Impact of aerobic exercise training on cognitive functions and affect associated to the COMT polymorphism in young adults

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A B S T R A C T

Physical fitness can serve as a means to enhance cognitive functioning by modulating particular aspects of brain functioning. However, mechanisms underlying this modulating effect remain widely unresolved. To examine the impact and to clarify the mechanisms of physical fitness training in a young and healthy population, it was investigated whether an increase in fitness would result in improvements in executive control processes and positive and negative affect. Moreover, genotype of the Val158Met polymorphism in catechol-O-methyltransferase (COMT) as an index of relative central dopamine bioavailability was determined to elucidate dopamine tuning efficiency and its association with performance in the applied cognitive tasks. Seventy-five individuals participated and underwent an incremental fitness test to assess physical fitness. An exercising group subsequently engaged in a 17 weeks running training consisting of three running sessions at moderate to high, individually adjusted intensities. Associated with increased fitness improved cognitive flexibility and cognitive control were observed, whereas working memory remained unaffected. In runners, Val/Val participants improved cognitive performance to a greater extent compared to individuals carrying a Met allele. From the present results it is concluded that an increase in physical fitness provides a means to improve cognitive functioning via dopaminergic modulation.

1. Introduction

Engaging in regular physical exercise has been associated with a number of beneficial effects on physical and mental health. Throughout the adult lifespan physical fitness is associated with reduced risk of chronic diseases including cardiovascular diseases, colon and breast cancer, arthritis and diabetes and has the potential reduce morbidity and mortality associated with these chronic diseases. Physically fit individuals show a reduced risk of age-related neurocognitive diseases like Parkinson and dementia compared to those exercising infrequently or not at all (Mensink, 2003; US Department of Health and Human Services, 2000). Furthermore, mental disorders such as depression and anxiety seem to benefit from increase in physical fitness which is in the meantime successfully utilized in therapy of affective disorders (Fox,

1999). Moreover, an emerging body of evidence suggests moderate to strong associations between physical fitness, cognition and human brain function. Previous research indicates that aerobically fit individuals perform better on a variety of tasks with the greatest benefits occurring for visuospatial processes and executive functions (Colcombe & Kramer, 2003; Hillman, Erickson, & Kramer, 2008). Executive functions represent a theorized "higher" cognitive system that controls and manages other cognitive processes such as concept formation, working memory, attentional control, cognitive flexibility and initiation of appropriate actions/inhibition of inappropriate actions. The executive functions rely on the prefrontal cortex (Royall et al., 2002). The association of acute exercise with cognitive, particularly prefrontal cortex functions is assumed to follow an inverted U-shape, with exercise improving performance as intensity increases up to an optimal level, followed by deterioration of performance as exercise intensity further increases (Kamijo et al., 2004).

The mechanisms underlying cognitive improvement associated with increase in physical fitness in humans are still unresolved and have not been comprehensively investigated, such that to date

results from animal studies serve as a basis for understanding the positive effects observed. For example, regular physical exercise (i.e. mainly wheel-running) has been shown to stimulate brain vascularization (Kleim, Jones, & Schallert, 2003; Kleim et al., 2007), increase levels of brain catecholamines, particularly dopamine and noradrenalin (Sutoo & Akiyama, 2003), and brain-derived neurotrophic factor (BDNF), which in turn increase neuronal survival (Berchtold, Castello, & Cotman, 2010; Vaynman & Gomez-Pinilla, 2005) and neurogenesis (Creer, Romberg, Saksida, van Praag, & Bussey, 2010; Van Praag, Kempermann, & Gage, 1999). In the animal, these changes create a basis for better learning, retention and performance, leading to a more efficient, plastic and adaptive brain (Van Praag, Shubert, Zhao, & Gage, 2005). In humans, on the other hand, the association between improvement in cognitive functions and exercise-induced changes in the brain has not yet been thoroughly investigated, but the prefrontal cortex and dopamine (DA) seem to play a critical role. Winter and colleagues (Winter et al., 2007) conducted a study using peripheral measures as indicators of central levels of catecholamines (e.g. dopamine, epinephrine) as well as brain-derived neurotrophic factor (BDNF), assuming that brain and systemic dopamine levels respond similarly to physical exercise. They observed a positive linear correlation of elevated levels of peripheral dopamine and epinephrine with better retention of learned verbal material. The authors conclude that BDNF, dopamine and epinephrine may be mediators through which physical exercise improves learning (Winter et al., 2007). However, other studies documented no changes in brain catecholamines, and the only study to examine dopamine during exercise in humans via PET scans reported no significant differences in striatal dopamine release immediately subsequent to exercise (Wang et al., 2000). Overall, the role of dopamine for learning has been studied extensively (Arias-Carrion & Poppel, 2007) and is supported by the observation that learning outcome in humans can be improved pharmacologically by administration of the dopamine precursor levodopa (Knecht et al., 2004). Dopamine modulates working memory by controlling the dorsolateral prefrontal cortical circuits (Tanaka, 2006), that is part of the internal reward system (Schultz, 2005), and modulates arousal and attention and the cognitive control of behavior (Cools, 2008). Beyond learning, affect has also been shown to depend on dopamine signalling, associating experiences of reward with positive affect (Ashby, Isen, & Turken, 1999). It has been proposed that positive affect is associated with elevated brain dopamine levels and that cognitive performance benefits from enhanced positive affect through altered dopamine signalling (Ashby et al., 1999). However, research linking positive affect and dopamine is still sparse. Research on impact of COMT genotype an mood and positive affect is limited to mood disorders and has so far yielded inconsistent results (for review see (Opmeer, Kortekaas, & Aleman, 2010). In the present study, positive affect was expected to benefit from the exercise intervention (Stroth, Hille, Spitzer, & Reinhardt, 2009).

