Daily memantine treatment blunts hedonic response to sucrose in rats

Psychopharmacology (Berl). 2020 Jan; 237(1), 103-114.
doi: 10.1007/s00213-019-05348-3. Epub 2019 Aug 14. PubMed PMID: 31414153.

Daily memantine treatment blunts hedonic response to sucrose in rats

Adriana Galistu, Paolo S. D'Aquila

Correspondence:

Paolo S. D'Aquila dsfpaolo@uniss.it

Dipartimento di Scienze Biomediche, Università di Sassari, Sassari, Italy

Viale San Pietro 43/b, 07100 Sassari, Italy

Funding The present study was funded by the Fondazione di Sardegna, Sassari, Italy.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

Abstract

Rationale Preclinical and clinical studies suggest the potential use of memantine in the treatment of binge eating disorder. The aim of this study was to further investigate the mechanisms by which memantine influences the motivational aspects of ingestion through the analysis of licking microstructure. To interpret treatment effects in relation to drug action at specific functionally relevant times, we compared the effect of two different administration schedules.

Methods Memantine was administered daily for a week, either 1 hour before or immediately after a 30-min daily session. The effects on the microstructure of licking for a 10% sucrose solution in rats were examined in the course of treatment and for 15 days after treatment discontinuation.

Results Treatment before testing reduced ingestion due to reduced burst size, and increased latency in the first session. However, a progressive increase in burst number across sessions led to a full recovery of ingestion levels by the end of treatment. Daily post-session administration induced a dramatic decrease of activation of licking behaviour, indicated by reduced burst number, accompanied to reduced burst size. A slow recovery of ingestion took place after treatment discontinuation.

Conclusion These results suggest a reduced hedonic/reward evaluation response, an effect likely due to NMDA receptor blockade occurring during the testing time, and support the hypothesis that memantine interferes with the hedonic/non-homeostatic mechanisms regulating food-intake and food seeking. The effect of post-session administration might be explained by the development of conditioned taste aversion.

Keywords: Memantine; Licking Microstructure; Anhedonia; Activation; Conditioned Taste Aversion.

Introduction

Memantine, along with dizocilpine, phencyclidine and ketamine, is an uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist belonging to the class of the "open channel blockers". However, memantine is devoid of the clinically unacceptable side effects displayed by the other open channel blockers, such as hallucinations, dissociative symptoms, drowsiness and coma (Chen and Lipton 2006; Parsons et al. 2007; Traynelis et al. 2010) and is currently indicated for the treatment of moderate to severe Alzheimer's type dementia (Dou et al. 2018; Kishi et al. 2017). The possible therapeutic use of this drug was investigated also for psychiatric conditions such as bipolar disorder, obsessive-compulsive disorder, post traumatic stress disorder, drug addiction and schizophrenia (Lu and Nasrallah 2018; Sani et al. 2012). Moreover, a few preclinical (Bisaga et al. 2008; Foltin et al. 2008; Popik et al. 2011; Smith et al. 2015) and clinical studies (Brennan et al. 2008; De Chiara et al. 2014; Hermanussen et al. 2005; Lu and Nasrallah 2018) suggest its potential use in the treatment of obesity and eating disorders, in particular binge eating disorder.

Systemic administration of memantine was reported to reduce binge-like consumption of highly palatable food in rats (Popik et al. 2011; Smith et al. 2015) and baboons (Bisaga et al. 2008; Foltin et al. 2008). Acute administration reduced operant binge-like overeating of a highly palatable sugary reward, but not food restriction-induced overeating of ordinary lab chow in rats. The effects of memantine were selective for the palatable food *versus* ordinary lab chow also in reducing food seeking under a second order schedule of reinforcement and in a behavioural paradigm of compulsive eating (Smith et al. 2015). Daily treatment in non-deprived rats reduced binge-like eating of a higly palatable fat diet. Interestingly, this effect persisted after treatment discontinuation (Popik et al. 2011). These observations were interpreted as evidence of the ability of memantine, *via* NMDA receptor blockade, to reduce the reinforcement related to the hedonic/non-homeostatic mechanisms governing food intake (Bisaga et al. 2008; Foltin et al. 2008; Popik et al. 2011; Smith et al. 2015). To account for the ability of memantine of preventing the relapse to binge-like eating of the fat diet (Popik et al. 2011), it was hypothesized the involvement of the mechanisms by which NMDA receptor antagonists block the expression of the neuroadaptations associated with the rewarding effect of drugs of abuse (Bisaga and Popik 2000; Popik et al. 2006).

To further elucidate the mechanisms by which memantine influences eating behaviour, we decided to investigate its effects on the microstructure of the ingestion of sucrose, which is highly rewarding, both for taste and for caloric content, and is capable to trigger neuroadaptations which relieve eating behaviour from the control of homeostatic need, thus leading to compulsive overeating (Freeman et al. 2018). In particular, we examined the microstructure of licking for an aqueous sucrose solution. The analysis of the lick pattern provides measures which show specific responses to manipulations involving either taste or hunger/satiety internal cues (D'Aquila and Galistu 2017; Davis 1989; Davis and Smith 1992; Schneider et al. 1990; Smith 2001; Spector et al. 1998), and which might reveal important aspects of the process by which reward evaluation regulates behavioural activation (D'Aquila 2010; D'Aquila and Galistu 2017; Dwyer 2012; Galistu and D'Aquila 2013; Johnson 2018a, 2018b). In more detail, rats ingesting fluids emit licks which cluster in bursts, i.e. discrete series of licks at the rate of about 5 to 7 licks *per* second (Davis 1989). Burst number corresponds to the number of times that subjects engage in licking, and is under the influence of stimuli that do not involve the orosensory contact with the reward, such as post-ingestive cues (Davis and Smith 1992; Smith 2001; Spector et al. 1998). In contrast, burst size, defined as the number of licks *per* burst, is dependent on stimuli involving the orosensory contact with the reward, such as quality and intensity of taste (D'Aquila and Galistu 2017; Davis 1989; Davis and Smith 1992; Schneider et al. 1990; Smith 2001; Spector et al. 1998). Therefore, the number and the size of licking bursts might be interpreted as measures revealing, respectively, (i) a process of activation of a reward-oriented response, possibly due to the attribution of incentive motivational properties to reward-related cues, and (ii) an evaluation process occurring during the consummatory transaction with the reward, reflecting palatability and possibly related to the experience of pleasure (D'Aquila 2010; D'Aquila and Galistu 2017; Dwyer 2012; Galistu and D'Aquila 2013; Higgs and Cooper 1998; Johnson 2018a, 2018b). A previous study in rats licking for a sucrose solution reported that acute administration of phencyclidine and dizocilpine reduced burst size, an effect which might be interpreted as a blunted hedonic/reward evaluation response, but did so only at doses producing signs of motor impairment (Lydall et al. 2010).

Since the effects of memantine on the intake of a highly palatable food were observed not only after acute treatment, but also in the course of a daily treatment, and persisted after its discontinuation (Popik et al. 2011), we decided to examine licking microstructure in daily sessions, both in the course of daily treatment

with memantine, and after treatment discontinuation. Moreover, in order to interpret treatment effects in relation to drug action at specific functionally relevant times, we compared the effect of two treatment schedules which differed as for administration time – hence as for brain drug levels – relative to the testing sessions.

In this study we examined the microstructure of licking for a 10% sucrose solution in rats. In particular, we investigated the effect of 10 mg/kg memantine, administered daily for a week either 1 hour before or immediately after testing in two separate groups of subjects. This implies that the experimental sessions were performed either 1 hour or 23 hours after drug administration. Moreover, the behaviour was observed for 15 days after treatment discontinuation.

