The active heroin metabolite 6-acetylmorphine has powerful reinforcing effects as assessed by self-administration in the rat

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Riccardo Avvisati*[1], Inger Lise Bogen[2,3], Jannike M. Andersen[2,4], Vigdis Vindenes[2,5], Jørg Mørland[5,6], Aldo Badiani[1,7], Fernando Boix[2].

[1]Sussex Addiction Research and Intervention Centre (SARIC), School of Psychology, University of Sussex, Brighton, UK; [2]Section of Drug Abuse Research, Department of Forensic Sciences, Oslo University Hospital, Norway; [3]Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Norway; [4]Department of Pharmaceutical Biosciences, School of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Oslo, Norway; [5]Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Norway; [6]Norwegian Institute of Public Health, Oslo, Norway, [7]Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy

*Current address: MRC Brain Network Dynamics Unit, University of Oxford, Oxford, UK

Corresponding Authors:

Fernando Boix: Section of Drug Abuse Research, Dept. of Forensic Sciences, Oslo University Hospital, PO Box 4950 Nydalen, N-0424 Oslo, Norway. E-mail: fernando.boix.escolan@ous-hf.no

Aldo Badiani: SARIC, University of Sussex, Sussex House, Falmer, Brighton, BN1 9RH, UK. E-mail: Aldo.Badiani@sussex.ac.uk

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ABSTRACT

Previous studies have suggested that at least some of the behavioral effects of heroin might be mediated by its active metabolite 6-acetylmorphine (6-AM). The aim of the present study was to investigate the reinforcing effects of 6-AM and its role in mediating those of heroin. We used an intravenous self-administration procedure in male Sprague-Dawley rats including four phases: acquisition, extinction, reinstatement of drug-seeking, and re-acquisition. Independent groups of rats readily learned to self-administer equimolar doses (0.135 µmol/kg) of either 6-AM (44.3 µg/kg) or heroin (50 µg/kg). Under a fixed ratio 1 (FR1) schedule of reinforcement, the rate of responding was the same for 6-AM and heroin, but it was significantly higher for 6-AM than for heroin under a FR2 schedule. A non-contingent infusion ('priming') of 0.068 µmol/kg of either 6-AM or heroin reinstated non-reinforced drug-seeking (relapse). The rats readily re-acquired self-administration behaviour when given access to one of two doses (0.068 and 0.135 µmol/kg) of 6-AM or heroin. Pretreatment with a specific monoclonal antibody (mAb) against 6-AM blocked the priming effect of 6-AM, and modified the rate of lever-pressing on re-acquisition of 6-AM self-administration in a manner compatible with a shift to the right of the dose-effect curve. The mAb did not affect heroin responding. The present results show that 6-AM possesses reinforcing effects similar to those of heroin. The lack of effect of 6-AM mAb on heroin priming and heroin self-administration calls for further studies to clarify the role of heroin and its metabolites in heroin reward.

Keywords:

Heroin, 6-acetylmorphine, self-administration, reinforcement.

Abbreviations:

6-AM: 6-Acetylmorphine

M6G: Morphine-6-glucuronide

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1. INTRODUCTION

Heroin (diacetylmorphine) is an acetylated derivative of morphine with a high addiction potential (Darke, 2011; Nutt et al., 2007). The past few years in particular have seen a sharp increase in heroin abuse and, as a consequence, an alarming rise in the number of heroin-related overdoses and deaths (Dowell et al., 2017; European Monitoring Centre for Drugs and Drug Addiction, 2018). Yet, despite the high abuse potential heroin, and the related health and social costs, the mechanisms responsible for its reinforcing effects are still poorly understood.

Heroin's estimated plasma half-life is less than 5 minutes, being rapidly transformed into 6-acetylmorphine (6-AM), which is further de-acetylated to morphine, both in rodents (Andersen et al., 2009; Gottas et al., 2013) and in humans (Rook et al., 2006a; Rook et al., 2006b). 6-AM, and morphine are μ-opioid (MOP) receptor agonists (Inturrisi et al. 1983, Selley et al. 2001). The very fast metabolism of heroin, and the very similar pharmacological profiles of heroin and morphine, led to hypothesize that the effects of the former are mostly mediated by the latter (Way et al., 1960; Wright, 1941). However, there is some evidence against this simplification. The euphorigenic effect of heroin (the so-called 'rush' or 'high') develops very rapidly after systemic administration (Smith and Beecher, 1962), whereas morphine concentrations in the plasma increase much more slowly (Rook et al., 2006a; Rook et al., 2006b), and, owing to its low lipophilicity, even more slowly in the brain (Gottas et al., 2014; Seleman et al., 2014). Thus, the attention of researchers has shifted to 6-AM, which binds MOP receptors with similar affinity as morphine (Inturrisi et al., 1983) but has greater efficacy (Selley et al., 2001). In humans, 6-AM is found at higher concentrations than morphine immediately after heroin administration, overlapping in time with heroin's high (Rook et al., 2006a; Rook et al., 2006b). In rodents as well, 6-AM represents the main metabolites for the first 30 min after heroin administration (Andersen et al., 2009; Gottas et al., 2014; Gottas et al., 2013). Thus, 6-AM is a clear candidate to mediate the reinforcing properties of heroin.