Measuring central dopamine levels in humans is problematic since dopamine cannot cross the blood–brain-barrier (Volkow et al., 1996). On the genetic level, a functional polymorphism related to dopamine metabolism – the catechol-*O*-methyltransferase (COMT) – can be used as an indicator of central dopamine availability. The COMT is an enzyme that plays a crucial role in the metabolism of DA by inactivating it in the synaptic cleft. The COMT gene contains a functional polymorphism resulting from a substitution of valine (Val) by methionine (Met) at codon 158 of the COMT gene (Val158Met) located at the q11 band of human chromosome 22. The Met allele is associated with a 3- to 4-fold reduction in activity of the COMT enzyme. Thus, the Val allele is associated with higher enzymatic activity and consequently lower extracellular dopamine levels, while heterozygotes (Val/Met genotype) have intermediate levels of COMT activity (Lachman et al.,

1996). Animal studies show that the COMT enzyme, by catabolizing DA regulates its neurotransmission in subcortical and cortical (particularly prefrontal) brain areas, thereby exerting influence on general levels of DA signalling (Bilder, Volavka, Lachman, & Grace, 2004). From these findings it is inferred that carrying the Val allele would result in lower prefrontal DA levels. Accordingly, an association between the Met allele and better performance in prefrontally processed executive functions (typically indexed by the Wisconsin Card Sorting Test, WCST) and working memory (evaluated with the N-back task) was hypothesized (Egan et al., 2001). This association should become apparent in Met/Met individuals performing best and Val/Val individuals performing poorest, as Met/Met individuals should have an optimal level of dopamine signalling (Tunbridge, Harrison, & Weinberger, 2006). The role of COMT functional variation in cognition is supported by several studies (Goldman, Weinberger, Malhotra, & Goldberg, 2009), whereas others do not find an association between the COMT genotype and cognitive performance (Barnett, Scoriels, & Munafo, 2008). In a task that requires working memory as well as inhibition of a prepotent response (as parts of the executive functions), it has been shown that results depend on the COMT genotype, with Met carriers significantly outperforming their Val/Val counterparts (Diamond, Briand, Fossella, & Gehlbach, 2004). Previous reports suggest that cognitive processes such as information updating and maintenance in the face of competing stimuli (interference) are sensitive to prefrontal dopamine levels which in turn are modulated by COMT activity (Goldman et al., 2009). On the other hand, dopamine does not always enhance cognitive performance and may in fact constrain it. The relationship of dopamine levels and cognitive performance seems to follow an inverted U-shaped form, with levels below as well as beyond an optimum impairing prefrontal function (Cools & Robbins, 2004). Given that the relationship between exercise intensity and cognitive performance as well as dopamine levels and cognitive performance are characterized by a U-shaped curves and given that dopamine plays an essential role in prefrontal brain functions and exercise having the potential to enhance prefrontal functioning, we hypothesized that positive effects related to an increase in physical fitness may be partly mediated by dopamine. This lead to the hypothesis, that exercise unfolds its beneficial effect on cognitive performance depending on the COMT genotype: we hypothesized that within cognitive tasks requiring prefrontal functioning, particularly Val/Val individuals would benefit from exercise training by elevation of prefrontal dopamine availability related to improvement in physical fitness, whereas Met carriers may not (or to a lesser extent) benefit from exercise as they are assumed to posses optimal levels of dopamine.

2. Method

2.1. Participants

The study sample consisted of 75 participants. Participants were recruited via placards and personal invitation and signed up autonomously to the running or the control group. Forty-seven participants assigned to the running group (41 female, 6 male) and 28 participants assigned to the control group (23 female, 5 male). The quasi-experimental approach was chosen after the majority of participants had declined randomization procedure due to a desire to engage in exercise training. Age of participants ranged from 17 to 47 years (M = 22.7 ± 5.7). Exclusion criteria comprised history of head trauma, drug or alcohol abuse, history of neurological or psychiatric medical diseases, as well as intake of medication affecting the central nervous system. One female student had to be excluded according to these criteria. All participants provided written informed consent, or written informed

consent was provided by the participants' legal guardian, in accordance with the institutional ethical review board at the University of Ulm.

For both treatment groups, Chi-sq test confirmed that the observed genotype distribution was consistent with that expected under Hardy–Weinberg-Equilibrium (Chi-sq < 3.84, $p > .05$). COMT alleles were distributed between groups as follows: Runners: 14 Val/Val, 7 Met/Met, 26 Val/Met (Chi-sq = .81, $p > .05$). Controls: 9 Val/Val, 4 Met/Met, 15 Val/Met (Chi-sq = .32, $p > .05$). Taking into account the small groups within the present population, we decided to group participants as Val/Val versus Met carriers, the latter including Met/Met and Val/Met genotypes) for further analyses, following previous studies that did not find significant differences in cognitive performance between Met/Met and Val/Met individuals (Bosia et al., 2007; Rosa et al., 2004).