Brain levels of memantine in rats following intra-peritoneal injection were reported to reach the maximal concentration (C_{max}) after 68.5 ± 3.4 minutes (T_{max}), with a half-life of 2.8 ± 0.5 hours (Spanagel et al. 1994). According to these pharmacokinetic measurements, the behavioural tests in the present study were performed at the time of maximal brain drug levels in the group receiving memantine before testing, and with drug levels of less than 1% with respect to brain C_{max} in the group receiving memantine after testing. Therefore, the effects observed in the "after testing" group cannot be explained by ongoing significant blockade of NMDA receptors (or to ongoing activity at sites other than NMDA receptors) during behavioural testing. The dose of 10 mg/kg was chosen taking into account previous studies examining the effect of memantine in binge eating-related animal models (Popik et al. 2011; Smith et al. 2015).

Materials and methods

Subjects and drug treatments

Experimentally naïve male Sprague-Dawley rats (Harlan, Italy) weighing 300-350 g at the beginning of the experiments were used as subjects. The animals were housed in groups of two-three per cage in controlled environmental conditions (temperature 22-24° C; humidity 50-60%; light on at 08:00, off at 20:00), with free access to food and water. Memantine HCl was purchased as an injectable commercially available

pharmaceutical form (Ebixa, Lundbeck, Denmark) in ampoules containing an aqueous solution at the concentration of 10 mg/ml and, after dilution with distilled water when appropriate, was administered intraperitoneally at the dose of 10 mg/kg, in a volume of 1 ml/kg. Vehicle treatment consisted in a 1 ml/kg distilled water i.p. administration.

All the experimental procedures were carried out in accordance with the regulatory requirement of the Italian law (D.L. 116, 1992) and Council Directive 2010/63EU of the European Parliament and Council, and were approved by the Independent Committee of Bioethics for Animal Testing of the University of Sassari and authorised by the Ministry of Health, Italy.

Apparatus, microstructural measures and testing conditions

Behavioural testing was carried out using a multistation lick analysis system (Habitest,Coulbourn Instruments, USA) connected to a computer. Rats were individually placed in a Perspex chamber with an opening in the centre of the front wall allowing access to a bottle spout. The recording period started either after the first lick or after 3-min that the animals were placed into the chambers, so that the latency to the first lick had a cut off time of 3-min. The interruptions of a photocell beam by each single tongue movement while licking the spout were recorded, with a temporal resolution to the nearest 20 milliseconds. The raw data were analysed through Graphic State 3.2 software (Coulbourn Instruments, USA) and, besides lick number, the following microstructural measures were obtained: number of bursts, time spent in bursts, latency to the first lick. A burst was defined as a series of licks with pauses no longer than 400 milliseconds (see D'Aquila 2010). Burst size (number of licks per burst) and intra-burst lick rate (lick/sec within bursts) were then calculated. The data were collected in time bins of 3-min in sessions of 30-min.

The experiments were performed between 09:00 and 13:00, i.e. during the light phase of the lighting cycle. All the experiments were perfomed in non-deprived animals.

Procedures

The subjects (N=36) were first familiarised with the test apparatus with 14 daily 30-min training sessions,

where they had access to a 10% sucrose solution. Based on the whole-session mean burst size of the 13th training session, they were allocated into four matched groups (n=9). In seven successive 30-min daily sessions (S1-S7), we compared the effect of memantine (10 mg/kg) *versus* vehicle of two different administration schedules, with the treatment performed either 1 hour before or immediately after testing. This implies that the experimental sessions were performed either 1 hour ("before testing" groups) or 23 hours ("after testing" groups) after drug treatment. The subjects of the "after testing" groups received their first injection after the last training session. Moreover, daily tests (except the weekends) were performed up to the 15th day of treatment discontinuation.

Due to occasional technical failure of the recording stations, the data from one subject in the treatment phase (vehicle-treated, belonging to the "after testing" group), and from two subjects in the post-treatment phase (memantine-treated, belonging to the "after testing" group) were lost.

Statistical analysis

Statistical analysis of all sets of data was performed with ANOVA, by the software Statistica 8.0 (StatSoft Inc.). When a significant interaction between factors was revealed, comparisons were performed by F-test for contrasts. Sample size was determined on the basis of previous studies investigating licking behaviour.

Body weight data were analysed by ANOVA, with *treatment* and *administration time* as between-group factors, and *time* as a within-group factor, with three levels corresponding to (i) the start and (ii) the end of treatment, and (iii) the end of the experiment.

ANOVA of the whole-session data involved two between-group factors: *treatment*, with two levels corresponding to memantine and vehicle treatment, and *administration time*, with two levels, *before testing* and *after testing*; and a within-group factor, *session*, with 7 (or 11) levels, corresponding to treatment (or post-treatment) sessions. Due to the numerous empty cells in the data of the number of lick *per* burst and of the intra-burst lick rate in the "after testing" group, "before testing" and "after testing" group data for these parameters have been subjected to separate analyses (see Results section for more details). Thus, the analysis of these parameters did not involve the between-group factor *administration time.*

In selected/representative sessions, the within-session burst number time-course was also analysed

by ANOVA, with *time* as a within-group factor, with 10 levels corresponding to the ten 3-min time bins within the session.

Results

Body weight

Due to differences between the groups at the beginning of the experiment which approached statistical significance [*treatment*×*administration time*: $F(1, 32)=4.11$, $P=0.0509$], in the analysis of the whole set of data, each individual datum was expressed as the percent value of the relative group mean body weight at the beginning of treatment. ANOVA showed only a significant effect of *time* [$F(2, 64)=128.9$, $P<10^{-6}$], due to the increase in body weight in all groups (Fig. 1). These results are consistent with the results of a previous study examining the effect of memantine, with a comparable dose and treatment duration (Popik et al. 2011).

Effect of treatment with memantine

A statistically significant *administration time*×*treatment*×*session* interaction [$F(6, 186)=4.33$, $P=0.0004$] was revealed (Fig. 2, top panels). The groups receiving vehicle either before or after testing showed a similar level of lick number, which remained stable across the seven daily sessions. The groups receiving memantine, either before or after testing, showed a similar reduction of lick number with respect to the corresponding vehicle-treated control group in the first session, with no statistically significant difference between them (remember that the "after testing" groups received the first injection immediately after the last training session, i.e. 23 hours before the first testing session). However, the lick number time-course across sessions showed an opposite direction depending on administration time: in the "before testing" group, a progressive increase across sessions was observed, so that in the last 3 sessions no statistically significant differences with respect to the corresponding vehicle group were present (Fig. 2, top left panel); in the "after testing" group, a further decrease across sessions was observed, which led to values very close to naught in

the last three sessions (Fig. 2, top right panel).

ANOVA of the number of licks *per* burst data of the "before testing" groups showed only a statistically significant effect of *treatment* [F(1, 16)=13.9, P=0.0017], due to reduced mean burst size in the group receiving memantine, which was apparent since the first administration (Fig. 2, mid left panel). The data relative to the "after testing" memantine-treated group presented numerous empty cells from the third session up till the end of the experiment, due to the high number of subjects which did not drink (n values of the sessions from 3 to 7 are reported on the legend of Fig. 2). Therefore, only the data of the first two sessions were subjected to analysis. ANOVA revealed a statistically significant effect of *treatment* [F(1, 15)=7.7, P=0.01], due to a reduced mean burst size in the group receiving memantine (Fig. 2, mid right panel).

ANOVA of the intra-burst lick rate data of the "before testing" groups (Fig. 2, bottom panel) showed only a statistically significant effect of *treatment* [F(1, 16)=10.13, P=0.0057], due to slightly reduced values in the group receiving memantine. As for the "after testing" groups, ANOVA of the data from the first two sessions failed to reveal statistically significant effects [*treatment*: F(1, 15)=0.276, n.s.; *session*: F(1, 15)=0.080, n.s.; *treatment*×*session*: F(1, 15)=0.001, n.s.].

