LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

EMVU Research Association of TUM/LMU

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Substudy:
Effects of chronic whole body exposure to GSM or UMTS on immune response and stress

DMF, "International Workshop on Long-Term Effects",
October 11 - 12, 2007

Objective

- Investigation of chronic effects of high-frequency fields associated with mobile communication on the immune and stress systems in an animal model (rats)
- Chronic exposure of 3 generations of Wistar rats (F0, F1, F2)
- Mobile telecommunication systems GSM (900 MHz) and UMTS (1900 MHz) with a SAR of 0.4 W/kg similar to human exposure.

Objective

Immune system

- Comparison of the immune competence of the individual animal under long-term GSM or UMTS exposure with a non-exposed control group by means of injection of various antigens (ovalbumin, chicken IgY)

Stress system (ACTH test)

- by means of an "artificial" stressor (adrenocorticotropic hormone) determinaton whether rats under long-term exposure to GSM or UMTS react adequately (increase of corticosterone) compared to a control group

Adaptive stress and chronic stress?

- Stress is the body's unspecific overall response to activation of the affective centers in the CNS by external (and internal) stimuli.
  Affective centers → limbic system
  (= "affective center" → "emotionality").
- affective activation does not have to be directly associated with environmental effects (e.g. "exam nerves").
- Stress reactions are always adaptive
- irrespective of the type of stress animals exhibit the same physiological response pattern

→ General Adaptation Syndrome

Adaptive stress and chronic stress?

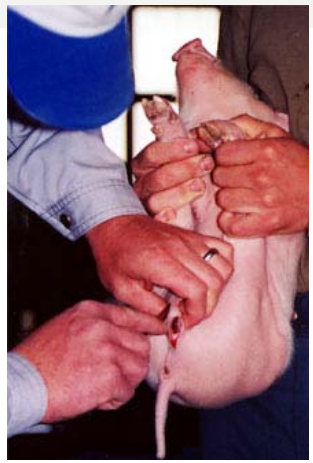
- an individual's ability to cope with stressors depends on
  - the current physiological condition (e.g. health, reproduction),
  - the individual "history" and experience
  - the genetic predisposition
- the extent of the stress reaction depends on how quickly and effectively the individual gains control

Literature

Stress reactions

- Flight
- Resistance
- Freezing
- Elimination (micturition/defecation)

acute



chronic

- Stereotypes
- Cannibalism
- Illness
- Reduced performance
- Fertility disorders

. . . .

?

Stress systems

- Two basic types of stress coping

Active *coping* = flight and fight syndrome, struggle for control
Passive *coping* = freezing

- Two main components of stress reaction:

Sympathetic-Adrenal-Medulla system (= SAM axis)
Hypothalamic-Pituitary-Adrenal system (= HPA axis)

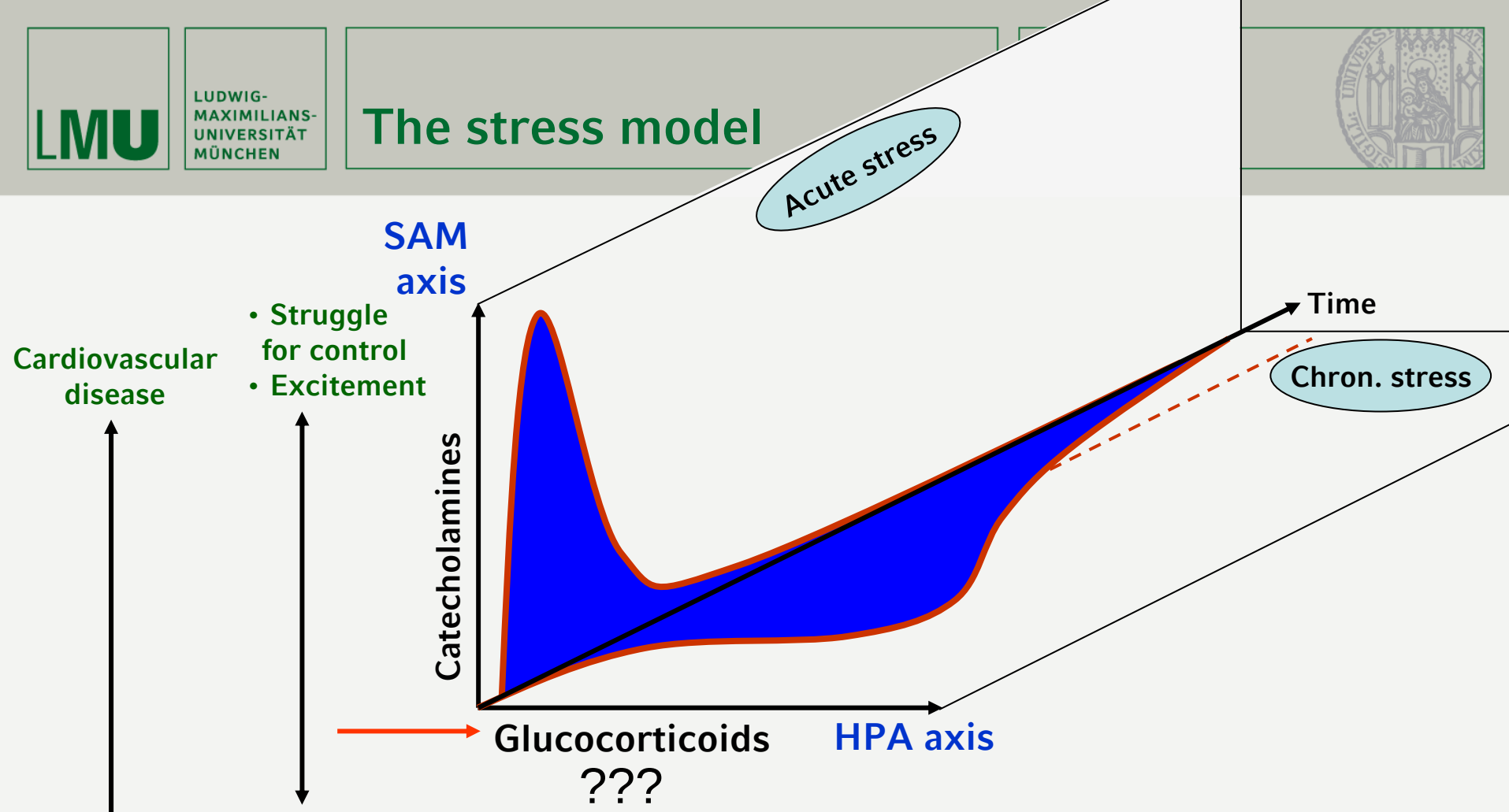
- Acute stress situation → *challenge* of both systems → preparation of the organism for (active or passive) coping with the stressor

Stress systems

If the individual is neither able to cope with the situation nor to avoid it successfully, this results in chronic stress, with either the one or the other stress axis remaining permanently activated depending on the stressor and the type of behavior.

Chronic stress is an expression of overchallenged adaptability!

The stress model



Cardiovascular disease

- Struggle for control
- Excitement

Emotional condition:
- Control
- Relaxation

- Loss of control
- Apathy, depression

???

Physical condition:
- Normal (healthy)

