-, irrespective of the emotional condition. The larger nP3 amplitudes might suggest that higher DA display allows 9R+ individuals to deploy a stronger activation to unexpected novel events as compared to frequent repetitive standard events, irrespective of the condition. Accordingly, a similar neuroimaging study revealed that human striatum reflects the level of saliency associated to a stimulus, providing a signal to reallocate limited resources to important events (Zink, Pagnoni, Chappelow, Martin-Skurski, & Berns, 2006), presumably reflected by the nP3. Moreover, similar DA-regulated frontocentral positive brain responses (i.e., P2a; (Potts, Martin, Burton, & Montague, 2006) has been proposed to reflect striatum DA signaling which implements a 'gating' mechanism controlling the access of information to neural systems for cognitive control (Montague, Hyman, & Cohen, 2004; O'Reilly, Braver, & Cohen, 1999; Potts et al., 2006);. This is consistent with a recent proposal postulating that the early positive subcomponent of nP3 reflects a general mechanism of activation of representation necessary for the subsequent motor response (Barceló, Perianez, & Nyhus, 2007). Accordingly, the administration of the D2 receptor antagonist haloperidol during an oddball paradigm resulted in lower amplitudes of the auditory P3 response (Kahkonen et al., 2002).

Therefore, putative larger DA tone in 9R+ individuals seems to illustrate a ceiling effect due to the stronger activation of unexpected auditory novel events, which would impede an enhancement of the nP3 brain response by a negative emotional context. We speculate that a stronger nP3 enhancement would be observed in absence of this ceiling effect.

The current study illustrated the way in which DAT1 genotype, and thus striatal DA display, is involved in reactivity to novel event during negative emotion. Individuals associated to lower striatal DA availability failed to show increased distraction during negative emotion, but experienced stronger orienting of attention towards novel events during negative emotion as revealed by larger early nP3 in the NEG as compared to the NEU condition. However, the polymorphism associated to larger striatal DA display showed increased distraction during negative emotion and larger amplitudes of nP3, probably reflecting stronger activation of mental representations by all novel events, reaching a ceiling with no further enhancement during negative emotion. Because during a threatening situation orienting of attention towards unexpected events results in adaptive advantage for survival as it may provide for rapid awareness of a potential harm, it is especially relevant to reveal the role of chemical neurotransmission on the effects of emotion on cognition. Importantly, these findings deepen in the neurobiological basis of the deficit in the emotion-cognition interaction revealed by blunted reactivity during negative emotion in PD patients with dysfunctional midbrain DA system. The relevant role of DAT1 gene on reactivity to novel events should be taken in account in future studies concerning the pharmacological

treatment of cognitive deficits of DA-depleted disorders, given the relevance of emotion in cognitive processes, and the individual variability in the response to the treatment according to the genotypic variation.

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Footnote 1:

IAPS identification numbers. Emotional pictures: 1050, 1051, 1052, 1120, 1200, 1270, 1300, 1301, 1930, 1931, 2710, 2750, 2800, 3000, 3010, 3015, 3022, 3030, 3051, 3053, 3061, 3062, 3063, 3071, 3080, 3100, 3102, 3110, 3120, 3130, 3140, 3150, 3160, 3168, 3170, 3230, 3250, 3261, 3266, 3400, 3550, 6250, 6260, 8230, 9040, 9265, 9405, 9490, 9570, 1019, 1111, 1201, 1274, 1275, 2141, 2352_2, 2691, 2730, 2900, 3060, 3064, 3301, 3500, 3530, 3550_1, 6212, 6213, 6312, 6313, 6350, 6360, 6540, 6550, 6560, 6821, 6831, 6838, 6940, 9050, 9250, 9252, 9253, 9400, 9402, 9410, 9420, 9421, 9433, 9520, 9910, 9920, 9921, 1022, 1101, 1113, 1121, 1240, 1280, 1321, 3190, 3181, 2753, 2900, 2900_2, 3350, 3180, 9592, 6190, 6200, 6250, 6210, 6243, 6510, 6830, 6836, 9611, 9911, 9490. Neutral pictures: 1450, 1560, 1590, 1600, 1620, 1640, 1660, 1670, 1740, 1810, 2190, 2200, 2206, 2210, 2214, 2215, 2221, 2372, 2383, 2410, 2440, 2480, 2487, 2495,

2500, 2570, 2600, 2749, 2751, 2752, 2840, 2880, 8300, 5395, 5410, 5460, 5470, 5480, 5500, 5660, 5700, 5740, 5870, 5875, 5890, 5900, 6150, 7000, 7002, 7004, 7006, 7009, 9830, 7010, 7020, 7025, 7030, 7034, 7035, 7040, 7050, 7060, 7080, 7090, 7095, 7096, 7100, 7110, 7130, 7140, 7150, 7170, 7175, 7184, 7185, 7190, 7211, 7233, 7235, 7490, 7491, 7705, 7820, 7950, 8021, 8041, 8090, 8130, 8160, 8200, 8250, 8260, 8280, 8340, 8465, 8510, 9210, 1601, 1604, 1721, 1812,1850, 1900, 1910, 1942, 4100, 2515, 2560, 2580, 2791, 2850, 4233, 4605, 5000, 5001, 5020, 5200, 5201, 5390, 5500, 5510, 5520, 5530, 5532, 5533, 5534, 5535, 5621, 5623, 5760, 4617, 5780, 5830, 5849, 5891, 5910, 5450, 5994, 5600, 5831, 7031, 2209, 7186, 7187, 7200, 7205, 7217, 7224, 7234, 7220, 7283, 7280, 7281, 7284, 7285, 7286, 7320, 7350, 7390, 7402, 7410, 7481, 7495, 7496, 7501, 7510, 7560, 7590, 7595, 7620, 7640, 7700, 7710, 8030, 8033, 8116, 8117, 8162, 8185, 8220, 8311, 8420, 8500, 7284, 7289, 9390, 9417, 9700.

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Study IV.

Garcia-Garcia, M., Barceló, F., Clemente, I.C., Escera, C. The role of DAT1 on the fast detection of task-relevance. *Submitted*

The role of DAT1 on the rapid detection of task-novelty

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Running title: DAT and rapid task-novelty detection

Abstract

Because humans are surrounded by a stimulus-rich environment, fast detection of relevant sensory events is crucial for the efficient control of attention. Here we tested the hypothesis that the rapid detection of relevance, known to modulate the auditory N1 potential (100 ms post-stimulus) might be mediated by the cortical-striatum dopaminergic (DA) pathway. Forty healthy volunteers were divided in two groups according to the presence or absence of the 9-repetition allele (9R) of the DAT1 gene for the dopamine transporter. Participants performed a cued task-switching paradigm in which the effects of exogenous sensory changes could be dissociated from those of endogenous task-set reconfiguration. Individuals with the 9R allele, related to larger striatal DA display, showed an amplitude enhancement of the auditory N1 elicited to sensory changes requiring a task-set reconfiguration as compared to sensory changes with no task-relevance. In contrast, individuals without the 9R allele did not have their N1 waveform modulated by task-relevance. The present results provide evidence for a role of the DAT1 genotype in the rapid detection of task-relevant sensory changes. We conclude that the auditory N1 potential might thus constitute an endophenotypic marker of DA functioning during the rapid detection of task-novelty.

Introduction

An adaptive behavior in everyday life requires the rapid detection and flexible integration of contextual information allowing for fine-grained adjustments to environmental demands. This rapid detection of task-relevant sensory changes has been proposed to depend on a fast route for capturing environmental changes which have immediate behavioral consequences (Barceló, Perianez, & Nyhus, 2007; Brass, Ullsperger, Knoesche, von Cramon, & Phillips, 2005). Such route involves phasic dopaminergic (DA) D2 responses regulated by loops between the PFC and the striatum (Seamans, Gorelova, Durstewitz and Yang, 2001). From this theoretical perspective, D2 DA receptor action might modulate the early detection of task-relevance in a sensory change. The dopamine transporter (DAT) is the most important regulator of DA at human striatum, where D2 DA receptors are mostly expressed (Camps, Cortes, Gueye, Probst, & Palacios, 1989). DAT mediates the active reuptake of DA from the synapse and critically regulates the extent to which DA diffuses in the extracellular space, and thus, the duration of cellular action of DA, especially in the striatum (Sesack, Hawrylak, Matus, Guido, & Levey, 1998).

In humans, the earliest electrophysiological brain response reflecting auditory processing at cortical level, known as the N1 auditory evoked potential, has been proposed as a marker of bottom-up sensory processes, such as attentional capture for subsequent access to consciousness

(Jaaskelainen et al., 2004; Näätänen & Winkler, 1999). However, the auditory N1 waveform does not represent an unitary stimulus-evoked process, but rather a compound response to several simultaneous active neural generators (Näätänen & Winkler, 1999). In fact, at least three exogenous (i.e., depending upon the physical characteristics of sensory stimulation) and three endogenous (i.e., depending upon subject's factors or informative value of the stimuli) components may be activated simultaneously to generate the auditory N1 wave (Näätänen & Picton, 1987). Some of these components are known to be sensitive to attention (Woldorff et al., 1993). In particular, a non-specific component of the N1 waveform was proposed to be related to the activation following the occurrence of a potentially relevant event, in order to facilitate appropriate sensory and motor responses (Näätänen & Picton, 1987). More recently cued task-switching studies have observed an early negative frontocentral response peaking around 100 ms post cue being modulated by the task relevance of the cue (Barceló et al., 2007; Brass et al., 2005). Moreover, a similar modulation by the task relevance of the cue has been found in the visual modality (Wylie, Javitt, & Foxe, 2003).

In the present study we tested the hypothesis that the DA display would promote the rapid detection of task-relevant changes, which would be carried out already at the level of generation if the auditory N1 evoked potential, i.e., circa 100 ms. For this purpose, a sample of healthy volunteers was divided into two groups according to a functional variable number of

tandem repeat (VNTR) polymorphism identified in the 3'-untranslated region of the DAT1 gene with repeat copy number ranging from 3 to 11, being 9- and 10-repeat (9R and 10R) the most frequent in population (Vandenbergh et al., 1992). The 10R allele has been associated with larger gene expression in vivo (Heinz et al., 2000; see however Jacobsen et al., 2000) and in vitro (Fuke et al., 2001; Mill, Asherson, Browes, D'Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005). Thus, individuals homozygous for the 10R allele (i.e., 9R-) show less DA available at the striatum, and those possessing the 9R allele (i.e., 9R+) have more striatal DA for D2 receptors binding.

In summary, we here hypothesized a predisposition of the 9R+ group (larger D2 binding) for a more effective rapid detection of task relevance, probably through a rapid DA pathway connecting the striatum to the prefrontal cortex (Redgrave & Gurney, 2006). This rapid pathway was measured as an early modulation of the fronto-central N1, previously related to task-relevance in task-switching paradigm (Barceló, Escera, Corral, & Perianez, 2006; Barceló et al., 2007); In order to test this hypothesis, participants with either high or low striatal DA activity (i.e., 9R+ or 9R- respectively) performed a cued task-switching paradigm where acoustic changes could be accompanied or not by a concurrent switch in task set, thus allowing us to dissociate the effects of sensory changes from those of task novelty.

Materials and Methods

Participants

Forty individuals (eight men, mean age 22 ± 4.2 years, range 18-29 years) participated in the study. They were recruited from a larger sample of volunteers which were interviewed according to an adapted version of the Clinical Interview of the Diagnostic and Statistical Manual (DSM IV-R), for exclusion of subjects with neurological and psychiatric illness, phobias, and drug consumption. All participants gave informed consent at each phase of the study (interview, buccal cells extraction and EEG recordings) according to the Declaration of Helsinki and the Ethic Committee of the University of Barcelóna. All subjects had normal or corrected-to-normal vision and normal audition. After exclusion by diagnostic criteria and after obtaining the DAT1 polymorphisms, the participants showing the most frequent genotypes (9R/9R, 9R/10R, 10R/10R; Vandenbergh et al., 1992) were selected for an EEG recording session. Participants genotyped as 10R/10R were assigned to the 9R- group associated with the functional effect of increased DAT expression (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005). Participants genotyped as 9R/10R and 9R/9R were included in the 9R+ group. Five participants were excluded from the analyses due to a large amount of artifacts in their EEG recordings. From the remaining 35 individuals, eighteen composed the 9R+ group and seventeen subjects were included in the 9R- group. Participants from each of the two genetic groups

did not differ significantly in age, gender and state or trait anxiety scores (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

DNA isolation and genotyping

In order to genotype the participants for the DAT1 gene, DNA was first collected with cheek cell swabs and extracted using the Epicentres® BuccalAmp™ DNA Extraction Kit (Epicentre, Madison, WS). Upon isolation of DNA, the 40-bp VNTR polymorphisms for the DAT1 gene (rs#28363170) were obtained for each DNA sample following similar procedures as those described by (Sano, Kondoh, Kakimoto, & Kondo, 1993), modified by amplifying PCR-VNTR using a fluorescently tagged primer. An initial 4-minutes denaturing at 95° C, 30 cycles of denaturing at 95° C during 30 seconds, annealing at 68° C for 30 second and extension at 72° C for additional 90 seconds performed in the presence of the primers DAT-F 5' 6-FAM TGTGGTGTAGGGAACGGCCTGAG 3' and DAT-R 5' CTTCCTGGAGGTCACGG-CTCAAGG, were then followed by a final extension at 72° C for another 10 minutes. Amplification products were analyzed using a capillary electrophoresis on the sequencer ABI Prism® 3730 (Applied Biosystems, Foster City, CA) and through the Fragments Analysis Technique with GeneMapper® Software Version 4.0 (Applied Biosystems, Foster City, CA). The resulting fragments consist of 280 bp for 5 repetitions, 320 for 6, 360 bp for 7, 400 bp for 8, 440 bp for 9, 480 bp for 10, 520 for 11 and 600 bp for 13 repetitions.

Behavioral procedure

A task-cueing protocol inspired by the Wisconsin Card Sorting Test (WCST; (Rubinstein, Meyer, & Evans, 2001) and adapted for measuring event-related brain potentials (ERPs; (Barceló, 2003) was administered to participants. Each trial consisted of a tonal cue followed by a target display with four key cards on top of one choice card, all centered on a computer screen. The target stimulus subtended a visual angle of 4º horizontally and 3.5º vertically, and remained on display until a response was given or up to a maximum of 3000 ms. Subjects were instructed to match the choice card with one of the four key cards following two possible task rules (color or shape). To ensure that all participants could see colors properly, the Test of Ishihara was applied for excluding participants with suspected color blindness. Before target onset, one out of four tonal cues explicitly informed the subject whether to sort the card according to either 'color' (500/1000 Hz) or 'shape' (2000/4000 Hz) rules. Binaural tones were delivered through Sehnheiser® HD202 headphones with a duration of 200 ms, 10 ms rise/fall times and 65 dB SPL. The meaning of the tonal cues was reversed for half of the subjects. All stimuli were presented with the stimulation program Presentation® (Neurobehavioral Systems Inc., Albany, CA). Three trial types were defined in order to dissociate the processing of changes in sensory and task representations. In *repeat* trials, both the tonal cue and the task were repeated relative to the previous trial. In *cue-switch* trials, only the cue changed but the task remained the same as in the previous trial. In *task-*

switch trials both cue and task changed. Responses were made using 4 keys on a keyboard, mapped onto the four fingers of the dominant hand, in an array corresponding to the layout of the four key-cards. The far left button designated the key card on the far left of the display, the far right button designated the key card on the far right, and so on. All three trial types were randomly presented with the same overall probability along the 200 trials of the experimental block, as well as during the 50 practice trials. The cues related to each criterion were employed five times during the instruction period of the practice block, and three more times during the instructions of the experimental block, in order to ensure that each participant had correctly learnt the cue-task association. Whenever the hit rate of the practice block was lower than 75%, an additional practice block was administered to ensure full assimilation of the correct cue-task association prior to the experimental run. All task sets declared in the instructions consisted of four-feature-stimulus to four-forced-response mappings. 'Task set' denotes here, in a broad sense, a set of rules that govern the mapping between sensory inputs and motor responses (Braver, Reynolds, & Donaldson, 2003). The cue-target interval randomly varied between 650 ±150 ms, thus minimizing the effects of a constant preparation interval (Rogers & Monsell, 1995), and the target remained on the screen until a response was given (up to a maximal of 3000 ms). Response-cue intervals also varied randomly around 1100 ±100 ms within the trial block.

EEG Data Acquisition

EEG activity was recorded (ANT Software b.v., Enschede, The Netherlands) during task performance from 64 scalp electrodes following the extended 10/10 convention in an electrically and acoustically shielded room. Horizontal and vertical electro-oculographic (EOG) recordings were obtained with electrodes placed at the outer cantus of the right eye and above the right eye. The common reference electrode was placed on the tip of the nose, and the ground was located at the chest. The EEG was amplified and digitized at a sampling rate of 512 Hz. Impedances were kept below 10 kΩ during the whole recording session, which lasted about 20 minutes.

Data processing

Cue-locked ERPs were averaged offline for each trial type (repeat, cue-switch and task-switch), for an epoch of 800 ms including a pre-stimulus baseline of 200 ms. Target-locked ERPs will not be reported as they did not account for any group-related behavioral cost. The first five trials of the block were excluded from analysis. Frequencies above 30 Hz were digitally filtered out from individual EEG epochs prior to ERP averaging. EOG correction was performed via a blind source separation technique with ASA 4.5 of ANT® Software (Enschede, The Netherlands), as described in Belouchrani, Abed-Meraim, Cardoso, & Moulines, (1997). After EOG correction, any epochs containing EEG activity exceeding ± 100 μV peak-to-peak amplitudes were

rejected from further analysis. The mean percentages of clean EEG epochs retained for ERP averages were 74.4%, 75.1% and 72.7% epochs from the repeat, cue-switch and task-switch conditions, respectively, and these did not differ among the three task conditions.

Data analysis

For behavioral analysis, any correct button press within 200-3000 ms after target onset was regarded as a hit, and the mean RT was computed for hit trials only. Hit rate and mean RT were submitted to a two-way mixed ANOVA with one repeated-measures factor (Trial type: repeat, cue-switch, task-switch), and one between-subject factor (Group: 9R+ and 9R-). Pair-wise post hoc comparisons were performed to examine significant difference between conditions.

