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Stress-induced hyperthermia is reduced by rapid-acting anxiolytic drugs independent of injection stress in rats

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ABSTRACT

Background: Stress-induced hyperthermia (SIH) is the transient rise in body temperature after encountering a stressor. The SIH response can be blocked by administration of various anxiolytic drugs prior to inducing stress. However, a drug injection involves handling and injection stress and therefore induces a SIH response itself. In the standard SIH test, drugs are therefore injected 60 min before stress induction to allow injection-induced hyperthermia to decline. This makes it difficult to study putative anxiolytic compounds with a short half-life. The present study therefore aimed to compare the effects of standard (stressful) and stress-free anxiolytic drug administration on the subsequent SIH response with a 10-minute injection-stressor interval.
Methods: Anxiolytic drugs with short half-lives (midazolam, 8-OH-DPAT, nicotine) were injected subcutaneously in rats using either a stressful (manual injection) or stress-free injection (subcutaneous cannula) method 10 min before novel cage stress. Body temperature and locomotor activity were measured using telemetric transmitters.
Results: Stressful and stress-free drug administration resulted in comparable drug effects on the stress-induced hyperthermia and locomotor responses in rats.
Conclusion: The present study shows that both stressful and stress-free drug injection shortly before a stressor results in reproducible attenuation of the SIH response in rats. In rats, a short injection-stressor interval can therefore be applied using the SIH model, enabling the study of putative anxiolytic drugs with short half-lives.

1. Introduction

Stress-induced hyperthermia (SIH) is a transient rise in body temperature in response to stress and is comparable across all species (Vinkers et al., 2008). Anxiolytic drugs including benzodiazepines and 5-HT_{1A} receptor agonists block the SIH response (Olivier et al., 2002, 2003). In contrast, non-anxiolytic drugs including dopaminergic and noradrenergic drugs do not affect the response, and the SIH model therefore possesses excellent predictive validity (Bouwknecht et al., 2007).

Administration of a drug involves handling and injection of animals and therefore induces a SIH response itself (Van der Heyden et al., 1997). In the classic SIH test using rectal temperature measurements, drugs are therefore injected 60 min before a stressor when injection-induced hyperthermia has sufficiently declined (Van der Heyden et al., 1997). In mice, an injection-stressor interval shorter than 60 min leads to a smaller SIH response because body temperature is still increased after injection stress (Van der Heyden et al., 1997). This makes it difficult to study putative anxiolytic compounds with a short half-life. For example, injection of nicotine ($t_{1/2}$ = 6 min (Petersen et al., 1984)) 10 min prior to stress led to false-positive results in the SIH test due to an elevated 'basal' temperature in vehicle-treated mice (Bouwknecht et al., 2007). In the same experiment, nicotine had no effects on the SIH response after an injection-stressor interval of 30 min, indicating that such an interval extension is not always possible (Bouwknecht et al., 2007).

It is therefore of interest to study the effects of injection stress on anxiolytic drug outcome in the SIH model. Also, the effects of injection stress on the subsequent stress response are unknown. We therefore aimed to compare the effects of standard (stressful) and stress-free administration of various anxiolytic drugs with short half-lives on the SIH and locomotor responses in rats using a 10-minute injection-stressor interval. Locomotor activity was measured to compare the temperature effects of injection stress to locomotor responses. Relatively stress-free drug injection was achieved using a tether–swivel combination connected to a subcutaneous catheter, minimizing handling and injection stress. The anxiolytic midazolam is a benzodiazepine with

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rapid onset of action and a high metabolic clearance ($t_{1/2}=27$ min) (Mandema et al., 1991; Reves et al., 1985). The 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) also possesses anxiolytic effects (Shields and King, 2008), and has a half-life of around 30 min (Yu and Lewander, 1997). Nicotine acts swiftly ($t_{1/2}=6$ min) on nicotinic receptors known to be involved in anxiety processes (Petersen et al., 1984; Picciotto et al., 2002).

