

Emotional activation in human beings: procedures for experimental stress induction

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Abstract: The study about the effects of stress on cognition and behavior is an area of special interest and research development for psychology and neurosciences. Stress induction is an essential element for these studies, because it enables the manipulation of this kind of emotional activation as an independent variable to prove its effect over behavior. Nevertheless, this induction must be enough to produce a significant increase in cortisol levels and, at the same time, to achieve the established ethical standards for experiments with human beings. This article aims to provide a general review about emotional activation in order to deepen the procedures used in the experimental induce stress in human beings. The conclusion is that the elaboration of more efficient protocols is required, and the use of simulation and other technologic tools could be very useful.

Keywords: stress, cortisol, emotional activation, cognition, behavior.

Introduction

The study of the relationship between emotions and cognitive and behavioral variables become a fundamental area of analysis for psychology and neurosciences, generating valuable contributions for the experimental behavior study (Blasco, Borrás, Rey, Bonillo, & Fernández, 1997). The study of the effects of stress on cognition arises in this context, especially on memory, as a fertile field for research with human and non-human subjects, which has built a body of multidisciplinary knowledge of high potential for basic and applied research.

Studies with non-human beings establish the mechanisms implied in the way that stress affects memory and make an objective evaluation of the phenomena involved in this influence relationship. However, studies with humans allow examining more complex cognitive processes by verbal reporting. Thus, it is possible question the experimental subjects about their subjective experience in the experimental situation (autobiographical information) and about the self-monitoring of their cognitive tasks and the cognitive control they perform (metacognition) (Qiu et al., 2018).

Research with human beings requires the design of non-invasive procedures, with great caution to avoid generating long-term negative effects on subjects, which is a challenge considering the need to create protocols for stress induction that meet all ethical standards and generate a sufficient level of stress to evaluate the effects of this variable on memory.

Thus, this article begins offering an introduction to the explanatory theories of emotions, approaching stress as a modality of emotional activation. Then it focuses on the relationship between stress and cognition and, finally, includes a review of the experimental protocols available most commonly used for stress induction in humans and an analysis of their usefulness, effectiveness and relevance, from the methodological point of view, with the aim of ensuring the internal validity of laboratory procedures performed in this area of knowledge.

Foundations of emotions

Emotions are defined as subjective phenomena, with multiple dimensions, of short duration, related to intentions and expressions, proving to be crucial in the processes of adaptation of the human being to environmental demands. These include four main elements that interact dynamically: feelings, body stimulation, the sense of intention and the social-expressive (Reeve, 2010). Emotions have three main functions (Gross, 2006; Siemer, Mauss, & Gross, 2007; Rolls, 2011, cited by Feldman, 2017): preparing for action, shaping future behavior and facilitating social interaction.

James-Lange's theory (James, 1890; 1894, cited by Reeve, 2010) states that emotion is experienced from a sequence involving the appearance of a stimulus, the emotion and, subsequently, the body's reaction. Therefore, the emotional experience would be prior to the appearance of physiological changes. Cannon-Bard's theory, argues that emotional and physiological activation are simultaneous reactions to the same stimulus (Canon, 1929, cited by Feldman, 2017), while

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Schachter-Singer's theory proposes that emotions are a product of physiological activation and cognitive processing made of a particular event (Schachter & Singer, 1962, cited by Feldman, 2017).

Moreover, the theory of differential emotions states that there are ten clearly identified basic emotions that respond to specialized motivational purposes, with a subjective experience, a facial expression and a physiological activation particular to each of them, constituting a system with the adaptive function of preparing the individual to act according to the demands of the environment (Izard, 1991; 1993). According to Ekman (1992), emotions are grouped in such a way that others can be derived from the basic ones, by variation or combination, broadening the range of possible emotional experiences, which should not be mistaken with moods, attitudes, personality traits, or mental disorders. Finally, the cognitive approach proposes that emotions arise from the processing of information, which is explained from the construct of assessment (Scherer, Schorr, & Johnstone, 2001; Smith, Haynes, Lazarus, & Pope, 1993). It consists of the calculation of the subjective importance of a process, based on two main postulates: firstly, cognitive assessment precedes emotion and, secondly, it would not be the success but the assessment that would produce the emotion (Lazarus, 1991).