For the analysis of the auditory frontocentral N1 component, the mean amplitudes were computed in the latency window from 110 to 140 ms and the latencies of local minimums from 70 to 150 ms. Both variables were computed at channels F3, F4, Fz, C3, C4, Cz, P3, P4 and Pz. Three-factor repeated-measures ANOVAs were performed including three within-subjects factors: Trial type (repeat, cue-switch and task-switch), Frontality (three levels for frontal, central and parietal channels) and Laterality (three levels for the left, middle and right channels), as well as the between-subject factor Group (9R+ and 9R-). Pair-wise post hoc comparisons were performed

between all trial types to examine whether any trial type effect was due to a switch in cue or in task. The Greenhouse-Geisser correction was applied to the degrees of freedom of the ANOVAs, and the corrected P-values were reported whenever appropriate. In order to parcel the location of the effect, an ANOVA was performed with Trial type (task-switch compared to cue-switch) and Laterality across all three levels of frontality for 9R+ individuals.

Results

Individuals from both groups showed reduced accuracy following any tonal change (main effect of Trial type: $F_{2,66}$=39.8, p<0.001) which was due to lower hit rates in task-switch compared to cue-switch trials ($F_{1,33}$=54.0, p<0.001; Fig. 1a). No effect of Group was found for the hit rate. Longer mean RTs after a tonal switch (main Trial type effect: $F_{2,66}$=100.7, p<0.001) were also observed, due to slower responses to cue-switch compared to repeat trials ($F_{1,33}$=123.6, p<0.001), as well as to task-switch compared to cue-switch trials ($F_{1,33}$=9.0, p=0.005). Although the two DAT1 groups did not differ significantly in their mean RT, the most striking behavioral result was the significant Trial type x Group interaction, which was due to the slower response in cue-switch compared to repeat trials in the 9R+ than in the 9R- group ($F_{1,33}$=6.6, p=0.015). Likewise, while 9R- individuals showed a further RT increase in task-switch as compared to cue-switch trials ($F_{1,16}$=16.5, p=0.001), this increase was not observed for the 9R+ group (fig. 1b).

The frontocentrally distributed N1 waveform peaked at 111, 111 and 116 ms in repeat, cue-switch and task-switch trials, respectively, for 9R+ individuals, and at 107, 113 and 112 ms in repeat, cue-switch and task-switch trials, respectively, for 9R- individuals. Although no effect nor interaction for peak latencies were observed, larger N1 amplitudes in task-switch relative to cue-switch and repeat trials were observed in the 9R+ ($F_{1,17}$=7.50, p=0.014) but not in the 9R- group, as supported by a significant Condition x Group interaction ($F_{1,33}$=5.12, p=0.030; Fig. 2a). This interaction was significant at frontal ($F_{1,17}$=14.2, p=0.002) and central channels ($F_{1,17}$=7.9, p=0.012), but not over more posterior scalp locations (Fig. 2b). Mean amplitudes of the frontocentral N1 waveform at Cz were of -4.6, -4.6 and -6.2 μV in repeat, cue-switch and task-switch trials, respectively, for 9R+ individuals, and of -4.4, -5.2 and -4.8 μV in repeat, cue-switch and task-switch trials, respectively, for 9R- individuals (Fig. 2c).

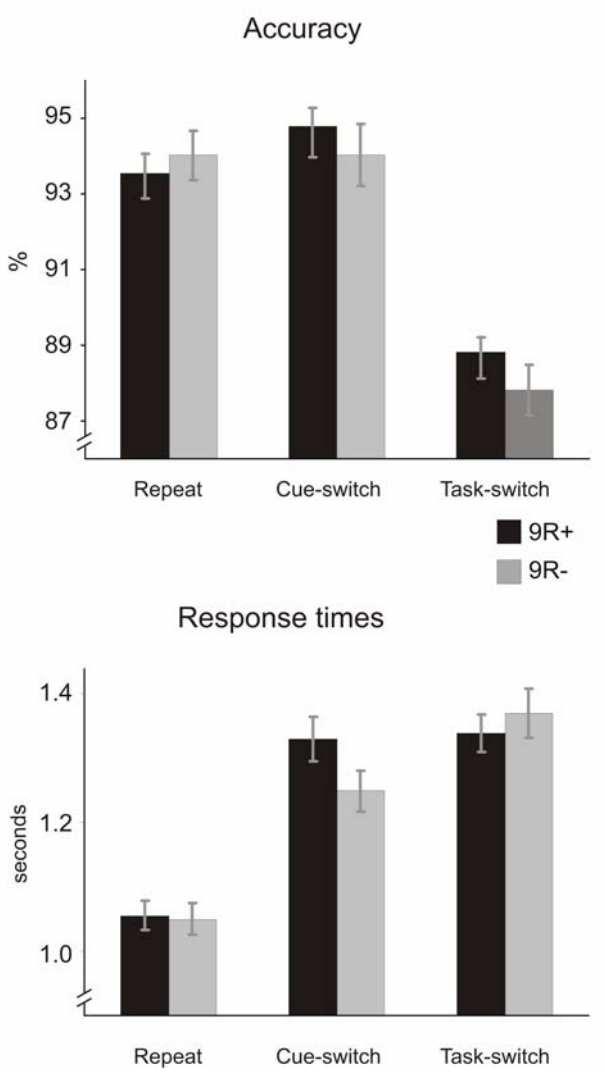

Figure 1. *Accuracy and response times (RT) for the 9R+ and 9R- groups across the three trial types. The hit rate was lower in task-switch trials as compared to the other two trial types, with no differences between the groups. The RT plot shows a delay in cue-switch trials for both groups; however, whereas the 9R- group showed larger RT in task-switch as compared to cue-switch trials, the 9R+ group showed similar RT for these two trial types.*

Discussion

The present study aimed at revealing the role of the DAT1 polymorphism on the rapid detection of task-novelty. The current results revealed that 9R+

individuals, presumed to have more striatal DA for D2 receptors binding, showed an enhancement of the frontocentral N1 waveform peaking as early as 110 ms following a cue signaling a switch compared to a cue signaling repetition in stimulus-response mapping of the ongoing task-set. These individuals also showed the largest RT in cue-switch trials and showed no further increase in task-switch trials. In contrast, the amplitude of this early frontocentral N1 waveform did not differ across conditions for 9R- individuals in spite of their significantly increased mean RTs in cue-switch as compared to repeat trials, as well as in task-switch as compared to cue-switch trials.

We proposed that the rapid detection of task-novelty would be carried out at the generation of the N1 waveform, the earliest brain response reflecting auditory processing at cortical level (Näätänen & Winkler, 1999). In fact, the 9R+ group displayed a enhancement of the N1 waveform for cues indicating task-novelty as compared to those signaling task-repetition. Several exogenous and endogenous components are involved in the generation of this N1 waveform (Näätänen & Picton, 1987), some of which are sensitive to attentional manipulations (Woldorff et al., 1993). The distribution of the amplitude enhancement found in the current study support a frontocentral N1 component sensitive to stimulus significance (Näätänen & Picton, 1987) and task-novelty (Barceló et al., 2007; Brass et al., 2005).

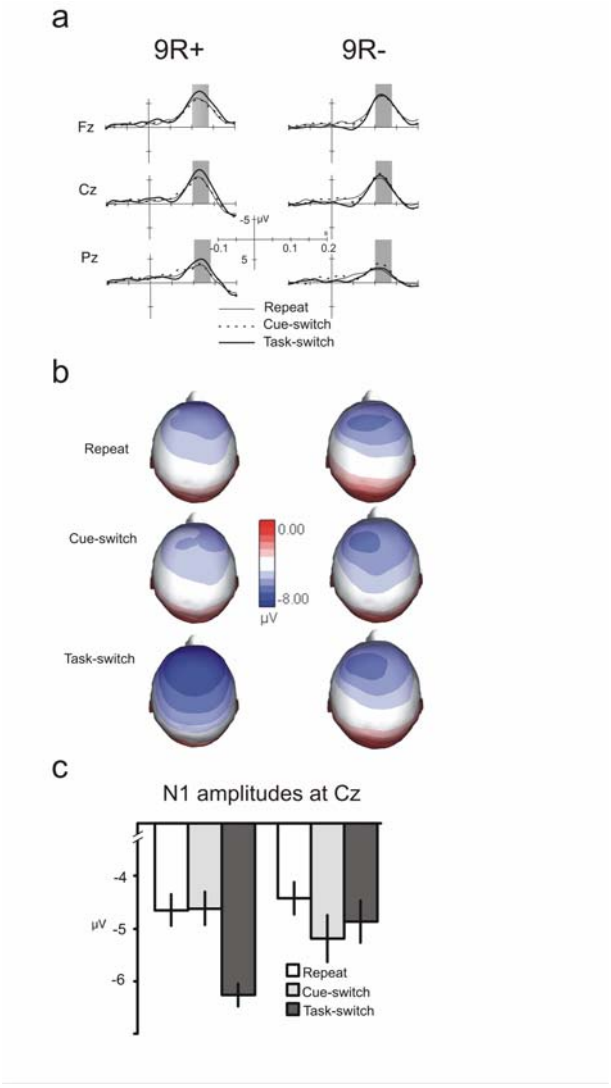

Figure 2. *The N1 auditory evoked potential. (a) Cue-locked brain waves at Fz, Cz and Pz locations for 9R+ and 9R- individuals across the three trial types. Notice that 9R+ individuals display an amplitude enhancement for task-switch as compared to cue-switch trials, which is not observed in 9R- individuals. (b) Scalp distribution of the brain response in the three trial types for both 9R+ and 9R- individuals. The effect of task-switching compared to cue-switching in the shadowed time windows displayed by 9R+ shows a frontocentral distribution. In contrast, 9R- individuals show no specific effect for task-switch in such subcomponent. (c) Amplitudes of the frontocentral N1 component at the Cz electrode in the three trial types and for the two groups. Larger amplitudes in task-switch trials relative to cue-switch and repeat trials were observed in the 9R+ but not in the 9R- group.*

Importantly, only individuals with presumed larger DA display at striatum (i.e., 9R+; (Heinz et al., 2000) showed such an early detection of task-relevance in sensory changes. A relevant role of DA in attentional processes such as the detection of salient stimuli (Wilson & Bowman, 2006), reward motivation (Niv, Daw, Joel, & Dayan, 2007) or task-switching (Cools, Barker, Sahakian, & Robbins, 2003; Cools, Clark, & Robbins, 2004; Cools, Ivry, & D'Esposito, 2006) has been widely evidenced by previous studies. Moreover, D2 DA receptor binding, which is mainly regulated by DAT, are centrally involved in reward-mediating mesocorticolimbic pathways (Neville, Johnstone, & Walton, 2004) and play a critical role in cognitive flexibility (Cools et al., 2003; Cools et al., 2004; Cools et al., 2006) involving detection of task-novelty. D2 responses are regulated by loops between the PFC and the striatum (Seamans, Gorelova, Durstewitz, & Yang, 2001) in the prefrontotectal route associated to capture of environmental changes which have immediate behavioral consequences (Barceló et al., 2007; Brass et al., 2005). These prefrontostriatal loops may account for the current findings arguing a crucial role of D2 activity on the early detection of task-novelty. Accordingly, recent studies evidenced a facilitation of very early novelty processing by reward-motivation (Bunzeck, Doeller, Fuentemilla, Dolan, & Duzel, 2009), possibly related to the elevated levels of DA in the context of reward (Niv et al., 2007). Moreover, due to D1/D2 reciprocal interaction, D2 activity regulation by D1 PFC activity (Akil et al., 2003; Meyer-Lindenberg et al., 2005) might account for disrupted brain responses to all novel information in PFC patients, which supports the hypothesis of PFC

structures as a switch operator for processing familiar and novel information at highest level in hierarchy of cognitive control (Miller & Cohen, 2001). It seems plausible that cortical-subcortical connections like prefrontostriatal pathways offer a potential circuit for the rapid detection of unexpected and potentially relevant sensory signals, as enough information can be conveyed through this route to detect a mismatch between sensory input and active PFC representations (Potts, Martin, Burton, & Montague, 2006; Redgrave & Gurney, 2006).

The similar mean RTs to cue- and task-switch trials in 9R+ individuals seem to indicate that they responded to each cue in a context independent manner, that is, irrespective of the meaning of the previous trial for switching or repeating the task. Interestingly, the amplitude of the N1 component in 9R+ individuals could dissociate between cues signaling task repetition and those signaling a task switch, suggesting that 9R+ individuals detected the need to reconfigure the current task-set at an earlier stage than 9R- individuals did as a consequence of their higher striatal DA display. However, this early dissociation is not reflected on the behavioral correlates of task-switch, in which 9R+ individuals fail to dissociate between cue-switch and task-switch trials. This temporal advantage could also result in slightly increased distractibility (i.e., longer RTs after a sensory change) as shown in cue-switch relative to repeat trials displayed in 9R+ as compared to 9R- individuals.

Although the current participants were healthy volunteers, these results can shed some light for understanding cognitive deficits in DA-related disorders, such as the rigid behavior revealed in Parkinson's disease (PD) patients by significant impairment in the WCST and other task-switching related protocols (Cools, Barker, Sahakian, & Robbins, 2001; Cools et al., 2003; Cools et al., 2004; Meiran, Friedman, & Yehene, 2004). These deficits have been generally attributed to problems in the flexible use of abstract rules (Meiran et al., 2004; Yehene, Meiran, & Soroker, 2008). Moreover, the Attention Deficit Hyperactivity Disorder (ADHD), also related to a poor ability to flexibly adjust behavior to environmental demands (Nigg & Casey, 2005), is associated to the 10-repeat allele (Yang et al., 2007) and its pharmacological treatment increases DA in the striatum in order to improve attentional functions by increasing the signal-to-noise ratio in target neurons (Volkow et al., 2001). The current results provide indirect evidence of the role of DAT1 genotype in the adaptive mechanism of rapid detection of task-relevant sensory changes which should be taken into account to design the pharmacological treatment of related disorders or neurological diseases, since drug responsiveness is variable in the population as a consequence of the genotype, and cognitive impairments might have different causes as well.

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Study V.

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The role of the Dopamine Transporter DAT1 genotype on the neural correlates of cognitive flexibility

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Running title: DAT1 and cognitive flexibility

Abstract

Cognitive flexibility, the ability to adapt goal-oriented behavior in response to changing environmental demands, varies widely amongst individuals, yet its underlying neural mechanisms are not fully understood. Neuropharmacological and human clinical studies have suggested a critical role for striatal dopaminergic function mediated by the dopamine transporter (DAT). The present study aimed at revealing the role of the DAT in the individual brain response stereotypy underlying cognitive flexibility. A task-switching protocol was administered to a sample divided according to the presence or absence of the 9 repetition (9R) allele of the DAT1 polymorphism, while registering behavioral and electrophysiological novelty-P3 (nP3) responses. The absence of the 9R (higher gene expression) is related to less striatal dopamine availability. Individuals lacking the 9R (9R-) showed specific response time (RT) increases for sensory change and task-set reconfiguration, as well as brain modulations not observed in participants with the 9R allele (9R+), suggesting that task performance of the former group depended on immediate local context. In contrast, individuals displaying high striatal DA showed larger RT costs than 9R- individuals to any sensory change, with no further increase for task-set reconfiguration, and a larger early positive brain response irrespective of the task condition, probably reflecting larger inhibition of any previous interference as well as stronger activation of the current task set. This distinct stereotypy of cerebral

responses reveals different patterns of cognitive control according to the DAT1 gene polymorphism.

Introduction

Cognitive flexibility, the ability to adapt goal-directed behavior in response to changing environmental demands, is one crucial factor in the executive control of attention, and yet it varies widely amongst individuals. The dopamine (DA) function might mediate such individual differences in the brain mechanisms of cognitive flexibility, as DA receptor binding in the human striatum has been shown to enhance cognitive flexibility by facilitating the updating of new relevant representations in working memory (Cools, 2008; Frank, 2005). Enhanced striatum DA function promotes exploratory and orienting behavior (i.e., attention orienting towards novel stimuli) and behavioral switching (i.e., novel actions; Kaplan & Oudeyer, 2007). On the other hand, DA depletion by experimental manipulation (Sawaguchi & Goldman-Rakic, 1994) or in clinical conditions such as Parkinson disease (S. J. Lewis, Dove, Robbins, Barker, & Owen, 2003) impairs performance on set-shifting tasks.

The dopamine transporter (DAT) is the most important DA regulator at the human striatum (Sesack, Hawrylak, Matus, Guido, & Levey, 1998), as it mediates DA active reuptake from the synapse (D. A. Lewis et al., 2001). A functional variable number of tandem repeat (VNTR) polymorphism was identified in the DAT1 gene with 9- and 10-repeat (9R and 10R) as the most frequent alleles in the population (Vandenbergh et al., 1992). The 10R/10R genotype results in increased DAT expression (Fuke et al., 2001; Heinz et

al., 2000; Mill, Asherson, Browes, D'Souza, & Craig, 2002; VanNess, Owens, & Kilts, 2005; see however Jacobsen et al., 2000) and putatively, decreased synaptic DA tone in cortico-striatal pathways (Wichmann & DeLong, 1996).

In the present study, we explored the role of the DAT1 genotype in the individual differences in cognitive flexibility by examining the influence of the 9-repeat allele in the behavioral and electrophysiological response to a task-cueing protocol inspired by the Wisconsin Card Sorting Test (Rubinstein, Meyer, & Evans, 2001) and adapted for measuring human event-related brain potentials (ERP; Barceló, 2003). Barceló, Escera, Corral, & Perianez, (2006) have recently demonstrated that auditory cues directing a switch in the mental set to a new task elicit a characteristic electroencephalographic (EEG) response known as the “novelty-P3” (nP3) complex. This nP3 response accounts for operations of context-updating involved in the processing of both sensory novelty (Escera, Alho, Winkler, & Näätänen, 1998; Escera, Alho, Schroger, & Winkler, 2000; Escera & Corral, 2007), and task novelty (Barceló, Perianez, & Knight, 2002) as reflected by a late fronto-posterior positivity, which is preceded by an early fronto-central positivity associated to mechanisms of task rule reactivation (Barceló, Perianez, & Nyhus, 2007).