2. Methods and materials

2.1. Animals

Male Wistar rats (Harlan Zeist, the Netherlands) were housed socially (four rats per cage) in a controlled environment with a non-reversed 12-hour light/dark cycle (white lights on from 7am to 7pm). Animals had unlimited access to food (standard lab chow) and water. One week after arrival, telemetry transmitters were implanted and a subcutaneous cannula was implanted. The implantations of a telemetric transmitter and a subcutaneous cannula were combined into one surgical procedure. After recovery from surgery, rats were singly housed in type III Macrolon® cages with a plastic tube as cage enrichment. Food (standard lab chow) and tap water were available ad libitum. Once a week, an experimental procedure was carried out. All experiments were carried out with approval of the ethical committee on animal experiments of the Academic Biomedical Center, Utrecht University, the Netherlands, and in accordance with the Declaration of Helsinki (6th revision, 2008).

2.2. Surgeries

2.2.1. Telemetry transmitter surgery

Telemetric devices (type ETA-F20, Data Sciences International, St Paul, MN, USA) were implanted in the abdominal cavity as described earlier (Pattij et al., 2001). Prior to surgery, rats received a subcutaneous (s.c.) injection (2 ml/kg) of the antibiotic Baytrill® (2.5% enrofloxacin). Rats were anaesthetized using O_2/NO_2/Isoflurane gas anesthesia. Carprofen (5 mg/kg, s.c.) was given as an analgesic immediately after surgery and twice daily for two days after surgery. After surgery, animals were housed individually and recovery from surgery was monitored (body weight). Also, all rats had access to wet food and solid drinks for two days after surgery. Wound recovery was regularly checked.

2.2.2. Subcutaneous cannula surgery

Rats were equipped with a cannula that was placed subcutaneously approximately 9 cm along the right flank of the animal. Cannulas were made of polyurethane tubing (Instech Laboratories, Plymouth Meeting, PA, USA), and the last 3 cm of each cannula was perforated with a needle at every 2 mm to allow fluid to spread evenly and to prevent cannula obstruction. The subcutaneous cannula was connected to a Vascular Access Harness (Instech Laboratories, Plymouth Meeting, PA, USA).

2.3. Radiotelemetry system

The radiotelemetry system consisted of an implantable transmitter with two flexible leads (type ETA-F20, Data Sciences International, St Paul, MN, USA), a telemetric receiver (model RPC-1) and a Data Exchange Matrix collecting input from the receivers, all purchased from Data Sciences International (St. Paul, MN, USA). The matrix was connected to a Compaq computer. Signals from the transmitters were passed on via a radio signal to the receiver, localized under the animal cage, transforming it into a digital signal. Digital information from the telemetry receivers was collected by the data matrix and provided to the computer where all raw data were stored. Data were collected using Dataquest Gold A.R.T. software (DSI, version 2.2). Raw data consisted of locomotor activity and body temperature responses collected for 10 s every 2 min.

2.4. Experimental procedure

2.4.1. General

Rats received a stressful or stress-free subcutaneous injection with vehicle or a certain drug dose 10 min before novel cage stress. Ten minutes later, rats were placed in a novel cage (clean cage with fresh bedding) and left undisturbed. To prevent habituation to the novel cage procedure, the interval between two experiments was set to be at least one week. Overall, rats generally received two different treatment with a testing interval of at least one week, in accordance with a one week testing interval in the SIH paradigm to wash out acute drug effects (Vinkers et al., 2008). Stress-free vehicle ($n=10$), midazolam ($n=8$), nicotine ($n=6$) and 8-OH-DPAT ($n=7$) were administered, as well as stressful vehicle ($n=6$), midazolam ($n=8$), nicotine ($n=3$) and 8-OH-DPAT ($n=5$).

2.4.2. Stress-free injection method

The vascular access harness of each rat was connected to a tether (Instech Laboratories, Plymouth Meeting, PA, USA) which was connected to a lever arm with a swivel that was mounted on top of the cage. This setup made it possible to inject drugs via the tubing extending from the swivel at some distance from the cage without any animal handling. All tethers were filled with physiological saline at room temperature before connecting. Rats were connected to the tethers at least 2 h before the SIH test.

