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Role of dopamine D_1-like and D_2-like receptors in the activation of ingestive behaviour in thirsty rats licking for water

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Compliance with ethical standards

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

Abstract

Rationale Analysis of lick pattern for sucrose and NaCl and of the forced swimming response after dopamine antagonist administration led us to suggest that dopamine on D_1-like receptors is involved in behavioural activation, and the level of activation is "reboosted" on the basis of an evaluation process involving D_2-like receptors. Although some studies investigated licking microstructure for water after dopamine antagonists, the within-session time-course of their effect was never investigated.

Objectives The aims of this study were to further investigate the role of dopamine receptors in the mechanisms governing water ingestion, focussing on the within-session time-course of the microstructure parameters, and to test the proposed hypothesis.

Materials and methods The effects of the dopamine D_1-like receptor antagonist SCH 23390 (0.01–0.04 mg/kg) and of the dopamine D_2-like receptor antagonist raclopride (0.025–0.25 mg/kg) on licking microstructure for water were examined in 20-h water-deprived rats in 30-min sessions.

Results As previously observed with sucrose and NaCl, SCH 23390 reduced licking by reducing burst number, suggesting reduced behavioural activation. Moreover, it resulted in an increased burst size. Raclopride reduced the size of licking bursts, while their number was either increased or decreased depending on the dose.

Conclusion The results support the suggestion that D_1 receptors are involved in behavioural activation and D_2 receptors are involved in a related evaluation process. Within the framework of the proposed hypothesis, the increased burst size after D_1-like receptor blockade might be interpreted as a pro-hedonic effect consequent to the increased cost of the activation of the licking response.

Keywords: Activation; Dopamine; Dopamine receptor; Ingestion; Motivation; Reward

Introduction

Microstructure analysis reveals that 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). The number of licking bursts corresponds to the number of times that the 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. In contrast, the size of licking bursts, 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 (Davis and Smith 1992; Johnson 2018; 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 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 and Galistu 2017; Davis 1989; Davis and Smith 1992; Dwyer 2012; Higgs and Cooper 1998; Schneider et al. 1990; Smith 2001; Spector et al. 1998).

Results from studies on the effect of dopamine D_1-like and D_2-like receptor antagonists on licking microstructure (Canu et al. 2010; D'Aquila 2010; D'Aquila et al. 2012; Galistu and D'Aquila 2012, 2013) and the forced swimming response (D'Aquila and Galistu 2012), led us to suggest that (i) dopamine on D_1-like receptors is involved in the activation of goal directed responses and (ii) the level of response activation (or the level of response effort allocation) is updated, or "reboosted", on the basis of an evaluation process requiring dopamine on D_2-like receptors. Indeed, dopamine D_1-like receptor antagonist administration reduced the number of licking bursts and of the subjects engaging in licking at all, and increased the latency to lick (D'Aquila 2010; D'Aquila et al. 2012; Galistu and D'Aquila 2012). In addition, in rats confined

in a cylinder filled with water, it resulted in a reduction of the attempts to climb the cylinder wall ("climbing"), which is the most effortful option in such a condition (D'Aquila and Galistu 2012). These results suggest that dopamine D_1-like receptors might be involved in "behavioural activation" (for a definition, see Robbins and Everitt 2007) and in "response effort allocation" (for a definition, see Salamone et al. 2005, 2007, 2018). In contrast, dopamine D_2-like receptor antagonist administration reduced the size of licking bursts (D'Aquila 2010; Galistu et al. 2011; Genn et al. 2003; Liao and Ko 1995; Schneider et al. 1990) and produced on the within-session burst number time course an effect similar to that observed after either antipsychotics or reward devaluation on operant responding for different rewards (D'Aquila 2010; Galistu and D'Aquila 2013), i.e. either a transient increase (low doses) or a steep decline (higher doses), which occur only after the contact with the reward (see Wise 2004). These observations might be interpreted as an involvement of dopamine D_2-like receptors in reward evaluation/hedonic impact.

The interpretation of the within-session decline induced by dopamine antagonists in operant responding as functionally equivalent to the effect of reward devaluation – "extinction mimicry" – was a crucial argument in support of the "anhedonia hypothesis" (Wise et al. 1978; see Wise 2004), according to which dopamine mediates pleasure. However, a number of studies provided compelling evidence inconsistent with this hypothesis (Berridge 2007, 2012; Berridge et al. 1989; Canon and Palmiter, 2003; Salamone et al. 1997, 2007; Wassum et al. 2011; see also Wise 2004, 2006). Among these findings, it is worth of particular attention here the observation that dopamine antagonist administration and lesioning of dopamine mesolimbic ascending pathways leave intact the appetitive taste/hedonic reactions in response to sucrose (Berridge 2007; Berridge et al., 1989). This is a serious challenge to the interpretation of the reduced burst size in response to dopamine antagonists as a sign of anhedonia or reduced reward evaluation (see D'Aquila 2010; Dwyer 2012). As for "extinction mimicry", a number of studies revealed important differences between extinction and the effect of dopamine antagonists, thus

questioning their functional equivalence and suggesting accounts focussing on motor aspects (Rick et al. 2006; Salamone 1986; Sanger 1986; Spivak and Amit 1986; Tombaugh et al 1980, 1982). Further support to these accounts was provided by evidence of within-session impairments of motor competence in response to antipsychotics, such as increases in operant response duration in rats (Liao and Fowler 1990) and micrographia in humans (Haase and Janssen 1985). Nonetheless, the question of "extinction mimicry" might still pose a problem for the theoretical accounts of dopamine function which rule out its involvement in reward evaluation, as in the case of the "incentive salience attribution" hypothesis (see Berridge 2007, 2012). Indeed, to account for this phenomenon, it was proposed the concept of "reboosting", a process whereby the contact with the reward updates ("reboosts") the level of incentive salience attribution to a reward-associated stimulus (see Berridge 2007). A tentative solution to this problem might come from the observation that raclopride treated rats subjected to forced swimming fail to reduce "climbing" levels within a 15-min session in spite of the lack of efficacy of this costly and effortful response (i.e. in spite of the failure of the escape attempts). This observation led us to suggest that, in fact, dopamine D_2-like receptors might be involved in a process of evaluation of "response efficacy", rather than in the assessment of the reward value (D'Aquila and Galistu 2012). This interpretation might fit also to the case of licking, since the efficacy of the licking response in terms of cost-benefit ratio is strictly dependent on the value of the reward, which, in the case of sucrose, is the caloric content of the solution signalled by the sweet taste. (In D'Aquila and Galistu 2012 we proposed a more detailed explanation of how evaluation of response efficacy might account for the response patterns observed both in the forced swimming condition and in response to reward devaluation.)

A great deal of experimental evidence demonstrates the involvement of dopamine transmission in the control of water ingestion (Dourish 1983; Dourish and Jones 1982; Fortin and Roitman 2018; Marshall and Ungerstedt 1976; Pal et al. 1992; Papp and Bal 1986; Ungerstedt 1971),

with dopamine agonists and antagonists influencing water intake, after either systemic administration, or infusion into discrete brain areas (Hsu et al. 2018; Stricker et al. 2012). The dopamine D_1-like receptor antagonist SCH 23390 (Didriksen et al. 1993; Galistu and D'Aquila 2012; Gilbert and Cooper 1987; Ljungberg 1989b, 1990; Liao and Ko 1995; Ukai et al. 1989), and the dopamine D_2-like receptor antagonists haloperidol (De Santis et al. 2014; Huang et al. 2010; Ljungberg 1990; Liao and Ko 1995) and raclopride (Canu et al. 2010; Clifton et al. 1991; Didriksen et al. 1993; Ljungberg 1989a; Schneider et al. 1990), were reported to reduce water intake in thirsty subjects after systemic administration (but see Gilber and Cooper 1987, reporting an increased water intake following administration of the dopamine D_2-like receptor antagonist sulpiride).

A few of these studies reported the results of a microstructure analysis, but none of them examined the within-session time course of drug effects on the lick pattern (Canu et al. 2010, Galistu and D'Aquila 2012; Huang et al. 2010; Liao and Ko 1995). Thus, we decided to investigate the effect of dopamine D_1-like and D_2-like receptor antagonists on the microstructure of licking for water, focussing on the analysis of the within-session time-course of drug effects. In support of this approach, we provided experimental evidence suggesting that the analysis of the within-session time course of burst number might be crucial for the interpretation of drug effects in behaviourally (and psychologically) meaningful functional terms (D'Aquila 2010; Galistu and D'Aquila 2013; D'Aquila and Galistu 2017). Moreover, microstructural analysis of water ingestion has shown that burst size in rats ingesting water, in contrast to the case of sucrose and NaCl ingestion, is sensitive to post-ingestional stimuli, which induce a within-session decrease (Davis et al. 1999).