Participants' characteristics are presented in Table 1.

2.2. Fitness testing

Fitness testing took place on a weekend at the beginning and end of the training period respectively. Participants were scheduled groupwise (six participants in each group) and the schedule of pre-intervention testing was repeated for the post-intervention testing at the same time of the day to avoid circadian distortions.

Individual physical fitness was assessed by a maximal field track test to determine the individual workload level at lactate threshold for each participant. The participants underwent an incremental step test until volitional exhaustion, performed on an outdoor track of 400 m laps. Starting at 4 km/h, running velocity was increased by 2 km/h for each step. Step duration was 3 min. Running velocity was controlled by an experimenter who signaled pace at given time intervals. Before and during the test, capillary blood samples were taken from the hyperaemic earlobe (Finalgon forte, Thomae, Biberach, Germany) within 15–30 s after each step of exercise intensity, as well as subsequent to the test within 1st, 3rd, and 5th min of recovery phase. Concentration of lactate in the blood samples was analyzed following the test procedure using a glucose-/lactate-analyzer (BIOSEN C_line Sport, EKF Diagnostics). Running velocities and heart rates at the fixed lactate concentration of 4 mmol/l were calculated by means of mathematic interpolation (Mader et al., 1976; Roecker et al., 2002). Heart rate was controlled by a monitor comprising a chest transmitter as well as a receiver unit worn on the wrist (Polar Electro®, Buettelborn, Germany, Model F6). Heart rate and concentration of lactate at every level of intensity were documented on an individual record sheet for each participant.

Table 1
Participants' characteristics.

	Genotype	Age (years)	Height (cm)	Weight (kg)	v4 mmol/l
Runners	Met (33)	21.6 ± 3.4	168.6 ± 5.7	64.0 ± 10.5	9.4 ± 1.5
			168.4 ± 5.7	63.9 ± 10.5	9.9 ± 1.4
	Val (14)	21.1 ± 3.1	165.5 ± 8.1	61.2 ± 10.1	8.6 ± 1.7
			165.5 ± 8.1	60.1 ± 9.4	9.0 ± 1.7
Controls	Met (19)	22.6 ± 5.7	171.0 ± 9.7	66.7 ± 13.2	8.4 ± 1.1
			171.0 ± 9.6	66.3 ± 14.6	8.5 ± 1.1
	Val (9)	23.5 ± 3.9	169.0 ± 7.3	70.4 ± 15.8	8.8 ± 1.7
			169.0 ± 7.3	71.8 ± 15.6	8.7 ± 1.5

Participants' characteristics including age, height, weight and running speed at individual lactate threshold (4 mmol/l lactate) for all participants. Groups of runners and controls are subdivided into Val and Met carriers at pre- and post-interventional testing ($t1/t2$). Depicted as means (M) and standard deviations (SD).

2.3. Running training

Participants were assigned to five "fitness groups" according to their baseline physical fitness level, with two low fit groups starting the training with walking instead of running sessions. Relating to performance in the fitness test, lactate thresholds and corresponding heart rates were used to prescribe a 4 months aerobic training program of progressively increasing intensity and duration. Training took place outdoors within a defined area, leaving the precise course to the participants' choice. Participants were required to complete 50 sessions within 4 months (about three sessions per week). They were requested not to exercise more than one session per day and not more than four sessions per week.

Of the essential training variables, exercise intensity and its distribution are probably the most critical. The workout was planned by an expert graduate sport scientist (MS) according to intensity at the lactate threshold, representing transition from aerobic to anaerobic workout (Roecker, Striegel, & Dickhuth, 2003; Wasserman & McIlroy, 1964). Referring to this threshold model, training zones have been recommended (Noakes, 2001), and standardized intensity-zone scales consisting of up to five different zones have been implemented (Zintl, 1994). Accordingly, national and international sports governing bodies have published exercise guidelines (e.g. see ACSM Position Stand, 1998) which served as the basis for the individual training plans.

In order to track the course of training attendance, intensities and progress, participants received a heart rate monitor comprising a chest transmitter as well as a receiver unit worn on the wrist. The latter was also used as a storing device for individual heart rate intervals and running session information, such as "time in zone" and "mean heart rate" (Polar Electro®, Buettelborn, Germany, Model F6). By means of this monitor, participants were able to control their training according to an individual heart rate zone ("own zone") and to document each training session in terms of a paper diary according to the stored data. This diary was gathered and evaluated at the end of the training intervention. Participants who fell behind with their training were encouraged to engage in training again afterwards and to carefully document the interruption within the diary. Program adherence was monitored via weekly email correspondence and participants were invited to contact the investigators at any time.