Stress as emotional activation

Stress is a widely used term and is defined in multiple ways, depending on the area of study that addresses it. An example of this are the different applied perspectives that handle this concept from the social context (Väänänen, Anttila, Turtiainen, & Varje, 2012), educational (Fares et al, 2016), medicine (Cutshalla, Bergstrom, & Kalish, 2016) and occupational health (Upadyaya, Vartiainen, & Salmela-Aro, 2016), among other areas of study, implying multiple ways of operationalizing it and defining it as a concept and the need to specify its approach when this term is used.

Selye (1936) adopted the term stress from its use in engineering to refer to an unspecific phenomenon represented by a series of symptoms produced by a wide variety of harmful agents. More modern approaches define stress as a perceived threat to homeostasis, which may or may not be conscious (Goldstein & McEwen, 2002) and whose response depends on the particular challenge affecting homeostasis, the interpretation of stress and the perceived ability to confront it (Goldstein & Kopin, 2007). The term stressor refers to "any environmental demand that creates a state of tension or threat (stress) and requires change or adaptation (adjustment)" (Morris & Maisto, 2014, p. 363).

From the neuroscience point of view, stress, seen as a kind of emotional activation, is a physiological

reaction that affects the properties of brain cells and can affect the nervous system and other systems, as well as behavioral and cognitive processes (Pruessner, Pruessner, Hellhammer, Picked, & Lupien, 2007). This reaction is produced by the effect of stressful stimuli, conditions that affect the homeostasis and physiology of an organism (Dickerson & Kemeny, 2004). For an environmental stimulus to become an emotional activator it must meet one or more of the following characteristics: novelty (Rose, 1980), unpredictability (Mason, 1968), lack of control (Sapolsky, 1993) and must represent a threat of potential harm or loss (Blascovich & Tomaka, 1996) which, in the case of humans, may be related to social assessment (Lupien, Maheu, Than Tu, Fiocco, & Schramek, 2007). Stress can be absolute, produced by real threats to the integrity of the person, or relative, threats perceived by the individual from the interpretation made of the situation (Pruessner et al., 2007). Stressors may consist of external disturbances, of the individual's environment or internal environmental disturbances, such as health problems affecting the individual (Oken, Chamine, & Wakeland, 2015).

The perception of an absolute or relative threat results in many hormonal events clearly characterized: initially, the hypothalamus releases the corticotropin releasing factor (CRF), which triggers the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland into the bloodstream, stimulating the release of glucocorticoids in the adrenal cortex (GC, cortisol in the case of humans and corticosterone in the case of rodents) in a process known as activation of the HPA axis (hypothalamus – pituitary – adrenal). The activation of the axis corresponds to a mechanism of adaptation of the organism that allows it to increase the availability of corporal energy as preparation to respond to the demands of the environment (Lupien et al., 2005).

In the brain, it is possible to find two types of receptors for glucocorticoids: mineralocorticoids (MR or Type I) and glucocorticoids (GR or Type II). There are two main differences between these receptors: first, MR have a greater affinity with GC, resulting in a greater occupation of these receptors for a large part of the day, so, in a stressful situation, most of the MR are saturated, generating a greater occupation of MR characteristic of emotional activation. The second difference is related to the distribution in the brain, MR are located exclusively in the limbic system, while GR are located in cortical and subcortical areas, especially in the prefrontal cortex (De Kloet, Oitzl, & Joels, 1999).

Stress and cognition: the effect of stress on memory

A high density of glucocorticoid receptors, released in situations with high emotional content, is found in the hippocampus and in the parahippocampal

region (De Kloet et al, 1999; Diamond, Fleshner, Ingersoll, & Rose, 1996), for this reason is possible to expect an influence of stress on memory.

