Genetic marker of norepinephrine synthesis predicts individual differences in post-error slowing: A pilot study

Lorenza S. Colzato a,*, Mischa de Rover b, Wery P.M. van den Wildenberg b, Sander Nieuwenhuis c

a Leiden University, Cognitive Psychology Unit & Leiden Institute for Brain and Cognition, Leiden, The Netherlands
b University of Amsterdam, Amsterdam Center for the Study of Adaptive Control in Brain and Behaviour (Acacia), Psychology Department, Amsterdam, The Netherlands
c Amsterdam University College, Amsterdam, The Netherlands

A B S T R A C T

When our brain detects the commission of an error, we slow down immediately thereafter: a phenomenon called post-error slowing (PES). Some researchers have speculated that slowing after unexpected errors or negative feedback is related to the activity of the neuromodulatory locus coeruleus—norepinephrine system. In the present pilot study, we tested whether individual differences in the size of PES are related to differences in genetic predisposition related to norepinephrine synthesis. In a sample of 100 healthy adults, we studied the dependency of an individual’s size of PES on the DBH5 Ins/del polymorphism—a variation in the DBH gene associated with the production of the enzyme dopamine b-hydroxylase, which catalyzes the conversion of dopamine to norepinephrine. DBH5 Ins/del heterozygotes, who have intermediate levels of plasma DBH activity and NE release, showed increased PES in a Simon task compared to del/del homozygotes and ins/ins homozygotes, who have low and high levels of plasma DBH activity, respectively. This outcome pattern presents preliminary evidence that the size of PES varies with DBH activity and, presumably, NE release according to an inverted U-shape: intermediate levels of DBH activity and NE release are associated with larger post-error adjustments.

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1. Introduction

People tend to slow down after they commit an error, a phenomenon known as post-error slowing (PES). Specifically, PES reflects longer reaction times (RTs) in trials following an error than in trials following a correct response (Rabbitt, 1966). PES has been observed in a wide range of tasks (Danielmeier & Ullsperger, 2011), including the Simon task (King, Korb, Van Cramon, & Ullsperger, 2010; Danielmeier, Eichele, Forstmann, Tittgemeyer, & Ullsperger, 2011), the flanker task (Debener et al., 2005; Krämer et al., 2007) and the Stroop task (Gehring & Fencsik, 2001). PES is particularly pronounced when the interval between the erroneous response and the subsequent stimulus is short (Jentzsch & Dudschig, 2009; Danielmeier & Ullsperger, 2011), and when errors are infrequent (Notebaert et al., 2009; Steinborn, Flehmig, Bratze, & Schröter, 2012).

The goal of the present research was to examine the hypothesis that PES is mediated by the activity of the locus coeruleus (LC)–norepinephrine (NE) system, one of the major neuromodulator systems. This hypothesis was motivated by two proposals in the literature. In recent work, Nunez Castellar and colleagues (Nunez Castellar, Kuhn, Fias, & Notebaert, 2010; see also Notebaert et al., 2009) have speculated that slowing after unexpected negative feedback may reflect an orienting response related to the activity of the LC–NE system. Indeed, as noted by Dayan and Yu (2006), unexpected events (such as errors) are associated with increases in phasic NE firing. Dayan and Yu propose that the phasic NE signal codes the unexpected uncertainty evoked by the unexpected event and serves as an “interrupt” signal, enabling the system to re-orient and react appropriately to the event. Accordingly, Ullsperger, Harsay, Wessel, and Ridderinkhof (2010) have suggested that errors may elicit a phasic NE response and corresponding autonomic orienting response, and that this may be a prerequisite for conscious perception of the error and PES. In line with this view, Carp and Compton (2009) found that errors were followed by decreased EEG alpha power, a cortical correlate of the orienting response and increased LC–NE activity (Swick, Pineda, & Foote, 1994; Swick, Pineda, Schacher, & Foote, 1994), and error-related reductions in alpha power predicted individual differences in PES.