Since reduced striatum DA levels have been shown to impair cognitive flexibility (Cools, Barker, Sahakian, & Robbins, 2001; Cools, Barker,

Sahakian, & Robbins, 2003; Cools, Clark, & Robbins, 2004; Cools, Ivry, & D'Esposito, 2006; Cools, 2008; S. J. Lewis et al., 2003), individuals genotyped 10R/10R are expected to show a more rigid behavior resulting in larger task-switch cost and a less effective gating mechanism for context update than their counter partners, both reflected on the putative endophenotypical nP3 brain response.

Materials and Methods

Participants

Forty individuals (eight men, mean age 22 ± 4.2 years, range 18-29 years) participated in the present study. They were recruited from a wider sample of volunteers which were interviewed according to an adapted version of the Clinical Interview of the Diagnostic and Statistical Manual (DSM IV-R), for exclusion of subjects with neurological and psychiatric illness, phobias, and drug consumption. All participants gave informed consent at each phase of the study (interview, buccal cells extraction and EEG recordings) according to the Declaration of Helsinki and the Ethic Committee of the University of Barcelóna. All subjects had normal or corrected-to-normal vision and normal audition. After exclusion by diagnostic criteria and obtaining the DAT1 polymorphisms, the participants showing the most frequent genotypes (9R/9R, 9R/10R, 10R/10R; Vandenbergh et al., 1992) were selected for an EEG recording session. Participants genotyped as 10R/10R were assigned

to the 9R- group associated with the functional effect of increased DAT expression (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005), and participants genotyped as 9R/10R and 9R/9R were included in the 9R+ group. Two participants were excluded from the ERP analyses due to a large amount of artifacts in their EEG recordings. From the remaining 38 individuals, twenty composed the 9R+ group and eighteen subjects were included in the 9R- group. Participants from each of the two genetic groups did not differ significantly in age, gender and state or trait anxiety scores (STAI; Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983).

DNA isolation and genotyping

A functional variable number of tandem repeat (VNTR) polymorphism was identified in the 3'-untranslated region of the DAT1 gene with repeat copy number ranging from 3-to-11, being 9- and 10-repeat (9R and 10R) the most frequent in population (Vandenbergh et al., 1992). The 10R/10R genotype results in increased DAT expression in vivo (Heinz et al., 2000; see however Jacobsen et al., 2000) and in vitro (Fuke et al., 2001; Mill et al., 2002; VanNess et al., 2005). In order to genotype the participants for the DAT1 gene, DNA was first collected with cheek cell swabs and extracted using the Epicentres® BuccalAmp™ DNA Extraction Kit (Epicentre, Madison, WS). Upon isolation of DNA, the 40-bp VNTR polymorphisms for the DAT1 gene (rs#28363170) were obtained for each DNA sample following the procedures described by Sano, Kondoh, Kakimoto, & Kondo (1993), and modified by

amplifying PCR-VNTR using a fluorescently tagged primer. An initial 4-minutes denaturing at 95° C, 30 cycles of denaturing at 95° C during 30 seconds, annealing at 68° C for 30 second and extension at 72° C for additional 90 seconds performed in the presence of the primers DAT-F 5' 6-FAM TGTGGTGTAGGGAACGGCCTGAG 3' and DAT-R 5' CTTCCTGGAGGTCACGG- CTCAAGG, were then followed by a final extension at 72° C for another 10 minutes. Amplification products were analyzed using a capillary electrophoresis on the sequencer ABI Prism® 3730 (Applied Biosystems, Foster City, CA) and through the Fragments Analysis Technique with GeneMapper® Software Version 4.0 (Applied Biosystems, Foster City, CA).The resulting fragments consist of 280 bp for 5 repetitions, 320 for 6, 360 bp for 7, 400 bp for 8, 440 bp for 9, 480 bp for 10, 520 for 11 and 600 bp for 13 repetitions.

Behavioral procedure

A task-cueing protocol inspired by the WCST (Rubinstein et al., 2001) and adapted for measuring ERPs (Barceló, 2003) was administered to participants. Each trial consisted of a tonal cue followed by a target display with four key cards on top of one choice card, all centered on a screen (Fig. 1). The target stimulus subtended a visual angle of 4º horizontally and 3.5º vertically, and remained on display until a response was given or up to a maximum of 3000 ms. Subjects were instructed to match the choice card with one of the four key cards following two possible task rules (color or

shape). To ensure that all participants could see colors properly, the Test of Ishihara was applied for excluding participants with suspected color blindness. Before target onset, one out of four tonal cues explicitly informed the subject whether to sort the card according to either the 'color' (500/1000 Hz) or 'shape' (2000/4000 Hz) rules. Binaural tones were delivered through Sehnheiser® HD202 headphones with a duration of 200 ms, 10 ms rise/fall times and 65 dB SPL. The meaning of the tonal cues was reversed for half of the subjects. All stimuli were presented with the stimulation program Presentation® (Neurobehavioral Systems Inc., Albany, CA). Three trial types were defined in order to dissociate the processing of changes in sensory and task representations. In the *repeat* trials, both the tonal cue and the task were repeated relative to the previous trial. In the *cue-switch* trials, only the cue changed but the task remained the same as in the previous trial. In the *task-switch* trials both cue and task changed. Responses were made using 4 keys on a keyboard, mapped onto the four fingers of the dominant hand, in an array corresponding to the layout of the four key-cards. The far left button designated the key card on the far left of the display, the far right button designated the key card on the far right, and so on (Fig. 1). All three trial types were randomly presented with the same overall probability along the 200 trials of the experimental block, as well as during the 50 practice trials. The cues related to each criterion were employed five times during the instruction period of the practice block, and three more times during the instructions of the experimental block, in order to ensure that each participant had correctly learnt the cue-task association. Whenever the hit

rate of the practice block was lower than 75%, an additional practice block was administered to ensure full assimilation of the correct cue-task association prior to the run of experimental block. There was no effect of the DAT1 genotype on the number of practice blocks administered. All the task sets declared in the instructions consisted of four-feature-stimulus to four-forced-response mappings. 'Task set' denotes here, in a broad sense, a set of rules that govern the mapping between sensory inputs and motor responses (Braver, Reynolds, & Donaldson, 2003). The cue-target interval randomly varied between 650 ±150 ms, thus minimizing the effects of a constant preparation interval (Rogers & Monsell, 1995), and the target remained on the screen until a response was given (up to a maximal of 3000 ms). Response-cue intervals also varied randomly around 1100 ±100 ms within the trial block.

EEG Data Acquisition

EEG activity was recorded (ANT Software b.v., Enschede, The Netherlands) during task performance from 64 scalp electrodes following the extended 10/10 convention in an electrically and acoustically shielded room. Horizontal and vertical electro-oculographic (EOG) recordings were obtained with electrodes placed at the outer cantus of the right eye and above the right eye. The common reference electrode was placed on the tip of the nose, and the ground was located at the chest. The EEG was amplified and

digitized at a sampling rate of 512 Hz. Impedances were kept below 10 kΩ during the whole recording session, which lasted about 20 minutes.

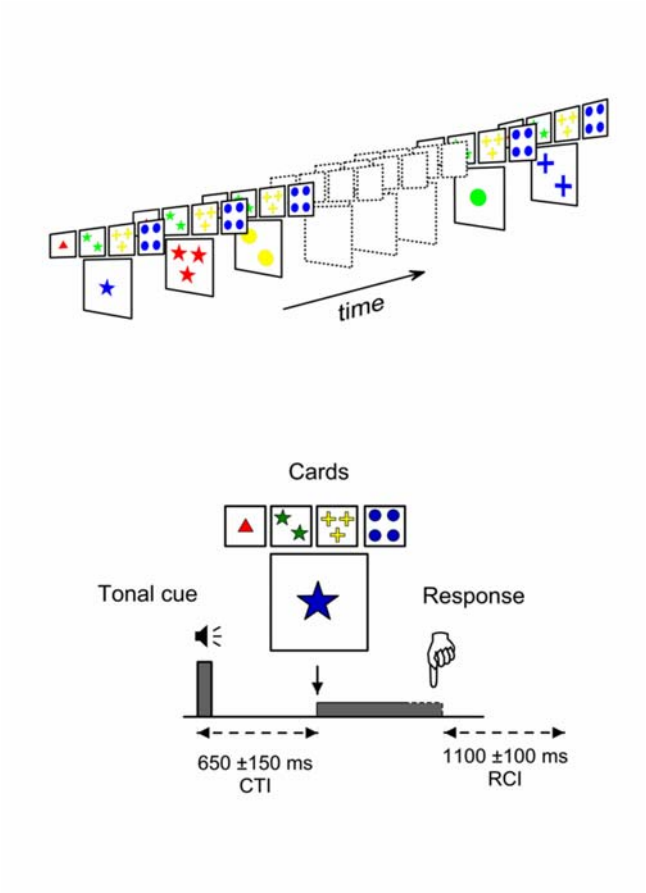

Figure 1. *Stimulus material and experimental design. Each trial consisted of a tonal cue followed by a visual target display with four key cards on top of one choice card. Subjects were instructed to classify targets according to their color or to their shape. Before target onset, a tonal cue (500/1000 and 2000/4000 Hz tones) informed whether to classify according to the color or the shape rules. The meaning of the two tones was counterbalanced across subjects. The length of the cue-target interval (CTI) and the response-cue interval were jittered.*

Data processing

ERPs were averaged offline for each trial type (repeat, cue-switch and task-switch), for an epoch of 1400 ms including a pre-stimulus baseline of 200 ms. The first five trials of the block were excluded from analysis. Frequencies above 30 Hz were digitally filtered out from individual EEG epochs prior to ERP averaging. EOG correction was performed via a blind source separation technique with ASA 4.5 of ANT® Software (Enschede, The Netherlands), as described in Belouchrani, Abed-Meraim, Cardoso, & Moulines, (1997). After EOG correction, any epochs containing EEG activity exceeding ± 100 μV peak-to-peak amplitudes were rejected from further analysis. The mean percentages of clean EEG epochs retained for ERP averages were 74.4%, 75.1% and 72.7% epochs from the repeat, cue-switch and task-switch conditions, respectively, which did not differ between any of the trial types.

Data analysis

For behavioral analysis, any correct button press within 200-3000 ms after target onset was regarded as a hit, and the mean RT was computed for hit trials only. Hit rate and mean RT were submitted to a two-way mixed ANOVA with one repeated-measures factor (Trial type: repeat, cue-switch, task-switch), and one between-subject factor (Group: 9R+ and 9R-). Pair-

wise post hoc comparisons were performed to examine any significant difference between conditions.

For the analysis of the auditory brain responses, the mean amplitudes of the following ERP components were computed in the specified latency windows: the early fronto-central positivity from 180 to 220 ms, the late fronto-posterior positivity from 300 to 340 ms, the late negative deflection from 420 to 440 ms. Likewise, the slow fronto-parietal negativity was computed in two latency windows, from 600 to 700 ms (SW1), and from 800 to 900 ms (SW2). All these brain responses were measured at channels F3, F4, Fz, C3, C4, Cz, P3, P4 and Pz. A three-factor repeated-measures ANOVA was performed on all these ERP measures including three within-subjects factors: Trial type (repeat, cue-switch and task-switch), Frontality (three levels for frontal, central and parietal channels) and Laterality (three levels for the left, middle and right channels), as well as the between-subject factor Group (9R+ and 9R-). Pair-wise post hoc comparisons were performed across all trial types to examine whether the trial type effect was due to a cue-switch or to a task-switch. The Greenhouse-Geisser correction was applied to the degrees of freedom of the ANOVAs, and the corrected P-values were reported whenever was appropriate. Target-locked ERPs were not reported as they did not account for any group-related behavioral cost.

Results

Behavioral Results

Both groups showed a decrease of hit rate after a tonal switch (main effect of Trial type: $F_{2,72}$=39.7, p<0.001) which was due to a decrease in hit rate in the task-switch compared to cue-switch trials ($F_{1,36}$=56.3, p<0.001). No effect of Group was found for the hit rate. An increase in RTs after a tonal switch (main Trial type effect: $F_{2,72}$=71.6, p<0.001) was also observed, due to an increase in RT in cue-switch compared to repeat trials ($F_{1,36}$=75.1, p<0.001). Although the two DAT1 groups did not differ significantly in their mean RT, the most striking behavioral result was the interaction Trial type x Group ($F_{1,36}$=4.4, p=0.033), which was due to the fact that task-switch trials caused a RT increase compared to cue-switch trials in the 9R- group ($F_{1,13}$=13.6, p=0.003), whereas the 9R+ reached the largest RT increase already in cue-switch trials with no further increase in task-switch trials (Fig. 2).

Electrophysiology

Auditory cues elicited a typical fronto-parietal nP3 consisting on an early fronto-central positivity followed by a late fronto-posterior positivity and a late negative deflection (Fig. 3A). Mean amplitudes of the early fronto-central positivity were substantially larger for the 9R+ group than for the 9R- group, particularly at central and parietal locations. This was supported by a

significant Frontality x Group interaction ($F_{2,72}$=5.2, p=0.023; Fig. 3A, B), there being a main Group effect at central ($F_{1,36}$=6.3, p=0.016) and parietal ($F_{1,36}$=6.0, p=0.019; Fig. 3B) locations. However, this brain response was not affected by Trial type. As for the late fronto-posterior positivity, a main effect of Trial type ($F_{2,72}$=13.1, p<0.001) revealed larger amplitudes after cue-switch compared to repeat trials ($F_{1,36}$=6.7, p=0.013), and for task-switch compared to cue-switch trials ($F_{1,36}$=7.0, p=0.012). However, this late positivity was not affected by the DAT1 polymorphism. The subsequent late negative deflection showed a Trial type x Group interaction ($F_{2,72}$=3.5, p=0.041) revealing smaller amplitudes in task-switch as compared to cue-switch trials in the 9R- group ($F_{2,34}$=4.5, p=0.028), while all three trial types elicited similar amplitudes in the 9R+ group (Fig. 4A).

Following the nP3 complex, the late slow fronto-parietal negativity showed a main Trial type effect (SW1: $F_{2,72}$=21.3, p<0.001; SW2: $F_{2,72}$=15.0, p<0.001) which was due to an amplitude decrease in cue-switch as compared to repeat trials (SW1: $F_{1,36}$=26.3, p<0.001; SW2: $F_{1,36}$=22.1, p<0.001). However, the SW2 reduction was largest in the 9R- than in the 9R+ group as supported by a significant Trial type x Group interaction ($F_{2,72}$=4.3, p=0.017). The mean amplitude difference between cue-switch *minus* repeat trials was 3.83 µV and 2.01 µV for the 9R- and 9R+ groups, respectively (Fig. 4B).

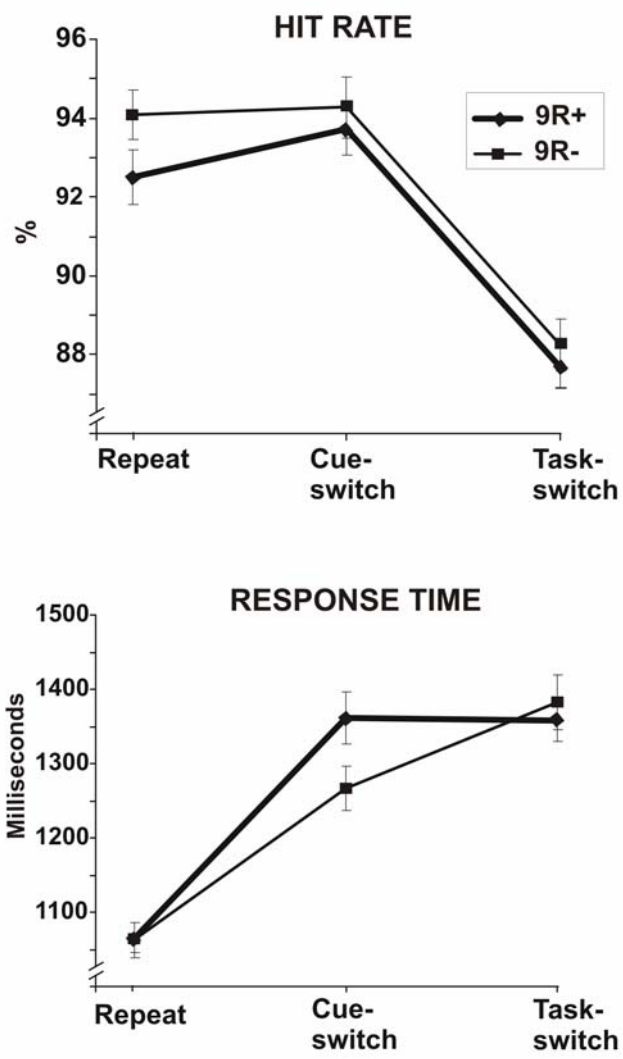

Figure 2. *Response times (RTs) and hit rate for the 9R+ and 9R- groups across the three trial types. The hit rate was lower in task switch trials as compared to the other two trial types, with no differences between the groups. The RT plot shows a delay in cue-switch trials for both groups; however, whereas the 9R- group showed larger RT in task-switch as compared to cue-switch trials, the 9R+ group showed similar RT for these two trial types.*

Discussion

A cued task switching paradigm was used in human subjects with different polymorphic variations for the DAT1, in order to reveal the role of the DAT in human variability in the brain response stereotypy underlying cognitive flexibility. The polymorphic groups showed different patterns of behavioral switch cost: whereas mean RT increased in cue-switch as compared to repeat trials, and in task-switch as compared to cue-switch trials for 9R- individuals, the 9R+ group reached their largest mean RTs in cue-switch trials with no further increase in task-switch trials. This behavioral profile was accompanied by larger early fronto-central positivity at central and parietal scalp regions in 9R+ as compared to the 9R- group. In contrast, task conditions modulated the amplitude of a late negative deflection for 9R-, but not for 9R+ individuals.

