Distribution of temperature changes and neurovascular coupling in rat brain following 3,4-methylenedioxymethamphetamine (MDMA,'ecstasy') exposure

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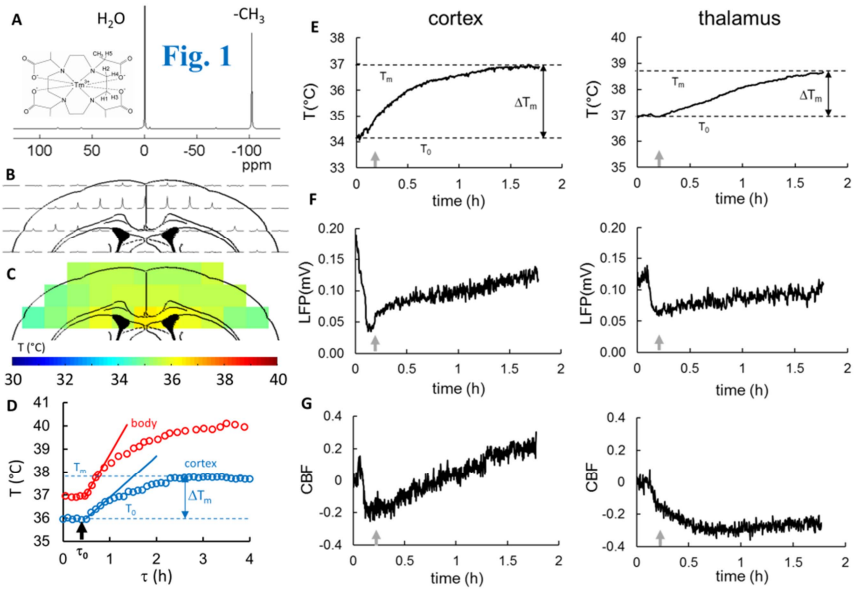
TARGET AUDIENCE Neurophysiologists interested in mechanisms and effects of MDMA action in the brain.

PURPOSE (+/-)3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy') is an abused psychostimulant [1] producing strong monoaminergic stimulation. However, the most severe and potentially fatal acute effects of MDMA involve extreme hyperthermia and its consequences on multiple organ systems, e.g., rhabdomyolysis, coagulopathy, kidney, heart, and liver failure [2, 3]. MDMA-induced thermogenesis involves activation of uncoupling proteins (UCP), primarily a type specific to skeletal muscle (UCP-3) and which is absent in brain, although other UCP types are expressed in brain (e.g., thalamus) and might contribute to thermogenesis. Since neuroimaging of brain temperature could provide mechanistic insights of MDMA action, we measured spatial distributions of MDMA-induced temperature changes and dynamics in rat cortex using a magnetic resonance method called Biosensor Imaging of Redundant Deviation of Shifts (BIRDS) [4], with an exogenous temperature probe (thulium ion and macrocyclic chelate 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetramethyl-1,4,7,10-tetraacetate ($DOTMA^{4-}$)) (Fig.1A). MDMA induced a fast and homogenous temperature rise throughout the cortex. MDMA-induced temperature changes and dynamics in cortex and body were correlated. In addition, simultaneous temperature (by thermocouple), neuronal activity (by microelectrodes), and tissue perfusion (by laser-Doppler) were measured in cortex and thalamus to investigate possible mechanisms of MDMA-induced warming across brain regions.



