ENDOCANNABINOID-MEDIATED PLASTICITY AT INHIBITORY SYNAPSES ONTO MIDBRAIN DOPAMINE NEURONS AS A POSSIBLE MARKER OF VULNERABILITY TO ADDICTION

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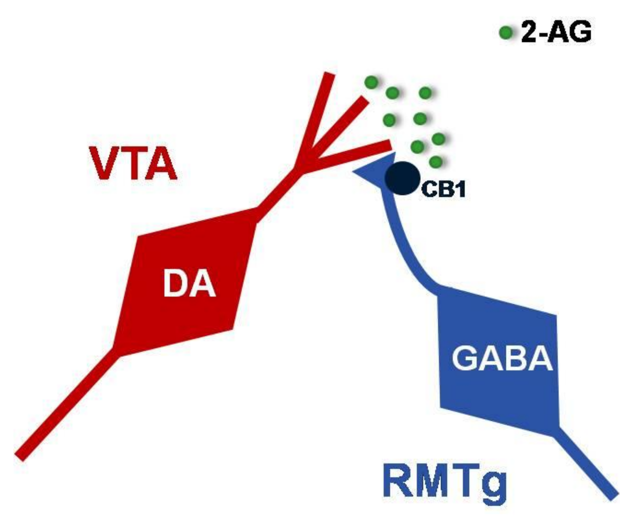


Addictive drugs share the properties of being self-administered by laboratory animals, and of activating the brain reward circuitry, which stems from the ventral tegmental area (VTA) where dopamine (DA) cells are located. The rostromedial tegmental nucleus (RMTg), located caudally to the VTA, is a GABAergic structure which sends inhibitory projections to the VTA DA neurons.

As a major brake for DA neurons, the RMTg is involved in the mechanisms of reward and aversion and in neurobiology of addiction. Endocannabinoids are a family of lipid signaling molecules that serve as retrograde messenger acting at cannabinoid receptor type 1 (CB1), which is localized on both excitatory and inhibitory synapses. One of the best characterized member of this family, 2-arachidonoylglycerol (2-AG), has been found in the VTA where it regulates different forms of synaptic plasticity providing a critical modulation of the DA neuron output and ultimately, of the systems driving and regulating motivated behaviors.

Sardinian alcohol-preferring (sP) and -non-preferring (sNP) rats are one of the few pairs of rat lines selectively bred for high and low alcohol preference and consumption, respectively. They have been widely used as model for studying predisposition to alcohol abuse.

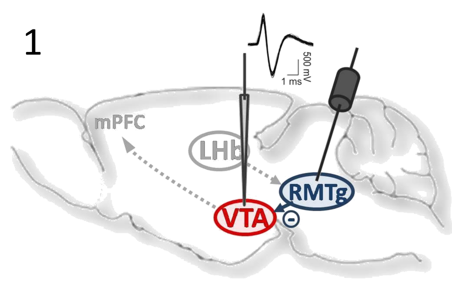
Here we investigated the functional role of endocannabinoid regulation of RMTg inputs to DA neurons in sP and sNP rats, in order to clarify the neurobiological basis of their alcohol preference and avoidance.

Methods

All experiments followed international guidelines on the ethical use of animals from the European Communities Council (EEC) (86/609/EEC).

IN VIVO ELECTROPHYSIOLOGY IN THE VTA

Male Sprague Dawley (SD), sP and sNP rats (250-350g); Urethane anesthesia: 1,3 g/Kg i.p. An extracellular recording electrode was inserted within the VTA (6.0 mm rostral-caudal, 0.3–0.6 mm medial-lateral, 7.0–8.0 mm ventral). To evaluate the inhibitory input arising from the RMTg to the VTA, a Formvar-coated stimulating stainless steel bipolar electrode (Ø 250 mm tip) was inserted in the ipsilateral RMTg (9.6 mm rostral-caudal, 0.8 mm medial-lateral, 7.0 mm ventral with an inclination of 20° anteroposterior on the coronal plane). Neurons located in The VTA were recorded and identified according to already published criteria such as firing rate <10 Hz, duration of action potential >2.5 ms, inhibitory responses to hindpaw pinching. Once a cell was selected, stimuli (0.5 mA) were delivered to the RMTg at 1.0 Hz.