DAT1 and task-switch cost

Even though both groups showed a RT cost following any change in acoustic stimulation (i.e., a distraction effect; (Escera, Alho, Winkler, & Näätänen, 1998; Escera, Alho, Schroger, & Winkler, 2000; Escera & Corral, 2007), larger RT costs were observed between cue-switch and repeat trials for 9R+ than for 9R- individuals, with no further increase in RT costs between cue-switch and task-switch trials. Conversely, 9R- individuals showed an additional RT cost between cue-switch and task-switch trials

(Karayanidis, Coltheart, Michie, & Murphy, 2003; Fig. 2). The importance of striatal DA in the flexible control of attention (Kaplan & Oudeyer, 2007; Montague, Hyman, & Cohen, 2004) has been revealed by larger task-switch RT costs in conditions of lesser DA display in human striatum (see Cools et al., 2001; Cools et al., 2003; Cools et al., 2004; Cools et al., 2006; Cools, 2008) accompanied by a task-switch cost in accuracy. However, in the current study the two groups showed similar mean RTs and hit rates, but nevertheless distinct patterns of switch cost according to their genetic profiles. Larger task-switch costs were predicted for the 9R- group due to their lesser striatal DA display (Cools et al., 2001; Cools, Barker, Sahakian, & Robbins, 2003; Cools, Clark, & Robbins, 2004; Cools, Ivry, & D'Esposito, 2006; Cools, 2008). However, the present results revealed similar mean RTs to task-switch cues in both groups, although 9R- individuals did discriminate between cue-switch and task-switch trials. Instead, the 9R+ group invested similar RTs for processing all cue switches, suggesting a context-independent processing of all auditory changes. 9R+ individuals invested about 100 ms extra for cue-switch trials than the 9R- did. This slower evaluation of a sensory change could be due to an excess of protection against interference (Cools et al., 2001). Larger DA display in frontostriatal circuits (9R+) might help to protect the current task-set in the presence of competing novel sensory or task demands (cf., Cools et al., 2001).Contrary to the 9R+, the 9R- group processed all auditory inputs in a context-dependent fashion, resulting in a comparatively more efficient sorting of cue-switches depending on whether these auditory changes signaled or not the

preparatory re-mapping between visual inputs and motor responses (i.e., task-set switching or repetition).

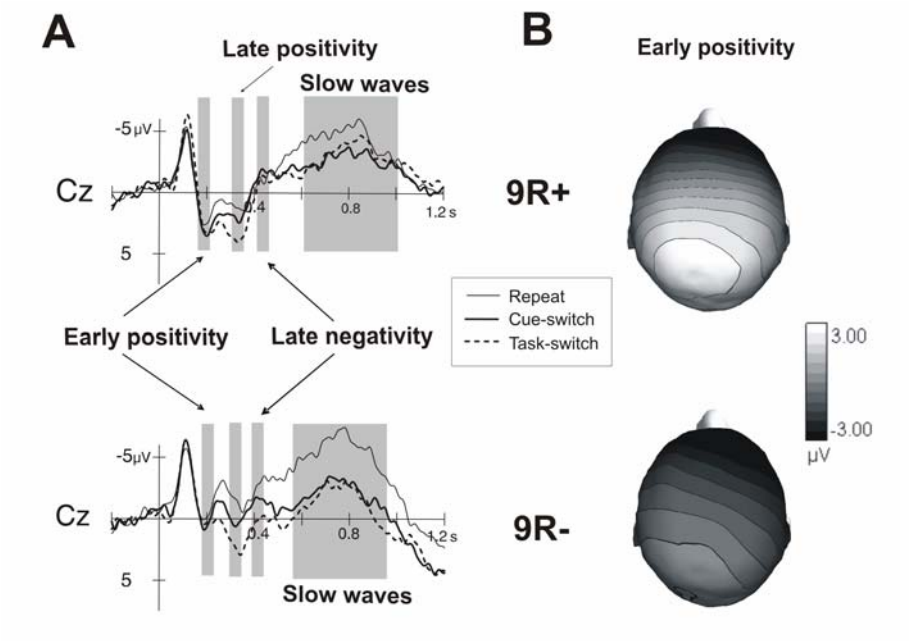

***Figure 3**. Event-related brain potentials (ERPs) elicited by the auditory cues in the three different trial types. (A) Relevant ERP waveforms for both groups across the three trial types have been shadowed at the Cz recording site. Notice the larger early positivity in the 9R+ group relative to the 9R- group. (B) Scalp distribution of the early fronto-central positivity for both groups in repeat trials. This brain potential was larger in the 9R+ than in the 9R- group mostly over central and parietal scalp regions.*

DAT1 and the early positivity of the nP3

Moreover, the 9R+ group showed larger amplitude of the early positivity of the nP3 as compared to the 9R-, irrespective of the task condition, reflecting an effect of the DAT1 genotype on auditory stimulation. In an auditory oddball paradigm, the administration of the D2 receptor antagonist haloperidol resulted in lower amplitudes of a brain response elicited by the

auditory target (Kahkonen et al., 2002). Accordingly, this early positivity of the nP3 presumably indicates that a higher DA display allows 9R+ individuals to deploy a stronger reactivation of the task rule, thus avoiding interference from a previous task-set (Cools et al., 2001), as suggested by the larger RT to cue-switch observed in 9R+ in comparison to 9R-. Although this is the first study suggesting a direct implication of the striatal DA display in the general mechanism of task-set activation reflected by the early positive component of the nP3, similar DA-regulated fronto-medial positivities (i.e., P2a; Potts, Martin, Burton, & Montague, 2006) has been proposed to reflect striatum DA signaling which implements a 'gating' mechanism controlling the access of information to neural systems for cognitive control (Montague et al., 2004; O'Reilly, Braver, & Cohen, 1999; Potts et al., 2006). This is consistent with a recent proposal that the early positive subcomponent of nP3 reflects a general mechanism of task-set activation necessary for the subsequent remapping of stimulus-response associations (Barceló et al., 2007). Accordingly, larger DA tone in 9R+ individuals facilitates a stronger activation of stimulus-response mappings following every cue. This early positivity failed to distinguish among task conditions. In turn, the late fronto-posterior positivity discriminated among trial types, although it was not influenced by the DAT1 polymorphism. Hence, this late positivity might be mediated by another neurotransmitter system, such as norepinephrine (Nieuwenhuis, Aston-Jones, & Cohen, 2005), or by the combined action of other DA receptors.

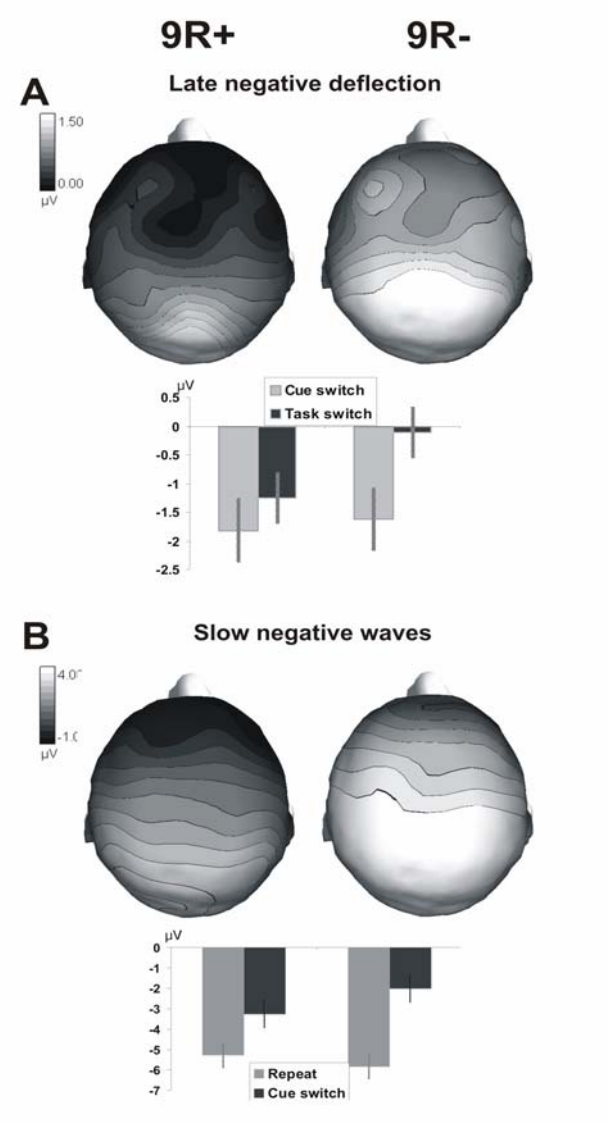

Figure 4. The frontal negative deflection and the late slow fronto-posterior negativities showed a different modulation by task condition in each of the groups. (A) The scalp distributions of the difference wave obtained by subtracting ERPs of task-switch minus ERPs of cue-switch trials at the late negative deflection. The underlying plot shows the amplitudes in both groups in cue-switch and task-switch trials. The 9R- group displayed an amplitude decrease in the task-switch as compared to the cue-switch condition, not present in the 9R+. (B) The scalp distributions of the differential response obtained by subtracting ERPs of cue-switch minus ERPs of repeat trials at the slow fronto-posterior negativity time range. The underlying plot shows the amplitudes in both groups in the repeat and cue-switch conditions. The 9R- group displayed a stronger amplitude decrease in the cue-switch as compared to the repeat condition than the 9R+.

DAT1 and the switch-related negative deflection

The late negative deflection decreased in task-switch as compared to cue-switch trials in the 9R- group, but showed similar amplitudes across all trial types in the 9R+ group. The negative deflection was followed in both groups by a slow fronto-posterior negative wave 600-1000 ms post-cue onset that was larger for repeat than for cue- or task-switch trials. These slow negative waves have been related to improved prediction following repetition (Birbaumer, Elbert, Canavan, & Rockstroh, 1990), and hence they were more enhanced after repetition in the 9R- compared to the 9R+ group (Fig. 4). Larger increase of the slow wave in the 9R- group may thus reflect stronger readiness (Walter et al., 1967) whenever there is a sensory match between new and old auditory stimuli, resulting in a more efficient integration of the meaning of a new sound stimulus into the ongoing task context. Moreover, the late negative deflection, which might reflect an early phase of the slow waves, present larger amplitudes in repeat than switch trials for 9R- as a reflection of more efficient readiness due to the acoustic match between the previous and the current trial. However, a decrease was found in task-switch trials as compared to other trial types, due to the acoustic and semantic mismatch while comparing the current and the preceding trials. In contrast, the late negativity was not modulated in 9R+ individuals by trial type, as they performed task in a context-independent fashion and did not seem to integrate the current cue into the ongoing context. Similar, switch-related late frontal negative deflections have been previously reported in

task-cueing paradigms (Mueller, Swainson, & Jackson, 2007), specifically linked to task switch trials (Brass, Ullsperger, Knoesche, von Cramon, & Phillips, 2005), and also during preparation for a switch in task after a Go response but not following a No-Go event (Astle, Jackson, & Swainson, 2008), perhaps reflecting interference from the previous task-set during motor readjustment (Mueller et al., 2007). These interactions between the trial types and the genotype are reflecting the role of DAT1 polymorphisms on dynamic trial-to-trial adjustments on the cognitive control. The current results indicate that this late negative component of the nP3 is mediated by DA and seems to reflect processes needed to integrate new sensory representations to the ongoing task context.

Conclusions and implications

The current study has revealed that the DAT1 gene plays a crucial role in human differences in cognitive flexibility. Performance in a task-cuing protocol was influenced by the presence of the allele 9R (9R+), leading to similar behavioral and electrophysiological responses to all auditory changes regardless of whether these prompted for a repetition or a change in the task rules. This outcome suggests a context-independent processing of such sensory changes, as supported by the similar negative deflection for all trial types in 9R+ individuals in contrast to the 9R- group showing a modulation by comparing the current trial type with the previous one. Arguably, larger DA display in the striatum, where DAT is mostly expressed (D. A. Lewis et

al., 2001), would allow 9R+ individuals to deploy a stronger activation of the current task-set by avoiding interference from a previous one. In contrast, individuals with a higher gene expression and thus lesser striatum DA availability (9R- group) showed a more context-dependent fashion of cognitive control, sorting cue-switches depending on whether they signaled preparatory control of action within the ongoing task-set or as part of a new task-set.

Even though the current participants were all healthy volunteers, these results can shed light on the understanding of cognitive disorders or pathologies resulting from striatal dysfunction like Parkinson disease. The rigid behavior of these patients, as revealed by a impairments in the WCST and other task-switching analogues (Cools et al., 2001; Cools et al., 2003; Cools et al., 2004; Cools et al., 2006; Cools, 2008; Meiran, Friedman, & Yehene, 2004) have been attributed to a deficit in the flexible use of abstract task rules (Meiran et al., 2004; Yehene, Meiran, & Soroker, 2008). Likewise, the Attention Deficit Hyperactivity Disorder (ADHD) has been related to a poor ability to flexibly adjust behavior to environmental changes (Nigg & Casey, 2005), and has also been associated to the 10-repeat allele (related to poor striatal DA). The pharmacological treatment of ADHD increases DA levels in the striatum in order to improve attentional functions by increasing the signal-to-noise ratio in target neurons (Volkow et al., 2001). The current results provide strong evidence of the role of the 9-repeat allele in the flexible control of human attention, and could improve our understanding of

pharmacological treatment of related disorders or neurological diseases, given individual variability in drug responsiveness as a consequence of the genotype. Furthermore, reported electrophysiological correlates to the switch costs might constitute an endophenotypic marker of such cognitive deficits, and could help to isolate in near future dysfunction in the human striatum dopaminergic system, even when accompanied by very subtle or no behavioral concomitants.

Acknowledgements

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Study VI.

Garcia-Garcia, M., Barceló, F., Clemente, I.C., Escera, C. COMT and DRD2 gene-gene interaction modulates contextual updating of mental representations.*Submitted*

COMT and DRD2 gene-gene interaction modulates contextual updating of mental representations

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Abstract

The differential expression of the dopamine transmitter through its D1 and D2 receptors on the modulation of prefrontal cortex pyramidal neurons has been proposed to account for individual differences in the updating of higher order task representations. Here we examined the interaction between two polymorphic variations of genes involved in the regulation of D1 and D2 activity (Catechol-O-Methyltransferase -COMT- and D2 dopamine receptor -DRD2-) on the neural mechanisms of task-set switching and maintenance. A task-cueing paradigm was employed to measure behavioral costs and a scalp-recorded specific brain potential (novelty-P3) associated to distinct context updating operations in the face of either sensory or task novelty. The interaction between the COMT and DRD2 genes was evidenced by corresponding behavioral costs and novelty-P3 enhancements reflecting task-set updating mechanisms. This effect was found only in individuals combining genes that yielded an optimal balance between D1 and D2 receptors action. Individuals with either sub- or supraoptimal D1/D2 balance showed enhanced novelty-P3 responses to all sensory changes, indicative of contextual task-set updating to sensory cues irrespective of the task context. These results support the epitasis of COMT and DRD2 phenotypes in the flexible control of contextual information in humans.

Introduction

The ability to flexibly adapt to constantly changing environmental demands requires selection and maintenance of appropriate -and suppression of inappropriate- mental representations for goal-directed behavior. This ability is dependent on the dopamine (DA) transmitter system (1-9) through a reciprocal relationship between DA D1 and D2 receptors binding (10,11). Indeed, the activation of D1 receptors in prefrontal cortex (PFC) results in larger stability of mental representations by inhibiting distracters (12), whereas D2 receptor (DRD2) activity at the striatum facilitates the flexible update of the mental set by allowing new motivationally relevant representations (13). This reciprocal relationship might be drawn to an inverted-U model of context updating and prefrontal dopaminergic activity (8,14,15), whereby middling levels of PFC DA activity result in optimal performance, whereas the highest and lowest levels lead to suboptimal efficiency in the updating of working memory contents. Yet, the genetic underpinnings of this hypothetical model have not been established.

Two well-known polymorphic variations of DA-related genes are involved in the regulation of PFC DA concentrations through D1 receptor binding, and DRD2 density, respectively. For the Met158/108Val single nucleotide polymorphism of the Catechol-O-Methyltransferase (COMT) gene (rs#116790;16), a Val to Met substitution is thought to increase the efficiency of the enzyme (17), which in turn inactivates DA diffused out of the synaptic

cleft (18). Val homozygous individuals are thus expected to have decreased synaptic PFC DA levels for D1 receptor binding as compared with Met homozygous individuals, and consequently, they are expected to sustain comparatively less stable task-set representations (18,19). On the other hand, the genetic polymorphism for the DRD2 Taq Ia (rs#126450) is expressed mainly in the human striatum (20). DRD2 A1 allele carriers show a 30-40% reduction in DRD2 density as compared to A2 homozygous individuals (21).

According to the functional significance of these two polymorphisms, individuals homozygous for Met and lacking the A1 allele (i.e., MetA1-) and those homozygous for Val and presenting the A1 allele (i.e., ValA1+) are expected to display a compensated balance of COMT levels and DRD2 density leading to middling levels of PFC DA activity through the interaction of D1 and D2 receptors (22). In contrast, individuals homozygous for Met and presenting the A1allele (i.e., MetA1+) would display supraoptimal PFC DA activity, while those homozygous for Val and A2 (i.e., ValA1-) would be expected to display suboptimal PFC DA activity. As a consequence, the combined effect of COMT and DRD2 genes foresees behavioral benefits in the manipulation of working memory contents (23). However, the combined effect of the COMT and DRD2 genotypes on the behavioral and electrophysiological responses associated with the updating of contextual information has not been addressed so far in humans.

In the present study, we tested the hypothesis that individuals with a putative optimal balance of D1 and D2 receptors stimulation (i.e., MetA1- ValA1+) would show a more efficient updating of task-set information compared to individuals presenting either the lowest or the highest levels (i.e., ValA1-, MetA1+), and that this differentiation should be paralleled by the scalp-recorded novelty-P3 (nP3) response, a neural signature derived from the human electroencephalogram (EEG) associated with context updating operations in the face of both sensory (24-26) and task (27,28) novelty. In order to do so, participants performed a task-cueing protocol inspired by the WCST (29) and adapted for measuring event-related brain potentials (ERPs; 30). This protocol is designed to segregate the behavioral and brain responses to sensory changes from those related to the updating of higher order task-set information in working memory. Three trial types were defined: *repeat* trials, in which both the tonal cue and the task repeated relative to the previous trial; *cue-switch* trials, in which only the cue changed, but the task remained the same as in the previous trial, and *task-switch* trials in which both the cue and task changed.