MATERIALS AND METHODS ***Animal preparation:*** Sprague-Dawley rats (200-300g) were tracheotomized and artificially ventilated (30% O_2). The animals were anesthetized with an intraperitonial injection of urethane (1.3 g/Kg). A subcutaneous line was inserted for administration of MDMA (20 mg/kg) and an intravenous line for administration of D-tubocurarine chloride (1 mg/kg/hr) or $TmDOTMA^-$ (150-200 µmol/hr). An arterial line was used for monitoring physiology (blood pH, pO_2, pCO_2) throughout the experiment. The anesthetized rats were prepared with renal ligation [4]. In both magnet (for BIRDS) and bench (for temperature, neuronal activity and tissue perfusion) experiments a water heating pad was used to maintain animal body temperature at physiological levels. Core body temperature was measured continuously with a rectal temperature probe. ***In vivo BIRDS (n=10):*** $TmDOTMA^-$ was continuously infused for ~2 hours, followed by the MDMA injection. CSI data (Fig.1B) were obtained on a modified 11.7 T Bruker horizontal-bore spectrometer (Billerica, MA) using a ^1H resonator/surface coil RF probe. A gaussian pulse of 200 µs was used for excitation of a 6 mm slice with FOV of 2.56 cm x 2.56 cm. The following parameters were used: 16x16 encode steps, TR=11 ms, 100 averages and 4 min 40s acquisition time. The temperature maps (Fig.1C) were calculated from the chemical shifts of $TmDOTMA^-$ methyl group according to the equation: $T=34.45+1.460\cdot(\delta_{CH3}+103)+0.0152\cdot(\delta_{CH3}+103)^2$ [4]. The maximum change in the temperature, ΔT_m, was estimated from the difference between the maximum temperature (T_m) and the temperature at the moment of MDMA administration (T_0) (Fig.1D). The MDMA-induced warming rate immediately after MDMA administration was calculated by fitting the temperature variation over time to a linear function to represent the initial rate of heating (Fig.1D). ***In vivo bench experiments (n=4).*** Anesthetized rats were mounted on a stereotaxic frame placed on a vibration-free table inside a Faraday cage. The scalp and the *galea aponeurotica* were removed and small burr holes were drilled for insertion of a multisensor probe, which was custom-designed to measure temperature (Fig.1E), neuronal activity (Fig.1F) and cerebral blood flow (Fig.1G). Recordings were localized to the middle cortical layers of the forelimb somatosensory cortex (4.4 mm lateral to bregma, 1.0 mm anterior to bregma, 0.9±0.1 mm depth from cortical surface) and ventral posterior lateral nucleus of the thalamus (3 mm lateral to bregma, 3 mm posterior to bregma, 5 mm depth from cortical surface).

RESULTS AND DISCUSSION The results from different animals show that the distribution of cortical ΔT_m was variable, spanning a relatively large range of values, from 0.8 to 2.4 °C. However, within the same rat, the cortical temperature changes spanned a much smaller range, with a standard deviation of less than 0.23 °C. The warming rates were quite similar in all rats investigated (~2 °C/h) and showed a relatively homogenous distribution across the entire cortex, spanning a range of values from 1.4 to 2.6 °C/h with standard deviations of less than 0.3 °C/h. The average cortical temperature change over all rats investigated was 1.6±0.4 °C, whereas the average cortical warming rate was 2.0±0.2 °C/h. Saline-induced temperature measurements, for cortex by BIRDS or for body by a rectal thermocouple, in 3 rats showed no significant changes. A strong linear correlation ($R^2 = 0.96$) was observed between the average cortical temperature change and the body temperature change induced by MDMA. However, when the brain and body warming rates are compared a much lower correlation is found ($R^2 = 0.43$), with the body warming rates spanning a larger range of values (2.1 to 3.5 °C/h) compared to cortical values (1.7 to 2.1 °C/h). Typical examples of temperature, neuronal activity and blood flow recordings from the cortex and thalamus from a rat are shown in Fig.1E-G. Similar multi-modal trends were observed for other rats investigated. Both the maximal temperature change and the warming rate were higher in the cortex than in the thalamus. While the cortical temperature increase seemed to follow immediately after the MDMA injection, the thalamic temperature increase was delayed by 10-15 minutes. MDMA injection resulted in a rapid decrease in the LFP within the initial 15 minutes, followed by a slower increase in both regions although the thalamic activity increase was much slower. The LFP recovery nearly reached pre-MDMA values for both regions. The MDMA-induced CBF dynamics were quite similar to the LFP patterns. While within the first few minutes of MDMA injection both regions showed a small but rapid CBF rise, there was a large CBF decrease in both regions within the initial 15-30 minutes followed by a slower increase in both regions although the thalamic CBF increase was much smaller and gradual. In summary, we studied acute hyperthermia induced by MDMA using BIRDS with $TmDOTMA^-$. This method provided a reliable and non-invasive method for temperature distribution and dynamics in the cerebral cortex. Independent of cortical temperature changes, a single bolus injection of MDMA led to a rapid initial temperature increase with a rate of 2 °C/h and a maximum temperature change of 2.4 °C observed at 2 h post injection. The correlation between the cortical and body temperature changes suggest that the heat produced in the body is carried by blood to the brain and contributes partially to cortical temperature increase. Moreover, multi-modal measurements of temperature, blood flow, and neuronal activity suggest differences in the MDMA-induced response in the cortex and thalamus with regard to lack of neurovascular coupling in the thalamus compared to the cortex.

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