An alternative hypothesis about the role of the LC–NE system in PES has been proposed by Cohen, Botvinick, and Carter (2000). Their argument is that following an error (in the context of overall good performance), the LC–NE system is driven toward a more
phasic firing mode (Aston-Jones & Cohen, 2005), characterized by somewhat lower tonic LC activity and larger task-related phasic LC responses. The resulting decrease in tonic NE release lowers overall neural gain (or responsivity) and thus increases decision thresholds (Servan-Schreiber, Printz, & Cohen, 1990). This diminishes erroneous responding and increases the duration of the decision process, resulting in a more cautious point on the speed-accuracy tradeoff. Importantly, the increased duration of the decision process is manifested in post-error slowing. Recent diffusion-model analyses have found support for the notion that PES reflects an increase in response caution (Dutilh et al., 2012).

So far, these hypotheses about the role of NE in PES have remained speculative, because in contrast to the role of dopamine, in general very little is known about the role of the LC–NE system in performance monitoring (Jocham & Ullsperger, 2009).

2. Purpose of study

To test the hypothesis that an individual’s size of PES is modulated by activity of the LC–NE system, we related PES to the genetic variability associated with dopamine β-hydroxylase, the enzyme that converts dopamine to NE in noradrenergic neurons. As suggested by Cubells et al. (2000), DjH activity in both serum and plasma are biochemical phenotypes that reflect allelic variation at the DBH locus. That is, DjH level is a stable, genetically controlled trait. Indeed, previous studies have shown that the DBH is the major locus in accounting for more than 80% of the genetically controlled trait. Indeed, previous studies have shown allelic variation at the DBH locus. That is, DjH levels in the plasma (Wilson, Elston, Siervogel, & Tran, 1988). Moreover, specific alleles of polymorphisms located near the 5’ portion of the DBH gene have been found to be associated with lower DjH levels in the plasma (Cubells et al., 1998).

For this reason we focused on the DBH5′-ins/del polymorphism, located approximately 3 kb 5′ to the DBH transcriptional start site, a choice that was driven by available in vitro and/or in vivo assays demonstrating significant impact of this polymorphism on aspects of biological function related to NE neurotransmission (Cubells et al., 2000).

In a European-American population DBH5′-ins/del heterozygotes are associated with an average level of plasma DjH activity while del/del homozygotes and ins/ins homozygotes are associated with low and high levels of plasma DjH activity, respectively (Cubells et al., 2000). Furthermore, evidence suggests a direct relationship between DjH activity and NE release: Hwang and colleagues looked at the DBH gene and the amount of NE, before and after pharmacological manipulation, namely the administration of forskolin to cultured cells (Hwang, Kim, & Lee, 1994). The results showed that the expression of the DBH gene was higher by forskolin, ie, more dopamine-β-hydroxylase protein was produced in the presence of forskolin, compared to placebo. Moreover, in the forskolin condition, there was more NE released by the cells, which suggests a clear association between the amount of protein and the levels of NE release. Other, strong evidence for a direct relationship between mutations in the DBH gene and levels of NE is the existence of DjH deficiency, a rare disease caused by a series of mutations in the DBH gene, leading to a complete absence of DjH and NE in plasma (Jepma et al., 2011; Senard & Rouet, 2006).

Based on the hypothesis that PES reflects an error-related response of the LC–NE system (Cohen et al., 2000; Nunez Castellar et al., 2010; Ullsperger et al., 2010), we predicted that an individual’s magnitude of PES in a Simon task is modulated by the DBH5′-ins/del polymorphism.

3. Methods

3.1. Participants

One hundred young Caucasian healthy adults (44 male/56 female), with a mean age of 22.5 years (SD = 2.4, range 18–30) and an estimated IQ of 1215 (SD = 3.1, range 100–130) served as participants for partial fulfillment of course credit or a financial reward. The sample was drawn from adults in the Leiden and Rotterdam metropolitan area (The Netherlands), who volunteered to participate in studies of behavioral genetics. Exclusion criteria were any major medical illness that could affect brain function, current and/or past substance abuse, neurological conditions, history of head injury, and personal history of psychiatric medical treatment. Participants were selected via a phone interview using the Mini International Neuropsychiatric Interview (MINI; Lecrubier et al., 1997). The MINI is a well established brief diagnostic tool in clinical and stress research that screens for several psychiatric disorders including schizophrenia, depression, mania, ADHD, and obsessive-compulsive disorder. Written informed consent was obtained from all participants; the protocol was approved by the ethics committee of the Institute of Psychology at Leiden University.