Results

Performance

Mean accuracy was over 90%. All groups showed a decrease in accuracy following a tonal switch (main effect of Trial type: $F_{2,62}$=33.0, p<0.001), which

was due to a decrease in hit rate in task-switch as compared to cue-switch trials ($F_{1,31}$=41.23, p<0.001). No effect of Group was found for accuracy data. As for response times (RT), a main Trial type effect ($F_{2,62}$=73.9, p<0.001) was due to an increase in mean RTs from repeat to cue-switch trials ($F_{1,31}$=73.5, p<0.001), with no differences between cue-switch and task-switch trials. The four groups did not differ in their mean RTs. However, a Trial type x COMT x DRD2 interaction ($F_{2,62}$=3.6, p=0.036) revealed larger mean RTs in task-switch as compared to cue-switch trials (Trial type x COMT x DRD2: $F_{1,31}$=5.7, p=0.023) apparent only for the ValA1+ ($F_{1,}$7=3.7, p=0.097) and Met A1- groups ($F_{1,8}$=8.7, p=0.018). In contrast, the ValA1- and MetA1+ groups did not show differences in their mean RTs between cue-switch and task-switch trials (Fig. 1).

Novelty-P3 brain response

A specific increase in the amplitude of the fronto-central nP3 positivity to tonal cues (main Trial type effect: $F_{2,62}$=11.9, p<0.001) was observed both in response to cue-switch relative to repeat tones ($F_{1,31}$=7.5, p<0.001), and in response to task-switch relative to cue-switch tones ($F_{1,31}$=6.3, p=0.017). Remarkably, a significant Trial type x COMT x DRD2 three-way interaction ($F_{2,62}$=3.9, p=0.029) revealed larger mean nP3 amplitude to task-switch tones as compared to cue-switch tones (Trial type x COMT x DRD2: $F_{1,31}$=7.5, p=0.010) in ValA1+ ($F_{1,7}$=17.3, p=0.004) and MetA1- individuals ($F_{1,8}$=6.8, p=0.004). In contrast, ValA1- and MetA1+ groups showed similar

mean nP3 amplitudes to cue-switch and task-switch tones, but larger nP3 amplitudes to cue-switch relative to repeat tones (Trial type x COMT x DRD2: $F_{1,31}$=4.8, p=0.036; Fig. 2a,b).

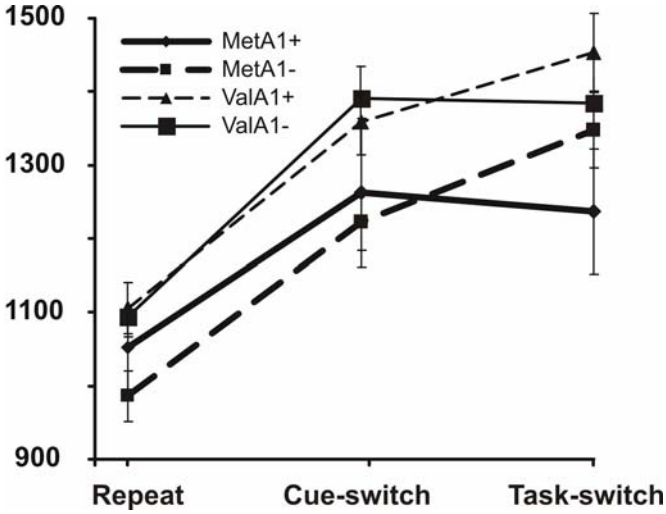

Figure 1. *Response times for all three trial types in the four groups. All groups experienced an increase in RT for cue-switch compared to repeat trials. However, ValA1+ and MetA1- groups showed an increase in task-switch as compared to cue-switch trials, whereas ValA1- and MetA1+ reached already their largest RT in cue-switch trials with no further increase in task-switch trials.*

Discussion

The present study explored the role of a gene-gene interaction related to DA regulation, namely COMT and DRD2, on the neural correlate of the updating of contextual information. Although all groups experienced an increase in mean RT following a sensory change, ValA1+ and MetA1- individuals revealed a task-specific switch cost, showing longer mean RTs when confronted with sensory changes that also demanded a change in higher-

order task representations (Fig. 2c). In contrast, the groups ValA1- and MetA1+ showed similar mean RTs in cue-switch and task-switch trials. Thus, ValA1+ and MetA1- individuals displayed increased amplitudes in the brain sign for updating of contextual information in task switch as compared to cue switch trials, although similar amplitudes were observed in repeat and cue-switch trials. In turn, ValA1- and MetA1+ groups showed increased nP3 amplitudes to cue-switch as compared to repeat trials, with no differences between cue-switch and task-switch trials. These results suggest that individuals with an optimal balance between PFC DA levels and DRD2 density (i.e., ValA1+, MetA1-) show a more efficient updating of contextual information within the ongoing task situation (Fig. 2d).

A crucial role of PFC in cognitive control consists of making the necessary adjustments in attentional bias in the face of ongoing environmental demands (31,32). D1/D2 receptor action is known to regulate the stability and flexibility of mental representations through the modulation of the firing of prefrontal pyramidal neurons (5,12). Hence, we might approach the role of PFC DA on the updating of task-representations by examining the effect of D1/D2 action-regulator genes on the updating of sensory and task information in working memory (33). Interestingly, the observation of maximal behavioral RT costs following any change in acoustic stimulation without any further increase in RT costs for task-switch trials in individuals with either the lowest or the highest PFC DA levels suggest that these groups reconfigured the current task-set following any tonal change and

irrespective of the ongoing task context. Differently, individuals displaying middling levels of PFC DA showed context-dependent behavioral correlates of the control of attention, consisting of sensory-specific RT costs related to the updating of perceptual representations during cue-switching, and also task-specific RT costs related to the updating of higher-order task-set representations (Fig. 2c,d). The epistasis between COMT and DRD2 genes had previously been observed in a stroop task (34) and during the manipulation of working memory contents (23), and hence, an optimal balance of COMT levels and DRD2 density seems to facilitate an efficient context-dependent updating of memory representations. The combined effects of COMT activity and DRD2 density might thus reflect the interaction between a gating mechanism for the updating to new task-set representations, and another mechanism for the online maintenance of task representations promoted by PFC DA concentrations (8,14).

The fronto-posterior nP3 positivity has been previously established as a brain signature of the updating of contextual information in working memory (28,33,35). In the two groups with either the lowest or the highest PFC DA levels, the amplitude of this brain nP3 potential was increased in cue-switch trials compared to repeat trials, although it did not differ between cue- and task-switch trials, in parallel with the observed behavioral switch costs. This electrophysiological pattern suggests that individuals with extreme PFC DA levels process every sensory change regardless of its significance for switching or repeating the previous task-set. Accordingly, these subjects

seemed to reconfigure the higher-order task-set representation after any cue switch, and irrespective of its task relevance. Several studies have reported a similar association between suboptimal and supraoptimal PFC DA levels, and an inefficient updating of contextual information (15,23,36), which could indicate a lesser capacity for integrating sensory changes into the ongoing task context. Reversely, individuals with genetically based middling levels of PFC DA activity were more efficient in integrating new sensory information into a constantly changing task context. Accordingly, they showed similar nP3 amplitudes for repeat and cue-switch trials. In turn, brain nP3 potentials were enhanced in task-switch trials, indicating task-specific context-dependent updating of task-set information in individuals with middling PFC DA levels (Fig. 2c,d).

The current results were obtained from healthy volunteers without any clinical or subclinical symptoms, and consequently, behavioral accuracy and mean RTs did not differ across groups. However, the different patterns of behavioral performance shown by individuals with diverse genetic background for the corticostriatal DA system shed light upon the bases of individual differences in cognitive control, and may help us to understand cognitive deficits associated with DA dysregulation in prefrontostriatal pathways. Indeed, the dysfunctional behavior observed in patients with Parkinson's disease, as revealed by impairments in the WCST and other task-switching analogues (6-9,37,38), have been attributed to a deficit in the flexible use of abstract rules (37,39). Likewise, the attention deficit and

hyperactivity disorder (ADHD) has been related to a poor ability to flexibly adjust behavior to environmental changes (40), and has been treated pharmacologically by increasing DRD2 binding in order to improve attentional functions (41).

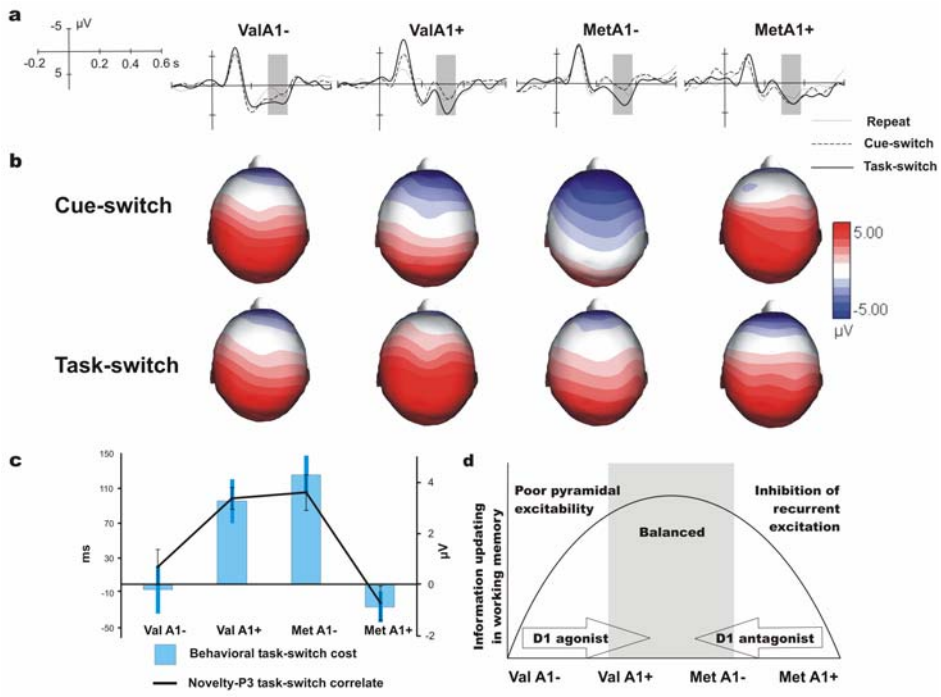

Figure 2. *a) ERPs for all three trial types in the four groups. Larger amplitudes of the nP3 (shadowed) were observed in cue-switch relative to repeat trials in ValA1- and MetA1+ groups but similar amplitude for cue-switch and task-switch trials. In contrast, ValA1+ and MetA1- displayed similar amplitudes for repeat and cue-switch trials, but larger amplitudes in task-switch compared to cue switch trials. b) Scalp distributions of the fronto-parietal positive response of nP3 for cue-switch trials and task-switch trials for all four groups. ValA1- and MetA1+ display a parietally distributed increase of the brain response in task-switch relative to cue-switch trials. c) Behavioral and electrophysiological costs in task-switch compared to cue-switch. The bars show RT in task-switch trials minus RT in cue-switch trials for all four groups. The lines plot the mean amplitude of the nP3 at Cz channel for task-switch trials minus amplitudes in cue-switch trials for all four groups. Notice that the behavioral RT costs displayed by the ValA1+ and MetA1- groups are paralleled by the modulation of the nP3; in contrast, no behavioral RT costs for task-set reconfiguration was observed in ValA1- and MetA1+ groups, as well as no modulation of the nP3 became evident. d) Inverted-U model of PFC DA activity. The four groups of the study combining polymorphisms for the COMT and DRD2 are disposed along the X-axis according to the levels of PFC DA activity reached through D1/D2 receptors binding. The multifaceted role of DA activity account to the efficient manipulation of information in working memory through an inverted-U function, whereby middling levels of prefrontal DA (shadowed) results in optimal performance, whereas highest and lowest PFC DA concentrations leads to suboptimal manipulation of information in working memory.*

Remarkably, the results obtained in the present study and summarized in the Figure 2c fit the inverted-U model of PFC DA activity and efficient updating of contextual information.

The present cued task-switching paradigm (42) has proved highly sensitive to cognitive control processes, such as online maintenance and updating of goal-representations facilitated by D1 and D2 receptors binding. Moreover, the current results support the hypothesis of an inverted-U function between PFC DA activity, task-switch costs and its neurophysiological correlates. The current results also provide evidence for the combined role of COMT and DRD2 phenotypes in the flexible control of human attention, and they could help improving our understanding of the pharmacological treatment of attentional disorders and related neurological diseases, given the individual variability in drug responsiveness as a consequence of the genotype. Furthermore, our results suggest that a well-known brain signature of contextual information processing (28) may serve as a trustable endophenotype for the functional activation of the corticostriatal DA system. The task-specific stereotypy disclosed here for this brain response makes it a good candidate to bridge the gap between genetics and behavior.

Materials and methods

Participants

Forty individuals (six men, two left-handed, mean ± SD age; 22 ± 4.2 years, range 18-29 years) participated in the study. They were recruited from a wider sample of volunteers after interview through to an adapted version of the Clinical Interview of the Diagnostic and Statistical Manual (DSM IV-R), for exclusion of subjects with neurological and psychiatric illness, phobias, and drug consumption. All participants gave informed consent at each phase of the experimental procedure (interview, buccal cells extraction and electroencephalographic -EEG- recordings) according to the Declaration of Helsinki and the Ethic Committee of the University of Barcelóna. All subjects had normal or corrected-to-normal vision and normal audition. After exclusion by diagnostic criteria and following analyses of the COMT and DRD2 polymorphisms, participants homozygous for the COMT gene (Met/Met, Val/Val), and those presenting the most frequent alleles for DRD2 (A1, A2) were selected for an EEG recording session. Participants genotyped as Met/Met were assigned to the MetA1+ group when they presented the A1 allele (A1/A1, or A1/A2) and to the MetA1- group when they were homozygous for the A2 allele of the DRD2 gene. Participants genotyped as Val/Val were assigned to the ValA1+ group when they presented the A1 allele (A1/A1, or A1/A2) and to the ValA1- group when they were homozygous for the A2 allele for the DRD2 gene. Five participants

were excluded from the analyses due to excessive artifacts in their EEG recordings. From the remaining 35 individuals, six composed the MetA1+ group, nine the MetA1- group, eight the ValA1+ group, and twelve were included in the ValA1- group. Participants from each of the genetic groups did not differ significantly in age, state or trait anxiety scores (STAI, 43).

DNA isolation and genotyping

DNA was collected with cheek cell swabs and extracted using the Epicentres® BuccalAmp™ DNA Extraction Kit (Epicentre, Madison, WS). Upon isolation of DNA, both single nucleotic polymorphisms (SNP) for the COMT Met108/158Val and DRD2 Taq IA were genotyping were performed by real time PCR using fluorescence detection technique by means of the Applied Biosystems TaqMan technology (Applied Biosystems, Foster City, CA, USA).

Procedure

A task-cueing protocol inspired by the WCST (29) and adapted for measuring event-related brain potentials (ERPs; 30) was administered to participants. Each trial consisted of a tonal cue followed by a target display with four key cards on top of one choice card, all centered on a computer screen, and subtending a visual angle of 4º horizontally and 3.5º vertically. Subjects were instructed to match the choice card with one of the four key

cards following two possible task rules (color or shape). Before target onset, one out of four tonal cues explicitly informed the subject whether to sort the card according to either the 'color' (500/1000 Hz) or 'shape' (2000/4000 Hz) rules. Binaural tones were delivered through Sehnheiser® HD202 headphones with a duration of 200 ms, 10 ms rise/fall times and 65 dB SPL. The meaning of the tonal cues was reversed for half of the subjects. All stimuli were presented with the stimulation program Presentation® (Neurobehavioral Systems Inc., Albany, CA). Three trial types were defined. In the *repeat* trials, both the tonal cue and the task were repeated relative to the previous trial. In the *cue-switch* trials, only the cue changed but the task remained the same as in the previous trial. In the *task-switch* trials both cue and task changed. Responses were made using 4 keys on a keyboard, mapped onto the four fingers of the dominant hand, in an array corresponding to the layout of the four key-cards. All three trial types were randomly presented with the same overall probability along the 200 trials of the experimental block, as well as during the 50 practice trials. The cues related to each criterion were employed five times during the instruction period of the practice block to ensure that each participant had correctly learnt the cue-task association. The cue-target interval (CTI) randomly varied between 650 ±150 ms, thus minimizing the effects of a constant preparation interval (44), and the target remained on the screen until a response was given (up to a maximal of 3000 ms). Response-cue intervals (RCIs) also varied randomly around 1100 ±100 ms within the trial block.

EEG Data Acquisition

Electroencephalographic activity was recorded (ANT Software b.v., Enschede, The Netherlands) during task performance from 64 scalp electrodes following the extended 10/10 convention in an electrically and acoustically shielded room. Horizontal and vertical electro-oculographic (EOG) recordings were obtained with electrodes placed at the outer cantus of the right eye and above the right eye. The common reference electrode was placed on the tip of the nose, and the ground was located at the chest. The EEG was amplified and digitized at a sampling rate of 512 Hz. Impedances were kept below 10 kΩ during the whole experimental recording session, which lasted about 25 minutes.

Data processing

ERPs were averaged offline for each trial type (repeat, cue-switch and task-switch), for an epoch of 800 ms including a pre-stimulus baseline of 200 ms. The first five trials of the block were excluded from analysis. Frequencies above 30 Hz were digitally filtered out from individual EEG epochs prior to ERP averaging. EOG correction was performed via a blind source separation technique with ASA 4.5 of ANT® Software (Enschede, The Netherlands), as described in Belouchrani et al. (45). After EOG correction, any epochs containing EEG activity exceeding ± 100 μV peak-to-peak amplitudes were rejected from further analysis. The mean percentages of

clean EEG epochs retained for ERP averages were 74.4%, 75.1% and 72.7% epochs from the repeat, cue-switch and task-switch conditions, respectively, which did not differ between any of the trial types.

Data analysis

For behavioral analysis, any correct button press within 200-3000 ms after target onset was regarded as a hit, and the mean RT was computed for hit trials only. Hit rate and mean RT were submitted to a two-way mixed ANOVA with one repeated-measures factor (Trial type: repeat, cue-switch, task-switch), and two between-subject factor (COMT: Val and Met; DRD2: A1+ and A1-). Pair-wise post hoc comparisons were performed to examine any significant difference between conditions.

