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ORIGINAL INVESTIGATION

Serotonin and dopamine differentially affect appetitive and aversive general Pavlovian-to-instrumental transfer

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Abstract
Rationale Human motivation and decision-making is influenced by the interaction of Pavlovian and instrumental systems. The neurotransmitters dopamine and serotonin have been suggested to play a major role in motivation and decision-making, but how they affect this interaction in humans is largely unknown.
Objective We investigated the effect of these neurotransmitters in a general Pavlovian-to-instrumental transfer (PIT) task which measured the nonspecific effect of appetitive and aversive Pavlovian cues on instrumental responses.
Methods For that purpose, we used selective dietary depletion of the amino acid precursors of serotonin and dopamine: tryptophan (n=34) and tyrosine/phenylalanine (n=35), respectively, and compared the performance of these groups to a control group (n=34) receiving a nondepleted (balanced) amino acid drink.
Results We found that PIT differed between groups: Relative to the control group that exhibited only appetitive PIT, we found reduced appetitive PIT in the tyrosine/phenylalanine-depleted group and enhanced aversive PIT in the tryptophan-depleted group.
Conclusions These results demonstrate a differential involvement of serotonin and dopamine in motivated behavior. They suggest that reductions in serotonin enhance the motivational influence of aversive stimuli on instrumental behavior and do not affect the influence of appetitive stimuli, while reductions in dopamine diminish the influence of appetitive stimuli. No conclusions could be drawn about how dopamine affects the influence of aversive stimuli. The interplay of both neurotransmitter systems allows for flexible and adaptive responses depending on the behavioral context.

Keywords Decision-making · Motivation · Pavlovian-to-instrumental transfer · Serotonin · Dopamine · Tryptophan depletion · Tyrosine depletion

Introduction

Humans are constantly faced with stimuli predictive of reward or punishment. Such predictive Pavlovian associations usually arise independent of voluntary motor responses. They can, however, also affect basic motivational tendencies that become apparent as approach and avoidance behavior: For example, the sight of a beloved person on the other side of the street might lead us to cross the street, but the sound of a honking car will stop us from doing so. At the same time, Pavlovian stimuli can raise or lower our level of activation depending on their positive or negative valence, which becomes apparent in the intensity or vigor with which we carry out our responses (Braver et al. 2014).

This Pavlovian system contrasts with a separate response-contingent, instrumental learning system (Rescorla and Solomon 1967), under which learned associations do not arise without an explicit and distinct behavioral response. While these systems may act separately and in parallel, they can also interact (Dayan et al. 2006; Guitart-Masip et al. 2014). One way to investigate this interaction is to probe the motivational influence of Pavlovian stimuli on unrelated instrumental responses. This influence is known as general Pavlovian-to-instrumental transfer (PIT); *general*, because the outcome following the Pavlovian cue is not related to the outcome of the instrumental response (in contrast to specific PIT where

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the outcome is the same; Dickinson and Balleine 2002; Corbit and Balleine 2005). Typically, PIT leads to a modulation of the instrumental response through the presence of a Pavlovian stimulus and is often thought to arise from a change in the motivational state of the observer (Estes 1943; Rescorla and Solomon 1967; Holland and Gallagher 2003). Motivation has both activational and directional functions (Salamone and Correa 2012; Braver et al. 2014), where activation is related to stimulus-unspecific invigoration, typically assessed by response rate (e.g., Niv et al. 2007), and direction is related to specific response biases, typically assessed by choices. This means that on the one hand, PIT might reflect an influence of the acquired (positive or negative) valence of the Pavlovian stimulus on instrumental behavior, where a positive value enhances and a negative value diminishes motivational activation of the subject. On the other hand, PIT might be understood more at the level of behavioral responses, where the response tendency evoked by the Pavlovian stimulus (approach/avoid) biases instrumental responding (Huys et al. 2011). Often such directional Pavlovian response tendencies go in hand with general activation, making these views difficult to distinguish. Either way, PIT serves as a paradigmatic tool for investigating motivation and its influence on decision-making behavior.

PIT has first been described in rats (Estes 1943; Lovibond 1983; Colwill and Rescorla 1988), but more recently was also demonstrated in humans (Paredes-Olay et al. 2002; Hogarth et al. 2007; Allman et al. 2010; Huys et al. 2011; Nadler et al. 2011). Structures such as the amygdala, the striatum, and the prefrontal cortex have been implicated as the neural locus at which PIT exerts its effect (Holmes et al. 2010), confirmed by human neuroimaging studies (Bray et al. 2008; Talmi et al. 2008; Prévost et al. 2012; Lewis et al. 2013). Only recently, aversive general PIT has been reported in humans, with similar brain structures involved as in appetitive general PIT (Geurts et al. 2013a). Expanding PIT to the aversive domain broadens the scope of this effect: While previous PIT studies have focused on increases in instrumental behavior through appetitive Pavlovian stimuli, it has now become clear that aversive Pavlovian stimuli can also decrease instrumental responding (Huys et al. 2011; Geurts et al. 2013a), in line with a demotivating function of the Pavlovian stimulus.

The brain regions mentioned above are among the primary targets of the neurotransmitters dopamine and serotonin. Indeed, among others, dopamine has been suggested to play a central role in regulating motivational states (Salamone and Correa 2002; Wise 2004). For example, previous research in rats demonstrated that silencing of the ventral tegmental area—a brain region densely populated with dopaminergic neurons—leads to a reduction of appetitive PIT (Murschall and Hauber 2006; Corbit et al. 2007), and directly blocking dopamine receptors in the nucleus accumbens also reduces appetitive PIT (Lex and Hauber 2008). These results suggest that dopamine may play a similar role for PIT in humans, but to date causal evidence for a relationship with PIT is scarce. In addition, it is unknown whether and how this neurotransmitter relates to aversive PIT. On the one hand, it has been suggested that dopamine is particularly involved in appetitive motivation and reinforcement (Schultz 2007a). According to this view, only appetitive, but not aversive PIT should be affected by dopamine. Others have ascribed dopamine a more general role irrespective of valence (Bromberg-Martin et al. 2010). In this case, dopamine would play a role both for appetitive and aversive PIT. Taken together, the involvement of dopamine in appetitive and aversive PIT remains an open issue.

The role of serotonin in motivation is less clear. Serotonin has often been described as a counterpart to dopamine, in that dopamine is related to reward and behavioral activation, while serotonin is related to punishment and behavioral inhibition (Boureau and Dayan 2011). More specifically, it has been suggested that serotonin enhances behavioral inhibition as a natural response to aversive events (Soubrié 1986; Crockett et al. 2009; Dayan and Huys 2009). Assuming that aversive PIT arises from the influence of inhibitory Pavlovian response tendencies as described above, then reducing serotonin should weaken these tendencies, thus reduce aversive PIT. Serotonin has also been shown to decrease aversive processing (Cools et al. 2008), possibly by inhibiting aversive thought (Dayan and Huys 2008). Since aversive stimuli can reduce the level of general activation, this means that reducing serotonin might more strongly reduce the vigor of responses in the presence of aversive stimuli. This is in line with lethargy often observed in major depression which can be treated by serotonin agonists, and with experiments demonstrating reduced incentive motivation for low serotonin (Cools et al. 2005; Roiser et al. 2006). According to this view, reducing serotonin should enhance aversive PIT, i.e., evoke even less responding because of even more strongly reduced motivation. Importantly, these two views are not mutually exclusive. For example, an (involuntary) behavioral response tendency—in line with the former view—might initially be elicited, but an effect of the valence of the context on behavioral activation might come into play later in responding (see "Discussion" for details). In line with the view that serotonin acts on aversive PIT by disinhibiting Pavlovian responses to aversive stimuli, a recent study demonstrated that lowering serotonin levels can affect choices of subjects in an aversive context (Geurts et al. 2013b). Results are mixed for the involvement in appetitive PIT, with a study in rats demonstrating reduced responding to appetitive stimuli (Sanders et al. 2007), while a study in humans found no effect (Geurts et al. 2013b). In addition, whether serotonin influences general PIT in humans and whether appetitive and aversive PIT are differentially affected have remained open questions.