3.2. Apparatus, stimuli, and task

The experiment was controlled by a Targa Pentium III computer. All stimuli were presented in a resolution of 800 by 600 pixels in 16-bit color on a 17″ CRT refreshing at 100 Hz. Participants were seated at a viewing distance of about 50 cm. Throughout each experimental block, a small (.5 cm) dark grey square was presented in the center of the computer screen and served as a fixation point. The stimulus on each trial was either a green or a blue circle (1.5 cm in diameter) that was presented to the left or right of fixation. The color and location of the circle varied randomly but both colors and locations appeared equally often across the experiment. Responses were made by pressing the “z” or “?” button of the computer keyboard with the left or right index finger, respectively.

3.3. Simon task

The experiment consisted of a 25-min session in which participants made speeded discriminative responses to the color of the circle. Participants operated both response keys by responding left to a green circle and right to a blue circle. Circles stayed on the screen until the response was given or 1500 ms had passed. Intervals between subsequent stimuli varied randomly but equiprobably, from 1750 to 2250 ms in steps of 100 ms. Participants were to ignore the location of the stimulus and to base their response exclusively on its color. Participants were to respond as quickly as possible while keeping average error rates below 15%; feedback was provided at the end of a trial block. The task consisted of 6 blocks of 60 trials, the first of which served as a practice block.

3.4. IQ

Individual IQs were determined by means of a 30-min reasoning-based intelligence test (Raven Standard Progressive Matrices: SPM) that participants completed before performing the Simon task. The SPM assesses the individual’s ability to create perceptual relations and to reason by analogy independent of language and formal schooling; it is a standard, widely-used test to measure Spearman’s g factor as well as fluid intelligence (Raven, Court & Raven, 1988).

3.5. DNA laboratory analysis

Genomic DNA was extracted from saliva samples using the OrageneTM DNA self-collection kit following the manufacturer’s instructions (DNA Genotek, Inc., 2006). DBH5′-ins/del polymorphism was genotyped using Applied Biosystems (ABI) TaqMan technology. Following Colzato, Pratt, and Hommel (2010), the genotype in 7 participants.

After denaturation at 94°C for 1 min, the mixture was submitted to 30 cycles each made of 30 s denaturation (94°C), annealing (55°C), and elongation (72°C).

Three genotype groups were established (see Table 1); ins/ins homozygotes, ins/del heterozygotes and del/del homozygotes. We were unable to determine the genotype in 7 participants.
3.6. Statistical analysis

First, repeated-measures ANOVAs were performed to examine age, sex, and IQ differences between genotype groups. Second, to test the effect of DBH5-\(ins/del\) polymorphism on performance in the Simon task, mean correct RTs and (square-rooted) error percentages were analyzed by means of ANOVAs using spatial stimulus-response correspondence as within- and genotype as a between-subjects factor. Finally, PES was analyzed using a separate univariate ANOVA with genotype as a between-subjects factor. PES was calculated as mean post-error RT minus mean post-correct RT. Post-correct trials were only included if they were preceded by at least two and followed by at least one other correct trial (cf. Danielmeier & Ullsperger, 2011). A significance level of \(p < .05\) was adopted for the ANOVAs. A significance level of \(p < .025\) (\(p = .05\), two factors) was adopted for the post-hoc comparisons, correcting \(p\) values for multiple comparisons (Bonferroni correction).

4. Results

4.1. Participants

Sample information and genotype-specific demographics are shown in Table 1. The genotype frequencies in our cohort of participants did not deviate from the Hardy–Weinberg equilibrium (\(p = .47\)). No significant differences were found among genotype frequencies with respect to age, sex, or IQ, \(F_s < 1\).

4.2. Simon task

The analyses of RT and error rate showed a main effect of correspondence, \(F(1,92) = 446.34, p < .0001, \text{MSE} = 168.901, \eta^2_p = .83\) (RTs) and \(F(1,92) = 138.08, p < .0001, \text{MSE} = 16.066, \eta^2_p = .60\) (errors). These main effects indicated that responses were faster and more accurate with stimulus-response correspondence (366 ms and 2.6\% than with non-correspondence (406 ms and 9.5\%). The main effects were not modified by the DBH5-\(ins/del\) polymorphism, \(F_s < 1\).