2.4.3. Stressful injection method

Drugs were injected using a standard subcutaneous injection method on the flank with a needle and syringe.

2.5. Drugs

Midazolam HCl, ±-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) and nicotine-di-tartrate were obtained from Sigma Aldrich (Zwijndrecht, The Netherlands) and dissolved in saline. The amount of nicotine-di-tartrate was adjusted to obtain the concentration of free base nicotine as indicated in the literature (Matta et al., 2007). An injection volume of 1 ml/kg was used and all drugs were injected subcutaneously. Fresh solutions and suspensions were prepared each testing day, and all drugs were injected at room temperature.

2.6. Data analysis

All data were collected in 2-minute blocks and are displayed as mean ± SEM. All experiments were carried out with a between-subject design. Drug effects on body temperature and locomotor activity were analyzed during the first 60 min after novel cage stress using a univariate repeated measures analysis of variance (ANOVA) with time as within-subject factor and drug as between-subject factor. In the vehicle conditions, stressful and stress-free injection methods were compared using a univariate repeated measures analysis of variance (ANOVA) with time as within-subject factor and injection method as between-subject factor. Cumulative activity levels were obtained by summation of locomotor activity either during the 10-minute period after injection (reflecting locomotor responses to injection stress) or during the first 60 min after the novel cage procedure (reflecting stress-induced locomotor responses), and were compared using a one way ANOVA. A probability level of $p<0.05$ was set as statistically significant, probability levels between $p=0.05$ and $p=0.1$ were regarded as trends. To ensure sufficient power of drug effects on the SIH response, a repeated measures power analysis was conducted based on literature (D'Amico et al., 2001). Using a standard deviation of 0.35 (based on our current results), the power during the first 60 min

after the novel cage stress was over 95%, independent of correlation between the time points (data not shown).

3. Results

3.1. Midazolam (3 mg/kg)

3.1.1. Body temperature

Midazolam did not influence basal body temperature (stressful: drug effect $F_{1,12}=1.05$, $p=0.33$, NS; stress-free: drug effect $F_{1,16}=2.07$, $p=0.17$, NS). Stressful injection of midazolam did not significantly reduce the SIH response (drug×time interaction $F_{29,348}=1.07$, $p=0.37$, NS), whereas stress-free injection did reduce the SIH response (drug×time interaction $F_{29,464}=4.51$, $p<0.001$) (Fig. 1).

3.1.2. Locomotor activity

Midazolam reduced stress-induced locomotor activity after both stressful and stress-free injection (*stressful*: drug×time interaction $F_{29,348}=2.92$, $p<0.001$; drug effect $F_{1,12}=20.64$, $p<0.001$; *stress-free*: drug×time interaction $F_{2}9,464=0.68$, $p=0.90$, NS; drug effect $F_{1,16}=5.92$, $p<0.05$). Midazolam also decreased cumulative locomotor levels after novel cage stress (*stressful*: $F_{1,13}=20.64$, $p<0.001$; *stress-free*: $F_{1,17}=5.92$, $p<0.05$), but not directly after injection (*stressful*: $F_{1,13}=0.11$, $p=0.74$, NS; *stress-free*: $F_{1,17}=2.30$, $p=0.15$, NS). (Fig. 2, right panel).

3.2. 8-OH-DPAT (0.4 mg/kg)

3.2.1. Body temperature

8-OH-DPAT reduced the SIH response and basal body temperature after both the stressful and the stress-free injection method (*stressful*: drug×time interaction $F_{29,319}=32.20$, $p<0.001$; drug effect $F_{1,11}=97.64$, $p<0.001$. *Stress-free*: drug×time interaction $F_{29,377}=18.11$, $p<0.001$; drug effect $F_{1,13}=21.09$, $p=0.001$) (Fig. 1).

