Neural correlates of memory for odor detection conditioning in adult rats

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(Received 21 June 1993; Revised version received 12 August 1993; Accepted 17 August 1993)

Key words: Memory; Olfaction; Olfactory bulb; Anterior olfactory nucleus; Pyriform cortex; Hippocampus; 2-Deoxyglucose

Adult male Wistar rats were trained in a simple odor detection task, with peppermint odor serving as either an S+, S− or as a randomly presented odor. Twenty-four hours after the last training session, rats were injected with [14C]2-deoxyglucose and exposed to the odor. Mean relative 2-deoxyglucose uptake to the odor was enhanced in the pars dorsalis of the anterior olfactory nucleus of S+ and S− trained rats compared to controls. In contrast, no differences in uptake were detected in either odor-specific focal regions of the olfactory bulb glomerular layer, the pyriform cortex, or the hippocampus.

Associative olfactory conditioning in preweanling rats produces marked changes in olfactory bulb neural response patterns to the learned odor [21, 27]. Although the olfactory bulb is not the single critical locus for olfactory learning in newborns [10, 22], there is strong evidence to suggest that plasticity in the bulb plays an important role in these simple early olfactory memories [23].

In the adult rat, lesion studies suggest that pathways critical for olfactory memories may be dependent on the exact nature of the learning paradigm. For example, extensive lesions of the olfactory bulb in the adult [12] or its primary output, the lateral olfactory tract (LOT) [17, 18], do not produce anosmia or impair memory of a simple odor detection task. Furthermore, secondary olfactory structures like the pyriform cortex and the hippocampus are involved in only some forms of olfactory memory. For example, lesions of the pyriform cortex are ineffective at blocking previously learned odor discriminations of simple odor cues, but produce a dramatic impairment in discrimination of complex, multiple component odor cues [20].

In fact, one of the few structures that may be required for even simple odor detection tasks in the adult is the anterior olfactory nucleus (AON). The AON receives input from the olfactory bulb and olfactory cortex and is the major relay for commissural connections between the olfactory bulbs [4, 11, 16]. Lesions of the olfactory peduncle producing damage to both the LOT and the AON severely impair odor detection memory, while lesions of the LOT alone do not [17].

In light of this neuroanatomical evidence for possible roles of the olfactory bulb, AON, pyriform cortex and hippocampal formation in some forms of olfactory memory, the present experiment examined regional differences in relative 2-deoxyglucose (2-DG) uptake in response to learned odors in adult rats. This study focused on the olfactory pathway from the olfactory bulb to the hippocampus and utilized a simple odor detection task. It was expected that those structures actively involved in memory for simple olfactory cues would be differentially activated by exposure to the learned odor.

The subjects were male Wistar rats (n = 21) from Hilltop Lab Animals (Scottsdale, PA). Colony rooms were maintained on a 12:12 light/dark schedule with lights on at 07.00 h. Animals were used for both behavioral and 2-DG testing. Due to dissection and histological problems, not all brain areas were analyzed in all animals. The animals were shaped to perform a standard barpress operant task for a 45-mg Bioserve food pellet and given 4 to 6 daily 1-h no-odor training sessions.

At the end of this preliminary training, the animals were randomly assigned to 1 of 3 groups for odor detection training: S+, S−, or control. S+ (n = 7), only bar presses (BP) made during odor presentation were rewarded. Animals were placed in the operant chamber for 1 h with 10, 2-min exposures to odor with pseudo-random inter-odor intervals. S− (n = 7), only BP made while the odor was off were rewarded. Control (n = 7), all BP were rewarded regardless of whether the odor was on or
off. The same times and order of odor presentation were used for all groups. Food intakes and weights did not differ between groups.

Subjects in all conditions received 7 daily training sessions lasting 1 h each, and all received the same amount of odor exposure. Odor stimuli consisted of a 1:10 concentration of saturated peppermint odor (Shilling), presented by a computer controlled flow dilution olfactometer and added to a continuous clean airstream (2 l/min). Data were calculated as: ln(BP during odor/BP no odor) and analyzed with a repeated measures ANOVA with subjects nested in groups.