A first objective of this study was to further elucidate the role of dopamine D_1-like and D_2-like receptors in the control of water ingestion. A second objective was to test the hypothesis that dopamine D_1-like and D_2-like receptors are involved, respectively, in “behavioural

activation" and in the related evaluation process (D'Aquila 2010; D'Aquila and Galistu 2017; Galistu and D'Aquila 2013). To these ends, we investigated the effects of the dopamine D_1-like receptor antagonist SCH 23390 (Iorio et al. 1983) and of the dopamine D_2-like receptor antagonist raclopride (Köhler et al. 1985) on the microstructure of licking for water in 20-h water-deprived rats in 30-min sessions.

Materials and methods

Subjects

Experimentally naïve male Sprague-Dawley rats (Harlan, Italy) weighing 350-450 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.

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 authorised by the Ministry of Health, Italy.

Drugs and treatments

The dopamine D_1-like receptor antagonist SCH 23390 [R(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride] and the dopamine D_2-like receptor antagonist raclopride [S(−)-raclopride-L-tartrate] (Sigma, St. Louis, USA) were dissolved in distilled water. SCH 23390 and raclopride were injected subcutaneously in a volume of 1 ml/kg at dose ranges which yielded, in previous studies on sucrose (D'Aquila 2010) and NaCl

ingestion (Galistu and D'Aquila 2013), comparable effects on lick number and frequency of licking within bursts . The doses, expressed in milligrams *per* kilogram, were: 0.025, 0.125 and 0.25 for raclopride; 0.01, 0.02 and 0.04 for SCH 23390. Vehicle treatment sessions consisted in a 1 ml/kg distilled water administration. The time interval between drug, or the respective vehicle treatments, and experimental testing was 30-min for raclopride and 15-min for SCH 23390. These time intervals were chosen on the basis of previous studies on licking microstructure (Canu et al. 2010; D'Aquila 2010; D'Aquila et al. 2012; Galistu and D'Aquila 2012, 2013; Smith and Smith 2010) and other experimental paradigms (see Missale et al. 1998).

Apparatus and microstructural measures

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 180-sec. 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 into 3-min time bins in 30-min sessions.

Experimental design and procedures

The subjects were first familiarised with the test apparatus in 4 training sessions – 2 *per* week for 2 weeks – where they had access to water for 30-min. Following the training phase, the effects of SCH 23390 (Experiment 1) and raclopride (Experiment 2) were examined. 40 subjects were allocated into two separate groups, SCH 23390 (N=20) and raclopride (N=20), matched according to the burst size of the last training session. Due to technical problems of a recording station, the data from one subject from Experiment 1 were excluded from the analysis. For both experiments, a repeated measure design was adopted, with each rat tested at every dose, including treatment with vehicle, in 4 experimental 30-min sessions. All sessions were carried out after 20-h water deprivation, and were performed 72-96-h apart to avoid carry over effects. The water deprivation regime was limited to the 20-h periods before each experimental or training session. The order of the treatment sessions was balanced across subjects according to a modified Latin square design. The subjects were tested in cohorts of four. The data were collected in 3-min time bins. All experiments were performed between 09:00 and 14:00, in the light phase of the lighting cycle.

Statistical analysis

Statistical analysis of all sets of data was performed with ANOVA, by the software Statistica 8.0 (StatSoft Inc.).

Lick number and burst number data analysis involved *treatment* (4 levels, corresponding to doses) and *time* (10 levels, corresponding to time bins) as within-group factors. Analysis of the whole-session number of licks *per* burst and intra-burst lick rate, and of the latency to the first lick data did not involve the factor *time*. When a significant main effect of

the factor *treatment* was present, post hoc comparisons between the whole-session value of each drug dose and the relative vehicle were made by the Dunnett's test. When a significant interaction between factors was revealed, comparisons were performed by F-test for contrasts. Further analyses were performed to deal with either empty cells or with dependent variables showing no variance due to subjects failing to engage in licking. Moreover, analyses were performed to investigate drug effects in relation to the expected within-session changes in burst size (see Results for details).

An additional analysis with a direct comparison between the effects of the dose ranges of the two antagonists on the whole session data of all the examined parameters was performed, with *drug* as a between-group factor (2 levels corresponding to SCH 23390 and raclopride) and *dose* as a within-group factor (four levels corresponding to vehicle, low, mid and high dose). For all the parameters, each individual value relative to each dose was expressed as the percent of the average of the values of the corresponding vehicle treatment.

Results

Experiment 1: effects of SCH 23390

Treatment with the SCH 23390 highest dose (0.04 mg/kg) resulted in a dramatic reduction of the activation of licking behaviour. Indeed, (i) most subjects failed to engage in licking behaviour (11 out of 19), (ii) seven subjects, out of the eight which did show licking behaviour, emitted the first lick after the 180-sec latency cut-off time, and (iii) in the within-session data of the lick number and burst number were present dependent variables relative to 2-3 time bins, respectively, showing no variance because no subjects emitted a single lick or a single licking burst. Therefore, in the analysis of the within-session lick number and burst number, and of the

whole-session number of licks *per* burst and intra-burst lick rate, the data relative to the highest dose were excluded. Further analyses including the data relative to the highest dose were conducted on the whole-session number of licks *per* burst and intra-burst lick rate data, and on the latency data, of the eight subjects which did show licking behaviour with this dose.

ANOVA of the whole session lick number data revealed a main effect of *treatment* [$F(3,54)=39.41$, $P<10^{-6}$] due to a dose-dependent decrease of this measure (Fig. 1, top left). ANOVA of the within-session data with the exclusion of the high dose revealed a statistically significant effect of *treatment* [$F(2,36)=16.73$, $P<10^{-5}$], *time* [$F(9,162)=27.82$, $P<10^{-6}$] and of the interaction between the two factors [$F(18,324)=8.25$, $P<10^{-6}$]. After treatment with vehicle, a lick number decline was observed. With the dose of 0.01 mg/kg, the lick number values were reduced with respect to vehicle from the second to the fourth 3-min time bin. With the higher doses, the reduced values were observed since the beginning of the session. Moreover, a statistically significant increase with respect to vehicle was observed with the dose of 0.02 mg/kg at the 21-min time bin (Fig.1, left panels).

ANOVA of the burst number whole-session data revealed a statistically significant effect of *treatment* [$F(3,54)=68.65$, $P<10^{-6}$] due to a dose-dependent decrease of burst number (Fig. 1, top right). ANOVA of the within-session data with the exclusion of the high dose revealed a statistically significant effect of *treatment* [$F(2,36)=53.35$, $P<10^{-6}$], *time* [$F(9,162)=23.69$, $P<10^{-6}$] and a significant interaction between the two factors [$F(18,324)=8.03$, $<10^{-6}$]. After treatment with vehicle, a burst number decline was observed. Treatment with all the three doses resulted in reduced values since the beginning of the session. However, while the burst number time-course with the low dose was parallel to the vehicle-treatment time-course – starting with relatively high values at the beginning of the session, then showing a decline –, the values observed with the mid and the high dose were very low since the beginning of the session, with the latter being very close to naught in the first half of the session (Fig. 1, right

panels).

The number of lick *per* burst data analysis, without the high dose data, showed a statistically significant effect of *treatment* [F(2,32)=5.42, P=0.009]. Further analysis (Dunnett's test) showed a statistically significant increase with the dose of 0.02 mg/Kg (Fig. 2, top left). The analysis including the high dose data, limited to eight subjects, showed a statistically significant effect of *treatment* [F(3,21)=7.17, P=0.0017]. Further analysis (Dunnett's test) showed a statistically significant increase with the dose of 0.04 mg/Kg, but not with the dose of 0.02 (Fig. 2, top right). As shown in Fig. 2 (top left), the increased average burst size value observed with this dose was determined by the presence of two subjects showing particularly high values, which, however, failed to engage in licking with the highest dose.

ANOVA of the intra-burst lick rate data, without the high dose data, showed a statistically significant effect of *treatment* [F(2,32)=8.23, P=0.0013], due to decreased values with the low and the mid dose (Fig. 2, left mid panel).The analysis including the high dose data, limited to eight subjects, showed a statistically significant effect of *treatment* [F(3,21)=4.8, P=0.01]. Further analysis (Dunnett's test) showed a statistically significant decrease with all the three doses (Fig. 2, right mid panel).

ANOVA of the latency to the first lick data showed a statistically significant effect of *treatment* [F(3,54)=32.8, P<10-6], due to a dose-dependent increase of latency values (Fig. 2, bottom left). The analysis limited to the eight subjects showed a main effect of *treatment* [F(3,21)=17.85, $P<10^{-5}$], with statistically significant increased values observed with the two highest doses.

