***Title page**

$GABA_A$ receptors in the ventral tegmental area control the outcome of a social competition in rats

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*Abstract

Abstract

Social dominance can be attained through social competitions. Recent work in both humans and rodents has identified trait anxiety as a crucial predictor of social competitiveness. In addition, the anxiolytic $GABA_A$ positive modulator, diazepam, injected either systemically or into the ventral tegmental area (VTA) was shown to increase social dominance. Here, we investigated the impact of pharmacologically targeting $GABA_A$ receptors in the VTA for the outcome of a social competition between two unfamiliar male rats, one of them infused with vehicle and the other one with the drug under study. We show that infusion of the $GABA_A$ receptor agonist, muscimol, reduced anxiety-like behaviors and enhanced social competition, the $GABA_A$ receptor antagonist, bicuculline had the opposite effects. Importantly, intra-VTA muscimol administration also counteracted the disadvantage of high anxious rats to win a social competition against low anxious rats. Furthermore, we assessed the effectiveness of targeting specific $GABA_A$ receptor subunits by infusing zolpidem (α1-subunit agonist) or TCS1105 (a benzodiazepine ligand with α2-subunit agonistic and α1-subunit antagonistic effects) into the VTA. While zolpidem infusion did not affect the outcome of the social hierarchy, TCS1105 enhanced social dominance. Our data highlights GABAergic mechanisms involving the engagement of α2-subunit containing $GABA_A$ receptors in the VTA for the attainment of dominance rank. The involvement of α2-subunit containing $GABA_A$ receptors in the VTA for the regulation of social competitiveness supports the potential therapeutic relevance of targeting these receptors to ameliorate anxiety-related social dysfunctions.

Keywords: Anxiety, GABA, Rats, Social competition, Ventral Tegmental Area

1. Introduction

Trait anxiety has been shown to relate to differences in social competitiveness in both humans (Goette et al., 2015) and rodents (Hollis et al. 2015; van der Kooij et al. 2017). In behavioral economic experiments performed in human subjects under stress, high anxious participants showed lower competitive self-confidence than low anxious ones (Goette et al., 2015).

Chronic subordinate colony housing, a psychosocial stressor, induced an anxiogenic phenotype in mice (Slattery et al. 2012), thus linking anxiety to social status. However, mice exhibiting an innate low anxiety profile appeared to be resilient from the stress-related consequences of chronic subordinate housing (Füchsl et al. 2014). In rats, high-anxious individuals typically lose the contest for a new territory when competing with low-anxious conspecifics (Hollis et al. 2015). Similarly, low-anxious rats tended to become dominant when animals cohabitated together for longer than a single social encounter, as reported for the visual burrow system (Davis et al. 2009). In line, the anxiolytic drug chlordiazepoxide temporarily boosted competition for sucrose pellets in underperforming rats (Gentsch et al. 1990) whereas peripheral administration of diazepam (Valium®), an anxiolytic drug that acts as a positive modulator at γ-aminobutyric acid $(GABA)_A$ receptors, not only reduced the rats´ anxiety levels, it also boosted their social dominance (van der Kooij et al. 2017). The anxiolytic- as well as dominance-enhancing effects of diazepam in rats could be reproduced by local drug infusion into the ventral tegmental area (VTA), while diazepam infusion into the NAc had no effect (van der Kooij et al. 2017). Therefore, the VTA appears as a pivotal brain region for the control of anxiety and social competition.

The most prominent cell types in the VTA are dopaminergic and GABAergic (Dobi et al. 2010). The majority of VTA dopaminergic neurons project to the nucleus accumbens (NAc) (Morales and Pickel, 2012) and those projections have been implicated in reward-motivated learning, addiction and effort (Adcock et al. 2006; Berridge, 2007; Schultz, 2002; Salamone and Correa, 2012). Benzodiazepines were shown to disinhibit the GABAergic control on dopaminergic projection neurons in the VTA,

resulting in dopamine release from the VTA (Tan et al. 2010). Specifically, preferential binding of benzodiazepines to α1-subunit containing $GABA_A$ receptors (rather than $GABA_A$ receptors containing α3-subunits) in VTA interneurons leads to local disinhibition, and thus stimulation, of dopaminergic neurons (Tan et al. 2010). Importantly, along with inducing an increase in accumbal dopamine levels in the NAc, diazepam microinfusion into the VTA enhances social dominance in rats (van der Kooij et al. 2017). This effect is blocked by antagonizing dopamine D1 receptors in the NAc, while intra-NAc infusion of a dopamine D1 receptor agonist enhances social competition (van der Kooij et al. 2017). These findings point to a critical role of the GABAergic regulatory control of VTA dopaminergic neurons projecting to the NAc for social rank attainment. However, the GABA receptor subtype involved in this process has not yet being identified.

Here, we aimed at exploring the relevance of specific $GABA_A$ receptor subtypes (α1- and α2-subunit containing $GABA_A$ receptors) in the VTA for the control of social competition between two male rats. To this end, we first validated that muscimol ($GABA_A$ agonist) and bicuculline ($GABA_A$ antagonist) infusions into the VTA had opposite effects in the outcome of a social competition; i.e., respectively enhancing vs. decreasing social dominance. Subsequently, we focused on the effect of intra-VTA infusion of zolpidem (a benzodiazepine agonist selective for α1-subunit containing $GABA_A$ receptors) and TCS1105 (a benzodiazepine ligand, with agonistic properties at α2- but antagonistic effects at α1-subunit containing $GABA_A$ receptors).

2. Methods

2.1 Animals

Adult male Wistar rats (Charles River, L'Arbresle, France) weighing 250-275g at the start of experiments were used. Animals were individually housed in polypropylene cages (57 x 35 x 20 cm) with abundant pine bedding in a temperature- (23°C) and light- (lights on from 0700-1900 hours) controlled room. All animals had *ad libitum* access to standard food and water. Upon arrival to the

facility, animals were allowed to habituate to the vivarium for one week and were then handled for 2 min/day during three days prior to the start of all experiments. All behavioral manipulations were performed during the light phase by experimenters blind to treatment groups. All experiments were performed with the approval of the Cantonal Veterinary Authorities (Vaud, Switzerland) and carried out in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU).