The goal of the present study was to investigate the relative contribution of serotonin and dopamine to appetitive and aversive general PIT in humans. For that purpose, we employed dietary depletion of amino acid precursors of the neurotransmitters serotonin and dopamine. More specifically, serotonin was temporally lowered by consumption of an amino acid drink lacking tryptophan (Young et al. 1985), and dopamine was reduced by consumption of an amino acid drink lacking tyrosine and phenylalanine (Moja et al. 1996; Sheehan et al. 1996). Both are well-established procedures (Mendelsohn et al. 2009; Young 2013): Tryptophan depletion has been shown to alter central serotonin synthesis and release in rats (Moja et al. 1989; Stancampiano et al. 1997) and mice (Biskup et al. 2012), and serotonergic metabolites are reduced after tryptophan depletion in humans (Carpenter et al. 1998). Combined tyrosine and phenylalanine depletion reduces central dopamine availability in rats without affecting noradrenaline or serotonin (McTavish et al. 1999; Le Masurier et al. 2013).

The behavioral task tested the influence of both appetitive and aversive Pavlovian conditioning on instrumental behavior. We expected that a reduction in tyrosine and phenylalanine would reduce appetitive PIT (Murschall and Hauber 2006; Corbit et al. 2007), whereas we were open with respect to all other effects. Although tryptophan depletion reduced aversive PIT in a previous study (Geurts et al. 2013b)—in line with a selective effect on behavioral inhibition—this previous study did not assess response vigor (i.e., the number or intensity of responses to a stimulus). As described above, choices might be more sensitive to short-lived inhibitory effects of aversive Pavlovian cues, while extended response vigor might be more sensitive to reduced levels of activation after these (possibly automatic) response tendencies. For that reason, it is interesting to test the generality of these results and, specifically, see if they extend to a selective manipulation of general PIT which uses response vigor, or whether serotonin might also serve to increase the demotivating effects of aversive Pavlovian cues. In addition to the effect of dopamine on appetitive PIT and serotonin on aversive PIT, the effect of dopamine on aversive and serotonin on appetitive PIT was investigated without specific expectations based on previous results.

Methods

Participants

One hundred and eight subjects participated in the study. Five subjects were excluded due to nausea and vomiting after consumption of the amino acid drink. We were unable to take blood samples from two subjects, but since there was no reason to assume they did not follow the diet, they were kept in the sample. The final sample consisted of 103 participants (44 female, 59 male) with an age of 24.22 (SD 3.25) years.

The study design was double-blind. Of the 103 participants, 34 subjects were assigned to receive the balanced amino acid drink (BAL group), 34 subjects the tryptophan depletion (TRP group), and 35 subjects the tyrosine/phenylalanine depletion (TYR group). The three groups were roughly counterbalanced with respect to age ($\chi^2(5)=0.80$, $p=0.9768$) and gender ($\chi^2(5)=1.90$, $p=0.8622$). Prior to participation in the study, all participants were screened in a standardized telephone interview for history of renal, hepatic, thyroid, gastrointestinal, or neurological disorder, as well as medication, drug use including nicotine use (no regular smoking within the last year, no cigarettes within the last month), excessive alcohol use (no more than 80 g/week) and regular caffeine consumption (no more than two cups of coffee/day), personal or first- and second-degree family history of psychiatric disorders including major depression, and pregnancy. To ensure that candidates were responding truthfully in the screening, they were told about the possible adverse effects of the procedure in case any of these conditions would be present. In addition, candidates were told that they did not have to provide details about the nature of a disease, should their response be positive. Only participants that did not fulfill any of the above exclusion criteria were allowed participation in the study. Menstrual cycle of female participants was not controlled for, but the absence of gender-specific effects (see below) argues against a specific hormonal influence on these results. Participants provided written informed consent to the participation of the study. This study was approved by the local ethics committee of the General Medical Council Hamburg (PV3661) and was conducted in accordance with the Declaration of Helsinki. All participants were financially compensated for the participation in the study.

Amino acid mixture

It has been shown that a combination of a 1-day low-protein diet with an amino acid mixture lacking tryptophan (TRP) or tyrosine/phenylalanine (TYR/PHE) can effectively reduce the availability of these amino acids in the brain (Hood et al. 2005; Young 2013). Male participants received an amino acid mixture (90 g) containing amino acids in a proportion similar to human breast milk. The mixture for the BAL group was: L-alanine 4.1 g, L-arginine 3.7 g, L-aspartic acid 9.8 g, L-cysteine 2.0 g, glycine 2.4 g, L-histidine 2.4 g, L-isoleucine 6.1 g, L-leucine 10.2 g, L-lysine 7.6 g, L-methionine 3.0 g, L-phenylalanine 4.3 g, L-proline 9.3 g, L-serine 5.3 g, L-threonine 4.3 g, L-tryptophan 3.0 g, L-tyrosine 5.3 g, and L-valine 6.8 g. Female participants received 20 % less due to lower body weight. The TYR group received the same mixture as the BAL group, but lacking the amino acids tyrosine and

phenylalanine. The TRP group received the same mixture as the BAL group, but lacking the amino acid tryptophan. The mixture was dissolved in 300 ml of tap water and contained lemon flavor to mask the unpleasant taste.

Depletion procedure

In each testing session, between three and six participants were tested in parallel to reduce the overall workload for the experimenters. The experiment presented in this article was conducted always by the same experimenter. The day before the experiment, subjects received a low-protein diet (2,500 kcal, <20 g of protein). The reasoning for this procedure is that reduced protein availability may contribute to the effect of dietary depletion because of reduced amino acid availability (Reilly et al. 1997; Hood et al. 2005). Subjects were also given a battery of personality tests and a depression scale to fill out at home and bring in on the next day. On the day of testing, subjects arrived at 8:45 am when blood samples (10 ml) were taken. The samples were centrifuged immediately after they were drawn, and the blood serum was stored at −35 °C and transferred to a −80 °C refrigerator on the same day. At a later point, the serum samples were sent to a medical laboratory (Medical Laboratory of Bremen, Bremen, Germany) for analysis of the amino acid composition. In the laboratory, amino acids of plasma samples were separated using high-performance liquid chromatography (HPLC), followed by triple quadrupole mass spectrometry to determine amino acid concentration. After blood samples had been taken and subjects had been transferred to the waiting room, subjects filled out a mood questionnaire. Following this, they received the amino acid drink. Participants were instructed to consume the drink at their own pace, but within 5 min and were allowed to mix the drink with further tap water. After consumption of the drink, they were given peppermint chewing gum or dragées free of amino acids and sugar against the unpleasant taste.

Subjects were given roughly 15 min to relax after the consumption of the drink. They then participated in an unrelated experiment of roughly 15–20 min, followed by the first part of this study (see "Experimental procedure"). Afterwards, they were seated in the waiting room and were allowed to carry out their study homework or watch DVDs provided by the experimenter. During this waiting period, they were also given a low-protein, low-calorie snack to reduce the feeling of hunger and were provided with water or fresh peppermint tea against the unpleasant taste. Roughly 4 h 30 min after the first mood assessment, subjects were given a second mood questionnaire, followed by an additional blood sample. Roughly 5 h after consumption of the amino acid drink, subjects participated in the second part of the study. After this, they took part in a range of other experiments investigating decision-making and motivation, which lasted until approximately 4:30 pm. These experiments were unrelated to the present task. Finally, to cancel the effects of depletion, subjects received a protein-carbohydrate meal and were allowed to leave. All subjects filled out a post-experimental questionnaire on the next day.

