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Neural correlates of olfactory and visual memory performance in 3D-simulated mazes after intranasal insulin application

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ABSTRACT

This fMRI study intended to establish 3D-simulated mazes with olfactory and visual cues and examine the effect of intranasally applied insulin on memory performance in healthy subjects. The effect of insulin on hippocampus-dependent brain activation was explored using a double-blind and placebo-controlled design. Following intranasal administration of either insulin (40 IU) or placebo, 16 male subjects participated in two experimental MRI sessions with olfactory and visual mazes. Each maze included two separate runs. The first was an encoding maze during which subjects learned eight olfactory or eight visual cues at different target locations. The second was a recall maze during which subjects were asked to remember the target cues at spatial locations. For eleven included subjects in the fMRI analysis we were able to validate brain activation for odor perception and visuospatial tasks. However, we did not observe an enhancement of declarative memory performance in our behavioral data or hippocampal activity in response to insulin application in the fMRI analysis. It is therefore possible that intranasal insulin application is sensitive to the methodological variations e.g. timing of task execution and dose of application. Findings from this study suggest that our method of 3D-simulated mazes is feasible for studying neural correlates of olfactory and visual memory performance.

1. Introduction

One of the most challenging tasks for the human brain in everyday life is to extract relevant information from a complex environment. Thus, it is very useful that the perceived information is not always novel and re-experienced sensory inputs might have a potential to awake associated memories. This is especially true when the sensory stimulus is an odorant. Smells are extremely potent to elicit emotional reactions, especially, when they are not consciously perceived as it has been already described a century ago as the "Proust phenomenon" (Proust, 1913). Odors are also powerful cues concerning autobiographical memory and they allow the human brain to react faster or more precisely (Larsson & Willander, 2009; Larsson, Willander, Karlsson, & Arshamian, 2014; Willander & Larsson, 2007). The strong connection between odors and memory is supported by human brain anatomy. Olfactory nerves in the nasal mucosa transmit molecular information from the external world to the olfactory bulb (OB), which is connected to the piriform cortex (pirC). From the pirC, the information is gated through the entorhinal cortex to the hippocampus, which are both crucial for memory function. In other words, within a few synapses, olfactory information reaches brain areas that are mediating memory processes. This happens on an unconscious level since information processing does not involve a thalamic relay (Courtiol & Wilson, 2015; Lundstrom, Boesveldt, & Albrecht, 2011). The close anatomical connections between brain structures for olfactory and memory processing privileges the sense of smell with a strong potential for evoking memories and a strong impact on memory function.

The brain regions hippocampus and olfactory bulb, which are responsible for memory functions and smell perception, are considered brain areas with the highest density of insulin receptors (Havrankova, Roth, & Brownstein, 1978; Havrankova, Schmechel, Roth, & Brownstein, 1978; Squire & Zola, 1996) and are specific targets for insulin effects. Research in humans has established enhancing effects of an intranasal insulin application of 160 IU per day over a course of eight weeks on declarative memory performance in healthy subjects for a delayed recall task (Benedict et al., 2004), which was based on word lists that had to be

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remembered immediately and one week later. Further, a study by Krug, Benedict, Born, and Hallschmid (2010), where hippocampus-dependent memory performance for spatial (15 card-pairs associated to locations) and working memory (digit span test) were tested, was improved. A magnetoencephalography study (whole-head MEG) revealed, that a single dose of intranasal insulin application (160 IU) involves a strong insulin modulation on global network communication efficiency by decreasing the characteristic path length and increasing signaling between different brain regions measured during resting state condition (Stingl et al., 2010). Here, not only brain areas related to satiation and whole-body energy metabolism, but also related to non-homeostatic pathways, like the hippocampus are involved. For the exploration of the neural basis of short- and long-term memory and impaired cognitive functions as well as visual associative memory under the influence of intranasal applied insulin (e.g. Claxton et al., 2015; Shemesh, Rudich, Harman-Boehm, & Cukierman-Yaffe, 2012), different experimental paradigms have already been implemented (e.g. Novak et al., 2014). Within two studies from our group using a single dose of intranasal insulin application (40 IU), we were able to demonstrate a reduction of olfactory sensitivity to n-butanol odor on the one hand (Brunner, Benedict, & Freiherr, 2013) and a boosting effect during an olfactory spatial memory task on the other hand (Brunner, Kofoet, Benedict, & Freiherr, 2015). Despite a notable number of studies, the effects and the precise mechanisms of enhanced insulin concentrations in the human CNS, also caused by different insulin doses, are not fully elucidated. Further, the specific mechanisms and brain regions involved in human olfactory memory, especially olfactory spatial memory processes are still poorly understood and have not been reported up to date.