- Immunodeficiency
- Infections
- Ulcers

Literature

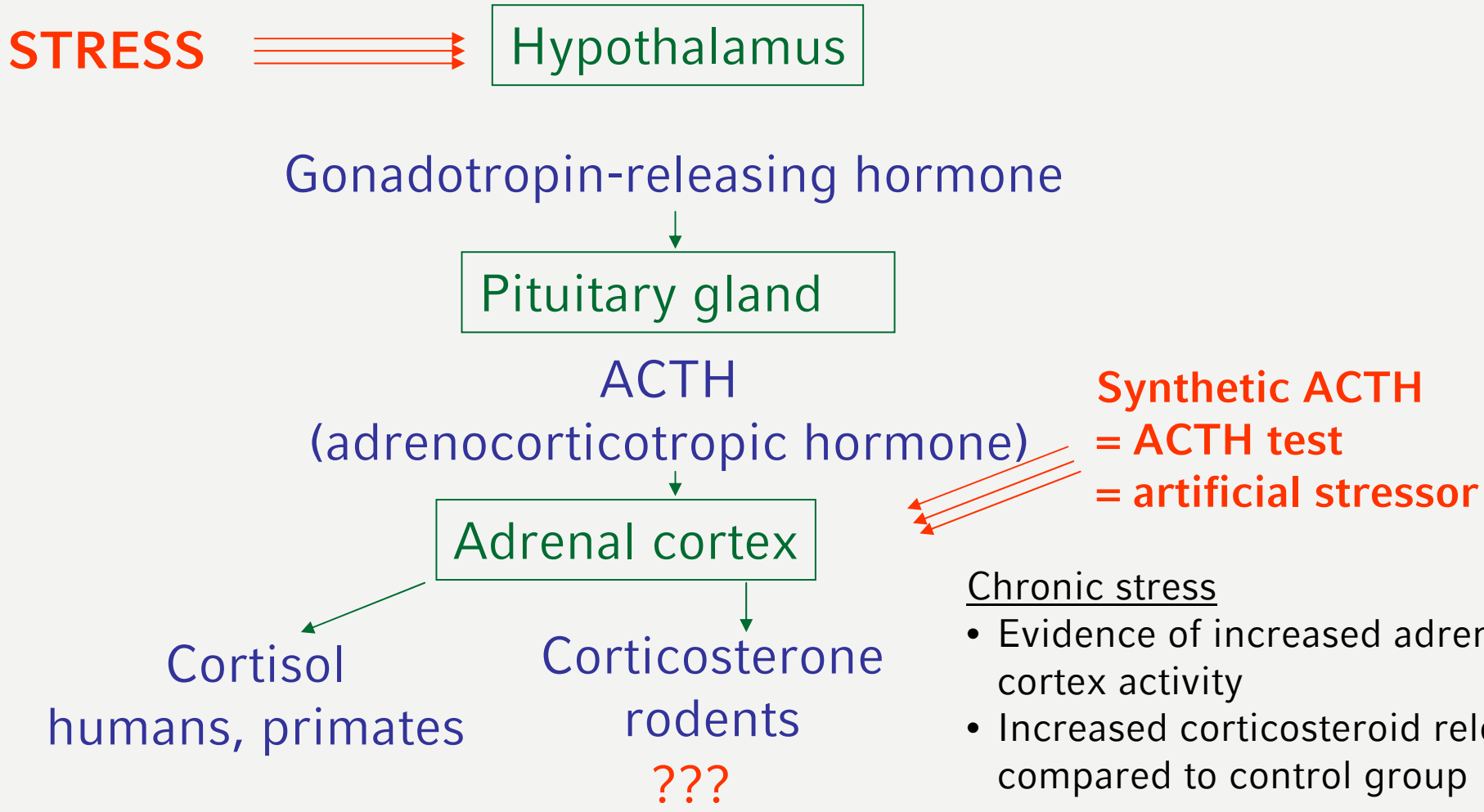
- Exposure of human leukocytes to electromagnetic field (830 MHz): increased occurrence of mitosis errors and chromosomal abnormalities (Mashevich et al., 2003)
- Irradiation of male rabbits with 2.1 GHz and 5 mW/cm^2
  ➡ Number but not function of T-lymphocytes reduced (Nageswari et al., 1991)

- Human blood cells exposed to 1950 MHz: no detectable influence (Tuschl et al., 2006)
- in mice no influence of GSM exposure on the production of antibodies against ovalbumin (Nasta et al., 2006)

Literature

- WENZEL et al. (2002): Under the influence of a high-frequency electromagnetic field of mobile radio antennae the salivary cortisol concentrations of cattle increase more markedly and decrease more slowly after ACTH application compared to an unexposed control group

Literature: Stress system



Chronic stress

- Evidence of increased adrenal cortex activity
- Increased corticosteroid release compared to control group

Animals, materials and methods

- Approval by the Government of Upper Bavaria under the file reference
  Az: 55.2-1-54-2531-91-04
- Study period: February 2005 to January 2007
- 294 female Wistar-Rats

- Generation F0 = Progeny of the mating of generation F1
  Generation F0 was divided into generations F0a and F0b
- Generation F0a = subjected to longest exposure of approx. 55 weeks
- Generation F0b = exposed for approx. 23 weeks
- Generation F1 = Progeny of the mating of generation F0a
  Generation F1 was not studied
- Generation F2 = Progeny of the mating of generation F1
  Exposed since procreation. Postpartum exposure approx. 23 weeks

Animals, materials and methods

	GSM (Global System for Mobile Communications, 900 MHz)	UMTS (Universal Mobile Telecom-munication System, 1966 MHz)	Sham (control)
F0a (84 animals)	23	26	35
F0b (84 animals)	27	24	33
F2 (126 animals)	42	42	42

- Double-blind study
- 294 female albino WISTAR rats, RjHAN strain
- Original animals supplied when aged 9 weeks
- The animals relevant for the main study were born in the chambers from our own breeding stock
- Animals marked using microchip transponder system (Alvic Transponder, ALVETRA GmbH)

Animals, materials and methods

- 3 specific fully air-conditioned high-frequency exposure chambers
  - GSM (900 MHz)
  - UMTS (1966 MHz)
  - Sham exposure

Animals, materials and methods

Immune system:

- Study day 0 — Blood sampling for control value
- Study day 7 and 28 — Immunization and boostering with the antigen
  - → Ovalbumin (OVA)
  - → Chicken immunoglobulin Y (IgY)
  - → Lipopeptide adjuvant (Pam3CysSerLys4)

- Study days 14 and 35
  - → Blood sampling to verify the success of vaccination and the increase in the specific antibody titer
  - → Determination of the antibodies anti-OVA, anti-IgY, total IgG by means of specific Enzyme-Linked Immunosorbent Assay (ELISA)

Animals, materials and methods

Immune system:

- Blood sampling from the awake animal
- Fixation of the animals in the restrainer
- approx. 0.5 ml blood per animal

Immunization and boostering:

- per animal 100 µg OVA, 100 µg IgY and 100 µg Pam_3-Cys-Ser-$(Lys)_4$ injected s.c. into the flank as adjuvant in 0.2 ml PBS (phosphate-buffered saline)

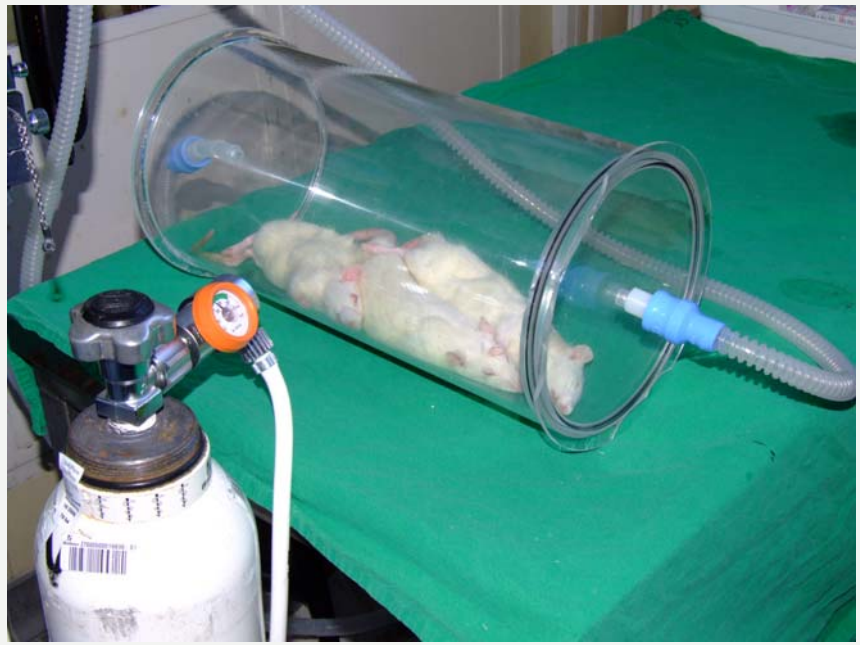
Determination of the antibodies anti-OVA, anti-IgY, total IgG by means of specific Enzyme-Linked Immunosorbent Assay.