ANOVA of the whole-session burst number showed a statistically significant *administration time*×*treatment*×*session* interaction [F(6, 186)=4.31, P=0.0004] (Fig. 3, top panels). The groups receiving vehicle, either before or after testing, showed a similar level of burst number, which remained stable across the seven daily sessions. In the first session, the groups receiving memantine, either before or after testing, did not show any statistically significant difference, either compared to the respective vehicle-treated control group, or between them. As with lick number, the burst number level time-course across sessions showed an opposite direction depending on administration time: in the "before testing" group, a progressive increase across sessions was observed, so that in the last 2 sessions a statistically significant increase with respect to the corresponding vehicle group was revealed (Fig. 3, top left panel); in the "after testing" group, a further decrease across sessions was observed, which led to values very close to naught in the last three sessions (Fig. 3, top right panel).

ANOVA of the latency to the first lick data showed a statistically significant *administration time*×*treatment*×*session* interaction [F(6, 186)=6.61, $P<10^{-5}$](Fig. 3, bottom panels). The groups receiving

vehicle, either before or after testing, showed a similar level of latency to the 1st lick, which remained stable across the seven daily sessions. The group receiving memantine before testing showed an increased latency in the 1st and in the 5th session with respect to the corresponding vehicle-treated group, while the group treated with memantine after testing showed an increased latency level in all the 7 sessions. As with lick and burst number, the latency time-course across the 7 experimental sessions of the two memantine-treated groups showed an opposite direction, depending on administration time, with the groups treated either before or after testing, showing either a decrease or a progressive increase, respectively, so that a statistically significant difference between them was apparent from the 3rd to the 7th session (with the exception of the 5th session).

Effects after the discontinuation of memantine treatment

ANOVA of the whole-session lick number (Fig. 4, top panels) showed a statistically significant *administration time*×*session*×*treatment* interaction [$F(10, 300)=6.6$, $P=10^{-6}$]. Both vehicle-treated groups showed similar and stable levels of lick number across the 11 sessions (in 15 days) after treatment discontinuation. The values of lick number across sessions of the "before testing" group treated with memantine were slightly higher with respect to the corresponding vehicle-treated group, with the differences reaching statistical significance in days 6-8 and 15. The values of the "after testing" group treated with memantine started from a very low level in the first day (as it was in the last days of treatment), and progressively increased to reach the same level of the corresponding vehicle-treated control group, so that, since day 9 on, no statistically significant differences were present. The difference between the two memantine-treated groups progressively decreased from the 1st to the last session, with the "after testing" group approaching (but not reaching) the level of the "before testing" group in the last few sessions.

ANOVA of the whole-session number of licks *per* burst data (Fig. 4, 3rd row panels) of the "before testing" groups failed to show any statistically significant effects [*treatment*: $F(1, 16)=0.11$, n.s.; *session*: $F(10, 160)=1.83$, n.s.; *treatment*×*session*: $F(10, 160)=0.45$, n.s.]. ANOVA of the data relative to the "after testing" groups revealed only a statistically significant effect of *session* [$F(10, 110)=2.5$, $P=0.008$], due to slight differences between session mean values.

ANOVA of intra-burst lick rate data either of the "before testing" or of the "after testing" groups (Fig. 2, bottom panel) failed to show any statistically significant effects ["before testing", *treatment*: F(1, 16)=0, n.s.; *session*: F(10, 160)=1.25, n.s.; "after testing", *treatment*: F(1, 11)=0.93, n.s.; *session*:F(10, 110)=0.68, n.s.; "before testing", *treatment*×*session*: F(10, 160)=0.92, n.s.; "after testing", *treatment*×*session*: F(10, 110)=0.7, n.s.].

ANOVA of the whole-session burst number (Fig. 4, 2nd row panels) showed a statistically significant *administration time*×*treatment*×*session* interaction [$F(10, 300)=6.18$, $P=10^{-6}$]. Both vehicle-treated groups showed similar and stable levels of burst number across the sessions after treatment discontinuation. The values of burst number across sessions of the "before testing" group treated with memantine failed to show statistically significant differences with respect to the corresponding vehicle-treated group. The values of the "after testing" group treated with memantine started from a very low level in the first day after treatment discontinuation (as it was in the last days of treatment), and progressively increased to reach the same level both of the corresponding vehicle-treated control group and of the "before testing" group treated with memantine.

ANOVA of latency to the first lick data (Fig. 4, bottom panels) showed a statistically significant *administration time*×*treatment*×*session* interaction [$F(10, 300)=5.39$, $P<10^{-6}$]. Both vehicle-treated groups showed similar and stable levels of latency values across the sessions after treatment discontinuation. The values of latency across sessions of the "before testing" group treated with memantine failed to show statistically significant differences with respect to the corresponding vehicle-treated group. The values of the "after testing" group treated with memantine started from a high level in the first post-treatment session (as it was in the last days of treatment), and progressively decreased to reach the same level both of the corresponding vehicle-treated control group and of the "before testing" group treated with memantine.

Within-session burst number time-course of selected/representative sessions

ANOVA of the first session data in the "before testing" groups showed a statistically significant *time*×*treatment* interaction [$F(9, 144)=2.03$, $P=0.039$](Fig. 5, top panel). F-tests for contrasts showed both groups declining without significant differences in the first 4 time bins. However, the memantine-treated

group showed a steeper decline with respect to the vehicle-treated group. Indeed, within-group comparisons between the first and the fourth time bin showed a statistically significant difference in the memantine-treated (P=0.0011) but not in the vehicle-treated group, with the between-group comparison in the fourth time bin approaching statistical significance (P=0.066). For the remaining part of the session, while a further decline was observed in the group treated with vehicle, a 2nd peak of burst number value was reached by the memantine-treated group in the 21 min time bin, with all other values not differing from the vehicle-treated group.

ANOVA of the 5th and 6th session data in the "before testing" groups showed a statistically significant *time*×*treatment* interaction [S5: F(9, 144)=2.02, P=0.04; S6: F(9, 144)=2.25, P=0.021](Fig. 5, 2nd and 3rd panels). F-tests for contrasts showed a similar time-course for both groups in the first part of the session, with a statistically significant difference in favour of the memantine-treated group with respect to the vehicle-treated one emerging in the last two time bins in session 5 and in the 15 min and 27 min time bins in session 6. The 7th session data analysis in the "before testing" group revealed a significant effect of *treatment* [F(1, 16)=5.95, P=0.026], due to the higher values of the memantine-treated group, and a significant effect of *time* [F(9, 144)=7.17, P<10^{-6}], due to the progressive decline of values regardless of group, with no interaction [F(9, 144)=1.6, n.s.](Fig. 5, bottom panel).

ANOVA of the first session data in the "after testing" group (Fig. 6, top panel) showed a statistically significant effect of the factor *time* [F(9, 135)=7.35, P<10^{-6}], due to the progressive decline of burst number regardless of treatment [*treatment*: F(1, 15)=3.7, n.s.; *treatment*×*time*: F(9, 135)=0.83, n.s.]. ANOVA of the second session data (Fig. 6, bottom panel) showed a statistically significant *treatment*×*time* interaction [F(9, 135)=4.21, P=0.000079]. F-tests for contrasts revealed a difference between the two groups at the beginning of the session, which persisted up to the 21 min time bin: indeed, the values of the vehicle-treated group showed a progressive decline along the session, while the memantine-treated group values were very low since the beginning of the session.

Discussion

The aim of this study was to compare the effects of two different treatment schedules on the microstructure of licking for sucrose, to further elucidate the mechanisms by which memantine influences the motivational aspects of eating behaviour.