3.2.2. Locomotor activity

8-OH-DPAT increased stress-induced and overall locomotor activity after both injection methods (*stressful*: drug×time interaction $F_{29,319}=2.99$, $p<0.001$; drug effect $F_{1,11}=20.35$, $p=0.001$. *Stress-free*: drug×time interaction $F_{29,377}=4.23$, $p<0.001$; drug effect $F_{1,13}=6.39$, $p<0.05$). 8-OH-DPAT also increased the calculated cumulative locomotor levels under both conditions after injection (*stressful*: $F_{1,12}=54.17$, $p<0.001$; *stress-free*: $F_{1,14}=19.78$, $p<0.001$) and after novel cage stress (*stressful*: $F_{1,12}=20.354$, $p<0.001$; *stress-free*: $F_{1,14}=6.39$, $p<0.001$) (Fig. 2, right panel).

3.3. Nicotine (1 mg/kg)

3.3.1. Body temperature

Nicotine reduced the SIH response and basal body temperature after stressful and stress-free injection (*stressful*: drug×time

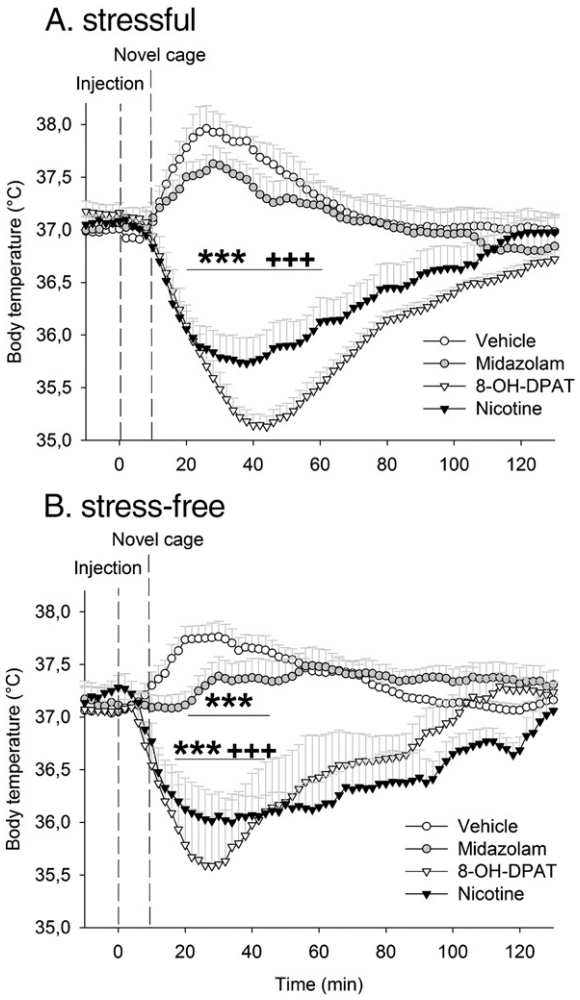

Fig. 1. Effects of stressful (A) and stress-free (B) subcutaneous injection of midazolam (3 mg/kg), 8-OH-DPAT (0.4 mg/kg) and nicotine (1 mg/kg) on the novel cage-induced stress-induced hyperthermia (SIH) response. *: time×drug interaction compared to vehicle (***: $p<0.001$). +: overall drug effect on body temperature compared to vehicle (+++: $p<0.001$).