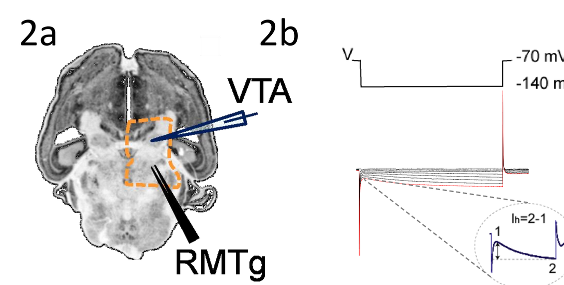
VTA SLICES PREPARATION

Male SD, sP and sNP rats (14-34 d) were anesthetized with halothane and killed. A block of tissue containing the midbrain was rapidly dissected and sliced in the horizontal plane (300 µm) (fig. 2a) with a vibratome in ice-cold low-Ca^{2+} solution containing (in mM): 126 NaCl, 1.6 KCl,1.2 NaH_2PO_4, 1.2 $MgCl_2$, 0.625 $CaCl_2$, 18 $NaHCO_3$, and 11 glucose). Slices were transferred to a holding chamber with artificial cerebrospinal fluid (ACSF, 37° C) saturated with 95% O_2 and 5% CO_2 containing (in mM): 126 NaCl, 1.6 KCl, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 2.4 $CaCl_2$, 18 $NaHCO_3$, and 11 glucose. Slices were allowed to recover for at least 1hr before being placed, in the recording chamber and superfused with the ACSF (34–36° C) saturated with 95% O_2 and 5% CO_2.

IN VITRO ELECTROPHYSIOLOGY

Cells were visualized with an upright microscope with infrared illumination. Whole-cell voltage-clamp experiments were made with electrodes filled with a solution containing (in mM): 144 KCl, 10 HEPES, 3.45 BAPTA, 1 $CaCl_2$, 2.5 Mg_2ATP, and 0.25 Mg_2GTP (pH 7.2-7.4, 275-285 mOsm). Experiments were begun only after series resistance had stabilized (typically 15-40 MΩ). Series and input resistance were monitored continuously on-line with a 5 mV depolarizing step (25 ms). Data were filtered at 2 kHz and digitized at 10 kHz.

DA neurons from the posterior VTA were identified by the presence of a large Ih current (>100 pA) that was assayed immediately after break-in, using a series of incremental 10 mV hyperpolarizing steps from a holding potential of -70 mV (fig. 2b). A bipolar stainless steel stimulating electrode was placed about 450 µm to the recording electrode (fig. 2a) and was used to stimulate at a frequency of 0.1 Hz.



Paired stimuli were given with an interstimulus interval of 50 ms, and the ratio between the second and the first IPSC was calculated and averaged for a 5 min baseline.

Each slice received only a single drug exposure. Drugs were applied in known concentrations to the superfusion medium. All the drugs were dissolved in DMSO. The final concentration of DMSO was < 0.01 %.

Depolarization-induced suppression of inhibition (DSI) protocol: the pulse used to evoke DSI was a 500 ms to 5 s step to +40 mV from -70 mV holding potential. The magnitude of DSI was measured as percentage of the mean amplitude of consecutive IPSCs after depolarization (acquired between 5 and 15 s after the end of the pulse) relative to that of five IPSCs before the depolarization.

DATA ANALYSIS

Averaged data from different experiments are presented as mean ± SEM. Statistical significance was assessed using the Student's t test one- or two-way analysis of variance (ANOVA) for repeated measures followed either by Dunnett's or t-test, where appropriate.

*P < 0.05, **P < 0.01, ***P < 0.001.