On the day following the seventh daily training session, animals were tested for local [14C]2-DG uptake to the odor. During testing for 2-DG uptake, the subjects were placed in an operant chamber identical to the one they were trained in with the exception that there was no response bar present. Peppermint odor concentration and flow was matched to that of training. The subjects were placed in the operant chamber for a 10-min habituation period and then injected with 40 μCi of [14C]2-DG and placed back in the chamber. A 45-min exposure period began in which odor was presented in 1-min presentations with 1-min inter-odor intervals. At the end of the odor presentation period, all animals were decapitated and the brains quickly removed and frozen in 2-methyl butanate at −45°C. Brains were then sliced in 20-μm coronal sections, and every third section was saved to be used for autoradiography. In the autoradiography procedure the brain sections were exposed to SB5 X-ray film for 8 days along with a set of precalibrated 14C standards.

Image analysis was done by computer assisted quantitative optical densitometry (Imaging Research Ltd.). To quantify 2-DG uptake, the computer determined a calibration curve that related grey value of the 14C standards to previously determined 14C tissue equivalents, then linearized this function to allow grey values of the brain autoradiographs to be translated to 14C levels. The autoradiographs were visually scanned for odor-specific olfactory bulb glomerular layer foci which are easily identified [9, 21] (Fig. 2). Other brain regions analyzed were located by comparison of counterstained sections with a stereotaxic atlas of the rat brain [15]. Optical density of each area was determined by outlining the area and determining an average density for that area (e.g. see Fig. 2). To correct for possible minute differences in section thickness, exposure or development times between groups, all readings were taken relative to a neighboring region of white matter, i.e. corpus callosum or anterior commissure [7]. For the olfactory bulb glomerular layer, readings were taken relative to the periventricular core of the bulb [2, 21]. No significant differences were found between conditioning groups in absolute 2-DG uptake in either the periventricular core of the olfactory bulb, the anterior commissure or the corpus callosum. In addition to the olfactory bulb, relative 2-DG uptake was measured in 5 other brain regions. Using the anterior commissure for relative measures, 2-DG uptake was measured in 3 nuclei of the anterior olfactory nucleus (AON) – the pars medialis (mAON), the pars dorsalis (dAON), and the pars lateralis (lAON). Using the corpus callosum for relative measures, 2-DG uptake was measured in the pyriform cortex and the ventral hippocampus. Optical density for each of the brain regions was determined in at least 2–3 sections/brain area/animal. Mean relative 2-DG uptake for each area was compared across groups with an ANOVA.

The behavioral data suggest that animals performed well at the odor detection task (Fig. 1. F2,18 = 102.25, P < 0.0001). Ryan’s multiple comparison on the three groups showed that S+ animals responded significantly more often during the odor than animals in the control group. Animals in the S− group responded significantly less often during the odor than either the control or S+ group.

Mean relative 2-DG uptake data are shown in Table I. The AON demonstrated a significant change in 2-DG uptake to the learned odor. Uptake in the dAON was significantly enhanced in both the S+ and S− groups compared to controls (F2,14 = 4.67, P < 0.05). Post-hoc tests revealed that relative 2-DG uptake to peppermint was enhanced in S+ and S− animals compared to controls (Fisher, P < 0.05). Uptake in the lAON and mAON was similarly enhanced in the S+ and S− groups, although these changes did not reach significance (mAON F2,14 = 2.15, P = 0.15; mAON F2,14 = 1.41, P = 0.28).

As shown in Table I, no other brain region showed a significant learning associated modification. Relative 2-DG uptake was increased in the pyriform cortex in both S+ and S− groups compared to controls, but this increase did not approach significance (F2,13 = 0.70, P = 0.52). Odor-specific glomerular layer foci in the ol-

Fig. 1. Ratio of responses while odor was on relative to while the odor was off. Note the S+ group showed an increase in the ratio of responses across days, while the S− group showed a decrease in the ratio of responses across days compared to randomly odor exposed controls.
factory bulb and the ventral hippocampal formation also showed no significant difference in relative 2-DG uptake between conditioning groups.

The present results suggest that odor detection conditioning is correlated with a modification of neural activity in the AON in adult rats. Interestingly, both odor signaling reward (S+) and odor signaling non-reward (S−) produced enhanced relative 2-DG uptake in the AON compared to uptake in odor experienced, control rats.