Memantine treatment before testing induced a reduction of sucrose ingestion in the first session, as indicated by the reduced lick number. This effect was exclusively due to reduced burst size and was accompanied by an increased latency to the first lick. The interpretation of the decreased burst size in terms of reduced palatability/hedonic response (or reduced reward evaluation) is in keeping with the observations of a few studies which reported that memantine selectively reduced binge-like eating of highly palatable food rewards (Bisaga et al. 2008; Foltin et al. 2008; Popik et al. 2011; Smith et al. 2015). However, it should be stressed that these observations stand on the measure of the whole-session food intake, which might be influenced by changes in reward evaluation (or palatability/hedonic impact) but also by changes in behavioural activation/incentive salience attribution (Berridge 2007; Higgs and Cooper 1998). Thus, the present observations – in particular the reduced burst size – provide an important piece of evidence in support of the hypothesis that the reduced binge-like eating observed after memantine administration in the cited studies might be due to a blunted hedonic response. This interpretation is consistent also with the observation that the NMDA receptor antagonist dizocilpine reduced sucrose preference, mimicking the effect of sucrose dilution (Vardigan et al. 2010).

The observation of an increase in latency in the first session is consistent with the observation that acute treatment with memantine fully blocked food-seeking in a second order schedule of reinforcement (Smith et al. 2015), an effect related to the salient environmental stimuli which trigger behavioural activation.

While the reduced burst size persisted up to the last treatment session, burst number, a measure representing activation of licking behaviour, showed a progressive increase across sessions, leading to a complete recovery of lick number, with latency values returning to the vehicle level since the second session (but an increased latency was present also in the fifth session). These observations suggest that memantine

induced a mild decrease in hedonic response/reward evaluation, successfully compensated by increased activation of the reward-oriented response – i.e. increased burst number – as previously observed in instrumental responding to mild reward devaluation (Wise 1982a, 1982b; Wise et al. 1978). The recovery in sucrose consumption observed in the present experiment is at variance with the observation by Popik and coll. (2011), which reported a reduced binge-like consumption of the fat diet for all the duration of memantine treatment and for a week after its discontinuation. Differences between the two studies, such as the nature of the reward (lard *versus* sucrose) and the duration of the testing session (2 hours *versus* 30 minutes), might account for this discrepancy.

As previously observed with relatively high doses of phencyclidine and dizocilpine (Lydall et al. 2010), a decrease of the intra-burst lick rate was present in all treatment sessions, suggesting the possibility that the observed reduction of burst size might be just the consequence of drug-induced motor impairment. However, several observations from this and previous studies run against this interpretation. Motor impairment is strictly related to reduced behavioural activation (Galistu and D'Aquila 2013; Keitz et al. 2003; Salamone et al. 1997, 2007; Shore et al. 2011), but the present results show increase in burst number and decrease in latency across sessions, i.e. a progressive increase in activation, in spite of the stably reduced intra-burst lick rate. Moreover, previous studies have shown that experimental manipulations more likely to be accounted for by motivational, rather than by motor mechanisms, resulted in changes of the intra-burst lick rate, with an increase of this measure after sucrose dilution (D'Aquila and Galistu 2017) and sodium depletion (D'Aquila et al. 2012), and a decrease with a more concentrated NaCl drink solution (D'Aquila et al. 2012) and with conditioned taste aversion to LiCl (Baird et al. 2005). It is also worth noting that memantine, at the same dose used in the present study, did not affect water intake or operant responding for lab chow after food deprivation, thus ruling out that general behavioural suppression or motor impairment might account for the reduction of operant binge-like eating of palatable food and palatable food-seeking (Smith et al 2015).

Figure 5 shows the results of "before testing" memantine administration in the first session and in the last three sessions, which correspond, respectively, to the sessions (i) when the reduced whole-session lick number in the memantine-treated group was due only to reduced burst size and (ii) when the increase in burst number fully compensated the reduced burst size in terms of whole-session lick number. The steeper decline

of the burst number observed in the memantine-treated rats with respect to the control group in the first session is reminiscent of the response pattern observed after sucrose dilution (D'Aquila and Galistu 2017). The slightly higher levels of burst number observed in the last three sessions are similar to the compensatory response to mild reward devaluation in instrumental behaviour (Wise 1982a, 1982b; Wise et al. 1978). These observations provide further support to the interpretation of the effects of "before testing" memantine in terms of anhedonia/reduced reward evaluation.

In the first test after treatment discontinuation no differences were present in any parameter. In the following tests the lick number in this group was tendentially increased, showing statistically significant differences with respect to the control group in some sessions, possibly due to a tendential increase in burst number. These observations, taken together with the results of the treatment phase, suggest that the effects of memantine before testing on burst size and intra-burst lick rate are related to brain drug concentration, while the effects on burst number are better interpreted as the result of an opponent process.

Also treatment with memantine after testing induced a reduction of lick number in the first session, mainly due to reduced burst size – observed also in the second session – with no reduction of the intra-burst lick rate. However, the lick number time-course across sessions followed an opposite direction with respect to the "before testing" group, showing a further decrease and reaching values very close to naught in the last sessions. The further decrease was due to reduced burst number, which showed a time-course paralleling that of lick number. The time-course of the latency to the first lick showed a progressive increase across sessions. These results indicate a dramatic reduction of behavioural activation following "after testing" memantine administration. Consistently with this interpretation, the analysis of the within-session time-course of burst number showed a reduced level of this measure since the beginning of the session, starting from the second session (see Fig. 6). After treatment discontinuation, lick number, burst number and latency time showed a progressive recovery until reaching the level displayed by the control group. Burst size did not show statistically significant differences with respect to the control group since the first post-treatment session. The most likely explanation to account for this response pattern is the development of a conditioned taste aversion (CTA). This interpretation is supported by previous studies showing the ability of different NMDA receptor antagonists, including dizocilpine, ketamine and phencyclidine, to induce CTA (Aguado et al. 1997; Jackson and Sanger 1989; Traverso et al. 2012) and by the observation of a reduced burst size, as observed

with CTA in previous reports (Arthurs et al. 2017; Baird et al. 2005; Gaillard and Stratford 2016; Lin et al. 2017).

Regardless of the mechanisms involved, the dramatically different effects yielded by the "before-testing" and the "after-testing" drug administration schedules demonstrate that the response to memantine daily treatment depends on drug action at specific functionally relevant times. However, since memantine half-life in humans is far longer than in rats, bringing about fairly stable drug levels in the course of chronic treatment (Parsons et al. 2007), this observation might have a limited relevance for the use of this drug in the treatment of binge-eating disorder.

It might be worth noting that the across session changes in burst number – i.e. the increases observed in the treatment phase in the "before testing" group and after treatment discontinuation in the "after testing" group and, on the other hand, the decreases observed in the treatment phase in the "after testing" group and after treatment discontinuation in the "before testing" group – were accompanied by changes in the opposite direction in latency values. This reflects the opposite relations of burst number on the one hand, and of latency to the first lick on the other hand, with the level of activation of a reward-oriented behavioural response, such as licking for sucrose. In support of this interpretation, in a previous study comparing the licking response to daily exposure to two different sucrose concentrations in two separate groups of subjects, it was observed a progressive increase in burst number across sessions accompanied by a progressive decrease of the latency values. Notably, the speed of the increase in burst number and of the decrease in latency was directly proportional to sucrose concentration, i.e. to the reward value (D'Aquila and Galistu 2017).

The consistency with previous studies examining the effect of different NMDA receptor antagonists on licking for sucrose (Lydall et al. 2010), palatable food consumption (Bisaga et al. 2008; Foltin et al. 2008; Popik et al. 2011; Smith et al. 2015) and conditioned taste aversion (Aguado et al. 1997; Jackson and Sanger 1989; Traverso et al. 2012) provides support to the interpretation of the present observations as the result of memantine activity at NMDA receptors. However, memantine blocks also 5-HT_3 receptors and different subtypes of cholinergic nicotinic receptors (α_7, α_4-β_2, α_9-α_{10}, α_3-β_2), with an affinity comparable to that for NMDA receptors (Lee et al. 2012; Parsons et al. 2007; Rammes et al. 2001). Previous evidence on the effect of 5-HT_3 receptor blockade (Hayes and Covasa 2005) and nicotinic receptor activation (Stojakovic et al.

