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EFFECTS OF THE DOPAMINE ANTAGONIST AMISULPRIDE ON 'WANTING' FOOD REWARDS

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1. Theoretical Background

Rewarding stimuli exert great power over behavior and mind. They possess the ability to motivate, that is to energize and guide behavior towards the satisfaction of an underlying need or the accomplishment of a goal (Berridge & Aldridge, 2008). Furthermore, rewards have properties that enable them to elicit positive affect or pleasure (Berridge, 2009).

The attractive forces of primary reward*s* such as food, water, sex and parental care act upon an innate biological preparedness (Schultz, 2015). Such rewards enable us to survive as individuals and ensure the survival of our species. Non-primary rewards, on the other hand, possess no intrinsic rewarding value but obtain it through experience as the result of associative learning (Schultz, Dayan, & Montague, 1997). *Motivation, pleasure and learning* thus appear to be closely interconnected in a system coding the rewarding properties of stimuli, adapting the organism to survive in an initially unpredictable environment (Berridge & Robinson, 1998). From this perspective, the main function of the reward system would seem to lie in protecting and advancing the organism (Ginane, Bonnet, Baumont, & Revell, 2015).

However, the reward system can also go awry and even act in opposition to the organism's integrity: In addiction, the attraction of certain rewards can become so strong that they are consumed in the face of strongly aversive consequences and even death (Robinson, 1993). This is especially puzzling when considering that addicts often report the pleasurable effects of drugs to diminish with prolonged abuse (Robinson, 1993).

Complementary to this, other pathologies are characterized by deficiencies where normally rewarding stimuli fail to exert their attractive force: In depression, a decrease of motivation and energy can be observed. However, new evidence hints at the relatively regular experience of pleasure in depressed individuals if they are provided with rewarding stimuli (Demyttenaere, De Fruyt, & Stahl, 2005; Szczypiński & Gola, 2018; Treadway & Zald, 2011). Reward processing is thus a key concept to understand and model behavior in its contradictive nature and to gain insight into certain pathologies connected to it.

Eating disorders are a prominent example of such pathologies, where reward processing is speculated to be causally involved (Finlayson, King, & Blundell, 2007b). In western societies, highly palatable food rewards are virtually ubiquitous. Perhaps not surprisingly, disorders such as obesity have been on the rise in the last decades, calling for sensible treatment approaches and providing grounds for emerging concepts such as food addiction (Ziauddeen & Fletcher, 2013). However, food intake can also be abnormally low,

indicating a potential disturbance of reward processing for food stimuli (Cowdrey, Finlayson, & Park, 2013). The goal of this paper is to investigate, whether a pharmacological manipulation in healthy human participants can induce changes in the rewarding value of food rewards with a focus on specific elements of reward processing discussed in detail further below. Such research bears the potential to inform theory about motivational processes connected to food intake and indicate sensible lines of treatment.

1.1. The dopaminergic system: Anatomy and pathways

The processing of rewarding stimuli has been repeatedly associated with the catecholaminergic neurotransmitter *dopamine* and the *dopaminergic pathways* where it unfolds its effects (Berridge & Robinson, 1998; Wolfram Schultz, 2015; Wise, 2006). The dopaminergic pathways that have long been in the focus of research include two major projections from the midbrain to the basal ganglia, referred to as the *nigrostriatal* and the *mesocorticolimbic dopamine system* (Arias-Carrión, Stamelou, Murillo-Rodríguez, Menéndez-González, & Pöppel, 2010; Baik, 2013; Wise, 2004).

The projections of the *nigrostriatal system* originate in the *pars compacta* of the *substantia nigra (SNc)* (Wise, 2004). It sends efferents to a part of the basal ganglia known as the *dorsal striatum*. This structure is comprised of two elements, the *caudate nucleus* and the *putamen*. The caudate nucleus is connected to the putamen by a range of grey matter bridges which give the entire formation a *striped* appearance in sagittal view (hence the name *striatum*) (Mink, 2013). The nigrostriatal pathway is generally believed to be primarily involved in motor function (Berridge & Robinson, 1998; Robbins & Everitt, 1992).

The dopaminergic pathway that has been the center of attention for the investigation of reward processing originates in the *ventral tegmental area* (VTA) and projects mainly to the *nucleus accumbens* (NAcc), as well as the *olfactory tubercle* (Robbins & Everitt, 1992). The nucleus accumbens is located where the caudate nucleus and the putamen meet ventrally. Collectively with the olfactory tubercle, it is also referred to as the *ventral striatum* (Robbins & Everitt, 1992). The VTA furthermore projects to limbic structures, such as the amygdala and the hippocampus (Wise, 2004). Thus, these projections form the *mesolimbic dopamine system*. Projections of the VTA to cortical structures such as the prefrontal or the cingulate cortex are referred to as the *mesocortical dopamine system* and collectively with the mesolimbic system as the *mesocorticolimbic dopamine system* (Wise, 2004). For a schematic overview of these pathways, see *Figure 1*.

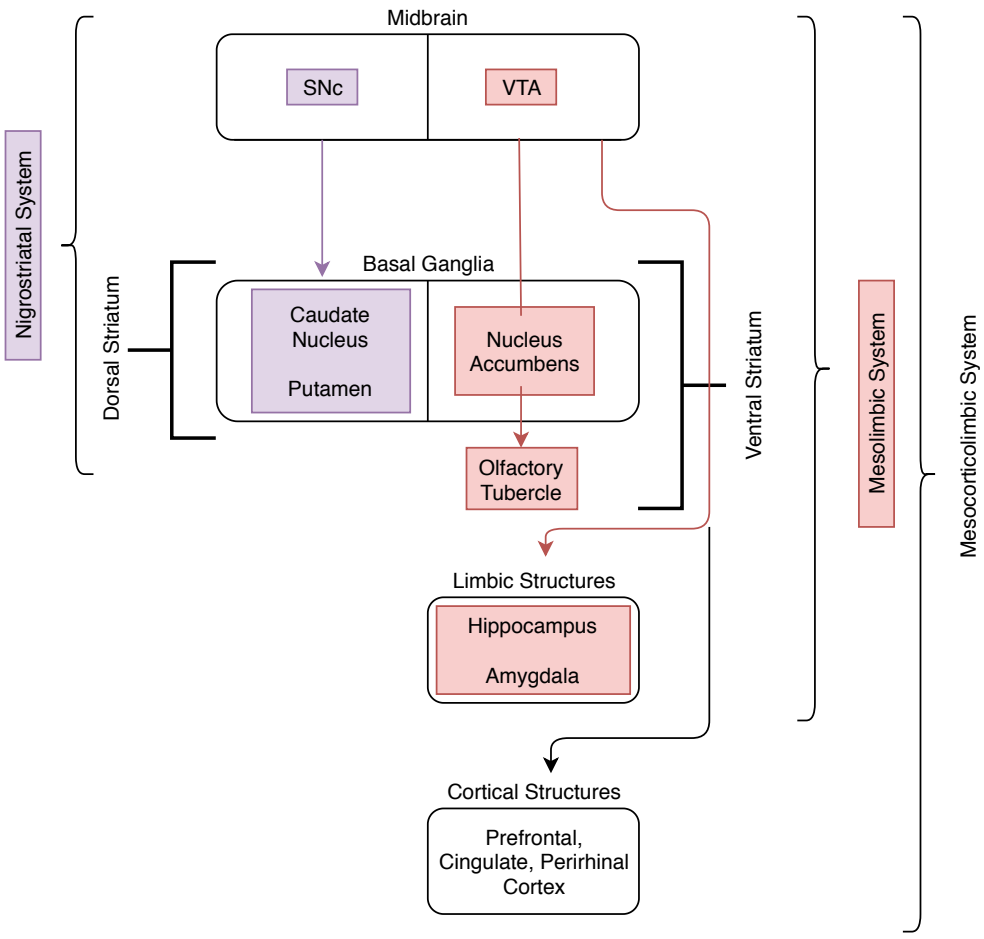

Figure 1: Dopaminergic Pathways

1.2. The functional role of mesolimbic dopamine activity

A number of propositions have been put forward concerning the functional role of mesolimbic dopamine and the debate is still not conclusively resolved. These perspectives are not necessarily mutually exclusive, but some have been gaining attention in recent years (Bergman, 2018), whereas others have meanwhile been discarded as largely inconsistent with more recent evidence. Three main perspectives have emerged over the years, that could be summarized under the terms "*liking*", "*learning*" and "*wanting*" (Berridge & Robinson, 1998; Blum, Gardner, Oscar-Berman, & Gold, 2012).

1.3. Motor function

Dopamine has important implications in *motor function*. This insight was built in part on a line of research connecting dopamine depletion in the nigrostriatal pathway to motor deficits characteristic of Parkinson (Bernheimer, Birkmayer, Hornykiewicz, Jellinger, & Seitelberger, 1973; Bernheimer & Hornykiewicz, 1965; Graybiel, Hirsch, & Agid, 1990; Smith & Villalba, 2008). Furthermore, long term treatment with neuroleptic drugs that act as dopamine antagonists entail impairments in motor function like tremor and difficulties to initiate voluntary movements, a disorder referred to as *drug-induced tardive dyskinesia* (Vijayakumar & Jankovic, 2016). Motor function and motivation are thus closely connected and the disentanglement of the two in terms of operationalization can be challenging: An animal that is not approaching food might *not be able* to do so due to motor impairment or it might *not be willing* due to motivational deficits.

1.4. 'Liking': The anhedonia hypothesis

Nonetheless, dopamine antagonists have been found to affect the execution of appetitive behavior without disturbing the underlying motor capacity necessary to perform it (Berridge & Robinson, 1998; Wise, 2004). That is, animals treated with neuroleptic drugs are able to perform well-learned movements leading to rewards. For example, they can traverse a runway and consume the reward that has been placed at its end (Wise, 2006). Obviously, the animal's motor capacity is not disturbed in a way that would hinder it from approaching rewarding stimuli. However, in the course of several trials, animals under the influence of dopamine antagonists will gradually change their behavior. Such animals will show less and less inclination to approach the reward or exert effort to obtain it (Wise, 2004). This pattern of behavior is mimicking an effect normally observed during the omission of reward for previously reinforced behaviors (extinction due to negative punishment) and is therefore known as *extinction mimicry* (Wise, 1982).

It has been interpreted as a *devaluation* of rewarding stimuli under the influence of dopamine antagonists. More specifically, it was believed that dopamine antagonists disturb the subjective *pleasurable impact* of rewards or its *hedonic value* (Wise, 2008). This devaluation would then lead to the observed effect of extinguishing learned behaviors. The *anhedonia hypothesis* of dopamine transmission has since solidified in the general knowledge of laypeople and a large amount of media reports still refer to dopamine as the "pleasure hormone". However, the conclusion that mesolimbic dopamine mediates the pleasurable

impact of rewarding stimuli is only one of several possible interpretations and later findings have challenged this assumption.

1.5. ‘Learning’: Reward prediction error

In a series of experiments, Schultz and colleagues (1997) found that dopamine neurons change their firing pattern when animals learn to associate a previously neutral stimulus with the delivery of a reward several seconds later. The authors used single-unit recording to monitor the activity of dopaminergic neurons in monkeys when receiving rewarding food stimuli like fruit juice or a morsel of apple, preceded by an initially neutral stimulus. Before learning the association, dopamine neurons fired shortly after the reception of the reward. After several trials, however, dopamine neurons fired in response to the now conditioned stimulus *in anticipation* of the reward and even stopped responding to the delivery of the reward itself. This finding stands in contrast to a central assumption of the anhedonia hypothesis: The anhedonia hypothesis would predict that dopamine neurons should be most active *during the consumption* of reward when the hedonic impact of the reward would be greatest. According to Schultz and colleagues, the role of dopamine instead lay in mediating the *associative learning* of reward predicting stimuli, not the hedonic impact of the reward itself. It would underlie the “stamping-in” of associations of stimuli with rewards that are predictably preceded by these stimuli. This perspective on dopamine activity has been termed the *prediction error hypothesis,* as it assumes phasic dopamine transmission to signal a difference between expected and received reward (Eisenegger et al., 2014). It has in turn been challenged by Berridge and Robinson, who proposed a dissociation of several components of reward processing*,* namely *‘wanting’* and *‘liking’.*

1.6. ‘Wanting’: Incentive salience

Berridge and colleagues (2009) have emphasized that reward processing is not a unitary construct but can be dissected into several components. Their concept distinguishes the *hedonic impact* of rewards (‘liking’) from their *motivational value* (‘wanting’), that is their ability to modulate the willingness to exert effort or spend resources in order to obtain a reward. (‘Wanting’ and ‘liking’ are theoretical constructs with important differences to what wanting and liking refer to in everyday language (Berridge, 2009). In order to make this distinction clear, these terms are written in quotation marks.)