2.4. Testing of affect and cognition

Testing of cognitive and affective variables took place during the same weekend as the fitness test. Runners as well as controls gathered regardless of group affiliation, leaving the investigators unaware of the group status of each participant until statistical analyses. Cognitive testing preceded the exercise testing to insure that no influence of the acute bout of exercise would bias cognitive performance. Cognitive measures were chosen based on the hypothesis that regular exercise induces performance improvements in tasks of executive functions and working memory. The following instruments were administered:

To assess working memory, participants performed a *2-back task* which is an established paradigm to study focal attention in working memory (McElree, 2001). The task was taken from the TAP (Test for Attentional Performance.) (Zimmermann & Fimm, 1995) and involved the comparison of a current stimulus with a stimulus that was presented two stimuli before in a sequential presentation. With every new item appearing, the focus of attention had to be switched to a new 2-back item. Thirty-four targets out of 200 stimuli were presented in random order with the only restriction that targets could not be presented back to back. Presentation time was 1500 ms and intertrial-interval was 1000 ms.

The *Stroop task* requires inhibition of a compelling response. The classic effect (known as the Stroop interference effect) is that the latency before naming the colour in which a word is printed increases when this word semantically represents a different colour, incongruent with the printed colour (i.e. the word blue printed in green), relative to the baseline condition where there is no incongruence (i.e. the word blue printed in blue). The Stroop effect provides evidence of difficulty in inhibiting an overlearned response, such as the automatic reading process (Stroop, 1935). Three categories of stimuli were presented to participants: congruent, neutral and incongruent, consisting of 33 stimuli each, appearing in random order. Presentation of stimuli was terminated by the participants' response, and remained on the screen for a maximum of 1500 ms. Intertrial-interval was 1000 ms.

The *Dots-mixed task* is a measure of the ability to ignore task-irrelevant stimuli, indicated by the difference between reaction time on response-compatible and response-incompatible trials (Diamond et al., 2004). Participants have to remember two rules (working memory) and inhibit the tendency to respond on the same side as the stimulus (interference) on one-half of the trials. In the present study, the task consisted of three blocks of 20 trials, with one block of congruent (same side), incongruent (opposite side) and mixed trials, respectively. Blocks were separated by a pause that was terminated by the participants. Stimuli were presented for 750 ms, intertrial-interval was 500 ms.

Measures of affect were chosen based on the hypothesis that exercise shows impact on affect, leading to enhanced positive and diminished negative affect.

The positive and negative affect schedule (PANAS), German Version (Krohne, Egloff, Kohlmann, & Tausch, 1996) is a 20-item self-report of positive and negative affect. The PANAS is meant to assess current moodstates. Participants are asked about their general feelings or emotions, for 10 positive (e.g. interested, excited) and 10 negative (e.g. distressed, guilty) adjectives. Each adjective is rated on a 5-point scale from "very slightly or not at all," to "extremely." Participants were asked to base their judgments on "last week". The internal consistencies of the PANAS scales are $\alpha = 0.89$ for the positive affect (PA) scale, and $\alpha = 0.85$ for the negative affect (NA) scale.

2.5. COMT genotyping

A venous blood sample was collected from each participant for genotypic analysis. Genomic DNA was extracted using a QIAamp DSP DNA Blood Mini Kit (QIAGEN, Germany). PCR was performed with the following primers: 5′-TCA CCA TCG AGA TCA ACC CC-3′ and 5′-GAA CGT GGT TGT AAC ACC TG-3′.

PCR amplification consisted of initial denaturation at 95 °C for 5 min followed by 40 cycles at 95 °C (30 s), 58 °C (30 s) and 72° (1 min) before a final extension step at 72 °C for 10 min. PCR products (176 bp) were digested using the enzyme NlaIII (New England Biolabs, Germany). Fragments were separated by 8% polyacrylamide gel electrophoresis (PAGE), stained with Ethidiumbromide and visualized by ultraviolet light. Homozygotes for the Val158 variant gave fragments of 83, 54 and 39 bp, heterozygotes gave fragments of 83, 65, 54 and 39 bp and homozygotes for Met158 gave fragments of 65, 54 and 39 bp.

2.6. Missing data

Initially 106 students participated in the pre-interventional tests to assess physical fitness as well as performance in cognitive measures. Thirty-one individuals dropped out during training. The observed drop-out rate of 29%, is considered acceptable, as it has been observed similarly in other interventional studies on exercise training (Annesi & Unruh, 2004; Baquet, van Praagh, & Berthoin, 2003; Flegal, Kishiyama, Zajdel, Haas, & Oken, 2007). After controlling for group differences between drop-outs and completers (no differences observed), all analyses were calculated on completers. Six participants did not yield interpretable results in genotyping and were also excluded.

3. Statistical analyses

Statistical analyses were carried out using STATISTICA for Windows, Version 7.1 (Statsoft Inc., Tusla, OK, USA, 2005). ANOVA for repeated measures was conducted to analyze the influence of aerobic training on cognition and affect in the running group compared to the control group, accounting for the two COMT genotypes (Val/Val and Met carriers). It contains two levels of the between-subject factor "group" (runners vs. controls), two levels of genotype (Val/Val vs. Met carriers) and two levels of the within-subject factor "time" (pre- and post-intervention). For dots-mixed and stroop tasks, the factor congruency (congruent vs. incongruent trials) was added. A series of 2(group) × 2(genotype) × 2(time) ANOVA for PANAS and 2-back tasks with repeated measures on the factor time was conducted. A series of 2(group) × 2(genotype) × 2(response mode) × 2(time) was conducted for dots-mixed and stroop task.