2017) on hedonic/non-homeostatic food-intake suggests that activity at these receptors cannot account for the effects of before session memantine administration. However, the 5-HT_3 receptor antagonist tropisetron, at a high dose, induced CTA (Briscione et al. 2013).

In conclusion, these results show that repeated treatment with memantine administered before licking sessions blunted the hedonic response to sucrose. This effect is likely due to NMDA receptor blockade occurring during the testing time. This observation provides support to the hypothesis that memantine reduces the intake of higly palatable food and dampens the related reward-oriented behaviours by interfering with the hedonic/non-homeostatic mechanisms regulating food-intake and food seeking, and might bear relevance in the explanation of the clinical effects of memantine in the treatment of binge-eating disorder. As for the dramatic reduction of the activation of licking behaviour observed with memantine administered after the licking sessions, the analysis of the microstructure parameters – especially the reduced burst size in the first two sessions – suggests as the most likely explanation the development of CTA. However, the design of the present study does not allow to draw definitive conclusions in this regard.

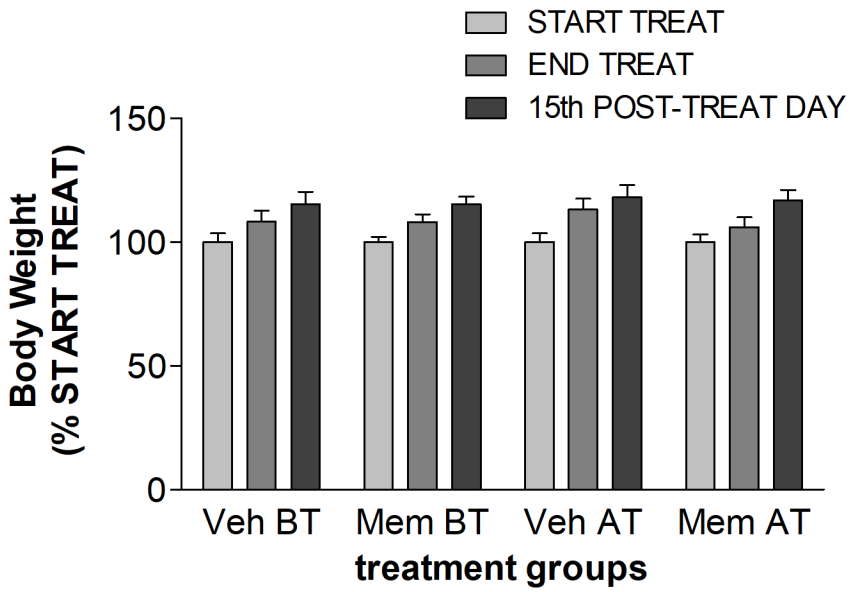

Fig 1 Body weights. START TREAT: start of treatment; END TREAT: end of treatment; POST-TREAT DAY: post-treatment day; Veh: vehicle; Mem: memantine 10 mg/kg; BT: "before testing" administration time condition groups; AT: "after testing" administration time condition groups. Values represent the mean ± S.E.M. from 9 subjects.

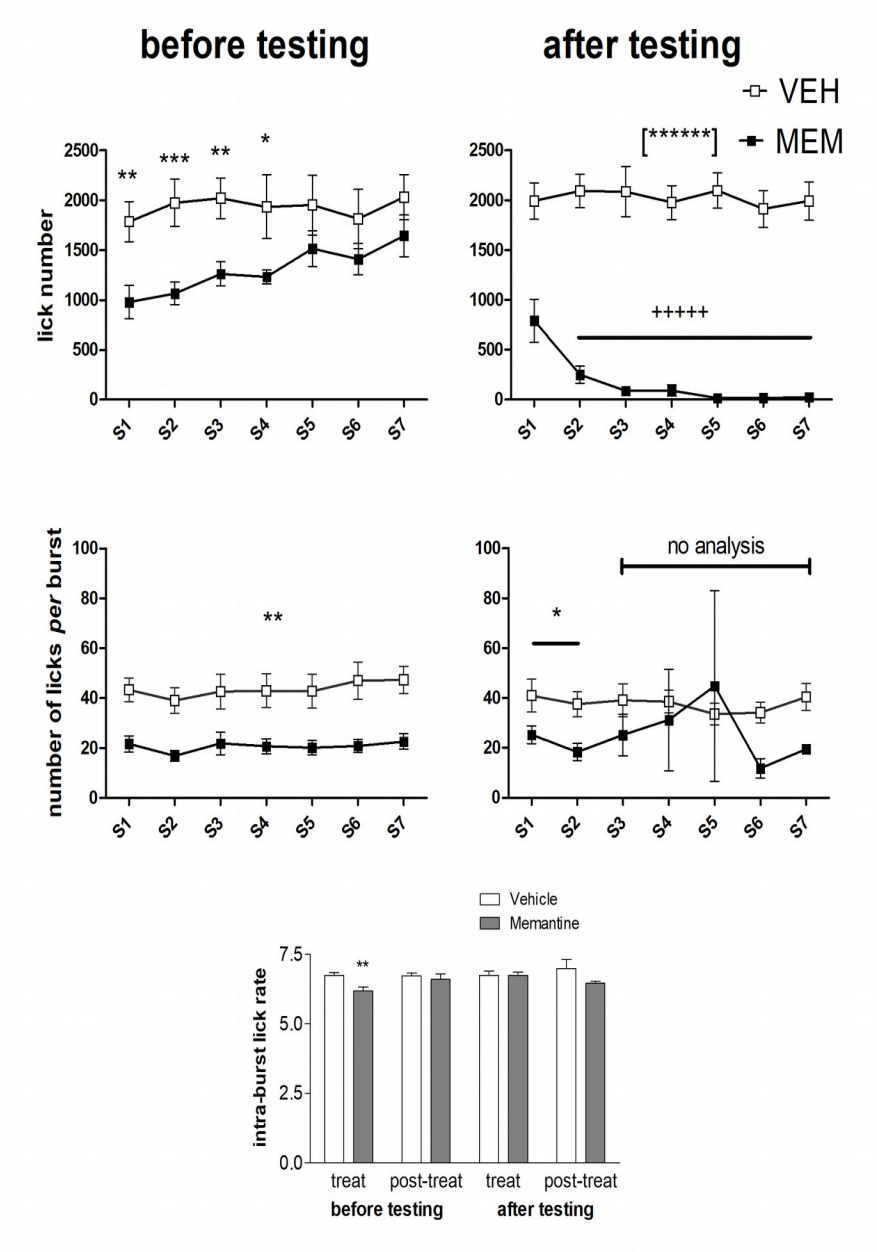

Fig 2 Lick number and burst size (number of lick *per* burst) across 7 daily sessions with a daily treatment with memantine 10 mg/kg, and intra-burst lick rate during treatment and after treatment discontinuation. VEH: vehicle; MEM: memantine; treat: treatment phase; post-treat: post-treatment phase. Left and right panels report, respectively, the data from the "before testing" and "after testing" administration time condition groups. Bottom panel: intra-burst lick rate of treatment- and post-treatment-phase. S(1-7): session (1-7). Mid right panel: due to the presence of numerous empty cells in the after testing memantine-treated group, only the data of the first two sessions were subjected to analysis (memantine-treated group: S3, n=6; S4, n=3; S5, n=2; S6, n=3; S7, n=2). Values represent the mean ± S.E.M. from 9 subjects. Memantine *versus* vehicle: *P<0.05, **P<0.01, ***P<0.001, ******P<10^{-6}; memantine "before testing" *versus* memantine "after testing": +++++P<10^{-5} (ANOVA followed by F-test for contrasts; straight lines indicate contrasts involving consecutive time points; symbols in square brackets refer to a comparison involving all the sessions).