In a series of experiments, Berridge and Robinson (1998) were able to show that primary affective reactions (in the form of facial expressions) to pleasurable food rewards are not disturbed by dopamine antagonists or lesions of dopaminergic brain areas. These primary affective reactions, i.e. tongue protrusions or mouth gapes, are a distinct reaction pattern to sweet or bitter tastes. They show large consistency across species (i.e. rats, monkeys, humans) and are displayed immediately after birth. Therefore, they are likely an innate and direct reflection of the subjective hedonic impact of rewarding stimuli.

In their experiments, Berridge et al. administered the neurotoxin 6-hydroxydopamine to rats, depleting dopamine in the striatum and nucleus accumbens almost completely (Berridge, Venier, & Robinson, 1989). However, these rats still displayed appropriate affective reaction patterns to sweet sucrose or bitter quinine. When sucrose was administered orally, dopamine depleted rats would appropriately exhibit hedonic reactions like tongue protrusions and paw licks. When bitter quinine was administered, they would display aversive reactions like gapes and chin rubs. Their affective reaction patterns did not differ from those of control groups. Conclusively, as affective facial reactions were not altered by the disturbance of dopamine transmission, the hedonic impact of rewarding or aversive stimuli apparently does not depend on it.

Importantly, mesolimbic dopamine transmission is supposedly also not crucial for reward learning, as those same dopamine depleted rats were able to learn the associative pairing of a sweet solution with the administration of illness-inducing lithium-chloride shortly after (Berridge & Robinson, 1998). In early trials, rats would display normal hedonic reactions to the taste of a sweet solution. However, this was changed to an aversive reaction when they learned that it was predictably followed by lithium-chloride induced nausea. Thus, associative learning was not mediated by dopamine transmission in this experiment, challenging the prediction error hypothesis.

Conversely, opioid agonists like morphine or benzodiazepines did influence affective reactions in these rats (Berridge & Robinson, 1998). Morphine enhanced hedonic reactions to sweet tastes, aversive reactions to bitter tastes were inhibited. Further supporting this perspective, brain damage to primarily opioidergic brain areas like the ventral pallidum produced aversive reactions to even sweet tastes and erased or diminished hedonic reactions (Berridge & Cromwell, 1990). Berridge and Robinson (1998) thus concluded that the hedonic impact, the ‘liking’ of rewards was processed distinctly from dopamine by an *accumbens opiod neural circuit*.

The notion of a dissociation of distinct reward processing components relying on either mesolimbic dopamine or opioid activity has become known as *Incentive Salience Theory*. The term "incentive salience" hints at the fact that both motivational and perceptual qualities are involved. When a stimulus becomes 'wanted' it grabs attention through its motivational value. Consequently, the amount of effort that is exerted to obtain a reward is not necessarily related to how pleasurable it is perceived. A powerful example of this, drug addiction, has already been mentioned in the introduction. However, in normal individuals 'wanting', 'liking' and 'learning' are closely connected and an orchestrated interaction of the three is necessary for intact reward processing (Berridge & Robinson, 2003).

As already pointed out, incentive salience theory is a very basic approach to motivational and affective mechanisms. It can be applied to a number of phenomena both non-pathological, i.e. personality and interindividual differences (M. D. Robinson, Moeller, & Ode, 2010; Smillie, 2013) and pathological, i.e. autism spectrum disorder (ASD) (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012). Another area of research where the application of incentive salience theory could lead to valuable insights is *food reward processing* (Epstein, Truesdale, Wojcik, Paluch, & Raynor, 2003; Finlayson, King, & Blundell, 2007).

1.7. Food 'wanting' and 'liking': Research on human participants

The incentive salience theory has largely been built on animal models. Many studies have used rodents (Pool, Sennwald, Delplanque, Brosch, & Sander, 2016). Methods that can be used in animal studies (such as lesions, intracranial self-stimulation or microdialysis) arguably produce convincing results that cannot be easily reproduced with human participants due to ethical considerations. In animals, activation of dopaminergic areas by food stimuli similar to those activated by drugs of abuse (NAcc) has been demonstrated (Bassareo & Di Chiara, 1997; Hernandez & Hoebel, 1988; Roitman, 2004). However, whether a dissociation of 'wanting' and 'liking' of food rewards can be safely assumed in humans and whether these processes are mediated by the same neural correlates as in animals is still subject to controversy (Finlayson & Dalton, 2012; Havermans, 2011).

A lot of research trying to dissociate 'wanting' and 'liking' in human participants has focused on abnormalities of these processes in pathological conditions such as binge-eating and obesity. This is explainable by the rise of these disorders in the western world, which has contributed to considerable scientific interest in developing sensible treatments for such conditions (Wang et al., 2001). In obese individuals, mesolimbic dopamine activity has been found to be related to the processing of rewarding food stimuli: For example, the expression

of dopamine receptors in striatal areas correlates with vulnerability to gain weight and body mass index (BMI) (Salamone & Correa, 2013). Furthermore, the amount of striatal dopamine receptors of obese individuals tends to be decreased (Wang et al., 2001). One possible interpretation of this finding is that pathological eating is caused by a long term deficiency of dopamine activity in reward circuits and a subsequent need to compensate by overeating (also known as *reward deficiency hypothesis*) (Blum et al., 2012).

In healthy participants, some research has been done using functional magnetic resonance imaging (fMRI), demonstrating the importance of mesolimbic dopamine activity for the processing of food rewards (Small, 2001). However, it has been found that rewarding food stimulates dopamine release in the dorsal (caudate and putamen) as opposed to the ventral striatum (NAcc) that has been implicated in the processing of rewarding stimuli in animal studies (Robbins & Everitt, 1992; Small, Jones-Gotman, & Dagher, 2003). Such findings highlight the potential differences in the neural correlates of food reward processing between animals and humans that still have to be further elucidated.

Some authors have argued that the distinction between 'wanting' and 'liking' is not a sensible approach for investigating human food reward processing, as these processes co-vary heavily under normal circumstances and the separation of the two in terms of operationalization tends to be blurred across studies (Havermans, 2011). Specifically, measures used for 'wanting' in one study sometimes are used for 'liking' in the other (Finlayson & Dalton, 2012; Havermans, 2011; Pool et al., 2016). Hence, findings of a separation of the two might "reflect poor construct validity rather than real effects" (Pool et al., 2016). Furthermore, Havermans (2011) argues that there would be no evidence that *neural sensitization*, a key process of the development of abnormal 'wanting' in substance addiction is caused by prolonged overconsumption of food, a problem also addressed by Ziauddeen and Fletcher (2013).

In a recent review, the measuring paradigms in the field of 'wanting' and 'liking' research have been criticized (Pool et al. 2016). The authors point out that many studies have ignored the *temporal nature* of reward processing and assessed 'wanting' and 'liking' at time points where these processes are either not happening or are confounded by other processes like memory or reward expectancy (Pool et al., 2016).

Nevertheless, there is now considerable evidence of a demonstrable distinction of 'wanting' and 'liking' in humans. Epstein, Truesdale, Wojcik, Paluch, & Raynor (2003) found that food stimuli were more reinforcing for healthy human participants in a deprived state, likely reflecting an increase of 'wanting' during physiological need-states. In contrast,

hedonic measurements of 'liking' were not influenced by deprivation, indicating a separation of these mechanisms in humans. Another study investigated the effects of stress on 'wanting' and 'liking' of food rewards in obese and healthy participants (Lemmens, Rutters, Born, & Westerterp-Plantenga, 2011). The authors were able to observe a dissociation of 'wanting' and 'liking' under stress: While 'liking' ratings were not influenced by stress for both normal-weight and obese participants, stress did influence ratings of 'wanting' and food intake for obese but not normal-weight participants. This points to the differential effects of stress on motivational processes and hedonic impact in healthy versus obese individuals.

Finally, Finlayson and colleagues have applied the distinction of 'wanting' and 'liking' successfully in a large number of studies investigating food processing in both healthy and pathological human participants (Cowdrey et al., 2013; Finlayson, Bryant, Blundell, & King, 2009; Finlayson, King, & Blundell, 2008; Finlayson et al., 2007b). Finlayson has also argued against the proposition of Havermans (2011) to abandon 'wanting' and 'liking' as theoretical constructs for the investigation of food reward processing in humans pointing to a number of studies using both reliable and valid measurement paradigms to successfully dissociate between these processes (Finlayson & Dalton, 2012). Clearly, the evidence regarding a distinction between 'wanting' and 'liking' in humans is still in parts contradictory. Further research is necessary to conclusively validate a dissociation of motivational and hedonic processes for human food reward processing.

As evidenced, there have been little studies trying to directly manipulate the underlying neural substrate of 'wanting'. Many studies have applied manipulations with more general effects on a large number of physiological parameters such as fasting, physical exercise, sleep or stress (Cameron, Goldfield, Finlayson, Blundell, & Doucet, 2014; Epstein et al., 2003; Lemmens et al., 2011; McNeil, Cadieux, Finlayson, Blundell, & Doucet, 2015). One study by McCabe, Huber, Harmer, & Cowen (2011) used a dopamine antagonist (sulpiride) as a manipulation in an fMRI study investigating the response of participants to the smell or sight of chocolate. They found that sulpiride reduced the BOLD response to food stimuli in the ventral striatum and the cingulate cortex, indicating an effect of dopamine antagonists on food reward processing. However, whether participants also experienced food rewards as less attractive cannot be determined by this type of study.

1.8. Dopamine receptor subtypes and their function

Dopamine binds to several different receptors, each involved in a variety of functions. Pharmacological manipulations of dopamine transmission tend to target only a subgroup of

receptor subtypes, thereby exerting different effects on behavioral and psychological processes. To anticipate the effects of a pharmacological manipulation, it is important to consider the different receptor subtypes.

So far, five dopamine receptor subtypes (D1 – D5) have been identified, belonging to two major classes: *D1-like* (D1 and D5) and *D2-like* (D2, D3, and D4) (Beaulieu & Gainetdinov, 2011). All dopamine receptors are G-protein-coupled receptors that rely on second messenger pathways to exert their effects.

Through G-protein activation, dopamine possesses the ability to modulate the enzyme adenylyl cyclase and thereby the intracellular breakdown of *adenosine triphosphate* (ATP) to *cyclic adenosine monophosphate* (cAMP) (Kebabian & Calne, 1979). Intracellular cAMP, in turn, acts as an important second messenger for the metabolism of cells and the modulation of the excitability of neurons. D1-like receptors stimulate intracellular cAMP, whereas D2-like receptors inhibit it (Baik, 2013). Thus, D1-like receptors can be said to strengthen signal transduction, whereas D2-like receptors weaken it (Gao & Gao, 2012).

Some evidence stemming from cocaine self-administration studies on rats demonstrate a role of D2-like dopamine receptors in enhancing cocaine-seeking behavior, while D1-like receptors were found to reduce it (Self, Barnhart, Lehman, & Nestler, 1996). However, in the case of intracranial self-stimulation of dopaminergic areas, both D1-like and D2-like receptor subtypes seem to mediate the reinforcing effects of electrical brain stimulation (Franklin & Vaccarino, 1983; Kornetsky & Esposito, 1981)

The functional role of D2-like receptors is very complex, as they are expressed both postsynaptically and presynaptically (Beaulieu & Gainetdinov, 2011). Presynaptically, they act as dopamine autoreceptors. Through a feedback signal, such autoreceptors can lead to inhibition and modulation of dopamine transmission (Baik, 2013). Smaller doses of some neuroleptics tend to bind preferably to presynaptic autoreceptors (Eisenegger et al., 2014; Rosenzweig et al., 2002). Therefore, a pharmacological manipulation aiming to antagonize dopamine activity has to be strong enough to bind to *postsynaptic* dopamine receptors utilizing a large enough dose (la Fougère et al., 2005).