The association between stress and memory is extensively studied and the contradictory results (facilitating and deleterious or harmful effects of stress on memory). Demonstrating the complexity of the interaction between these variables, which would be measured by the level of stress generated, the duration of the stressor, the kind of task and the phase of the memory evaluated (Joëls, Pu, Wiegert, Oitzl, & Krugers, 2006; Pruessner et al., 2007). In this respect, an inverted U-shaped relationship between glucocorticoids and cognitive performance is suggested, where there would be a facilitating effect with moderately elevated glucocorticoid levels (Lupien & McEwen, 1997; De Kloet et al., 1999) and a deleterious effect with very high or very low levels (Lupien & Lepage, 2001). This interaction would mainly involve the hippocampus, an important structure in memory consolidation and recovery, with a high density of glucocorticoid receptors and a major role in the negative feedback of HPA axis activity (Pruessner et al., 2007).

Previous studies show that, in rodents, exposure to brief periods of stress after training in spatial memory tasks improves performance in recovery tests (Cerón, 2015) and that this effect is accompanied by increases in the activity of structures such as the hippocampus, amygdala and prefrontal cortex (Urueña, 2016).

Animal experiments deepened these effects of stress on consolidation, confirming its facilitating effect (Roosendaal, 2002; Roosendaal & McGaugh, 2011) which would be mediated by beta-adrenergic and glucocorticoid mechanisms (McGaugh, 2000; Roosendaal, McEwen, & Chattarji, 2009). Specifically, stress leads to an activation of the beta-adrenoreceptor within the basolateral amygdala, which would strengthen memory consolidation by its efferences to other brain regions such as the hippocampus (McGaugh, 2004; Roosendaal & McGaugh, 2011).

This via is also validated in humans, by the exogenous administration of beta-adrenergic transmission triggers after training, revealing that this procedure also produces a facilitating effect of performance in memory tasks (Cahill & Alkire, 2003; Southwick et al., 2002). In addition, there is evidence that post-acquisition stress induction in facial recognition memory tasks has a facilitating effect on recovery in humans (Larra et al., 2014).

Induction of stress in humans

The study of the effects of stress on cognition and behavior involves the design and implementation of stress induction protocols that guarantee its manipulation as an independent variable in a variable

control context that allows the establishment of influence and causality relationships within the framework of the explanatory scope of the research. The development of experiments with humans requires the design of non-invasive procedures that guarantee limited effects that do not transcend in the long term and that generate moderate levels of stress, sufficient to observe their effects without generating damage in the participants of studies. Thus, it should include the elements of novelty (Rose, 1980), unpredictability (Mason, 1968), lack of control (Sapolsky, 1993), representing a threat of damage or potential loss (Blascovich & Tomaka, 1996) and, in some cases, social assessment (Lupien et al., 2007) cited above.

Traditional protocols for stress induction

In this context, protocols such as the Cold Pressor Task (CPT) and the Trier Social Stress Test (TSST) was designed to induce stress in humans, with high acceptance by the scientific community and widespread use in many kinds of experiments in psychology and neurosciences.

Cold Pressor Task

CPT is a sufficiently validated protocol for stress manipulation in humans. It showed to produce significant increases in the liberation of cortisol, known as stress hormone, whose action, as previously mentioned, is mediated by the activity of the HPA axis (Goldfarb, Froböse, Cools, & Phelps, 2017; Buser, Dreber, & Mollerstrom, 2017; Raio, Orederu, Palazzolo, Shurick, & Phelps, 2013; McRae et al., 2006).

The task for the participants was the immersion of the non-dominant hand in cold water (0 °C – 4 °C) during one to three minutes (Buser, Dreber, and Mollerstrom, 2017; McCullough, Ritchey, Ranganath, & Yonelinas, 2015; McRae et al., 2006). The control condition consists of applying the same procedure, with a modification in the water temperature as the only variation in the protocol, using warm water around 38 °C (Goldfarb et al., 2017). Some studies use the dominant hand in this procedure (Buser et al., 2017).

This task consistently generates activation in the sympathetic nervous system, producing increases in blood pressure and skin conductance. However, it does not always generate an increase in cortisol levels, so activation of the HPA axis is not evident in all cases (Mitchell, MacDonald, & Brodie, 2004; Smeets et al., 2012).

