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Research report

Activation of cortical and striatal regions during the expression of a naturalistic compulsive-like behavior in the rabbit

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ABSTRACT

Nest building behavior in the pregnant rabbit (*Oryctolagus cuniculus*) can serve as a model for compulsions in obsessive compulsive disorder (OCD). Previous work showed that the "straw carrying" phase of nest building (during which the rabbit repeatedly collects straw in its mouth, carries it into the nest box and deposits it there, and then returns to collect more) is associated with increased c-FOS expression (a marker of neuronal activity) in the orbitofrontal, anterior cingulate, and piriform cortices. In the present study, we quantified c-FOS expression in the caudate and putamen, as well as in the primary motor, somatosensory, and prefrontal cortices of: (1) pregnant rabbits given straw (PREG + STRAW); pregnant rabbits not given straw (PREG); (3) estrous rabbits given straw (ESTROUS + STRAW); and (4) estrous rabbits not given straw (ESTROUS). We found that straw carrying was associated with increased c-FOS expression in the dorsal putamen, ventral caudate, primary motor cortex, and somatosensory cortex. Additionally, a correlational analysis of PREG + STRAW animals revealed that these regions, along with the premotor and prelimbic cortices, were significantly intercorrelated with respect to c-FOS expression, suggesting their "coactivation" during repetitive straw carrying. By contrast, behavioral interactions of non-pregnant (ESTROUS) rabbits with straw (e.g., sniffing, nibbling it) were associated with a distinct pattern of c-FOS expression that included the medial and ventral putamen. c-FOS expression in PREG + STRAW rabbits is similar to patterns of regional brain activity in OCD patients exposed to obsession-provoking stimuli, as well as to those observed in healthy human mothers responding to infant-associated stimuli.

1. Introduction

Many types of neuropsychiatric symptoms occur along a continuum of severity that includes non-pathological, "normal" experience [e.g.,1, 2]. Thus, otherwise adaptive behavioral, cognitive, or emotional responses ("psychophysiological" responses) become pathological when they are exaggerated in intensity, prolonged in duration, or increased in frequency. Anxiety symptoms are a particularly clear example of this idea: acute fear (for example, of a spider or snake) or tonic anxious arousal can be adaptive and non-pathological in certain circumstances. However, acute and tonic fear responses that are unusually intense, prolonged, or out of context are non-adaptive, and comprise the core symptoms of anxiety disorders.

Therefore, an understanding of the neurobiological underpinnings of neuropsychiatric symptoms requires an understanding of the neural mechanisms that control the frequency, duration, and intensity of the corresponding adaptive psychophysiological response, and how certain stimuli (environmental, social, or internal cues, for example) optimally interact with these mechanisms at the neural level. According to this conceptualization of neuropsychiatric symptoms, alterations in neurobiological mechanisms that underlie the triggering of an adaptive psychophysiological response would determine symptom frequency and context of its occurrence, while negative regulatory systems would determine its intensity and duration.

We have proposed that maternal nest building in the domestic rabbit (*Oryctolagus cuniculus*) can serve as a useful model for elucidating the neurobiology underlying obsessive-compulsive symptoms, a core characteristic of obsessive compulsive disorder (OCD) and other disorders of the obsessive-compulsive spectrum [3–5]. Obsessions are intrusive, persistent, and repetitive thoughts, while compulsions are repetitive, often stereotyped, behaviors performed ostensibly in order to reduce anxiety associated with obsessions. An intriguing characteristic of OCD, one that may provide clues about the neurobiological origins of its symptoms, is that the content of obsessions and compulsions is most often related to one or more of the following themes: contamination/cleaning, danger/checking, symmetry/counting, and hoarding.

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Notably, each of these symptom categories involves evolutionarily conserved, adaptive cognitive-behavioral responses [6–8].

Maternal nest building in the rabbit comprises three behavioral phases, orchestrated by hormonal changes of pregnancy, that are expressed in a reliable temporal sequence: (1) digging a nest burrow, which typically occurs during mid-pregnancy; (2) collecting nest material (e.g., dry grass or straw) and depositing it into the nest burrow in order to form a nest, which typically occurs on a single evening during the final week of pregnancy; and (3) plucking hair from the ventrum and lining the nest with it, which typically occurs on the night of parturition (31 or 32 days after mating) [9–12].

The second phase of maternal nest building, the collection of nest material or "straw carrying", is the focus of the present study. In the laboratory, we have described this behavior in terms of individual components that comprise a straw carrying "cycle" [3–5]. Thus, the rabbit collects straw in her mouth by means of stereotyped head-bobbing motions, carries the straw into the nest box and deposits it there (often accompanied by digging into or scratching the floor of the nest box), and then returns to collect more straw. Approximately 20–40 such cycles are displayed during the straw carrying phase. In the laboratory, the introduction of straw into the rabbit's cage during late pregnancy (e.g., 28 days after mating) quickly triggers straw carrying, and this behavior is negatively modulated by the quantity of straw that is inside the nest box: more straw inside the nest box inhibits ongoing nest building behavior; less straw prolongs it [3]. Importantly, once the straw nest is completely formed inside the nest box, its removal does not provoke a second straw carrying phase: therefore, external stimuli associated with a completed straw nest apparently engage neurobiological processes that satiate the motivation to collect and carry straw. This behavior is adaptive only in a specific context (late pregnancy), and the signals that control the correct contextual expression are the hormones of pregnancy, the presence of nest building material, and the absence (or presence) of a completed straw nest inside the nest box, all of which can be experimentally controlled. Thus, straw carrying provides a unique opportunity to investigate neurobiological mechanisms that control the initiation and termination of a complex, repetitive behavioral response.