Experiment 2: effects of raclopride

ANOVA of the whole session lick number data revealed a statistically significant effect of

treatment [*treatment*: $F(3,57)=34.84$, $P<10^{-6}$]. Further analysis (Dunnett's test) showed a statistically significant decrease after treatment with the two highest doses (Fig. 3, top left).

Due to a dependent variable showing no variance (all values=0), corresponding to the 12-min time bin (9-12 min), two separate analyses were performed on the within-session data of lick number and burst number, the first involving the first three time bins (0-9 min) and the second involving the last six (12-30 min).

ANOVA of the first 9-min lick number data revealed a statistically significant effect of *treatment* [$F(3,57)=60.62$, $P<10^{-6}$], *time* [$F(2,38)=88.02$, $P<10^{-6}$] and a significant interaction between the two factors [$F(6,114)=8.39$, $P<10^{-6}$]. After treatment with vehicle, a lick number decline was observed. No effects were observed with the dose of 0.025 mg/Kg. A statistically significant decrease with respect to vehicle in the first 9-min of the session was observed with the doses of 0.125 and 0.250 mg/kg, with a more marked effect with the highest dose (Fig.3, left panels). ANOVA of the last 18-min revealed a statistically significant effect of *treatment* [$F(3,57)=3.89$, $P=0.013$], with no significant effect of *time* [$F(5,95)=1.43$, n.s.] or significant interaction between the two factors [$F(15,285)=0.95$, n.s.]. F-test for contrast revealed an increased level of lick number in the last 18-min of the session with the dose of 0.125 mg/kg (Fig. 3, left mid panel).

ANOVA of the whole session burst number data revealed a statistically significant effect of *treatment*, [$F(3,57)=8.41$, $P=0.0001$]. Further analysis (Dunnett's test) showed a statistically significant decrease after treatment with the high dose (Fig. 3, top right). ANOVA of the first 9-min revealed a statistically significant effect of *treatment* [$F(3,57)=19.08$, $P<10^{-6}$] and *time* [$F(2,38)=49.63$, $P<10^{-6}$], with no significant interaction between the two factors [$F(6,114)=0.97$, n.s.]. After treatment with vehicle, a burst number decline was observed. In the first 9-min of the session a significant increase of burst number was observed with the low dose of raclopride, while a significant decrease was observed with the high dose. No effects were observed with the

mid dose (Fig.3, right panels). ANOVA of the last 18-min revealed a statistically significant effect of *treatment* [F(3,57)=6.13, P=0.001], with no significant effect of *time* [F(5,95)=1.13, n.s.] or significant interaction between the two factors [F(15,285)=0.77, n.s.]. F-test for contrast revealed an increased level of burst number in the last 18-min of the session with the dose of 0.125 mg/kg (Fig. 3, right mid panel).

ANOVA of the number of lick *per* burst data showed a main effect of *treatment* [F(3,48)=17.67, $P=10^{-6}$] due to a dose-dependent decrease of burst size (fig. 4, top panel).

ANOVA of intra-burst lick rate data failed to show a statistically significant effect of *treatment* [F(3,48)=1.13, n.s.] (Fig. 4, mid panel).

ANOVA of the latency to the first lick data showed a main effect of *treatment* [F(3,57)=10.9, $P<10^{-5}$] due to an increase of latency with the highest dose (Fig. 4, bottom panel).

Whitin-session burst size changes and drug effects

On the bases of previous evidence showing that water post-ingestional stimuli exert negative feed-back on the size of licking bursts (Davis et al. 1999), we decided to perform an analysis on the within-session time course of this measure. In the cited study, it was reported that, in a real-drinking condition, burst size for water was reduced with respect to the sham-drinking condition, in a time interval from 5-min to 20-min after the beginning of the session (Davis et al. 1999). Thus, for this analysis, we split the sessions into three time intervals: 0-6 min, 6-21 min and 21-30 min (which is the best fitting approximation given the basic measure of 3-min time bins used in our study). Since the number (and the identity) of the subjects engaging in licking behaviour varied considerably between the different time-intervals and between the different drug dose sessions (see Fig. 5), a repeated measures analysis involving all the data for

each experiment would have led to too many empty cells. Thus, we performed paired samples analyses comparing the data of each later time interval (6-21 and 21-30) to the same session early time interval (0-6 min).

In the session with vehicle, with the subjects from both experiments included in the analysis, burst size was reduced with respect to the 0-6 time interval both in the 6-21 min [$F(1,38)=10.29$, $P=0.002$] and in the 21-30 min time interval [$F(1,31)=24.28$, $P=0.000026$](Fig. 5, bottom panel).

In the session with the low SCH 23390 dose, a reduction of burst size was observed only in the last time interval [$F(1,6)=12.85$, $P=0.011$](Fig. 5, top left). In the sessions with the mid and high SCH 23390 doses, the number of subjects engaging in licking in each time interval, and in particular in the first one, was too low to allow meaningful comparisons (Fig. 5, left, 2nd and 3rd panel).

In the session with the low raclopride dose, burst size was reduced with respect to the 0-6 time interval both in the 6-21 min [$F(1,18)=10.1$, $P=0.005$] and in the 21-30 min time interval [$F(1,12)=17.22$, $P=0.0013$](Fig. 5, right, top panel). In the session with the mid raclopride dose, a reduction of burst size was observed only in the last time interval [$F(1,11)=8.09$, $P=0.051$] (Fig. 5, right, 2nd panel). In the session with the high raclopride dose, no statistically significant differences were detected (Fig. 5, right, third panel).

Effect of SCH 23390 and raclopride on burst size in relation to the ingested amount of water

Since burst size is reduced by water post-ingestional stimuli (Davis et al. 1999), and, consistently, we observed a within-session reduction of this measure in the vehicle sessions (see Fig. 5), it is possible that the ability of SCH 23390 to increase burst size (see Fig. 2, top panels)

might be just the consequence of reduced ingestion (see Fig. 1, top left). To deal with this problem, burst size in the first 3-min time bin of the vehicle treatment session, was compared to burst size of each SCH 23390 dose session, within a time interval showing a similar level of lick number, which was 0-15 min for the low dose, 0-18 min for the mid dose, and 30-min, i.e. the whole session, for the high dose (Fig. 6, left panels). The comparisons involved only the subjects which engaged in licking behaviour in the analysed time intervals (low dose: N=16; mid dose: N=10; high dose: N=8). Repeated measures ANOVA revealed a statistically significant increase of burst size with the high SCH 23390 dose [$F(1,7)=7.01$, $P=0.033$]. The effects of the two lower doses were not statistically significant, in spite of the *ictu oculi* increased value of the mid dose (Fig. 6, right panels).

The same analysis was performed also on raclopride data. The time interval showing a similar level of lick number with respect to the first 3-min time bin of the vehicle treatment session was 0-3 min for the low dose (N=20), 0-15 min for the mid dose (N=17), and 30-min, i.e. the whole session, for the high dose (N=18). With the high dose, a lick number reduction approaching statistical significance was observed [ANOVA: $F(1,17)=3.64$, $P=0.073$](Fig. 7, left panels). Repeated measures ANOVA revealed a statistically significant decrease of burst size with the mid [$F(1,16)=30.1$, $P=0.00005$] and the high [$F(1,17)=57.59$, $P<10^{-5}$] raclopride doses. No statistically significant effect was observed with the low dose (Fig. 7, right panels).

Comparison between SCH 23390 and raclopride effects

ANOVA of the lick number whole-session data revealed a significant effect of *drug* [$F(1,37)=8.08$, $P=0.007$] and *dose* [$F(3,111)=70.7$, $P<10^{-6}$], with a significant interaction between the two factors [$F(3,111)=3.1$, $P=0.029$], based on which F-tests for contrasts were performed. The results showed a statistically significant difference between SCH 23390 and

raclopride with the low dose, with SCH 23390 showing a lower value (Fig. 8, top left).

ANOVA of the burst number whole-session data revealed a significant effect of *drug* [$F(1,37)=30.03$, $P<10^{-5}$] and *dose* [$F(3,111)=24.46$, $P<10^{-6}$], with a significant interaction between the two factors [$F(3,111)=11.1$, $P<10^{-5}$]. F-tests for contrasts showed statistically significant differences with all drug doses, with SCH 23390 showing lower values compared to raclopride (Fig. 8, top right).

ANOVA of the number of licks *per* burst whole-session data, excluding the data of the high doses, revealed a significant effect of *drug* [$F(1,34)=14.44$, $P=0.00057$], but not *dose* [$F(2,68)=0.96$, n.s.], with a significant interaction between the two factors [$F(2,68)=13.05$, $P=0.000016$], based on which F-tests for contrasts were performed. The results showed a statistically significant difference between SCH 23390 and raclopride at low and mid dose, with SCH 23390 and raclopride showing increased and decreased values compared to the vehicle level, respectively. The high dose data were analysed separately by one way ANOVA, which revealed a highly statistically significant difference in the same direction [$F(1,24)=62.6$, $P<10^{-6}$] (Fig. 8, mid left).