2.2 Elevated Plus Maze

Prior to the testing for social hierarchy, animals were tested for anxiety-related behavior in the EPM (Herrero et al. 2006). The EPM is elevated 65 cm above floor level, contains two open- (45 x 10 cm) and two closed arms (45 x 10 cm with 50 cm high walls) that emanate from a central platform (10 x 10 cm). The test starts after the animal is placed on the central platform and lasts 5 min. Behavior is analyzed using a computerized tracking system (Ethovision 3.1.16, Noldus, Wageningen, The Netherlands). Depending on the amount of time spent on the open arm, animals were classified as high- (HA, <5% open arm duration) intermediate (IA, 5-20 % open arm duration) or low-anxious (LA, >20% open arm duration) as earlier described (Hollis et al. 2015). Before and in between testing the apparatus was cleaned with a 5% EtOH solution.

2.3 Open Field

Anxiety-like behavior was also tested in the open field. The open field consisted of a black circular arena (1 m ∅ and 32 cm high). For analysis, the time spent in the rim zone (defined as the outer 20 cm area along the side-walls) was taken into account. Animals were placed in the center of the arena and their behavior was monitored for 20 min using a video camera that was mounted from the ceiling above the center of the arena. The light was adjusted to 8-10 lx in the center of the arena.

2.4 Social preference test

The test was performed in a rectangular, three-chambered box that included a central compartment where the rat was initially placed. Thereafter, retractable doors were removed and the rat could explore the left- and right compartment for 5 min. Both compartments were equipped with a floor-fixed transparent perforated Plexiglas cylinder that contained either an unfamiliar male juvenile rat or an unfamiliar object. The time spent sniffing either the juvenile (social target) or the novel object (inanimate target) was manually scored from videotapes by an experimenter who was blinded to the treatment groups.

2.5 Intracerebral cannulation surgery

Rats subjected to pharmacological experiments were implanted bilaterally with stainless steel guide cannulae targeting the VTA. Rats were anesthetized by isoflurane inhalation (induction 4% isoflurane for 4 min and maintenance 2.5% isoflurane in O_2 at a flow of 4L/min) and placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Small holes were drilled through the skull for bilateral placement of stainless steel 22 gauge guide cannulae (Plastics One, Roanoke, VA, USA) fitted with a removable dummy cannula. Coordinates were based on the atlas of Paxinos and Watson (1986) and were relative to bregma (in mm, A.P. -5.8, M.L. ±2.2, D.V. -7.45 at an 11° angle to avoid damaging the superior sagittal sinus). Cannulae were fixed to the skull with two anchoring screws and dental acrylic (Duralay 2244; Reliance, Worth, IL). After behavioral experiments animals were sacrificed by i.p. pentobarbital injection and correct cannula placement was routinely verified with Evans blue histology.

2.6 Drug infusions

Behavioral experiments were performed 5- or 30 min after drug- or vehicle administration. We randomly assigned animals to their respective treatment. For intra-cerebral infusions the dummy

was removed and an injector was inserted that extended 1 mm from the tip of the cannulae. Drugs (all from Tocris, Bioscience, Zug, Switzerland) were bilaterally infused at a 25 ng dose in a total volume of 0.3 μL during 1 min of constant flow. The injector remained in place for one additional minute after infusion to allow proper diffusion. Muscimol ($GABA_A$ agonist) and bicuculline methiodide ($GABA_A$ antagonist) were dissolved in saline (0.9%), which was also infused for their respective controls. Both muscimol and bicuculline have been infused cerebrally in other studies. Muscimol has been infused into the VTA of rats where it affected maternal behavior (Numan et al. 2009). Bicuculline methiodide has been infused in the brain (tectum) at a 25 ng dose before and effectively halted electroshock-induced convulsions (Weng and Rosenberg, 1992). In another study where the effects of bicuculline on brain activation were studied, bicuculline effects commenced almost immediately following administration but lasted no more than 10 minutes (Mueggler et al. 2001). Zolpidem (benzodiazepine agonist selective for α1-subunit containing $GABA_A$ receptors) and TCS1105 (benzodiazepine ligand, agonist at α2- and antagonist at α1-subunit containing $GABA_A$ receptors) were dissolved in 100% dimethyl sulfoxide (DMSO). Thus, DMSO was also used as vehicle-treatment for animals infused with zolpidem or TCS1105.

2.7 Social hierarchy test

Rats were pair-wise matched for bodyweight and for similar anxiety profile, with the exception of section 3.3 (figure 3) where animals that exhibited an opposite anxiety-profile (HA-LA) were matched. For pairs in which animals were matched for anxiety, the rats in each dyad were considered equal in their probability (= 50%) to become dominant or subordinate during their encounter. Using HA-LA pairs we investigated whether intra-VTA muscimol infusion would overcome the role of anxiety in the formation of a social hierarchy. Animals were marked on their body for identification and placed in pairs in a clean (neutral) cage without food or water for 20 min. During the social hierarchy test both rats displayed spontaneously offensive behavior, but this balance typically shifted

in the favor of one animal towards the end of the test. Social dominance was estimated by summation of the total duration of offensive behaviors for each rat in the dyad (offensive upright, lateral threat and keeping-down behavior, see Timmer and Sandi. 2010; Koolhaas et al. 1980). Thus, in more detail: Offensive upright (s) + Keeping down (s) + Lateral threat (s) = Total offensive behavior (s). The relative social dominance (%) for each pair was then calculated as follows: (Total offensive behavior by drug-treated rat / Total offensive behavior of the pair) * 100. During pilot studies, we found that infrequently offensive behavior was virtually absent during social encounter (no rat displaying >10s of total offensive behavior); these pairs were excluded from analysis as the relative social dominance in these pairs cannot be reliably measured. In total 5 pairs (= 10 animals) were excluded from the analyses on the basis of low aggressive behavior seen during the social encounter; this number corresponds to 7.4% of the pairs tested. Auto-grooming and social investigation was taken into account to determine the specificity of the drug-effects on offensive behavior.

