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Muscarinic and glutamatergic regulation of self-grooming behavior and ultrasonic vocalizations in the context of open-field habituation in rats

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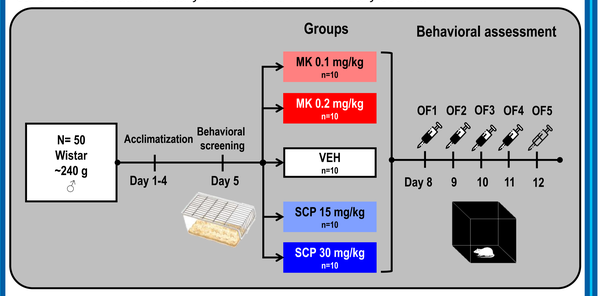
Introduction

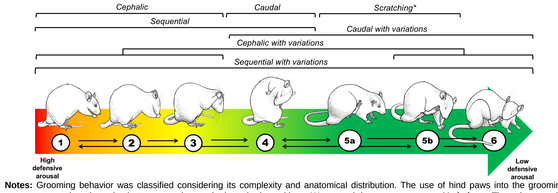
Habituation is a form of non-associative learning characterized by the reduction of a given response after repeated or prolonged exposures to the eliciting stimuli[1,2]. In rats, habituation is typically assessed by means of a single or repeated exposure to an unconditioned anxiety test such as the open field (OF)[3,4]. The OF consists of an open, illuminated arena surrounded by walls that naturally elicits a set of defensive responses in rodents (i.e., locomotion and rearing). Once the aversiveness of the context reduces, these defensive responses progressively decay within and between tests. This phenomenon is called novelty habituation[3,4]. We have reported that self-grooming progressively increases as habituation is taking place, particularly those subtypes characterized by long and complex sequences, which require the animals to disengage from exploratory and defensive responses. The appearance of those grooming subtypes has been interpreted as a post-stress reaction that is part of or contributes to coping with the aversiveness of the situation. We have proposed that the mechanisms controlling self-grooming are integrated within a system enabling emotional de-arousal during and after stress[3,5]. As the facilitation of habituation after repeated OF exposures increases complex forms of grooming, the prevention or disruption of the OF habituation should alter the emergence of grooming. Thus, we aimed to retard the habituation process by administering the muscarinic antagonist scopolamine (SCP) and the NMDA antagonist, MK-801 (MK). We also studied the concomitant emotional responses to the drugs by recording ultrasonic vocalizations (USVs) in the OF. When rats experience emotional distress, they typically emit 22-KHz calls to prevent conspecifics of a possible threat. Under novel or rewarding situations, animals produce 50-KHz calls as a form of social re-engagement or as signals of positive affect. Then, 22-KHz and 50-KHz USVs are interpreted as indicators of negative and positive emotionality, respectively[6,7,8]. which might be affected by the drugs.

Materials and Methods

Subjects: Fifty male Wistar (~250g) rats were behaviorally screened in a spontaneous activity test (cage-test). Then, they were assigned to the groups in a counterbalanced manner based on their locomotion, rearing, grooming, and ultrasonic vocalization. **Groups:** a) Vehicle (VEH; saline at 0.9%), b) MK-801 0.1 (MK 0.1 mg/kg), c) MK-801 0.2 (MK 0.2mg/kg), d) scopolamine 15 (SCP 15 mg/kg), and e) scopolamine 30 (SCP 30 mg/kg). **General procedure:** Animals were individually transported from the vivarium to a separated room for drug administration. After 15 min, rats were transported to the testing room and immediately placed in the center of an OF apparatus (70 x 70 x 40 cm) for a 15-min evaluation performed on four consecutive days. On the fifth day, rats from all groups were injected with vehicle and assessed in the OF as before (gray area). **Behavioral analysis**: Locomotion was automatically assessed using the AnyMaze® software. Rearing (Biped postures elevated ≥ 45° from the floor) and grooming (see below) were manually scored by trained observers with the free software SolomonCoder®. Grooming was classified exactly as previously described[5]. Twenty-two kHz USVs were automatically scored using the AviSoft SAS Lab Pro® software. Fifty-kHz calls were semi-automatically detected and then manually scored.