Animals, materials and methods

Stress system:

- Blood sampling from the anesthesized animal
- always at the same time (start: 9:00 am)
- Inhalation anesthesia using isoflurane (Isoba®, Essex Tierarznei Munich) and oxygen as carrier substance

Induction of anesthesia:
all animals from one experimental cage (n=3) are placed in a plexiglass tube primed with the anesthetic

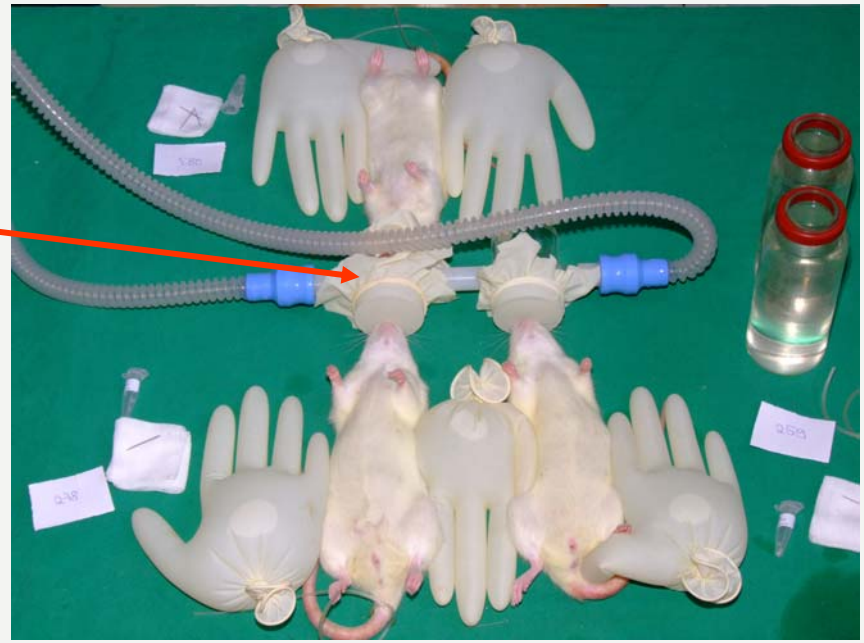


Animals, materials and methods

Stress system:

- Blood sampling from the anesthesized animal
- always at the same time (start: 9:00 am)
- Inhalation anesthesia using isoflurane (Isoba®, Essex Tierarznei Munich) and oxygen as carrier substance.

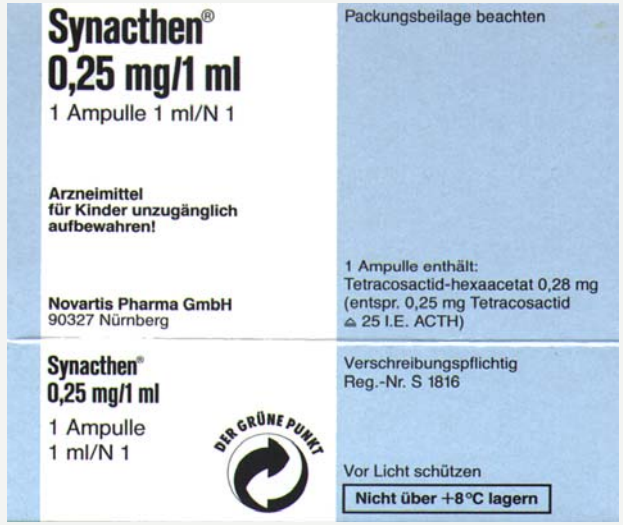
Maintenance of anesthesia:
supply to the animals via individual plexiglass head chambers



Animals, materials and methods

Stress system:

- Blood sampling from the tail veins (Vv. coccygeae)
- Synthetic ACTH (Synacthen®, Novartis Pharma GmbH)
  - 100 µg/kg body weight i.p.



Blood sampling

- 1st blood sample at time t0 prior to ACTH application
- immediately followed by ACTH application
- all other samples were taken 15 (t15), 30 (t30), 45 (t45), 60 (t60), 90 (t90) and 120 (t120) minutes after ACTH application.
  Corticosterone was also measured using ELISA.

Statistical evaluation

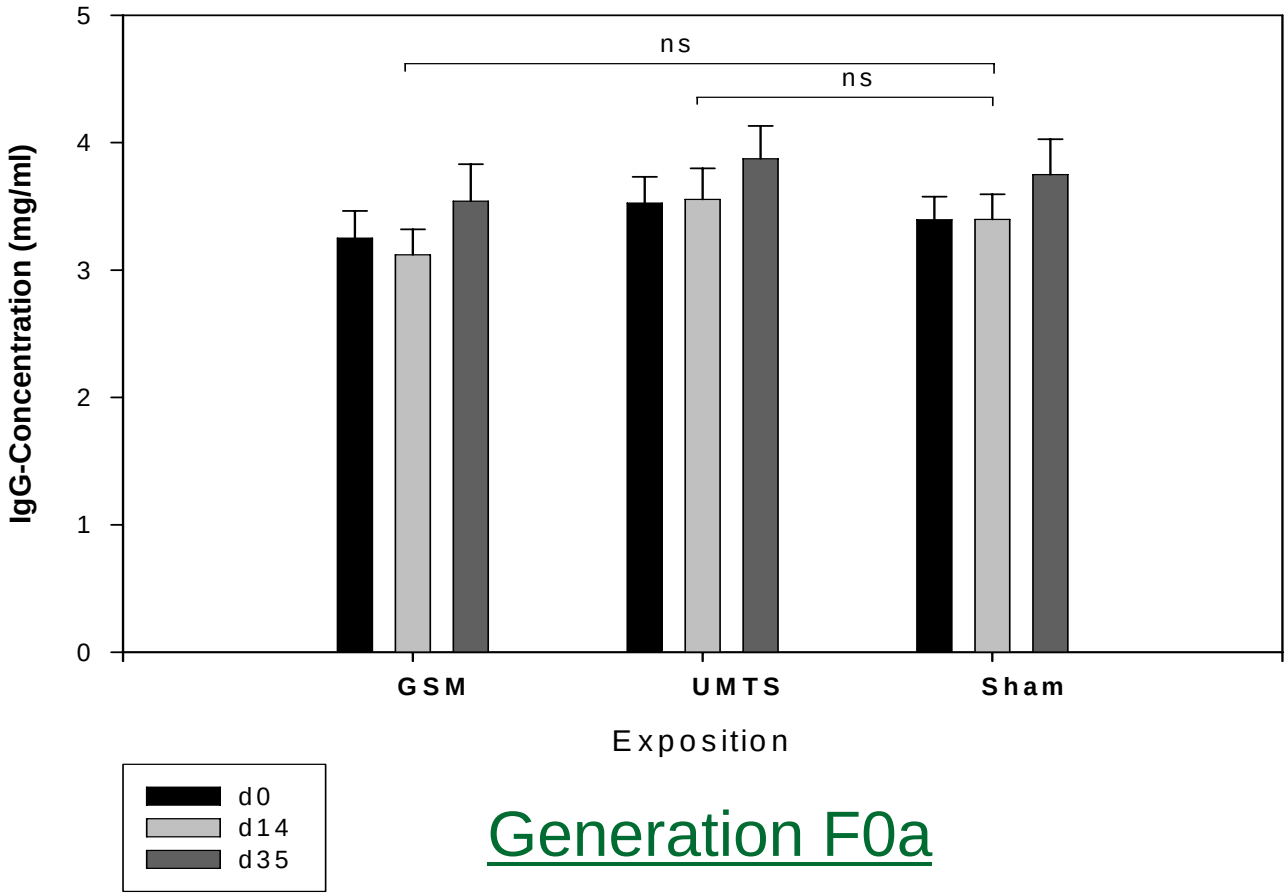
STA|B|LAB Statistisches Beratungs Labor

In cooperation with the Institute for Statistics of LMU Munich

- Statistical tests using SPSS 14
- the probability of error was rated as significant with $p < 0.05$
- the diagrams were created using the program SigmaPlot 9.0
  - they always contain mean value and SEM (standard error of the mean)