2.8 Statistical analyses.

Animals tested for social dominance were not re-tested to avoid putative carry-over effects of drug treatment and dominance outcome. Sample sizes are indicated in the figure legends. One way ANOVA with Bonferroni´s post-test or unpaired two-tailed Student's t-tests were used to compare sets of data obtained from independent groups of animals. Within-pair amounts of offensive behavior in the social competition test were compared using paired two-tailed Student's t-tests. In sets of data in which animals were matched for anxiety, relative social dominance scores were compared using one-sample t-tests against chance (50%). All data were analyzed using Prism version 5.01 (Graphpad software Inc., San Diego, CA). P-values are reported in the results section with the second decimal rounded to the nearest figure. Statistical significance was considered at the $p<0.05$ level.

3. Results

3.1 Pharmacological targeting of VTA $GABA_A$ receptors affects anxiety-like behaviors

We first evaluated whether pharmacological activation vs. inhibition of $GABA_A$ receptors in the VTA would affect anxiety-like behaviors in opposite directions, as assessed in the elevated plus maze. We found that, indeed, at 30 min post infusion, the $GABA_A$ receptor agonist muscimol led to a reduction in anxiety, as indicated by an increased time spent on the open arms of the maze. Conversely, at this time point, the $GABA_A$ receptor antagonist bicuculline had no effect (Figure 1A,B, one-way ANOVA $F_{2,38}$= 3.57, p=0.038 with post-tests: t= 2.53, p<0.05 for muscimol versus vehicle and t= 0.27, not significant for bicuculline versus vehicle). We reasoned that this lack of effect might be due to the short half-life of bicuculline under physiological conditions (Johnston et al. 1984) and performed an additional experiment in which rats were tested 5 min after infusion. Indeed, at this time point, bicuculline had anxiogenic effects as indicated by a reduction in the time animals infused with the compound spent in the open arms of the elevated plus maze (Figure 1C, t= 3.62, df= 14, p= 0.003). To validate the temporal anxiogenic bicuculline effects we exposed another set of rats to an open field test 5 min following intra-VTA infusions of bicuculline or vehicle. We observed that during the first 10 min bicuculline-infused rats spent more time in the outer edge of the open field (rim zone) but that during the following 10 min there was no difference between the two treatment groups (Figure 1D, effect of drug, $F_{1,22}$= 3.70, p= 0.067, subjects, $F_{1,22}$= 2.56, p= 0.016 and significant post-hoc test for 0-10 min: t= 2.50, p<0.05).

3.2 Pharmacological targeting of $GABA_A$ receptors affects social dominance

Then, we investigated the effect of the treatments identified above for their effectiveness to modulate anxiety-like behaviors on the outcome of a social competition (Figure 2A). Animals in each dyad of competing male rats were matched for body weight and anxiety-like levels in the elevated plus maze. One animal in each pair was treated with vehicle and the other with the targeted treatment. The social competition test started at relevant times after drug infusion (i.e., 30 min for muscimol and 5 min for bicuculline, see Figure 1). As hypothesized, intra-VTA muscimol increased the

expression of offensive behavior (Figure 2B, t= 2.346, df= 10, p= 0.041), enhancing social dominance above chance levels (Figure 2C, t= 2.412, df= 10, p= 0.037). The higher social dominance scores of intra-VTA muscimol treated animals remained relatively constant throughout the course of the encounter (Figure 2D, 0-5 min t= 1.907, df= 10, p= 0.086; 5-10 min t= 2.075, df= 10, p= 0.065; 10-15 min t= 1.718, df= 10, p= 0.117; 15-20 min t= 2.474, df= 10, p= 0.033). Conversely, intra-VTA bicuculline infusion did not affect overall offensive behavior (Figure 2E, t= 0.3558, df= 18, p= 0.726) or social dominance (Figure 2F, t= 0.9939, t= 18, p= 0.333) when interactions taking place during the complete 20 min encounter were analyzed. However, it should be noted that bicuculline-infused rats had social dominance levels initially reduced, during the first 10 min of the encounter (Figure 2G, 0-5 min t= 3.566, df= 18, p= 0.002; 5-10 min t= 1.887, df= 18, p= 0.075), a time point after bicuculline-infusion in which modulation of anxiety-like behaviors was apparent as well (see Figure 1C). Similar to the early testing of intra-VTA bicuculline, we also tested rats early after intra-VTA muscimol (5 min post-infusion) but this treatment was without effect (Supplementary Figure 1, t= 0.50, df= 7, p= 0.63).

3.3 Activation of $GABA_A$ receptor in the VTA enhances social dominance in high-anxious rats

In previous experiments in which we included anxiety-matched dyads covering the full spectrum of anxiety, intra-VTA administration of the $GABA_A$ receptor agonist, muscimol, reduced anxiety-like behaviors (Figure 1B) and facilitated social dominance (Figure 2B-D). Here, we assessed whether the same treatment would be effective to boost social dominance in high anxious rats competing against low anxious ones, a contest that is typically lost by high anxious individuals (Hollis et al. 2015; van der Kooij et al. 2017) (Figure 3A). Indeed, high-anxious rats that received intra-VTA muscimol, but not intra-VTA vehicle, displayed higher levels of offensive behavior than their low-anxious opponents (Figure 3B, t= 2.518, df= 8, p= 0.036). Thus, whereas intra-VTA vehicle-treated high-anxious rats displayed low levels of social dominance (Figure 3C, now 35.4 ± 10.6 % versus 34.7 ± 4.6%, as

reported in Hollis et al. 2015), intra-VTA muscimol treatment enhanced social dominance in high-anxious animals (Figure 3C t= 2.634, df= 16, p= 0.018).