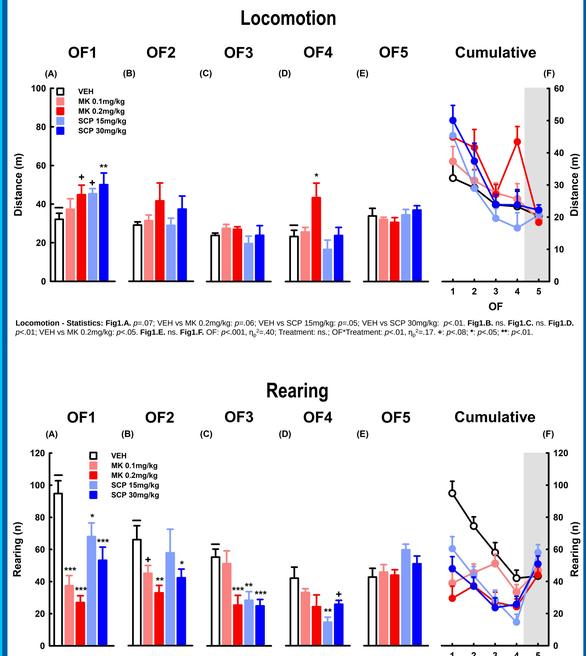


Notes: Behavioral screening (Cage-test): animals were tested in individual home cages (22x37.5x18 cm). All animals were group housed (5 per cage), and had free access to food and water during the experiment.



Notes: Grooming behavior was classified considering its complexity and anatomical distribution. The use of hind paws into the grooming sequences requires the animals to engage in very intricate body positions. We named those sequences as **Variations.** The subtypes with variations are thought to be more complex than those without them. We also considered if the grooming was directed to the head and fore paws (**Cephalic**), to the body (**Caudal**), or if it appears as a chain including both cephalic and caudal regions of the body (**Sequential**). During the sequential grooming with variations the rat´s capacity to respond promptly to threats is considerably diminished. For this reason, we suggest that this grooming subtype is related to the habituation and the emotional de-arousal process.

Results – MK-801 and scopolamine inhibited rearing behavior without affecting substantially the locomotor activity



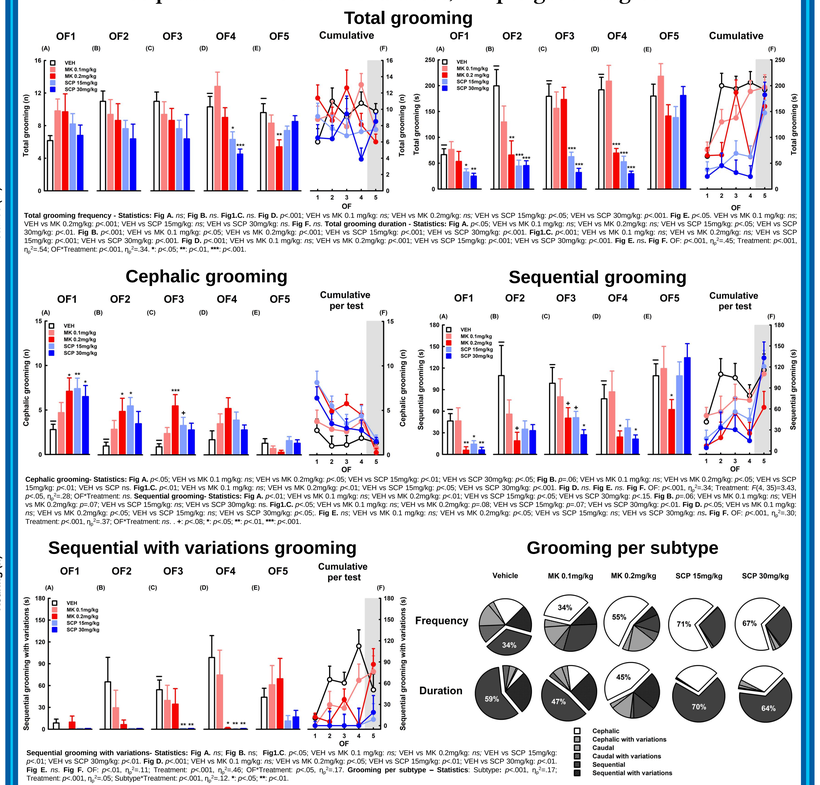
Locomotion - Statistics: Fig1.A. p=.07; VEH vs MK 0.2mg/kg: p=.06; VEH vs SCP 15mg/kg: p=.05; VEH vs SCP 30mg/kg: p<.01. **Fig1.B.** ns. **Fig1.C.** ns. **Fig1.D.** p<.01; VEH vs MK 0.2mg/kg: p<.05. **Fig1.E.** ns. **Fig1.F.** OF: p<.001, η_p^2=.40; Treatment: ns.; OF*Treatment: p<.01, η_p^2=.17. +: p<.08; *: p<.05; **: p<.01.