Results: Immune system

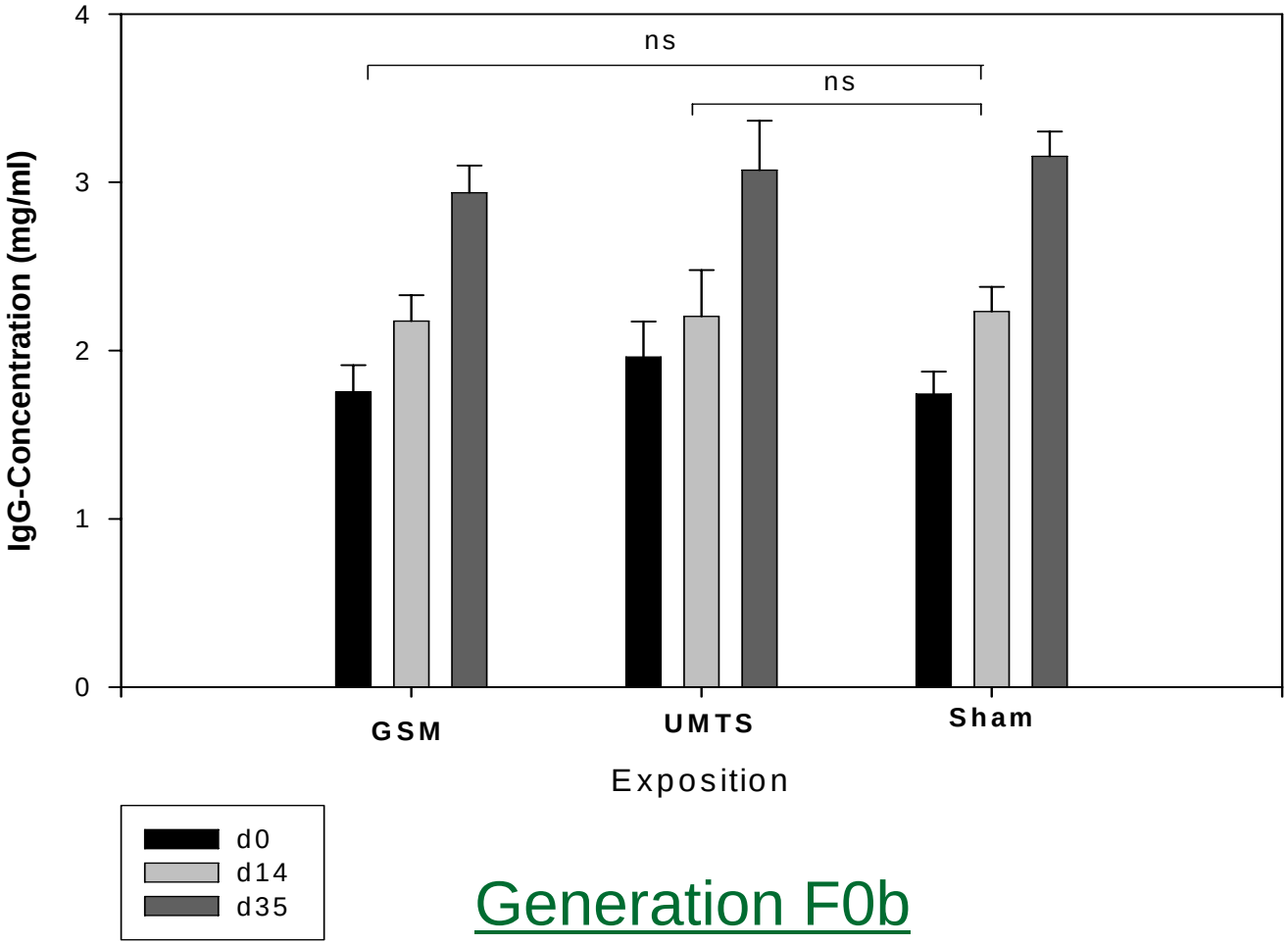
Total IgG concentration



Generation F0a

- as expected, the total IgG concentration increases significantly ($p<0.0001$) from day 0 (3.25 ± 0.21 mg/ml) via day 14 up to day 35 (3.54 ± 0.29 mg/ml).
- this effect is an organism's anticipated reaction to immunization.

Results: Immune system



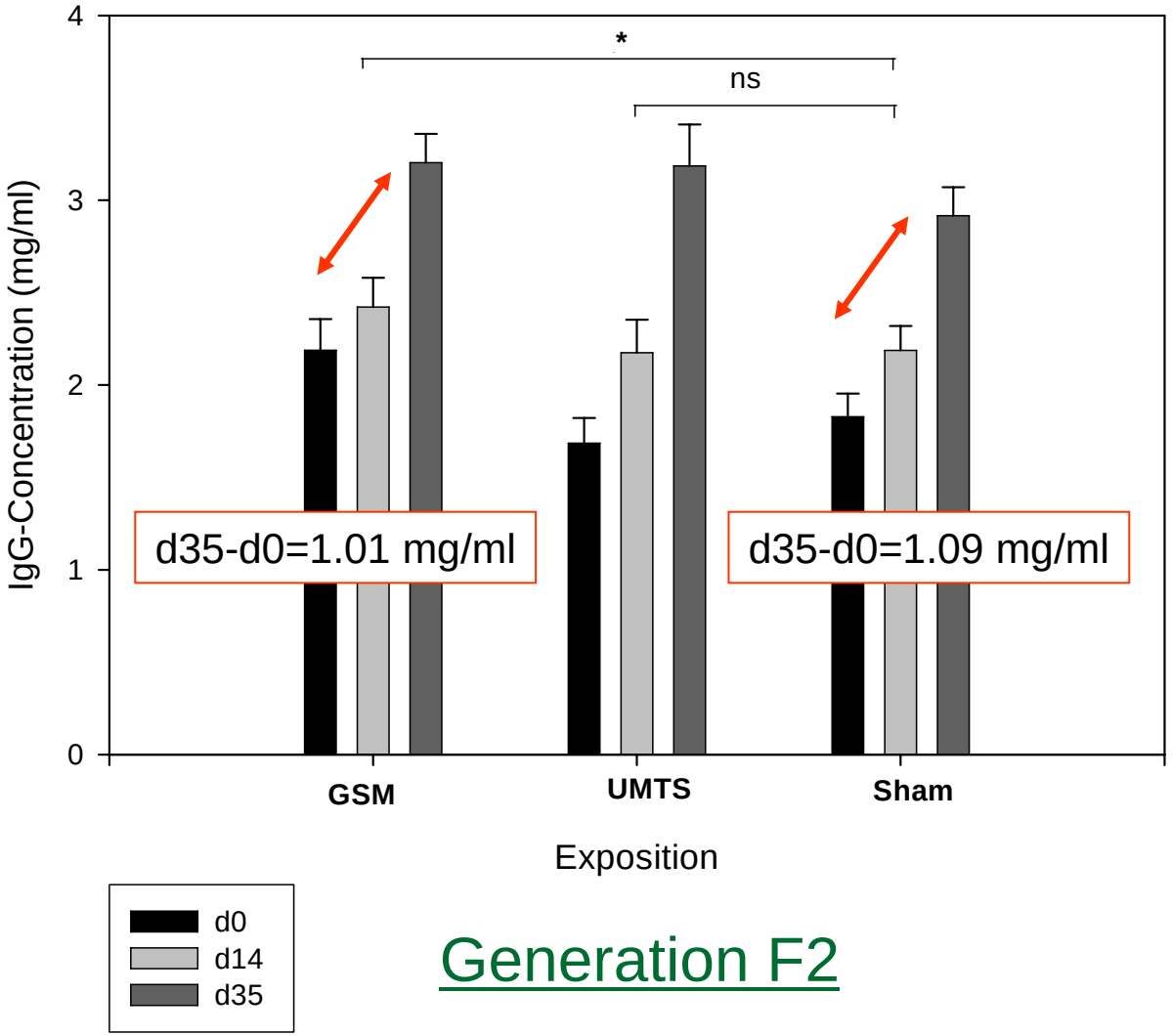
Generation F0b

- as expected, the total IgG concentration increases significantly ($p<0.0001$) from day 0 (3.25 ± 0.21 mg/ml) via day 14 up to day 35 (3.54 ± 0.29 mg/ml)
- this effect is an organism's anticipated reaction to immunization

In summary:

- no detectable influence of the type of exposure on the total serum IgG concentrations both in generation F0a and in generation F0b