3.4 Activation of $GABA_A$ α2-subunit receptor subunit in the VTA boosts social dominance

Following confirmation in former experiments that activation of VTA $GABA_A$ receptors boosts social dominance, we explored whether specifically targeting the activation of α1- or α2- $GABA_A$ receptor subunit would reproduce the effect. Thus, in pairs of rats matched for anxiety levels, one animal was infused in the VTA with vehicle and the other with either zolpidem (agonist for α1-subunit containing $GABA_A$ receptors) or TCS1105 (agonist for α2-subunit containing $GABA_A$ receptors and antagonist for α1-subunit containing $GABA_A$ receptors) (Figure 4A). Whereas zolpidem did not affect offensive behavior (t=1.013, df= 7, p= 0.345) nor social dominance (t= 0.5368, df= 7, p= 0.608), TCS1105 strongly enhanced offensive behavior (t= 3.381, df= 6, p= 0.015) and, hence, social dominance (t= 4.109, df= 6, p= 0.006) (Figure 4B,C). Neither intra-VTA zolpidem, nor intra-VTA TCS1105 infusions were associated with altered levels of social investigation or auto-grooming (Supplementary Table 1).

4. Discussion

Recently we identified the VTA as a decisive site of action for the effects of diazepam on anxiety and social dominance, and the NAc as a critical effector region (Hollis et al., 2015; van der Kooij et al. 2017). Since diazepam acts as a positive modulator for $GABA_A$ receptors, we hypothesized that GABAergic mechanisms at the level of the VTA could underlie the effects seen on social competition. Here we demonstrated that social competition is enhanced after intra-VTA infusion of a $GABA_A$ receptor agonist but reduced after infusion of a $GABA_A$ receptor antagonist. Subsequent experiments pointed to $GABA_A$ receptor α2-subunit in the VTA as a critical mechanism in the control of the outcome of a social competition.

Specifically, we found that intra-VTA infusion of the specific $GABA_A$ receptor agonist muscimol was anxiolytic and increased social dominance in animals matched for anxiety-like levels. Additionally, intra-VTA muscimol infusion also enhanced the typically low social dominance for high-anxious animals during social competition with low-anxious conspecifics. These findings obtained by infusing muscimol mirror those recently published involving diazepam, where anxiolytic effects were also matched by dominance-enhancing effects (van der Kooij et al. 2017). Conversely, intra-VTA infusion of the specific $GABA_A$ antagonist bicuculline had anxiogenic effects, though only for a limited duration. During this relevant time window, bicuculline also reduced social competition. These findings with respect to anxiety are in line with a previous study in which intra-VTA bicuculline (100ng/hemisphere) infusion in rats increased anxiety-like behavior in the open field and the elevated plus maze (Frye and Paris, 2009). Taken together, these results support the idea that a reduction in anxiety coincides with a boost in social dominance and that these effects can be modulated through $GABA_A$ receptors in the VTA.

The specific type of α-subunits present in the heteropentameric $GABA_A$ receptors dictate the behavioral effects induced by benzodiazepines (Rudolph and Knoflach, 2011). Our findings indicating social dominance enhancement by TCS1105, but not zolpidem, suggest that social competition is under the regulation of α2-containing $GABA_A$ receptors in the VTA. We cannot rule out the possibility that the zolpidem dose used (25ng/hemisphere) was low to observe functional effects. Given that $GABA_A$ α1 subunit mediates the sedative properties of benzodiazepines (Rudolph and Möhler, 2006), we were cautious not to increase the dose beyond the one we previously used for diazepam infusions (25 ng/hemisphere), where clear effects on anxiety and social dominance were observed (van der Kooij et al. 2017). Moreover, since TCS1105 also inhibits α1-subunit containing $GABA_A$ receptors, our findings point to a crucial role of $GABA_A$ α2-subunits, rather than $GABA_A$ α1-subunits.

To our knowledge, the distribution of the various α-containing $GABA_A$ receptors throughout VTA subregions has not been described. A study was conducted, however, that described the expression

of α1-containing $GABA_A$ receptors and its colocalization with TH at different anatomical levels of the rat VTA (Ciccarelli et al. 2012). When comparing the anatomical data provided by Ciccarelli et al. to the coordinates we used in the current study, our cannulae were targeting the border of the middle- and caudal VTA with the injectors terminating in the paranigral subregion of the VTA. Interestingly, for the paranigral VTA subregion the relative number of cells stained identified with α1-containing $GABA_A$ receptors were lower in the middle- and caudal VTA in comparison to the rostral part (rostral: 5.1 ± 1.0; middle: 2.7 ± 0.5; caudal: 4.4 ± 1.4). This relatively low expression of α1-containing $GABA_A$ receptors in our targeted area may have contributed to the lack of effect on social dominance we saw after infusion of zolpidem, but this remains speculative. Importantly, the intra-VTA infusion of zolpidem or TCS1105 was not associated with side-effects as determined by social investigation or auto-grooming during the social interaction tests (Supplementary Table 1).

Interestingly, $GABA_A$ α2-subunits have been linked to the anxiolytic effects of diazepam; mice in which the $GABA_A$ α2 subunits were rendered insensitive to diazepam by a knock-in point mutation did not express the anxiolytic or myorelaxant effects of diazepam (Löw et al. 2000; Crestani et al. 2001). Additionally, peripheral injections of TCS1105 to mice reduced anxiety-like behavior in the light-dark box (Taliani et al. 2009; TCS1105 is referred to as compound ´1c´ in this paper). Intra-VTA infusion of TCS1105 did not affect anxiety-like behavior on the EPM in our hands (Supplementary Figure 3), which suggests that anxiety might tap on to the mesolimbic system to modify motivated behaviors and social dominance. However, manipulations in the VTA do not necessarily revert on modifications in anxiety-like behaviors.

Importantly, intra-VTA muscimol has been associated with enhanced dopamine release in the NAc (Doherty and Gratton, 2007; Kalivas et al. 1990; Xi and Stein, 1998). The conventional view of muscimol as an agent that can be exploited unconditionally in order to silence a particular brain region may thus need revision in a cell-type and circuit-dependent manner. At least for the VTA, it is unlikely that a simple ´shut down´ is achieved via infusion of muscimol, possibly owing to the circuit

characteristics of the VTA (GABAergic inhibition of dopaminergic neurons). The situation might be different in other brain regions such as the NAc or the basolateral amygdala, where local muscimol infusions disrupted region-mediated behaviors (Hollis et al. 2015; van der Kooij et al., 2017).

