Medizinsche Fakultät

Institut für Medizinische Psychologie und Verhaltensimmunbiologie

UNIVERSITÄT DUISBURG ESSEN

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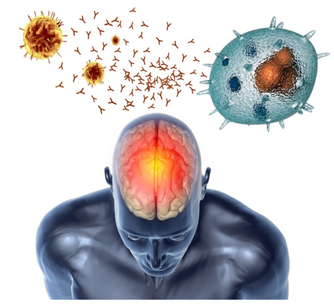


Repeated recall, extinction and reconsolidation in behaviorally conditioned immunosuppression

Introduction

Behavioral (classical Pavlovian) conditioning can be utilized to alter immune system functions. Behaviorally conditioned immunosuppression using the immunosuppressive drug cyclosporine A (CsA) has been documented in rodents and humans, highlighting the bidirectional communication between the central nervous system and the peripheral immune system. Potential applications of such learned immunosuppression in clinical situations can be linked to the placebo response which involves conditioning components as well as expectation-induced mechanisms. It is unclear however, whether and to what extent the learned immune response can be repeatedly recalled over time and finally whether conditioned immunosuppression can be controlled by extinction and reconsolidation procedures.

Overview

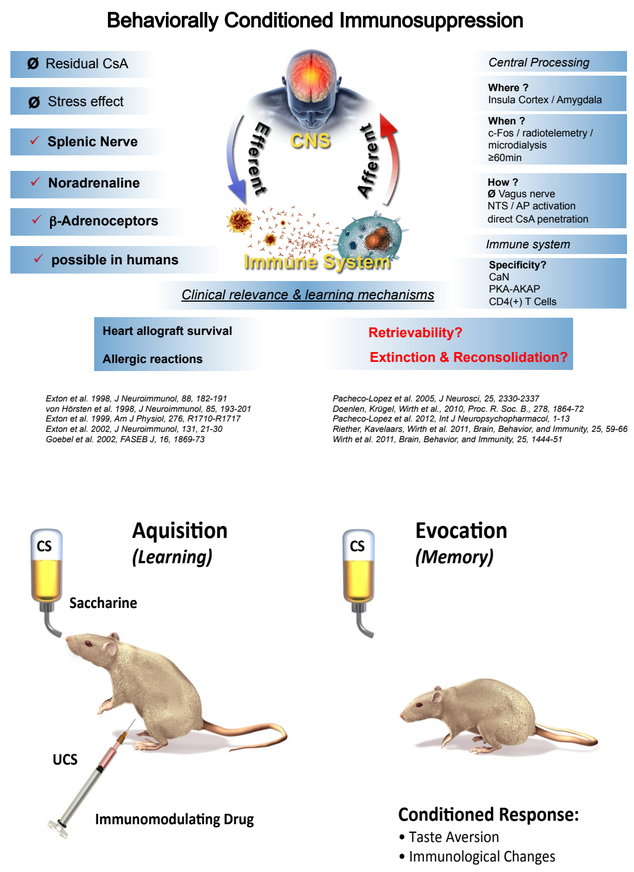


Fig. 1: Basic concepts of behaviorally conditioned immunosuppression.

During the *acquisition phase* the gustatory stimulus (saccharine; conditioned stimulus, CS) is paired with an immunomodulating agent (unconditioned stimulus, US; e. g. cyclosporine A). The CS / US association is stored in the brain and can be subsequently recalled during an *evocation phase*. Through re-exposure to the CS, stored information is re-enlisted and transmitted via efferent pathways and induces behavioral (taste aversion) and immunological changes mimicking the actual effect of the drug that served as the US.