Rearing - Statistics: Fig1.A. p<.001; VEH vs SCP 15mg/kg: p<.05; VEH vs MK 0.1 mg/kg: p<.001; VEH vs SCP 30mg/kg: p<.01; VEH vs MK 0.2mg/kg:p <.001. **Fig1.B.** p=.05; VEH vs MK 0.1 mg/kg: p=.05; VEH vs MK 0.2mg/kg: p<.01; VEH vs SCP 15mg/kg: ns; VEH vs SCP 30mg/kg: p<.05. **Fig1.C.** p<.001; VEH vs MK 0.1 mg/kg: ns; VEH vs MK 0.2mg/kg: p<.01; VEH vs SCP 15mg/kg: p<.01; VEH vs SCP 30mg/kg: p<.001. **Fig1.D.** p<.01; VEH vs MK 0.2mg/kg: ns; VEH vs MK 0.1 mg/kg: ns; VEH vs SCP 15mg/kg: p<.01; VEH vs SCP 30mg/kg: p=.05. **Fig1.E.** p=.06. **Fig1.F.** OF: p<.001, η_p^2=.36; Treatment: p<.001, η_p^2=.53; OF*Treatment: p<.001, η_p^2=.38. +: p<.08; *: p<.05; **: p<.01, ***: p<.001.

Main results:

- The administration of MK and SCP increased locomotor activity during the OF1 without affecting consistently the locomotion on the following OF assessments.
- Over days, locomotion decreased gradually in all animals irrespective of the treatments, indicating that MK and SCP caused no alteration on locomotion.
- VEH animals showed a progressive reduction of rearing over tests. All treatments, in contrast, produced low but irregular rates of rearing even from the OF1. On OF5, rearing returned almost to the initial levels and even out with the VEH group, suggesting that the reduction of rearing resulted from a direct pharmacological effect of the treatments and not from an impairment in the novelty habituation process.

Results – MK-801 and scopolamine suppressed complex grooming sequences while increased short, simple grooming bouts



Total grooming frequency - Statistics: Fig A. *ns*; **Fig B.** *ns*. **Fig1.C.** *ns*. **Fig D.** p<.001; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: *ns*; VEH vs SCP 15mg/kg: p<.05; VEH vs SCP 30mg/kg: p<.001. **Fig E.** p<.05. VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.001; VEH vs SCP 15mg/kg: *ns*; VEH vs SCP 30mg/kg: p<.01. **Fig F.** *ns*. **Total grooming duration - Statistics: Fig A.** p<.05; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: *ns*; VEH vs SCP 15mg/kg: p<.05; VEH vs SCP 30mg/kg: p<.01. **Fig B.** p<.001; VEH vs MK 0.1 mg/kg: p<.05; VEH vs MK 0.2mg/kg: p<.001; VEH vs SCP 15mg/kg: p<.001; VEH vs SCP 30mg/kg: p<.001. **Fig1.C.** p<.001; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: *ns*; VEH vs SCP 15mg/kg: p<.001; VEH vs SCP 30mg/kg: p<.001. **Fig D.** p<.001; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.001; VEH vs SCP 15mg/kg: p<.001; VEH vs SCP 30mg/kg: p<.001. **Fig E.** *ns*. **Fig F.** OF: p<.001, η_p^2=.45; Treatment: p<.001, η_p^2=.54; OF*Treatment: p<.001, η_p^2=.34. *: p<.05; **: p<.01, ***: p<.001.