To control for moderating effects of positive affect (PA), analyses of covariance with gain of PA as covariate were conducted for each measure of cognitive performance. Since no significant influence of PA was found in any of the analyses (p's > .40), we only report results from the ANOVAs.

To control for potential group differences between drop-outs and completers, simple comparisons of means were conducted for all measures of cognitive performance at baseline. Since no deviant score was found for any of the variables (all t's < 1.5, p's > .21), further analyses were conducted on completers only. Due to a large range of age a 2(group) × 2(genotype) ANOVA was conducted to control for potential differences between groups.

Level of statistical significance was set at $p < 0.05$. Significant interactions were post-hoc decomposed into their simple effects, using a Bonferroni alpha correction for multiple testing.

4. Results

Analyzing age, no difference between runners and controls or Val/Val and Met carriers was observed (all p's > .12). For participants' cognitive performance scores see Table 2.

4.1. Physical fitness data

Fitness was analyzed as running speed at the lactate threshold (4 mmol/l). Non-significant main effects of group and genotype indicated absence of differences in fitness between runners and controls as well as Val/Val and Met carriers (p's > .10). A significant interaction between group and time showed greater fitness-improvement in runners compared to controls ($F(1, 73) = 11.4$, $p < .01$). Post-hoc analyses revealed a non-significant trend of higher fitness in runners as compared to controls at baseline assessment ($p > .08$) and a significant difference post training ($p < .05$). There was a significant improvement in fitness in Val/Val as well as Met runners ($p < .01$) observed, but no such improvement in controls (p's > .70).

For *positive affect* (PA) a significant interaction of time and group indicated that runners increased positive affect compared to controls ($F(1, 73) = 6.16$; $p < .05$). The post-hoc analysis showed improved positive affect only in runners ($p < .001$) but not controls ($p > .40$). All effects involving the factor genotype were not significant (p's > .40).

Table 2
Participants' cognitive performance scores.

		Runners								Controls							
		MET carriers		VAL/VAL		MET/MET		MET/VAL		MET carriers		VAL/VAL		MET/ME		MET/VAL	
		(M)	(SD)	(M)	(SD)	(M)	(SD)	(M)	(SD)	(M)	(SD)	(M)	(SD)	(M)	(SD)	(M)	(SD)
PANAS	PA ($t1$)	32.8	6.4	32.3	5.3	35.0	7.1	32.0	6.0	33.2	5.4	32.0	5.2	30.5	5.2	34.3	5.3
	PA ($t2$)	35.5	6.8	36.5	7.6	36.5	8.8	35.1	6.0	32.3	5.0	32.4	5.8	30.5	5.3	33.1	4.9
	NA ($t1$)	18.0	4.7	18.1	3.0	18.6	4.2	17.7	4.9	19.6	4.9	17.8	3.2	21.3	5.7	18.9	4.6
	NA ($t2$)	18.1	5.7	18.2	5.2	21.4	6.3	17.0	5.1	18.1	6.0	17.3	4.6	23.3	8.5	15.8	2.6
2-back (RT)	2-back ($t1$)	6046.4	1186.7	6701.5	935.8	5707.0	1173.4	6170.9	1186.6	5727.2	965.8	6712.1	1210.6	5481.9	864.2	5836.2	1037.2
	2-back ($t2$)	5877.0	1384.0	5676.0	901.0	5532.0	1032.6	6003.5	1487.4	5911.2	933.1	5954.1	1524.5	5775.0	781.0	5971.7	1031.5
Stroop task (RT)	Con ($t1$)	7273.7	1228.8	7871.3	996.0	7060.7	1126.1	7351.8	1273.6	6798.0	989.8	7709.5	1278.4	6162.1	682.3	7080.6	1001.5
	Con ($t1$)	6495.5	768.7	6177.2	546.8	6434.1	679.2	6518.0	808.7	6151.1	470.9	6845.2	841.6	5901.1	306.7	6262.2	502.1
	Neutral ($t1$)	7298.1	1260.8	7629.4	927.8	7088.7	1066.3	7374.8	1333.4	7156.8	914.2	7839.2	1183.7	6575.6	662.1	7415.1	919.4
	Neutral ($t2$)	6558.1	695.1	6544.1	611.5	6398.9	617.4	6616.4	722.5	6191.3	627.5	6901.2	779.7	6105.0	263.2	6229.6	747.9
	Incon ($t1$)	8291.6	1397.1	8355.3	1343.4	8319.2	1597.0	8281.5	1346.2	7586.8	1073.3	8404.2	1307.6	6892.9	1166.2	7895.1	932.8
	Incon ($t2$)	6843.0	870.0	6916.5	719.6	6739.4	900.9	6881.0	871.0	6558.2	586.6	7068.1	798.0	6213.4	172.9	6711.4	647.4
Dots-mixed (RT)	Con ($t1$)	3873.7	576.3	3931.4	394.6	3923.6	488.1	3855.4	612.1	3675.2	374.6	3858.4	459.9	3737.1	556.4	3647.7	302.7
	Con ($t2$)	3662.8	496.7	3653.1	360.0	3733.4	396.1	3636.9	532.5	3499.9	321.2	3901.4	694.8	3334.8	323.2	3573.3	309.6
	Incon ($t1$)	4315.2	951.1	4422.0	498.3	4257.0	646.5	4336.5	1049.6	3973.9	457.3	4451.8	605.5	3898.7	559.7	4007.4	438.4
	Incon ($t2$)	4063.1	737.5	3887.4	415.8	4083.4	585.6	4055.6	794.8	3943.5	365.3	4307.7	782.1	3866.6	443.5	3977.8	349.4