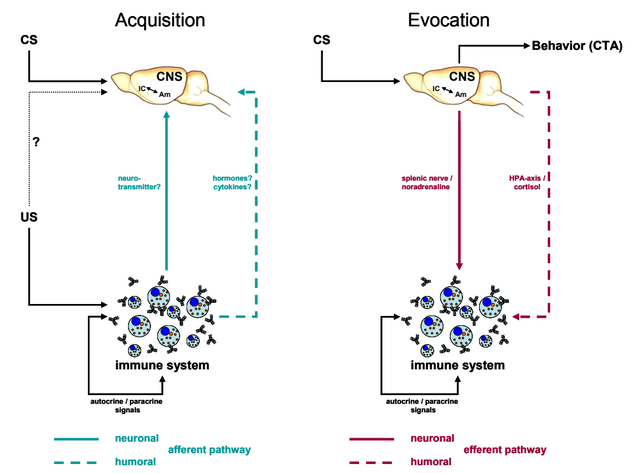
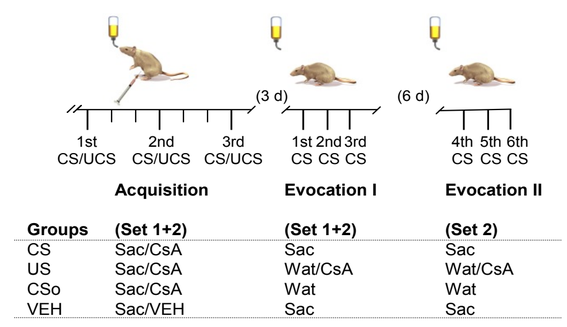


Fig. 2: Mechanisms of behaviorally conditioned immunosuppression.

In behaviorally conditioned immunosuppression using cyclosporine A as an unconditioned stimulus there are several possible afferent pathways for CsA mediated effects on the brain. CsA might alter immune functions, with those alterations being transmitted via afferent humoral pathways and neuronal pathways, or could directly act on the brain, permeating the blood brain barrier. Which of these afferent routes is activated after i. p. CsA injection is still undetermined. Using lesioning experiments, the insular cortex (IC) and the amygdala (Am) have been identified as two brain regions directly involved in neuro-immune processing of behavioral immunoconditioning. At evocation, the sympathetic nervous system seems to be the major pathway mediating conditioned immunosuppressive effects in saccharine-CsA conditioning paradigms. Behaviorally conditioned immunosuppression of IL-2 and IFN-γ production in this model has been shown to be dependent on calcineurin inhibition through noradrenaline and β-adrenoceptor mediated mechanisms.

Repeated recall of behaviorally conditioned immunosuppression



Groups	(Set 1+2)	(Set 1+2)	(Set 2)
CS	Sac/CsA	Sac	Sac
US	Sac/CsA	Wat/CsA	Wat/CsA
CSo	Sac/CsA	Wat	Wat
VEH	Sac/VEH	Sac	Sac

Fig. 3: Experimental design: repeated recall of behaviorally conditioned immunosuppression.

After an acquisition phase with 3 CS/UCS trials, 3 days later rats were either re-exposed to the CS just once (*evocation I, set 1*) or in addition re-exposed to the CS a second time after a break of 6 days (*evocation II, set 2*).

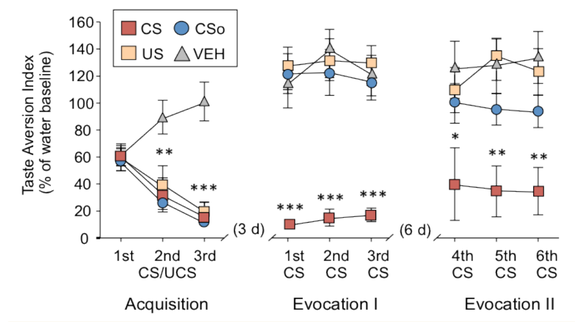


Fig. 4: Repeated recall: conditioned taste aversion data.

Since the taste avoidance data did not differ between groups in set 1 and set 2, these data were pooled (n=18/group for acquisition and *evocation I*). During the first acquisition trial animals in all groups showed a neophobic response to the CS that normalized in the vehicle treated animals (△VEH) after the 3rd acquisition trial. In contrast, the conditioned (■CS), the conditioned not-evoked (●CSo) as well as animals in the pharmacological control group (□US) displayed pronounced taste aversion against the CS. When rats in the conditioned group (CS) were re-exposed to the saccharin they showed pronounced aversion against the taste stimulus not only after the first evocation (*evocation I*) but also during the 2nd evocation (*evocation II*). (n=9 in each single group). Bonferroni post-hoc tests; ***p<0.001; **p<0.01; *p<0.05. Data are shown as mean ±SEM.