Experimental procedure

General structure of the experiment

The experiment was carried out in a dimly lit room on three to six participants in parallel. Each participant was seated approximately 50 cm from a 19-in. TFT screen and was wearing headphones throughout the entire experiment. The participants were seated in separate cubicles within the same room, so they would not distract each other. After the experiment was over, subjects participated in a number of other, unrelated experiments.

The experiment was set up as a spaceship game in which participants were pilots of a spaceship flying through different galaxies (contexts, Pavlovian cues). In all parts of the experiment, the spaceship was shown at the bottom of the screen and 60 % of the screen width was filled by the image of a galaxy. A cloud of dots moving downward was used to induce the feeling of the spaceship moving forward through space. The only interaction with the stimuli was possible by "firing" a shot (see below), which was used in the instrumental and the PIT task. We were interested in general PIT, i.e., the effect that a classically conditioned stimulus has on an *unrelated* instrumentally acquired response. For that reason, we used different rewards or punishments for Pavlovian and instrumental conditioning, respectively. The experiment consisted of six parts which will be explained in more detail below (see Fig. 1, for the main procedure). The first three parts took place in the morning before depletion was effective and consisted of (1) familiarization with the stimuli, (2) the instrumental learning phase, and (3) the Pavlovian learning phase. The last three parts were carried out in the afternoon during depletion and consisted of (4) a brief refresh of the Pavlovian phase, (5) a rating of contexts, and (6) the critical PIT task in which the Pavlovian and instrumental phases were combined under extinction, i.e., without receipt of rewards or punishments.

Familiarization

In the first part, the subjects were familiarized with the different stimuli they would encounter during the course of the experiment, including all rewards and punishments (see sections "Instrumental conditioning" and "Pavlovian conditioning" for details of familiarization with these stages). All stimuli were presented separately, i.e., no association between contexts and rewards/punishments was created at this point. However, subjects were familiarized with the task for the

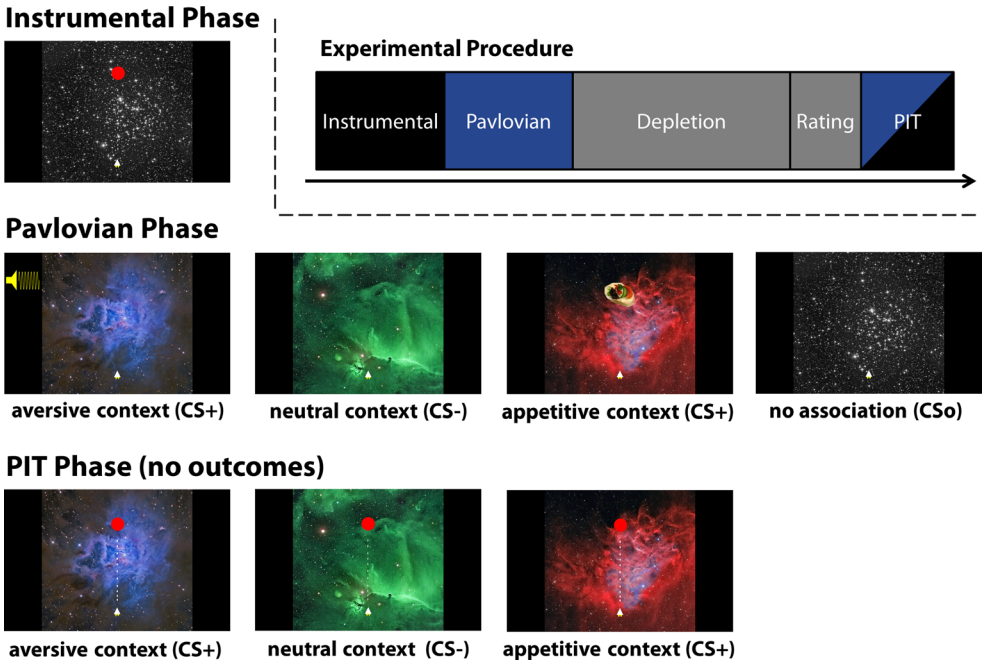

Fig. 1 Experimental procedure. The general procedure of the study is shown on the *top right*, with the detailed explanation in the remaining figure. Stimuli were scaled in this figure for better readability. The PIT task was framed as a spaceship game. The experiment started with the instrumental phase in which subjects learned to "shoot" repeatedly on a *red circle* to gain monetary reward. In the Pavlovian phase, different contexts consisting of images and sounds were paired with an aversive high-pitch sound (aversive context), with no outcome (neutral context), or with a food stimulus that was delivered to the subjects after the experiment (appetitive context). After selective amino acid depletion and a rating of Pavlovian stimuli, the PIT phase was carried out where the number of button presses was assessed in the three different contexts to reveal appetitive and aversive PIT

instrumental phase. Pilot experiments had shown that without further instructions subjects carried out the instrumental task extremely vigorously, probably because subjects did not want to risk missing a single reward. This could have led to a ceiling effect in the PIT phase. In addition, it has been suggested that the PIT effect can be increased when the baseline response rate is lowered (Holmes et al. 2010). For that purpose, subjects were given five trials of the instrumental task without winning or losing anything. They were instructed that they should use these trials to get a rough feeling for how often they would need to press to receive a monetary reward and that they could use this knowledge to "save their energy." Since this number was varied from trial to trial between 30 and 35 presses and the number of practice trials was limited, there was still sufficient ambiguity with respect to the number of button presses necessary.

Instrumental conditioning

The instrumental conditioning phase consisted of 30 trials, each lasting 13 s. The background was a grayscale galaxy throughout. Two seconds after the beginning of each trial, a red circle entered on the top left of the galaxy and moved to the center within 0.5 s, stayed there for 7.5 s, and disappeared within another 0.5 s (Fig. 1, top left). This red circle had been introduced to the subjects as a space object that could carry a treasure which served as a reward. This treasure would be revealed if the space object was hit often enough by shots fired at it. Importantly, subjects were instructed truthfully that they could not shoot "off" the space object, that the space object would appear and disappear independent of their shots, and that the object would only reveal its treasure when it was hit a sufficient number of times. This was done to ensure subjects would perceive the space object itself as appetitive (signaling potential to gain reward) or neutral, rather than as an aversive stimulus the subjects would need to fight, which could influence PIT (Huys et al. 2011). A shot could be fired by pressing the down arrow on the keyboard. On each trial, a random number between 30 and 35 shots were required to receive the reward which was not known to the subject. The treasure was an image of a 1 € coin which—if revealed—moved toward the spaceship until the two collided and then disappeared. Subjects were told that each treasure was worth 5 € cent which they would receive as additional financial compensation for participation in the study. To increase the rewarding effect of the treasure, it was paired with the sound of joyful cheering.

Pavlovian conditioning

The third part was the Pavlovian conditioning phase in which subjects learned an association of different contexts with food rewards and punishments. An experimental context consisted

of a colorful image of a galaxy which appeared in the background, paired with five short sounds that appeared every 2 s from the start of a trial. Each experimental context was shown for 12 s, with an additional 1 s transition period from one context to another. Between two experimental contexts, a baseline context was shown for 5 s which consisted of a grayscale galaxy without sounds (CSo), also with a 1-s transition period.