Participants' cognitive performance scores for working memory (2-back), inhibitory control (Stroop task), cognitive flexibility (dots-mixed) and affect (PANAS) separately. Groups of runners and controls are subdivided into Val/Val and Met carriers at pre- and post-interventional testing ($t1/t2$). Scores of Val/Met and Met/Met individuals are supplemented for descriptive purposes. Depicted as means (M) and standard deviations (SD).
Val = valine variant allele.
Met = methionine variant allele.
Con = congruent trials.
Incon = incongruent trials.
Neutral = neutral trials.

Negative affect (NA) was not significantly modulated by any factor (all p's > .35).

Analysis of the *2-back working memory task* was conducted for correct responses (hits) and reaction time (RT) of correct responses. Number of correct responses increased numerically, but this main effect of time reached only trend level ($F(1, 73) = 2.77$, $p < 0.10$). Analysis of reaction times (RT) revealed a significant main effect of time showing an overall reduction of RT ($F(1, 73) = 9.1$, $p < .01$). Val/Val significantly reduced RT compared to Met carriers as indexed by a significant interaction between genotype and time ($F(1, 73) = 8.41$, $p < .05$). As revealed by post-hoc tests, only runners carrying a Val allele decreased RT significantly ($p < .05$), while no effect was observed in Val/Val or Met controls or Met runners (p's > .50) (see Fig. 1).

Analysis of the *Stroop Task* included number of correct responses (hits) and reaction times of hits for congruent, neutral and incongruent trials, respectively. Trends of genotype ($F(1, 73) = 3.1$, $p < .08$) and response mode ($F(1, 73) = 2.4$, $p > .09$) indicated that Met individuals performed better than Val/Val and that participants' scored best in congruent trials. Bonferroni corrected post-hoc test did not reveal any significant differences between groups. Regarding RT, Val/Val showed numerically larger RT compared to Met carriers, but this main effect of genotype reached only trend level ($F(1, 73) = 3.6$, $p < .06$). A significant main effect of response mode indicated significantly larger RT in incongruent trials compared to neutral and congruent trials ($F(1, 73) = 67.2$, $p < .001$). A significant interaction of response mode, time and group revealed that in congruent trials only runners showed reduction of RT compared to neutral and incongruent trials ($F(1, 73) = 4.8$, $p < .01$). A significant interaction of response mode, time and genotype indicated that especially Val/Val showed a reduction in RT in congruent trials compared to neutral and incongruent trials ($F(1, 73) = 4.1$, $p < .05$). A significant fourfold interaction showed that particularly Val/Val runners significantly reduced RT in congruent and neutral trials ($F(1, 73) = 5.8$, $p < .01$). Post-hoc analyses revealed reduction of RT from pre- to post-intervention testing in congruent trials for Met runners ($p < .05$) as well as Val/Val runners ($p < .01$). In neutral trials, a trend was observed for both genotypes (p's < .10), whereas in incongruent trials no reduction of RT was found for either genotype (p's < .30) (see Fig. 2).

Analysis of the *dots-mixed-task* included number of correct responses (hits) and reaction times of correct responses for congruent and incongruent trials respectively. Analysis of hits revealed a significant interaction of time and group ($F(1, 73) = 4.3$, $p < .05$) indicating gain in correct responses in runners, absent in controls. However, Bonferroni corrected post-hoc test did not show significant differences between groups (p's > .27). Analysis of RT showed a main effect of response mode with shorter RT in congruent compared to incongruent trials ($F(1, 73) = 45.2$, $p < .001$). Main effect of time revealed significantly shorter RT in post- compared to pre-intervention assessment ($F(1, 73) = 14.3$, $p < .001$). A significant interaction of time and group indicated reduction of RT only in runners, but not in controls ($F(1, 73) = 5.3$, $p < .05$). Greatest
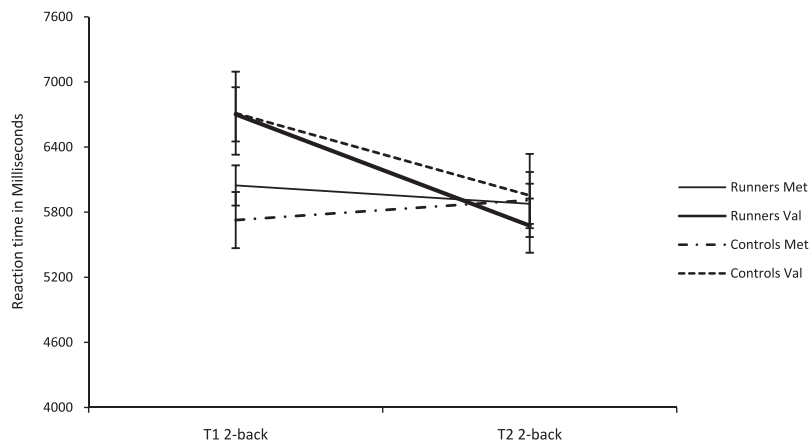