Because the dominance-promoting effects of intra-VTA diazepam are likely related to their function at the anxiety-related α2-, but not at the addiction-linked α1-, subunit containing $GABA_A$ receptors, our findings imply that treatments acting specifically on α2-subunit containing $GABA_A$ receptors may boost social competition devoid of concomitant addictive properties. This future perspective, albeit untested as of yet, may improve the subordinate status of high-anxious individuals, which often emerges under competitive settings (Gilbert et al. 2009; Goette et al. 2015).

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References

Adcock RA, Thangavel A, Whitfield-Gabrieli S, Knutson B, Gabrieli JD. Reward-motivated learning: mesolimbic activation precedes memory formation. *Neuron* 2006, **50**: 507-517.

Berridge KC. The debate over dopamine´s role in reward: the case for incentive salience. *Psychopharmacology* 2007, **191**: 391-431.

Crestani F, Löw K, Keist R, Mandelli M, Möhler H, Rudolph U. Molecular targets for the myorelaxant action of diazepam. *Mol Pharmacol* 2001, **59**: 442-5.

Ciccarelli A, Calza A, Panzanelli P, Concas A, Giustetto M, Sassoè-Pognetto M. Organization of GABAergic synaptic circuits in the rat ventral tegmental area. *PLOS ONE,* 2012, **7**: e46250.

Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC. Dominant rats are natural risk takers and display increased motivation for food reward. *Neuroscience* 2009, **162**: 23-30.

Dobi A, Margolis EB, Wang, HL, Harvey BK, Morales M. Glutamatergic and nonglutamatergic neurons of the ventral tegmental area establish local synaptic contacts with dopaminergic and nondopaminergic neurons. *J Neurosci* 2010, **30**: 218-229.

Doherty M, Gratton A. Differential involvement of ventral tegmental area GABA-A and GABA-B receptors in the regulation of the nucleus accumbens dopamine response to stress. *Brain Res* **1150**: 62-68.

Frye CA, Paris JJ. Infusions of bicuculline to the ventral tegmental area attenuates sexual, exploratory, and anti-anxiety behavior of proestrous rats. *Pharmacol Biochem Behav* 2009, **93**: 474-81.

Füchsl AM, Neumann ID, Reber SO. Stress resilience: a low-anxiety genotype protects male mice from the consequences of chronic psychosocial stress. *Endocrinology* 2014, **155**: 117-26.

Gentsch C,Lichtsteiner M, Feer H. Competition for sucrose-pellets in triads of male Wistar rats: effects of acute and subchronic chlordiazepoxide. *Psychopharmacology* 1990, **100**: 530-4.

Gilbert P, McEwan K, Bellew R, Mills A, Gale C. The dark side of competition: how competitive behaviour and striving to avoid inferiority are linked to depression, anxiety, stress and self-harm. *Psychol Psychother* 2009, **82**: 123-136.

Goette L, Bendahan S, Thoresen J, Hollis F, Sandi C. Stress pulls us apart: anxiety leads to differences in competitive confidence under stress. *Psychoneuroendocrinology* 2015, **54**: 115-123.

Herrero AI, Sandi C, Venero C. Individual differences in anxiety trait are related to spatial learning abilities and hippocampal expression of mineralcorticoid receptors. *Neurobiol Learn Mem* 2006, **86**: 150-9.

Hollis F, van der Kooij MA, Zanoletti O, Lozano L, Cantó C, Sandi C. Mitochondrial function in the brain links anxiety with social subordination. *Proc Natl Acad Sci* 2015, **112**: 15486-15491.

Johnston GA, Allen RD, Skerritt JH. Receptors in the Nervous System, 2nd Edition. Ed. Abel Lajtha. Chapter 8, page 215.

Kalivas PW, Duffy P, Eberhardt H. Modulation of A10 dopamine neurons by gamma-aminobutyric acid. *J Pharm Exp Ther* 1990, **253**: 858-866.

Koolhaas JM, Schuurman T, Wiepkema PR. The organization of intraspecific agonistic behaviour in the rat. *Prog Neurobiol* 1980, **15**: 247-68.

Löw K, Crestani F, Keist R, Benke D, Brünig I, Benson JA, Fritschy JM, Rülicke T, Bluethmann H, Möhler H, Rudolph U. Molecular and neuronal substrate for the selective attenuation of anxiety. *Science* 2000, **290**: 131-4.

Morales M, Pickel VM. Insights to drug addiction derived from ultrastructural views of the mesocorticolimbic system. *Ann N Y Acad Sci* 2012, **1258**: 71-88.

Mueggler T, Baumann D, Rausch M, Rudin M. Bicuculline-induced brain activation in mice detected by functional magnetic resonance imaging. *Magn Reson Med* 2001, **46**: 292-8.

Numan M, Stolzenberg DS, Dellevigne AA, Corenti CM, Numan MJ. Temporary inactivation of ventral tegmental area neurons with either muscimol or baclofen reversibly disrupts maternal behavior in rats through different underlying mechanisms. *Behav Neurosci* 2009, **123**: 740-51.

Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates* 1986. San Diego, Academic Press.

Rudolph U, Möhler H. GABA-based therapeutic approaches: GABAA receptor subtype functions. *Curr Opin Pharmacol* 2006, **6**: 18-23.

Rudolph U, Knoflach F. Beyond classical benzodiazepines: novel therapeutic potential of GABAA receptor subtypes. *Nat Rev Drug Discov* 2011, **10**: 685-97.

Salamone JD, Correa M. The mysterious motivational functions of mesolimbic dopamine. *Neuron* 2012, **76**: 470-485.

Sapolsky RM. The influence of social hierarchy on primate health. *Science* 2005, **308**: 648-652.

Schultz W. Getting formal with dopamine and reward. *Neuron* 2002, **36**: 241-263.

Slattery DA, Uschold N, Magoni M, Bär J, Popoli M, Neumann ID, Reber SO. Behavioural consequences of two chronic psychosocial stress paradigms: anxiety without depression. *Psychoneuroendocrinology* 2012, **37**: 702-14.