There is a significant overlap of the pattern of expression of dopamine receptor subtypes throughout the brain. The highest level of D1-like and D2-like is found in the striatum, the Nucleus accumbens and olfactory tubercle, in lower levels also in the VTA and the substantia nigra (Beaulieu & Gainetdinov, 2011).

Assigning a particular function to a single receptor subtype is difficult and such an attempt would be reductive in the face of the high complexity dopamine's functional role

(Missale, Nash, Robinson, Jaber, & Caron, 1998). Most receptor subtypes are involved in several of the vast amount of dopamine functions. Furthermore, targeting individual receptor subtypes by pharmacological manipulation is not always possible (Missale et al., 1998). Nonetheless, there is now mounting evidence that the D2-like receptor subtype, while not exclusively involved, plays an important role in mediating the rewarding properties of food reward (Baik, 2013; Eisenegger et al., 2014; Johnson & Kenny, 2010; Salamone & Correa, 2013; Volkow, Wang, Fowler, Tomasi, & Baler, 2011).

1.9. Amisulpride as a dopamine antagonist

Amisulpride is a dopamine antagonist belonging to the class of atypical neuroleptics. It binds selectively to the D2 and D3 receptor and is used in the treatment of psychotic symptoms in schizophrenia (Rosenzweig et al., 2002). Like most atypical antipsychotics, amisulpride shows a more favorable profile of adverse side effects compared to first-generation antipsychotics (i.e. chlorpromazine or haloperidol), particularly in regards to extrapyramidal symptoms such as tremor, hypertonia, and rigidity (Nasrallah, 2003). However, atypical neuroleptics have been found to induce other adverse side effects like weight gain and metabolic syndrome (Coccurello & Moles, 2010; Newcomer, 2005).

The daily dose of amisulpride in treatment ranges from 50mg up to 1200mg (Curran & Perry, 2001). At low doses (50mg-300mg), it ameliorates negative symptoms of schizophrenia (low affect and reduced motivation), likely achieved by blockade of presynaptic D2 autoreceptors, effectively enhancing dopamine transmission. At higher doses (>400mg) it is used to manage positive symptoms (Rosenzweig et al., 2002).

In a study by Ramaekers et al. (1999), a single dose of 400 mg administrated to healthy subjects did not entail adverse side effects on psychomotor or cognitive performance on the first of several days of treatment. However, on the fifth day of consecutive drug administration, amisulpride did produce extrapyramidal symptoms (Ramaekers et al., 1999). One of the possibilities of combatting extrapyramidal side effects elicited by neuroleptic drugs are anticholinergic drugs like biperiden (Dayalu & Chou, 2008; König et al., 1996; van Harten, Hoek, & Kahn, 1999).

A number of studies have used amisulpride or similar neuroleptics in the investigation of reward processing in humans. One study by Weber and colleagues (2016) used a 400 mg dose of amisulpride in order to investigate reward processing in terms of cue reactivity (responding to reward-related stimuli) and reward impulsivity (tolerance of reward delay). These constructs could be argued to be related to incentive salience. The authors found that

blockade of D2 and D3 dopamine receptors significantly reduced cue reactivity and reward impulsivity on a monetary instrumental transfer task and a reward delay task.

Other studies investigating reward processing have used similar doses of amisulpride (Jocham, Klein, & Ullsperger, 2014; Kahnt, Weber, Haker, Robbins, & Tobler, 2015). Another study that has already been mentioned has used sulpiride (McCabe et al., 2011). As a result, the authors found a reduced neural response to rewarding food stimuli in the ventral striatum and the anterior cingulate cortex.

Lastly, Eisenegger and colleagues (2014) have also used sulpiride to compare reward prediction error models with the incentive salience perspective on striatal dopamine activity. Using what they referred to as a "reinforcement learning task", they found the administration of sulpiride to disrupt choice performance selectively for stimuli indicating reward, whereas loss avoidance and learning were not affected. These studies support the view that blockade of striatal D2 and D3 receptors by amisulpride or a similar neuroleptic will disrupt 'wanting' for rewarding food stimuli in line with the incentive salience hypothesis.

1.10. Implications of incentive salience theory for successful operationalization

When referring to wanting in everyday language, the term usually involves cognitive aspects of motivation, such as the representation of a goal and the steps it would take to reach it. However, not all 'wanting' (in the sense of incentive salience) is conscious (Anselme & Robinson, 2016). Specifically, incentive salience is established largely by conditioning and does not necessarily involve any cognitive understanding of causal relationships. It can also be activated subliminally and unconsciously (Winkielman, Berridge, & Wilbarger, 2005). Thus, measuring 'wanting' requires a task that taps into these partly unconscious mechanisms by employing objective measures of effort, rather than relying only on rating questions that depend on participants' insight into and awareness of their current motivational state.

Furthermore, a research paradigm investigating reward processing in humans needs to take into account the *temporal nature* of reward processing (Pool et al., 2016). As mentioned before, 'wanting' is brought about by conditioning and is triggered by cues associated with reward delivery. Thus, neutral cues or unconditioned stimuli need to be first established as learned predictors of reward. 'Liking' on the other hand happens *during the consumption* of reward. Consequently, when measuring these processes, it needs to happen during the appropriate time window: 'Wanting' needs to be measured *after* the presentation of a conditioned stimulus predicting reward delivery but *before* consumption of the reward.

‘Liking’ needs to be assessed *during* or *shortly after* reward consumption. Only by measuring ‘wanting’ and ‘liking’ in this way can they be properly separated (Pool et al., 2016).

2. Research Question & Hypotheses

As mentioned above, the incentive salience hypothesis has largely been built on animal studies, particularly rats (Berridge & Robinson, 1998; Mahler & Berridge, 2012; Peciña, Cagniard, Berridge, Aldridge, & Zhuang, 2003; Wyvell & Berridge, 2000). The methodology that can be used in rodents to study the role of dopamine transmission in incentive salience is naturally limited in humans due to ethical considerations. Hence, the dissociation of ‘wanting’ and ‘liking’ of food reward in humans is still partly controversial (Havermans, 2011).

Some reviewers point out the difficulties in clearly separating the two mechanisms while simultaneously revealing major confounds like reward expectancy and memory (Pool et al., 2016). Others hint at the relative scarcity of attempts to discriminate food ‘wanting’ and liking in humans, relating it to the difficulty of assessing ‘wanting’ by classical measuring paradigms due to the problem of incentive salience involving unconscious mechanisms (Mela, 2006).

Furthermore, a lot of research has focused on abnormal food reward processing in obese or otherwise pathological participants (Baik, 2013; Volkow et al., 2011; Wang et al., 2001). Whether findings in pathological participants can be generalized to the healthy population is not clear.

Such limitations of earlier studies demonstrate the need for validation of the dependence of food ‘wanting’ on the neurological substrate of mesolimbic dopamine in healthy participants to further corroborate the conclusions drawn mainly from animal models or pathological participants. Importantly, such attempts should use diverse methods for operationalizing the constructs of ‘wanting’ to demonstrate the robustness of the hypothesis in human participants.

As indicated, administration of the atypical neuroleptic amisulpride or a similar dopamine antagonist leads to a blockade of striatal D2 and D3 receptors and in turn to a disturbance of reward processing mainly in line with the incentive salience hypothesis (Eisenegger et al., 2014; McCabe et al., 2011; Weber et al., 2016). An antagonistic pharmacological manipulation of striatal dopamine transmission should thus entail reduced ‘wanting’ in response to cues indicative of food rewards expressed both on implicit

(unconscious) and explicit (conscious) measures. Thus, the hypotheses of this paper are as follows:

H1: Selective blockade of postsynaptic striatal D2 and D3 receptors by amisulpride in healthy participants will lead to reduced ‘wanting’ in response to cues indicating food rewards, operationalized by:

H1a: reduced subjective ratings of ‘wanting’ a cued food reward

H1b: reduced exerted effort on a dynamometer measuring the strength of muscle contraction.

3. Methods

The hypotheses as described above have been investigated as part of a study by the clinical and social neuroscience unit (CSNU) at the University of Vienna led by Giorgia Silani and colleagues. The "Taste and Touch" study was conducted as a basic research study (monocentric, randomized, double-blind, placebo-controlled, three armed) at the Department of Psychiatry and Psychotherapy of the General Hospital Vienna (Allgemeines Krankenhaus/AKH, Währinger Gürtel 18-20, 1090 Wien). One of its main goals was the comparison of social rewards as a potentially distinct class of rewarding stimuli to food rewards (*common currency hypothesis*). The research questions of this paper aim to investigate only a part of the entire study, namely 'wanting' of food reward and dopamine blockade. However, I will describe a larger amount of the study and its main task in order to present a complete picture of how the data were collected.

3.1. Participants

The acquired sample consisted of 48 subjects, aged 18-33 (M = 22.94, SD = 3.31). Drug group sizes were equal with 24 participants receiving a dopamine antagonist and 24 a placebo. Gender distribution was not equal: only 7 of the 48 participants were males. Three males and 21 females received the drug, compared to four males and 20 females that were given a placebo. See Figure 2 for a visualization of these sample characteristics. Average BMI was 22.58 (SD = 2.74, range = 18.40 – 29.30).

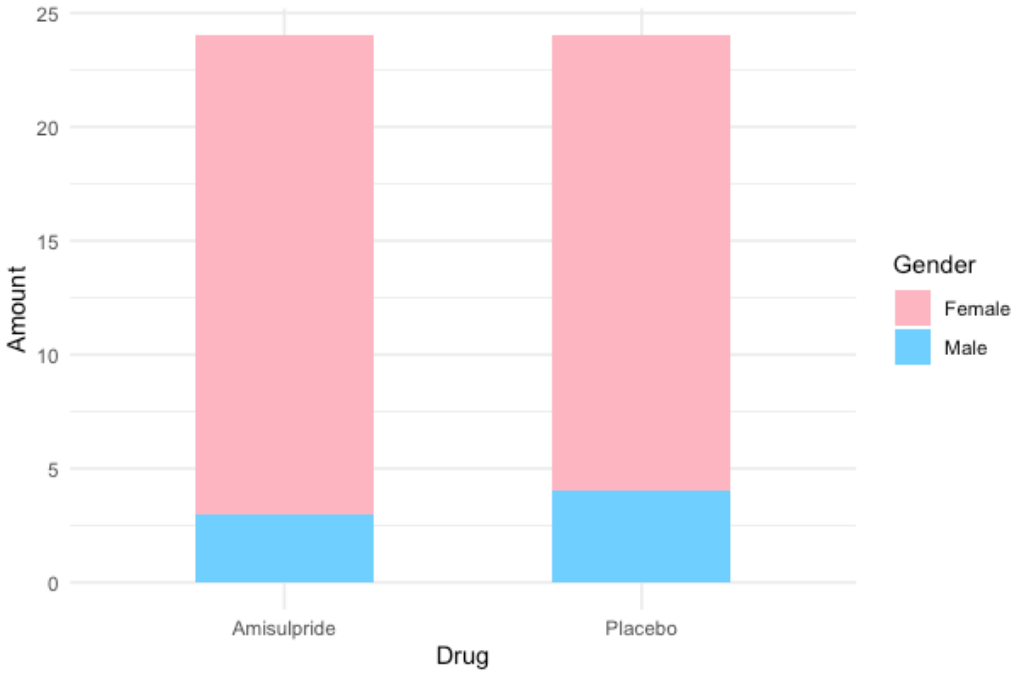

Figure 2: Gender and drug distribution

The “Taste and Touch study” was advertised by postings in social media and flyering in front of well-visited public buildings like university libraries. Participants had to fulfill certain criteria in order to be included in the study. The most important criteria are listed below (non-exhaustive list):

- Heterosexuality (to exclude sexual arousal during the social reward condition)
- Age between 18 and 35
- Right-handedness
- Fluency in German
- No history of alcohol or drug abuse
- Non-smoker (less than 5 cigarettes a day)
- No psychiatric or severe physical disorders
- Body-Mass-Index between 17 and 35

3.2. Study procedure

Testing of participants took place on two separate dates, referred to as *T0* and *T1* respectively. The main goal of T0 was to establish eligibility for the study. The main task (also termed *reward task*) and several additional short tasks took place on T1. T0 took roughly 1 hour in total, T1 required an average of 6 hours. Both dates were separately financially compensated: Subjects received 10 € plus a small amount of 2 – 10 € (5 € on average) won on a short task for completion of T0. For the second appointment, participants received 80 € plus the winnings of two small tasks, both on average 5 €. Conclusively, participants received a minimum of 92 € for the entire study.