There were three experimental contexts in the Pavlovian phase (Fig. 1, middle). Each context was shown six times, and all contexts were shown in random order, with the limitation that they were never repeated twice in a row and shown the same number of times in the first half of the Pavlovian phase as in the second half. The task of the subjects consisted in actively observing the galaxies and "trying to understand" them. No response to the stimuli was used to reduce the possibility of cross talk between Pavlovian conditioning and instrumental responses. In the *neutral context*, a colorful galaxy and sounds were shown, but not paired with any reward or punishment (CS−). In the *aversive context*, a different colorful galaxy and sound were always associated with an aversive high-pitch sound (CS+ aversive). The sound, consisting of a sine wave at 11,000 Hz and at 85 dB, was presented for 4 s, including a fade-in of 0.5 s. These features of the sound ensured that the stimulus was neither perceived as painful nor elicited startle. The onset of the aversive sound was at a random time between 5.5 and 8.5 s after the beginning of the trial to reduce the expectation of an exact time point at which the unconditioned stimulus is shown (Delamater and Holland 2008). In the *appetitive context*, another colorful galaxy and sound were always associated with a picture of a food reward (CS+ appetitive) shown at the top of the screen for 4 s. The onset of the food reward was also at a random time between 5.5 and 8.5 s after the beginning of the trial. The picture of the food reward depicted a sandwich. Subjects were told in the familiarization phase that these sandwiches were collected automatically once "discovered" and would be received at the end of all experiments. In addition, subjects were told to already imagine receiving them immediately. The rationale behind using food stimuli as appetitive conditioning stimuli was that subjects had fasted overnight and hence were in a state of food deprivation. To increase the effectiveness of the unconditioned stimuli, subjects received their favorite toppings for the sandwiches. They had been told prior to all experiments that they might receive sandwiches as rewarding stimuli and were asked to name their favorite toppings which they would receive in case they won any food. All subjects received the same amount of food reward at the end of all experiments.

The colorful galaxies were red, green, or blue, and the sounds were the sound of a sonar at 565 Hz, the sound of a vibraphone at 490 Hz, and a buzzing sound at 415 Hz. The association of galaxies and sounds with rewards and punishments was counterbalanced between subjects to control for effects caused by the presentation of these stimuli alone. The punishments and rewards were received on every trial.

Refresh and rating

The refresh phase was identical to the Pavlovian phase, but consisted only of two trials per experimental context. Then a rating phase was introduced to measure the effectiveness of Pavlovian conditioning. Subjects were told that the galaxies would be simulated and that they should rate how much they liked flying through these galaxies. In addition, subjects were asked how much they would recommend other people to fly through these galaxies. These separate ratings were carried out for an assessment both of the subjective feeling associated with the galaxies and an evaluation of the galaxies from a third person perspective. After each galaxy, no reward or punishment was shown. Ratings were carried out on the keyboard numbers 1 to 9, where a 1 indicated a very negative rating and 9 a very positive rating. Each galaxy was shown twice to get a more reliable estimate, and both ratings were averaged.

Pavlovian-to-instrumental transfer phase

Finally, the PIT phase was a combination of the Pavlovian and the instrumental phases (Fig. 1, bottom). It consisted of eight repetitions of each experimental context lasting 12 s, which all were separated by 4 s of the baseline context and 1 s of transition period between galaxies. Importantly, subjects did not receive any rewards or punishments during this phase. Pilot experiments indicated that full extinction confused subjects, leading some participants to even contact the experimenter suggesting that the experiment was broken. To prevent this, subjects were told in the instructions that the spaceship radar was broken and that they would not be shown any rewarding or punishing stimuli. In that way, it was left open whether subjects would still receive rewards for button presses, because they could not be seen. This instruction has the advantage that subjects do not search for causes of the change. At the same time, it left them in a more uncertain state about the presence of rewards than in nominal extinction where subjects knew that they would not see any rewarding or punishing stimulus, but would still receive them after the experiment.

Questionnaires

All participants completed a number of questionnaires which were provided in a German version. During the study, participants filled out the Multidimensional Mood State Questionnaire (Mehrdimensionaler Befindlichkeitsbogen, MDBF-A, Steyer 1997), with the added question regarding the level of appetite of the participant. The personality

questionnaires and the depression scale that had been filled out the day before the experiment included the Center for Epidemiologic Studies Depression Scale (CES-D, Radloff 1977; Hautzinger et al. 1993); the Cloninger Temperament and Character Inventory (TCI) with the subscales novelty seeking, harm avoidance, and persistence (Cloninger et al. 1994; Richter et al. 1999); the Sensation Seeking Scale (SSS, 5th version, Zuckerman 1996; Roth and Hammelstein 2003); and the NEO Five-Factor Inventory (NEO-FFI, Costa and McCrae 1992; Borkenau and Ostendorf 1993). The results of these questionnaires are reported in Supplementary Table 1.

Results

Biochemical measures

Blood samples were analyzed with respect to the percentage reduction in total tryptophan levels and total tyrosine/phenylalanine levels between the first and the second blood sample. In addition, the change in the ratio of tryptophan to the sum of large neutral amino acids (TRP to ΣLNAA) and tyrosine/phenylalanine (TYR/PHE) to ΣLNAA was investigated which is a better indicator of amino acid availability in the brain (Fernstrom 1978). The LNAAs were defined as tryptophan, tyrosine, phenylalanine, isoleucine, leucine, and valine. The results for the change in the ratio of TRP to ΣLNAA, TYR/PHE to ΣLNAA, and TYR to ΣLNAA-PHE are shown in Table 1. The third ratio accounts for the fact that PHE can only be converted to TYR outside of the brain, which means that the TYR to ΣLNAA-PHE ratio is a better indicator of TYR availability. As can be seen in Table 1, the results are quite comparable.

The reduction in TRP was different between the groups investigated ($F(2, 99)=104.31$, $p<0.001$). The reduction was significantly stronger in the TRP group than both the TYR group ($T(66)=14.24$, $p<0.001$) and the BAL group ($T(66)=14.58$, $p<0.001$), but not different between the TYR group and the BAL group ($T(66)=0.69$, $p=0.491$). The TYR/PHE reduction was also different between the groups investigated ($F(2, 99)=169.97$, $p<0.001$) and significantly stronger in the TYR group than the TRP group ($T(66)=18.00$, $p<0.001$) or the BAL group ($T(66)=18.78$, $p<0.001$), but not different between the TRP group and the BAL group ($T(66)=1.31$, $p=0.195$, two-sided).

Table 1 Results of blood sample analysis. Results reflect the change of the ratio of TRP to ΣLNAA, and the change of the ratio of TYR and PHE to ΣLNAA, separately for each group. Relevant depletion effects are highlighted as bold entries. The error reflects the standard error of the mean change

	BAL group (%)	TYR group (%)	TRP group (%)
Δ(TRP to ΣLNAA ratio)	+24.07±4.28	+40.15±5.43	**−94.65±0.57**
Δ(TYR/PHE to ΣLNAA ratio)	−22.61±2.75	**−90.57**±0.64	−0.39±3.09
Δ(TYR to ΣLNAA-PHE ratio)	−15.91±4.96	**−89.77**±0.59	+16.78±5.12

A similar pattern of results was obtained for the change in the ratio of TRP to ΣLNAA and TYR/PHE to ΣLNAA. The TRP to ΣLNAA ratio change was different between groups ($F(2, 99)=337.53$, $p<0.001$). The reduction was larger in the TRP than the TYR group ($T(66)=24.67$, $p<0.001$) or the BAL group ($T(66)=27.50$, $p<0.001$) and also significant for a larger reduction in the BAL group than the TYR group ($T(66)=2.32$, $p=0.023$, two-sided). Similarly, the TYR/PHE to ΣLNAA ratio change was different between groups ($F(2, 99)=235.61$, $p<0.001$). The reduction was larger in the TYR than the TRP group ($T(66)=28.55$, $p<0.001$) or the BAL group ($T(66)=24.04$, $p<0.001$) and also larger in the BAL group than the TRP group ($T(66)=5.37$, $p<0.001$). The results were very similar for the TYR to ΣLNAA-PHE ratio.