4.3. PES

All three groups slowed down after errors (Table 1). Averaged across genotypes, the size of PES was 27 ms. This was not accompanied by a decrease in the percentage of errors; 6.3\% on post-correct trials vs. 6.6\% on post-error trials (\(F < 1\)).

As predicted, DBH5-\(ins/del\) polymorphism affected the size of PES, \(F(2,90) = 4.88, p < .010, \eta^2_p = .10\): Post-hoc multiple comparisons revealed that \(ins/del\) heterozygotes showed a greater PES size than \(del/del\) homozygotes (\(p = .022\)) and \(ins/ins\) homozygotes (\(p = .006\)), while no difference in PES size was observed between \(del/del\) homozygotes and \(ins/ins\) homozygotes (\(p = .68\)) (Fig. 1). The groups did not differ in the post-error increase in accuracy, perhaps because of a ceiling effect: post-error accuracy was close to 100\%.

5. Conclusions

In this pilot study we investigated the dependency of an individual’s size of PES on the DBH5-\(ins/del\) polymorphism—a variation in the DBH gene associated with the production of the enzyme dopamine beta-hydroxylase, which catalyzes the conversion of dopamine to NE. We found that DBH5-\(ins/del\) heterozygotes (associated with average level of plasma D\(\beta\)H activity) showed increased PES compared to \(del/del\) homozygotes and \(ins/ins\) homozygotes (associated with low and high level of plasma D\(\beta\)H activity, respectively). These group differences in PES occurred in the context of otherwise similar task performance. Thus, we propose that PES is determined, at least in part, by differences in genetic predisposition related to NE synthesis, suggesting a modulatory role for NE in causing PES.

Our results provide the first direct, although preliminary, evidence for previous suggestions that PES may be related to the activity of the LC–NE system (Cohen et al., 2000; Nunez Castellar et al., 2010; Ullsperger et al., 2010). At first blush, our results seem inconsistent with a pharmacological study that found no effect on PES of the noradrenergic alpha-2 receptor antagonist yohimbine, which stimulates LC firing and increases NE release (yohimbine: 21 ms; placebo: 15 ms; Riba, Rodriguez-Fornells, Morte, Munte, & Barbanoj, 2005). However, the current results, as well as previous pharmacological studies (De Rover et al., 2012; Lukys, Gerstner, & Sandi, 2009) suggest that the effect of noradrenergic drugs on PES might go in opposite directions, depending on the baseline level of NE activity. For example, \(del/del\) homozygotes, with lower D\(\beta\)H activity and (presumably) lower basal NE levels may show an increase in PES when their NE levels are pharmacologically enhanced up to the

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N</th>
<th>Sex</th>
<th>Age</th>
<th>IQ</th>
<th>Post-error RT (ms)</th>
<th>Post-correct RT (ms)</th>
<th>Post-error % errors</th>
<th>Post-correct % errors</th>
<th>% Errors</th>
<th>PES (ms)</th>
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</tr>
<tr>
<td>DBH5-(ins/del)</td>
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</tr>
<tr>
<td>ins/ins</td>
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<td>10</td>
<td>15</td>
<td>22.0</td>
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<tr>
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<td>6.3</td>
<td>39.9</td>
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<tr>
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<td>15</td>
<td>22.5</td>
<td>123</td>
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<td>4.6</td>
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</tr>
<tr>
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<td>52</td>
<td>22.5</td>
<td>122</td>
<td>408</td>
<td>6.6</td>
<td>6.3</td>
<td>6.3</td>
<td>27.1</td>
</tr>
</tbody>
</table>

5. Conclusions

In this pilot study we investigated the dependency of an individual’s size of PES on the DBH5-\(ins/del\) polymorphism—a variation in the DBH gene associated with the production of the enzyme dopamine beta-hydroxylase, which catalyzes the conversion of dopamine to NE. We found that DBH5-\(ins/del\) heterozygotes (associated with average level of plasma D\(\beta\)H activity) showed increased PES compared to \(del/del\) homozygotes and \(ins/ins\) homozygotes (\(p = .022\)) and \(ins/ins\) homozygotes (\(p = .006\)). Vertical capped lines atop bars indicate standard error of the mean.
level of ins/del heterozygotes. In contrast, ins/del heterozygotes, with intermediate DjH activity and intermediate basal NE levels may show a decrease in PES when their NE levels are pharmacologically enhanced up to the level of ins/ins homozygotes. If a pharmacological study has a heterogeneous subject sample, these changes in opposite directions will, on average, cancel out. This suggests that future pharmacological studies interested in PES should investigate the interaction between drug and measures of basal noradrenergic activity.