Summary

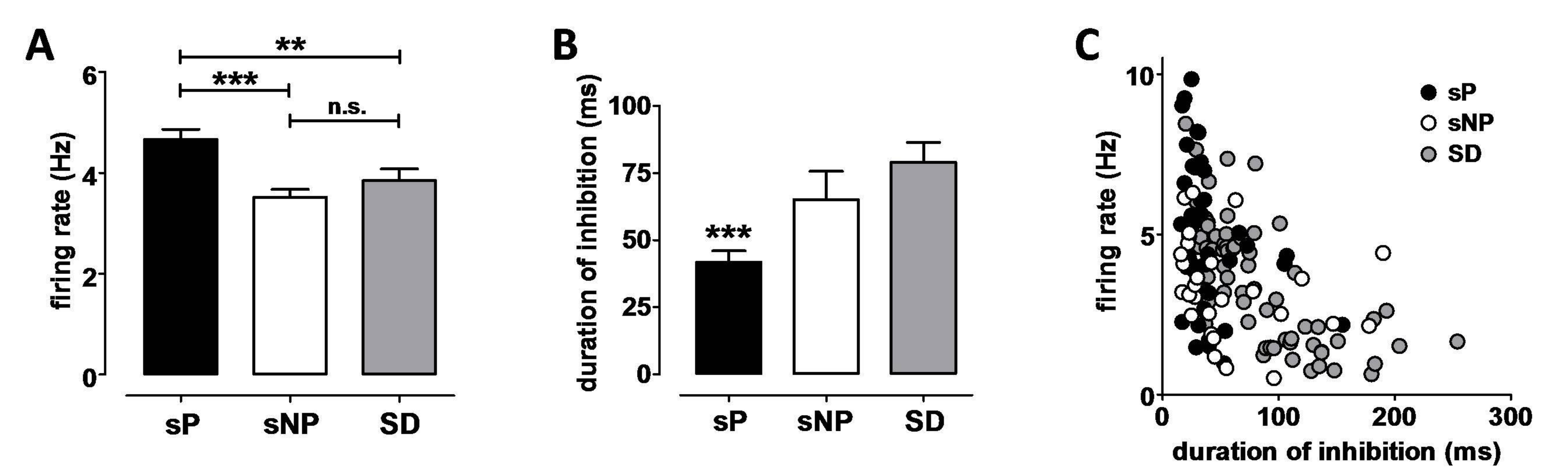
- In vivo, the increased firing activity of VTA DA neurons in sP rats negatively correlates with the duration of inhibition evoked by stimulation of the RMTg.
- In vitro, 2-AG mediates DSI at GABAergic RMTg-VTA synapses.
- sP rats display an enhanced DSI on RMTg afferents to DA neurons.
- Difference in DSI amplitude between sP and sNP rats depends on MAG lipase efficiency, which may differently regulate 2-AG levels at RMTg-VTA.

Conclusions

Given that sP rats are a vulnerable phenotype with predisposition to alcohol abuse, our results suggest that differences in the endocannabinoid system machinery might control specific sources of vulnerability.