The ratio of TRP to ΣLNAA and of TYR/PHE to ΣLNAA changed in all groups (all $T(33)>3.21$, all $p=0.003$), except for the ratio of TYR/PHE to ΣLNAA in the TRP group ($T(33)=0.13$, $p=0.901$, two-sided). Taken together, the analysis of the blood sample results demonstrates the successful reduction of TRP and TYR/PHE in the respective groups and additionally consistent, but moderate loading or depletion effects in the other groups.

Results of instrumental training

As expected, instrumental conditioning was effective (see Supplementary Fig. 1 for all button presses of all subjects, sorted by group). Subjects responded on all instrumental trials (M 43.86, SD 6.73). To investigate if there were a priori differences in the number of presses between groups, we subjected the results to a one-factor ANOVA. There was no effect of group on the number of presses ($F(2, 100)<1$), demonstrating that subjects in one group were not generally biased in responding more vigorously than subjects in other groups.

Results of Pavlovian training

To investigate whether subjects successfully associated Pavlovian stimuli with the respective outcomes, we assessed the ratings of the different contexts (aversive, neutral, appetitive). We were also interested if these contexts were evaluated differently by the three groups (BAL, TRP, TYR), because it has been shown previously that Pavlovian learning can be affected by tryptophan depletion (Hindi Attar et al. 2012), and even though learning took place before depletion, the effects

might have changed after depletion. The results are shown in Fig. 2. For self-evaluation of the contexts, a 3×3 repeated-measures ANOVA revealed a significant main effect of context ($F(2, 200)=263.37$, $p<0.001$), but neither a main effect of group ($F(2, 100)<1$) nor an interaction of context and group ($F(4, 200)<1$). The appetitive context was rated more positive than the neutral context ($T(102)=11.14$, $p<0.001$), and the aversive context was rated more negative than the neutral context ($T(102)=14.468$, $p<0.001$). The same result was found when subjects evaluated how much they would recommend the contexts to a third person (main effect of context: $F(2, 200)=246.68$, $p<0.001$, all other $F<1$). These results indicate that subjects associated the contexts with the corresponding valence and that this association was not different between groups.

Measurement of Pavlovian-to-instrumental transfer

To evaluate the PIT effect, we first looked at the number of button presses of each subject during each trial in the PIT phase of the experiment. An increase in the number of button presses relative to neutral would indicate invigoration caused by a specific context, and a decrease would indicate attenuation through the context. Since there are a priori differences between the subjects in the overall number of button presses, we normalized the number of presses per trial in the PIT phase to the mean number of presses per trial in the instrumental phase. This is similar to a calibration procedure used in a previous PIT study in humans investigating the vigor of responses (Talmi et al. 2008). Post hoc analyses demonstrated that this normalization procedure did not change the main pattern of results (see Supplementary Fig. 2 and the supplementary results for individual nonnormalized results). It is noteworthy that the overall number of button presses increased relative to the instrumental phase, irrespective of group ($T(102)=8.46$, $p<0.001$, two-sided), probably due to the increased uncertainty induced by the absence of feedback

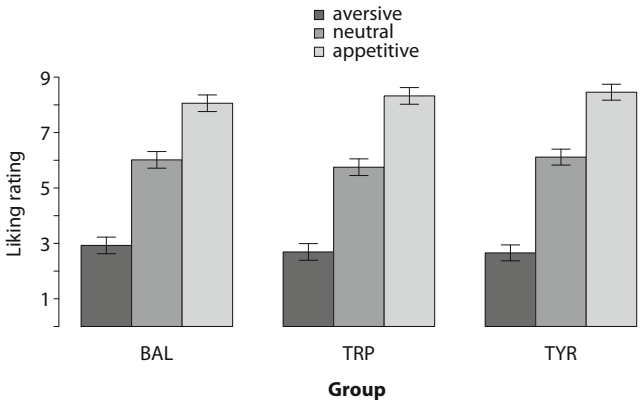

Fig. 2 Rating of Pavlovian stimuli across groups and contexts. *Error bars* reflect the standard within-subject error term for the factor context (Loftus and Masson 1994), separately for each group

in the PIT phase (see also "Methods"). In addition, responses in the neutral condition did not differ between groups ($F(2, 100)=2.10$, $p=0.128$), i.e., any comparisons relative to neutral are not confounded by differences in the mean response rate between groups. In the following, post hoc *t* tests are reported one-sided when the contrast tests the hypothesis that appetitive PIT is present (i.e., positive>neutral context) or that aversive PIT is present (i.e., neutral>negative context) and two-sided when there is a comparison between groups or the direction of the effect is not predefined.

The results of the PIT phase are displayed in Fig. 3. We ran a 3×3 repeated-measures ANOVA on the number of button presses in the PIT phase with the factors context (aversive, neutral, appetitive) and group (BAL, TRP, TYR). The results revealed a main effect of context ($F(2, 200)=4.96$, $p=0.008$, $\eta^2=0.04$), no main effect of group ($F(2, 100)=2.40$, $p=0.096$) and, importantly, an interaction of context and group ($F(4, 200)=3.01$, $p=0.019$, $\eta^2=0.06$). The nature of the main effect could not be interpreted due to the presence of a disordinal interaction. To investigate the source of the interaction, we followed this up with two 2×3 repeated-measures ANOVAs. There was a significant interaction of BAL vs. TRP×context ($F(2, 132)=4.02$, $p=0.020$, $\eta^2=0.05$), explained by increased aversive PIT in the TRP group ($T(66)=2.355$, p=0.022, two-sided), but no difference in appetitive PIT ($T(66)=0.00$, $p=1.00$, two-sided). In addition, there was a significant interaction of BAL vs. TYR×context ($F(2, 134)=3.13$, $p=0.047$, $\eta^2=0.04$), explained by larger appetitive PIT in the BAL group ($T(67)=2.72$, $p=0.008$, two-sided), but no difference in aversive PIT ($T(67)=1.673$, $p=0.099$, two-sided).

In addition, we ran three separate repeated-measures ANOVAs, one for each group. The BAL group showed a main effect of context ($F(2, 66)=3.41$, $p=0.039$, $\eta^2=0.09$), with more button presses in the appetitive than the neutral context ($T(33)=2.38$, $p=0.012$, one-sided), but no difference between appetitive and aversive ($T(33)=1.58$, $p=0.062$, one-sided) or neutral and aversive ($T(33)=1.15$, $p=0.13$, one-sided). This demonstrates that in the BAL group, only appetitive PIT is present. The TRP group also exhibited a main effect of context ($F(2, 66)=4.61$, $p=0.013$, $\eta^2=0.12$), with less button presses in the aversive than in the neutral context ($T(33)=2.17$, $p=0.019$, one-sided) or the appetitive context ($T(33)=2.19$, $p=0.018$, one-sided), but no difference between neutral and appetitive ($T(33)=1.47$, $p=0.076$, one-sided). This indicates the presence of aversive PIT, but not appetitive PIT. Finally, the TYR group revealed no main effect of context ($F(2, 66)<1$), demonstrating the absence of any PIT effect in this group.

