APP/PS1和SAMP8小鼠记忆关联实验中脑功能连接研究

【实验背景】

阿尔茨海默病(Alzheimer`s disease, AD)，通常称为老年性痴呆，是痴呆最常见的一种形式。临床上以记忆障碍、失语、失用、失认、视觉空间能力损害、执行功能障碍以及人格和行为改变等全面性痴呆表现为特征，病因迄今未明。其临床症状主要为进行性的记忆损伤以及其他认知功能的下降，主要包括：散发型阿尔茨海默病（sporadic Alzheimer`s disease，SAD）和家族型阿尔茨海默病（familial Alzheimer`s disease，FAD）。SAD主要发生在65岁之后，约占全部AD发病率的95%，而FAD的发病时间要早于SAD(Beach, 2008)。目前用于AD药物研发的动物模型主要包括4类，分 别是脑损伤造模(McGonigle, 2014)、药物造模(Ebert and Kirch, 1998; Sunderland et al., 1986)、转基因模型(Barrett et al., 2015; Holcomb et al., 1998; Lv et al., 2015; Oakley et al., 2006; Xuan et al., 2015)以及老化模型(Del Valle et al., 2010; del Valle et al., 2011; Manich et al., 2011; Porquet et al., 2013)。

前两种的AD模型虽然能够部分模拟AD的认知功能下降，但不能模拟AD复杂的病理特征。因此，目前使用较多的AD动物模型是转基因和老化模型。而在转基因模型中，APP/PS1转基因小鼠是使用最为广泛的模型之一。APP/PS1转基因小鼠出现AD样认知损伤的直接原因是，人源β淀粉样蛋白前体蛋白（β-amyloid precursor protein，APP）-695基因和外显子9缺失的变种早老素-1（presenilin-1，PS1）基因的转入，致使小鼠脑中Aβ水平升高(Radde et al., 2006)。进行性的Aβ沉积大约会在APP/PS1转基因小鼠出生后6~8周出现(Manook et al., 2012; Poisnel et al., 2012)，但是其行为学的下降大约会在Aβ沉积出现的2个月后才出现，并不与病理变化同步发生(Arendash et al., 2001; Jankowsky et al., 2004)。除了APP和PS1的转入外，APP/PS1转基因小鼠认知功能下降也与伴随着Aβ沉积出现的神经胶质过度增生(Jardanhazi-Kurutz et al., 2011; Malm et al., 2007; Yan et al., 2009)、神经炎症(Leroy et al., 2012)、以及兴奋性突触损伤(Mitew et al., 2013)相关。

SAMP8小鼠是目前认为较理想的老化模型，主要作为SAD的模型使用(Pallas et al., 2008)。由于SAMP8小鼠认知下降的直接原因是快速老化，因此SAMP8小鼠较之转基因小鼠能够更接近于AD的复杂性(Morley et al., 2012)。SAMP8小鼠除具有与AD病人相似的认知功能损伤外，最主要的特征即是不可逆的快速老化以及与老年人相似的表观改变，如：生存期短、脱毛、脊柱前凸和活动度下降(Hosokawa et al., 1984)。此外SAMP8小鼠还具有神经退行性变的特征，包括情绪异常和神经元丢失，但12月龄之前在SAMP8小鼠脑中都无法检测出Aβ沉积斑块(Morley et al., 2000)。SAMP8小鼠认知功能下降的机制较为复杂，与神经元变性(Kawamata et al., 1997)、氧化应激引起的脑部炎症(Morley et al., 2012)、外周性激素增龄性的下降(Kang et al., 2014; Novella et al., 2010)、髓细胞触发受体-2水平升高(Jiang et al., 2014)和促炎因子的分泌增多(Tha et al., 2000)都具有一定的相关性。

在一定程度上，APP/PS1和SAMP8小鼠可以分别代表FAD和SAD，但这两种小鼠在脑功能连接方面的对比研究较少。

【实验目的】

研究20月龄APP/PS1和SAMP8小鼠在记忆关联实验中的脑功能连接差异。

【实验内容】

1. 对APP/PS1和SAMP8小鼠及其各自对照组进行静息态fMRI检测；
2. 连续10天对APP/PS1和SAMP8小鼠及其各自对照组进行足底电刺激，使其将足底电刺激与回避行为产生关联；
3. 对APP/PS1和SAMP8小鼠及其各自对照组进行任务态fMRI检测（0.2mA足底电刺激）。
4. 对APP/PS1和SAMP8小鼠脑组织进行免疫荧光染色（Aβ、tau蛋白、小胶质细胞（CD16）、星形胶质细胞（C3）、SYP、PSD95、胆碱能神经元、谷氨酸能神经元）。
5. 对APP/PS1和SAMP8小鼠外周血进行生化指标检测。

【实验分组】

APP/PS1转基因小鼠，雄性，20月龄，3只，SPF级；C57小鼠雄性，20月龄，5只，SPF级；SAMP8小鼠，雄性，20月龄，5只，SPF级；SAMR1小鼠，20月龄，5只，SPF级。

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| --- | --- | --- | --- |
| **序号** | **组别** | **品系** | **只数** |
| 1 | FAD | APP/PS1 | 3 |
| 2 | C57 | 5 |
| 3 | SAD | SAMP8 | 5 |
| 4 | SAMR1 | 5 |

【实验方法】

1. **静息态fMRI**

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1. **记忆关联实验**
2. 实验设备：每个箱体为26cm×16cm×30cm的方形结构，侧壁为医用有机板、铝型材，箱体内部被带有门洞的黑色有机板分离为两个13cm×16cm的小室，底部为可通电的栅栏，使用电流加非条件刺激（Unconditioned Stimulus，US），电击动物足底，顶部配置有噪声发生器或光源，用来产生条件刺激（Conditioned Stimulus，CS）。SuperMaze软件记录数据。
3. 实验步骤：开启软件，实验条件见下表

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| --- | --- | --- | --- |
| **刺激序列** | **刺激类别** | **刺激强度** | **刺激时间** |
| 1-1 | 声音（0~255级） | 100（18000 Hz） | 5 |
|  | 光照（0~255级） | 100 | 5 |
| 1-2 | 电流 | 0.2（mA） | 5 |
| 1-3 | 刺激间隔 |  | 10 |
| 2-1 | 声音（0~255级） | 100（18000 Hz） | 5 |
|  | 光照（0~255级） | 100 | 5 |
| ······ |  |  |  |
| 50-2 | 电流 | 0.2（mA） | 5 |
| 50-3 | 刺激间隔 |  | 10 |
| 背景白噪音强度（0~255级） | | 50 | 持续刺激 |

将动物放入箱内，开启程序。若动物在开始的5s内，从所在一端穿到另一端，则灯光和噪音消失，电击也消失，此种反应记为回避；若动物在5s内没有穿到另一端就要接受电击，5s后电击停止，小鼠在5s内穿到对侧，记为逃避；若动物整个过程中均没有穿到对侧，记为逃避失败。每只小鼠每天接受训练50次（约20分钟），以对照组小鼠在达到80%的回避次数作为终止训练的标准。

重设软件，将噪音刺激和光刺激时间设为5s，而后电刺激取消，刺激间隔为15s。将动物放入穿梭箱内，开启程序。若动物在开始的5s内，从所在一端穿到另一端，则灯光和噪音消失，此种反应记为回避；若动物在5s内穿到另一端，记为逃避；若动物整个过程中均没有穿到对侧，记为逃避失败。

1. **任务态fMRI（0.2mA电刺激）**

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