Cephalic grooming- Statistics: Fig A. p<.05; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.05; VEH vs SCP 15mg/kg: p<.01; VEH vs SCP 30mg/kg: p<.05; **Fig B.** p=.06; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.05; VEH vs SCP 15mg/kg: p<.01; VEH vs SCP *ns*. **Fig1.C.** p<.01; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.01; VEH vs SCP 15mg/kg: p<.05; VEH vs SCP 30mg/kg: p<.001. **Fig D.** *ns*. **Fig E.** *ns*. **Fig F.** OF: p<.001, η_p^2=.34; Treatment: $F(4, 35)$=3.43, p<.05, η_p^2=.28; OF*Treatment: *ns*. **Sequential grooming- Statistics: Fig A.** p<.01; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.01; VEH vs SCP 15mg/kg: p<.05; VEH vs SCP 30mg/kg: p<.15. **Fig B.** p=.06; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p=.07; VEH vs SCP 15mg/kg: *ns*; VEH vs SCP 30mg/kg: *ns*. **Fig1.C.** p<.05; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p=.08; VEH vs SCP 15mg/kg: p=.07; VEH vs SCP 30mg/kg: p<.01. **Fig D.** p<.05; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.05; VEH vs SCP 15mg/kg: *ns*; VEH vs SCP 30mg/kg: p<.05. **Fig E.** *ns*; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.05; VEH vs SCP 15mg/kg: *ns*; VEH vs SCP 30mg/kg: *ns*. **Fig F.** OF: p<.001, η_p^2=.30; Treatment: p<.001, η_p^2=.37; OF*Treatment: *ns*. +: p<.08; *: p<.05; **: p<.01, ***: p<.001.

Sequential with variations grooming

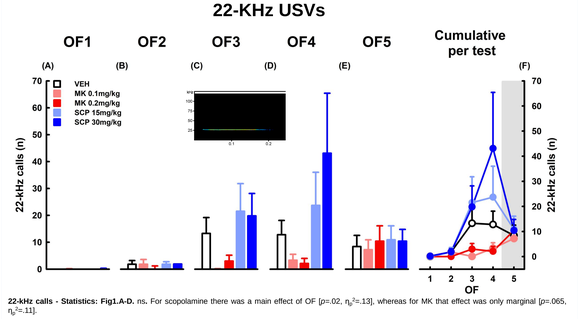
Grooming per subtype

Sequential grooming with variations- Statistics: Fig A. *ns*; **Fig B.** *ns*. **Fig1.C**. p<.05; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: *ns*; VEH vs SCP 15mg/kg: p<.01; VEH vs SCP 30mg/kg: p<.01. **Fig D.** p<.001; VEH vs MK 0.1 mg/kg: *ns*; VEH vs MK 0.2mg/kg: p<.05; VEH vs SCP 15mg/kg: p<.01; VEH vs SCP 30mg/kg: p<.01. **Fig E.** *ns*. **Fig F.** OF: p<.01, η_p^2=.11; Treatment: p<.001, η_p^2=.46; OF*Treatment: p<.05, η_p^2=.17. **Grooming per subtype – Statistics**: Subtype: p<.001, η_p^2=.17; Treatment: p<.001, η_p^2=.05; Subtype*Treatment: p<.001, η_p^2=.12. *: p<.05, **: p<.01.