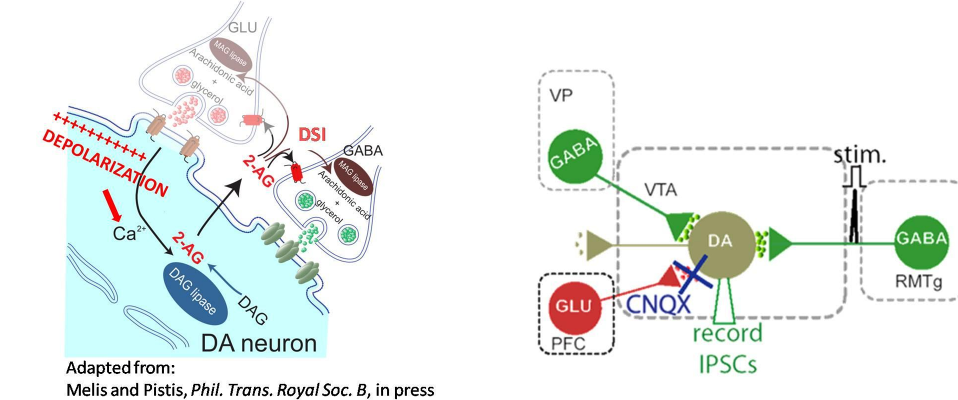
Results

In vivo

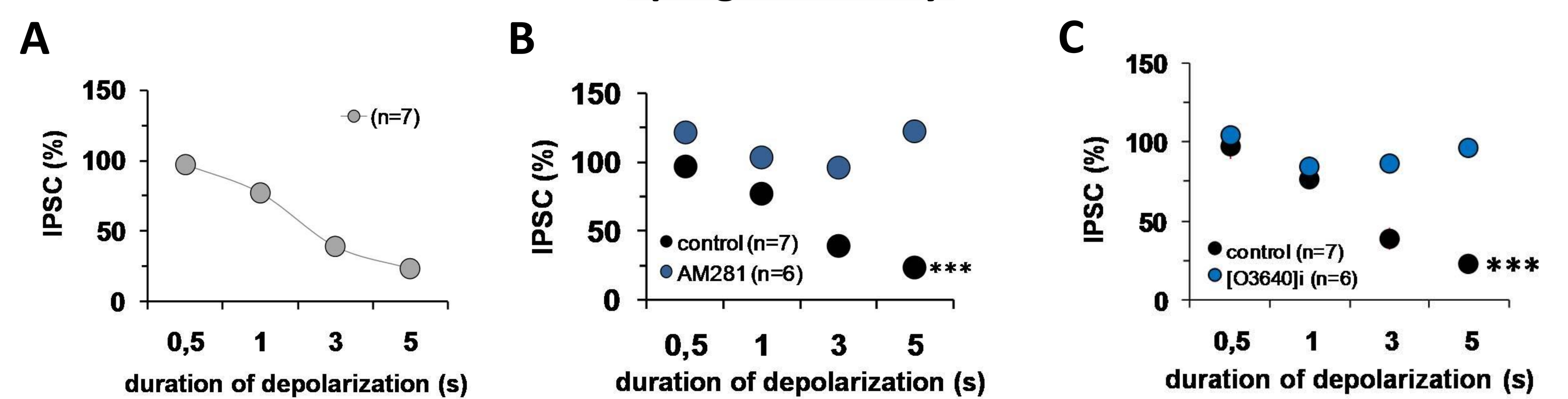


A) Mean firing rates of VTA DA neurons recorded from SD, sP and sNP rats. The graph shows an increased spontaneous firing activity in sP rats compared to sNP and SD rats.
B) Mean inhibitory response to RMTg stimulation of VTA DA neurons recorded from SD, sP and sNP rats. The bar graph illustrates a reduced duration of inhibition evoked by RMTg in sP rats.
C) Graphic representation of the negative correlation between spontaneous firing rate and the duration of inhibition elicited by electrical stimulation of the RMTg.

Ex vivo

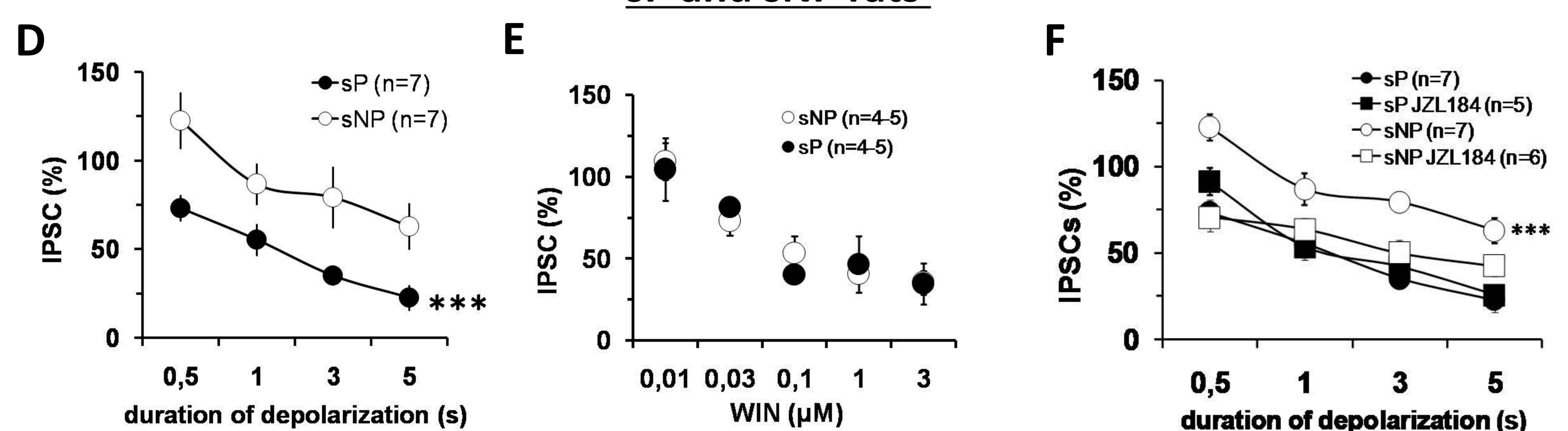


Sprague Dawley



A) DSI, an endocannabinoid-mediated form of short term plasticity is expressed by inhibitory synapses arising from RMTg afferents in SD rats .
B) The CB1 antagonist AM281 abolishes the DSI, thus demonstrating that DSI requires activation of CB1 receptors.
C) Pharmacological blockade of diacylglycerol lipase (DAGL, the enzyme for 2-AG synthesis) by O3640, inhibits the DSI, suggesting that DSI is selectively mediated by this endocannabinoid.

sP and sNP rats



D) DSI at RMTg-VTA synapses in sP rats is increased compared to sNP rats.
E) Dose-response curve for CB1 agonist WIN 55212 does not differ in sP and sNP rats, suggesting that their differences in DSI do not depend on CB1 number or function.
F) Pharmacological blockade of monoacylglycerol lipase (MAGL, the major enzyme for 2-AG degradation) by JZL184, increases DSI in sNP but not in sP rats. This result indicates that in sNP rats there is a higher baseline degradation of 2-AG in RMTg afferents.

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No potential conflict of interest

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