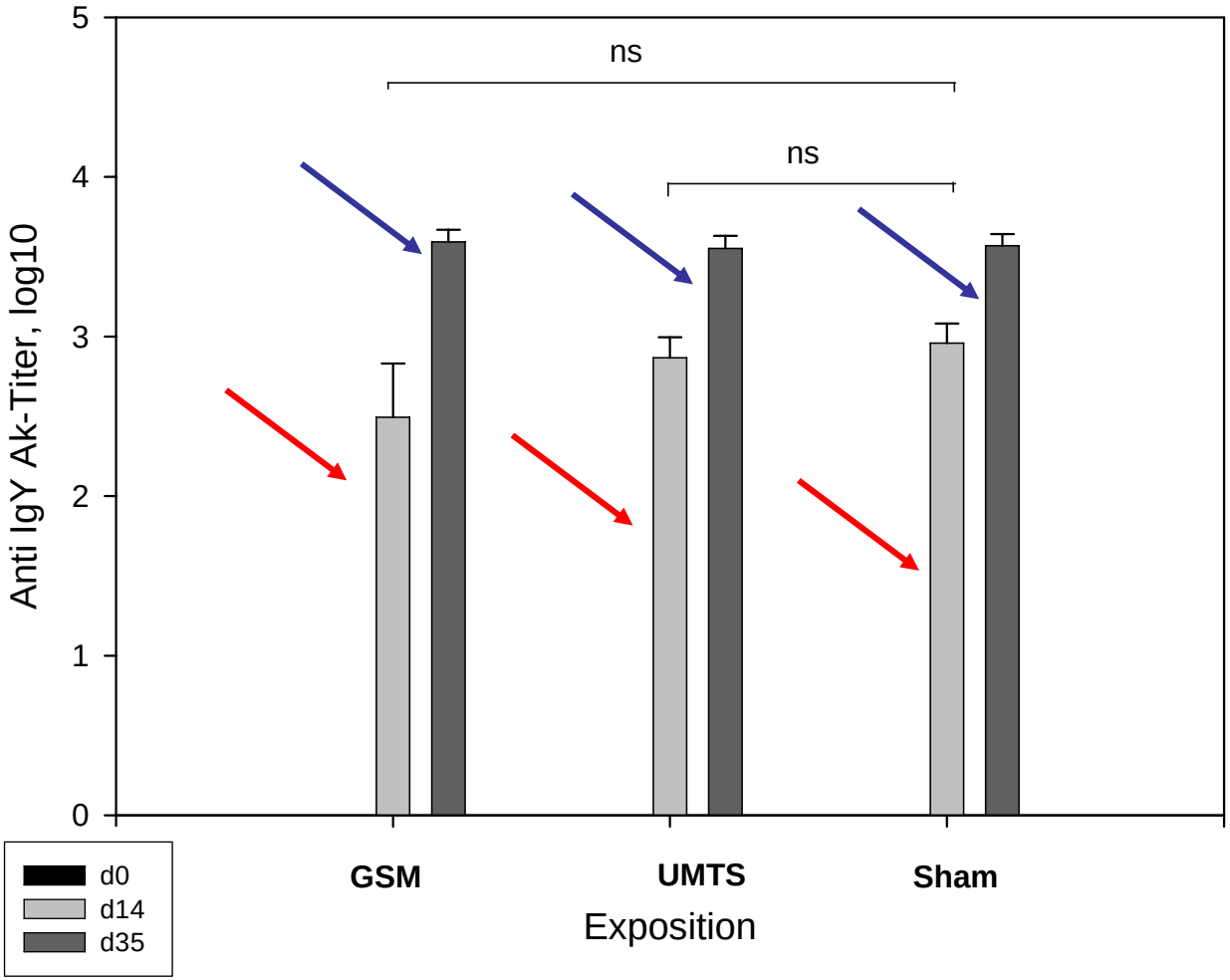
Results: Immune system



Generation F2

- in generation F2 the total IgG concentrations of the GSM group are significantly higher than in the control group (*p=0.04)
- however, the difference between the baseline level (day 0) and day 35 after immunization is the same

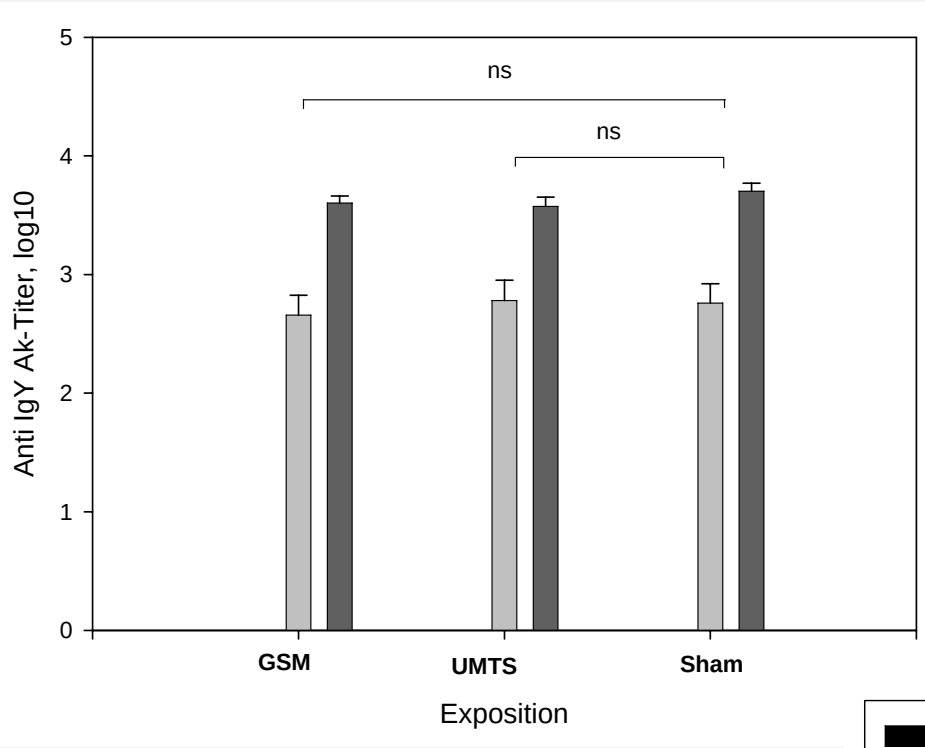
Results: Immune System

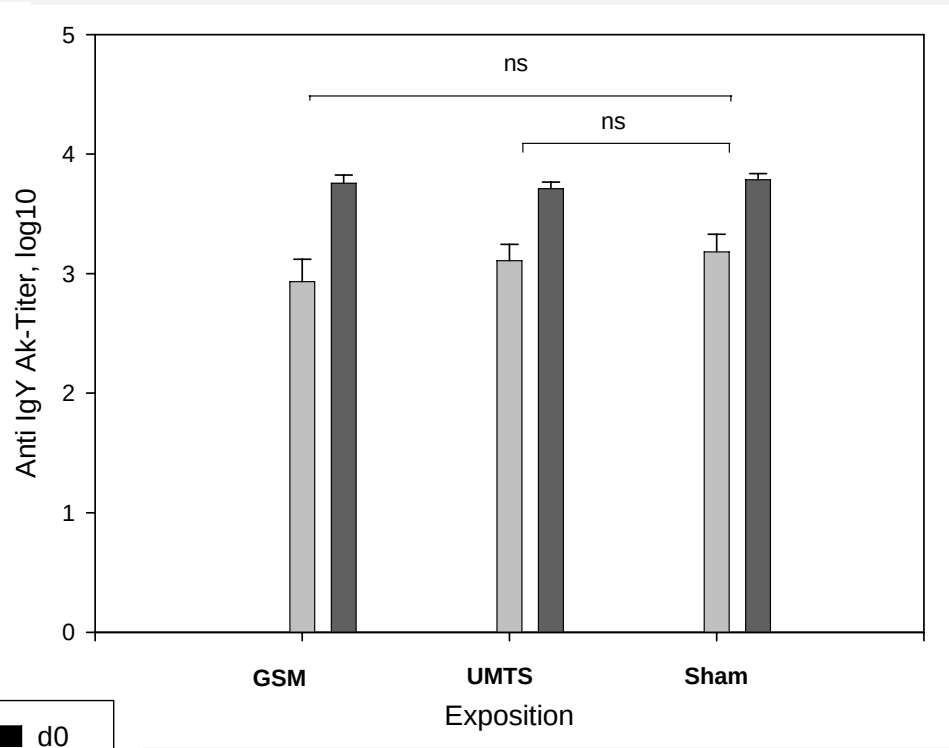


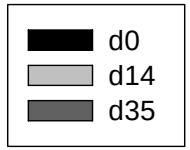
Anti-IgY: Generation F0a

- following immunization with IgY specific antibodies were already detectable in all generations after basic immunization on day 14
- due to the booster reaction the antibody titers (day 35) increased significantly in all animals ($p<0.0001$)
- as expected, no specific antibodies were detectable on day 0 prior to immunization