Before any testing was done, participants were handed a written informed consent document along with comprehensive information about the study procedure and risks of unwanted side effects on their first visit to the laboratory. A further medical examination was done by a study doctor, who completed an electrocardiogram, a blood test and the Mini-International Neuropsychiatric Interview *(MINI)* (Sheehan et al., 1998). The MINI is a short diagnostic interview for the diagnosis of psychiatric disorders in the biography of participants. If no abnormalities were detected, the second appointment was scheduled, on which the reward task took place.

At T1, a urine test was done on arrival at the laboratory to exclude any drug use prior to the experiment or pregnancy. If the test results were negative, participants received the

medication. To raise the rewarding value of food stimuli, participants had been asked to fast in the two hours before the experiment. Instead, they received a small snack (a granola bar of 400kcal) immediately after drug administration. Subjects were then asked to wait for three hours, during which they were allowed to read magazines. On average, the time in between pill and start of the reward task was 183 minutes (*SD* = 12.50). The waiting time was owed to the time it took for the drugs to reach maximum receptor occupancy.

3.3. Reward Task

The study employed a mixed design for its main task, where type of medication served as a between-subject factor and type of reward (social/non-social) as a within-subject factor. Participants orally received one of three medications 3 hours prior to the start of the reward task: *Solian* (400 mg amisulpride), *Dependex* (50 mg naltrexone) or a placebo, forming two experimental groups and one control group to which participants were randomly assigned. Naltrexone acts as an antagonist for the μ-opoid-receptor and was used as a pharmacological manipulation of 'liking'. This part of the study is not analyzed in this paper.

Regarding the within-subject factor, two types of reward were administered, each in blocks of several trials: Either a food reward, operationalized as a sweet drink (chocolate milk) or a social reward, operationalized as caressing of the forearm by the experimenter. Again, the social reward condition is not analyzed in this paper.

3.4. Stimuli

Several levels of reward were realized to allow for the comparison of 'wanting' and 'liking' in response to variously rewarding stimuli: Pure chocolate milk served as the most rewarding stimulus. A mixture of 25% chocolate milk and 75% sweetened milk of the same caloric value represented a lower level of reward and pure sweetened milk acted as the lowest level of reward ('high' = pure chocolate milk, 'low' = chocolate / sweet milk mix, 'very low' = pure sweet milk). 52g of sugar was added to the milk in order to raise it to identical caloric value. Both chocolate milk and sweetened milk were of 1.5 g/100ml fat content.

Drinks were delivered by a set of three plastic tubes (approved for human food consumption by the Food and Drug Administration) connected to three syringes containing the respective liquid. The syringes were operated by a set of pumps (PHD Ultra by Harvard Apparatus) to standardize liquid delivery: Two milliliters of milk were administered over a time window of two seconds. Two tubes were used to deliver the food stimuli, one was used

to rinse subjects' mouths with water in between trials. The tubes were placed in the mouth of participants via a mechanical arm that was manually adjustable.

3.5. Task Procedure

As mentioned in the theory section, a paradigm investigating the distinction of 'wanting' and 'liking' has to consider the temporal succession of reward processing: 'Wanting' of a rewarding stimulus precedes its delivery and is triggered by cues predicting it (Pool et al., 2016). 'Liking' on the other hand coincides with reward delivery or follows it immediately.

This temporal sequence was taken into account by first presenting a visual cue, announcing the delivery of the respective reward level. After presentation of the cue for 3 seconds, 'wanting' was measured in two ways: First, participants were asked to rate how much they wanted the announced stimulus by moving a continuous slider on the screen (no time limit), converted for analysis to a 20-point Likert scale (range -10 to +10). This served as a subjective measurement of 'wanting' that involved cognitive elements of incentive salience. Furthermore, it relied on participants' insight into and truthful reporting of their current motivational state.

Subjects were also given a dynamometer (HD-BTA, Vernier Software & Technology, USA) in their right hand to measure maximum muscle contraction over a time window of 4 seconds. This acted as a more objective operationalization of 'wanting', able to also capture subconscious components of motivation not accessible by rating questions. Visual feedback of muscle contraction was given by displaying a red bar on the screen, the height of which represented the exerted force.

Simultaneously to measuring 'wanting', the dynamometer was also used to determine the probabilities with which rewards were delivered. The height of the bar represented a spectrum of probabilities ranging from 0% to 100%: If subjects pressed their individual maximum (measured before the start of the task), the announced reward was delivered with a probability of 100%. If participants pressed half their maximum, the announced reward was delivered in 50% of cases. In the other 50%, the subjectively lowest reward (determined before the start of the task) was delivered.

This setup led to only the two highest reward levels ever being cued ('high'/ 'low'). The 'very low' reward level was never cued but only delivered if subjects pressed less than their maximum and the lowest reward was chosen by the program. Three pictures were used, representing cues of the respective reward levels. Pure chocolate milk was represented by a

black bottle, chocolate/sweet milk mix by a black-and-white striped bottle (not shown in Figure 3) and pure sweet milk by a white bottle.

After reward delivery (two seconds), participants were asked to relax for three seconds and then rate their 'liking' of the stimulus on a continuous slider, similar to rating 'wanting' (no time limit). For a visualization of the individual steps of the task, see Figure 3.

Figure 3: Steps of the food condition including cues and display time

To assess individual preferences before the start of the task, subjects completed a short task in which all reward levels were experienced and ranked. The order of individual preference was then adopted for the task. In most cases (69% of all participants) reward level preference was consistent with the prediction made above. The delivery of rewards in the assessment of preferences was accompanied by cues (pictures of bottles). This also served a first formation of an association between cue and reward. Furthermore, subjects completed training trials, in which the entire procedure was experienced and cue-reward associations were strengthened.

Individual differences in muscle strength were determined by calibrating the dynamometer in a short task called *MVC* (*maximum voluntary contraction*). The dynamometer was pressed by participants three times for three seconds. The mean value of these three trials was used to represent individual maximum strength. Force was then measured as a percentage of MVC. Average MVC scores were 292.93 N for males (*SD* = 52.62) and 162.66 N for females (*SD* = 44.81).

In total, the reward task consisted of 4 Blocks à 16 trials, two blocks per reward type (non-social/social). Reward type blocks were counterbalanced with the initial block determined randomly. In between blocks, participants could take a short break. In total, the task lasted for a duration of 90 minutes, including introduction, preference rating and MVC measurement.

3.6. Data analysis

To analyze the results, *linear mixed models (LMMs)* were fitted to the data with the open source programming language R (R Core Team, 2019) using both the package *lmerTes*t (Kuznetsova, Brockhoff, & Christensen, 2017) and the package *lme4* (Bates, Mächler, Bolker, & Walker, 2015). Residual analysis was carried out with the lme4 package due to easier accessibility of residuals. The R-Code used to carry out analysis can be found in the supplementary material.

Two predictors were included in the analysis (factor names are written in quotation marks): 'drug group' was treated as a fixed effect (between-subjects), whereas 'reward level' was included as a random effect, because measurements of different reward levels were done on the same subjects (within-subjects), leading to dependency in the data.

LMMs were chosen over a repeated-measures ANOVA because LMMs provide several advantages over traditional ANOVA approaches: treating factors as random effects allows for the generalization of a sample of conditions to a population of conditions (Barr, 2013). It can be argued that the conditions of 'reward level' are just a random sample of a population of possible reward level conditions. Thus, modeling them as random effects enables generalizing the effect beyond the conditions of 'high' and 'low' rewards used in the experiment.

Also, LMMs are more robust towards unbalanced and missing data (Boisgontier & Cheval, 2016) and information loss when averaging over participants (Judd, Westfall, & Kenny, 2012). Measurements were averaged across participants for both dependent variables. Moreover, the decision for LMMs is in line with the call by Boisgontier and Cheval (2016) to use LMMs in the field of neuroscience to avoid spurious results of ANOVA approaches.

4 LMMs were set up per dependent variable (written in cursive and quotation marks): '*force'* (contraction of the dynamometer in Newton) and '*wanting'* (ratings on a Likert scale of -10 - 10). Outliers in both dependent variables (more than 2 SDs above or below the mean) were excluded.

As described by Field (2012), LMMs were set up with increasing complexity, starting from a baseline model with just the intercept and adding in predictors successively in subsequent models. These models were then tested on differences in explaining variance in the data. The model that explained the most variance was then tested on the statistical assumptions of linear mixed models (linearity, homoskedasticity, and normality of residuals). Sphericity was not assessed, as there were only two levels for each factor, rendering testing for this assumption obsolete.

4. Results

Plotting the data by the predictors ‘reward level’ and ‘drug group’ and visually assessing trends reveals noticeable differences in both dependent variables for the factor ‘reward level’ but less so for the factor ‘drug group’ (see Figure 4). Descriptive statistics can be found in the tables below.

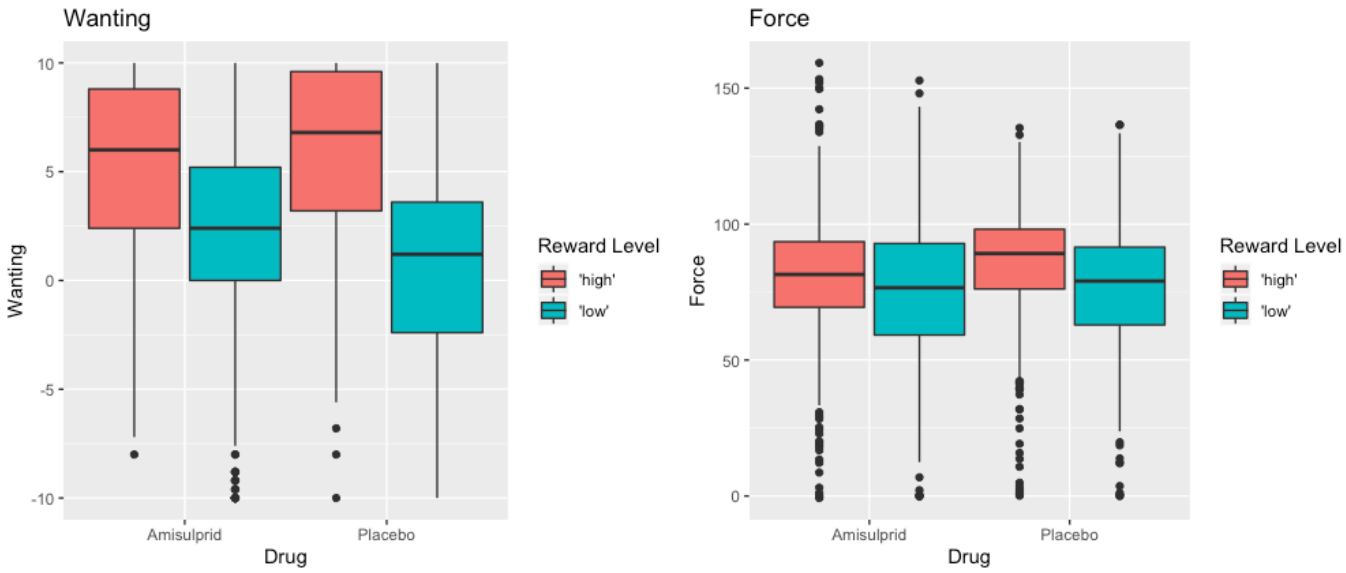

Figure 4: Box plots of ‘wanting’ and ‘force’ by ‘reward level’ and ‘drug group’

Table 1. Means and standard deviations for *'wanting'* as a function of a 2 X 2 design

	Reward Level			
	'high'		'low'	
Drug Group	*M*	*SD*	*M*	*SD*
Amisulpride	5.45	3.94	1.75	5.09
Placebo	5.92	4.06	0.74	5.06

Table 2. Means and standard deviations for *'force'* as a function of a 2 X 2 design

	Reward Level			
	'high'		'low'	
Drug Group	*M*	*SD*	*M*	*SD*
Amisulpride	80.45	25.87	75.34	28.67
Placebo	85.13	22.00	76.15	24.67

Note: M and *SD* represent mean and standard deviation, respectively.