Given, that odorants have a strong memory potential and that hippocampus and olfactory bulb are likely targets for insulin effects, the present study aimed to explore human brain activation during olfactory and visual cue presentation in 3D-simulated mazes after intranasal insulin application in comparison to a placebo in healthy men. The reason to investigate both, olfactory and visual spatial memory in this fMRI design, was to explore if intranasally applied insulin is generally involved in sensory memory processes or if it is specifically involved in manipulation of chemosensory processes. We used intranasal insulin administration as a non-invasive method to apply substances through the nasal cavity to the brain. This memory maze paradigm allows us to represent olfactory and visual cues in a realistic "map-like" environment that is a relevant simulation of daily occurrence in human navigation, which is mirrored in hippocampal activity.

2. Materials and methods

2.1. Participants

The protocol for the current fMRI study was approved by the local ethic review board. Prior to the experiment all participants were informed about the aim of the study and possible incidental findings. All participants signed a written consent form. Only healthy, right-handed male subjects were included. All subjects were non-smokers. They underwent a screening session with measurements of standard clinical blood parameters, and scored normally in three psychometric questionnaires (BSI - Brief Symptom Inventory, BDI - Beck Depression Inventory II, MOCA - Montreal Cognitive Assessment), and an olfactory identification test (16 items Sniffin' Sticks identification test, Burghart Medizintechnik GmbH, Wedel, Germany). During the timeframe of the study, none of the participants was taking medication or showed any abuse of substances. Based on the screening session three out of nineteen subjects were excluded before the MRI experiment started. After the scanning session five out of sixteen participants were excluded from imaging data analysis because of technical problems. The final sample consisted of eleven healthy, normosmic males for analysis of the fMRI data (n = 11; age: M = 24.91, SEM = 1.30 years; BMI: M = 23.69, SEM = 0.22 kg/m^2), and sixteen healthy normosmic males for the behavioral data (n = 16; age: M = 24.69, SEM = 1.04 - years; BMI: M = 23.11, SEM = 0.37 kg/m^2).

2.2. Experimental setting

Each subject underwent two MRI sessions: during one session they received intranasally applied insulin (40 IU equaling 0.4 ml Actrapid; Novo Nordisk, Mainz, Germany), during the second session they received 0.4 ml placebo solution (HOE 31 dilution buffer; Aventis Pharma, Bad Soden; Germany). Treatment conditions were counterbalanced and double-blind. Insulin and placebo solution were intranasally applied by the study leader and were stored in identical vials, so that neither participants nor the experimenter were aware of the respective experimental condition. The MRI sessions started in the morning (at 0800 or at 1100) after participants had fasted for 12 h. Right before and 20 min after intranasal administration of either insulin or placebo, blood was drawn for analysis of the concentrations of glucose, insulin, cortisol, leptin, and acetylcholine level (blood assays were analyzed by the local clinic's chemistry laboratory). During the 20 min time period subjects received experimental instructions regarding the paradigm. Then, participants were positioned in the scanner, the olfactometer tubing was inserted in both nostrils and a response box was positioned so that subjects were able to provide feedback with their index, middle and annular finger of the right hand.

2.3. Experimental paradigm

In each MRI session (insulin and placebo) four different mazes (two olfactory and two visual mazes) were presented in alternating order (one MRI session is illustrated in Fig. 1). The mazes were programmed with the open-source 3D computer graphics software Blender (Blender Institute BV, Amsterdam). The olfactory and visual stimuli were implemented in 3D-simulated mazes in an event-related design using E-prime 2.0 presentation software (Psychology Software Tools). MRI scanner, presentation software, response box, and olfactometer were synchronized during the event-related stimulation paradigm. The mazes differed in their structure (two sample mazes in bird's-eye perspective are presented in Fig. 2) and in their duration (from 3.5 min to 9 min) during the automated navigation. For this reason, all mazes were pre-evaluated by an independent test group regarding their level of difficulty and were rated as similarly difficult. The navigation through the mazes was accomplished by a PC, so that subjects had no influence on starts and stops or on the navigation itself. Each olfactory and visual maze contained two separate parts. The **first part** of the experimental design was the **encoding maze** during which subjects were automatically guided through the maze. The automatic guiding stopped at eight different spatial locations where the subjects received either eight olfactory cues (olfactory encoding maze) or eight visual cues (visual encoding maze). At a single spatial location, the subjects were only presented one olfactory cue or respectively one visual cue. Odor cues were presented via the olfactometer for 1.2 s and visual cues were presented on the mirrored screen for 2 s. The utilized black and white schematic visual icons did not contain many details, so that memorizing odors and visual cues were similarly difficult (links to the purchased visual icons: http://de.123rf.com for fruity icons number 127156838 and http://www.shutterstock.com for flowery icons number 22521869). The subjects' task was to learn and associate the eight odors or pictures with the respective spatial locations. The