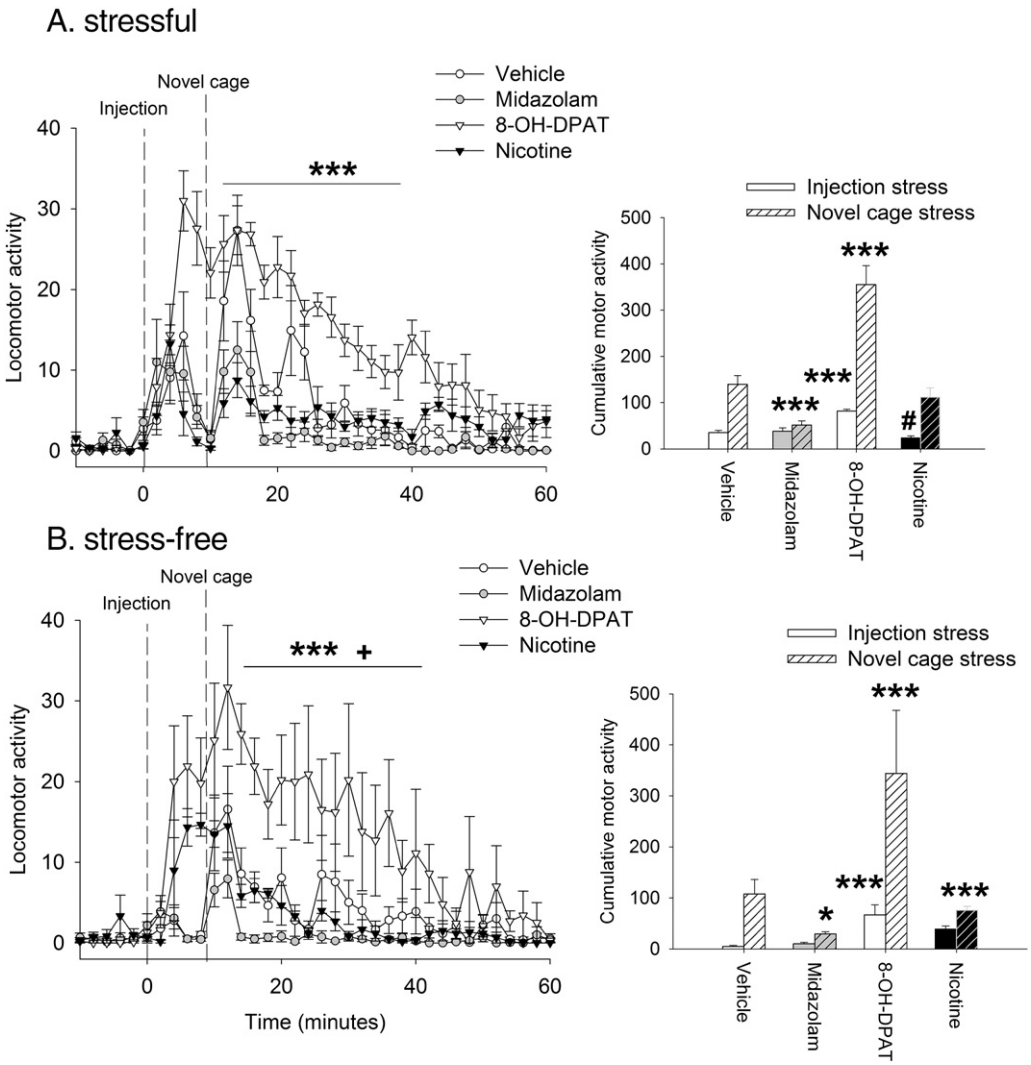

Fig. 2. Effects of stressful (A) and stress-free (B) subcutaneous injection of midazolam (3 mg/kg), 8-OH-DPAT (0.4 mg/kg) and nicotine (1 mg/kg) on the novel cage-induced locomotor response. *: time × drug interaction compared to vehicle (***: 8-OH-DPAT, $p<0.001$). +: overall drug effect on body temperature compared to vehicle (+: midazolam, $p<0.05$). Inset A and B: cumulative activity response after injection and after novel cage stress. ***: $p<0.001$; *: $p<0.05$; #: $p=0.09$ compared to vehicle.

interaction $F_{29,290}=16.70$, $p<0.001$; drug effect $F_{1,10}40.87$, $p<0.001$. *Stress-free*: drug × time interaction $F_{29,390}=4.56$, $p<0.001$; drug effect $F_{1,11}=38.55$, $p<0.001$) (Fig. 1).