4 LMMs were set up for each dependent variable, successively adding predictors to increase complexity: The first model contained a random intercept by subject only, for the second one, 'drug group' was added as a fixed effect. In the third model, 'reward level' was included as a random effect. The last model contained the interaction between 'reward level' and 'drug group'. Models were fitted using restricted maximum likelihood (REML).

For '*wanting'*, successive comparison of the variance explained by the models revealed the third model as the one with the lowest AIC (*Akaike's information criterion*). This model was fitting the data significantly better than the model with only 'drug group' as a predictor (ΔAIC = 1467.9). The subsequent interaction model did not differ significantly from this model (ΔAIC = -1.3). Thus, only values pertaining to the model with the lowest AIC ('reward level' as a random and 'drug group' as a fixed effect, no interaction) are reported.

H1a: Selective blockade of striatal dopamine activity entails *reduced subjective ratings of 'wanting'* a cued food reward

Examining the appropriate model for ratings of '*wanting'* more closely, only 'reward level' was a significant predictor ($t(47.01) = -4.76$, $p < 0.001$), whereas 'drug group' was not significant ($t(45.99) = -0.13$, $p = 0.90$).

Table 3. Summary of *'wanting'* model

Predictor	*b*	*SE*	*df*	*95% CI*	*t*	*p*
Intercept	5.69	0.64	59.17	[4.45, 6.95]	8.91	<0.001
Drug Group	-0.1	0.74	45.99	[-1.56, 1.37]	-0.13	0.90
Reward Level	-4.35	0.91	47.01	[-6.16, -2.54]	-4.76	<0.001

Note: b=unstandardized regression coefficient, SE=standard error, df=degrees of freedom, CI=confidence intervals

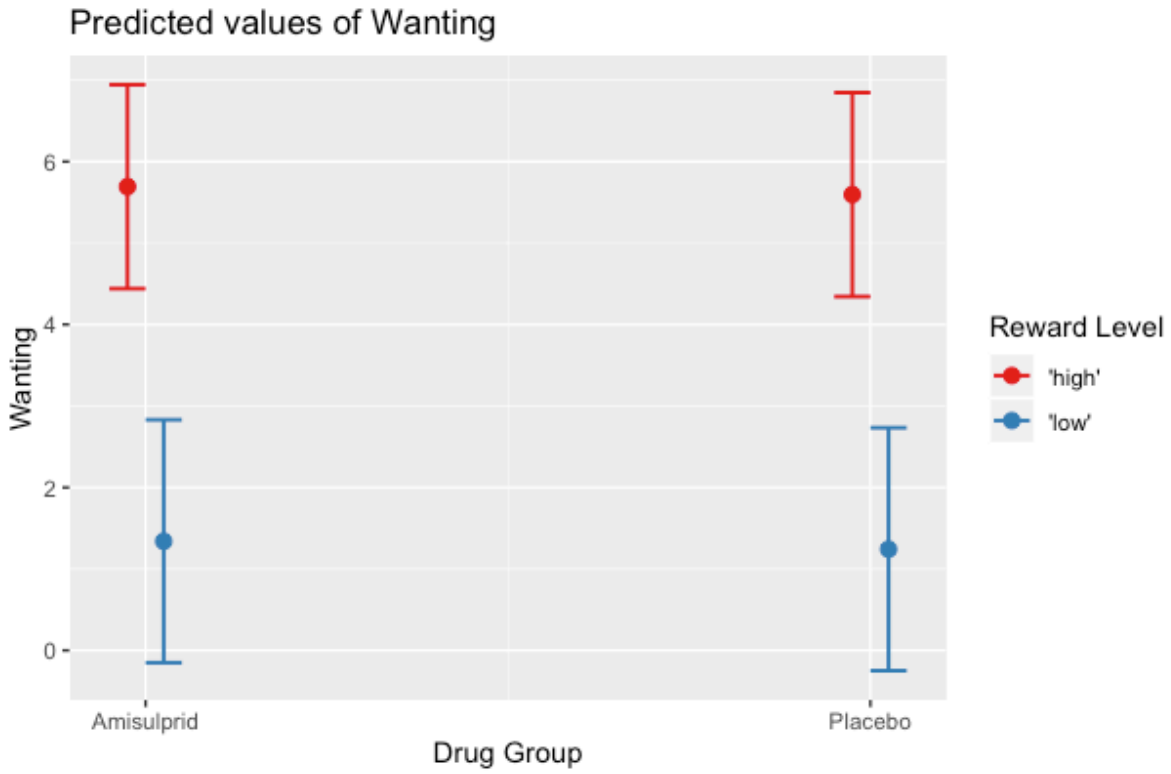

Figure 5: Predicted values of the best fitting model for ratings of 'wanting', error bars representing 95% confidence intervals

Further looking into the other models set up for the analysis confirmed that this pattern of results did not change across models: In the interaction model, only 'reward level' was a significant predictor of wanting. Even when 'gender' was added as a fixed effect, both the factor 'drug group' and the interaction were non-significant. 'Gender' itself was also a nonsignificant predictor (t (44.96) = 0.49, p = 0.62).

H1b: Selective blockade of striatal dopamine activity will lead to *reduced exerted effort* on a dynamometer measuring the strength of muscle contraction.

The same procedure was carried out for the second dependent variable, namely the amount of voluntary contraction of the dynamometer. Successive comparison similarly revealed the model with both 'drug group' and 'reward level' to fit significantly better than a model with only 'drug group' as a predictor (ΔAIC = 423). The interaction model did not fit the data significantly better (ΔAIC = -2). Thus, only values for the best fitting model are reported.

Again, only 'reward level' was a significant predictor of '*force'* (t (47.10)= -2.24, p = 0.03). The factor 'drug group' was not significant (t (45.99) = 0.65, p = 0.52).

Table 4. Summary of '*force'* model

Predictor	*b*	*SE*	*df*	*95% CI*	*t*	*p*
Intercept	81.14	3.63	50.81	[74.04, 88.23]	22.33	<0.001
Drug Group	3.16	4.87	45.99	[-6.41, 12.73]	0.65	0.52
Reward Level	-7.00	3.12	47.11	[-13.18, -0.81]	-2.24	0.03

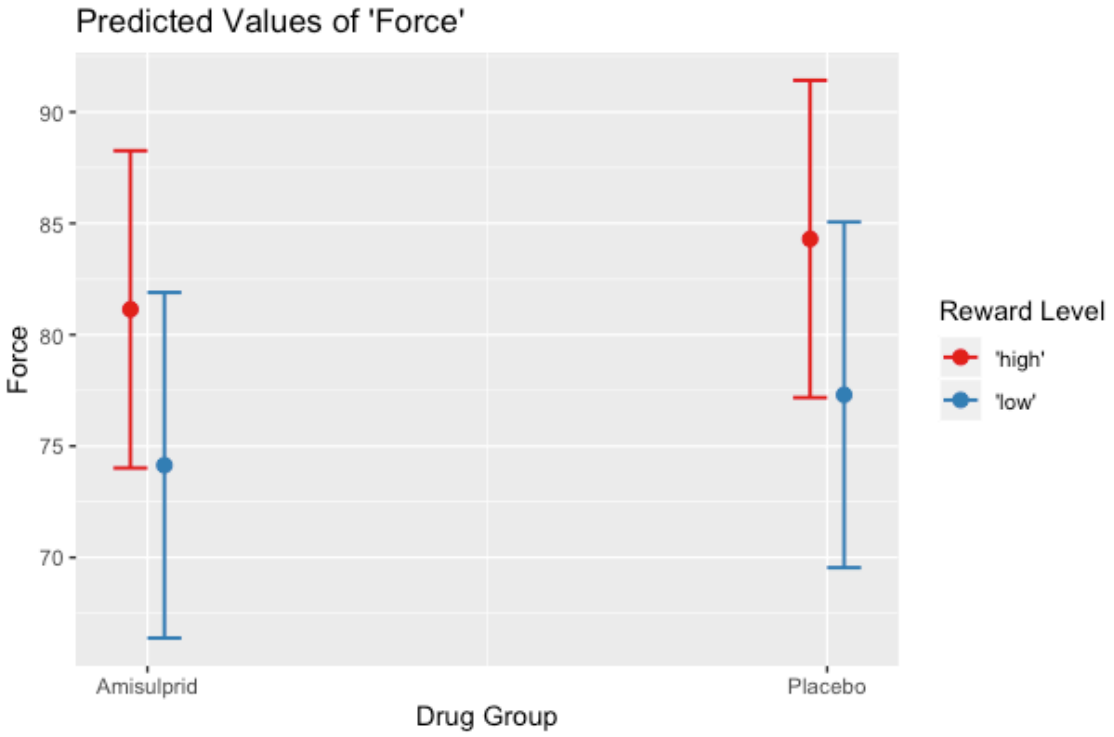

Figure 6: Predicted values of the best fitting model for 'force'

However, adding in further predictors changed this pattern of results. Including either the interaction of 'drug group' and 'reward level' or 'gender' removed any statistical significance for any predictor. Yet, the model presented above had the lowest AIC, therefore a significant effect of 'reward level' can be assumed.

According to Lorah (2018), the *Intraclass Correlation Coefficient (ICC)* is an appropriate effect size for random effects in a mixed model. The ICC for 'reward level' was calculated in R using the package *psychometric* package (Fletcher, 2010). For 'reward level', the ICC was 0.32. In other words, the proportion of variance in '*wanting'* scores accounted for by 'reward level' was 0.32. The ICC for force was 0.03, indicating a smaller effect of 'reward level' for '*force*' than for '*wanting*'. As the fixed effect 'drug group' was not significant in the analysis, no effect sizes are reported for this factor.

4.1. Assumption Checks

Linearity was assessed visually by plotting the residuals of the model against the observed values. The resulting plot seemed sufficiently randomly distributed for linearity of the relationship to be assumed. This was the case for both models.

Homoskedasticity was checked by plotting residuals against fitted values of the model. The plot was evenly distributed. No differences of variance for the individual factor levels could be detected. Using boxplots to plot residuals by factors ('drug group' and 'reward level') lead to a similar visual pattern. Variance appeared roughly equal for levels of both 'reward level' and 'drug group' in both models. Using *Levene's test* to test for unequal distribution of variance in factor levels revealed equal variance in levels of 'drug group' for the '*wanting'* model ($F(1) = 0.003$, $p = 0.95$). However, in levels of 'reward level', Levene's test was significant ($F(1) = 17.42$, $p < 0.001$). This pattern was also found when Levene's test was implemented manually (see supplementary material). For the '*force*' model, Levene's test was significant in both levels of 'reward level' and 'drug group' ($F(1) = 12.32$, $p < 0.001$) and ($F(1) = 17.42$, $p < 0.001$).

Normal distribution of residuals was first assessed visually by plotting residuals with both a QQplot and a histogram. On the QQplot, residuals diverged from the line predicting a normal distribution, mostly in the upper right and lower left of the diagram. Similarly, in the histogram, the distribution of residuals appeared slightly right-skewed. A Shapiro-Wilk test of residuals was also significant for both models ($W = 0.92$, $p < 0.001$) and ($W = 0.94$, $p < 0.001$).

However, as the results of such tests are dependent on sample size (N), significance in both the Levene Test and the Shapiro-Wilk test might be caused by the high amount of observations in the model ($N = 1506$). Because of this and because of the general robustness of linear mixed models against violation of assumptions, results of the analysis done above should be interpretable.