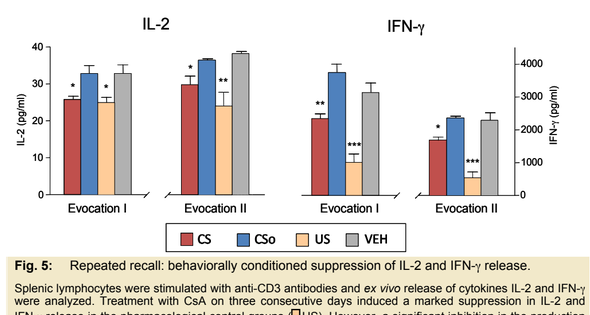


Fig. 5: Repeated recall: behaviorally conditioned suppression of IL-2 and IFN-γ release.

Splenic lymphocytes were stimulated with anti-CD3 antibodies and *ex vivo* release of cytokines IL-2 and IFN-γ were analyzed. Treatment with CsA on three consecutive days induced a marked suppression in IL-2 and IFN-γ release in the pharmacological control groups (□US). However, a significant inhibition in the production of both cytokines was observed after the 1st (*evocation I*) as well after the 2nd evocation (*evocation II*) in the conditioned animals (■CS) compared to the conditioned but not evoked rats (●CSo) and the vehicle control (△VEH). Bonferroni post-hoc tests; ***p<0.001; **p<0.01; *p<0.05. Data are shown as mean ±SEM.

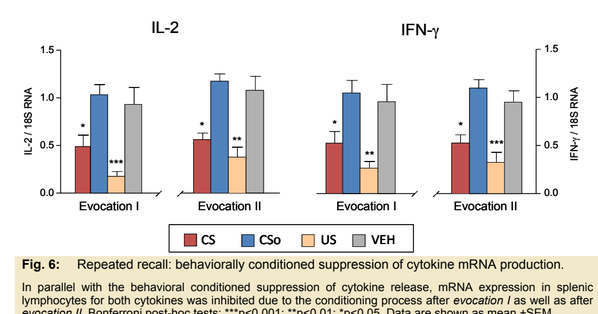


Fig. 6: Repeated recall: behaviorally conditioned suppression of cytokine mRNA production.

In parallel with the behavioral conditioned suppression of cytokine release, mRNA expression in splenic lymphocytes for both cytokines was inhibited due to the conditioning process after *evocation I* as well as after *evocation II*. Bonferroni post-hoc tests; ***p<0.001; **p<0.01; *p<0.05. Data are shown as mean ±SEM.

Extinction & Reconsolitation of behaviorally conditioned immunosuppression

Groups	CS -UCS Morning	CS -UCS Evening	CS Morning	CS Evening
Extinction (CSext)	Sac/CsA	Wat	Sac	Wat
Reconsolidation (CSrec)	Sac/CsA	Wat	Sac/CsA (low)	Wat
Non -contingent reconsolidation (CSncrec)	Sac/CsA	Wat	Sac	Wat/CsA (low)
CsA -treated (US)	Sac/CsA	Wat	Wat/CsA	Wat
Sham conditioned (CSo)	Sac/CsA	Wat	Wat	Wat
Vehicle (VEH)	Sac/Veh	Wat	Sac	Wat

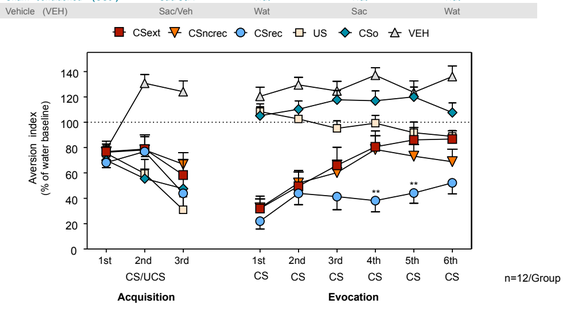


Fig. 7: Extinction and reconsolidation: conditioned taste aversion data.