Taliani S, Cosimelli B, Da Settimo F, Marini AM, La Motta C, Simorini F, Salerno S, Novellino E, Greco G, Cosconati S, Marineli L, Salvetti F, L'Abbate G, Trasciatti S, Montali M, Costa B, Martini C. Identification of anxiolytic/nonsedative agents among indol-3-ylglyoxylamides acting as functionally selective agonists at the gamma-aminobutyric acid-A (GABAA) alpha2 benzodiazepine receptor. *J Med Chem* 2009, **52**: 3723-34.

Tan KR, Brown M, Labouèbe G, Yvon C, Creton C, Fritschy JM, Rudolph U, Lüscher C. Neural bases for addictive properties of benzodiazepines. *Nature* 2010, **463**: 769-74.

Tan KR, Rudolph U, Lüscher C. Hooked on benzodiazepines: GABAA receptor subtypes and addiction. *Trends Neurosci* 2011, **34**: 188-97.

Timmer M, Sandi C. A role for glucocorticoids in the long-term establishment of a social hierarchy. *Psychoneuroendocrinology* 2010, **35**: 1543-52.

Van der Kooij MA, Hollis F, Lozano L, Zalachoras I, Abad S, Zanoletti O, Grosse J, Guillot de Suduiraut I, Cantó C, Sandi C. Diazepam actions in the VTA enhance social dominance and mitochondrial function in the nucleus accumbens by activation of dopamine D1 receptors. *Mol Psychiatry*, 2018, **23**: 569-578.

Weng X, Rosenberg HC. Infusion of bicuculline methiodide into the tectum: model specificity of pro-and anticonvulsant actions. *Epilepsy Res* 1992, **12**: 1-8.

Xi Z, Stein EA. Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptor- an in vivo electrochemical study. *Brain Res* 1998, **798**: 156-65.

Figure legends

Figure 1. Infusion of a $GABA_A$ receptor agonist or -antagonist in the ventral tegmental area affects anxiety-like behavior. (**A,B**) At 30 min post-infusion, the $GABA_A$ receptor agonist muscimol (MUS) reduced anxiety on the elevated plus maze whereas the $GABA_A$ receptor antagonist bicuculline (BIC) showed no effect (n= 10-16 per group). (**C**) Bicuculline enhanced anxiety when animals were tested 5 min post-infusion (n= 7-9 per group). (**D**) Rats infused with bicuculline spent more time in the rim-zone of the open field during the first 10 min as compared to controls (n= 11-13/group). Data are presented as mean + SEM (*p<0.05, **p<0.01, one- (**B**) or two-way (**D**) ANOVA plus bonferroni post-tests and (**C**) Student's non-paired t-test).

Figure 2. Infusion of a $GABA_A$ receptor agonist or –antagonist in the ventral tegmental area (VTA) affects social dominance. (**A, B**) Muscimol (MUS) infused intra-VTA 30 min prior to testing enhanced social offensive behavior, which was reflected by (**C**) a dominance level above chance (50%, n= 11 pairs). (**D**) The effect of intra-VTA muscimol on social dominance was constant throughout the social encounter. (**E**) Bicuculline (BIC) infused intra-VTA 5 min prior to testing did not affect overall offensive behavior or (**F**) social dominance (n= 19 pairs). (**G**) However, social dominance levels were reduced below chance during the first 10 min of the social encounter for bicuculline-treated animals. Data are presented as mean + SEM (t<p0.1, *p<0.05, **p<0.01, (**B,E**) Student's paired t-test and (**C,D,F,G**) one-sample t-test against chance-level).

Figure 3. Infusion of a $GABA_A$ receptor agonist into the ventral tegmental area enhances social dominance in high-anxious animals. (**A**,**B**) Muscimol (MUS) infused intra-VTA 30 min prior to social encounter enhanced offensive behavior in high-anxious which, (**C**) significantly boosted their default low social dominance (n= 9 pairs per group). Data are presented as mean + SEM (*p<0.05). (**A**) Paired and (**B**) unpaired student's t-test.

Figure 4. $GABA_A$ receptors containing alpha-2 subunits appear responsible for the effects of $GABA_A$ stimulation on social dominance. (**A**) Zolpidem (ZOL) and TCS1105 were infused intra-VTA 30 min prior to social encounter. (**B**) Zolpidem did not affect offensive behavior while TCS1105 enhanced offensive behavior (n= 7-8 pairs per group). (**C**) Social dominance level did not differ from chance level after intra-VTA zolpidem infusion but was enhanced by TCS1105 treatment. Data are presented as mean + SEM (**p<0.01, (**B**) Paired student's paired t-test and (**C**) one-sample t-test against chance-level).

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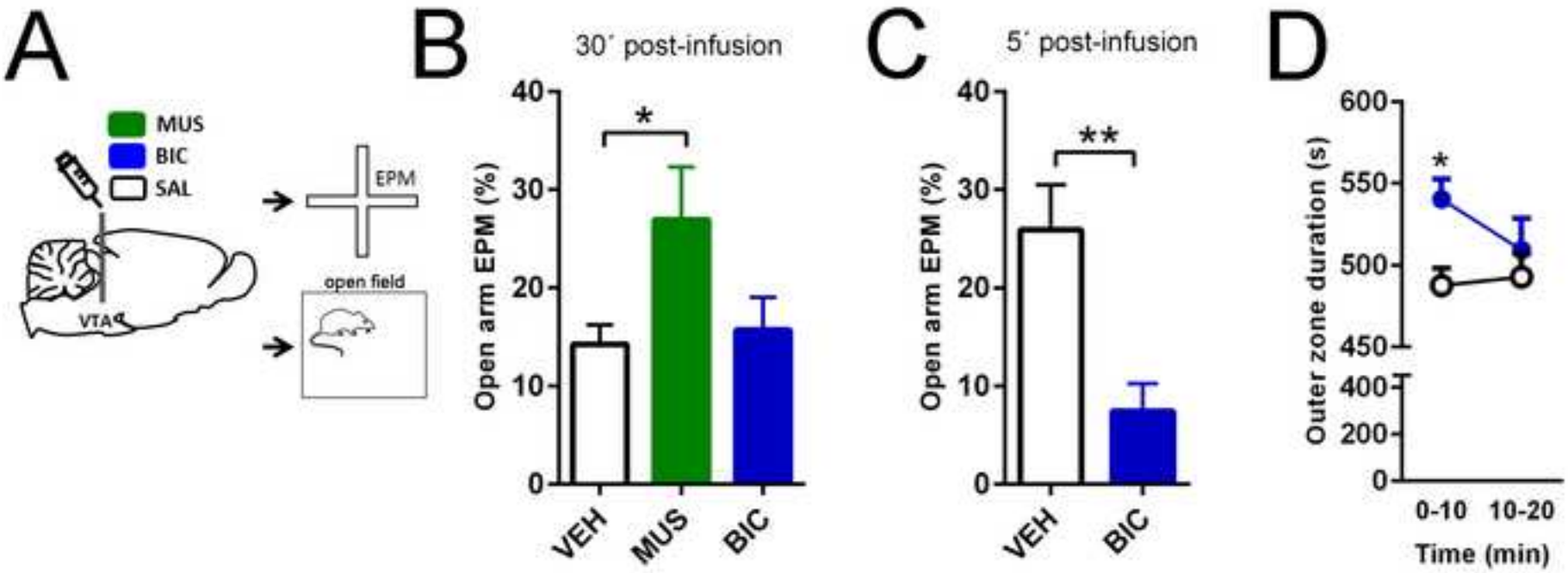