ANOVA of the intra-burst lick rate whole-session data, excluding the data of the high doses [*drug*: $F(1,34)=0.42$; *dose*: $F(2,68)=2.8$, n.s.; interaction: $F(2,68)=0.6$], and a separate one way ANOVA of the high dose data [$F(1,24)=3.08$, n.s.], failed to reveal statistically significant effects (Fig. 8, mid right).

ANOVA of the latency to the first lick data revealed a significant effect of *drug* [$F(1,37)=60.5$, $P<10^{-6}$] and *dose* [$F(3,111)=40.29$, $P<10^{-6}$], with a significant interaction between the two factors [$F(3,111)=13.47$, $P<10^{-6}$], on the bases of which F-tests for contrasts were performed. The results showed statistically significant differences between SCH 23390 and raclopride at all doses, with SCH 23390 showing higher values (Fig. 8, bottom panel).

Discussion

Consistently with the results of previous studies examining the ingestion of sucrose (D'Aquila 2010), NaCl (Galistu and D'Aquila 2012, 2013), and water (Galistu and D'Aquila 2012; Liao and Ko 1995), the dopamine D_1-like receptor antagonist SCH 23390 reduced the ingestion of water in thirsty rats, as indicated by the reduced lick number. The reduced ingestion was exclusively due to reduced activation of licking behaviour, as indicated by reduced number of licking bursts, increased latency to the first lick, and reduction of the number of subjects engaging in licking with the highest doses. All drug doses reduced the burst number level since the beginning of the session, as previously observed in different groups of subjects exposed to two different sucrose concentrations, i.e. in a condition which does not involve changes in the reward value (D'Aquila and Galistu 2017), thus suggesting that the reduced burst number is not the consequence of a failure of the "reboosting" of response activation occurring during the consummatory transaction with the reward. Moreover, a significantly decreased intra-burst lick rate was observed with all doses, suggesting that motor impairment was to some extent involved in the observed effects. Finally, an increased whole-session burst size was observed with the mid and high SCH 23390 doses, an observation which hardly can be explained as a direct consequence of motor impairment, and which might suggest a pro-hedonic effect.

As previously observed in rats ingesting sucrose (D'Aquila 2010; Galistu et al. 2011; Genn et al. 2003; Schneider et al. 1990), NaCl (Canu et al. 2010; D'Aquila et al. 2012; Galistu and D'Aquila 2013), and water (Canu et al. 2010; Liao and Ko 1995), the present data show that the dopamine D_2-like receptor antagonist raclopride reduced licking for water mainly through the reduction of burst size, with no effects on the intra-burst lick rate, which suggests the absence of a relevant motor impairment. Thus, within the framework of the proposed hypothesis, this effect might be interpreted as a failure of the "reboosting" process (see

Introduction). However, raclopride effect on burst number was reminiscent of the effects of either neuroleptics or reward devaluation in instrumental responding for different rewards (Wise et al. 1978; Wise 1982a,b) only to a limited extent. Indeed, it resulted either in a compensatory increase of this measure with the low doses or in a decrease with the high dose, but, at variance with our previous observations in rats licking for sucrose (D'Aquila 2010) and NaCl (Galistu and D'Aquila 2013), these changes in the level of burst number were present since the beginning of the session, rather than taking place only after a few minutes of contact with the reward. Thus, we failed to observe the characteristic extinction-like response pattern. In order to explain this discrepancy, it might be relevant to recall that the mechanisms governing water intake show important peculiarities as for the role played by the taste system. Water is sensed by specific taste receptor cells (TRCs), namely the acid-sensing TRCs, the activation of which – in a state of dehydration – results in the activation of drinking. However, while the appetitive intake of NaCl and sugars is dependent on the integrity of the taste system, normal water drinking – both spontaneous and water deprivation-induced – occurs even in the absence of taste signals (Zocchi et al. 2017). Thus, it is likely that taste might play a role in the "reboosting" of the licking response in the case of sucrose and NaCl solutions but not in the case of water.

The dose-ranges of SCH 23390 and raclopride used in this study were chosen because they yielded comparable effects on lick number and intra-burst lick rate in two previous studies, examining sucrose (D'Aquila 2010) and NaCl ingestion (Galistu and D'Aquila 2013). In the present study, the direct comparison between the two drugs revealed a statistically significant difference in lick number between SCH 23390 and raclopride at the low dose level. However, the relevant differences between the effects of the two drugs on the number and size of licking bursts were observed also at the dose levels – mid and high – showing similar effects on the whole-session lick number and intra-burst lick rate. Therefore, meaningful comparisons can be made both between the effects of the two drugs in the present study, and between the present

results and our previous findings.

Previous evidence demonstrated that water exerts a post-ingestional inhibitory effect on burst size (Davis et al. 1999). Consistently with this finding, we observed a within-session decrement of this measure in the vehicle and low dose sessions from both experiments, and in the mid dose raclopride session. No within-session changes in burst size were observed in the high raclopride dose session – possibly due to a floor effect –, while no statistical analysis could be performed of the mid and high SCH 23390 session data (see Results section for details). Thus, the possibility must be considered that the increased burst size observed with the two SCH 23390 highest doses might be due to the dramatic reduction of water post-ingestional stimuli consequent to reduced ingestion. The present results show that, with the highest SCH 23390 dose, but not with the mid dose, an increased burst size was still present comparing the whole-session data of the drug-treatment session to the first 3-min time bin data of the vehicle session, i.e. between time intervals showing a similar level of overall ingestion. These observations suggest that the most parsimonious explanation for the increased burst size observed with the mid dose is the reduction of the post-ingestional inhibitory feed-back. However, a further explanation *might* be required for the effect observed with the high dose (the possibility cannot be excluded that not only quantity but also density in time might be crucial in determining the negative post-ingestional feed-back). Within a cost-benefit ratio interpretative framework (Salamone et al. 1997, 2007, 2018), this effect might be interpreted as an increased hedonic/evaluation response in consequence of the increased cost of the licking response, due to the additional effort required to overcome the reduced activation level and/or the impaired motor function induced by dopamine D_1-like receptor blockade. This interpretation might be supported by the observation that an increase of the effort demand of an instrumental task for a less-preferred low-caloric sucrose solution led to an increased burst size in a subsequent free-access consumption test, which was interpreted as an increased reward evaluation (Johnson and

Gallagher, 2011; Lydall et al. 2010).

An important question to consider is the possible contribution of motor impairment to the effects of dopamine antagonist administration on lick pattern. Previous studies have shown evidence of specific motor effects, with reduced lick number accompanied by decreased force of tongue protrusion, increase of the individual lick duration, and of the inter-lick intervals (Fowler and Mortell 1992; Gramling et al., 1984; Gramling and Fowler, 1986), the combined effect of which should result in the reduction of the intra-burst lick rate, an effect that we observed here with all doses of SCH 23390, but not with raclopride. The dose-ranges of SCH 23390 and raclopride used in this and all the previous studies from our laboratory can be considered "border-line" as for the ability to reduce the intra-burst lick rate, with some studies failing to show significant effects on this measure (SCH 23390: D'Aquila et al. 2012, Galistu and D'Aquila 2012, 2013; Raclopride: Galistu and D'Aquila 2013; but see Canu et al. 2010, D'Aquila 2010, D'Aquila et al. 2012, with raclopride reducing the intra-burst lick rate). Relevant effects on the other parameters, especially burst number for SCH 23390 and burst size for raclopride, were observed even at doses which failed to affect the intra-burst lick rate (e.g. D'Aquila 2010). As for raclopride, increases in burst number were observed at doses resulting in reduced intra-burst lick rate (e.g. Canu et al. 2010). Thus, the effects of these two antagonists on the licking microstructure measures and especially the differences between them cannot be easily accounted for by deficits in motor competence. 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 might be worth noting also that we have shown that both SCH 23390 and raclopride failed to affect lick efficiency at doses

which resulted in reduced intra-burst lick rate (D'Aquila 2010).