It has been shown that systemic administration of 6-AM can produce analgesia and constipation (Umans and Inturrisi, 1981), as well as psychomotor activation (Andersen et al., 2009) similar to that produced by heroin. Interestingly, the time-course of brain concentration of 6-AM parallel those of locomotor activity (Andersen et al., 2009) and striatal dopamine levels (Gottas et al., 2014) after heroin administration. Still, despite some early work (Hubner and Kornetsky, 1992), relatively little is known about the rewarding properties of 6-AM. Thus, the main aim of the present study was to compare the reinforcing effects of equimolar doses of 6-AM and heroin using an intravenous (i.v.) self-administration procedure in the rat, which provides a direct index of the reinforcing effects of drugs (Badiani et al., 2011) and a valuable model of drug addiction (Spanagel, 2017). The second goal was to assess the effects of a pretreatment with a specific monoclonal antibody (mAb) raised against 6-AM (Bogen et al., 2014; Kvello et al., 2016) on the reinforcing effects of heroin, and in particular on its ability to affect reinstatement of drug-seeking in an animal model of relapse (for a review, see Shaham et al. (2003) and to sustain self-administration.

2. MATERIAL AND METHODS

2.1 Animals

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A total of 89 male Sprague-Dawley rats (ENVIGO, Huntingdon, United Kingdom) weighting 250-275 g at the beginning of the experiment were used in this study. Rats were housed in a temperature- and humidity-controlled room (21 ± 1° C and 55 ± 5%, respectively) on a 12/12 hours reverse light cycle (lights off at 8:00 am) for the entire duration of the experiments. Upon arrival, rats were housed in triplets in grid-top plastic cages (52 cm length, 40 cm width and 27 cm height) with wood shavings bedding and brown paper nesting material and allowed to acclimatize to the animal facility for a week. After this period, the animals underwent i.v. catheterization surgery. Eleven to sixteen animals were tested simultaneously, representing the different treatment groups, during one entire self-administration procedure. The rats had *ad libitum* access to food and water throughout the experiment, except during the self-administration sessions. All experimental procedures on animals were conducted in accordance with UK Animals (Scientific Procedures) Act 1986 (www.legislation.gov.uk/ukpga/1986/14/contents).

2.2 Surgery

On the day of surgery, the rats received an intraperitoneal injection of 2 mg/kg of xylazine hydrochloride (Rompun, Bayer HealthCare) and 100 mg/kg of ketamine (Anesketin, Dechra). The surgical procedures were similar to those previously described by Caprioli et al. (2008). Briefly, an 11 cm silicone catheter (0.37-mm inner diameter and 0.94-mm outer diameter), sheathed at 3.4 cm from its proximal end by a silicone bead, was inserted into the right jugular vein and secured to the surrounding soft tissues with silk thread. Its distal end was externalized through a small incision at the nape of the neck and connected to an L-shaped 22-gauge cannula, which was secured to rat's skull using dental cement and stainless steel screws. The catheters were flushed daily (at 6 pm) with 0.1 ml of sterile saline.

2.3 Apparatus

The apparatus, previously described in detail by Caprioli et al. (2007), consisted of self-administration chambers (28.5 cm length, 27 cm width and 32 cm height) made of transparent plastic (front and rear walls), aluminium (sidewalls and ceiling) and stainless steel (grid floor). Plastic trays covered with pinewood shaving were placed under the cage floors. Each cage was equipped with a counterbalanced arm, holding a liquid swivel above the chamber, and two retractable levers positioned 12.5 cm apart and 9 cm above the floor on the left-hand wall. Cue lights, consisting of a set of triple (green, red and yellow) LED lights, were positioned above each of the two levers. The self-administration cages were placed within sound-attenuating and light-attenuating cubicles. Each cage was connected via an electronic interface to a syringe pump (Razel Scientific Instruments, St. Albans, VT, USA) and to a programmable logic controller (PLC; Allen Bradley, Milwaukee, WI, USA). Finally, the PLCs were connected to PCs running custom control software developed by Aries Sistemi S.r.l. (Rome, Italy).

2.4 Drugs

Heroin hydrochloride (Johnson Matthey-Macfarlan Smith, Edinburgh, UK) and 6-AM hydrochloride (Lipomed, Arlesheim, Switzerland) were dissolved in saline. Equimolar doses of heroin and 6-AM, in an infusion volume of 40 µl and an infusion time of 4 seconds, were used throughout the experiment.

The following doses were used: 0.017, 0.034, 0.068, and 0.135 µmol/kg, corresponding to 2.5, 12.5, 25, and 50 µg/kg of heroin and 2.21, 11.1, 22.2, and 44.3 µg/kg of 6-AM, respectively.

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2.5 Self-administration procedure

Immediately after surgery, rats were assigned to the heroin or 6-AM group and individually housed in the self-administration chamber, where they remained for the rest of the experiment. The rats were allowed to recover from the surgery for 7–10 days before the start of self-administration training. Prior to the beginning of each session, food, water and nesting material were removed from the self-administration chamber and infusion lines were sterilized with ethanol, flushed with sterile saline, and then loaded with the appropriate drug solutions. At the beginning of each session, the two levers were extended (one 'active' and one 'inactive', counterbalanced across rats for the right vs. left position) and the cue light (all three LEDs) above the active lever was switched on. The schedule requirement, the number of consecutive lever presses, to obtain an infusion was increased from fixed ratio 1 (FR1) during the first 5 sessions, to FR2 for the rest of the experiment. Each animal received the same drug, 6-AM or heroin, throughout the experiment. The experiment included 17 consecutive daily sessions distributed four phases: i) acquisition of self-administration (sessions 1-7), ii) extinction of lever pressing during abstinence (sessions 8-14), iii) reinstatement of non-reinforced drug seeking by drug priming (session 15), iv) re-acquisition of self-administration (sessions 16 and 17). All sessions lasted 6 hours.

Figure 1 illustrates the timeline of the experimental protocol.