5. Discussion

5.1. Summary

The goal of this paper was to elucidate the functional role of mesolimbic dopamine activity in food reward processing by pharmacologically manipulating dopamine transmission in healthy human participants. The experimental procedure that has been used was based on a theory distinguishing the 'wanting' of food rewards from their hedonic impact or 'liking' (Berridge, 1996; Berridge & Kringelbach, 2015). The derived hypotheses stated that 'wanting', operationalized by two dependent variables of subjective rating questions and muscle contraction measured by a dynamometer, should be decreased in participants treated with the dopamine antagonist amisulpride compared to a control group, that had been given a placebo.

Contrary to expectations, amisulpride did not significantly decrease ratings of 'wanting' or contraction of the dynamometer. The only factor that reached significance in the analysis was 'reward level', that is the difference in reward value of variously rewarding stimuli (i.e. chocolate milk and milk mix). This factor was significant for both dependent variables. Other predictors considered in the statistical analysis, namely 'gender' or a 'drug group' by 'reward level' interaction were also not significant in any of the models set up for the analysis.

This finding stands in contrast to a large number of animal studies that have related the process of 'wanting' to mesolimbic dopamine activity (Salamone, 2007; Salamone & Correa, 2002). Some studies have been able to successfully manipulate 'wanting' using dopamine antagonists such as amisulpride or dopamine antagonists with similar pharmacological profiles (Jocham et al., 2014; Kahnt et al., 2015; McCabe et al., 2011). However, some authors point to studies, where attempts of a dissociation of 'wanting' and 'liking' have not been successful and even argue for the abandonment of these concepts (Havermans, 2011, 2012). This paper has not tried to distinguish 'wanting' from 'liking' but failing to pharmacologically manipulate 'wanting' in line with the incentive salience hypothesis adds to the controversy concerning the sensibility of this theoretical construct in the investigation of human food reward processing. There are several possible explanations, why findings in this experiment diverged from theoretical expectations, which do not necessarily invalidate the incentive salience hypothesis.

5.2. Possible Explanations

Above all, it has to be noted that this paper analyzed only half of the data of the entire study due to administrative reasons. The results of this paper, therefore, might not represent the full picture and non-significant results might be caused in part by small sample size and subsequently an insufficient power to detect effects of relatively small size along with a distortion of trends in the data.

The amount of evidence supporting the assumption that treatment with neuroleptic medication should lead to disturbances of reward processing (e. g. Berridge, 2007; Wolfram Schultz, 2015; Wise, 2006) makes it likely that not the theory underlying this investigation is flawed, but that the experimental setup might not have been sensitive enough to detect differences of subjective and objective 'wanting' of food rewards between experimental groups.

First, one might argue that the stimuli being applied might not have been strong enough. People in western societies are nowadays used to a wide range of highly palatable foods in nearly infinite supply. Subjects might have been more or less indifferent to two milliliters of chocolate milk or milk mix due to habituation to a high caloric diet. This stands in contrast to lab animals held on a hardly palatable and low caloric diet that are more easily stimulated by sweet and nutritious food rewards. At the very least, participants' indifference might make them not use the dynamometer meaningfully to achieve the cued reward. Such indifference towards a rewarding stimulus would naturally also be reflected in rating questions. However, such arguments seem to be falsified by the significant effect of 'reward level'. The statistical significance of this effect points to the fact that the experimental paradigm is generally able to detect differences in 'wanting' variously rewarding stimuli. It should therefore also be able to detect differences in 'wanting' if it has been successfully impaired in one group.

However, the problem of consciousness or awareness of one's motivational state might have affected results. As mentioned further above, incentive salience comprises elements that are not necessarily conscious (Anselme & Robinson, 2016). Whereas the difference in subjective incentive value of different reward levels can be consciously experienced, the effect of the dopamine antagonist on 'wanting' might not have reached these stages of high-level cognitive processing. Rating questions might not be able to capture differences in 'wanting' between groups, because they do not tap into unconscious mechanisms. This would make any effect of a dopamine antagonist in such a variable undetectable.

The dynamometer represents a more objective measure that should not rely on consciousness. However, this goes under the presumption that a learned association has been established between the force exerted on the dynamometer and the delivery of a reward. The probabilistic relationship between exerted effort and receival of a high or low food reward level was explained to the participants by the experimenters. Such an explanation would lead to a cognitive understanding of the relationship. As Berridge (2009) emphasizes, however, incentive salience relies on conditioning and is in large parts independent from cognitive understanding of causal relationships. Therefore, the squeezing of the dynamometer could have been subject to conscious control rather than being an automatically executed operant behavior established by conditioning. Consequently, to establish a strong association between squeezing the dynamometer and reward receival, a more extensive learning procedure might have to be realized that would not rely primarily on cognitive understanding of probabilistic relationships but on operant conditioning.

This account of differences in conscious accessibility of effects would explain the pattern of results of this paper without necessarily refuting the incentive salience hypothesis. The notion that dopamine antagonists exert their effect on food reward processing unconsciously is not unlikely. Food 'wanting' is a very complex process that requires the integration of both internal stimuli, pertaining to systemic bodily functions such as energy balance, physical activity, etc. *and* external reward-associated stimuli such as food sight, smell or other cues associated with food consumption. Manipulating only one parameter of this process in isolation might not influence 'wanting' enough to make it consciously accessible and thereby detectable with the experimental paradigm that was used.

Both internal and external stimuli could be used to refine the experimental procedure in order to allow for the detection of differences in 'wanting' between groups in future endeavors. First, effects of satiety might have distorted results in this experiment. Everyone can easily empathize with a situation of overeating, where a food reward such as chocolate that would be highly 'wanted' in a physiological need state loses its rewarding value or even induces feelings of aversion when satiety is reached. This phenomenon is known as alliesthesia and the effect of decreased incentive value of a stimulus during prolonged consumption is referred to as *sensory specific satiety* (Havermans, Janssen, Giesen, Roefs, & Jansen, 2009). During the 32 trials of the experiment, participants might experience oversaturation with chocolate milk or milk mix, which would reflect in rating questions and in the force applied to the dynamometer. Participants would also sometimes report to the experimenter that they "now had enough of chocolate milk" at the end of the experiment or

report a “plastic taste”, indicating a state of sensory specific satiety and potential influences of the plastic tubes on palatability. Taken together, the rewarding stimulus might even have had an aversive effect on participants towards the end of the procedure.

Importantly, such an effect might interact with medication, causing a decrease of ‘wanting’ in the placebo group, whereas the amisulpride group could be less affected. This would lead to similar means of ‘wanting’ in both groups and cover up any differences between groups caused by the dopamine antagonist.

A possible solution to this problem would lie in inducing a strong physiological need state so that the incentive value of the stimulus stays high for a prolonged time until completion of the experiment. This is especially important since participants received a relatively normal-sized meal in the form of a granola bar prior to the experiment, which might have reduced feelings of hunger. Naturally, the amount of fasting human participants can be subjected to is limited by ethical considerations. In addition, doing so would restrict conclusions to food ‘wanting’ during physiological need states and questions whether findings in such a setup could be generalized to a state of relative satiety would arise.

Alternatively, the statistical analysis could account for a decrease in ‘wanting’ over time in both groups and a potential interaction of sensory specific satiety and medication. Additionally, any influences of the experimental setup on palatability should be minimized as far as possible: due to costs, plastic tubes were not changed for sometimes long periods of time. This obviously influences palatability and exerts an effect on ‘wanting’ that should be avoided in further studies.

Regarding external or environmental stimuli that can trigger ‘wanting’, it has to be noted that the setting in which the experiment took place was highly artificial. Participants received the chocolate milk through a set of three plastic tubes. The artificiality of the situation might have hindered experiencing the stimulus as rewarding and in turn affect also the ‘wanting’ of the stimulus. Most rewards are learned and environmental cues play a great role in triggering the preparatory processes that precede consumption of food rewards (Schultz, 2015). Cues normally associated with eating can trigger ‘wanting’ (Berridge, 1996). The integration of environmental cues might even be a precondition for successfully triggering ‘wanting’ in a way that can be reported in rating questions or be acted out on the dynamometer.

Such environmental cues were not present for participants. Instead, they were placed in an unfamiliar, highly artificial environment not associated with the consumption of food rewards. The stimulus that was used (pictures of bottles) was learned only minutes prior to the

experiment and thus might not have been associated strongly enough with the receival of a reward to trigger unconscious elements of ‘wanting’. This might have affected ‘wanting’ to a degree that effects of the dopamine antagonist were concealed in both rating questions and effort. A possible approach to tackle this problem would lie in establishing a strong learned association of both cues and effort (and environmental cues such as the lab setting itself) with the delivery of food rewards. Such an undertaking would probably require the entire experimental procedure to be conducted several times. Of course, this would also increase the complexity and costs of the study.

Another important consideration involves the way in which dopamine antagonists exert their effect on reward processing over time. Specifically, dopamine antagonists might not influence reward processing as immediate as the hypotheses set up for this paper suggest. As mentioned earlier, Wise (2004) points out that the effects of neuroleptics on approach behavior play out over the course of several trials with animals responding normally in early trials and gradually responding less in later ones, a pattern known as extinction mimicry. He also uses these patterns of results in an attempt to disprove Berridge’s incentive salience theory (Wise, 2004). This would mean that neuroleptic suppression of incentive motivation by amisulpride would not be instantaneous but rather reflected in the data as a steady decline of ‘wanting’ over the course of many trials.

Consequently, a certain amount of trials would be necessary to make the effect of neuroleptics on ‘wanting’ visible. Theoretically, the 32 trials that were realized in total should be high enough to detect a reasonably large effect on ‘wanting’ given an appropriate sample size. Nonetheless, such a trend of a steady decline was not accounted for in the statistical analysis and it might necessary to model the data in this way to clearly disentangle the differences between groups.

Berridge (2007) has argued against Wise that findings of extinction mimicry would falsify the incentive salience account. He describes a *reboosting effect* that would be necessary to upkeep incentive salience over the course of several trials. Without reboosting, a reward would become less and less ‘wanted’ with repeated exposure. According to Berridge, such reboosting effects would be especially vulnerable to neuroleptic suppression. This would mean that well-established rewards and their cues remain ‘wanted’ under low- to-moderate neuroleptic influence for a certain amount of exposures. Less well-established cues would lose their incentive value more quickly. As the association of cue and reward in the conducted experiment could be argued to be not well-established, it should be subject to the effect of neuroleptic medication, contrary to the results of this paper.

Notwithstanding, this goes under the presumption that an unconsciously operating association of cue and reward, as well as force (in the sense of an operant behavior), and reward has been established at all and the assumption that the dependent variables used in this experiment have not been able to tap into potentially unconscious effects of dopamine antagonists still holds.

5.3. Limitations

One important limitation of this study is the artificiality of the testing situation and its influence on external validity. Whether findings that have been gained in such a laboratory setting can be generalized to real world rewards and more complex environments is debatable. Naturally, conclusions that can be drawn from this study are also limited by the inclusion criteria and sample characteristics, potentially posing threats to the generalizability of the findings to the population. Importantly, this study investigated healthy subjects. Samples drawn from pathological populations might show entirely different results. Dissociations of 'wanting' and 'liking' could be more pronounced in pathological (i.e. obese) participants: Studies that have demonstrated a dissociation of 'wanting' and 'liking' have often focused on participants with problematic eating behavior (Baik, 2013; Cowdrey et al., 2013). The attempt to distinctly manipulate these mechanisms in isolation might produce more pronounced results in pathological participants, where reward processing is supposedly disturbed, but lead to less decisive results in healthy participants.

In line with this assumption, neuroleptics have very unexpected effects on reward processing in participants suffering from certain pathologies relatively unconnected to food reward processing. In schizophrenia, where neuroleptics are commonly used to counteract positive psychotic symptoms, one of the most prominent side effects of such treatment is weight gain, which poses great obstacles for medication adherence in treating schizophrenic patients (Coccurello & Moles, 2010; Nasrallah, 2003; Nielsen, Rostrup, Wulff, Glenthøj, & Ebdrup, 2016). Weight gain under neuroleptic treatment is seemingly not only caused by metabolic disturbances but by disturbances of food reward processing (Nielsen et al., 2016). This would indicate a rise in 'wanting' food rewards under neuroleptic influence in schizophrenic patients. Obviously, such an effect of dopamine antagonism is diametrically opposed to the incentive salience hypothesis. This highlights the variability of effects of dopamine antagonists under different conditions. The mesolimbic reward system might be altered in schizophrenia in a way, that causes dopamine antagonists to reverse their effect pattern.