3.3.2. Locomotor activity

Nicotine reduced stress-induced locomotor levels after stressful injection (drug × time interaction $F_{29,290}=5.35$, $p<0.001$) but not after stress-free injection (drug × time interaction $F_{29,319}=0.21$, $p=0.97$, NS). Overall locomotor activity levels after novel cage stress were however not affected by nicotine (*stressful*: $F_{1,10}=1.00$, $p=0.34$, NS; *stress-free*: drug effect $F_{1,11}=0.36$, $p=0.56$, NS). Nicotine did not also affect the calculated cumulative activity after novel cage stress relative to vehicle (stressful: NC: $F_{1,11}=1.00$, $p=0.34$, NS; stress-free: $F_{1,12}=0.36$, $p=0.56$, NS) (Fig. 2, inset). In contrast, cumulative locomotor activity levels were increased immediately after nicotine injection independent of injection method (stressful: $F_{1,11}=3.11$, $p=0.09$, trend; stress-free: $F_{1,12}=53.60$, $p<0.001$) (Fig. 2, inset).

3.4. Stressful and stress-free vehicle injection compared

Stressful and stress-free vehicle injection did not differ in basal body temperature during the 10 min after injection (method effect $F_{1,14}=0.92$, $p=0.35$, NS; time × method interaction $F_{1,19}=0.02$, $p=0.23$, NS), whereas locomotor activity levels were increased only after stressful injection (method effect $F_{1,14}=44.26$, $p<0.001$; time × method interaction $F_{2,26}=3.38$ $p=0.05$). Cumulative activity levels confirmed that stressful injection led to increased locomotor activity after injection (method effect $F_{1,15}=44.26$, $p<0.001$). Although the SIH response in the stressful injection group was larger after novel cage stress (time × method interaction $F_{29,406}=1.77$, $p=0.01$), both groups had a similar basal body temperature (method effect $F_{1,14}=0.01$, $p=0.98$, NS). Stressful vehicle injection led to higher locomotor activity levels after novel cage stress relative to the stress-free vehicle injection (method × time interaction $F_{29,406}=3.39$, $p<0.01$), although overall locomotor levels were not different (method effect $F_{1,14}=0.63$, $p=0.44$, NS). Cumulative activity levels confirmed that overall activity was similar after novel cage stress (method effect $F_{1,15}=0.63$, $p=0.44$, NS).

4. Discussion

The present study compared standard (stressful) and stress-free drug injection shortly before novel cage stress in the stress-induced hyperthermia (SIH) model. The SIH model uses the transient body

temperature increase in response to stress that can be blocked by various anxiolytic drugs. However, administration of a drug involves handling and injection of animals and thus induces an autonomic stress response itself in both rats and mice (Van der Heyden et al., 1997; Vinkers et al., 2009). This makes it difficult to study the autonomic stress response when putative anxiolytic drugs are injected shortly before a stressor (Bouwknecht et al., 2007). Using a swivel–tether combination connected to a subcutaneous catheter, we were able to reduce the stress associated with manual (stressful) drug injections as stress-free injections did not increase locomotor responses and led to no apparent behavioral responses in the rat (Fig. 2B).

Both stressful and stress-free injection of anxiolytic drugs with a short half-life (8-OH-DPAT and nicotine) resulted in a robust attenuation of the SIH response in rats (Fig. 1). This indicates that a short injection-stressor interval can be used to study the effects of anxiolytic drugs on the autonomic stress response. In this way, compounds with a short half-life or lower doses of a compound can be assessed. In contrast, midazolam did not reduce the SIH response in the stressful injection method, although comparison by the eye might suggest otherwise (Fig. 1). This suggests that the stress-free injection method may be more sensitive to register anxiolytic effects on the SIH response. The $GABA_A$ receptor agonist midazolam, the nicotine receptor agonist nicotine and the 5-HT_{1A} receptor agonist 8-OH-DPAT all led to a robust decrease in stress-induced and basal body temperature. The effects of both 8-OH-DPAT and nicotine on body temperature are in line with known hypothermic and stress-induced hyperthermia reducing effects at similar doses in rats (Gordon et al., 2002; Rusyniak et al., 2007). Furthermore, we found that nicotine at a dose of 0.25 mg/kg reduced the SIH response without causing hypothermia independent of injection method (data not shown), which is again in line with nicotine effects on body temperature at lower doses (Gordon et al., 2002).