The CPT has received significant criticism about its true role in stress induction. It is not clear whether this procedure actually models a threat or activates a homeostatic temperature-regulating mechanism, which would not correspond to stress. In addition to its use as a pain inducer, which would generate ambiguity

regarding its true effect on the body (Lazarus, 1963), since this task has not only been used to study stress, but constitutes a protocol of great tradition for the study of pain and plays a crucial role in the understanding of its mechanisms, assessment and management (Birnie, Parker, & Chambers, 2016). This would confirm the approaches of the critics in relation to the ambiguity in the effects produced by this task, making it difficult to interpret the results of the studies that include it as a protocol for stress induction.

Trier Social Stress Test

The Trier Social Stress Test (TSST) is a widely validated protocol for the induction of psychosocial stress under laboratory conditions (McRae et al., 2006; Villada, Hidalgo, Almela, & Salvador, 2016; Bershad, Miller, & De Wit, 2017). The TSST includes a period of preparation of an oral presentation, a period for the execution of the presentation and one for performance in an arithmetic mental challenge (successive subtractions with intermediate level of difficulty). All in the presence of an audience or jury, some protocols include a recording of the session, in order to increase the level of psychosocial stress produced. Each of the phases (preparation, exposure and arithmetic task) has a duration of five minutes (Lupien et al., 2007; Lupien et al., 2005; McRae et al., 2006).

There are multiple variations of the TSST application protocol, so Romero-Martínez, Nunes-Costa, Lila, González-Bono, and Moya-Albiol (2014) designed an adjusted version of TSST for a study with a population of perpetrators of intimate partner violence. The entire experimental session lasted approximately 75 minutes, beginning with a 15-minute rest period with a continuous electroencephalogram record, in order to establish the baseline. Subsequently, the preparatory stage of the oral presentation was carried out, with a duration of 15 minutes. Then, the stressor application stage was implemented, with five minutes for the oral presentation, including a speech about the person point of view regarding domestic violence and opinions about the Spanish legislation applied to the case and in general and five more minutes for the arithmetic task. Finally, a recovery stage was implemented with a duration of 15 minutes. The findings of the study indicate particular patterns of cardiovascular response to psychosocial stress by perpetrators of violence against their partners.

Childs, Vicini, and De Wit (2006) designed a group version of the protocol to contrast it with the original version of individual tasks, in order to find strategies to reduce the costs for the application of TSST, which are usually high due to the requirements for its application in terms of time and personnel. Cortisol levels in saliva and reported anxiety increased

in both conditions and the subjects evaluated in group presented a more pronounced increase in their heart rate.

Recent studies have implemented the use of emerging technologies for the use of the TSST protocol, as the pilot study conducted by Wallergård, Jönsson, Österberg, Johansson, and Karlson (2011), in which a computerized version of the TSST was designed, making use of an immersive virtual reality system. In this case, the audience consisted of an evaluation committee made up of three virtual humans. The measurements made were physiological (electrocardiogram and respiratory frequency) and subjective, in both a stress response was detected induced by the virtual reality version of the TSST.

TSST showed significant increases in cortisol and ACTH levels in humans, higher than produced by CPT, which allows deducing a direct relationship of this task with the activity of the HPA axis, therefore, with stress processes, and a higher effectiveness in stress induction, compared to CPT (McRae et al., 2006).

TSST is widely used as a stress inducer in experimental studies, also receiving strong criticism, because it is questioned if it really generates stress or if the activation produced in the individual is due to cognitive effort, which involves the tasks to be performed (Lazarus, 1963). Other authors report methodological difficulties regard TSST use in its traditional version. Such as the cost, in relation to the time and personnel required (Childs et al., 2006), and the difficulty to maintain constant experimental conditions, because the audience is made up of humans that could change the severity exhibited towards the different experimental subjects, which constitutes a peculiar variable habitual in this protocol (Wallergård et al., 2011).