The attempt to parse the effects of dopamine antagonist into motor *versus* motivational effects involves the risk of circularity, since motivation is operationally defined in terms of likelihood to result in a goal-directed motor output (see Salamone et al., 2005, for a discussion on this point). Thus, the dichotomy between motivation and motor function was challenged from different perspectives. Indeed, it was suggested that "behavioural activation" is an area of overlap between the two (see Salamone et al. 1997, 2007), and that "an impaired motivational background might be the basis for the existence of slowness of movement in dopamine deficiency conditions" (Keitz et al. 2003). In the same line of thought is the expression "incentive-related motor activity" used in a study comparing the effects of a dopamine antagonist with extinction on instrumental behaviours (Salamone 1986). Consistently, a single neurocomputational model of basal ganglia function can account for a range of phenomena spanning from the Parkinsionian-like effects of neuroleptics to reinforcement learning (Cohen and Frank 2009). Finally, the categories of "activation" and "evaluation" (of response efficacy?), which might help to account for the striking qualitative differences between the effects of dopamine D_1- and D_2-like receptor antagonists in ingestive behaviour – but also in other behavioural paradigms such as the forced swimming response (D'Aquila and Galistu 2012) and other models investigating the reinforcing properties of food (Chausmer and Ettemberg 1997; Ettemberg and Camp 1986b; McFarland and Ettemberg 1998), water (Ettemberg and Camp 1986a) and addictive drugs (McFarland and Ettemberg 1995) –, can make sense within either the "motivational" or the "motor" interpretative framework.

A number of studies support the involvement of meso-striatal dopamine pathways in the regulation of water intake. Earlier studies showed that 6-OHDA lesions of the nigro-striatal dopamine pathway induce aphagia and adipsia (Dourish 1983; Dourish and Jones 1982; Marshall and Ungerstedt 1976; Pal et al. 1992; Papp and Bal 1986; Ungerstedt 1971). More

recently, it was reported that intra-oral infusion of water in thirsty rats induced phasic increases of dopamine concentration in the nucleus accumbens (NAc) shell (Fortin and Roitman 2018). Moreover, forebrain structures along the lamina terminalis, which are involved in sensing and processing information on body fluid balance, were shown to modulate neuronal activity in the ventral tegmental area (VTA)(Hurley et al. 2018). These observations suggest that the dopamine antagonist effects observed in the present study might be accounted for, at least in part, by the blockade of dopamine D_1- and D_2-like receptors in the meso-striatal dopamine pathways, with the possible involvement of the VTA-NAc pathway (i.e. the mesolimbic dopamine system) in the motivational aspects of the observed effects.

In conclusion, these results are consistent with the results of previous studies suggesting a different role for dopamine D_1- and D_2-like receptors in the control of water ingestion (Canu et al. 2010; Galistu and D'Aquila 2012; Liao and Ko 1995). It should be stressed that the analysis of the within-session time-course of the number and size of licking bursts was crucial in revealing the peculiarities of the dopamine antagonist effects on water intake with respect to the otherwise similar effects observed in our previous studies examining licking for sucrose and NaCl (D'Aquila 2010; Galistu and D'Aquila 2013). The present results show also consistency with the results of previous studies on licking and the forced swimming response which led to suggest the hypothesis that dopamine D_1-like receptors are involved in behavioural activation, while dopamine D_2-like receptors are involved in a process of evaluation (of response efficacy?) on the basis of which the level of "reboosting" of response activation is determined.

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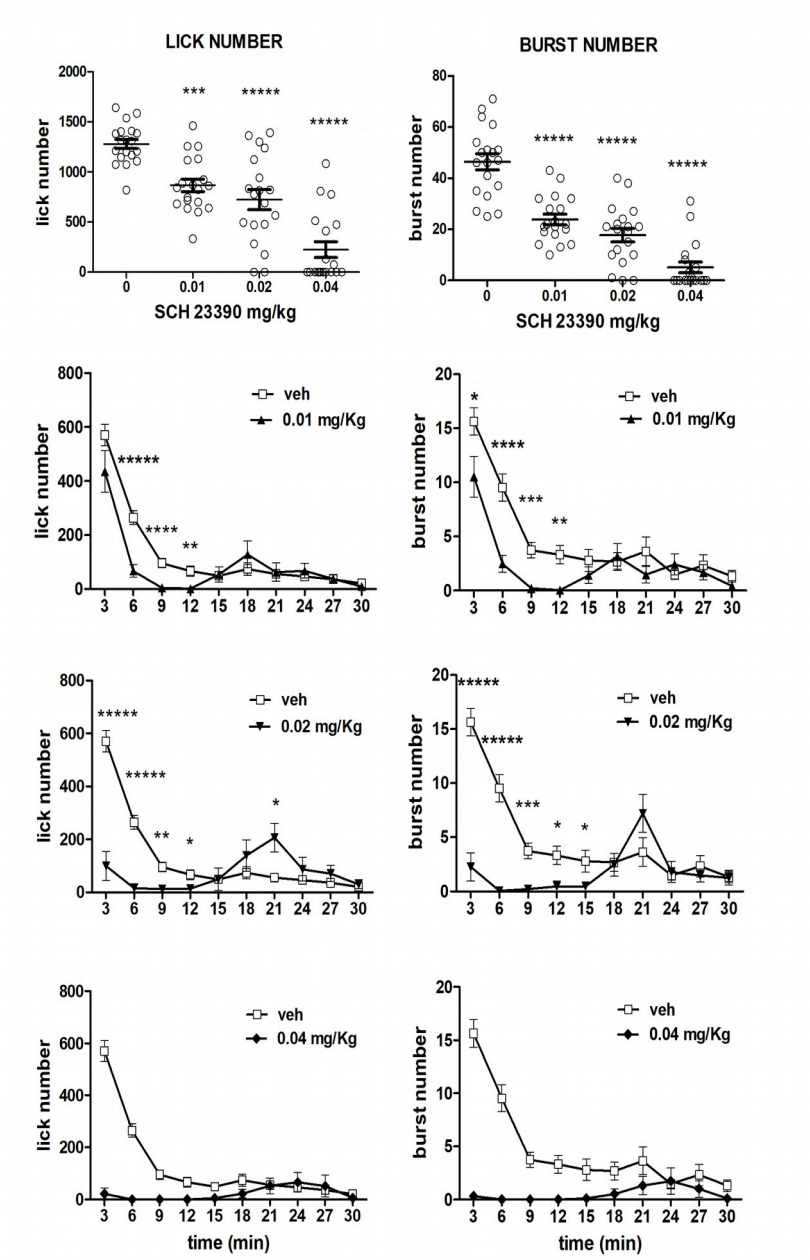

Fig. 1 Effect of SCH 23390 on lick number (left) and burst number (right). On top are shown the whole-session values, below the time course of each dose compared to vehicle. Values represent the mean ± S.E.M. from 19 subjects. Circles represent individual data. The data of the 0.04 mg/kg dose were excluded from the within-session data analysis due to 2 (LN)-3 (BN) dependent variables with no variance (all values=0). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, *****P<10^{-5} (ANOVA followed by Dunett's test or F-test for contrast).