In general, anxiolytic drugs that attenuate the SIH response generally also lead to hypothermia and disturb thermoregulatory processes (Vinkers et al., 2008). Therefore, in the current study, a complete distinction between an attenuation of the SIH response and a general reduction of the basal body temperature cannot be made. Stressful drug injection led to an overall less variable response, probably due to a better and more consistent drug delivery after manual injection (Figs. 1 and 2). In contrast to our study, injection stress in mice results in an almost maximal hyperthermia after 10 min (Van der Heyden et al., 1997). This difference may be attributed to a more controlled thermoregulation in rats, leading to a less reactive and less pronounced SIH response in reaction to injection stress. In support, we earlier found that handling stress in three different mouse strains led to a consistent SIH response of around 2 °C (van Bogaert et al., 2006), whereas handling stress in a rat leads to a SIH response of maximally 1 °C (Vinkers et al., 2008). The fact that stress does not immediately increase body temperature cannot be ascribed to physical transmitter delay as in the aforementioned study, various stressors increased body temperature in three different mouse strains within 2 min using identical telemetry transmitters (van Bogaert et al., 2006).

To our knowledge, this is the first study in which nicotine reduced the SIH response. In an earlier study in mice that used a similar injection-stressor interval, nicotine did not attenuate the SIH response (Bouwknecht et al., 2007). In this study, injection stress itself increased baseline temperature in mice and, as body temperature had not returned to baseline values, consequently reduced the SIH amplitude after vehicle treatment. 8-OH-DPAT was also able to reduce the SIH response, an effect that was already earlier found in mice using 6- to 25-fold higher doses which were injected 30 min before a stressor (Borsini et al., 1989). The effects of the benzodiazepine midazolam and the 5-HT_{1A} receptor agonist 8-OH-DPAT in the present study are in general agreement with known SIH-attenuating effects of similar acting drugs with longer half-lives, such as diazepam and flesinoxan (Vinkers et al., 2009, 2008). Midazolam led to overall sedation regardless of injection method (Fig. 2), which is in line with known sedative effects (Lau et al., 1996). Also, both stressful and stress-free injection of 5-HT_{1A} receptor agonist 8-OH-DPAT resulted in direct locomotor stimulant effects (Fig. 2), which is attributed to presynaptic 5-HT_{1A} receptor activation (Chen and Reith, 1995; Karamanakos et al., 2004).

Interestingly, stressful vehicle injection subsequently led to a larger SIH and locomotor response to novel cage stress compared to stress-free vehicle injection (Figs. 1 and 2). This is an interesting phenomenon, which may be explained by the fact that a stressful event (manual injection) is followed by another (relative) stressful event (novel cage stress). In animals, stress exacerbates subsequent anxiety-like responses in a number of anxiety models even immediately after an acute stressor (MacNeil et al., 1997; Vinkers et al., 2008; Zangrossi and File, 1992), and also in humans, unconditioned anxiety is enhanced by prior stress (Lissek et al., 2005). In addition, there is a link in rodents between prior stress and increased subsequent locomotor responses to psychostimulants (de Jong et al., 2005).

In conclusion, the present study shows that both stressful (manual) and stress-free administration of anxiolytic drugs with short half-lives shortly before novel cage stress reduce the SIH response. Thus, manual drug administration combined with a short injection-stressor interval can be applied to study in the SIH model in rats. This opens up possibilities to study lower doses of anxiolytic drugs or to assess putative anxiolytic drugs with short half-lives in the SIH model.

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