**TABLE 1**

**MEAN RELATIVE 2-DG UPTAKE IN EACH CONDITIONING GROUP AND EACH BRAIN AREA (± S.E.)**

Columns on right display percent change in uptake compared to control. Asterisks signify significant difference (*P < 0.05*) from control.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>S+</th>
<th>S−</th>
<th>Control</th>
<th>S+ % change</th>
<th>S− % change</th>
</tr>
</thead>
<tbody>
<tr>
<td>O.B. glom. foci</td>
<td>3.89 ± 0.71</td>
<td>3.70 ± 0.39</td>
<td>3.45 ± 0.31</td>
<td>13%</td>
<td>7%</td>
</tr>
<tr>
<td>mAON</td>
<td>1.53 ± 0.07</td>
<td>1.55 ± 0.15</td>
<td>1.32 ± 0.02</td>
<td>16%</td>
<td>16%</td>
</tr>
<tr>
<td>dAON</td>
<td>2.03 ± 0.09</td>
<td>2.09 ± 0.16</td>
<td>1.56 ± 0.13</td>
<td>30%**</td>
<td>34%**</td>
</tr>
<tr>
<td>IAON</td>
<td>3.17 ± 0.12</td>
<td>2.94 ± 0.31</td>
<td>2.51 ± 0.18</td>
<td>26%</td>
<td>17%</td>
</tr>
<tr>
<td>Pyriform</td>
<td>4.69 ± 0.55</td>
<td>4.26 ± 0.82</td>
<td>3.41 ± 0.98</td>
<td>38%</td>
<td>25%</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>2.08 ± 0.15</td>
<td>1.88 ± 0.23</td>
<td>1.85 ± 0.31</td>
<td>12%</td>
<td>2%</td>
</tr>
</tbody>
</table>

Fig. 2. Representative autoradiographs, cresyl violet counterstained sections and corresponding stereotaxic atlas drawings [15] through each of the areas analysed in the present study: olfactory bulb (A), anterior olfactory nucleus (B), pyriform cortex (C), and ventral hippocampus (D). Arrows and cross-hatching delineate regions analysed.
Several previous studies, using a variety of techniques, have examined olfactory bulb neural responsiveness to learned odors. Similar to the present findings, no learning associated change in olfactory bulb activity to learned odors was found in adult rats using either focal relative 2-DG uptake [3] or multi-unit mitral/tufted cell recordings [14]. However, spatial EEG patterns recorded from the olfactory bulb are modified by odor conditioning in the mature rabbit [1, 6, 24]. Similarly, while no change in 2-DG uptake in the pyriform cortex was detected here, pyriform cortex spatial EEG patterns are modified by aversive conditioning in rabbits [1]. It is unclear whether these differences in results reflect a true difference in olfactory bulb plasticity in rabbits and rats, or in conditioning paradigms, or whether they reflect a difference in sensitivity between activity assay techniques.

Interestingly, associative conditioning to odors in newborn rats produces marked changes in relative focal 2-DG uptake and in mitral/tufted cell single-unit responses to the learned odor [21, 27]. This plasticity in olfactory bulb function with experience, however, may only occur during a sensitive period during development [26, 28], similar to plasticity observed in other sensory systems [8, 25]. Perhaps, as the olfactory bulb becomes less plastic with age, the AON begins to replace the bulb memory functions.

Finally, the hippocampus has been shown to play a critical role in some forms of olfactory conditioning [5, 13, 19]. For example, lesions or denervation of the hippocampus produce rapid forgetting of olfactory memories [19] and impair performance on discrimination of simultaneously presented odor cues [13]. The hippocampus does not, however, appear to be critical for successive odor discriminations or simple odor detection. Thus, the present results, which found no significant change in hippocampal function with odor detection discrimination, correspond well with these lesion studies.

In summary, these results suggest that memory for very simple odor detection conditioning may involve changes in primary olfactory sensory system structures such as the AON. It is expected that more complex conditioning, such as simultaneous odor discriminations, may activate additional brain regions more traditionally tied to memory such as the hippocampal formation.

This work was performed in partial fulfillment of the requirements for an M.S. degree for W.D.H. who was supported by a minority supplement to NIDCD Grant DC00866 to D.A.W. and R.M.S. Additional support was provided by NSF Grants BNS8819189 to D.A.W. and BNS 9110506 to R.M.S.

26 Wilson, D.A., Pham, C., Smart, R.S. and Sullivan, R.M., Developmental changes in the neural correlates of odor avoidance conditioning, submitted.