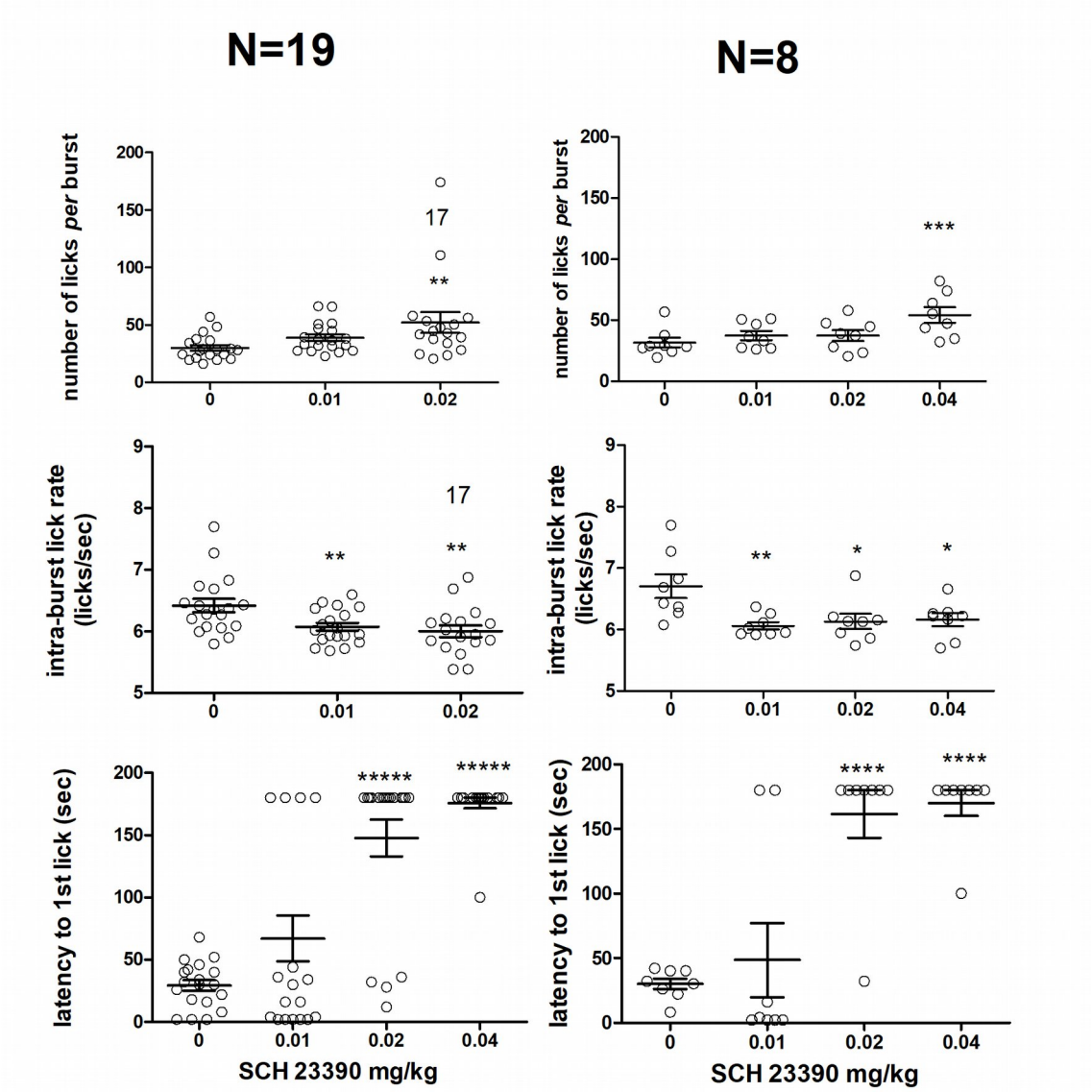

Fig. 2 Effect of SCH 23390 on number of lick *per* burst (top panels), intra-burst lick rate (mid panels) and latency to the first lick (bottom panels). In the right panels are reported the data relative to the eight subjects which engaged in licking behaviour with the highest dose (see text for more details).Values represent the mean ± S.E.M. from 19 or 8 subjects, unless otherwise indicated due to subjects failing to lick. Circles represent individual data. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, *****P<10^{-5} (ANOVA followed by Dunnett's test).

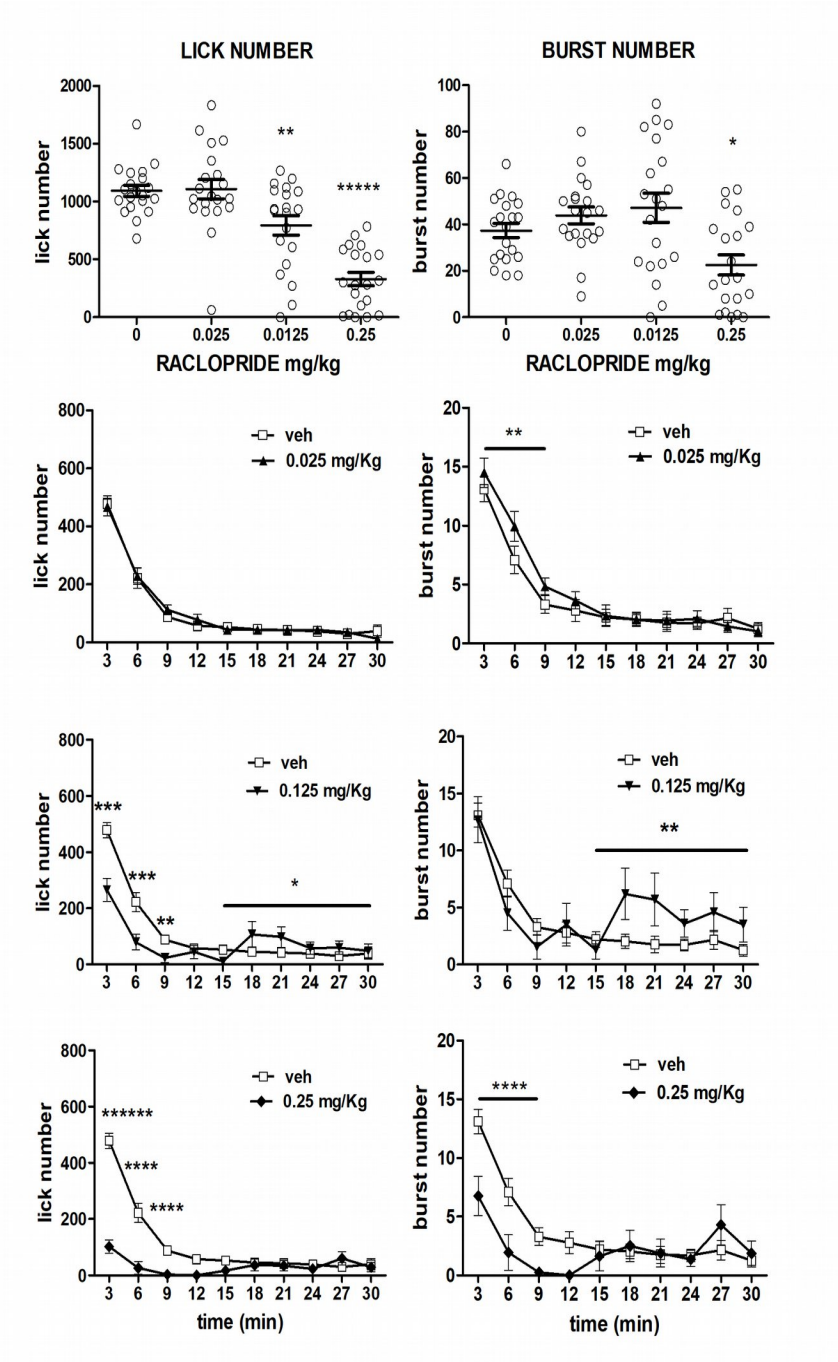

Fig. 3 Effect of raclopride on lick number (left) and burst number (right). On top are shown the total values, below the time course of each dose compared to vehicle. Values represent the mean ± S.E.M. from 20 subjects. Circles represent individual data. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, *****P<10^{-5}, ******P<10^{-6} (ANOVA followed by Dunnett's test or F-test for contrast; straight lines indicate comparisons involving consecutive time points based on the main effect of *treatment*).

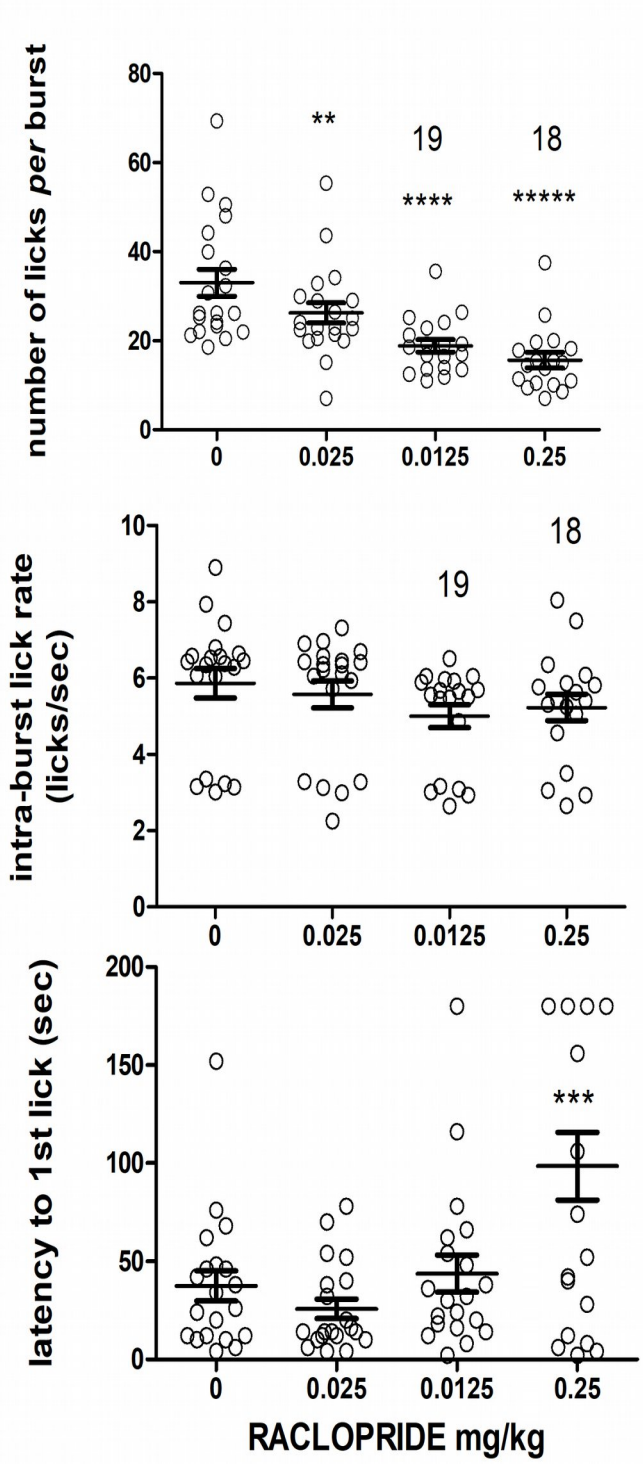

Fig. 4 Effect of raclopride on number of lick *per* burst (top panel), intra-burst lick rate (mid panel) and latency to the 1st lick (bottom panel). Values represent the mean ± S.E.M. from 20 subjects, unless otherwise indicated due to subjects failing to lick. Circles represent individual data. **P<0.01, ***P<0.001, ****P<0.0001, *****P<10^{-5}, (ANOVA followed by Dunnett's test).