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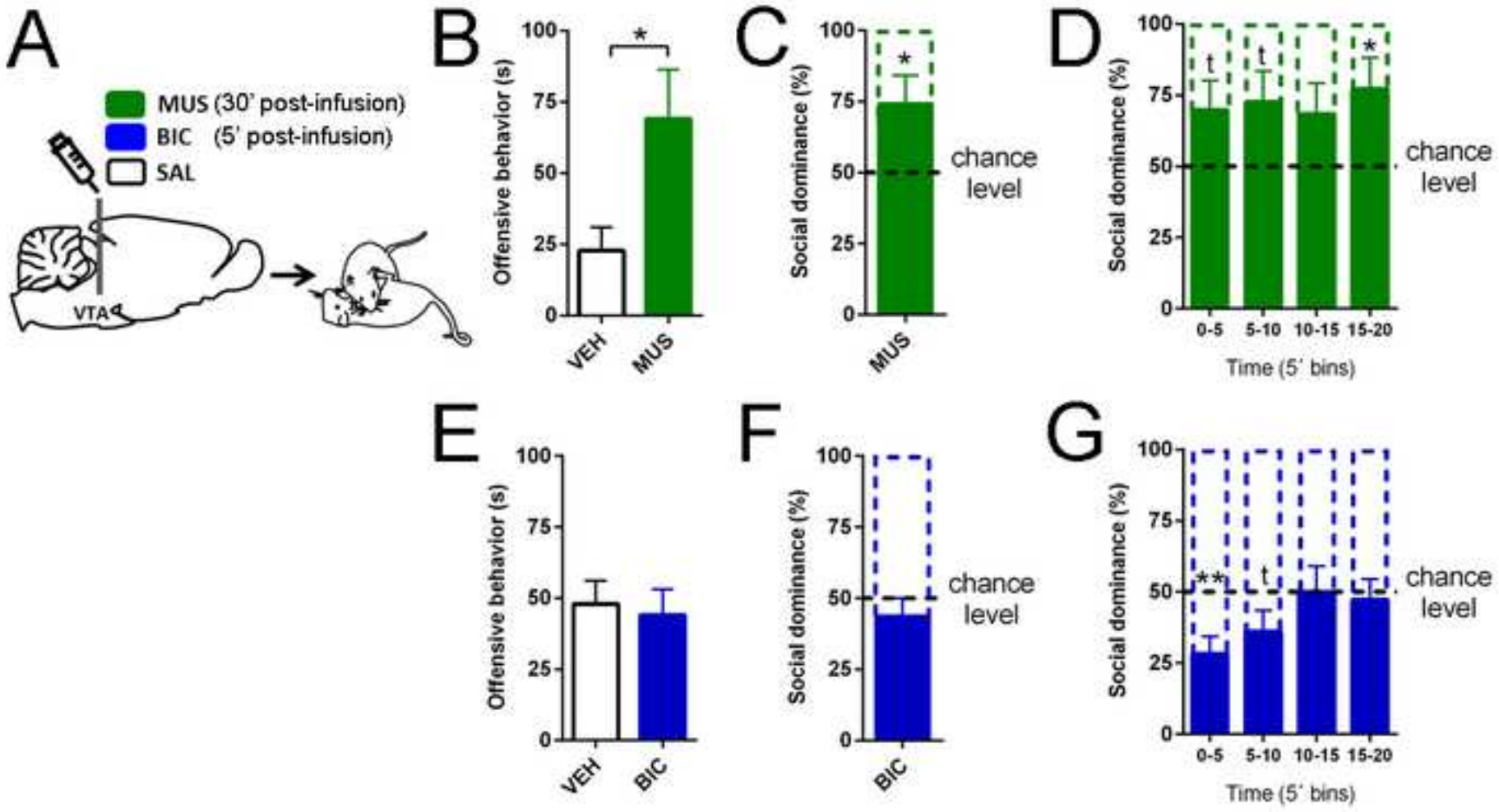

