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Evaluation of the Emotional Responsiveness of Splanchnicectomized Laboratory Rats Using the Elevated Plus-Maze

Heather A. Finkle

Abstract

The goal of this study was to ascertain whether emotional responsiveness is attenuated when a subject undergoes bilateral splanchnicectomy. To accomplish this, the splanchnic nerve was severed bilaterally, below the branches to the adrenal glands. The majority of visceral information reaches spinal and medullary centers via the splanchnic, vagus, and pelvic nerves. Therefore, when severing the splanchnic nerve one eliminates one of the major afferent pathways transmitting visceral information to the central nervous system. Thus, we were testing the hypothesis that decreased feedback from portions of the gastrointestinal system would decrease emotional reactivity in a mildly arousing situation. No significant differences in plus-maze behavior were observed between the splanchnicectomized, control and sham-operated groups, however. This indicates that splanchnic afferent fibers do not represent a significant pathway by which visceral sensation modulates central nervous system arousal.

Evaluation of the Emotional Responsiveness of Splanchnicectomized Laboratory Rats Using the Elevated Plus-Maze

In 1890, William James proposed that the perception of a stimulus or event elicits physiological changes and that these bodily changes are transformed to feelings, which we call emotions (James, 1890/1950b). This theory was elaborated over the years and came to be known as the James-Lange theory of emotions. Autonomic responses are not the only factors included in this concept of emotional activation. Expressive behaviors such as crying, and instrumental acts such as hitting, also contribute to the emotional experience (Laird & Bresler, 1990). Afferent impulses generated from the striated and smooth muscles which activate the emotion result from the perception of a stimulus or event (Izard, 1990). Thus, in the Jamesian view, afferent impulses activate the emotion.

Walter Cannon later proposed a more centralist viewpoint. He theorized that subjective experience as well as peripheral reactions are thalamic processes (Cannon, 1927). This theory, called the Cannon-Bard theory, hypothesizes that the thalamus not only channels sensory input into the cerebral cortex, but it also sends messages via the peripheral nervous system to activate viscera and skeletal muscles, leading to the physiological and behavioral reactions that typically accompany emotions. Winton (1990) outlined five major points of disagreement that Cannon had with the earlier James-Lange theory:

1) Complete separation of the viscera from the central nervous system does not alter emotional behavior.

2) Visceral changes are not specific for different emotional states and can be present during nonemotional states as well.

3) The viscera are relatively insensitive.

4) Visceral changes are too slow to cause emotional responses.

5) Artificial induction of a physiological state which is similar to the visceral state caused by an emotional experience does not produce the same perception of emotion.

Cannon's critique was extremely damaging to the James-Lange theory of emotions, and with the advent of behaviorism, the influence of feelings tended to be discounted altogether.

In the 1950s and 1960s James's philosophy reemerged in theories such as those of Schachter and Singer (1962). Schachter's theory of cognitive arousal shares key concepts with the Jamesian approach, while accounting for many of Cannon's arguments against it. In particular, Schachter agreed that the perception of bodily changes is necessary for an emotional experience to occur and granted Cannon's argument that visceral changes can be identical for a number of different emotional states as well as some nonemotional states (Winton, 1990). Schachter and Singer's point of view is that physiological changes do not inform the individual of emotional quality; rather, they induce the individual to search for a source to which the physiological reaction can be attributed. In a classic experiment they tested the hypothesis that if subjects were able to attribute an artificially induced physiological state to a drug injection, for example, then they would not attribute those feelings to emotional arousal (Schachter & Singer, 1962). They reasoned that subjects interpret feedback from their bodies with respect to their present situation. Therefore, physiological states may not affect the qualitative emotional experience, but they may affect the

intensity of that emotional experience (Schachter, 1971). Further, arousal may play a role in some, but not all, emotions.

Schachter (1971) responded to Cannon's argument that the viscera are insensitive by maintaining that the argument is irrelevant. He argued that any level of sensation, as long as there is sensation, is adequate for the evaluation and labeling of an emotional experience to occur. Schachter also provided a response to Cannon's point that complete separation of the viscera from the central nervous system does not alter emotional behavior. He did so by emphasizing that emotional behavior need not necessarily be accompanied by an emotional experience. However, Schachter did not provide a direct response to Cannon's argument concerning speed of visceral change (Winton, 1990).

Winton, Putnam, and Krauss (Winton, 1990) proposed a bidimensional model of emotional arousal suggesting that internal information is used to determine both the intensity and the evaluative quality of the emotion being experienced. This model provides an integration of the theories of both James and Schachter. Winton, et al. propose that the role of cognition is to identify the specific category of emotional experience based on the nature of the surrounding circumstances. Perception of the situation therefore leads to initiation of central and peripheral reactions that are subjectively bidimensional (evaluative with respect to the nature of the experience and informative with respect to intensity). As a result of the peripheral reaction, a cognitive search begins for something to which to attribute the perception. During this search, further appraisal of the situation and concomitant physiological changes continue to occur. In this model, autonomic processes will be perceived, and as a result, contribute to the affective reaction,

even though they occur only a short time after the central mechanisms. Winton does not claim that this model is applicable to all emotional situations, but suggests that it might be useful in some cases.