Fig. 1. Reaction times (RT) of correct responses in 2-back working memory task. Mean RT of runners and controls subdivided into Met and Val carriers are shown. Errorbars depict standard error of means. T1 = pre training, T1 = post training.
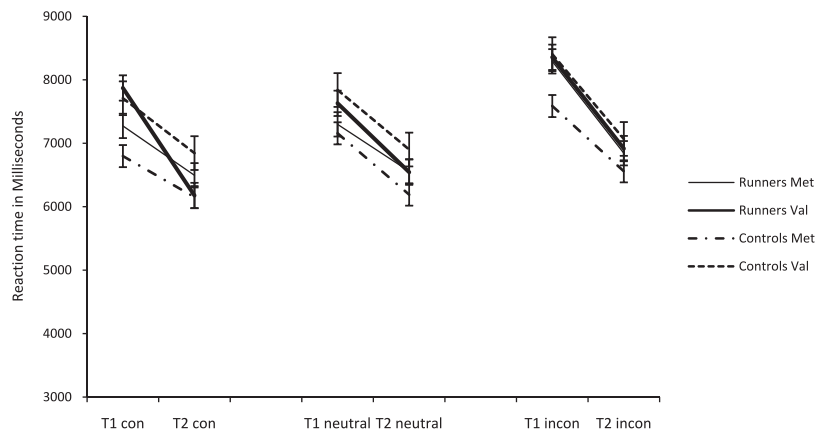

Fig. 2. Reaction times (RT) in congruent, neutral and incongruent trials of the *Stroop task*. Mean RT of runners and controls subdivided into Met and Val carriers are shown. Errorbars depict standard error of means. T1 = pre training, T1 = post training. Con = congruent trials, incon = incongruent trials.
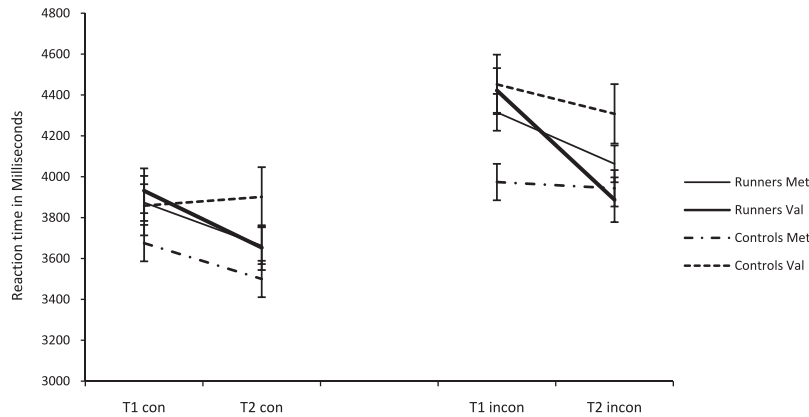
Fig. 3. Reaction times (RT) in congruent and incongruent trials of the *dots-mixed task*. Mean RT of runners and controls subdivided into Met and Val carriers are shown. Errorbars depict standard error of means. T1 = pre training, T1 = post training. con = congruent trials, incon = incongruent trials.

improvement was observed in Val/Val runners in incongruent trials ($p < .001$), but also significant improvements in Met and Val/Val runners in both response modes were seen (p's < .01). Neither main effect of group nor genotype reached significance (p's > .20), and although Met as well as Val/Val runners significantly improved performance, post-hoc analyses revealed no significant differences between runners and controls (p's > .54) or Val/Val and Met carriers (p's > .44), respectively (see Fig. 3).

5. Discussion

In the present study we found that a four-month exercise training enhanced physical fitness of runners compared to non-running controls, associated with a rise in positive affect and an increase in cognitive control as well as cognitive flexibility, whereas working memory remained unaffected. For the first time, we here compared individuals homozygote for the Val158 variant to individuals carrying a Met allele who engaged in a running training regimen with respect to effects on cognitive performance. We found a pronounced benefit of increased physical fitness in Val/Val individuals. To facilitate interpretation of our findings, we will discuss the results separately for each test and will then offer an integrated discussion of the postulated modulatory effects of exercise and the COMT genotype on cognition and affect, as well as the interaction of both.

In the present study, the exercising group significantly increased physical fitness within a four-month training period. Although improvement of fitness was evident, it did not show linear correlation with enhancement of cognitive performance. The present results are in line with the conclusion of a recent meta-analysis: Neither in cross-sectional nor long-term studies a linear relationship between exercise and cognition was observed, leading to the conclusion that mediators of the relationship need to be found (Etnier, Nowell, Landers, & Sibley, 2006).