2.5.1 Acquisition

Independent groups of rats were trained to self-administer 0.135 µmol/kg 6-AM (42 animals) or heroin (40 animals) during 7 consecutive daily sessions. This dose was selected on the basis of previous heroin self-administration studies (Caprioli et al., 2008; Montanari et al., 2015). After pressing the active lever at the programmed schedule, a drug infusion was delivered, the levers retracted, and the cue light switched off for a 40 seconds timeout period (to prevent self-administration of multiple consecutive infusions and overdosing). The rats that did not spontaneously self-administer at least one infusion within the first 5 minutes of the session were placed with their forepaws on the lever to prime an infusion. This was repeated at times 60 and 120 minutes for rats that did not self-administer at least one infusion in the time periods 5–60 and 60–120 minutes. The number of rats that received at least one priming infusion decreased progressively over sessions, from 31 for the 6-AM group and 27 for the heroin group in the first session to 9 animals in each group on the last acquisition session at day seven. These priming infusions were not included in the data analysis.

2.5.2 Extinction

The extinction phase consisted of 7 consecutive daily sessions, during which the rats received, upon completion of the task (FR2), an i.v. saline infusion instead of the drug solution. All other conditions during the sessions were kept identical. No priming infusions were given during extinction.

At the end of session 14, the rats were administered an intraperitoneal injection of either 10 mg/kg of a human mAb raised against 6-AM dissolved in saline (Bogen et al., 2014; Moghaddam et al., 2003) or 1 ml/kg saline, and left undisturbed in their cages until the following day.

2.5.3 Reinstatement of drug-seeking

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On session 15, independent groups of rats received one of three priming doses (0.017, 0.034, or 0.068 µmol/kg) of the same drug administered during acquisition. These doses were selected based on a study showing that 25 µg/kg (0.068 µmol/kg) of heroin was a more effective priming dose than 50 or 100 µg/kg for inducing relapse into heroin-seeking. Before the beginning of the session, the infusion lines were first filled with sterile saline, and their distal end (to be directly connected to the catheter) was then backfilled with 50 µl of the appropriate drug solution. , At the beginning of the session, the rats received a 10 seconds non-contingent infusion (i.e., triggered by the experimenter) al infusion consisting of 50 µl of the drug solution followed by 50 µl saline solution. During the rest of the session, lever pressing on the active lever resulted in the self-administration of saline, as during the extinction sessions. The session lasted for 6 hours, but only the data from the first hour was used for data analysis since responding attenuates over time as the animals are tested under extinction conditions (Shalev et al., 2002).

2.5.4 Re-acquisition of self-administration

During sessions 16 and 17 the rats were returned to a schedule of self-administration identical to that of sessions 6 and 7 (i.e., on an FR2 schedule of reinforcement), with the difference that on one session they received the dose used during training (0.135 µmol/kg) and on the other one half this dose (0.068 µmol/kg), in a counterbalanced fashion across sessions.

2.6 Quantification of IgG1.

At the end of session 17, the rats were sacrificed with an i.v. infusion of pentobarbital (4 mg in 20 µl), which also served to assess catheter patency. The animals that did not become ataxic and die within 5 seconds (a total of 7 rats) were excluded from the data analysis. After cervical dislocation and death confirmation, blood samples were taken by cardiac puncture with a heparinized syringe and put in 0.5 ml low protein binding Eppendorf tubes held in ice. The tubes were then stored in a -80°C freezer and later shipped to Oslo University Hospital (Oslo, Norway) for quantification of blood IgG1 levels.

Blood samples were diluted and analyzed for human IgG1 using a Novex ELISA kit (Thermo Fisher Scientific Inc., Waltham, MA). Two duplicates of each standard and control were used. Absorbance was measured (450 nm) within 1 hour after adding the stop solution using an ELx808 Absorbance Microplate Reader (BioTek Instruments Inc., Winooski, VT). Samples from all animals receiving the 6-AM mAb were tested, but samples from only 11 animals pretreated with saline were analysed as control.

2.7 Data analysis

The data from sessions 1-5 (acquisition of self-administration on FR1), sessions 6-7 (acquisition of self-administration on FR2) and sessions 8-14 (extinction) were analysed separately using a two-way mixed ANOVA for the between-subject factor *drug* (two levels: heroin vs. 6-AM) and the within-subject factor *session* (one level for each session). Further statistical analysis was conducted on, sessions 4-7, using a three way mixed ANOVA for the between-subject factor *drug* (two levels: heroin vs. 6-AM) and the within-subject factors *sessions* (two levels, one for each session) and *schedule* (two levels: FR1 vs. FR2).

For the reinstatement session only the data concerning the first hour were analyzed (see Montanari et al. (2015); Shalev et al. (2002)). Reinstatement was defined as a significant increase on the number

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of presses on the active lever or infusions during the first hour of session 15 relative to the first hour of the last extinction session (session 14). These data were analyzed using a three-way mixed ANOVA for the between-subject factors *drug* (two levels: heroin vs. 6-AM) and *antibody* (saline vs. mAb) and the within-subject factor *reinstatement* (two levels: session 14 vs. session 15).

Re-acquisition of self-administration was indicated by a significant increase in the number of presses on the active lever and/or in the number of infusions during session 16 or 17 relative to the last extinction session (session 14). Re-acquisition data were analysed using a four-way mixed ANOVA for the between-subject factors *drug* (two levels: heroin vs. 6-AM), dose (two levels: 0.068 vs 0.135 µmol/kg) and *antibody* (saline vs. mAb) and the within-subject factor *re-acquisition* (two levels: session 14 vs. session 16 or 17). Student's t-tests for independent or repeated measures were used, as required, for post-hoc comparisons of interest. The statistical analysis was run separately for active lever presses, inactive lever presses, and drug infusions. All statistical analyses were conducted using SPSS statistical software version 23 (IBM Corp, Armonk, NY, USA).