Alternative protocols for stress induction

The study of the effects that psychological stressors have on the physiology of individuals at the experimental level generated variable results and a high inconsistency. Only in some cases, it was possible to obtain the expected increases in cortisol levels, which makes evident the need to establish and delimit the essential elements that constitute the enabling environments of produce the increase in cortisol levels and the stress response (Dickerson & Kemeny, 2004).

Considering the criticisms and methodological difficulties identified in these protocols, which are the most common for stress induction in humans, arises the need to design new procedures to ensure the manipulation of the variable, with proper control and greater precision. In such a way that stress induction

is clear and allows for more conclusive findings regarding the influence of stress on cognition and behavior.

As a result of this search for alternative protocols for the induction of stress in humans, different procedures were developed, some use images, films, interference tasks, tasks for which individuals are required to perform specific and unusual activities, tasks involving the use of video games, virtual reality and augmented reality.

Protocols designed from a combination of traditional procedures

Socially Evaluated Cold Pressor Test (SECPT)

This procedure results from a combination of CPT and TSST, designed to generate the activation of the sympathetic nervous system and the HPA axis simultaneously, which produce these two protocols (Schwabe, Haddad, & Schachinger, 2008). Before starting the experiment, participants are informed that a video record of their facial expressions will be made for further analysis. Then, they are instructed to put the right hand in cold water (0-4 °C) while looking at the camera and maintain their hand in the water for as long as possible, with a maximum duration of three minutes, at the same time they are observed by the investigator (of the opposite sex). This procedure showed an increase in heart rate similar to that induced by CPT and a significant increase in cortisol levels in saliva, which is only observed when the subject is exposed to cold (not warm) water, suggesting that the effect on the HPA axis depends on the combination of physical and social stressors (Schwabe et al., 2008).

Although this protocol has demonstrated consistency in the activation of the HPA axis (Schwabe & Wolf, 2010; 2011), its results are still lower than the generated by the TSST (Smeets, 2011). Besides this, because it is a combination of CPT and TSST, this procedure receives the same criticisms as for these protocols, so it is not clear if the physiological reaction that it generates corresponds to stress or is only a product of the pain produced by cold water. Furthermore, the effectiveness of the social assessment component depends on the vulnerability of the participant to this type of stress and on the characteristics of the investigator responsible for the assessment. Finally, it is proposed that the choice of an evaluator specifically of the opposite sex to generate stress would not consider aspects of gender diversity.

Maastricht Acute Stress Test (MAST)

This procedure also consists of combining physical stress with social evaluation, from the selection of the most stressful characteristics of

TSST and CPT. (Smeets et al., 2012). The protocol begins with a five-minute preparation phase, when instructions are presented, followed by five SECPT tests (at a constant temperature of 2 °C) with a variable duration of 60 to 90 seconds. At inter-assay intervals, participants dry their hands and immediately begin an arithmetic task similar to that used in the TSST, of successive subtractions, receiving negative feedback each time they make a mistake; once the interval is over, they start a SECPT test again. This protocol demonstrated similar levels of sympathetic activation and more significant increases of cortisol in saliva compared to CPT; related to TSST, equivalent levels of sympathetic activation and cortisol are found, making it a much simpler and shorter-lasting effectiveness instrument similar to TSST (Smeets et al., 2012).

A variation of this protocol (imaging Maastricht Acute Stress Test – iMAST) was designed to perform stress induction procedures on the functional magnetic resonance scanner (fMRI) (Quaedflieg, Meyer, & Smeets, 2013). In this case, since it is not possible to use cold water in the fMRI chamber, a thermal stimulator is used and the negative evaluation is done through an intercom system equipped with microphone and headphones to allow the participant to communicate with the researchers. This variation in the procedure showed to generate significant increases in cortisol levels in saliva and on subjective stress scales, making it a useful alternative for neuroscience studies involving real-time observation of localized brain activity (Quaedflieg et al., 2013). In this case, the criticisms previously presented, related to the ambiguity between the effects of stress and pain produced by the low temperature in the hand and the subjectivity of the social evaluation component, would also be applied.