Performance in the Stroop task showed a trend towards association with the COMT genotype, with Met-carriers outperforming their Val/Val counterparts, although this did not reach statistical significance. Depending on the genotype, performance was modulated by increase in physical fitness. Subsequent to training, runners homozygous for Val showed greater facilitation effects as indexed by shorter reaction times. Facilitation represents a priming effect with the word (irrelevant stimulus dimension) priming the colour. This effect involves attentional processes and opposes the interference effect (Roelofs, 2010). Although runners exhibited increased task performance in terms of faster reactions times compared to controls, the initially proposed effect that particularly incongruent trials, placing highest demands on executive functions

(i.e. inhibitory control), show the greatest benefit from exercise, was not confirmed. Kramer and colleagues (Kramer et al., 1999) had shown in an elderly sample that physical training was accompanied by decreased reaction times only for incompatible task material, whereas no positive exercise-related effects were observed in compatible trials. To our knowledge, no observations on exercise in relation to Stroop performance in a young population have been reported to date. We therefore propose that in the elderly, where an age-related decline of cognitive functioning is assumed to occur, fitness-related benefits on task performance may differ substantially in young and healthy subjects. The fact that Val/Val in particular showed a reduction in RT in stroop tasks corroborates our hypothesis that dopamine serves as a mediator in the relationship between exercise and cognitive functioning.

As a measure of inhibitory control and working memory, the dots-mixed task (Diamond et al., 2004) revealed an improvement in runners' performance as compared to controls. As hypothesized, particularly within the incongruent task condition, runners were able to improve performance significantly. Findings of the present study support the hypothesis of "selective improvement" introduced by Kramer and colleagues (Colcombe & Kramer, 2003) showing that particularly higher cognitive functions (requiring executive control and relying on the prefrontal cortex) benefit from increase in physical fitness and exercise training, respectively. Beyond the finding that runners improve cognitive performance it was observed that Val/Val runners exhibited greatest augmentation of performance.

Results of the present study indicate that genotype and to a lesser extent exercise training impact RT in a working memory task. At baseline, Val/Val exhibited numerically longer RT on a trend level, but by repeating the test, only Val/Val, particularly within the running group, were able to significantly improve performance. Regarding correct responses, we did not find significant effects of either exercise training or genotype. Although functional neuroimaging suggests that in Met carriers prefrontal working memory functions operate on a more efficient basis (Egan et al., 2001), findings from behavioural studies do not allow for conclusive association of working memory and the COMT genotype (Bruder et al., 2005). There are numerous studies reporting an advantageous effect of the Met allele on prefrontal functioning as revealed by neuropsychological as well as neuroimaging data (Goldman et al., 2009; Tunbridge et al., 2006). Weinberger and colleagues describe the relationship between cortical dopamine and prefrontal cortex function as an inverted U-shape, with the effect of COMT activity depending on the individual basic dopaminergic tone on this curve. The authors suggest that in healthy individuals, homozygous Met carriers are located around the peak

of the curve and Val/Val located further down along the curve's rising "left" arm, accounting for the observed positive influence of the Met allele on cognitive functions (Tunbridge et al., 2006). In the present study, we found a trend towards an association between COMT genotype and performance in cognitive tasks, with Met-carriers outperforming their Val/Val counterparts on tasks of inhibitory control (Stroop task) and working memory (2-back task). Furthermore, greater increase in performance related to an increase in physical fitness in Val/Val as compared to Met runners was observed suggesting that exercise may entail optimization of central dopamine availability.

Confirming previous work (Stroth et al., 2009), runners showed significantly enhanced scores on positive affect within the PANAS subsequent to exercise training compared to controls, who did not report such an increase in positive affect. Negative affect remained unaffected. Thus, findings of the present study suggest that increased physical fitness does not lead to a reduction of dysphoric symptoms, but rather to a benefit in well-being through enhancement of positively valenced aspects of mood and self perception, such as perception of "strength" and "vitality". We did not find any influence of the COMT genotype on measures of affect (either positive or negative).

In the present study, we were able to include a large number of participants who completed a four-month exercise training, allowing us to collect data from a reasonably large sample, necessary for the investigation of genetic impact on cognitive functioning. There are however, a number of limitations to our study. One shortcoming lies in the lack of randomization of the study population. Studies of physical activity and exercise in humans usually have to deal with a self-selection problem of participants: people willing to participate in a long-term aerobic fitness training experiment will probably show a positive attitude towards exercise. They may even exhibit a satisfactory physical fitness status and therefore may not be representative of the general population. In the present study, we did not find a statistically significant difference of aerobic fitness at baseline, but potential self-selection biases cannot be ruled out. Therefore the results have to replicated, ideally within a randomized sample, to validate the observed association of exercise training, cognitive functioning and dopamine as one mediating variable. Furthermore, cognition is a complex trait and is therefore likely to be underpinned by many genes, each with a relatively small effect. Associations between single genes (polymorphisms) and single cognitive processes are clearly a simplification of genetic, neurobiological and cognitive subsystems that interact in complex ways, making further investigation necessary.

6. Conclusion

In summary, subsequent to a running training, associated with increased physical fitness, we found improved cognitive flexibility and cognitive control. Also working memory was partly influenced by increased physical fitness. Alteration of cognitive performance was furthermore related to the COMT genotype, with Val/Val runners improving cognitive performance to a greater extent compared to individuals carrying a Met allele. Increase in physical fitness was also accompanied by a gain of positive affect, irrespective of the COMT genotype. From the present results we conclude that the potential benefit an increase in physical fitness provides for cognitive functioning at least partly mediated by dopaminergic modulation.

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