Several outstanding questions remain to be answered. First, why would PES be largest in DBH5′-ins/del heterozygotes? This result is an instance, somewhat uncommon, of heterosis: the finding that subjects heterozygous for a specific gene polymorphism show a significantly larger or smaller effect for a quantitative or dichotomous trait than subjects homozygous for either allele. In their review on heterosis, Comings and MacMurray (2000) propose that one of the most likely principles underlying heterosis is the inverted U-shaped response curve, and as a prime example they describe the relationship between tonic and phasic firing rates of the LC–NE system: phasic LC responses are largest during intermediate levels of tonic LC firing (Aston-Jones & Cohen, 2005). This suggests that the relationship between PES and DjH (and hence tonic NE levels) is mediated by the strength of LC phasic activity, perhaps that elicited by errors. This account would be consistent with the finding that subjects with large error-related skin-conductance responses, a potential correlate of phasic LC activity (Nieuwenhuis, de Geus, & Aston-Jones, 2011), slow down more following errors (Hajcak, McDonald, & Simons, 2003). In contrast, a relationship between PES and phasic LC activity seems inconsistent with the increased-threshold account of PES (Dutilh et al., 2012), at least on the assumption that phasic LC responses reflect post-decision signals (i.e. signals happening after the decision process of which PES is a manifestation: Aston-Jones & Cohen, 2005).

A second outstanding question is whether the relationship between DjH activity and PES may be mediated (in part) by dopamine, given that DjH converts dopamine to NE. Neurological studies have found that DjH-deficient patients, who lack DjH and NE, have increased plasma concentrations of dopamine (e.g., Jepma et al., 2011). Cubells and Zabetian (2004) have proposed that a similar principle may hold for DBH gene polymorphisms: genotypes associated with higher DjH activity are characterized by higher NE and lower dopamine levels, and vice versa. However, to our knowledge there is very little empirical evidence regarding the effect of DBH polymorphisms on dopamine levels, and the little available evidence suggests that DBH genotype effects on NE and dopamine levels are not necessarily inversely related (Jönsson et al., 2004). Furthermore, previous research has found that PES is not significantly modulated by d-amphetamine (de Bruijn, Hulstijn, Verkes, Ruitg, & Sabbe, 2004) and the dopamine D4 receptor (DRD4) gene and catechol-O-methyltransferase (COMT) gene (Krämer et al., 2007). However, Krämer et al. (2007) did find an effect of the DRD4 polymorphism on slowing following failed attempts to stop a response. Finally, mixed results have been reported for the dopamine antagonist haloperidol (de Bruijn, Sabbe, Hulstijn, Ruitg, & Verkes, 2006; Wardle, Yang, & de Wit, 2012), possibly due to differences in dose. Altogether, we cannot fully exclude the possibility that our main finding reflects genetic differences in dopamine levels.

The tentative relationship we observed between PES size and DBH5′-ins/del polymorphism is consistent with the hypothesis that post-error adjustments are related to the activity of the LC–NE system.

5.1. Limitations

One main limitation of the current study is that it employs a very small sample size with regards to studies that examine association with genetic variation. As a result, it will be important to replicate these preliminary results with an independent sample, that is larger in size. In addition, future behavioral-genetic studies may attempt to isolate different causes of PES, for example by varying response-stimulus interval (Jentzsch & Duschig, 2009) and error likelihood (Notebaert et al., 2009), and examine if DjH genotype causes differential sensitivity to these factors.

A second major limitation is that we did not examine potential physiological correlates of post-error slowing. Future studies, including physiological measures such as EEG or pupil diameter, need to investigate the neural mechanism by which NE affects PES. Informative scalp-EEG measures include the P300 (Nunez Castellar et al., 2010) and EEG alpha power, two cortical correlates of the orienting response and increased LC–NE activity (Nieuwenhuis et al., 2011; Swick et al., 1994; Swick et al., 1994).

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