For the analysis of brain responses, the mean amplitudes of the fronto-central positive component of the nP3 was computed in the latency window from 300 to 340 ms, measured at channels F3, F4, Fz, C3, C4, Cz, P3, P4 and Pz. A three-factor repeated-measures ANOVA was performed on all these ERP measures including three within-subjects factors: Trial type (repeat, cue-switch and task-switch), Frontality (three levels for frontal, central and parietal channels) and Laterality (three levels for the left, middle and right channels), as well as the two between-subject factors COMT (Met and Val) and DRD2 (A1+ and A1-). Pair-wise post hoc comparisons were performed between all trial types to examine whether any specific effect was

due to the updating of either sensory or task representations during cue-switching or task-switching, respectively. The Greenhouse-Geisser correction was applied to the degrees of freedom of the ANOVAs, and the corrected P-values were reported whenever was appropriate. Target-locked ERPs will not be reported here as they did not account for any group-related behavioral effects nor interactions in the present study.

Acknowledgements

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5. General discussion

The present studies aimed at revealing the role of the polymorphic variability associated to the genes for the COMT, DAT and DRD2 in operations of updating of sensory and task novel contextual information.

First of all, we confirmed that GBRs are modulated by attentional processes (Herrmann & Mecklinger, 2000; Herrmann & Knight, 2001; Tiitinen et al., 1993) through an increase of the PS of GBRs after NOV relative to STD sounds with a clear mid-central scalp distribution for oscillations around 35 Hz, and an anterior distribution at around 40 Hz. The results observed in studies I and III confirm that the PS of auditory gamma oscillations reflect an enhancement of attentional resources re-allocated for processing novel events (Tiitinen et al., 1993; Yago et al., 2003; Yordanova et al., 1997; Yordanova et al., 2000; Yordanova et al., 2001), as supported by the behavioral results. Moreover, we have shown the PS of GRBs is sensitive to the modulation of novelty processing by a negative emotional context (Dominguez-Borras et al., 2008; Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Garcia-Garcia et al., 2008), through larger increase of the PS of GBRs in a NEG as compared to a NEU context over mid-line scalp distributions.

The amount of DAT regulated the mechanisms involved in the cognitive processes of updating contextual information, as it was elucidated on studies

III-V. Studies III and V revealed that individuals presumed to display lesser amounts of DAT (Fuke et al., 2001; Heinz et al., 2000; Mill et al., 2002; VanNess et al., 2005), and thus larger amounts of DA in the striatum, had a substantially larger nP3 response to all auditory stimuli. The larger nP3 amplitudes seem to indicate that higher DA display allows 9R+ individuals to deploy a stronger activation to auditory stimuli, irrespective of the condition as a general mechanism of activation of the representation necessary for the subsequent motor response (Barceló et al., 2007). Accordingly, a neuroimaging study revealed that human striatum reflects the level of saliency associated to a stimulus, providing a signal to reallocate limited resources to important events (Zink et al., 2006), presumably reflected by the nP3. Moreover, similar DA-regulated frontocentral positive brain responses (i.e., P2a; Potts, Martin, Burton, & Montague, 2006) has been proposed to reflect striatum DA signaling which implements a 'gating' mechanism controlling the access of information to neural systems for cognitive control (Montague, Hyman, & Cohen, 2004; O'Reilly, Braver, & Cohen, 1999; Potts et al., 2006).

Therefore, the larger DA tone in 9R+ individuals seems to illustrate a ceiling effect due to the stronger activation, which impeded an enhancement of the nP3 brain response by a NEG emotional context in study III. However, study III showed increased behavioral distraction in a NEG as compared to a NEU context indicating stronger processing of unexpected novel events in a threatening situation (Dominguez-Borras et al., 2008; Dominguez-Borras,

Garcia-Garcia, & Escera, 2008a; Garcia-Garcia et al., 2008). We, therefore, speculate that they would show an increase of nP3 amplitudes in NEG relative to NEU context in absence of the reported ceiling effect. However, 9R- individuals did display larger nP3 in NEG relative to NEU context in absence of the expected behavioral concomitants. This absence of increased distraction in NEG contexts might reflect diminished reactivity to distraction in threatening situation, similar to the blunted reactivity found in patients with midbrain DA dysfunction (Bowers et al., 2006) during unpleasant events.

Interestingly, studies IV and V revealed that DAT regulates the mechanisms of cognitive control during a task-cueing protocol. Behavioral and electrophysiological concomitants of the cognitive control of attention indicated that 9R+ individuals reconfigured the stimulus-response map after any switch in acoustic stimulation, as displayed by similar RTs after a tonal switch indicating either task-novelty or task repetition. However, the RT increase after a tonal switch relative to a tonal repetition (i.e., a distraction effect; Escera et al., 1998; Escera et al., 2000; Escera & Corral, 2007) was larger in 9R+ than in 9R-, probably reflecting a stronger protection against interference of the previous contextual information (Cools et al., 2001). Differently, 9R- individuals seemed to invest their more limited resources (i.e., striatal DA) in a more efficient way by reconfiguring the stimulus-response map only when required, as shown by the RT increase in task-switch relative to cue-switch trials (Karayanidis et al., 2003). Moreover, a

dissociation was observed in the late brain responses (i.e., the late negative deflection reflecting interference from the previous task-set during motor readjustment -Mueller, Swainson, & Jackson, 2007- and the late slow waves) of a tonal switch indicating task-novelty from a tonal switch indicating task-repetition in the 9R- but not in 9R+ group. This different stereotipy of the late brain responses support the notion of a context-dependent processing of sensory changes in 9R- individuals, but a context-independent one in 9R+ individuals.

However, study IV revealed that 9R+ individuals showed a very early dissociation (i.e., frontocentral N1 component) of task-novelty or repetition. It seems plausible that PFC-striatum dopaminergic pathways offer a potential circuit for the rapid detection of unexpected and potentially relevant sensory signals, as enough information can be conveyed through this route to detect a mismatch between sensory input and active PFC representations (Barceló et al., 2007; Redgrave & Gurney, 2006). Paradoxically, 9R+ individuals do not take behavioral advantage of the early detection of task-novelty.

Furthermore, a very interesting epistatic interaction was found in the polymorphic variations of the genes of the COMT and the DRD2 for cognitive processes of updating contextual information. Interestingly, parallel results were found for the two different paradigms we used in studies II and VI. This parallelism revealed that the PFC-striatum dopaminergic system is involved in the common neural network shared in the cognitive processes of

updating contextual information by processing task-irrelevant novel events, or by processing task-relevant sensory changes signaling a reconfiguration of the stimulus-response map (Barceló et al., 2006). The interaction of the two polymorphisms for COMT and DRD2, according to the functional relationship of D1 and D2 receptors action, had been hypothesized to display different levels of PFC DA activity. Hence, Individuals homozygous for Met and lacking the A1 allele (i.e., MetA1-), and those homozygous for Val and presenting the A1 allele (i.e., ValA1+) are expected to display a compensated balance of COMT levels and DRD2 density leading to middling levels of PFC DA. In contrast, individuals homozygous for Met and presenting the A1allele (i.e., MetA1+) would display highest PFC DA activity, while those homozygous for Val and A2 (i.e., ValA1-) would be expected to display suboptimal PFC DA activity.

ValA1- and MetA1+ groups were not distracted by novel events in an auditory-visual distraction paradigm and did not show a RT delay for sensory changes indicating task-novelty relating to those signaling a repetition. Differently, ValA+ and MetA1- groups, associated to middling levels of PFC DA activity, displayed the reported distraction by novel events compared to STD sounds (Escera et al., 1998; Escera et al., 2001; Escera & Corral, 2007), and paralelly, displayed reported RT task-switch costs when a task-set reconfiguration was required (Karayanidis et al., 2003). The reciprocal interaction between D1 and D2 receptors activity, known to regulate the stability and flexibility of mental representations through the modulation of

the firing of prefrontal pyramidal neurons (Durstewitz et al., 2000; Sawaguchi & Goldman-Rakic, 1994), might account for this epistasis between COMT and DRD2 polymorphisms. Therefore, the combined effects of COMT activity and DRD2 density might reflect the interaction between a gating mechanism allowing the updating to new mental representations and the maintenance of those representations promoted by PFC DA concentrations (Arnsten, 1998; Cools et al., 2004).

However, different electrophysiological concomitants of the cognitive processes of interest were correlated to the behavioral results. While the amplitudes of the nP3 paralleled the RT task-switch costs during task-set reconfiguration, the PS of neural oscillatory activity around 40 Hz was corresponded with the behavioral distraction effect along the groups. The differences found in the processing of task-irrelevant novel events according to different levels of PFC DA activity seem to be reflected at faster frequencies of the EEG, than those found in the processing of task-relevant sensory changes.

In the distraction paradigm, enhanced amplitudes of oscillatory activity at 40 Hz and 100 ms post-stimulus in NOV as compared to STD in all four groups suggest increased attention directed to novel events as compared to standard ones (Busch et al., 2006; Debener et al., 2003; Herrmann et al., 2004; Tiitinen et al., 1993; Yordanova et al., 1997; Yordanova et al., 2000; Yordanova et al., 2001). Involuntary attention capture is an ecologically

critical mechanism for detecting potentially important stimuli in the environment (Escera et al., 2003) and it is, hence, adaptive that all four groups increase the attentional resources for potentially relevant events. However, high temporal preciseness of particular neural firing patterns reflected by the phase of brain oscillations (Buzsaki & Draguhn, 2004). suggests a relevant regulation of phase resetting of neural firing (Klimesch, Sauseng, & Hanslmayr, 2007) during stimulus-driven control of attention in ValA1- and MetA1+ groups that might account for the behavioral benefit showed by the absence of distraction in comparison with the ValA1+ and MetA1- groups.

Moreover, ValA1- and MetA1+ groups showed increased nP3 amplitudes in cue-switch trials compared to repeat trials, although similar ones between cue- and task-switch trials, paralleling the behavioral switch costs. This electrophysiological pattern suggests these individuals reset and reconfigure the higher-order task-set representation after any cue switch irrespective of its task relevance. Reversely, ValA1+ and MetA1- individuals with genetically based middling levels of PFC DA activity seemed to be more efficient in integrating new sensory information into a constantly changing task context, as they showed similar nP3 amplitudes for repeat and cue-switch trials, but increased brain nP3 potentials in task-switch trials.

Similar COMT/DRD2 interaction have shown better manipulation of contents in working memory for ValA1- and MetA1+ individuals (Gosso et al., 2008;

Stelzel et al., 2009), supporting the model claiming that working memory performance needs an optimal level of DA signaling in the PFC, which depend on enzymatic activity controlling DA levels as well as DA receptor sensitivity.

DAT expression is not limited to subcortical regions, but it is also involved in the extrasynaptic regulation of DA diffusion in the cortex (Cragg & Rice, 2004), although much less abundant (D. A. Lewis et al., 2001). Therefore, in order to infer the interaction between PFC and striatum DA activity, relevant in the control attention (Cools, 2008), we preferred to focus on the epistasis between the COMT (enzyme which degrades intracellular postsynaptic DA in cortical regions; Sesack et al., 1998), and the DRD2 (mostly expressed in the human striatum; Camps et al., 1989) for a more accurate analysis.

Implications

The findings reported in this PhD thesis work were obtained from healthy volunteers without any clinical or subclinical symptoms, and hence, behavioral accuracy and mean RTs did not differ across groups. However, the genetic base of the diverse patterns of brain and behavioral responses shown during stimulus-driven control of attention in the present research work partially fulfill the incognita of the huge individual variability in cognitive processes. Moreover, these outcomes may help us to understand disruption in attentional control associated to DA dysregulation in prefrontostriatal

pathways. Indeed, the dysfunctional behavior observed in patients with Parkinson's disease shown by impairments in the WCST and other task-switching analogues (Cools et al., 2001; Cools et al., 2003; Cools et al., 2004; Cools et al., 2006; Cools, 2008; Meiran et al., 2004) have been attributed to a deficit in the flexible use of abstract rules (Meiran et al., 2004; Yehene et al., 2008). Likewise, the ADHD has been related to a poor ability to flexibly adjust behavior to environmental changes (Nigg & Casey, 2005), and has been pharmacologically treated by promting DRD2 binding in order to improve attentional functions (Volkow et al., 2001) to increase the signal-to-noise ratio in target neurons (Volkow et al., 2001). Moreover, the current findings may help to clarify deficits in emotional processing observed in patients with a dysfunction in the DA system (i.e., Parkinson 's disease; (Lawrence, Goerendt, & Brooks, 2007; Troisi et al., 2002). These results may also improve our understanding of pharmacological treatment of related disorders or neurological diseases, given individual variability in drug responsiveness because of the genotype.

Furthermore, the paradigms used in these studies have shown a high sensitivity to cognitive control processes such as sustained information and updating of goal-representations facilitated by D1 and D2 receptors binding. Moreover, the current results support the hypothesis of an inverted-U function of PFC DA activity and behavioral and neurophysiological correlates of operations of updating contextual information. Furthermore, the well-known signature of contextual information nP3 (Barceló et al., 2006) may act

as a trustable endophenotype for the corticostriatal DA system, as the stereotypy of this brain response seem to dissociate DA related genotypes resulting as a good link in the gap between the gene and behavior. All reported electrophysiological correlates might constitute reliable endophenotypic markers of cognitive deficits, and could help to isolate in near future dysfunction in the human corticostriatal dopaminergic system, even when accompanied by very subtle or no behavioral concomitants.

6. Conclusions

The general aim of the present research work was to investigate the role of three DA-related polymorphic variations on updating contextual information, such as attentional control during task switching and processing of novel events. The results clearly show a strong involvement of DA-related genes in the control of attention for updating contextual information. The expression of the DAT regulated the strength activation of mental representations, as well as the rapid detection of task-novelty. It played a relevant role in mechanisms of novelty processing during a negative emotional context and partially determined the dependency of the context while evaluating a sensory change. Differently, the epistasis between COMT and DRD2 fitted the inverted-U model of updating contextual information, and regulated behavioral and neurophysiologichal mechanisms of distraction and novelty processing, as well as task-switch costs and the subsequent reconfiguration

of mental representations. According to the specific goals of the thesis, the conclusions are as follow:

In study I, we aimed at tapping the brain mechanisms supporting enhanced orienting of attention to novel auditory events under emotional threat. We found that auditory involuntary attention was reflected by an increase of gamma-band PS. More important, we showed that a negative emotional context tunes novelty processing by means of the PS of brain activity in the gamma frequency band around 40 Hz in specific neural networks.

In study II, we explored the role of gene-gene interaction between COMT and DRD2 polymorphisms on stimulus–driven control of attention, such as processing novel environmental events. The outcome established an inverted-U function of PFC DA activity and behavioral distraction by a novel event, by which middling levels of PFC DA activity account for shifting of attention towards novel events to be accompanied by a delay in RT to the task. Groups with either the lowest or the highest PFC DA activity performed the task with no delay in RT in NOV as compared to STD, presumably through a neural mechanism of time-resetting of neural firing for compensating gene-based suboptimal neurochemical patterns for context-updating.

In study III, we aimed at revealing the role of DAT genotype on the modulation of novelty processing by an emotional context. A lack of

enhancement of distraction by the emotional context in 9R- individuals was compensated by an increase of the early nP3 in the negative context revealing that processing of unexpected auditory novel events was enhanced in 9R- individuals as well. Differently, larger striatal DA display in 9R+ individuals led them to strongest activation of auditory novel events reaching largest early nP3 amplitude after any novel sound, with no further increase during a threatening situation.

In study IV, we intended to reveal the role of the DAT1 polymorphism on the rapid detection of task-relevant sensory changes. The findings suggested that 9R+ individuals detect the need of task-set reconfiguration at an earlier stage than 9R- individuals did. We provided evidence of the role of DAT1 genotype in the adaptive mechanism of rapid detection of task-relevance in sensory changes.

In study V, we investigated the role of the DAT in the individual brain response stereotypy underlying cognitive flexibility. 9R- individuals showed specific RT increases and brain response modulations for sensory change and task-set reconfiguration suggesting that task performance depended on immediate local context. 9R+ individuals showed larger inhibition of any previous interference as well as stronger activation of the current task set.

In study VI, we examined the role of interaction between two polymorphic variations of genes (COMT and DRD2) involved in the regulation of D1 and D2 activity on the neural mechanisms of task-set switching and maintenance. The interaction between the COMT and DRD2 genes was evidenced by a corresponding behavioral costs and novelty-P3 enhancements following a task-switch found only in individuals combining genes for a putative optimal balance between D1 and D2 receptors action. Individuals with a different combination of polymorphisms showed, however, such enhancement after any sensory change, thus resetting and reconfiguring task-set representations after any cue switch irrespective of the preceding context.

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ANNEX 1. CATALAN SUMMARY

El nostres gens tenen un paper important en la manera que enfrontem els canvis de l'ambient i adaptem la nostra conducta adequadament. El present treball de recerca se centra en el paper de tres gens relacionats amb la dopamina (DA) sobre processos cognitius, com el canvi de l'atenció quan és requerit per les demandes ambientals o el processament d'esdeveniments inesperats però potencialment rellevants. L'activitat dopaminèrgica al còrtex prefrontal (PFC) i a l'estriat semblen tenir papers diferents en el processament atencional i interaccionen per a regular l'estabilitat i flexibilitat de l'actualització de la informació contextual. Per això, vam estudiar l'acció de gens que regulaven l'acció de la dopamina del PFC (i.e. Catechol-O-Methyltransferase; COMT), la resposta de dopamina difosa en l'espai extrasinàptic estriatal (i.e. Transportador de Dopamina; DAT) i la concentració de receptors de dopamina de tipus D2 (i.e. receptors D2 de dopamina; DRD2).