Results: Immune System







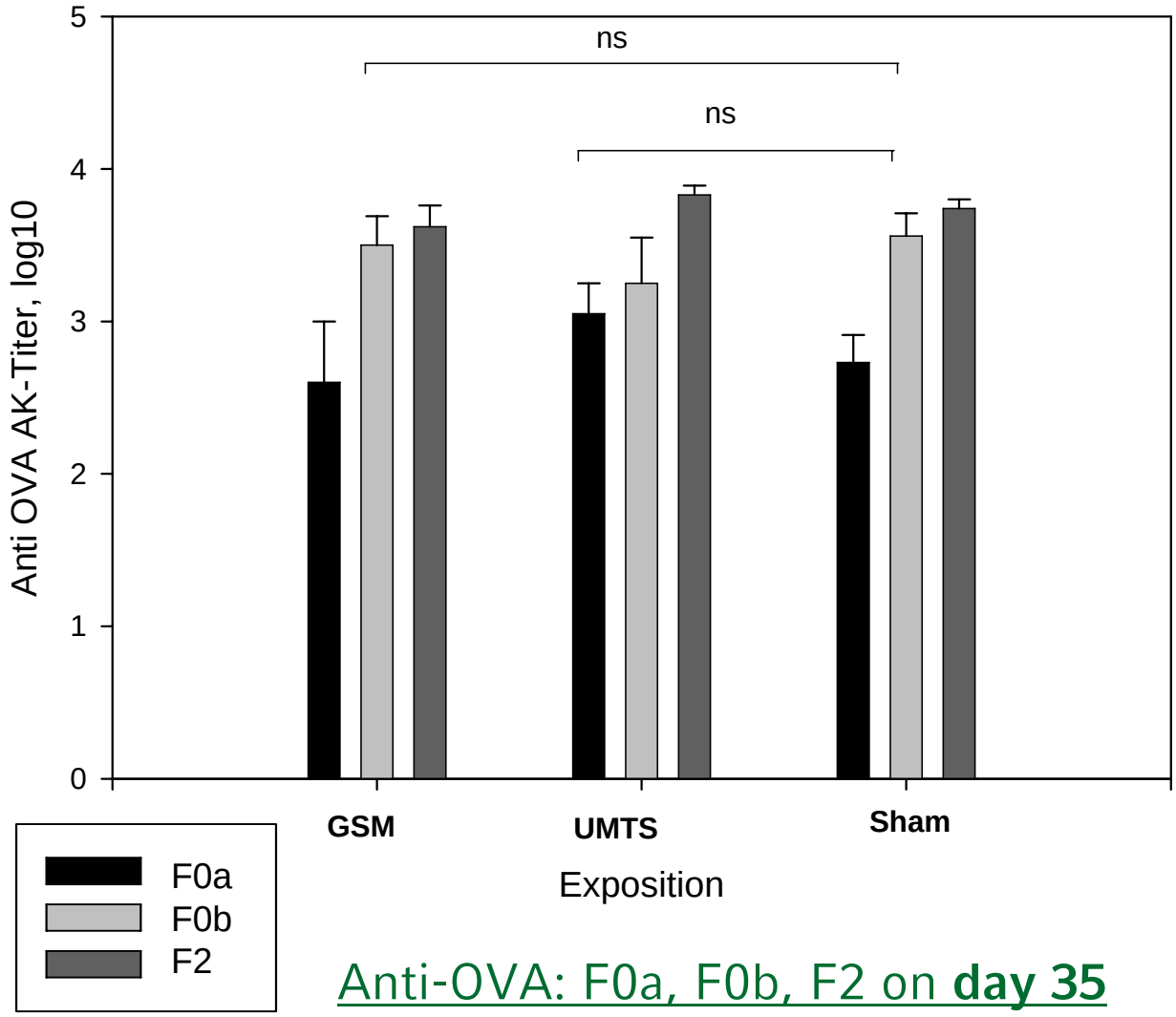
Anti-IgY: Generation F0b

Anti-IgY: Generation F2

In summary:

no detectable influence of the type of exposure on the Anti-IgY concentrations in generation F0a, F0b and F2

Results: Immune system



Anti-OVA: F0a, F0b, F2 on **day 35**

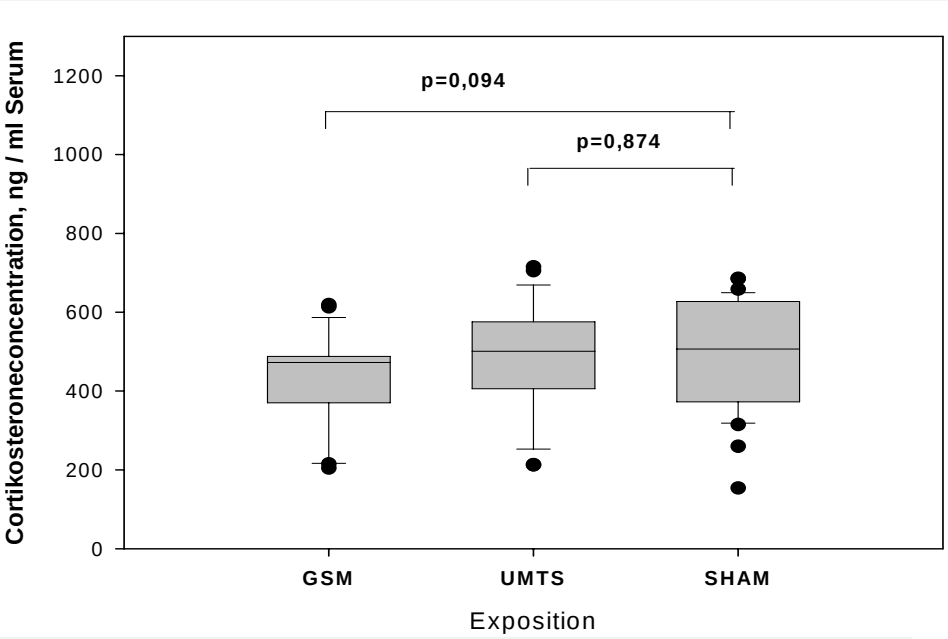
- following immunization with OVA no specific antibodies were detectable on day 14 after basic immunization on day 0
- specific antibody titers were not detectable in any group until the booster reaction (day 35), ($p<0.0001$)
- as expected, no specific antibodies were detectable on day 0 prior to immunization either

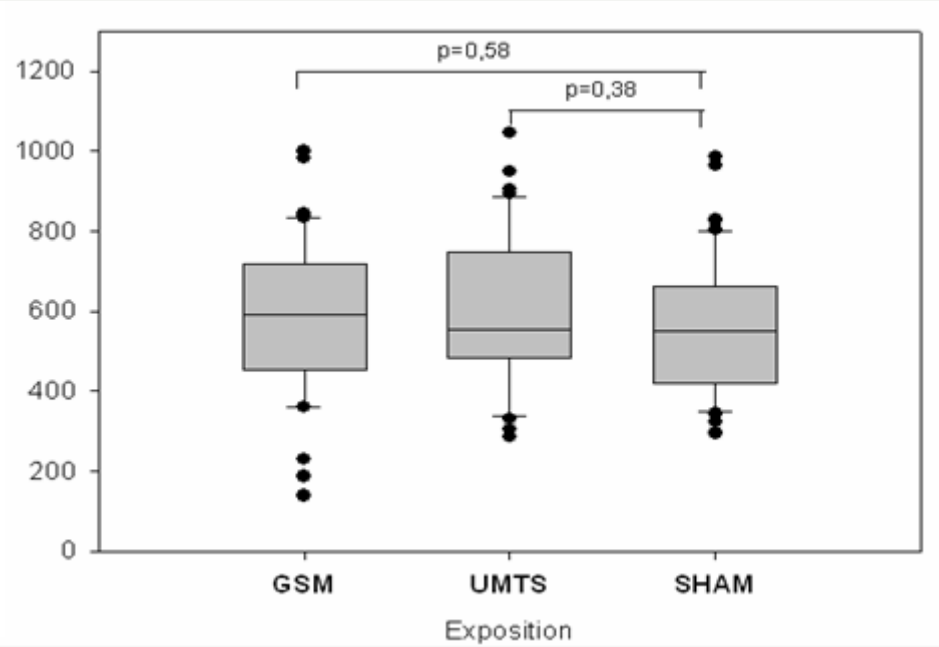
In summary:

no detectable influence of the type of exposure on the Anti-OVA concentrations in generation F0a, F0b and F2

Results: Stress system

Corticosterone basal concentration (t0):