Protocols involving the execution of specific tasks

Sing-a-Song-StressTest (SSST)

The task of singing a song (SSST) is a procedure designed to induce stress in an ethical manner that does not generate lasting negative effects on subjects (Brouwer & Hogervorst, 2014). This protocol begins with the selection of a participant and two “accomplices” (members of the research team) to enter into the experimental room, where the experiment leader presents the instructions. One at a time must remain seated in front of a monitor, reading the messages that appear (with intervals between rehearsals of 60 seconds, consisting of a countdown from 60 on the same monitor) while being recorded by a video camera. It is indicated that one of the messages may contain a task to be performed (participants do not know that this is an experiment

for stress induction or that it involves a chant task). The true participant is the first to carry out the task, exposed to a presentation of neutral messages with a final message indicating that a song must be sung loudly once the interval ended. Heart rate and skin conductance measurements show a significant increase after the singing task, compared to neutral messages (Brouwer & Hogervorst, 2014). It is evident that this protocol generates sympathetic activation, its role in the production of stress by activating the HPA axis is not clear, for which cortisol measurement is required after the execution of the task. Besides this, it is possible that the activation produced by this protocol depends on the performance of the participants. Thus, it is possible that a person who frequently sings in public, or has the ability to sing is not affected in the same way by this kind of procedure.

Montreal Imaging Stress Task (MIST)

The Montreal Image Stress Task (MIST) is a protocol derived from the mental challenge segment of the Trier Social Stress Test, designed to induce moderate psychological stress (Dedovic et al., 2005). This procedure consists of a series of computerized mental arithmetic challenges (with time limitation and manipulated to be at the limit of the individual's mental capacity, complemented with the presentation of the individual's average performance and expected performance), combined with social evaluation components. This protocol shows to increase cortisol levels in saliva significantly, and is useful in studies requiring the use of techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET). In this case, the model proves to be effective in activating the HPA axis. However, the social evaluation component can generate differential results, depending on the characteristics of the participant, due to its subjective nature. An important advantage of this protocol is its adaptability to equipment that requires scanning such as fMRI and PET.

Mannheim Multicomponent Stress Test (MMST)

This is a five-minute protocol designed to cause relatively high levels of stress by the simultaneous use of four different modalities of stressors (cognitive, emotional, acoustic, and motivational), not including the social assessment component (Reinhardt, Schmahl, Wüst, & Bohus, 2012). The procedure begins with a baseline where subjects are invited to relax in an empty room. Then, five minutes of stress induction begin with the presentation, for one minute, of a white noise (acoustic stressor mode) and photographs of negative affective value (emotional mode), some of them repeated. Participants are asked to indicate which are the photographs that appear more than once, after five photographs, one of positive value is presented

to avoid effects of habituation. After the first minute of stress induction, the arithmetic task (cognitive mode) begins, consisting of an arithmetic task in which numbers are presented sequentially on a screen. The participants must add the most recent number with the previous one and repeat this task consecutively, while the photographs of negative images continue to appear in the background on the screen and the white noise continues to sound in the hearing devices. Since the beginning of the study, it is indicated to the participants that they will receive a sum of money for their participation in the study, starting with 100 € and decreasing each time a mistake is made in the arithmetic task, which will be informed by an acoustic signal (motivational mode). This procedure showed significant changes in the electrodermal activity of the skin, as well as increased cortisol levels in saliva, heart rate, and subjective stress reporting presented by participants. With these results in mind, this protocol is considered an economical, short-term alternative for studies seeking to evaluate stress reactions without the social evaluation component (Reinhardt et al., 2012). While this procedure demonstrates effectiveness in activating the HPA axis, it is possible for participants with differential abilities for cognitive challenge to develop differential levels of stress.