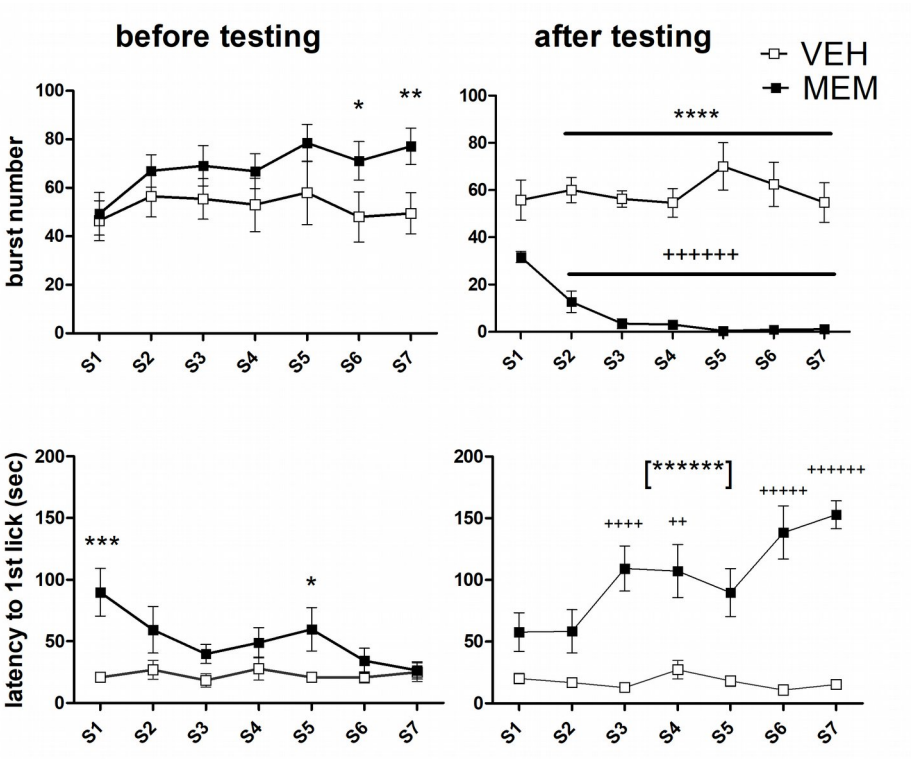

Fig 3 Burst number and latency to the first lick across 7 daily sessions with a daily treatment with memantine 10 mg/kg. VEH: vehicle; MEM: memantine. Left and right panels report, respectively, the data from the "before testing" and "after testing" administration time condition groups. S(1-7): session (1-7). Values represent the mean ± S.E.M. from 9 subjects. Memantine *versus* vehicle: *P<0.05, **P<0.01, ***P<0.001, ****P<0.00001, ******P<10^{-6}; memantine "before testing" *versus* memantine "after testing": ++P<0.01, ++++P<0.00001, +++++P<10^{-5}, ++++++P<10^{-6} (ANOVA followed by F-test for contrasts; straight lines indicate contrasts involving consecutive time points; symbols in square brackets refer to a comparison involving all the sessions).

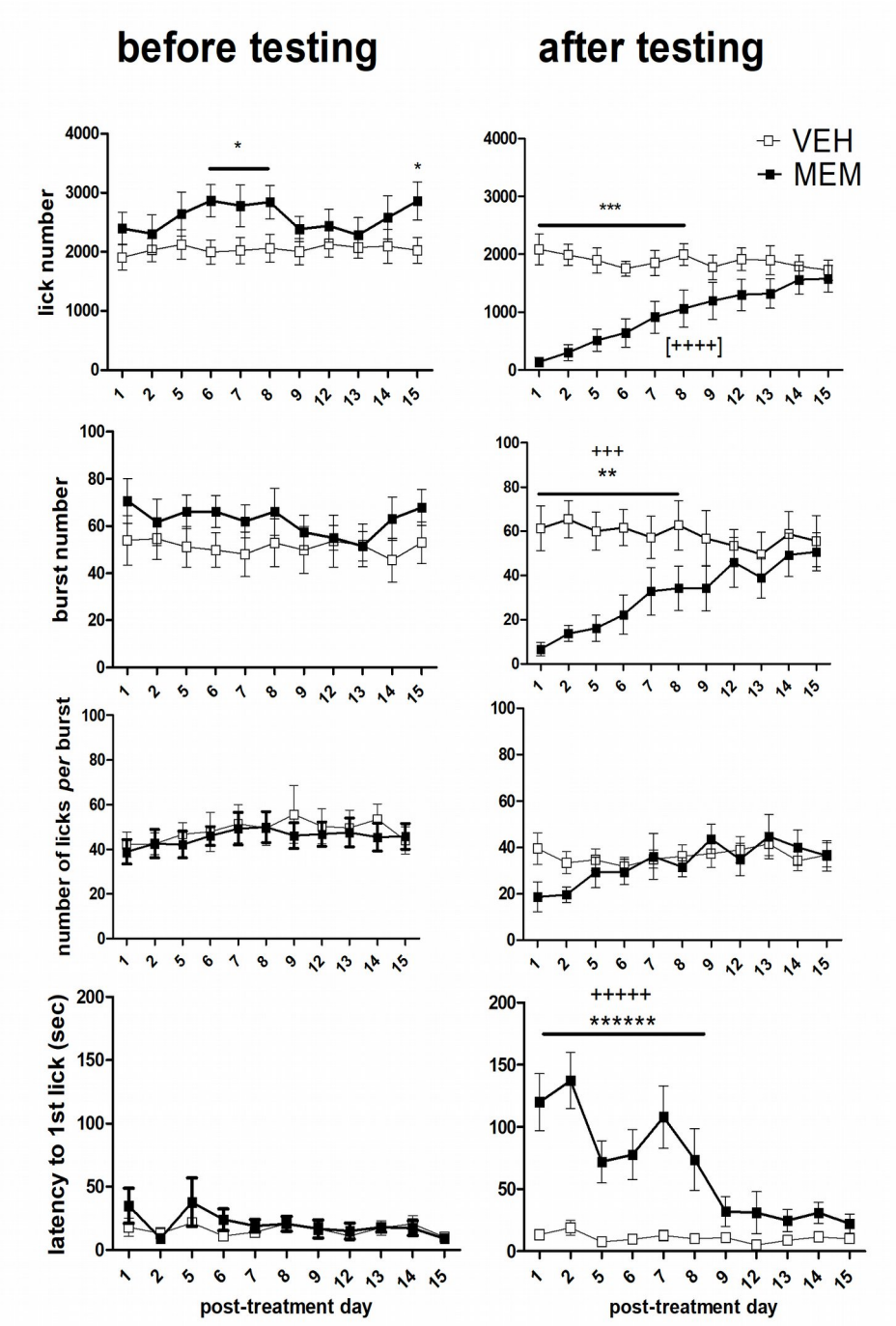

Fig 4 Lick number, burst number, burst size (number of lick *per* burst), and latency to the first lick after discontinuation of a 7 days daily treatment with memantine 10 mg/kg. VEH: vehicle; MEM: memantine. Left and right panels report, respectively, the data from the "before testing" and "after testing" administration time condition groups. Values represent the mean ± S.E.M. from 9 subjects. Memantine *versus* vehicle: *P<0.05, **P<0.01, ***P<0.001, ******P<10^{-6}; memantine "before testing" *versus* memantine "after testing": +++P<0.001, ++++P<0.00001, ++++ +P<10^{-5} (ANOVA followed by F-test for contrasts; straight lines indicate contrasts involving consecutive time points; symbols in square brackets refer to a comparison involving all the sessions).