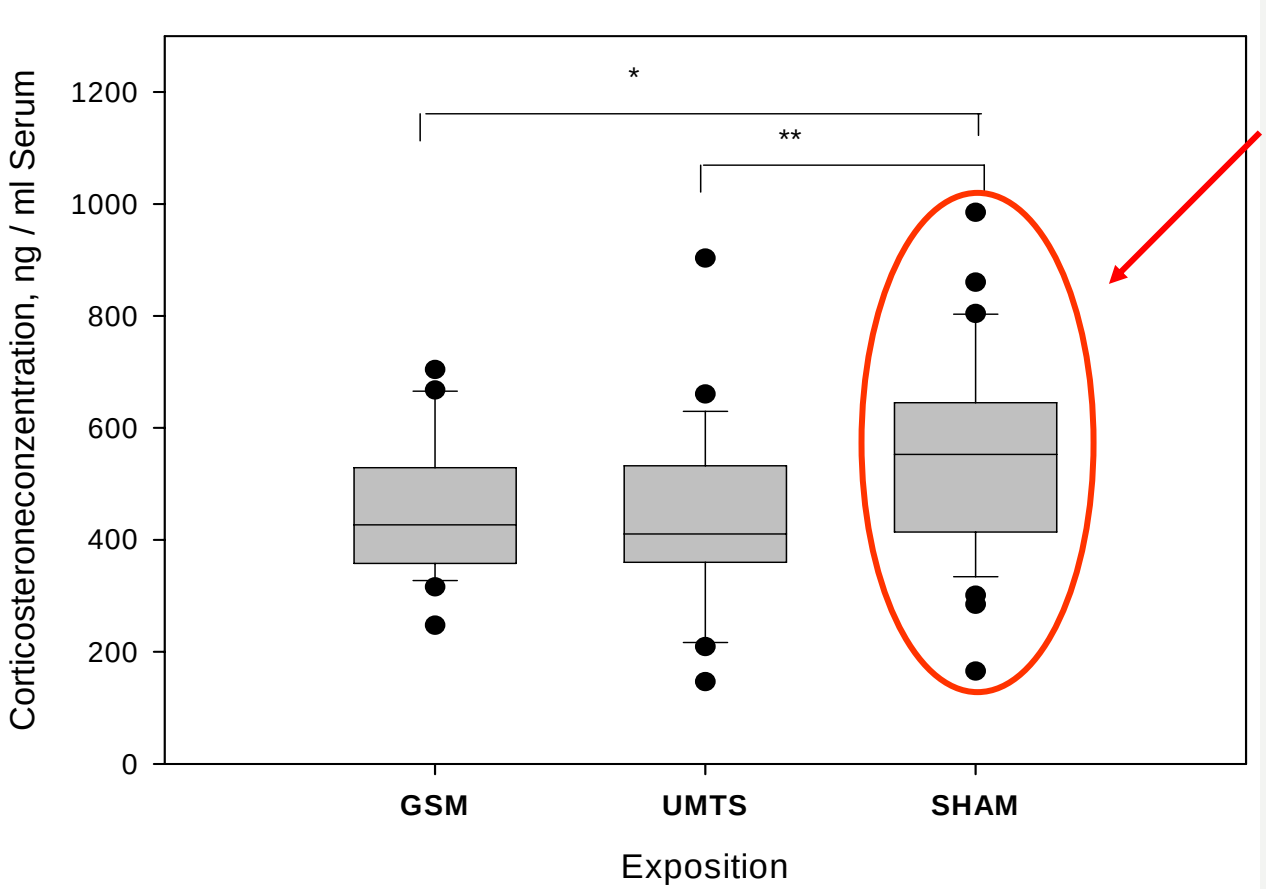


Generation F0a
n = 84, 23 GSM, 26 UMTS and 35 Sham animals

Generation F2
n = 124, 41 GSM, 42 UMTS and 41 Sham animals

Results: Stress system

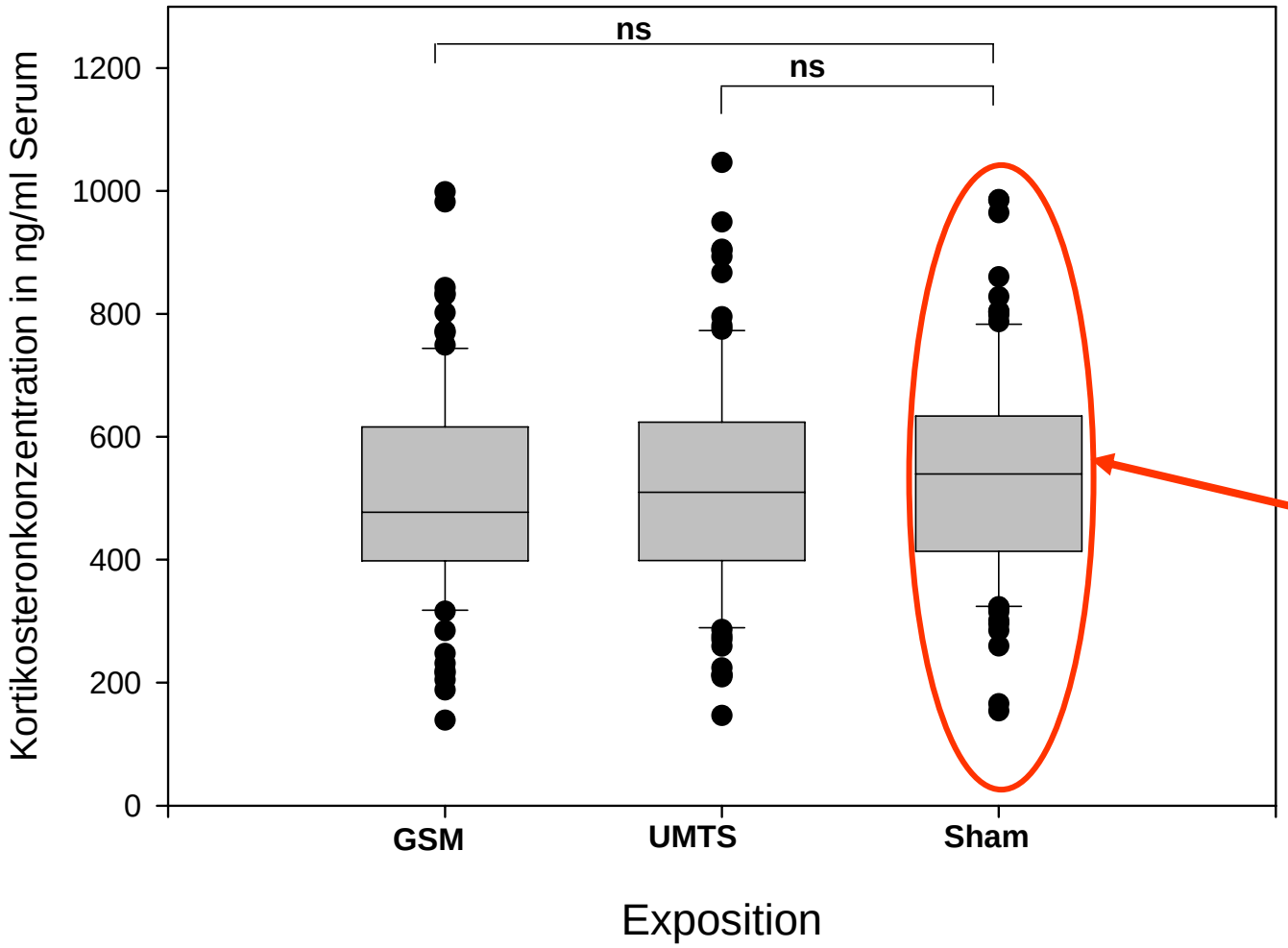
Corticosterone basal concentration (t0): Generation F0b



- the basal concentrations of the sham exposed animals are significantly higher than those of the GSM (*p=0.015) and of the UMTS exposed (**p=0.005) animals

Results: Stress system

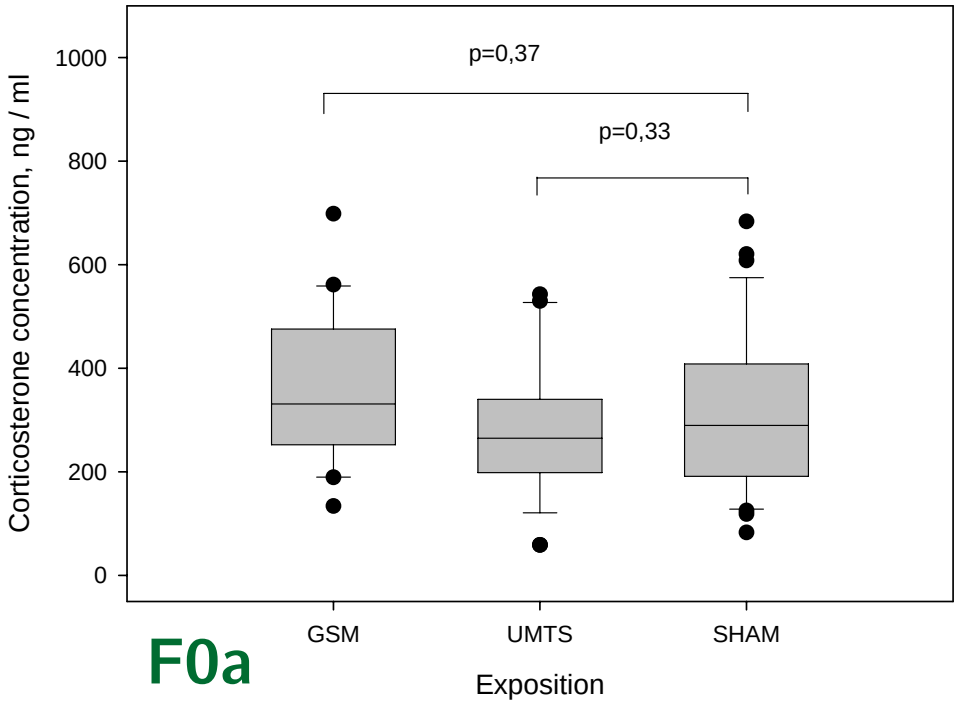
Comparison of corticosterone basal concentrations irrespective of the generation