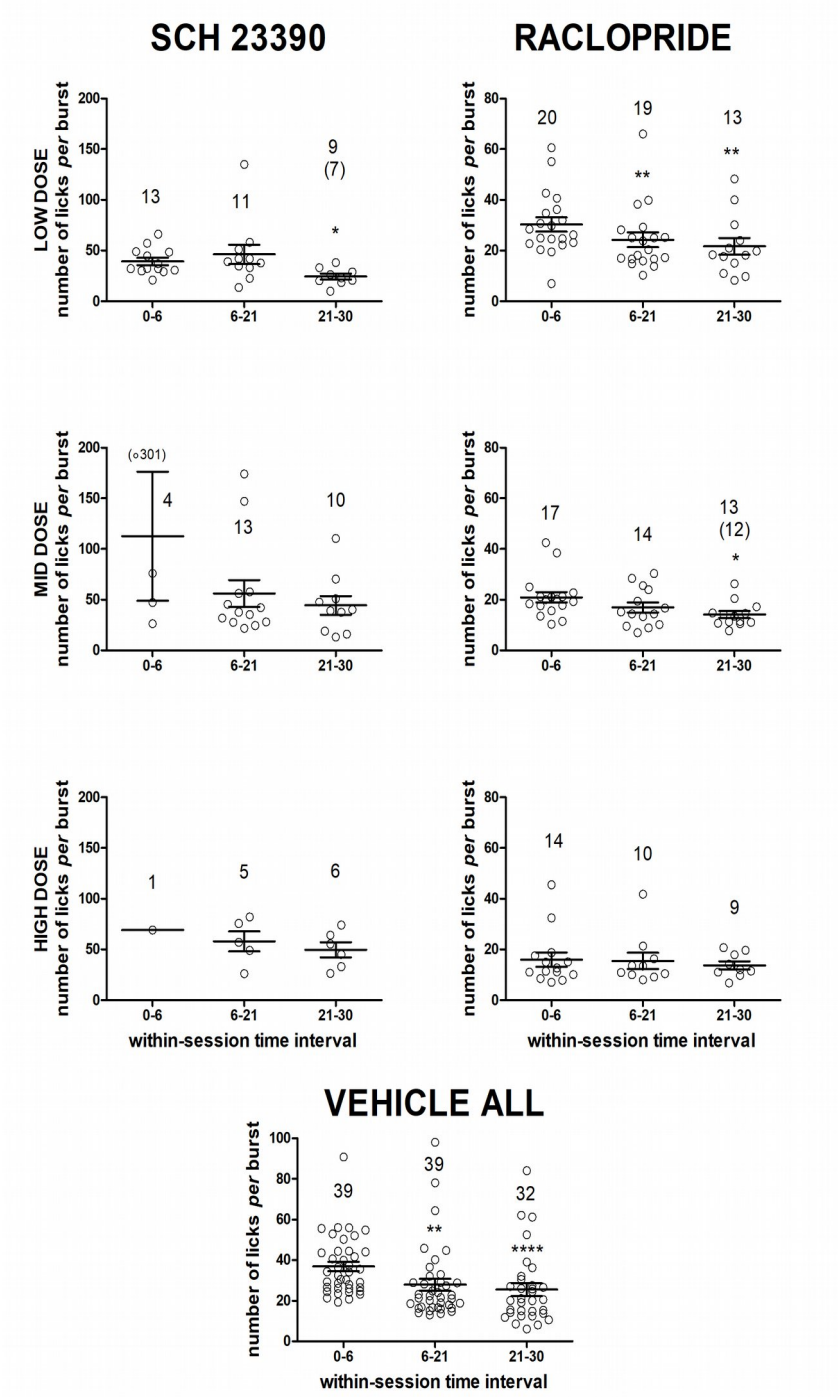

Fig. 5 Within-session time course of burst size (number of licks *per* burst). Values represent the mean ± S.E.M. from the number of subjects indicated for each time interval. If different, the number of subjects involved in the paired samples comparison with the 0-6 min time interval is indicated in parentheses. Circles represent individual data. *P<0.05, **P<0.01, ****P<0.0001 (ANOVA).

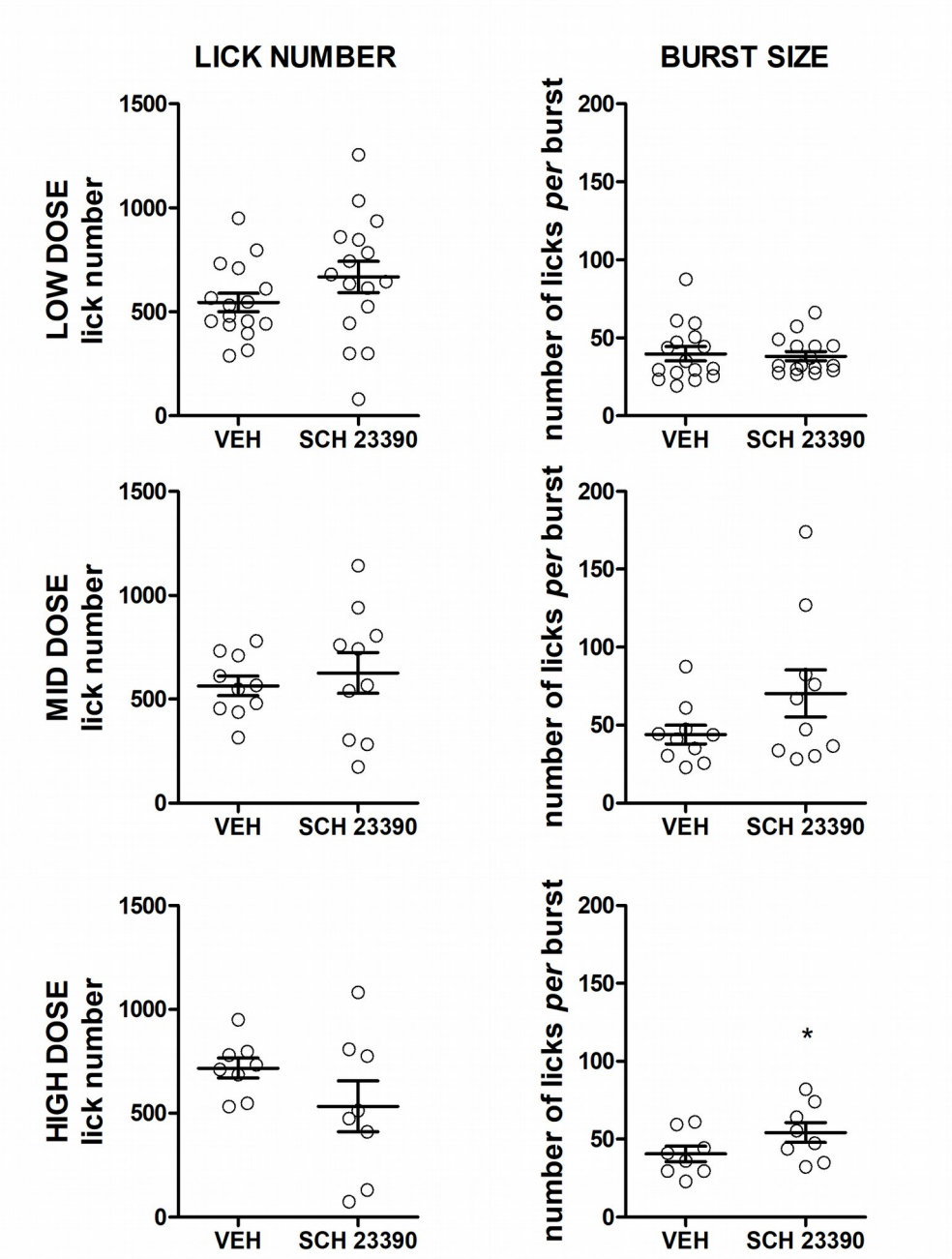

Fig 6 Comparison between burst size (number of licks *per* burst) of the first 3-min time bin of the vehicle-treatment session *versus* burst size with each SCH 23390 dose, in a time interval showing a similar level of ingested volume (lick number). Values represent the mean ± S.E.M. from the subjects which, after treatment with each SCH 23390 dose, engaged in licking behaviour in the specified time interval. Circles represent individual data. The number of subjects and the specified time intervals were, respectively, 16 subjects and 15-min (0-15 min interval) for the low dose session, 10 subjects and 18-min (0-18 min interval) for the mid dose session, and 8 subjects and 30-min (the whole session) for the high dose session. *P<0.05 (ANOVA).