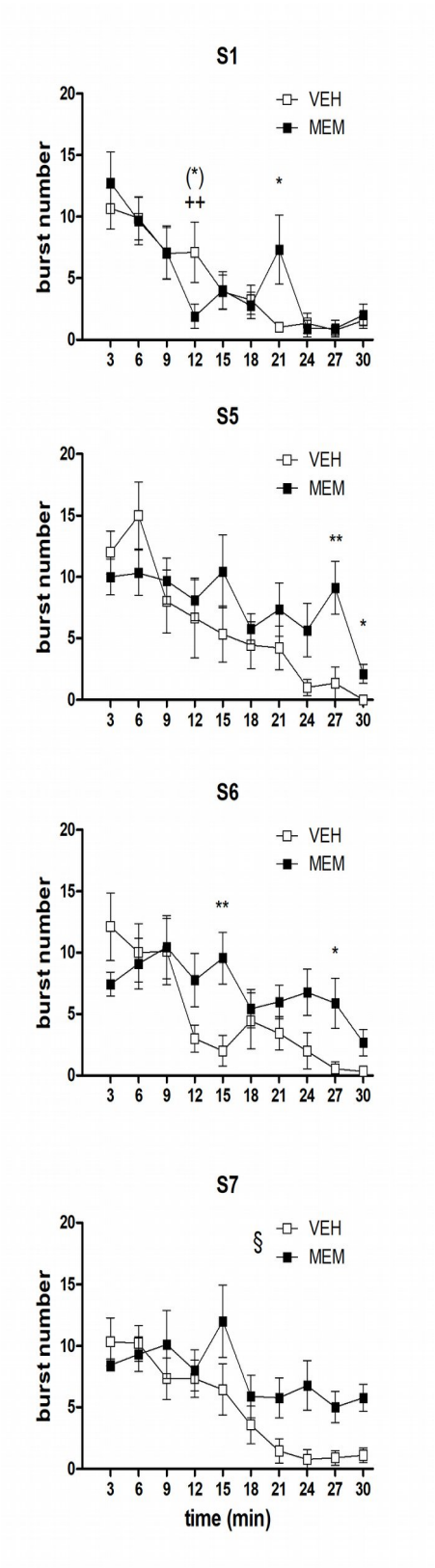

Fig 5 Within-session burst number time course in the first and last three sessions of a 7 days daily treatment with memantine “before testing”. VEH: vehicle; MEM: memantine; S: session. Values represent the mean ± S.E.M. from 9 subjects. Memantine *versus* vehicle: (*)P<10, *P<0.05, **P<0.01; within-group comparison *versus* first time bin in memantine-treated group: ++P<0.01 (ANOVA followed by F-test for contrasts); §P<0.05 (ANOVA, main effect of *treatment*).

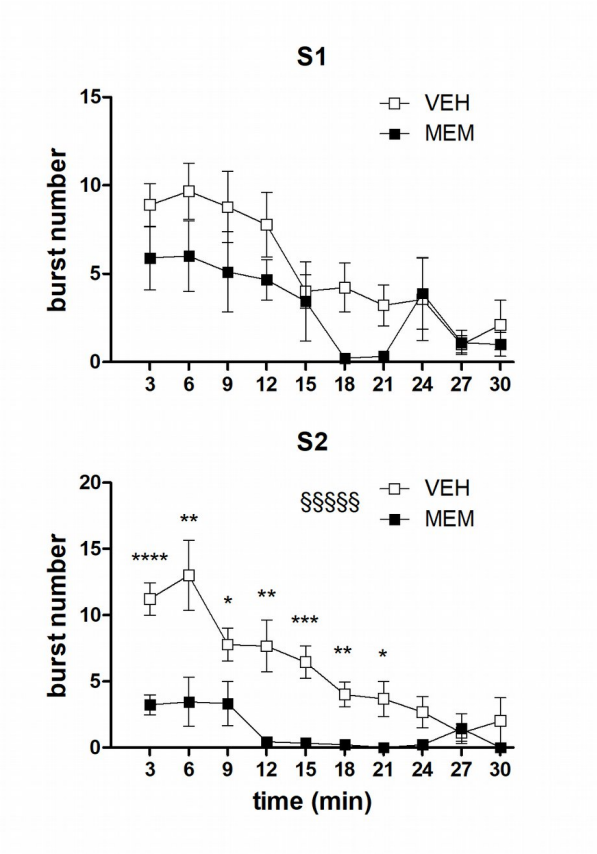

Fig 6 Within-session burst number time course in the first two sessions of a 7 days daily treatment with memantine "after testing". VEH: vehicle; MEM: memantine; S: session. Values represent the mean ± S.E.M. from 9 subjects. Memantine *versus* vehicle: *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (ANOVA followed by F-test for contrasts); §§§§§P<10^{-5} (ANOVA, main effect of *treatment*).

References

Aguado L, del Valle R, Pérez L (1987) The NMDA-receptor antagonist ketamine as an unconditioned stimulus in taste aversion learning. Neurobiol Learn Mem 68:189-196

Arthurs J, Lin JY, Ocampo R, Reilly S (2017) Lactose malabsorption and taste aversion learning. Physiol Behav 180:39-44

Baird JP, St John SJ, Nguyen EA (2005) Temporal and qualitative dynamics of conditioned taste aversion processing: combined generalization testing and licking microstructure analysis. Behav Neurosci 119:983-1003.

Berridge KC (2007) The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology 191:391-431.

Bisaga A, Danysz W, FoltinRW (2008) Antagonism of glutamatergic NMDA and mGluR5 receptors decreases consumption of food in baboon model of binge-eating disorder. Eur Neuropsychopharmacol 18: 794-802.

Bisaga A, Popik P (2000) In search of a new pharmacological treatment for drug and alcohol addiction: N-methyl-D-aspartate (NMDA) antagonists. Drug Alcohol Depend 59:1-15

Brennan BP, Roberts JL, Fogarty KV, Reynolds KA, Jonas JM, Hudson JI (2008) Memantine in the treatment of binge eating disorder: an open-label, prospective trial. Int J Eat Disord 41:520-526.

Briscione MA, Serafine KM, Merluzzi AP, Rice KC, Riley AL (2013) The effects of the 5-HT3 receptor antagonist tropisetron on cocaine-induced conditioned taste aversions. Pharmacol Biochem Behav 105:112-

Chen HS, Lipton SA (2006) The chemical biology of clinically tolerated NMDA receptor antagonists. J Neurochem 97:1611-1626.

D'Aquila PS (2010) Dopamine on D2-like receptors "reboosts" dopamine D1-like receptor-mediated behavioural activation in rats licking for sucrose. Neuropharmacology 58:1085-1096.

D'Aquila PS, Galistu A (2017) Within-session decrement of the emission of licking bursts following reward devaluation in rats licking for sucrose. PLoS One 12(5):e0177705.

D'Aquila PS, Rossi R, Rizzi A, Galistu A (2012) Possible role of dopamine D1-like and D2-like receptors in behavioural activation and "contingent" reward evaluation in sodium-replete and sodium-depleted rats licking for NaCl solutions. Pharmacol Biochem Behav 101:99–106.

Davis JD (1989) The microstructure of ingestive behavior. Ann N Y Acad Sci 575:106-119; discussion 120-121.

Davis JD, Smith GP (1992) Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. Behav Neurosci 106:217-228.

De Chiara L, Serra G, Koukopoulos AE, Koukopoulos A, Serra G (2014) Memantine in the treatment and prophylaxis of bipolar type II mood disorder and co-morbid eating disorder: a case report. Riv Psichiatr 49:192-194.

Dou KX, Tan MS, Tan CC, Cao XP, Hou XH, Guo QH, Tan L, Mok V, Yu JT (2018) Comparative safety and effectiveness of cholinesterase inhibitors and memantine for Alzheimer's disease: a network meta-analysis of 41 randomized controlled trials. Alzheimers Res Ther. 10(1):126.