As an additional measure of invigoration, we also investigated the response time of the first button press in the trial. For this, we ran another 3×3 repeated-measures ANOVA. The analysis revealed a main effect of context ($F(2, 200)=3.44$, $p=0.034$, $\eta^2=0.03$), but no main effect of group ($F(2, 100)<1$)

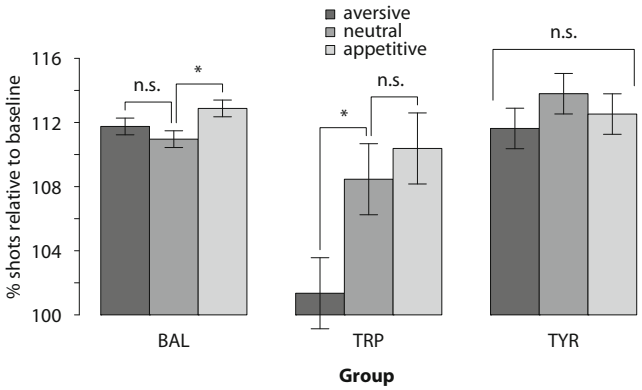

Fig. 3 Main results of the PIT task. The results reflect the mean number of button presses in context separately for each group, scaled by the average number of button presses in the instrumental phase to account for between-subject variability in the baseline number of presses. *Error bars* reflect the standard within-subject error term for the factor context (Loftus and Masson 1994), separately for each group

and no interaction ($F(4, 200)=1.01$, $p=0.401$). Collapsing across groups, we found an earlier button press onset in the appetitive context as compared to the neutral ($T(102)=2.30$, $p=0.012$, one-sided) or aversive contexts ($T(102)=2.06$, $p=0.021$, one-sided), but no difference between the neutral and aversive contexts ($T(102)=0.18$, $p=0.429$, one-sided). This result indicates that the onset of firing rate is affected by the context, but that this measure is not affected by amino acid depletion.

Influence of blood serum levels on PIT effects

We additionally investigated whether changes in TRP to LNAA ratio and TYR/PHE to LNAA ratio mirrored the results of this study. Importantly, if the interaction remains after accounting for group membership, this would indicate that individual depletion or loading effect contributed significantly to the results obtained here, irrespective of group membership. Conversely, if the interaction of group and context remained after accounting for serum level, this would indicate an independent contribution of group membership to the results (e.g., through a sampling bias). We first ran two repeated-measures ANOVAs with the factor context and the covariate TRP to LNAA reduction and TYR/PHE to LNAA reduction (the results were the same for TYR to LNAA-PHE reduction). There were a significant TRP×context interaction ($F(2, 200)=4.541$, $p=0.012$) and a marginally significant TYR/PHE×context interaction ($F(2, 200)=2.853$, $p=0.060$). The interaction disappeared completely when accounting for the effect of group (both $F(2, 196)<1$). In addition, the group×context interaction also disappeared when accounting for serum levels (TRP: $F(4, 196)<1$; TYR/PHE: $F(4, 196)=1.916$, $p=0.110$). This demonstrates that neither group membership nor serum level can alone account for the interaction effect found in the present study. Please note that alternatively, similar analyses could be carried out within each group, but would be less sensitive to addressing this issue.

Control analysis: intrinsic valence of pictures of galaxies and sounds

It is possible that the specific combination of galaxy pictures and sounds led to the PIT effects reported in the present study. For example, participants could prefer one galaxy and the corresponding sound over another, leading to more button presses in the presence of that context, independent of the association of that galaxy with appetitive or aversive stimuli. To investigate this effect, we sorted the results of ratings and the PIT task according to the galaxies and sounds that subjects were presented with, independent of the association with appetitive or aversive stimuli. Since there was no main effect of group in the previous analysis, we collapsed across group. The rating and PIT results were subjected to three separate repeated-measures ANOVAs (two for the ratings, one for the PIT effect). No main effect was found for the ratings of galaxies and sounds (both $F(2, 204)<1$), and there was no difference in the PIT effect that depended on galaxies or sounds ($F(2, 204)=1.08$, $p=0.341$). This demonstrates that our results cannot be explained by the intrinsic valence of the unconditioned stimuli.

Influence of depletion on mood and relationship between depressiveness scores and PIT effect

To investigate whether there was a selective influence of tryptophan or tyrosine/phenylalanine depletion on mood, we ran a 2×3 repeated-measures ANOVA with the factors time (before depletion, after depletion) and group (BAL, TRP, TYR) on the scores of the scale "mood" of the MDBF-A (no other scales showed significant main effects or interactions). The analysis revealed a main effect of time ($F(1, 100)=31.40$, $p<0.001$), no main effect of group ($F(2, 100)=2.08$, $p=0.131$), and an interaction of time and group ($F(2, 100)=5.53$, $p=0.005$). Mood levels were generally lower after depletion than before depletion (before 16.48, after 14.90, difference −1.57). Follow-up analyses revealed no difference between the BAL group and the TRP group in reduced mood scores (TRP −0.59, BAL −1.29, $T(66)=1.03$, $p=0.308$), but significantly lower mood in the TYR group (TYR −2.80) than either other groups (TYR vs. BAL: $T(67)=2.10$, $p=0.040$; TYR vs. TRP: $T(67)=3.47$, $p=0.001$). To investigate if mood affected motivation in the TYR group, we ran three additional analyses. First, we repeated the ANOVA with the subjective rating of hunger before and after depletion. The analysis showed no significant main effect or interaction (all $F<1$), demonstrating that reduced mood did not directly affect the motivational function of the food stimulus. Second, we added the change in mood as a covariate to the 3×3 repeated-measures ANOVA

investigating the PIT effect between contexts and groups. The pattern of results remained unchanged and there was no interaction of mood difference with context ($F(2, 198)<1$). Third, for the TYR group, we correlated the size of aversive and appetitive PIT with the change in mood through depletion. There was no significant correlation between the size of PIT and reduction in mood (aversive PIT: $r=-0.23$, $p=0.180$; appetitive PIT: $r=-0.06$, $p=0.720$). Taken together, these results demonstrate that mood did not affect the PIT results.

Finally, we assessed whether the scores from the CES-D measuring depressive symptoms in participants influenced the pattern of results we found. In addition, it has been suggested that depletion can differentially affect male and female participants (Booij et al. 2002). We repeated the 3×3 repeated-measures ANOVA investigating the PIT effect between contexts and groups and added the CES-D score and gender as between-subject covariates. There were no main effect of CES-D score ($F(1, 98)<1$), no main effect of gender ($F(1, 98)=1.542$, $p=0.217$), and neither an interaction of context with CES-D score ($F(2, 196)<1$) nor with gender ($F(2, 196)=1.773$, $p=0.173$). The main effect of context was significant ($F(2, 196)=4.676$, $p=0.010$), and importantly, the interaction between context and group remained ($F(4, 196)=3.041$, $p=0.018$), demonstrating that depressive symptoms as measured with the CES-D and gender did not affect the results.

Influence of personality differences on PIT effect

To investigate whether personality differences could account for the results of the present study, we checked for differences in personality scores between groups. A one-factor ANOVA with the factor group demonstrated significant differences between the groups in the subscale harm avoidance in the TCI ($F(2, 100)=6.170$, $p=0.003$) and significant differences in neuroticism of the NEO-FFI ($F(2, 100)=6.534$, $p=0.002$), both explained by on average smaller values in the tryptophan group than either other groups (smallest $T(67)=2.411$, $p=0.019$). Repeating the above 3×3 repeated-measures ANOVA investigating the PIT effect between contexts and groups and adding harm avoidance, the interaction between context and group remained significant ($F(2, 100)=2.427$, $p=0.049$) and was marginally significant when accounting for neuroticism ($F(4, 198)=2.363$, $p=0.055$). Additional analyses revealed no correlation between either appetitive PIT or aversive PIT and neuroticism, neither across all groups, nor within each group (most significant $r=-0.160$, $p=0.106$, referring to appetitive PIT across all groups).