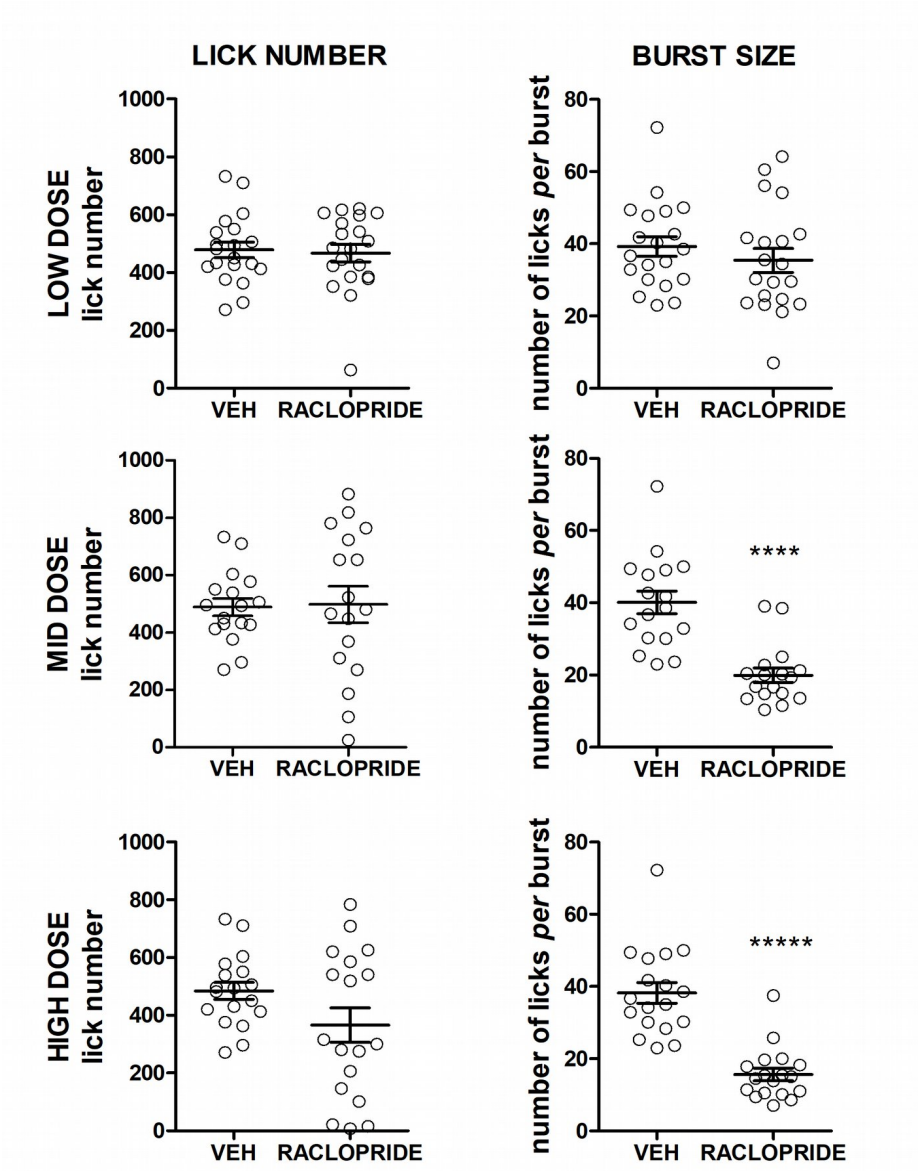

Fig 7 Comparison between burst size (number of licks *per* burst) of the first 3-min time bin of the vehicle-treatment session *versus* burst size with each raclopride dose, in a time interval showing a similar level of ingested volume (lick number). Values represent the mean ± S.E.M. from the subjects which, after treatment with each raclopride dose, engaged in licking behaviour in the specified time interval. Circles represent individual data. The number of subjects and the specified time intervals were, respectively, 20 subjects and 3-min (0-3 min interval) for the low dose session, 17 subjects and 15-min (0-15 min interval) for the mid dose session, and 18 subjects and 30-min (the whole session) for the high dose session. ****P<0.00001, *****P<10^{-5} (ANOVA).

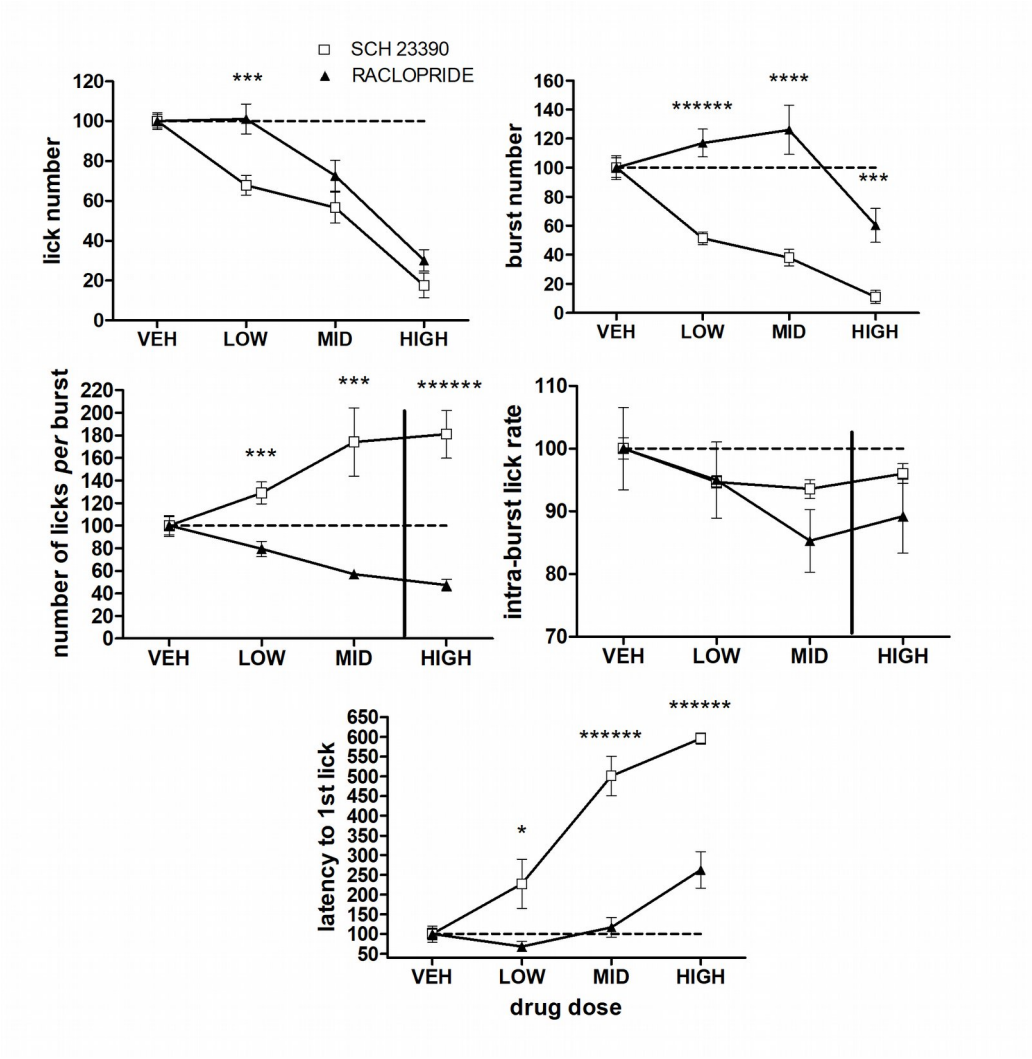

Fig. 8 Comparison between the effects of SCH 23390 and raclopride on lick number (top left), burst number (top right), burst size (mid left), intra-burst lick rate (mid right) and latency to the first lick (bottom panels). Drug doses (mg/kg): VEH, vehicle; LOW, SCH 23390 0.01, raclopride 0.0125; MID, SCH 23390 0.02, raclopride 0.125; HIGH, SCH 23390 0.04, raclopride 0.250. The values are expressed as percent of the mean value of the relative vehicle treatment. The dashed line indicates vehicle level (100%). A vertical line separates segments or points subjected to separate statistical analysis. All values represent the mean ± S.E.M. from 8 to 20 subjects. *P < 0.05; ***P < 0.001; ****P < 0.0001; ******P<10^{-6} (ANOVA followed by F-test for contrasts).