Conversely, there's a range of studies that demonstrate how treatment with dopamine agonists in Parkinson's disease increases the incentive value of a wide array of rewards (such as gambling or sexual rewards) (Merims & Giladi, 2008). Such findings again support the incentive salience hypothesis.

To summarize, there is still contradictory evidence not only in this study but also in the field as a whole. It is therefore important to consider how the mesolimbic reward system interacts with different pathological conditions to fully understand how dopamine agonists or antagonists influence reward processing.

5.4. Main insights

Since the initial hypotheses had to be rejected, the essential gain in knowledge lies in determining the experimental paradigms that are or are *not* able to manipulate processes of incentive motivation in healthy human participants. In animals, the destruction of mesolimbic reward circuitry has pronounced effects on incentive motivation (Berridge & Robinson, 1998). Apparently, evoking the same pattern of results by pharmacological manipulation in human participants is harder than expected.

This study is one of the few that attempted to elicit differences in 'wanting' by directly and separately manipulating the underlying neural substrate. Some studies that have used food stimuli as rewards have adopted other physiological manipulations like fasting, stress or physical exercise (Cameron et al., 2014; Epstein et al., 2003; Lemmens et al., 2011; McNeil et al., 2015). Such manipulations have more widespread effects on a vast array of systemic bodily functions that are perhaps necessary to evoke detectable differences in 'wanting'. Manipulating only the underlying neural substrate without also manipulating other, systemic parameters of incentive motivation for food rewards seems to fall short of having any significant effects on 'wanting' measured in this way.

Interestingly, the factor 'reward level', pertaining to subjective differences in the incentive value of variously rewarding stimuli, was significant. It thus seems that the experimental paradigm is generally able to detect differences in 'wanting' and that participants meaningfully interacted with both rating questions and the dynamometer. This makes it likely that drug group differences should also be detectable, given a refinement of the experimental conditions and the exclusion of additional effects that might have distorted and confounded the data. As already mentioned above, such refinements would involve the induction of a physiological need state to upkeep incentive motivation over a prolonged period of time and avoid satiety or even aversion towards the rewarding stimulus, the

establishment of a strong association between cue and reward as well as force and reward that is independent from cognitive understanding of probabilistic relationships and a statistical approach that accounts for a steady decrease in ‘wanting’ rather than assuming an instantaneous effect.

5.5. Practical Relevance

The findings of this paper carry several implications for practical application, mainly for the treatment of eating disorders such as obesity or anorexia. In his article, Berridge (2009) discusses several possibilities, how incentive salience might be involved in pathological food intake. In short, he proposes three possibilities: First, abnormal eating behaviors might be *caused* by reward dysfunction, namely anomalous ‘liking’ and subsequently disturbed ‘wanting’ in obesity or anorexia. Second, reward processing distortions could also be viewed as a *consequence* of abnormal physiological feedback signals, caused by periods of food over- or underconsumption. Third, reward processing might not be affected in eating disorders, instead, other causes outside of mesolimbic reward processing would have to be assumed as causes.

If the results of this paper are substantiated by further studies, it should be assumed that abnormal food intake is not caused by alterations of incentive salience. Specifically, if food ‘wanting’ cannot be manipulated by suppressing dopamine activity, it is unlikely that an elevation of mesolimbic dopamine alone would be sufficient to cause food overconsumption. Instead, reward processing alterations might rather be a consequence of said eating behaviors, such as binge eating or strict dieting. Consequently, the use of medication to correct reward processing in the hope of correcting eating behaviors would not be recommendable. In treatment, it might be more sensible to target other possible causes of eating disorders outside of reward processing.

The findings of this study also bear implications for the concept of *food addiction*. There is still a large debate in the literature, whether a phenomenon such as food addiction actually exists (Ziauddeen & Fletcher, 2013). One key feature of food addiction on a neurobiological level is that drugs of abuse and food act on the same neural substrate and facilitate increased stimulation of this substrate after repeated exposure, a process known as *neural sensitization* (Berridge, 2009). Some studies suggest that such changes in dopamine activity might indeed be caused by food intake regimens, mainly when altering between periods of overeating and dieting, albeit such studies have often used rodents as test subjects (Avena & Hoebel, 2003; Bello, Sweigart, Lakoski, Norgren, & Hajnal, 2003; Carr, 2002). In

the present study, using human participants, alterations of food 'wanting' could not be brought about by manipulating the underlying neural substrate in isolation. Consequently, it is unlikely that eating disorders such as obesity involve sensitization of the mesolimbic reward system as a primary cause. Therefore, food addiction as a concept should be handled with caution and treatment of eating disorders should not be conceived in the same way as substance abuse disorders.

Naturally, these implications are subject to the reliability and validity of the findings of this paper and future studies should first try to exclude alternative explanations for the lack of results before refuting concepts such as food addiction, neural sensitization in response to food stimuli and excessive or deficient food 'wanting' in eating disorders.

5.6. Future Research

First and foremost, the possible causes for the lack of results in this paper mentioned above should be addressed in future studies using this paradigm. If such a refinement should lead to reliable and valid differences in 'wanting' food rewards between drug groups, it would substantiate the incentive salience hypothesis and open up further possibilities for the investigation of incentive motivation. Instead of healthy participants, subjects suffering from disorders connected to food reward processing could serve as participants. Determining whether such pathologies are characterized in terms of elevated or decreased food 'wanting' compared to controls would inform theory about potential underlying causes and suggest appropriate lines of treatment. Similar studies have already been done, indicating that reward processing indeed may be disturbed in anorexia (Cowdrey et al., 2013; Watson, Werling, Zucker, & Platt, 2010). Instead of dopamine antagonists, agonists could be used, investigating whether normal food reward processing could be reestablished in anorectic participants.

However, such speculation goes under the presumption that a refinement of the experimental procedure does produce valid and reliable results. Should this not be the case, the incentive salience hypothesis would have to be revised to account for discrepant outcomes such as the one in this paper or refuted entirely. Perhaps other perspectives on mesolimbic dopamine activity are more fit to analyze the data appropriately. Ultimately, if relating processes of incentive salience to dopamine transmission in humans consistently fails, research would have to follow Haverman's recommendation of abandoning these concepts.

For now, it is too early to determine whether the incentive salience hypothesis can be considered falsified. There are several reasonable explanations to account for the lack of results in this paper that do not necessarily contradict the original theory. Possible solutions to

exclude confounding effects have been discussed. Future investigations that build on these solutions will hopefully resolve, whether ‘wanting’ of food rewards in human participants can be brought about by pharmacological manipulation and further our understanding of motivational processes and pathologies connected to it.

6. References

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7. Supplementary material

7.1. Abstract (English)

Reward processing is a key concept to gain insights into pathologies characterized by deficits in motivation and anhedonia. One branch of disorders, where abnormal reward processing is speculated to be involved, is eating disorders. A large amount of evidence links reward processing to mesolimbic dopamine activity. The most influential contemporary perspective on the functional role of dopamine activity is the incentive salience hypothesis. It proposes mesolimbic dopamine to mediate the cognitive process of incentive salience, which consists of attributing subjective rewarding value or 'wanting' to a stimulus. However, there is controversy as to whether this perspective is applicable to humans. To investigate this presumption, an experiment including 48 healthy participants was conducted. Subjects were randomly assigned to two groups, receiving either a dopamine antagonist (400 mg amisulpride) or a placebo. Then, subjects completed a task in which they were administered rewarding food stimuli of differing reward value (referred to as 'reward level'). The reward value of the applied food stimulus was dependent on muscle contraction on a dynamometer. This also served as a dependent variable for 'wanting', along with rating questions. The effect of 'reward level' was significant for both dependent variables, whereas amisulpride was not found to have a significant effect on either variable, contrary to expectations. Several explanations for this result and potential refinements of the experimental paradigm are discussed. Most likely, the experimental setup was not sensitive enough to detect differences of 'wanting' between drug groups due to problems such as unconscious elements of 'wanting', delayed effects of dopamine antagonists on reward processing and the lack of an appropriate statistical approach to account for such confounds.

Keywords: Incentive Salience, Amisulpride, Reward Processing, Mesolimbic Dopamine, Obesity, Anorexia, Food Addiction

7.2. Abstract (German)

Belohnungsverarbeitung ist ein zentrales Konzept, um Pathologien zu verstehen, die mit Motivationsdefiziten und Anhedonie zusammenhängen. Im Bereich der Essstörungen werden Veränderungen der Belohnungsverarbeitung als mögliche Ursache dieser Pathologien angenommen. Eine ganze Reihe von Studien hat gezeigt, dass der Prozess der Belohnungsverarbeitung mit Dopaminausschüttung im mesolimbischen System zusammenhängt. Die einflussreichste zeitgenössische Perspektive auf die funktionale Rolle mesolimbischer Dopaminausschüttung ist die Anreiz-Salienz-Hypothese. Anreiz-Salienz ist ein kognitiver Prozess, der einem Stimulus einen subjektiven Belohnungswert zuweist und wird auch ‚Wollen' genannt. Ob diese Theorie auf Menschen anwendbar ist bleibt jedoch Gegenstand von Kontroversen. Um die Übertragbarkeit der Anreiz-Salienz-Hypothese zu untersuchen wurde ein Experiment an 48 gesunden menschlichen Probanden durchgeführt. Teilnehmer wurden zufällig zu zwei Gruppen zugeteilt, von denen eine Gruppe 400mg des Dopamin-Antagonisten Amisulprid erhielt, die andere ein Placebo. Daraufhin wurde eine Aufgabe durchgeführt, in welcher Teilnehmer Nahrungsstimuli von unterschiedlichem Belohnungswert (genannt ‚reward level') erhalten konnten. Der Belohnungswert des verabreichten Nahrungsstimulus hing von der Kraft ab, mit der Teilnehmer einen Dynomometer (Druckmesser) zusammendrückten. Diese Messung stellte gleichzeitig eine abhängige Variable für ‚Wollen' dar. Zusätzlich wurde ‚Wollen' mit Ratingfragen erfasst. Der Faktor ‚reward level' war für beide abhängigen Variablen signifikant. Amisulprid hatte hingegen keinen signifikanten Effekt auf diese Variablen, was den Erwartungen widerspricht. Mehrere mögliche Erklärungen für diese Ergebnisse werden in der Arbeit besprochen. Mit großer Wahrscheinlichkeit war das Experiment nicht geeignet, Unterschiede zwischen den Gruppen zu entdecken, da ‚Wollen' auch unbewusste Elemente enthält, Dopamin-Antagonisten ihre Wirkung möglicherweise nicht unmittelbar entfalten und die statistische Herangehensweise daher nicht geeignet war, diesen konfundierenden Einflüssen gerecht zu werden.