Discussion

The goal of this study was to investigate the influence of serotonin and dopamine on general Pavlovian-to-instrumental transfer (PIT) in humans. In particular, we were interested to see whether the two neurotransmitters differentially affect the influence of appetitive and aversive Pavlovian cues on instrumental behavior. To this end, we used dietary depletion of the respective amino acid precursors in a PIT task measuring the vigor of responses. Our findings are threefold: First, we demonstrate appetitive general PIT, but no aversive general PIT in subjects receiving a balanced amino acid mixture. Second, tryptophan depletion enhanced aversive general PIT while not affecting appetitive general PIT. Third, tyrosine/phenylalanine depletion led to reduced appetitive general PIT, while no conclusions could be drawn regarding its role in aversive PIT. Our findings indicate that serotonin and dopamine differentially modulate the motivational influence of appetitive and aversive stimuli on instrumental behavior.

Appetitive and aversive general PIT in the control group

The results in the control group receiving a balanced amino acid mixture replicate previous results demonstrating appetitive general PIT in humans (Talmi et al. 2008; Nadler et al. 2011; but see Geurts et al. 2013a). Since general PIT involving response vigor has not consistently been reported in these studies, our result is encouraging and shows that our task was effective in evoking appetitive general PIT. Food reward was not delivered immediately, but only after the experiment due to the depletion procedure, so the image of food must have exerted its effect as a secondary reward.

In accordance with previous findings (Huys et al. 2011; Geurts et al. 2013a), we expected to also find aversive PIT in the control group, i.e., a reduction in the number of button presses through the punished context. Although Pavlovian conditioning was effective and the relevant context was rated as aversive, the effect was probably not strong enough to evoke aversive PIT in nondepleted individuals. This is supported by the marginally significant difference in the number of button presses between the appetitive and aversive context. Also, the presence of aversive PIT in the tryptophan-depleted group demonstrates that the procedure was in general effective, though possibly not strong enough to find an effect in the control group. In addition, contrary to previous studies (Huys et al. 2011; Geurts et al. 2013a; Geurts et al. 2013b), aversive PIT was examined as a change in the vigor of responses, not the choices that subjects made (see below). Taken together, these results indicate that the aversive Pavlovian influence on response vigor is more subtle and possibly needs more or stronger aversive events to elicit successful aversive PIT in the control group. Alternatively, aversive Pavlovian events that are more strongly related to withdrawal than the aversive sounds used in the present study, for example painful stimuli, could lead to enhanced aversive PIT.

The role of serotonin in appetitive and aversive general PIT

Subjects under tryptophan depletion exhibited an increase in aversive general PIT, while appetitive PIT was not affected. This latter result agrees with a previous study in humans finding no effect on appetitive PIT under tryptophan depletion (Geurts et al. 2013b; but see Sanders et al. 2007). The effects of serotonin reported in the present study are not perceptual, i.e., stimuli with negative valence are not merely perceived as more negative, since valence ratings of the Pavlovian phase did not differ between groups. Rather, our results point toward a modulatory influence of serotonin on motivation.

The currently dominant view of serotonin suggests that it plays a dual role in negative affect and behavioral inhibition (Cools et al. 2008; Dayan and Huys 2009; Tops et al. 2009; Cools et al. 2011). More specifically, it has been suggested that serotonin serves to enhance the coupling of aversive stimuli with (reflexive) behavioral inhibition (Crockett et al. 2009; Crockett et al. 2012; Geurts et al. 2013b; but see Deakin and Graeff 1991). A recent PIT study is in line with this account, demonstrating that tryptophan depletion leads to an increased proportion of choices to go in the presence of aversive Pavlovian cues (Geurts et al. 2013b). This result supports the idea that low serotonin leads to behavioral disinhibition in aversive PIT, which is in seeming contrast with the present results reporting the opposite finding.

However, as pointed out in the "Introduction," it is unclear if the results of Geurts et al. (2013b) extend to cases when response vigor is used, rather than choices. Importantly, choices and response vigor reflect different aspects of motivation (Salamone and Correa 2012), the former more closely related to response biases, and the latter more to generally activating functions (Niv et al. 2007). While the direction of these components in general PIT is usually the same and is difficult to distinguish, the influence of serotonin might be different for the two. Indeed, low serotonin can also increase aversive processing in humans (reviewed in Cools et al. 2008). In that way, low serotonin might lead to more strongly reduced general activation through aversive stimuli, i.e., a stronger *de*motivating effect.

Importantly, these views are not mutually exclusive: Reflexive Pavlovian response tendencies may primarily affect whether to respond or which response to choose, not how vigorously to carry out responses. Conversely, the demotivating effect of aversive Pavlovian stimuli might lead to reduced response vigor after a response has been elicited. For example, an observer may choose a rewarding option as often as before, but may approach it with less effort. Without this distinction, the finding of Geurts et al. (2013b) might also be interpreted as serotonin increasing or reinstating the motivation of subjects to respond in the presence of aversive stimuli. The present results may help explain this (somewhat counterintuitive) interpretation as reflecting only one particular motivational influence of serotonin.

One additional explanation for this seeming discrepancy is that behavioral inhibition only affects instrumental behavior directly when there actually is a motivation in the instrumental task to inhibit a behavior (Tops et al. 2009). Outcome-specific PIT suggests that Pavlovian response tendencies also contain a goal-directed component (Holland 2004; Corbit et al. 2007; but see Allman et al. 2010), because here PIT effects are larger when Pavlovian and instrumental outcomes are shared than when they are different. In addition, a recent study suggested that Pavlovian stimuli can lead to behavioral inhibition in an avoidance task and to behavioral activation in an approach task although the outcome remained unchanged (Huys et al. 2011). Previous, possibly conflicting tryptophan depletion studies were carried out in a task-setting in which it was sometimes better to withhold one choice (Crockett et al. 2009; Geurts et al. 2013b) or withhold one and choose the other option (Crockett et al. 2012), while in the present instrumental task, there was no incentive to inhibit behavior. In other words, the present instrumental task might not have been sensitive to potential Pavlovian effects of behavioral inhibition. In this view, only when behavioral inhibition becomes task relevant, the pattern of results reflects behavioral disinhibition when serotonin is reduced. Additional studies investigating PIT with a serotonin challenge are needed to provide more definite answers to how serotonin affects the interaction of Pavlovian and instrumental systems.

The role of dopamine in appetitive and aversive general PIT

We found that tyrosine/phenylalanine depletion reduced appetitive PIT, in line with a reduced impact of appetitive Pavlovian stimuli on instrumental behavior. Previous studies demonstrated that blockade of dopamine receptors in the nucleus accumbens reduces appetitive PIT (Lex and Hauber 2008), dopamine-agonist amphetamine injections enhance appetitive PIT (Wyvell and Berridge 2000), and nucleus accumbens lesions or inactivation reduces appetitive PIT (Corbit et al. 2001; Hall et al. 2001; Corbit and Janak 2007). In addition, also inactivation of the ventral tegmental area (Murschall and Hauber 2006; Corbit et al. 2007) reduces appetitive PIT. Our results demonstrating reduced appetitive PIT by tyrosine/phenylalanine depletion are in line with these findings and extend them to humans. In addition, the results indicate that the effect of dopamine reduction—as measured by tyrosine/phenylalanine depletion—is also found for general PIT, a distinction that was made only in one of these previous studies (Corbit et al. 2007).

What is more difficult to interpret is the finding that for aversive PIT we found no difference between tyrosine/phenylalanine depletion and the control group. Since we found no aversive PIT in the control group in the first place,

we cannot know if the absence of an interaction is related to the groups responding the same in aversive PIT or to the fact that differences in aversive PIT were too small to be detected. What the results do show, however, is that aversive PIT is not enhanced by tyrosine/phenylalanine depletion, a result we found for tryptophan depletion. To our knowledge, there are no animal studies that have investigated the effect of dopamine reduction on aversive PIT.