3. RESULTS

3.1 Acquisition (Figure 2)

During sessions 1-5, animals learned to self-administer 6-AM or heroin (on FR1), as indicated by a significant increase in lever presses over session (Session: $F_{4,320}$=43.98, p<0.0001), with no significant effect of drugs (Session x Drug: $F_{4,320}$=1.94, p=0.104; Drug: $F_{1,80}$=2.83, p=0.096). Also for sessions 6-7 (on FR2) there was a significant effect of session ($F_{1,80}$=138.53, p<0.0001), but in this case also a significant effect of drug ($F_{1,80}$=4.70, p=0.033) and a drug x session interaction ($F_{1,80}$=4.53, p=0.036), as lever pressing for 6-AM was greater than for heroin. The same pattern was observed for the number of infusions. Besides, there was a significant effect of *schedule* ($F_{1,80}$=138,5, p<0.0001) and a *schedule* x *drug* interaction ($F_{1,80}$=4,53, p=0.036). There were no significant changes in lever pressing on the inactive lever.

3.2 Extinction (Figure 2)

During the extinction sessions, there was a significant decrease in the number of presses on the active lever ($F_{6,480}$=17.87, p≤0.0001) with no main effect of drug ($F_{1,80}$=0.39, p=0.843) nor session x drug interaction ($F_{6,480}$=0.779, p=0.527). The same pattern was observed for the number of saline infusions.

3.3 Reinstatement of drug-seeking (Figure 3)

After having tested 21 saline pretreated rats with 0.017 or 0.034 µmol/kg priming doses, it was observed that neither 6-AM (4 and 5 rats respectively) nor heroin (6 rats for each dose) induced reinstatement on these animals (data not shown). Due to the low number of subjects, no statistical analysis was carried out. The use of these two doses was discontinued and only the dose of 0.068 µmol/kg was used for the remainder of the experiment.

Priming with 0.068 µmol/kg reinstated lever pressing on the drug-paired lever (reinstatement: $F_{1,41}$=18.11, p=0.000) and the number of infusions (reinstatement: $F_{1,41}$=17.70, p=0.000) during the first hour after priming, without affecting presses at the inactive lever (data not shown). The post-

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hoc tests showed that priming with both drugs reestablished self-administration in the saline pretreated animals, but in the animals pretreated with the 6-AM mAb only the group receiving heroin showed reinstatement.

3.4 Re-acquisition of self-administration (Figure 4)

When the drugs were made available for the re-acquisition of self-administration on sessions 16 and 17, there was a significant effect in the number of active lever presses ($F_{1,74}$=89,52, p<0.0001) and in the number of infusions ($F_{1,74}$=85,05, p<0.0001) relative to the last extinction session (session 14). This effect was depending on which drug the animals received, which dose, as well as pretreatment with the 6-AM antibody, both for the number of presses at the active lever (re-acquisition x drug x dose x pretreatment : $F_{1,74}$=9,45, p=0.003) and number of infusions (re-acquisition x drug x dose x pretreatment : $F_{1,74}$=8,08, p=0.004).

Post-hoc analysis showed that the rats pre-treated with saline resumed lever pressing on the active lever and received a number of infusions significantly greater than on the last extinction session and at least similar to those of the last session of acquisition (session 7), independently of the drug or dose received. However, the animals receiving 0.068 µmol/kg 6-AM exhibited a nearly statistical significant (p=0.065) higher number of presses on the active lever and a significant (p<0.05) higher number of infusions than observed on the last acquisition session.

In the animals pretreated with the 6-AM mAb, the effects differed depending on the drug and dose received. In the animals self-administering 0.068 µmol/kg 6-AM and pretreated with the mAb, the number of active lever presses and infusions were not significantly different from either the last acquisition or extinction sessions (session 7 and 14 respectively). On the contrary, the animals pretreated with the mAb and self-administering 0.135 µmol/kg 6-AM exhibited a significant (p<0.05) increase in the number of presses on the active lever and the number of infusions, relative to both the last session of acquisition and the last session of extinction. They showed also a significant higher number of presses on the active lever and infusions when compared against the saline pretreated animals in the same re-acquisition session. Pretreatment with the 6-AM mAb did not affect the re-acquisition of heroin self-administration relative to the saline pretreated animals. However, in the animals self-administering 0.135 µmol/kg of heroin, the number of infusions was statistically significant higher (p<0.05) compared to the last acquisition session, despite the effect size being small. During the re-acquisition phase, no significant differences were observed between groups in the number of presses on the inactive lever. No significant differences between saline and 6-AM mAb pretreatment were observed during the second re-acquisition session for any of the two drugs and doses used (data not shown).

3.5 IgG levels

As expected, IgG levels were significantly (p<0.001) higher in animals pretreated with 6-AM mAb (35.2 ± 2.8 µg/ml) compared with animals pretreated with saline (0.18 ± 0.09 µg/ml). No differences were observed in IgG levels between animals self-administering 6-AM or heroin (33.3 ± 3.5 µg/ml and 35.2 ± 2.8 µg/ml, respectively).

4. DISCUSSION

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To the best of our knowledge, the present study is the first demonstration of reinforcing and addictive effects of 6-AM, as indicated by self-administration procedures. In particular, we report three major findings. First, the rats easily learned to self-administer 6-AM intravenously, which is considered a direct index of the reinforcing effects of drugs (Badiani et al., 2011). Second, after a period of abstinence, a single non-contingent i.v. infusion of 6-AM reinstated non-reinforced drug-seeking, which is considered an animal model of relapse (Shaham et al. 2003; Spanagel et al. 2017). Third, the rats rapidly re-acquired self-administration when given access to 6-AM after a period of abstinence.