In the present study, we examined the pattern of c-FOS expression (a marker of neuronal activity) in the striatum, somatosensory cortex, and motor cortex associated with the display of repetitive straw carrying behavior. We correlated c-FOS expression in these regions with prefrontal regions, as well as with specific behavioral parameters of repetitive straw carrying behavior. We predicted that repetitive straw carrying would be associated with increased c-FOS expression in the striatum (caudate, putamen), in part because of human studies that observed fMRI activation of this region in association with OCD symptom provocation. Our working model is that straw carrying behavior in the rabbit is an adaptive, compulsive-like behavior that can serve to model the neurobiology of compulsions of OCD.

2. Materials and methods

2.1. Animals

Twenty-six adult (6–24 months), maternally-experienced female New Zealand rabbits (that had at least one prior full-term pregnancy, in which a complete maternal nest was constructed, and in which pups were raised to weaning), from the colony maintained at the *Centro de Investigación en Reproducción Animal*, Tlaxcala, México), were used in these experiments. They were maintained at ambient temperature (15–25 °C), on a long-day light cycle (lights on at 06:00, lights off at 20:00 h), and given rabbit chow (Purina) and water *ad libitum*.

Rabbits (n = 14) were mated with a sexually experienced buck, and placed individually in large wire "maternal" cages (90 cm long × 60 cm wide × 40 cm high), which contained a wooden nest box (49 cm × 28 cm × 27 cm) having an entrance (a hole of diameter 22 cm) cut in the front. The other rabbits of the study ("estrous"; n = 12) were not mated but were placed individually in wire cages containing nest boxes for 28 days prior to the experiment, under the conditions described above. Throughout this work, animal care adhered to the Law for the Protection of Animals (Mexico), and the protocol was approved by the Animal Ethics Committee of the *Universidad Autónoma de Tlaxcala*.

2.2. Experimental design

The experiments were initiated between 08:30 – 10:00 h on the 28th day of pregnancy, or in the case of estrous rabbits, 28 days after being moved into a maternal cage with a nest box. All females were denied access to nest material (straw) until the day of the experiment. For half of the rabbits, at t = 0 min, straw was placed inside the cage, but outside the nest box; all straw was removed from the nest box and cage at t = 30 min. The other half of the rabbits did not receive straw. In all cases, the behavior of the rabbits was subsequently recorded for 60 min. There were four comparison groups, as follows: (1) PREG + STRAW (n = 7): pregnant females were allowed to interact with straw for 30 min; (2) PREG (n = 7): pregnant females that did not receive straw; (3) ESTROUS + STRAW (n = 6): estrous females that were allowed to interact with straw for 30 min, (4) ESTROUS (n = 6): estrous females that did not receive straw. Immediately after the 1 h observation period, the rabbit was sacrificed and perfused.

2.3. Sacrifice, perfusion and immunohistochemistry

Females were anesthetized with a combination of Xilazine (i.m., 30 mg, in 1.5 mL saline) and Ketamine (i.m., 150 mg, in 1.5 mL saline), and then sacrificed with an overdose of sodium pentobarbital (i.v.), 2 min after receiving an i.v. injection of 10,000 IU heparin sulfate, the latter in order to inhibit the formation of blood clots during the perfusion. Rabbits were perfused transcardially, first with physiological saline (NaCl, 0.9%) and then 4% paraformaldehyde in 0.1 M phosphate buffer. The brains remained within the skull overnight, at room temperature (RT). The next day, brains were removed from the skull and cryoprotected by successive overnight incubations, at 4 °C, in solutions of increasing sucrose concentration (10, 20, and 30%, 0.1 M phosphate buffer). Brains were then frozen at −20 °C and sectioned coronally using a cryostat (40 μm), from the rostral pole to the beginning of the thalamus. Sections were processed for c-FOS immunohistochemistry, using a protocol previously described [5]. Free floating sections were washed three times in phosphate buffer (PB) 0.1 M, pH 7.4 in order to remove excess aldehydes, and then for 10 min in 0.05% hydrogen peroxide in order to eliminate endogenous peroxidase activity. After preincubation for 1 h in 1% normal donkey serum (Santa Cruz Biotechnology, Santa Cruz, CA) sections were incubated for 48 h at 4 °C in the polyclonal antibody SC-52-G (Santa Cruz Biotechnology), diluted at 1:2000 with 0.1% Triton X-100 (Sigma, St. Louis, MO) in 0.1 M PB. This antibody has been used successfully to visualize the c-FOS protein in the adult female rabbit [5]. The antibody solution was removed and sections were washed 3 times in 0.1 M PB, and then processed with the Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA (biotinylated donkey anti-goat serum IgG, 1 h at RT; avidin and biotinylated horseradish peroxidase solution, 1 h at RT). Sections were rinsed 3 times in 0.1 PB, and c-FOS labeling was visualized using diaminobenzidine/nickel substrate working solution (DAB peroxidase substrate SK-4100; Vector Laboratories). Sections were mounted onto gelatin subbed slides, dehydrated and then cover-slipped with Entelan mounting medium (Merck; Darmstadt, Germany).

2.4. c-FOS cell quantification

Processed sections were examined under bright-field illumination in an Olympus CH2 microscope and 2 microphotographs were taken