This means our results can either be interpreted as reflecting a selective influence of dopamine on appetitive PIT or a nonselective influence on PIT in terms of motivational salience (e.g., arousal). The former view is in line with a larger literature on the involvement of dopamine in reinforcement and appetitive motivation (reviewed in Wise 2004; Schultz 2007a; Bromberg-Martin et al. 2010). For example, blockade of the dopamine system can render normally potent rewards ineffective (Wise 2004). Tonic dopamine has been linked to increased motivation by affecting the behavior to minimize opportunity cost and thus maximize reward rate (Niv et al. 2007). In this view, dopamine depletion might act selectively on appetitive PIT, by decreasing the effect of a rewarding Pavlovian context on the number of instrumental responses.

There is, however, also evidence for a more general role of dopamine in motivation, irrespective of valence (reviewed in Bromberg-Martin et al. 2010; Boureau and Dayan 2011), and a large literature relates dopamine to learning of tasks involving aversive motivation (Salamone et al. 2007). Patients with psychic akinesia experience a general reduction of motivational drive, caused by bilateral lesions of the basal ganglia (Habib 2004). Also major depression and chronic fatigue—syndromes where motivational drive is reduced irrespective of rewarding or punishing consequences—have been associated with dopaminergic function (Salamone et al. 2006). Together, these results indicate that dopamine can also signal the motivational salience of stimuli, irrespective of valence. In this view, the influence of both appetitive and aversive Pavlovian cues on instrumental responses should be reduced by tyrosine/phenylalanine depletion. In other words, both appetitive and aversive PIT might be reduced by dopamine depletion if dopamine acts on the motivational influence of Pavlovian stimuli on instrumental behavior, but would not merely reduce the overall number of responses in the motivationally salient conditions.

In this view, one might alternatively expect that the overall number of button presses in the instrumental task should be reduced by dopamine depletion, irrespective of context, because in this view dopamine plays a generally activating role in motivated behavior (Robbins and Everitt 2007). However, the dopaminergic influence of the PIT effect should be mirrored by the *modulation* of the influence of Pavlovian cues on instrumental behavior, not by an adaptation of instrumental responses per se. Thus, dopamine depletion might exert its effect on PIT through a reduced influence of Pavlovian cues on instrumental behavior, irrespective of valence. On top of this PIT effect, one might expect an overall reduction of instrumental responses, because the instrumental task was carried out in a rewarded context. Nevertheless, this is not a requirement. For example, in our instrumental task, the subject can only affect the reward delivery, not the reward rate, by adapting the overall number of instrumental responses (Niv et al. 2007). Thus, it is plausible that we found a selective influence of (tonic) dopamine reduction on how Pavlovian cues affect instrumental behavior. In other words, a general reduction of instrumental responses is not a necessary requirement for this second view.

Although there is evidence for a general involvement of dopamine in motivated behavior, there is a predominance of dopamine neurons that respond toward reward, with only a minority of neurons responding to punishment (Schultz 2007a; Schultz 2007b). It has been argued that this bias evolved, because there is an asymmetry in the environmental pressure toward discovering rewards rather than avoiding punishment (Dayan and Huys 2009). In light of these findings, it is likely that the influence of dopamine is stronger on appetitive PIT than on aversive PIT, but this hypothesis awaits further testing.

Possible limitations of the present study

There are a number of possible limitations that have not been mentioned above and deserve further discussion. First, we did not collect autonomic measures of conditioned responses to Pavlovian cues. While it is not uncommon in the Pavlovian condition in human PIT tasks to measure ratings (Bray et al. 2008; Talmi et al. 2008), preference choices (Huys et al. 2011; Geurts et al. 2013a; Geurts et al. 2013b), or explicitly query the association of Pavlovian cues with outcomes (Nadler et al. 2011; Prévost et al. 2012), it is possible that some of our results mirrored cognitive evaluation, rather than innate Pavlovian responses. However, the presence of PIT and modulation through depletion argues that responses in the Pavlovian condition are not explained merely by cognitive evaluation. Still, this topic deserves further study.

In addition, the Pavlovian outcomes used in the present study were of different nature. While a primary Pavlovian outcome was used in the aversive condition (aversive noise), a secondary Pavlovian outcome was used in the appetitive condition (food picture). This difference might lead to differential impact of Pavlovian cues on the PIT task. While one would expect a larger influence of a primary cue than a secondary cue, the influence of a secondary cue might also decay differently under extinction, which might explain the absence of aversive PIT in the control group. However, when analyzing results based on the first and second half of the PIT trials, there was no interaction of this effect containing

context, indicating that PIT did not decay differently (results not shown). Nevertheless, ideally, only primary or secondary Pavlovian outcomes should be used in the same experiment.

Third—related to the above points—we did not directly compare the relative value of the appetitive and aversive Pavlovian stimuli. This makes a direct comparison of appetitive and aversive conditions in the PIT task difficult, because the stimuli could be differently effective. The presence or absence of an interaction between appetitive and aversive conditions with group could for that reason be caused by different effectiveness of the stimuli or by effects of depletion, which cannot be teased apart. This also prevented us to test the effect of valence (i.e., appetitive and aversive) against the neutral control condition. Thus, the presence of appetitive PIT and the absence of aversive PIT in one group—or vice versa—do not necessarily imply that an effect is valence-specific, because this could be caused by the lower effectiveness of one Pavlovian stimulus. Additional research is needed for more conclusive evidence about the valence specificity of tryptophan and tyrosine/phenylalanine depletion in PIT.

Fourth, although depletion protocols are well established and have been reported to have rather specific effects (Ardis et al. 2009; Le Masurier et al. 2013), it is always possible that tryptophan depletion did not selectively affect serotonin and that tyrosine/phenylalanine depletion did not selectively affect dopamine.

A final caveat is that all effects of appetitive and aversive PIT needed to be calculated relative to the neutral condition, which would ideally remain identical across groups. Also appetitive/aversive conditions cannot be compared meaningfully between groups without reference to neutral, because differences in appetitive/aversive responding between groups could be caused by overall differences between groups (as evidenced by the trend level effect of group), an effect that is accounted for by comparing appetitive/aversive only relative to neutral. One might argue that a change in baseline responding contributed to the reduced appetitive PIT in the tyrosine/phenylalanine-depleted group. However, the neutral condition alone was not significantly different between groups, arguing against a specific bias through different baselines, and this condition is the best approximation of a baseline. Future studies might introduce a group that does not receive amino acids as an additional control to strengthen the conclusions drawn from similar studies.

Summary and conclusions

In the present study, we used dietary depletion of amino acid precursors of dopamine and serotonin in a general PIT task and demonstrated that these neurotransmitters are differentially involved in appetitive and aversive general PIT. Reduced serotonin as measured by tryptophan depletion increased the influence of aversive Pavlovian cues on instrumental behavior, while leaving the appetitive influence unaffected. Reductions in dopamine (through tyrosine/phenylalanine depletion) on the other hand reduced the size of appetitive general PIT, while no conclusions could be drawn for aversive PIT. We have pointed out a motivational account that distinguishes between activation (invigoration vs. quiescence) and direction (approach vs. avoid) and which might explain seemingly conflicting findings regarding the role of serotonin. Finally, we have argued that the effect of dopamine on motivation is not necessarily restricted to the appetitive domain, but could also be modulated by aversive contexts. However, more studies are needed to elucidate the valence-dependent contribution of dopamine to motivation. Together, the differential involvement of serotonin and dopamine found in the present study demonstrates the interplay of both neurotransmitter systems in shaping our motivation and decision-making, depending on the valence of the context the observer experiences.

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Conflict of interest The authors declare no conflict of interest.

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