Schlüsselwörter: Anreiz-Salienz, Amisulprid, Belohnungsverarbeitung, Mesolimbisches Dopamin, Fettleibigkeit, Anorexia, Nahrungssucht

7.3. List of Figures

7.4. List of Tables

7.5. List of Abbreviations

AIC	Akaikes information criterion
ANOVA	Analysis of Variance
ASD	Autism spectrum disorder
ATP	Adenosine triphosphate
BMI	Body-Mass-Index
cAMP	Cyclic adenosine monophosphate
fMRI	Functional magnetic resonance imaging
ICC	Intraclass correlation coefficient
LMM	Linear mixed model
MVC	Maximum voluntary contraction
NAcc	Nucleus Accumbens
SNc	Pars compacta of the substantia nigra
VTA	Ventral tegmental area

7.6. R Code

```
#---------------------LOADING PACKAGES

#Adjust these if they don't load

library("ggplot2",
library("lme4",
library("gridExtra",
library("pastecs",
library(dplyr)
library(car)
library(lattice)
library(lmerTest)
library(sjPlot)
library(sjlabelled)

#--------------------READ AND SELECT DATA-----------------

#Please adjust according to the location of the data file

url_long <- "~/Library/Mobile
Documents/com~apple~CloudDocs/Psychologie Studium/Master/Semester
3/MAII Silani/Statistische
Auswertung/Behavior_social_reward_task_71_subs_LONG.txt"
url_wide <- "~/Library/Mobile
Documents/com~apple~CloudDocs/Psychologie Studium/Master/Semester
3/MAII Silani/Statistische
Auswertung/Behavior_social_reward_task_71_subs_WIDE.txt"
data_long <- read.delim(url_long)
data_wide <- read.delim(url_wide)

# select just nonsocial, then select only Amisulprid and Placebo
data_long_ns <- subset(data_long, condition == "'nonsocial'")
data_long_ns_Ami <- subset(data_long_ns, data_long_ns$Drug_group !=
2)

#Turn Drug_group to factor and rename values
data_long_ns_Ami$Drug_group <-
as.factor(data_long_ns_Ami$Drug_group)
data_long_ns_Ami$Drug_group <-
plyr::mapvalues(data_long_ns_Ami$Drug_group, from=c(1, 3),
to=c("Amisulprid", "Placebo"))

#Excluding outliers for wanting and renaming dataframe for easier
access
data_new <- subset(data_long_ns_Ami,
data_long_ns_Ami$outlier_wanting==0)
```

```
#Creating Dataframe for plotting gender distribution and barplotting
it
GenderData <- data.frame('Gender' = c('Female', 'Female', 'Male',
'Male'), 'Drug' = c('Amisulpride', 'Placebo', 'Amisulpride',
'Placebo'),

'Amount' = c(21, 20, 3, 4))
ggplot(GenderData, aes(x=Drug, y=Amount, fill=Gender)) +
geom_bar(stat = 'identity', width = 0.5) +
scale_fill_manual(values=c("#ffb6c1", '#73d0ff')) + theme_minimal()


#Ploting data by Drug_group and reward type, no gender
grid.arrange(
  ggplot(data_new, aes(x=Drug_group, y=wanting, fill=reward_type)) +
    geom_boxplot(width = 1) + labs(title = "Wanting", x = 'Drug', y
= 'Wanting', fill = 'Reward Level'),
  ggplot(data_new, aes(x=Drug_group, y=max_mean_percentage_force,
fill=reward_type)) +
    geom_boxplot(width = 1) + labs(title = "Force", x = 'Drug', y =
'Force', fill = 'Reward Level'),
  ncol=2)

#Descriptive Stats for each level of Drug_group and reward_type
by(data_new$wanting, list(data_new$reward_type,
data_new$Drug_group),
   stat.desc, basic = FALSE)

#-----------------------------------------Trying LMMs
#Basic Formula:
# model_1 <- lmer(DV ~ fixedEff1 * fixedEff2 + (1 + randomSlopes |
randomIntercepts), data=nameofdatafile)



#---------WANTING

#random intercept by subject, no predictors
baseModel <- lme4::lmer(wanting ~ 1 + (1|sub), data_new)
#plotting
ggplot(subset(data_long_ns_Ami, outlier_wanting == 0),
aes(y=wanting, x=reward_type, fill=Drug_group)) +
  geom_boxplot()  + geom_point(aes(y=fitted(baseModel), colour =
'baseModel')) +
  labs(title = "Wanting", x = 'Reward Level', y = 'Wanting', fill =
'Drug Group', colour = 'Predicted') + facet_wrap(~sub)
#intercept varies across subjects, no slope

#adding fixed effect Drug_group, note that baseModel is also
included in the plot
```

```
ModelDrug_group <- lme4::lmer(wanting ~ Drug_group + (1|sub),
data_new)
ggplot(subset(data_long_ns_Ami, outlier_wanting == 0),
aes(y=wanting, x=reward_type, fill=Drug_group)) +
  geom_boxplot()  + geom_point(aes(y=fitted(ModelDrug_group), colour
= 'ModelDrug_Group')) + geom_point(aes(y=fitted(baseModel), colour =
'baseModel')) +
  labs(title = "Wanting", x = 'Reward Level', y = 'Wanting', fill =
'Drug Group', colour = 'Predicted') + facet_wrap(~sub)
#almost complete overlap of baseModel and ModelDrug_group, zooming
would reveal very small differences (has been tested)


#adding reward_type as a fixed effect
Modelreward_typefix <- lmerTest::lmer(wanting ~ Drug_group +
reward_type + (1|sub), data_new)
ggplot(subset(data_long_ns_Ami, outlier_wanting == 0),
aes(y=wanting, x=reward_type, fill=Drug_group)) +
  geom_boxplot()  + geom_point(aes(y=fitted(Modelreward_typefix),
colour = 'Modelreward_typefix')) +
  labs(title = "Wanting", x = 'Reward Level', y = 'Wanting', fill =
'Drug Group', colour = 'Predicted') + facet_wrap(~sub)
#slope has changed, but stays fixed across subjects

#letting slopes of reward_type vary by subject
Modelreward_typerand <- lmerTest::lmer(wanting ~ Drug_group +
reward_type + (1+reward_type|sub), data_new)
ggplot(subset(data_long_ns_Ami, outlier_wanting == 0),
aes(y=wanting, x=reward_type, fill=Drug_group)) +
  geom_boxplot()  + geom_point(aes(y=fitted(Modelreward_typerand),
colour = 'Modelreward_typerand')) +
  labs(title = "Wanting", x = 'Reward Level', y = 'Wanting', fill =
'Drug Group', colour = 'Predicted') + facet_wrap(~sub)
#slope now varies based on subject. best fit visually

Modelreward_typerand2 <- lme4::lmer(wanting ~ Drug_group +
reward_type + (1 + reward_type|sub), data_new)
summary(Modelreward_typerand2)

#adding interaction
Modelreward_typeint <- lmerTest::lmer(wanting ~ Drug_group *
reward_type + (1+reward_type|sub), data_new)
ggplot(subset(data_long_ns_Ami, outlier_wanting == 0),
aes(y=wanting, x=as.factor(reward_type), color=Drug_group)) +
  geom_boxplot()  + geom_point(aes(y=fitted(Modelreward_typeint),
color = 'red')) +
  labs(title = "Wanting", x = 'Drug', y = 'Wanting', fill = 'Reward
Level') + facet_wrap(~sub)
#no visible changes
```

```
#Comparing all models successively
anova(baseModel, ModelDrug_group, Modelreward_typefix,
Modelreward_typerand, Modelreward_typeint)
#best fit = Modelreward_typerand, interaction model not significant

anova(Modelreward_typerand)
#reward_type significant, drug_group not significant

summary(Modelreward_typerand)

#excluding Drug_group as a predictor and comparing it to
Modelreward_typerand
ModelnoDrug <- lme4::lmer(wanting ~ reward_type + (1 +
reward_type|sub), data_new)
anova(ModelnoDrug, Modelreward_typerand)
#no significant difference --> drug_group could be excluded

# changing labels (necessary for plot)
label_names <- c("max_mean_percentage_force", "Drug_group",
"reward_type")
names(label_names) <- c("Force", "Drug Group", "Reward Level")

set_label(data_new$max_mean_percentage_force) <- "Force"
set_label(data_new$Drug_group) <- "Drug Group"
set_label(data_new$reward_type) <- "Reward Level"
set_label(data_new$wanting) <- "Wanting"

#plotting predicted values of the best fitting model
plot_model(Modelreward_typerand, type="pred", terms =
c("Drug_group", "reward_type"), title = "Predicte Values of
'Wanting'")


#For further analysis: adding gender as a fixed effect
ModelGender <- lmerTest::lmer(wanting ~ Drug_group * reward_type +
gender + (1 + reward_type|sub), data_new)
anova(ModelGender)
summary(ModelGender)
anova(Modelreward_typeint, ModelGender)
#Pattern of results does not change

#confidence intervals for wanting model
confint(Modelreward_typerand, oldNames = FALSE)

#Calculating the ICC

ICC1.lme(wanting, reward_type, data_new)


#--------------FORCE
```

```
#Setting up models, this time without plotting them
baseModelForce <- lme4::lmer(max_mean_percentage_force ~ 1 +
(1|sub), data_new)
ModelDrug_groupForce <- lme4::lmer(max_mean_percentage_force ~
Drug_group + (1|sub), data_new)
Modelreward_typeForceRes <- lme4::lmer(max_mean_percentage_force ~
Drug_group + reward_type + (1+reward_type|sub), data_new)
Modelreward_typeForce <- lmerTest::lmer(max_mean_percentage_force ~
Drug_group + reward_type + (1+reward_type|sub), data_new)
Modelreward_typeIntForceRes <-
lme4Test::lmer(max_mean_percentage_force ~ Drug_group * reward_type
+ (1+reward_type|sub), data_new)
Modelreward_typeIntForce <- lmerTest::lmer(max_mean_percentage_force
~ Drug_group * reward_type + (1+reward_type|sub), data_new)
ModelGenderForce <- lmerTest::lmer(max_mean_percentage_force ~
Drug_group * reward_type + gender + (1 + reward_type|sub), data_new)

#Comparing models
anova(baseModelForce, ModelDrug_groupForce, Modelreward_typeForce,
Modelreward_typeIntForce, ModelGenderForce)
#Best fitting: Modelreward_typeForce


#confidence intervals
confint(Modelreward_typeForce, oldNames = FALSE)

#Plotting Model
plot_model(Modelreward_typeForce, type="pred", terms=c("Drug_group",
"reward_type"), title = "Predicted Values of 'Force'")

#Calculating ICC
ICC1.lme(max_mean_percentage_force, reward_type, data_new)

#---------------------Checking Assumptions: RESIDUAL ANALYSIS
#Only for Modelreward_typerand and Modelreward_typeForce

#ASSUMPTION 1: LINEARITY

#Plot residuals vs observed

plot(data_new$wanting, resid(Modelreward_typerand))
plot(data_new$wanting, resid(Modelreward_typeForce))
#looks random

#ASSUMPTION 2: HOMOSKEDASTICITY

#plotting residuals against fitted values
plot(Modelreward_typerand)
plot(Modelreward_typeForce)
```

```
#looks blob-like, which is a good thing

#boxplotting
boxplot(residuals(Modelreward_typerand) ~ data_new$Drug_group)
boxplot(residuals(Modelreward_typerand) ~ data_new$reward_type)

boxplot(residuals(Modelreward_typeForce) ~ data_new$Drug_group)
boxplot(residuals(Modelreward_typeForce) ~ data_new$reward_type)
#Variance looks roughly equal for both Drug group and reward type
for both models

#Levene Test
#in levels of Drug_group for Wanting
leveneTest(residuals(Modelreward_typerand),data_new$Drug_group)
#not significant --> Homogeneity of Variance for levels of
Drug_group can be assumed
#For Force
leveneTest(residuals(Modelreward_typeForce),data_new$Drug_group)
#significant


#in levels if reward_type for Wanting
leveneTest(residuals(Modelreward_typerand),data_new$reward_type)
#significant
#For Force
leveneTest(residuals(Modelreward_typerand),data_new$reward_type)
#significant
#-->due to high number of observations, N=1504?

#MANUAL LEVENE TEST FOR WANTING MODEL

#save residuals in original dataframe
data_new$ModelResiduals <- residuals(Modelreward_typerand)
data_new$AbsModelResiduals <- abs(data_new$ModelResiduals)
data_new$AbsModelResiduals2 <- data_new$ModelResiduals^2

# predict Residuals by subject in a linear model and anova
LeveneModel <- lm(AbsModelResiduals2 ~ sub, data = data_new)
anova(LeveneModel)
#significant

#ASSUMPTION 3: Normal distribution OF RESIDUALS
qqmath(Modelreward_typerand, id = 0.05)
qqmath(Modelreward_typeForce, id = 0.05)

hist(residuals(Modelreward_typerand))
hist(residuals(Modelreward_typeForce))

qqPlot(residuals(Modelreward_typerand))
qqPlot(residuals(Modelreward_typeForce))
```

```
shapiro.test(residuals(Modelreward_typerand))
shapiro.test(residuals(Modelreward_typeForce))
#highly significant --> Normality of residuals cannot be assumed
#--> high sample size and robustness of LMMs
```