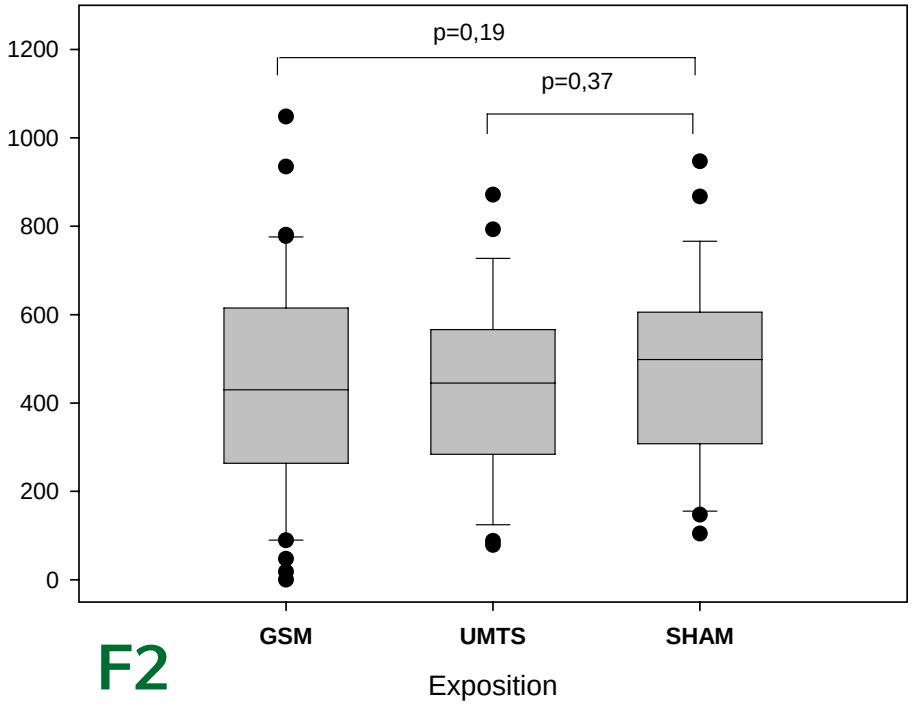
- distribution of the baseline concentration as a function of exposure is almost the same in all 3 groups
- no significant differences
- the values responsible for the significant changes in generation F0b are all found in the collective

Results: Stress system

Difference in corticosterone concentrations between maximum and basal value



F0a



F2

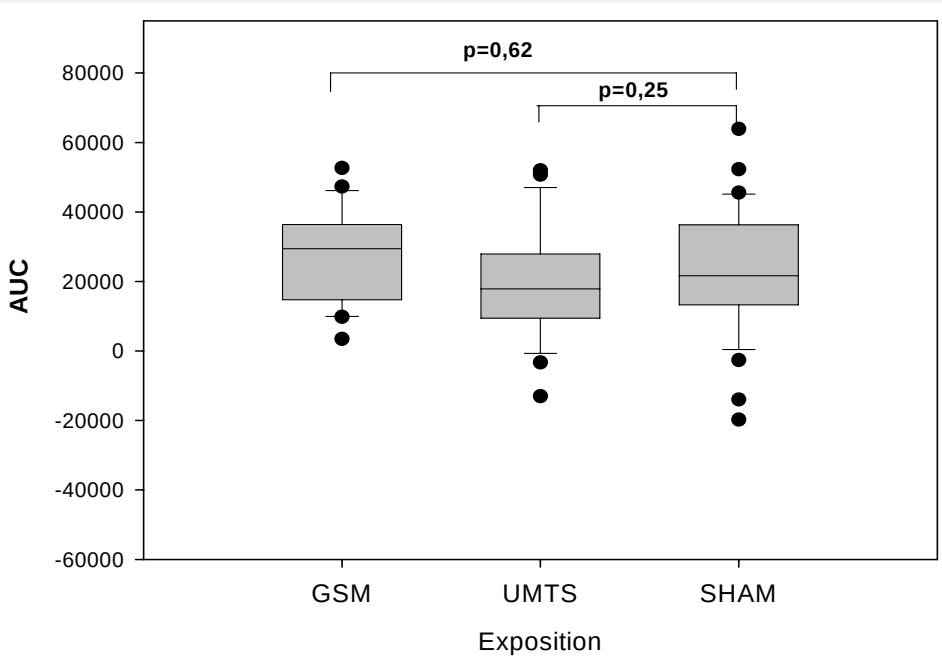
Time to reach maximum value:

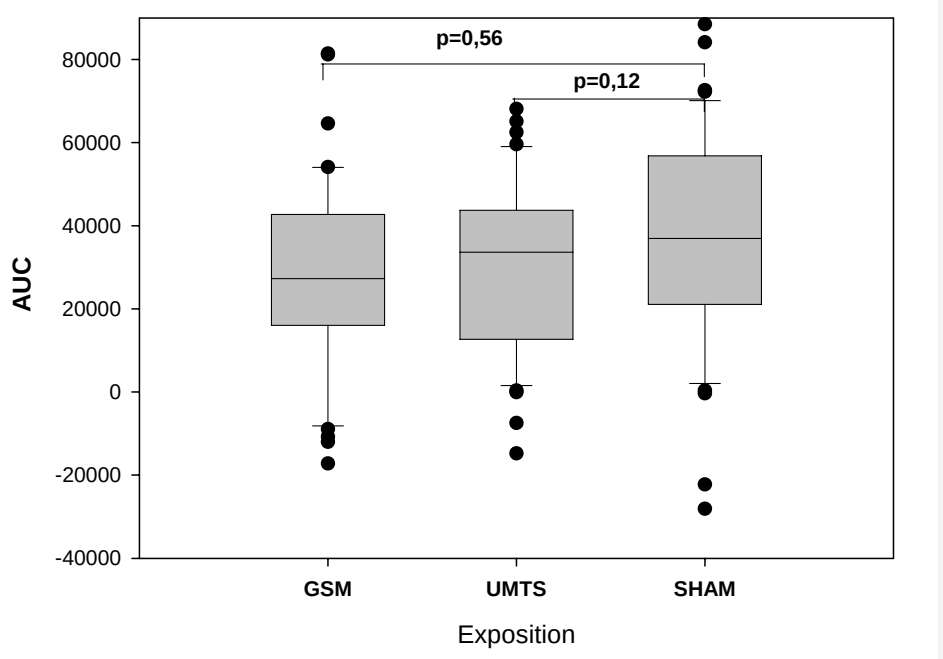
F0a	86,6 min
F0b	78,0 min
F2	86,5 min

} n.s

Results: Stress system

Area under the Curve (AUC)





F0a **F2**

The AUC in this case is the area defined on the one hand by the curve of the corticosterone concentration, and on the other hand by a parallel to the x axis through the y axis intersection (serum corticosterone concentration at time t0).

Summary and discussion of results

1. In **generation F2** the total IgG concentrations are significantly higher than in the control group (p=0.04).
   - however, the difference between the baseline level (day 0) and day 35 after immunization is the same.
   - this difference was not confirmed by the specific antibody titers
   - as generation F0b was exposed analogously the results of generation F2 were not confirmed

2. There is no detectable influence of exposure with regard to IgY and OVA

Summary and discussion of results

3. In order to rule out any effect of manipulation despite anesthesia, such as the duration of blood sampling, on the stress reaction, this variable was recorded during each blood sampling procedure (in minutes and seconds), and any potential effect was statistically analyzed:
   - → overall, no influence of the duration of blood sampling on the results is detectable

Summary and discussion of results

4. The corticosterone basal concentrations both of the GSM- and of the UMTS-exposed animals of **generation F0b** are significantly lower than the basal concentrations of the Sham-animals:
    - however, in the comparison of corticosterone basal concentrations irrespective of the generation the values responsible for the significant changes in generation F0b are all found in the collective
    - the distribution of the basal concentrations as a function of exposure (irrespective of the generations) is almost identical in all three groups so that the joint evaluation of representation of the variation range yields no significant differences between the basal concentrations

Summary and discussion of results

5. No influence of the exposure on the
   - corticosterone basal concentration,
   - AUC,
   - for the Difference in corticosterone concentrations between maximum and basal value
   - and for the time to reach the maximum corticosterone concentration after ACTH-injection

were found for the long-time exposed generation F0a and the „short-time“-exposed generation F2

Fazit

The results presented therefore permit the conclusion that chronic high-frequency magnetic fields characteristic of GSM or UMTS ...

1. do not constitute a situation of permanent stress
2. and have no relevant influence on measured immune parameters

...in rats.

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Thank you for your Attention!