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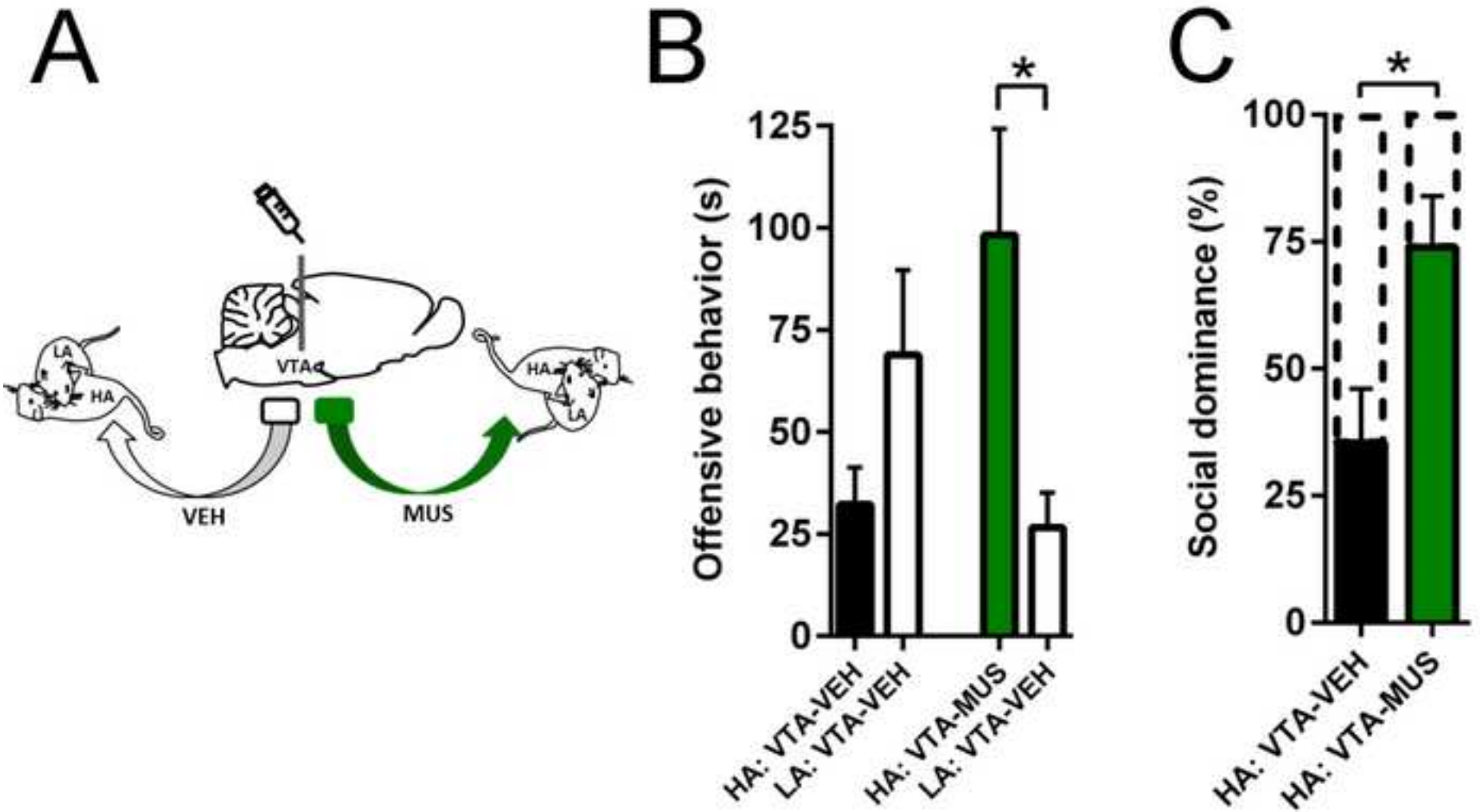

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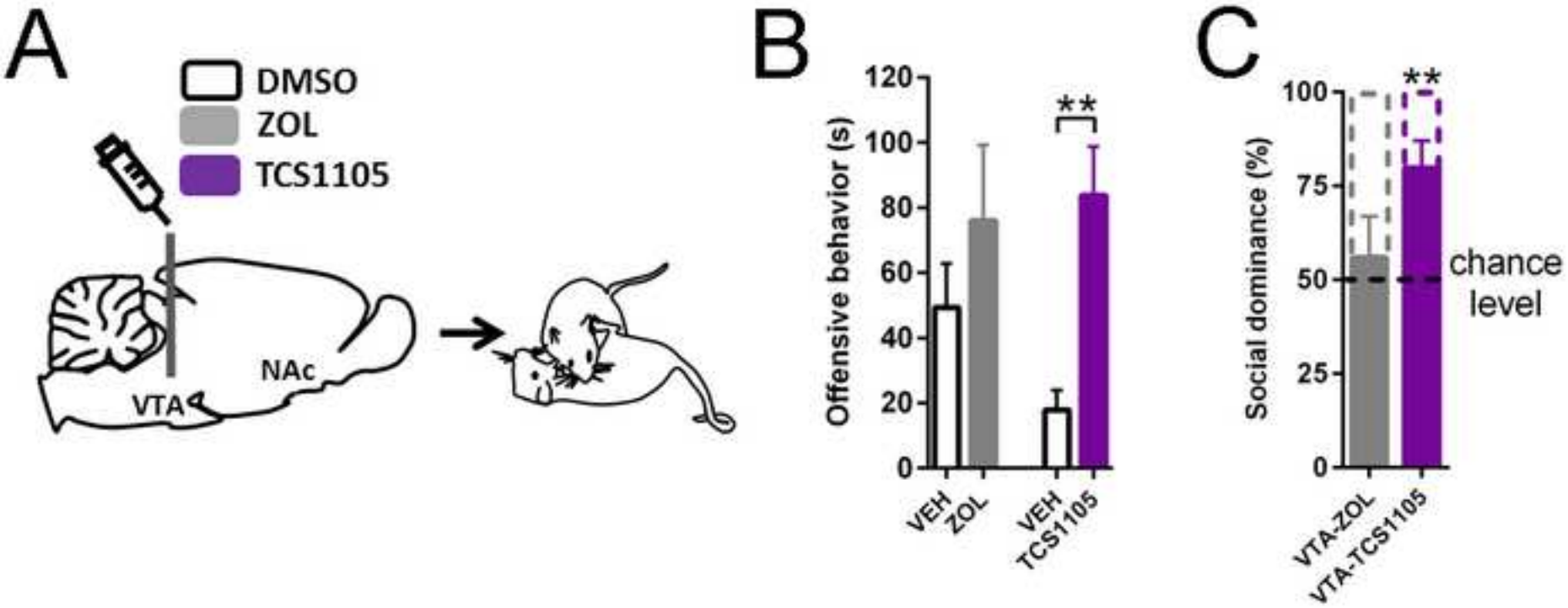

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