4.1 Reinforcing effects of 6-AM vs. heroin

These reinforcing effects of 6-AM were similar to those of heroin, including the ability to trigger relapse into drug-seeking after a period of abstinence. However, the patterns of self-administration for 6-AM presented interesting differences from that of heroin. Although during the acquisition phase there were not statistical differences in the rate of responding on FR1, there were a significantly higher rate and greater number of infusions for 6-AM versus heroin on FR2. Furthermore, during the re-acquisition phase, the response rate and number of infusions for rats self-administering 0.068 µmol/kg of 6-AM was nearly twice as high as that of rats self-administering 0.135 µmol/kg (the dose used during the acquisition phase). On the contrary, the response rate and number of infusions for the two heroin doses did not differ from each other. A possible explanation for these results is that, when administered systemically, 6-AM is more potent than heroin with regard to their reinforcing effects. However, further self-administration studies, including more doses and/or break point procedures, are needed in order to conclusively elucidate the relative reinforcing potency of heroin and 6-AM when injected. Besides, previous reports that heroin is more potent than 6-AM in lowering the threshold for brain self-stimulation (Hubner and Kornetsky, 1992) and in producing conditioned place preference (Kvello et al., submitted).

4.2. Role of 6-AM in the reinforcing effects of heroin

The ability of 6-AM to sustain self-administration is consistent with the notion that 6-AM might represent the main mediator of the reinforcing effects of heroin. Raleigh and colleagues (Raleigh et al., 2014), for example, in reporting that a morphine-conjugated vaccine can alter heroin self-administration in the rat, attributed this effect mainly to a reduction in brain levels of 6-AM. Thus, we hypothesized that a pre-treatment with specific mAb against 6-AM would block, or at least impair, heroin self-administration. Yet, we found that while mAb was effective in blocking 6-AM-induced relapse of drug seeking during the reinstatement session and altered the re-acquisition of 6-AM self-administration (in a way compatible with a shift to the right in the dose-effect curve), it had a negligible effect on heroin-induced relapse and on the re-acquisition of heroin self-administration, even though at the end of the experiment there were no differences in IgG levels between 6-AM and heroin groups.

This finding does not appear to be consistent with the hypothesized role of 6-AM in the reinforcing effects of heroin. In a preliminary experiment conducted in preparation for the present study (data not shown), an intraperitoneal injection of 7.5 mg/kg of mAb 15 min before an i.v. infusion of 0.135 µmol/kg of heroin reduced brain levels of 6-AM by 30-50% (measured 5 min after heroin administration). Previous experiments have shown that, after heroin administration in mice, this mAb reduces 6-AM levels in the brain, as well as heroin-induced psychomotor activity (Bogen et al., 2014; Kvello et al., 2016). In a recent study, pretreatment with this mAb blocked conditioned place

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preference induced by heroin in mice (Kvello et al., Submitted), indicating that blockade of 6-AM is able to affect at least some aspects of the rewarding effects of heroin. Thus, the lack of effect of the mAb on heroin self-administration cannot be attributed to a possible general lack of action on 6-AM generated from heroin.

There are several possible reasons why anti-6-AM mAb failed to affect heroin self-administration and heroin priming. The first one concerns the kinetics of heroin deacetylation. Most of the 6-AM found in the brain shortly after heroin administration originates from heroin deacetylation in the blood (Boix et al., 2013). Still, 6-AM levels in the brain are much higher and rise faster after administration of heroin than after administration of equimolar doses of 6-AM (Andersen et al., 2009; Gottas et al., 2014). This 'additional' amount of 6-AM most likely derives from the deacetylation of heroin in the brain after its rapid passage through the blood-brain barrier (Oldendorf et al., 1972) and is obviously out of the reach of the mAb. Indeed, brain 6-AM levels are reduced by mAb to a lesser extent after heroin administration than after 6-AM administration (Kvello et al., 2016). The mAb is also much less effective in reducing heroin-induced behavior immediately after heroin administration than later in time (i.e figure 3 in Bogen et al., 2014). Thus, the rapid intracerebral formation of 6-AM from heroin could be fast enough to sustain self-administration at the infusion dose used (Comer et al., 1999; Marsch et al., 2001; Samaha and Robinson, 2005) and would explain the lack of effect of the mAb. However, it would be then necessary to assume that the 6-AM formed in the brain is sufficient to drive heroin self-administration but not heroin-induced CPP or locomotor sensitization. This discrepancy is not entirely surprising, given that the underpinnings of self-administration, locomotor sensitization, and CPP have been shown to be largely independent (Ahmed and Cador, 2005; Bardo and Bevins, 2000; Caprioli et al., 2008; Dietz et al., 2007; Huston et al., 2013; Shabat-Simon et al., 2008). In this respect, it is important to point out that, although the mAb used here has very low affinity for heroin (Bogen et al., 2014), a higher dose of the mAb might have been effective (Kvello et al., Submitted).

Alternatively, it is possible that the reinforcing effect sustaining heroin self-administration is mediated by other active metabolites of heroin. Heroin has been considered to be only an effective vehicle to deliver morphine to the brain. However, the euphorigenic effect of heroin (the so-called 'rush' or 'high') develops very rapidly after systemic administration (Smith and Beecher, 1962), whereas morphine concentrations in the plasma increase much more slowly (Rook et al., 2006a; Rook et al., 2006b), and, owing to its low lipophilicity, even more slowly in the brain, (Gottas et al., 2014; Seleman et al., 2014). This explains why higher doses of morphine than of heroin, are necessary to produce comparable behavioral responses (Andersen et al., 2009; Bardo et al., 1995; Eriksen et al., 2014; Hubner and Kornetsky, 1992; Hutto and Crowder, 1997), and in particular to sustain self-administration (Walker et al., 1999). Besides, the levels of morphine reached in the brain after systemic heroin administration are not high enough to achieve an equivalent behavioral response as the one induced by heroin (Andersen et al., 2009). Another candidate for mediating the effects of heroin is morphine-6-glucuronide (M6G) (Vindenes et al., 2006; Vindenes et al., 2008). Although very little M6G is formed after heroin administration to drug-naïve rats, increased synthesis is observed after repeated exposure to heroin (Antonilli et al., 2005; Antonilli et al., 2003) as well as after heroin-self-administration (Meringolo et al., 2012). Yet, the time-course of M6G formation is even more delayed than that of morphine, making it less likely that it could significantly contribute to the rapid onset of heroin rewarding effects.