Main results:

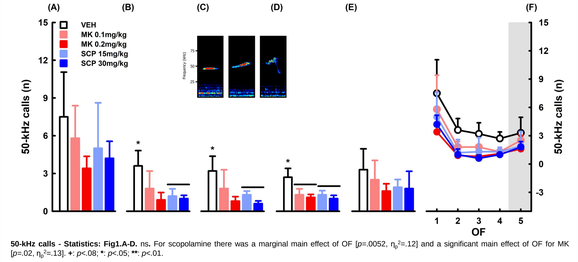
- In the VEH group, total grooming duration showed a stepped increase from the first to the second OF. In the MK 0.1mg/kg group, such an increase was rather progressive over the OF assessments. Conversely, the administration of SCP and MK at 0.2mg/kg strongly reduced grooming duration, an effect that fully restored when treatments were withdrawn on OF5.
- The administration of SCP and MK at 0.2mg/kg increased the emission of cephalic grooming during the first three OF assessments, while prevented the increase of complex grooming sequences over tests.
- When treatments were withdrawn, only MK 0.2mg/kg did not fully restore to the levels of sequential grooming observed in the other groups. In the case of sequential grooming with variations, both scopolamine doses produced a carry-over effect on OF5.

Results – MK-801 and scopolamine had opposite effects on the frequency of 22-kHz calls



22-kHz calls - Statistics: Fig1.A-D. ns. For scopolamine there was a main effect of OF (p=.02, η_p^2=.13], whereas for MK that effect was only marginal [p=.065, η_p^2=.11].

Results – Both MK-801 and scopolamine reduced the rate of 50-kHz calls



50-kHz calls - Statistics: Fig1.A-D. ns. For scopolamine there was a marginal main effect of OF [p=.0052, η_p^2=.12] and a significant main effect of OF for MK [p=.02, η_p^2=.13]. +: p<.08; *: p<.05; **: p<.01.

Main results:

- After two consecutive OF, 22-kHz calls increased spontaneously in VEH rats, which maintained a similar call rate until OF5. On the contrary, the 50-kHz calls reduced after OF1, and remained almost the same thereafter. The kinetic seen for both subtypes of USVs may have resulted from the stress associated with the repeated injections and the OF environment.
- The administration of scopolamine produced a non-significant increase in 22-kHz calls, especially on OF3 and OF4. Such an effect restored on OFT5, when no drug was given.
- Scopolamine significantly decreased the emission of 50-kHz calls, especially from OF2 to OF4. On OF5, the rate of USVs slightly restored without reaching the levels seen in VEH-treated rats.
- MK, in contrast, produced a general suppression of call rate. This effect was more pronounced for the 22-kHz than for 50-kHz calls because the rate of the latter was really low in all animals.

Summary and conclusions

- After repeating administration, MK-801 and scopolamine had no effects on traditional parameters of OF habituation. Rearing behavior, however, was pharmacologically inhibited by both treatments without evidencing carry-over effects when drugs were withdrawn.
- The subtypes of grooming were differentially affected by the treatments. Both MK-801 and scopolamine increased cephalic grooming, while withheld the expected increase in sequential grooming seen after repeated OF testing. Although sequential grooming did not fully restore after MK-801 administration at the high dose, only scopolamine produced a complete carry-over effect at both doses. On USVs, 22-kHz calls tended to increase with scopolamine treatment, but did not show a carry-over effect when the drug was withdrawn. On the contrary, the 50-kHz calls were rather attenuated by scopolamine, whereas MK-801 produced a general inhibition on both USVs subtypes.
- Cholinergic signaling through muscarinic receptors seems to regulate differentially the distint grooming subtypes and the affective responses likely involved in the habituation process without affecting the traditional OF parameters.
- In contrast to our expectations, the antagonism of NMDA receptors had almost no effects on non-associative learning and produced a rather overall behavioral inhibition seen only under the acute effects of the drug. The increase in cephalic grooming after MK-801 administration suggests, however, that glutamatergic signaling is part of the neural mechanisms controlling the motor sequences but not those involved in the emotional de-arousal process. In fact, shifting pharmacologically the naturally-ocurring sequences of grooming in the OF did not compromise habituation between-tests.

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