Dwyer DM (2012) EPS Prize Lecture. Licking and liking: the assessment of hedonic responses in rodents. Q J Exp Psychol 65:371-394.

Foltin RW, Danysz W, Bisaga A (2008) A novel procedure for assessing the effects of drugs on satiation in baboons: effects of memantine and dexfenfluramine. Psychopharmacology (Berl) 199:583-592.

Freeman CR, Zehra A, Ramirez V, Wiers CE, Volkow ND, WangGJ (2018) Impact of sugar on the body, brain, and behavior. Front Biosci 23:2255-2266.

Gaillard D, Stratford JM (2016) Measurement of Behavioral Taste Responses in Mice: Two-Bottle Preference, Lickometer, and Conditioned Taste-Aversion Tests. Curr Protoc Mouse Biol 6:380-407

Galistu A, D'Aquila PS (2013) Dopamine on D2-like receptors "reboosts" dopamine D1-like receptor-mediated behavioural activation in rats licking for a isotonic NaCl solution. Psychopharmacology (Berl). 229:357-366.

Hayes MR, Covasa M (2005) CCK and 5-HT act synergistically to suppress food intake through simultaneous activation of CCK-1 and 5-HT3 receptors. Peptides 26:2322-2330.

Hermanussen M, Tresguerres JA (2005) A new anti-obesity drug treatment: first clinical evidence that, antagonising glutamate-gated Ca^{2+} ion channels with memantine normalises binge-eating disorders. Econ Hum Biol 3:329-337.

Higgs S, Cooper SJ (1998) Evidence for early opioid modulation of licking responses to sucrose and intralipid: a microstructural analysis in the rat. Psychopharmacology (Berl) 139:342-355.

Jackson A, Sanger DJ (1989) Conditioned taste aversions induced by phencyclidine and other antagonists of

N-methyl-D-aspartate. Neuropharmacology 28:459-464

Johnson AW (2018a) Characterizing ingestive behavior through licking microstructure: Underlying neurobiology and its use in the study of obesity in animal models. Int J Dev Neurosci 64:38-47.

Johnson AW (2018b) Examining the influence of CS duration and US density on cue-potentiated feeding through analyses of licking microstructure. Lear Motiv 61:85-96.

Keitz M, Martin-Soelch C, Leenders KL (2003) Reward processing in the brain: a prerequisite for movement preparation? Neural Plast 10:121-128.

Kishi T, Matsunaga S, Oya K, Nomura I, Ikuta T, Iwata N (2017) Memantine for Alzheimer's disease: an updated systematic review and meta-analysis. J Alzheimers Dis 60:401-425.

Lee RH, Tseng TY, Wu CY, Chen PY, Chen MF, Kuo JS, Lee TJ (2012) Memantine inhibits α3β2-nAChRs-mediated nitrergic neurogenic vasodilation in porcine basilar arteries. PLoS One 7(7):e40326.

Lin JY, Arthurs J, Reilly S. Anesthesia-inducing drugs also induce conditioned taste aversions. Physiol Behav 177:247-251

Lu S, Nasrallah HA (2018) The use of memantine in neuropsychiatric disorders: An overview. Ann Clin Psychiatry 30:234-248.

Lydall ES, Gilmour G, Dwyer DM (2010) Analysis of licking microstructure provides no evidence for a reduction in reward value following acute or sub-chronic phencyclidine administration. Psychopharmacology (Berl) 209:153-162.

Parsons CG, Stöffler A, Danysz W (2007) Memantine: a NMDA receptor antagonist that improves memory

by restoration of homeostasis in the glutamatergic system—too little activation is bad, too much is even worse. Neuropharmacology 53:699-723.

Popik P, Kos T, Zhang Y, Bisaga A (2011) Memantine reduces consumption of highly palatable food in a rat model of binge eating. Amino Acids 40:477-485.

Popik P, Wrobel M, Bisaga A (2006) Reinstatement of morphine-conditioned reward is blocked by memantine. Neuropsychopharmacology 31:160-170.

Rammes G, Rupprecht R, Ferrari U, Zieglgänsberger W, Parsons CG (2001) The N-methyl-D-aspartate receptor channel blockers memantine, MRZ 2/579 and other amino-alkyl-cyclohexanes antagonise 5-HT(3) receptor currents in cultured HEK-293 and N1E-115 cell systems in a non-competitive manner. Neurosci Lett 306:81-84.

Salamone JD, Cousins MS, Snyder BJ (1997) Behavioral functions of nucleus accumbens dopamine: empirical and conceptual problems with the anhedonia hypothesis. Neurosci Biobehav Rev 21:341-359.

Salamone JD, Correa M, Farrar A, Mingote SM (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology (Berl) 191:461-482.

Sani G, Serra G, Kotzalidis GD, Romano S, Tamorri SM, Manfredi G, Caloro M, Telesforo CL, Caltagirone SS, Panaccione I, Simonetti A, Demontis F, SerraG, Girardi P (2012) The role of memantine in the treatment of psychiatric disorders other than the dementias: a review of current preclinical and clinical evidence. CNS Drugs 26:663-690.

Schneider LH, Davis JD, Watson CA, Smith GP 1990. Similar effect of raclopride and reduced sucrose concentration on the microstructure of sucrose sham feeding. Eur J Pharmacol 186:61-70.

Shore DM, Rafal R, Parkinson JA (2011) Appetitive motivational deficits in individuals with Parkinson's disease. Mov Disord 26:1887-1892.

Smith GP (2001) John Davis and the meanings of licking. Appetite 36:84-92.

Smith KL, Rao RR, Velázquez-Sánchez C, Valenza M, Giuliano C, Everitt BJ, SabinoV, Cottone P (2015) The uncompetitive N-methyl-D-aspartate antagonist memantine reduces binge-like eating, food-seeking behavior, and compulsive eating: role of the nucleus accumbens shell. Neuropsychopharmacology 40:1163-1171.

Spanagel R, Eilbacher B, Wilke R (1994) Memantine-induced dopamine release in the prefrontal cortex and striatum of the rat--a pharmacokinetic microdialysis study. Eur J Pharmacol 262:21-26.

Spector AC, Klumpp PA, Kaplan JM (1998) Analytical issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat. Behav Neurosci 112:678-94.

Stojakovic A, Espinosa EP, Farhad OT, Lutfy K (2017) Effects of nicotine on homeostatic and hedonic components of food intake. J Endocrinol 235(1):R13-R31.

Traverso LM, Ruiz G, De la Casa LG (2012) MK-801 induces a low intensity conditioned taste aversion. Pharmacol Biochem Behav 100:645-651

Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, Hansen KB, Yuan H, Myers SJ, Dingledine R (2010) Glutamate receptor ion channels: structure, regulation, and function. Pharmacol Rev 62:405-96.

Vardigan JD, Huszar SL, McNaughton CH, Hutson PH, Uslaner JM (2010) MK-801 produces a deficit in

sucrose preference that is reversed by clozapine, D-serine, and the metabotropic glutamate 5 receptor positive allosteric modulator CDPPB: relevance to negative symptoms associated with schizophrenia? Pharmacol Biochem Behav 95:223-229

Wise RA (1982a) Common neural basis for stimulation reward, drug reward and food reward. In: Hoebel BG, Novin D (Eds), The Neural Basis of Feeding and Reward. Haer Institute for Electrophysiological Research, Brunswick, ME, pp 445-454.

Wise RA (1982b) Neuroleptics and operant behaviour: the anhedonia hypothesis. Behavioral and Brain Sciences 5:39-87.

Wise RA, Spindler J, deWit H, Gerberg GJ (1978) Neuroleptic-induced “anhedonia” in rats: pimozide blocks reward quality of food. Science 201:262-264.