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Finally, it is possible that the pharmacodynamics properties of heroin cannot be reduced to that of its metabolites. While heroin has lower affinity for MOP receptors than 6-AM or morphine (Inturrisi et al., 1983), its efficacy is similar to that of 6-AM and greater than that of morphine (Selley et al., 2001). Heroin and its metabolites differ not only in terms of receptor affinity and efficacy but also in their ability to enhance dopamine release in the striatum. Indeed, Gottas et al. (2014) have shown that the temporal and quantitative pattern of dopamine release after systemic 6-AM is very different not only from that of heroin but also from that of morphine. On the one hand, the differences in the ability to increase dopaminergic transmission, which is thought to play a major role in motivation (Berke, 2018), might be invoked to explain the differences in the reinforcing effects of 6-AM relative to heroin. On the other hand, it is not easy to explain the mechanisms responsible for these differences if the mechanisms of actions of heroin and of its metabolites are the same. Thus, it is possible that the intrinsic reinforcing effects of heroin, 6-AM, morphine, and M6G are mediated at least in part by distinct substrates. For example, since there is evidence that heroin self-administration is largely independent of striatal dopamine release (Badiani et al., 2011), it would be important to determine if the same is true for 6-AM.

5. CONCLUSION

The present study shows that the heroin metabolite 6-AM resembles heroin in the ability to sustain self-administration behavior and to precipitate relapse into drug seeking after a period of abstinence. Therefore, we conclude that 6-AM has reinforcing effects and addictive potential. However, contrary to our working hypothesis, a 6-AM specific monoclonal antibody failed to affect heroin-induced relapse into drug-seeking and the re-acquisition of heroin self-administration after a period of relapse. It is possible that heroin deacetylation in the brain yielded sufficient 6-AM out of the reach of mAb to sustain self-administration. These findings indicate that more studies are required to clarify if and to what extent the reinforcing effects of heroin depend, as commonly held, on its metabolites.

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Figure 1. Time line of the experimental protocol used through the self-administration study.

Figure 2. Number (mean ±s.e.m.) of lever presses (upper panel) on the active (circles) or inactive (triangles) lever and infusions (lower panel, circles) during the acquisition and extinction of self-administration of 0.135 µmol/kg 6-AM (filled symbols) or heroin (open symbols). A FR1 schedule was used during the first five sessions of acquisition, and a FR2 schedule for the rest of the experiment (see Material and Methods for further detail).

Figure 3. Number (mean ± s.e.m.) of active lever presses (upper panel) and infusions (lower panel) during the first hour of the last extinction session (black columns) and the reinstatement session (grey columns). After the last extinction session, animals were treated ip with saline or 10 mg/kg of a monoclonal antibody against 6-AM (6-AM mAb). At the start of the reinstatement session, the animals were primed with a single infusion of 0.068 µmol/kg 6-AM or heroin. *p<0.05 versus last extinction session (post-hoc paired Student's t-tests).

Figure 4. Number (mean ± s.e.m.) of active lever presses (upper panel) and infusions (lower panel) during the last acquisition session (0.135 µmol/kg 6-AM or heroin), last extinction session, and the first re-acquisition session. After the last extinction session, animals were administered ip either with saline or 10 mg/kg of a monoclonal antibody against 6-AM (6-AM mAb). During the re-acquisition session, the animals self-administered 0.068 or 0.135 µmol/kg 6-AM or heroin. Whereas the columns for last day of acquisition and extinction represent data for all the animals together, two columns are used to represent data from animals pretreated with saline or 6-AM antibody. *p<0.05 versus Last Day Extinction (post-hoc paired Student's t-tests); # p<0.05 versus Last Day Acquisition (post-hoc paired Student's t-tests); ¤ p<0.05 versus 0.068 µmol/kg (post-hoc independent Student's t-tests); & p<0.05 versus re-acquisition Saline (post-hoc independent Student's t-tests).

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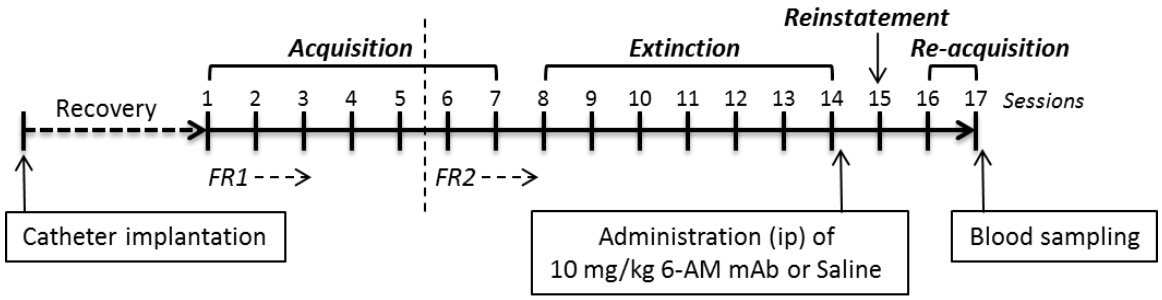