During the first acquisition trial all groups showed a neophobic response to the CS that normalized in the vehicle treated animals (△VEH). In contrast, all other groups displayed conditioned taste aversion against the CS. When rats in the extinction group (■CSext) were re-exposed to the saccharin they showed pronounced aversion against the taste stimuli after the first evocation trials which was extinguished after the 5th and 6th evocation. Animals receiving a contingent sub-therapeutic (2 mg/kg) CsA injection together with the CS at evocation (○CSrec) displayed a constantly strong CTA compared to the CSext group during the last three evocation trials. Non-contingent pairing of the sub-therapeutic CsA injection with CS re-exposure (▽CSncrec) had no effect on CTA compared to the CSext group (*n=12* in each group). Bonferroni post-hoc tests; ***p<0.001; **p<0.01 vs. VEH; #p<0.05 vs. CSo; **p<0.01; *p<0.05 vs. CSext; Data are shown as mean ±SEM.

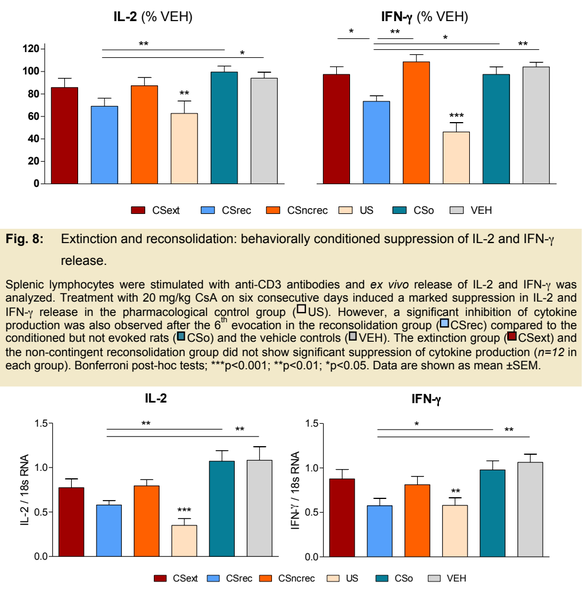


Fig. 8: Extinction and reconsolidation: behaviorally conditioned suppression of IL-2 and IFN-γ release.

Splenic lymphocytes were stimulated with anti-CD3 antibodies and *ex vivo* release of IL-2 and IFN-γ was analyzed. Treatment with 20 mg/kg CsA on six consecutive days induced a marked suppression in IL-2 and IFN-γ release in the pharmacological control group (□US). However, a significant inhibition of cytokine production was also observed after the 6th evocation in the reconsolidation group (□CSrec) compared to the conditioned but not evoked rats (■CSo) and the vehicle controls (□VEH). The extinction group (■CSext) and the non-contingent reconsolidation group did not show significant suppression of cytokine production (*n=12* in each group). Bonferroni post-hoc tests; ***p<0.001; **p<0.01; *p<0.05. Data are shown as mean ±SEM.

Fig. 9: Extinction and reconsolidation: behaviorally conditioned suppression of IL-2 and IFN-γ mRNA production

In parallel with the behaviorally conditioned suppression of cytokine release, mRNA expression in splenic lymphocytes for both cytokines was inhibited due to the reconsolidation process after the 6th evocation trial. The CSext (■) and CSncrec (■) groups showed no significant suppression of cytokine mRNA production compared to the control (■CSo and □VEH) groups (*n=12* in each group). Bonferroni post-hoc tests; ***p<0.001; **p<0.01; *p<0.05. Data are shown as mean ±SEM.

Conclusions

After pairing CsA as an unconditioned stimulus (US) with a novel taste as a conditioned stimulus (CS) at acquisition, sole presentation of the CS at evocation caused a significant inhibition of interleukin-2 (IL-2) and interferon-γ (IFN-γ) production by splenic T-cells in rats. More importantly however, an immunosuppressive effect of comparable magnitude was retrievable during a second, unreinforced evocation separated from the first evocation by an interval of 6 days. Furthermore, six CS re-exposures on consecutive days led to an extinction of conditioned immunosuppression that could be reinstalled by a reconsolidation procedure, applying contingent sub-therapeutic CsA injections together with the CS at evocation. Together these data demonstrate that conditioned immunosuppression can be repeatedly recalled and modulated by extinction and reconsolidation processes, thus implying new therapeutic approaches via conditioning procedures for the treatment of diseases where a suppression of the immune system is required

Timo Wirth
Dr. rer. medic.
Betreuer: Prof. Dr. Manfred Schedlowski