It is therefore clear that visceral sensation plays some role in modulating emotional arousal, whether qualitatively or quantitatively. The physiological mechanisms by which this modulation occurs are not well understood. A variety of investigations have been made with respect to feedback via the parasympathetic nervous system (Adam, 1967) and the general consensus is that parasympathetic afferent pathways do play some modulatory role. The present study was aimed at investigating whether responses involving the sympathetic division of the autonomic nervous system play a similar role in modulating emotional arousal.

In this study, the splanchnic nerves were severed, reducing, although not eliminating, visceral sensation primarily from the gastrointestinal tract, which travels via this pathway. Emotional responsiveness was then measured using the plus-maze task. In this task, entries into the open arms of the maze are thought to produce anxiety in laboratory rats, and therefore normal subjects typically spend more time in the closed arms of the maze (see Pellow, Chopin, File & Briley, 1985). If visceral sensation mediated by aspects of the sympathetic system contributes to the perception of anxiety, one would expect more open-arm entries from the splanchnicectomized subjects than from control or sham-operated subjects. This would be expected because subjects with reduced visceral sensation are likely to show an attenuated fear response to the open arms of the maze. If there is no significant increase in open-arm entries observed in the splanchnicectomized animals compared to controls or sham-operated animals, then two

alternative conclusions can be drawn. First, the afferent splanchnic fibers from visceral organs of the gastrointestinal tract do not carry information pertaining to emotional state to the central nervous system. Alternatively, it is possible that visceral sensation from the gastrointestinal system does not significantly contribute to emotional responsiveness in stressful situations.

Method

Subjects

Ninety male Sprague-Dawley rats acquired from Harlan Industries were obtained for this study. Their ages ranged from 50 to 70 days and their weight from 200 to 250 grams. Upon arrival, the rats were quarantined for 10 days prior to being relocated in the laboratory. In the laboratory, the rats were housed in individual cages with food and water freely available at all times. A 12-hour light-dark schedule (lights on at 7:00 A.M.) was adhered to. Temperature was maintained at 22°C $\pm$ 2°C. Before any experimental treatment, each animal was handled to accustom it to the experimenter. Handling, gently holding and stroking the animal, occurred for a minimum of 5 minutes a day on 3 consecutive days or until the animal appeared comfortable with the experimenter.

Apparatus

The plus-maze consisted of two open arms and two enclosed arms, all with measurements of 28 x 9 cm. The walls of the closed arms were 47 cm high, and the maze was open at the top. The two open arms were opposite each other, and the four arms were positioned in a cross shape. The maze was elevated to a height of 38 cm from the floor. A 15-watt light in one corner of the room was used to produce dim illumination.

Procedure

In the primary experiment, each rat had a 7-day period of rest and recovery following surgery. Then all rats were trained in an inhibitory avoidance task and given a posttraining injection of 4-OH-amphetamine. Following retention testing, each rat had another 24-hour rest period before beginning the plus-maze test. A description of the surgical procedure and method for verification of splanchnicectomy is included in Appendix B.

Rats were placed individually in the center of the maze facing an open arm and were removed after the 15-minute test period was completed. During this time they were allowed to explore freely. An observer scored (a) the number of open and closed-arm entries (arm entry defined as all four feet in an arm), (b) total rears and (c) the time spent in various sections of the maze including the center platform. In addition, the occurrence of waste elimination (fecal boli) was recorded. The apparatus was wiped clean between each test.

Data were collected on a portable computer using custom-written tracking software. The computer program recorded position and behavior along with a time stamp (time of occurrence of each behavior or event). The data were then translated into an easily understandable format and a data file with totals was generated.

Statistical Analysis

Data concerning time spent in the various arms of the plus-maze and the number of rears were analyzed using a nonparametric ANOVA procedure (Kruskal-Wallis) to determine if there were any significant between-group differences. If between-group differences were found, the

ANOVA procedure would be followed by individual comparisons using Mann-Whitney U-tests. The fecal boli data were analyzed using a one-way ANOVA.

Results

Data and related statistics are summarized in Table 1 of Appendix A. The data from six of the laboratory rats in the surgical group was rejected because of failure to verify the completeness of the splanchnicectomy. One of those rats also belonged to the first set of six subjects which was dismissed due to observational error.

Bilateral splanchnicectomy had no significant effect on time spent in the various sections of the elevated plus-maze. Kruskal-Wallis analysis revealed that time spent in the open arms, $H(2) = 0.7301$, time spent in the closed arms, $H(2) = 1.5957$, and time spent in the center of the maze, $H(2) = 2.8646$ were all non-significant with respect to condition. The number of rears by condition was also nonsignificant, $H(2) = 0.0087$. One-way ANOVA analysis of the number of fecal boli by condition yielded $F(2,79) = 1.6863$, NS.