Figure 1

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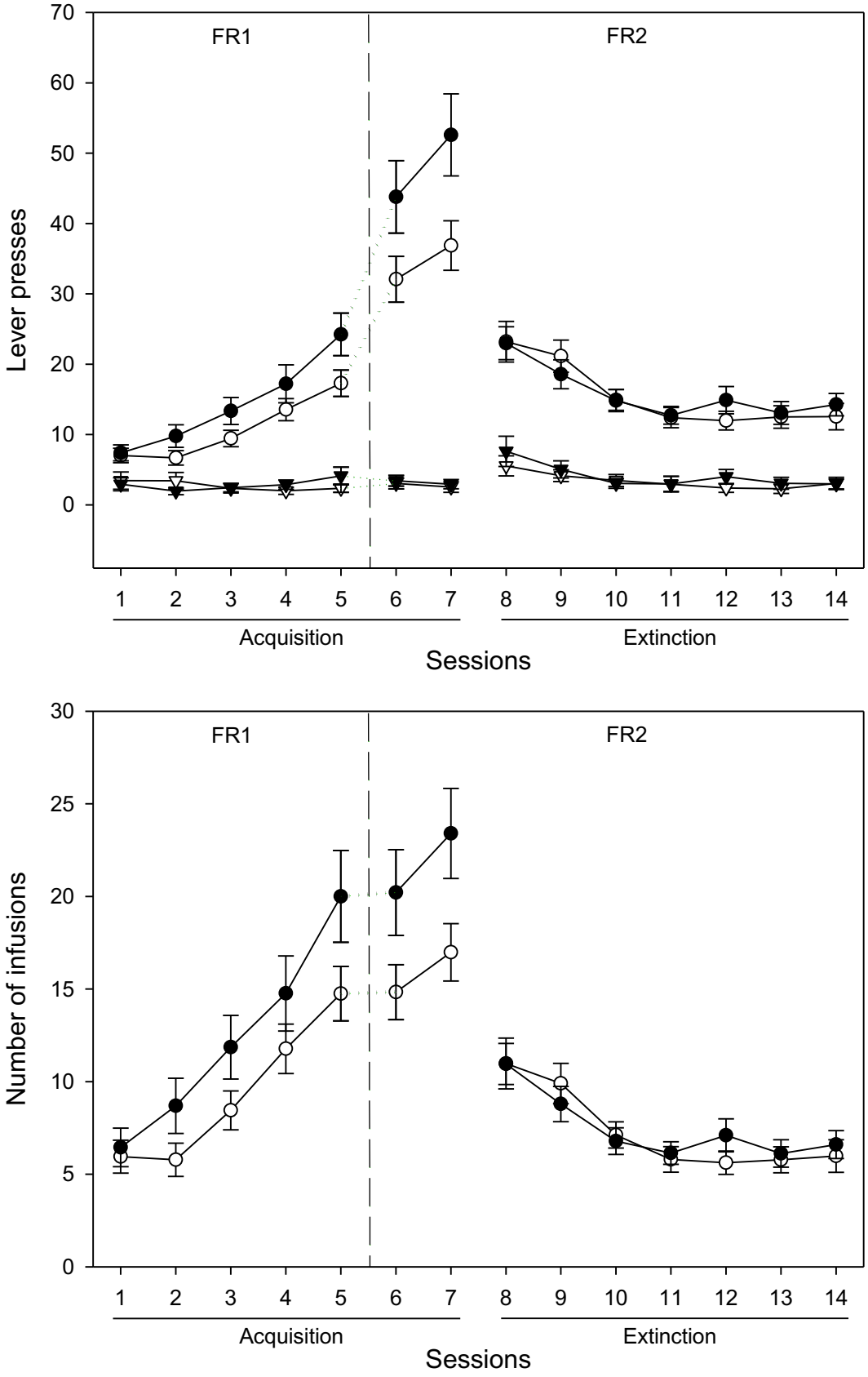

Figure 2

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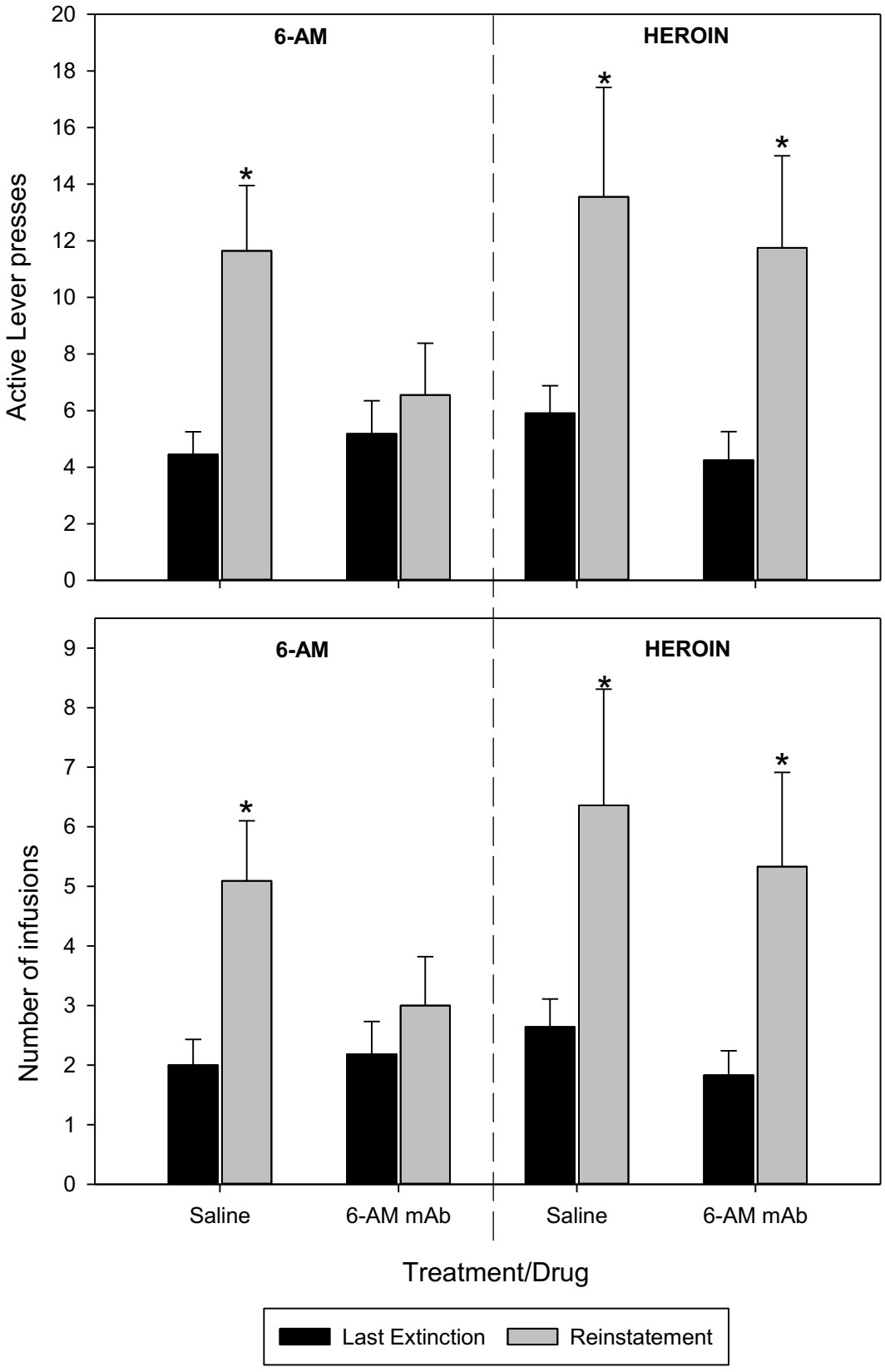