Els participants van realitzar dues versions diferents d'un paradigma de distracció auditiu-visual, en el qual se'ls demanava que ignoressin tons estàndards freqüents i sons ambientals nous rars que precedien els objectius pertinents de la tasca. En dos estudis, vam manipular l'efecte d'un context emocional sobre el processament d'esdeveniments nous inesperats, donada la potencial rellevància d'un esdeveniment nou durant una situació d'amenaça en la qual pot ser nociu. En tres estudis, els participants amb

diferents al·lels o combinacions d'al·lels van realitzar un paradigma de commutació de tasca, en el qual l'actualització d'informació sensorial i de tasca es podria dissociar. Al llarg dels sis estudis, es van emprar mesures conductuals i electrofisiològiques enregistrades al cuir cabellut, com les anàlisis de l'electroencefalograma (EEG) al domini del temps promitjant els potencials cerebrals relacionats a esdeveniments (ERP), i l'activitat oscil·latòria cerebral al domini temps- freqüència.

Tres estudis van mostrar el paper del gen del DAT en el control cognitiu de l'atenció, suggerint així la pertinència de la DA estriatal en la flexibilitat cognitiva. Els nostres resultats suggereixen un processament independent del context dels canvis sensorials per la reconfiguració del set de la tasca en individus amb l'al·lel de 9 repeticions de DAT (9R+), relacionats amb una major disponibilitat de dopamina estriatal. Tanmateix, aquests individus van mostrar una detecció primerenca de la rellevància per la tasca dels canvis sensorials. El gen del DAT regulava la modulació del processament de la novetat per un context emocional. Els individus sense l'al·lel de 9 repeticions (9 R-), i així menor disponibilitat de dopamina estriatal, van mostrar una resposta cerebral augmentada als esdeveniments nous en un context amenaçador però cap modulació conductual. Per contra, els individus 9R+ van mostrar un realçament conductual de distracció en una situació amenaçadora però una resposta cerebral més gran a la novetat en una situació neutra sense un major augment en una de negativa.

Dos estudis van mostrar el paper de la interacció dels gens de la COMT i del DRD2 sobre processos atencionals. S'ha suggerit que els individus amb l'al·lel de COMT Val i l'al·lel de DRD2 A1 (A1+) i COMT Met sense l'al·lel de DRD2 A1 (A1-) mostren una interacció equilibrada de dopamina prefrontal i estriatal. Aquests grups van mostrar distracció conductual, mentre que els individus ValA1- i els individus MetA1+ no van ser distrets per sons nous en un paradigma de distracció visual auditiu. Tanmateix, els grups no-distrets resultaven processar esdeveniments nous a través de la restauració d'activitat neuronal a 40 Hz. A més, aquells amb una interacció equilibrada semblaven tornar a configurar la informació de la tasca quan era necessari, mentre que aquells amb dopamina PFC o estriatal extremes tornaven a configurar el set de la tasca després de cada canvi sensorial.

Els resultats dels estudis de la tesi proporcionen una evidència del paper rellevant dels gens de la COMT, el DAT1 i el DRD2 en processos cognitius, i ajuden a entendre els dèficits cognitius associats a la disregulació de la dopamina en trastorns psiquiàtrics.

Introducció

L'ocurrència d'un estímul nou inesperat durant la realització d'una tasca provoca distracció, com mostren els temps de resposta (RT) augmentats després d'estímuls nous en comparació amb esdeveniments estàndards en "paradigmes de distracció" auditius visuals (Escera, Alho, Winkler, &

Näätänen, 1998; Escera, Alho, Schroger, & Winkler, 2000; Escera, Yago, Corral, Corbera, & Nunez, 2003; Escera & Corral, 2007). Sobre els mecanismes que controlen l'atenció, hi han evidències recents que suggereixen una interacció constant entre control atencional exogen per esdeveniments ambientals nous i control cognitiu endogen (Pashler, Johnston, & Ruthruff, 2001).

Per exemple, resultats electrofisiològics van mostrar un realçament del processament de novetat quan els esdeveniments nous es processaven durant la realització d'una tasca que implicava fotografies emocionalment negatives (Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Dominguez-Borras, Garcia-Garcia, & Escera, 2008b; Garcia-Garcia, Dominguez-Borras, SanMiguel, & Escera, 2008). Estímuls nous inesperats en una situació emocionalment negativa, com una amenaçant, adquireixen una importància vital perquè són potencialment nocius, per tant, un processament més fort es torna crucial i té un valor adaptatiu obvi.

Els individus normalment han de prestar atenció seqüencialment a molts estímuls diferents en resposta a l'ambient que canvia, exigint una adaptació flexible a tasques cognitives noves. Aquesta adaptació implica una reconfiguració apropiada del set mental per donar una resposta adequada a la tasca nova. Els paradigmes de commutació de tasca s'utilitzen per a investigar mecanismes de reconfiguració del set de la tasca. En paradigmes de commutació de tasca, cada tasca exigeix atenció

a, i classificació d'un atribut diferent de l'estímul, o recuperació a la memòria de treball d'una propietat diferent de l'estímul. El cost de canvi és conductualment reflectit per una disminució en el rendiment, evidenciada per un augment en RT i l'índex d'error (Karayanidis, Coltheart, Michie, & Murphy, 2003; Rogers & Monsell, 1995) Un paradigma de commutació de tasca inspirat en la prova clàssica de PFC, la Wisconsin Card Sorting Test (Rubinstein, Meyer, & Evans, 2001), i implementat per a proporcionar una eina fiable per estudiar la dinàmica d'ERP subjacent al control executiu d'atenció i, en particular, les operacions cognitives d'actualització del set de tasca en memòria de treball (Barceló, Muñoz-Cespedes, Pozo, & Rubia, 2000;Barceló, Periañez, & Knight, 2002) va trobar que un estímul auditiu dirigint un canvi en el set mental a una tasca nova provocava un potencial cerebral distribuït fronto-posteriorment que s'assemblava a les respostes cerebrals a distractors nous en paradigmes de distracció auditiu-visuals (Escera et al., 1998; Escera et al., 2000; Escera & Corral, 2007) suggerint que el processament de novetat sensorial i de tasca podria compartir una xarxa neuronal comuna (Barceló, Escera, Corral, & Perianez, 2006)

La implicació del sistema de la dopamina (DA) en el control de l'atenció ha estat àmpliament evidenciat en les darreres dècades (Kaplan & Oudeyer, 2007; D. A. Lewis et al., 2001; S. J. Lewis, Dove, Robbins, Barker, & Owen, 2003; Sawaguchi & Goldman-Rakic, 1994) Per exemple, els estudis han informat que la DA és crucial en el control d'atenció quan és dirigida

per un estímul exogen (Cools, Barker, Sahakian, & Robbins, 2001; Cools, Barker, Sahakian, & Robbins, 2003; Cools, 2008), així com quan és regulada pels sistemes de recompenses (Montague & Berns, 2002; Schultz, 2002; Stefani & Moghaddam, 2006). Hi ha una relació recíproca entre els receptors D1 i D2 de la DA (Akil et al., 2003; Meyer-Lindenberg et al., 2002). Mentre que es creu que l'estimulació de receptors D1 en el PFC promou l'estabilitat de representacions per resistència a la distracció (Durstewitz, Seamans, & Sejnowski, 2000), l'estimulació de receptors D2 es relaciona amb la facilitació de representacions flexibles permetent l'actualització de representacions (Frank, 2005). El paper polifacètic de l'activitat de la DA en el PFC regulada per les accions dels receptors D1/D2 explica l'actualització eficaç de representacions en memòria de treball mitjançant una funció d'U invertida, on nivells mitjans de DA prefrontal resulten en un rendiment òptim (i.e., tasca de N-back; Callicott et al., 1999), mentre que concentracions de DA al PFC més altes i més baixes porten a una actualització subòptima d'informació en memòria de treball (Arnsten, 1998; Cools, Clark, & Robbins, 2004; Williams & Castner, 2006). Per això, l'equilibri entre accions de D1/D2 podria estar implicat en control, com el processament d'esdeveniments nous ambientals (Birkas et al., 2006), o l'actualització del context de tasca.

Així, les diferències individuals en el control de l'atenció, i en l'atenció involuntària poden ser determinades per la possibilitat de senyalització de DA als receptors D1 i D2. Això és regulat per uns quants polimorfismes

funcionals genètics que s'expressen en llocs diferents del sistema dopaminèrgic de cervell.

La DA difosa fora de l'espai sinàptic i lligada als receptors de D1 en PFC és inactivada principalment per la Catechol-O-Methyltransferasa (COMT) (Bilder, Volavka, Lachman, & Grace, 2004). S'ha descrit un polimorfisme funcional per al gen que sintetitza COMT (rs#116790; Lachman et al., 1996), resultant d'una substitució de Val a Met al locus de 108/158 en la seqüència de pèptids. La substitució d'al·lels Val augmenta l'eficiència de l'enzim en comparació amb l'al·lel Met (Mannisto & Kaakkola, 1999). Així, als individus Val/Val s'espera que presentin nivells disminuït de DA sinàptic al PFC, mentre als individus Met s'espera que tinguin alt un nivell DA al PFC. L'al·lel Val (activitat alta) dona lloc a una menor habilitat per mantenir informació en memòria de treball (Bilder et al., 2004), però major habilitat per actualitzar el seu contingut amb informació nova (Cools et al., 2004). Per l'al·lel Met, relacionat amb nivells de DA elevats al PFC i més estimulació de receptors D1, a canvi, es pronostica millor rendiment en tasques cognitives que impliquen el manteniment d'informació, però una flexibilitat cognitiva disminuïda (Egan et al., 2001; Winterer et al., 2004).

El transportador de dopamina (DAT) aconsegueix la recaptació activa de DA de la sinapsi i és el principal regulador de la neurotransmissió dopaminèrgica al cervell mitjà. El DAT regula l'extensió a la qual la DA es difon en l'espai extracel·lular, i així, la duració de l'acció cel·lular de DA,

especialment a l'estriat (Sesack, Hawrylak, Matus, Guido, & Levey, 1998). Un polimorfisme de número variable de repeticions de tàndem (VNTR) va ser identificat al gen de DAT1 amb 9- i 10 repeticions (9R i 10R) com els al·lels més freqüents en la població (Vandenbergh et al., 1992). El genotip de 10R/10R ocasiona una major expressió de DAT (Fuke et al., 2001; Heinz et al., 2000; VanNess, Owens, & Kilts, 2005; vegi tanmateix Jacobsen et al., 2000) i, putativament, menor DA als tractes cortico-estriatals (Wichmann & DeLong, 1996) modulant els receptors de D2 implicats en tractes mesocorticolimbics (Neville, Johnstone, & Walton, 2004).

El receptor dompaminèrgic D2 (DRD2), localitzat en neurones dopaminèrgiques postsinàptiques, està implicat centralment al sistemes de recompensa mesocorticolimbics (Neville, Johnstone, & Walton, 2004) i s'expressa principalment a l'estriat humà (Camps, Cortes, Gueye, Probst, & Palacios, 1989). El DRD2 té un polimorfisme funcional que ha mostrat funcionalitat en uns quants estudis respecte a funcionament cognitiu. El polimorfisme DRD2 TAQ IA és un polimorfisme de fragment de restricció, que és també provocat per una mutació en un ùnic nucleòtid (rs#126450). Per al gen de DRD2, els portadors d'al·lels d'A1 mostren una reducció de 30-40% en densitat de DRD2 en comparació amb homozigots A2 (Ritchie & Noble, 2003). Pels portadors de l'A1, les baixes concentracions de receptors de D2 promourien una atenuació de la resposta de DA estriatal, augmentant així el *firing* de neurones piramidals de PFC (Seamans &

Yang, 2004), millorant així el rendiment sobre tasques de retard de resposta (Seamans, Gorelova, Durstewitz, & Yang, 2001).

Els "paradigmes de distracció" auditiu-visuals (revisions en Escera et al., 2000; Escera et al., 2003; Escera & Corral, 2007) són àmpliament utilitzats en l'estudi del processament de novetat i de l'atenció involuntària. Durant un paradigma de distracció auditiu-visual, els ERPs enregistrats al cuir cabellut mostren una resposta prominent, l'anomenada P3 de novetat (nP3) o P3a, associada amb l'avaluació d'esdeveniments nous per a la subsegüent acció conductual (Escera & Corral, 2007; Friedman, Cycowicz, & Gaeta, 2001). Pel potencial nP3 s'han descrit dos subcomponents, el primerenc i el tardà, clarament revelats sobre la base de la seva latència temporal, distribució sobre el cuir cabellut i concomitants psicològics (Escera et al., 1998; Yago, Escera, Alho, Giard, & Serra-Grabulosa, 2003). A més, el nP3 reflexa la interacció entre el control atencional exogen per esdeveniments ambientals nous i el control cognitiu endogen (Pashler et al., 2001). Un realçament de la primera fase de P3 de novetat es va observar quan es van processar sons nous durant la realització d'una tasca que implicava fotografies emocionalment negatives (Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Dominguez-Borras, Garcia-Garcia, & Escera, 2008b; Garcia-Garcia et al., 2008). A més, durant un paradigma de commutació de tasca inspirat per la prova clàssica de PFC, la Winsconsin Card Sorting Test (Rubinstein et al., 2001), un estímul auditiu dirigint un canvi en el set mental a una tasca nova provoca un

potencial cerebral distribuït fronto-posteriorment i associat a operacions d'actualització contextual (Barceló et al., 2000; Barceló et al., 2002). Aquest potencial s'assembla al component de nP3 a estímuls nous en paradigmes de distracció auditiu-visuals (Barceló et al., 2002) i, a més, va revelar que el processament de novetat sensorial i de tasca podrien compartir una xarxa neuronal comuna (Barceló et al., 2006).

Segons Näätänen & Picton, (1987), com a mínim tres components exògens (i.e., depenent de característiques físiques d'estimulació sensorial) i tres endògens (i.e., depenent del valor informatiu de l'estimulació) es poden activar simultàniament a l'ona N1 auditiva que culmina al voltant de 70-100 ms després de l'estimulació auditiva. Alguns d'aquests components són sensibles a l'atenció (Woldorff et al., 1993). Un component no específic de l'ona N1 va ser relacionat amb l'ocurrència d'un esdeveniment potencialment rellevant, per a facilitar respostes sensorials i motores apropiades (Näätänen & Picton, 1987).

El propòsit general del treball d'aquesta tesi és mostrar alguns dels factors implicats en la variabilitat interindividual observats en els mecanismes neuronals d'actualització d'informació contextual durant processament de novetat i la reconfiguració del set de tasca. Tres polimorfismes genètics dopaminèrgics van ser seleccionats donada l'evidència del paper del sistema de DA en el control de l'atenció. S'espera que les variacions polimòrfiques del DAT estiguin implicades en la detecció ràpida de

rellevància de tasca, i juguin un paper en els processos d'actualització contextual tant del processament de la novetat com de reconfiguració del set de la tasca. A més, la interacció dels dos polimorfismes per a COMT i DRD2, segons la relació funcional de l'acció dels receptors de D1 i D2, s'hipotetitza que mostraran nivells diferents d'activitat de DA al PFC: els individus homozigòtics per a Met i sense l'al·lel de A1 (i.e., MetA1-), i aquells homozigòtics per a Val i presentant l'al·lel de A1 (i.e. ValA1+) s'espera que mostrin un equilibri compensat de nivells de COMT i densitat de DRD2 que condueix a nivells mitjans d'activitat dopaminèrgica al PFC; per contrast, els individus homozigòtics per a Met i amb l'allele A1 (i.e. MetA1+) mostrarien activitat de DA al PFC més alta, mentre d'aquells homozigòtics per a Val i A2 (i.e., ValA1-) s'esperaria que mostressin una activitat de DA al PFC subòptima. Conseqüentment, l'efecte combinat de la variabilitat genètica per la COMT i el DRD2 es reflectiria en els mecanismes neuronals i les operacions d'actualització de context pel canvi de tasca i el processament de la novetat.

Mètode

Cent cinquanta voluntaris van ser entrevistats segons una versió adaptada de l'Entrevista Clínica del Manual de Diagnosi i Estadístic (DSM IV-R), per a l'exclusió d'individus amb possibles malalties neurològiques i psiquiàtriques, fòbies, i consum de drogues. Els participants homozigòtics per a la COMT i aquells amb els al·lels més freqüents pel DRD2 (A1, A2), i

els que mostraven els genotips més freqüents pel DAT (9R/9R, 9R/10R, 10R/10R; Vandenbergh et al., 1992) van ser seleccionats per una sessió d'enregistrament d'EEG. Els participants genotipats com a 10R/10R es van assignar al grup de 9R- associat amb un efecte funcional de major expressió del DAT (Fuke et al., 2001; VanNess et al., 2005), i els genotipats com 9R/10R i 9R/9R es van incloure en el grup de 9R+.

El DNA es va recollir amb escobillons de cèl·lules de mucosa bucal i se va extreure utilitzant l'Equipament d'Extracció d'Epicentres® BuccalAmpTM DNA (Epicentre, Madison, WS). Després de l'aïllament del DNA, els dos polimorfismes de nucleòtids ùnics per a la COMT Met108/158Val i el DRD2 Taq IA van ser realitzats per la reacció en cadena de la polimerasa (PCR) en temps real que utilitzava tècnica de descoberta de fluorescència per mitjà de la tecnologia de TaqMan Biosystems Aplicada (Biosystems aplicat, CA, els USA). Pel que fa el gen del DAT1, el 40-bp VNTR els polimorfismes van ser obtinguts per a cada mostra de DNA seguint els procediments descrits a Sano, Kondoh, Kakimoto, & Kondo, (1993), i modificat mitjancant l'amplificació de la PCR-VNTR que utilitza unes tècniques de detecció fluorescent.

Dues versions diferents del paradigma de distracció visual-auditiu (Escera et al., 1998; Escera et al., 2003; Escera & Corral, 2007) van ser administrats. Els estímuls auditius eren un to estàndard de 700 Hz (STD) i 100 sons nous complexos ambientals únics (NOV). Al llarg de la seqüència

la probabilitat d'ocurrència del to estàndard era 0.8, i els sons nous ocorrien amb el complementari 0.2, de manera que cada so nou ocorria només una vegada dins de cada condició.