Discussion

The physiological component of emotional arousal plays an important role in the modulation of emotional states. William James first proposed that afferent impulses generated by the smooth and striated muscles activate emotion (1890/1950b). The James-Lange theory of emotion proposed that a specific combination of autonomic and muscular feedback caused a specific emotional feeling, something which we now know is only partially correct. Although discredited by the work of Walter Cannon and others, the essence of James's theory survives and is still being examined by researchers today.

Sixty years after James first put forth his theory of emotions, Schachter and Singer (1962) reemphasized that the perception of physiological changes within the body is a necessary component of an emotional experience. They theorized that the physiological changes that occur as a result of an emotional experience cause an individual to search the environment for the source of these changes. Further, physiological changes have the potential to affect the intensity of the emotional experience. Specific autonomic and muscular changes do not lead to specific emotions, however. Rather, general physiological changes contribute to a nonspecific feeling of arousal that prompts the individual to search the environment for some context to which the arousal can be attributed.

Today, theorists such as Winton, Putnam and Krauss (Winton, 1990) are continuing to research the role that physiological changes play in modulating emotional arousal. They suggest that physiological arousal is not undifferentiated and have proposed a modified version of Schachter's and Singer's hypothesis. Whereas Schachter and Singer stated that physiological changes provided information with respect to emotional intensity only, Winton et al. have reintroduced James's idea that bodily changes can cause affective reactions. Physiological reactions in turn contribute to the commencement of evaluative behaviors.

Although it is recognized that visceral sensation does modulate emotional responsiveness in some manner, it is not clear by what biological mechanisms such modulation is effected. Parasympathetic afferent pathways represent one likely mechanism by which neural messages associated with changes in physiological state are transmitted to the central nervous system. This study attempted to ascertain whether sympathetic afferent pathways might play a similar role. We

hypothesized that bilateral splanchnicectomy would reduce the intensity of visceral sensation from the gastrointestinal tract, thereby causing an attenuated fear response in laboratory rats subjected to a mildly arousing situation (the open arms of a plus-maze).

This hypothesis was not supported by the data collected in this study. The absence of a significant difference in plus-maze behavior between the splanchnicectomized animals and the two groups of control subjects indicates that the splanchnic afferent fibers do not represent a significant pathway by which visceral sensation modulates central nervous system arousal. Although the splanchnic nerve is one of the major afferent sympathetic pathways that transmits visceral information to the central nervous system, it is premature to presume that the sympathetic division of the autonomic nervous system does not play a similar role to that of the parasympathetic division in modulating arousal. Afferent fibers comprise only around 10-20% of the greater splanchnic nerve, whereas the vagus nerve, which also carries peripheral information to the central nervous system, is comprised of a much higher percentage of afferent fibers (Kuo, Yang, Yamasaki, & Krauthamer, 1982).

The outcome of the present study is consistent with the results obtained from the concurrent study investigating whether the effects of peripherally administered 4-OH-amphetamine on memory are mediated by the splanchnic afferents (Noyes, 1995). It is well established that 4-OH-amphetamine enhances memory retention, but is unable to pass the blood-brain barrier in significant amounts. This implies the presence of an intermediary process. The 4-OH-amphetamine stimulates the release of adrenal catecholamines such as epinephrine, which also cannot freely cross the blood-brain barrier. It is possible that epinephrine modulates memory by

activating the receptors for peripheral afferents that affect noradrenergic systems in the brain (Williams & McGaugh, 1993). Vagotomy does attenuate the effects of such peripherally acting drugs on memory, indicating that the vagus nerve is likely to play such an intermediate role (Williams & Jensen, 1993). In the concurrent study, no significant differences in memory retention were found between the splanchnicectomized and control subjects (Noyes, 1995), suggesting that the splanchnic nerve does not have a similar function to that of the vagus nerve. Taken together the findings of the present research project indicate that the splanchnic afferents do not represent a major pathway by which peripheral information reaches the central nervous system.

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Appendix A

TABLE 1

Behavior of splanchnicectomized, sham-operated and control rats in the elevated plus-maze.

	Mean Time (sec)			Mean #	Mean # of
	Open Arms	Closed Arms	Center	of Rears	Fecal Boli
Control	117.43±44.81	644.11±44.96	138.36±12.20	40.25±2.24	0.93±0.21
Splanchnicectomized	103.65±46.83	692.57±49.48	103.78±12.12	39.09±3.15	0.78±0.23
Sham-Operated	99.64±34.53	677.43±38.80	122.93±15.15	39.86±2.50	0.46±0.11
	H(2)=0.73, NS	H(2)=1.60, NS	H(2)=2.86, NS	H(2)=0.009, NS	F(2,79)=1.6, NS

Appendix B

This section includes the method section from M. Noyes's "The Effects of Splanchnicectomy on Memory Storage Processes Produced by 4-OH-Amphetamine", Masters thesis submitted to the Graduate School, Southern Illinois University at Carbondale, 1995.