Figure 3

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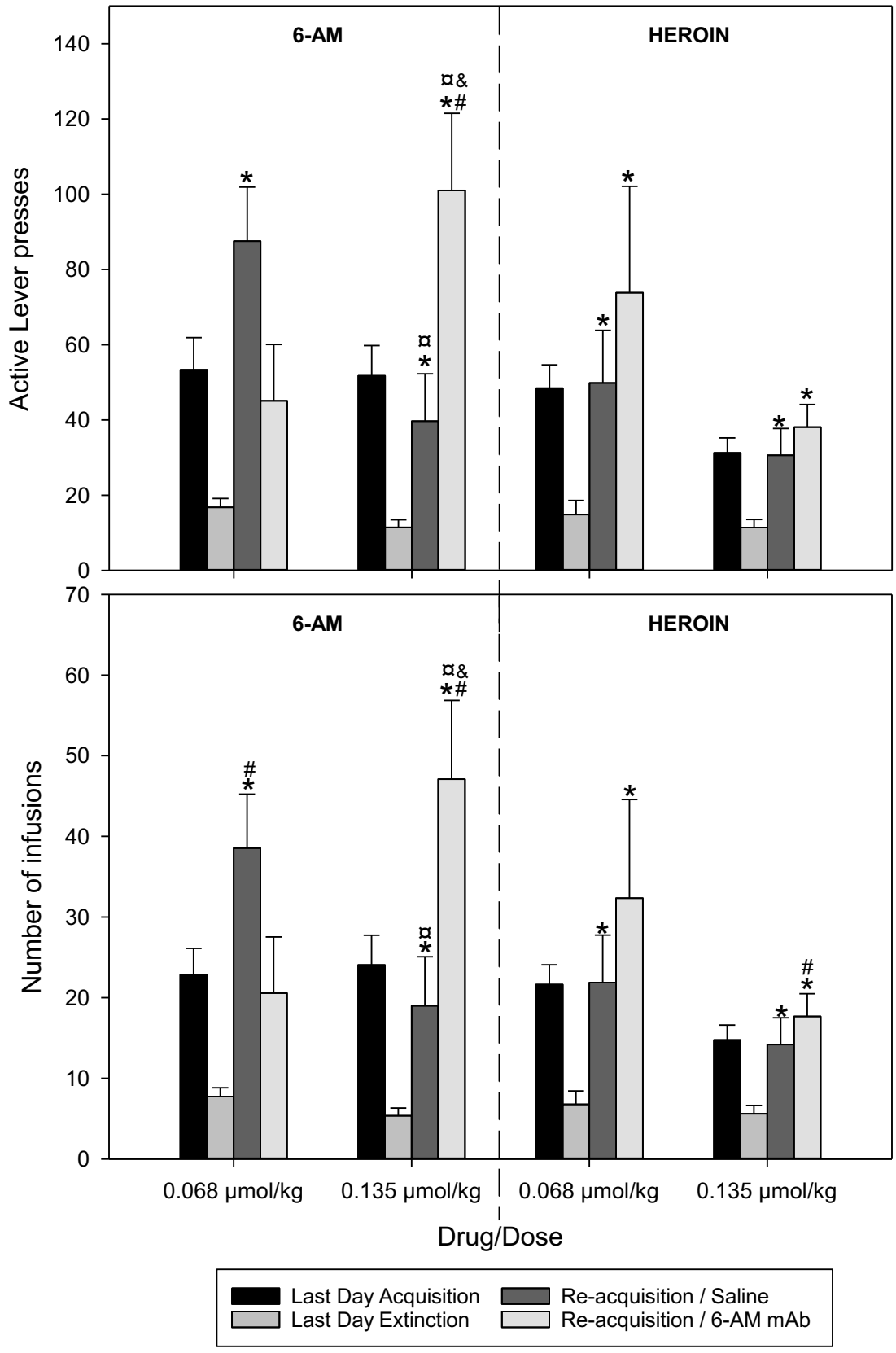

Figure 4

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