Un protocol de commutació de tasca inspirat pel WCST (Rubinstein et al., 2001) i adaptat per mesurar ERPs (Barceló, 2003) va ser administrat als participants. Se'ls manava que fessin correspondre la carta d'elecció amb una de les quatre cartes clau segons dues regles de tasca possibles (color o forma). Abans de la ocurrència de la carta, un de quatre tons informava explícitament si la carta s'havia de classificar segons el 'color' (500/1000 Hz) o segons la 'forma' (2000/4000 Hz). Tres tipus de trials es definien per tal de dissociar el processament de canvis de representacions sensorials i de tasca. Per tant, als trials de repetició, tant els tons com la tasca es repetien en relació al trial previ. En els trials de canvi de senyal, només el to canvia però la tasca romania igual que en el trial previ. En el canvi de tasca canviaven ambdós, el to i la tasca.

L'activitat d'EEG va ser enregistrada (ANT Software b.v., Enschede, Els Països Baixos) durant la realització de la tasca des de 64 elèctrodes sobre el cuir cabellut segons la convenció estesa de 10/10 en un espai elèctricament i acústicament aïllat.

Una premsa de botons correcta dins de 100-1200 ms després del començament de l'estímul visual era considerat com un encert, i els RTs

mitjans es computaven per a encerts només. Es va fer una mitjana d'ERPs fora de línia per a cada tipus de trial o condició de tasca, d'una època de 1400 ms incloent-hi una línia de base preestímul de 200 ms. Les freqüències damunt de 30 Hz es filtraven digitalment als EEGs individuals abans de fer una mitjana dels ERP. La nP3 es mesurava com l'amplitud mitjana en les finestres de temps de 200-290 i 290-370 ms per les fases primerenca i tardana respectivament. Les amplituds del N1 auditiu van ser computades a la finestra de temps de 110 a 140 ms. Les amplituds dels components d'ERP següents es van computar a les finestres de temps especificades: la positivitat primerenca fronto-central de 180 a 220 ms, la positivitat fronto-posterior des de 300 a 340 ms, la desviació negativa tardana de 420 a 440 ms. Igualment, les negativitats fornto-parietal lentes es van computar a dues finestres de temps, des de 600 a 700 ms (SW1), i des de 800 fins a 900 ms (SW2). Les amplituds mitjanes del subcomponent positiu fronto-central del P3 de novetat van ser computades a la finestra de temps de 300 a 340 ms. Totes aquestes respostes del cervell es van mesurar en canals F3, F4, Fz, C3, C4, Cz, P3, P4 i Pz.

Per obtenir els components de temps- freqüència de les respostes en banda gamma, les èpoques de les quals es feia una mitjana per a cada condició i participant es van descompondre per mitjà d'una transformació Wavelet contínua. Les transformacions de temps- freqüència van ser obtingudes per l'aplicació de *wavelets Morlet* complexament valorades, que són gaussians en els dominis de temps i freqüència. La sincronització de

fase es va calcular per mitjà de la fase de l'activitat oscil·latòria mesurada utilitzant el factor de coherència de fase (PLF) proposat per Tallon-Baudry, Bertrand, Delpuech, & Permier, (1997). Les amplituds màximes i els valors de PLF dels GBRs es van obtenir en la finestra de temps de 100 a 200 ms i els anàlisis es van realitzar per freqüències al voltant de 40 Hz (35-45 Hz).

Resultats

A l'estudi I, un realçament de l'efecte de distracció es va trobar en un context emocional negatiu en comparació amb un de neutre. Aquests efectes conductuals van trobar efectes paral·lels en la PS d'oscil·lacions neuronals en la banda de freqüències de gamma en una regió específica: l'augment de PS després de sons nous era més gran quan ocorrien en context emocional negatiu en comparació al neutre en distribucions centrals.

A l'estudi II, els individus amb un equilibri entre la disponibilitat de DA al PFC i la densitat de DRD2 (i.e., ValA1+, MetA1-) van ser distrets per esdeveniments nous auditius (i.e. experimentat un retard en RT després de l'ocurrència d'un esdeveniment nou en comparació a sons estàndards), mentre que els individus amb altres distribucions d'al·lels per la COMT i el DRD2 mostren RTs similars en NOV i STD (i.e., cap distracció). Les oscil·lacions cerebrals van mostrar similars PS per als dos tipus d'estímuls

auditius en grups amb l'equilibri de COMT/DRD2, però la PS de tal oscil·lacions neuronals de 40 Hz es van veure augmentades a NOV en comparació amb STD per a individus amb l'activitat dopaminèrgica PFC més baixa o més alta.

A l'estudi III, els individus amb més DA estriatal (i.e. 9R+) van mostrar un augment de RT provocat pel so nou inesperat mostrant l'efecte conductual de distracció, que era més gran dins d'un context emocional NEG que en un de NEU. Diferencialment, els individus de 9R- van mostrar un retard similar de RTs provocat pels NOV tant al NEU com al context emocional NEG. Tanmateix, els individus de 9R+ no van mostrar cap modulació del component de nP3 pel context emocional, mentre que el grup de 9R- va mostrar un realçament de l'amplitud de la fase primerenca de nP3 en NEG en comparació amb NEU. A més, l'amplitud mitjana del component de nP3 era substancialment més gran al grup 9R+ que al 9R-.

Als estudisi IV I V, els individus de 9R+ van mostrar un increment de la resposta cerebral de N1 auditiva a uns 110 ms després del començament d'una senyal indicant un canvi en la informació de tasca en comparació amb una senyal indicant no canvi en tasca. També van arribar a RTs més grans en els trials de canvi de senyal sense major augment en trials de canvi de tasca. Per contrast, els individus de 9R- van mostrar amplituds similars de la resposta cerebral de N1 en totes tres condicions, i van mostrar RTs augmentats en trials de canvi de senyal en comparació amb

trials de repetició, així com en trials de canvi de tasca en comparació amb trials de canvi de senyal. A més, els 9R+ van presentar una nP3 primerenca més gran en regions centrals i parietals en comparació amb el grup 9R-. Per contrast, les condicions de tasca modulaven l'amplitud de la desviació negativa tardana per a 9R-, però no per als individus 9R+.

A l'estudi VI, els individus que combinaven ValA1+ i MetA1- pels polimorfismes par la COMT i el DRD2 van experimentar un cost de commutació de tasca específic, mostrant RTs mitjans més llargs quan afrontaven canvis sensorials que també demanaven un canvi en la representació de la tasca. Per contrast, els grups ValA1- i MetA1+ van mostrar RTs mitjans similars en trials canvi de senyal i trials de canvi de tasca. Així, els individus ValA1+ i MetA1- van mostrar amplituds augmentades del nP3 en trials de canvi de tasca en comparació amb trials de canvi de senyal, encara que van mostrar amplituds similars en trials repetició i de canvi de senyal. En canvi, els grups de ValA1- i MetA1+ van mostrar amplituds més grans en trials de canvi de senyal en comparació amb aquells de repetició, però amplituds similars en trials canvi de senyal i de canvi de tasca.

Discussió

La present tesi va mostrar un paper important de la interacció espitàtica entre els polimorfismes per la COMT i el DRD2 en l'ocurrència de l'efecte

de distracció I els processos cognitius d'actualització de la informació contextual. Com que l'estimulació dels receptors D2 facilita a l'actualització de la informació en memòria de treball (Cools, 2008), l'efecte combinat de l'activitat de la COMT i la densitat de DRD2 podria reflectir la interacció entre un mecanisme de *gating* que permet les funcions d'actualització necessàries per l'activació d'un nou set de tasca durant el manteniment de representacions de tasca promogudes per les concentracions de DA al PFC (Arnsten, 1998; Cools et al., 2004). L'augment d'amplituds, tanmateix, de l'activitat oscil·latòria a 40 Hz a 100 ms post-estímul pel NOV en comparació amb l'STD en tots quatre grups indica que s'ha produït un augment en l'atenció dirigida als esdeveniments nous en comparació amb els estàndards (Busch, Herrmann, Muller, Lenz, & Gruber, 2006; Debener, Herrmann, Kranczioch, Gembris, & Engel, 2003; Herrmann, Munk, & Engel, 2004; Tiitinen et al., 1993; Yordanova, Kolev, & Demiralp, 1997; Yordanova et al., 2000; Yordanova, Banaschewski, Kolev, Woerner, & Rothenberger, 2001), donat que la captura involuntària de l'atenció és un mecanisme ecològicament necessari per detectar estímuls potencialment importants en l'ambient (Escera et al., 2003). Tanmateix, mentre les amplituds d'EEG reflecteixen força patrons d'activació sostinguts de parcel·les cortical grans, la fase de les oscil·lacions cerebrals s'ha relacionat amb una alta precisió temporal de patrons del *firing* neuronals particulars (Buzsaki & Draguhn, 2004). El realçament de PS mostrat només pels grups MetA1+ i ValA1- pels NOV en comparació als STD probablement reflecteix un mecanisme de *resetting* neuronal per

compensar les seves característiques neuroquìmiques subòptimes per l'actualització de context sense cap empitjorament conductual pels esdeveniments nous ambientals, ja que paradoxalment ocasionen un millor rendiment, donat que el processament d'esdeveniments nous no resulta en el retard de RT.

L'acció dels receptors D1/D2 regulen l'estabilitat i la flexibilitat de representacions mentals durant la modulació del *firing* de les neurones piramidals prefrontals (Durstewitz et al., 2000; Sawaguchi & Goldman-Rakic, 1994). Per això, podríem inferir un paper de DA al PFC sobre l'actualització de representacions de tasca examinant l'efecte de gens reguladors de l'acció de D1/D2 sobre l'actualització d'informació sensorial i de tasca en memòria de treball (Perianez & Barceló, 2009). Els dos grups amb els nivells més baixos o més alts de DA al PFC van mostrar que les amplituds de nP3 eren augmentades en trials de canvi de senyal en comparació amb trials de repetició, encara que eren similars entre trials de canvi de senyal i de canvi de tasca amb coherència amb els costos de canvi conductuals observats. Aquest patró electrofisiològic suggereix que els individus amb nivells de DA al PFC extrems processen cada canvi sensorial similarment sense tenir en compte la seva importància per canviar o repetir el set de tasca previ. D'acord amb això, semblava que aquests grups restauressin i tornessin a configurar la representació de tasca després d'algun canvi de senyal sense tenir en compte la seva rellevància per la tasca. Amplituds més grans del nP3

s'observaven per a trials de canvi de tasca relatiu a canvis de senyal, indicant una actualització dependent-de-context específica pel set de tasca als grups amb nivells de PFC DA mitjans.

En un paradigma de distracció auditiu-visual, els individus amb l'al·lel de 9R per al genotip de DAT (9R+) van mostrar una amplitud més gran de la nP3 en comparació amb el 9R-. La major amplitud del nP3 de novetat pot indicar que una major disponibilitat de DA permet als individus 9R+ desplegar una activació més forta de la novetat en comparació a esdeveniments estàndards repetitius. Aquesta explicació es coherent amb una proposta recent de que el primer subcomponent positiu de nP3 reflecteix un mecanisme general d'activació de representacions necessaris pel subseqüent remapatge d'associacions d'estímul- resposta (Barceló, Perianez, & Nyhus, 2007).

Un atenuació de la reactivitat a estímuls en un context d'emoció negativa en pacients amb disfunció dopaminèrgica a l'estriat (Lawrence, Goerendt, & Brooks, 2007) suggereix que la DA estriatal esta implicada en la interacció entre els processaments atencionals i d'emoció; aquesta atenuació pot explicar l'absència d'efectes conductuals de la modulació de processament de novetat auditiu pel context emocional negatiu en individus amb menys DA estriatal. Tanmateix, encara que cap efecte conductual de la modulació no s'observa per a individus de 9R-, mostraven un realçament de la fase primerenca del nP3 en NEG en comparació amb

NEU que reflectia un processament més fort d'esdeveniments nous inesperats durant emoció negativa (Dominguez-Borras, Garcia-Garcia, & Escera, 2008a; Dominguez-Borras, Garcia-Garcia, & Escera, 2008b; Garcia-Garcia et al., 2008) quan podria constituir un perill. El DAT juga un paper important en mecanismes que regulen la modulació de processament de novetat per un context emocional.

En un paradigma de commutació de tasca, el grup de 9R+ pel DAT invertia RTs similars per processar tots els canvis de senyals, suggerint un processament independent de context de tots els canvis auditius. Els individus de 9R+ invertien aproximadament 100 ms extra per a trials de canvi de senyal que els 9R-. Aquesta avaluació més lenta d'un canvi sensorial podria ser conseqüència d'un excés de protecció contra la interferència (Cools et al., 2001). La major disponibilitat de DA en circuits frontostriatals (9R+) podria ajudar a protegir el set de tasca actual en presència de novetat que competeix pels recursos atencionals (cfr. Cools et al., 2001). Al contrari del 9R+, el grup de 9R- va processar totes els estímuls auditius de manera dependent del context, ocasionant una ordenació més eficaç dels canvis de senyal depenent de si els canvis auditius indicant o no el remapatge preparatori entre estímuls visuals i respostes motores (i.e., commutació de tasca o repetició). A més, només els individus amb més DA inferida a l'estriat (i.e., 9R+; Heinz et al., 2000) van mostrar un realçament del component N1 per trials de canvi de tasca en comparació amb trials de canvi de senyal, reflectint una detecció molt

primerenca de la rellevància per la tasca dels canvis sensorials. Els bucles prefrontoestriatals dopaminèrgics estan involucrats en la detecció ràpida de la novetat per la tasca, i advoquen a favor d'un paper crucial de l'acció dels receptors D2 en aquests mecanismes.

Un augment més gran de l'ona lenta en el grup 9R- pot així reflectir una preparació més forta (Walter et al., 1967) quan hi ha un *match* sensorial entre l'estímul auditius recent i el previ, ocasionant una integració més eficaç del estímul nou en el context de tasca en curs. A més, la desviació negativa tardana, que podria reflectir una primera fase de les ones lentes, presenta amplituds més grans en la repetició que els trials de canvi per a 9R- com a reflexa de més disponibilitat eficaç a causa del *match* acústic entre el trial previ i l'actual. Per contrast, la negativitat tardana no es modula en individus de 9R+ pel tipus de trials, perquè van realitzar la tasca d'una manera independent de context i no van semblar integrar les senyals al context en curs.

Els paradigmes utilitzats en aquests estudis han mostrat una alta sensibilitat als processos de control cognitius com són la informació sostinguda i l'actualització de representacions d'objectiu facilitades per estimulació de receptors de D1 i D2. A més, els resultats actuals donen suport a la hipòtesi d'una funció d'U invertida d'activitat de DA al PFC i variables conductuals i neurofisiològiques relacionades amb operacions d'actualització d'informació contextual. Els patrons electrofisiològics

mostrats podrien constituir marcadors endofenotípics fiables de dèficits cognitius, i podria ajudar a aïllar una disfunció en el sistema de corticostriatal dopaminèrgic humà, fins i tot quan es acompanyada per molt subtil o cap concomitant conductual, resultant en un bon enllaç pel buit entre el gen I la condnucta.

Conclusions

En l'estudi I, vam estudiar els mecanismes cerebrals que donen suport a l'augment de l'orientació de l'atenció cap a esdeveniments auditius nous sota amenaça emocional. Vam trobar que l'atenció involuntària auditiva era reflectida per un augment de PS de banda de gamma. Més important, vam mostrar que un context emocional negatiu afina el processament de la novetat per mitjà de la PS de l'activitat cerebral en la banda de freqüència gamma al voltant 40 Hz en xarxes neuronals específiques.

En l'estudi II, vam explorar el rol de la interacció dels gens per la COMT i el DRD2 en el control exogen d'atenció. El resultat establia una funció d'U invertida de l'activitat de DA al PFC i la distracció conductual per un esdeveniment nou, per la qual uns nivells mitjans de DA al PFC condueixen a una orientació de l'atenció cap a esdeveniments nous amb un retard en RT a la tasca. Els grups amb l'activitat de DA al PFC més baixa o més alta van realitzar la tasca sense retard en RT pel NOV en comparació amb l'STD, probablement a través d'un mecanisme neuronal

de reposició de fase del *firing* neuronal que els permet l'actualització d'informació context sense distreure's de la tasca rellevant en curs.

En l'estudi III, vam intentar revelar el paper del genotip del DAT sobre la modulació de processament de la novetat per un context emocional. Una manca d'augment de la distracció pel context emocional en individus de 9R- va ser compensada per un augment de la fase primerenca del nP3 en context negatiu, mostrant que el processament d'esdeveniments nous auditius inesperats va ser realçat també en individus de 9R-. Contrariament, un major disponibilitat de DA estriatal en individus 9R+ els va portar a una activació més forta dels esdeveniments nous auditius que es va reflectir en uns amplitud més gran de la nP3 després de qualsevol so nou, sense major augment durant una situació amenaçadora.

En l'estudi IV, vam examinar el paper del polimorfisme del DAT1 sobre la detecció ràpida de canvis sensorials rellevants per la tasca. Els descobriments van suggerir que els individus 9R+ detecten la necessitat de reconfiguració del set de la tasca en una etapa anterior que els individus de 9R-. Vam proporcionar una evidència del paper de genotip de DAT1 als mecanismes adaptatius de detecció ràpida de rellevància de tasca en canvis sensorials.

En l'estudi V, vam investigar el paper del DAT en la estereotípia de resposta cerebral subjacent a la flexibilitat cognitiva. Els individus de 9R-

van mostrar augments i modulacions de les respostes cerebrals per a canvis sensorials i per a la reconfiguració de set de tasca, suggerint que el rendiment en la tasca depèn del context local immediat. Els individus 9R+ van mostra una major inhibició de qualsevol interferència prèvia així com una activació més forta de la tasca actual.

En l'estudi VI, vam examinar el paper de l'interacció entre dues variacions polimòrfiques d'uns gens (COMT i DRD2) implicats en la regulació d'activitat dels receptors D1 i D2 sobre els mecanismes neuronals de commutació i el manteniment del set de la tasca. La interacció entre els gens de la COMT i del DRD2 es va evidenciar en un paral·lelisme entre els patrons conductuals de costos de RT i els realçaments de nP3 quan havien de fer un canvi de tasca. Aquests costos i correlats electrofisiologics es van trobar només en individus que combinen gens relacionats amb un equilibri òptim entre l'activitat dels receptors D1 i D2. Els individus amb una combinació diferent de polimorfismes van mostrar, tanmateix, tal realçament després de qualsevol canvi sensorial, així restaurant i tornant a configurar representacions de tasca sense tenir en compte el context anterior.

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