Stroop test

Stroop test is an individual administration task with an average duration of five minutes, consisting of the presentation of plates on which the words "RED", "BLUE", "GREEN" and "YELLOW" are written in different colors that do not correspond to the one expressed in the text, generating an interference. The participant should read the text, avoiding mentioning the color in which it is written. This test is applied in different clinical and experimental contexts; in the case of the study of the effects of stress, it is used to demonstrate that it produces increases in the reactivity of blood pressure (Gianaros et al., 2005). In this case, it is still necessary to demonstrate the activation of the HPA axis resulting from the execution of the task. So studies are required to measure cortisol levels for this purpose. This is a procedure of easy and economic application that, if its effectiveness in activating the HPA axis is demonstrated, could constitute a useful alternative, especially for studies with massive participation of experimental subjects.

Protocols based on the use of audiovisual material

Trauma film paradigm

The procedures for the induction of stress from audiovisual material arise from the reflection made initially by Lazarus (1963), who questioned the

pertinence of existing protocols up to that moment to induce stress in humans. He proposed the need to use alternative paradigms constituted by simulations that lead the subject to be in contact with potentially dangerous situations without real risks and without producing long-term negative collateral effects on the subjects. The initial studies using this technique were made with documentary videos of ablations made in Australian tribes, generating emotional activation measured by skin conductance and heart rate (Lazarus, 1963). Subsequently, these procedures led to the Trauma film paradigm, a model of post-traumatic stress using videos of real traffic accidents (Holmes, Brewin, & Hennessy, 2004; James et al., 2016). Experiments using these protocols often define as a control condition the exposure to neutral films including natural scenes, wild animals in their habitat (Kreibig, Wilhelm, Roth, & Gross, 2007) and marine life with relaxing music (Kunze, Arntz, & Kindt, 2015).

Another alternative for this kind of paradigm is the induction of acute stress by watching films of suspense or terror, generating significant elevations in cortisol levels in contrast to films of emotionally neutral content (Hubert & de Jong-Meyer, 1989, 1992; Kirschbaum & Hellhammer, 1989). The trauma film paradigm constitutes a useful alternative for the induction of stress, without exposing the subjects to potentially dangerous situations. However, its long-term consequences are discussed, since the exposure to audiovisual material so shocking, could produce emotional effects in the participants after the end of the experiment.

International Affective Picture System (IAPS)

International Affective Picture System (IAPS) is a widely validated protocol. It is composed of more than a thousand color photographs of everyday situations, evaluated and categorized into affective dimensions based on the level of control over the image, the level of activation or calmness it provokes and its value, or the level of liking or displeasure produced by the observation of the image (Lang, Bradley, & Cuthbert, 2008). Specifically, as a technique for stress induction, the images that generate the stimulation and values that correspond to this kind of emotion are used. This protocol demonstrated measurable changes in the three channels of emotional response: behavioral, physiological and verbal (Bradley & Lang, 2000). It is validated in different countries and used in a large number of experimental studies aimed at exploring different emotional states (Moltó et al., 2013). This protocol demonstrated a high effectiveness. However, it is necessary to identify its role in the activation of the HPA axis, by measuring cortisol levels after exposure to photographs. It is a general procedure in the exploration of emotions, so its effectiveness

in inducing stress could be proven in more specific variations of the protocol, aimed specifically at studying this emotion.

Set of Fear Inducing Pictures (SFIP)

The set of fear-inducing photographs (SFIP) consists of a group of 400 color photographs, taken from different sources, divided into five categories: blood or injection, small animals, social exposure, angry and neutral faces (Michałowski et al., 2017). This procedure was designed to study fear reactions and as an instrument to deepen the study of phobias. The effectiveness of this procedure was tested using self-report scales, which evaluate the intensity and valence of the emotions produced. However, it is necessary to measure the levels of cortisol after exposure to the protocol images, in order to identify the relationship of this procedure with the activation of the HPA axis.

FilmStim

FilmStim (Schaefer, Nils, Sanchez, & Philippot, 2010) consists of a set of video clips taken from famous films, selected and classified during their validation, to cause basic emotions, including fear. This method generates emotional activation in 24 classification criteria, such as the subjective stimulation, significantly higher scores in different scales of emotional measurement and differential oscillatory patterns in electroencephalogram (Gärtner & Bajbouj, 2014). In this case, although the procedure generates increases in subjective emotional scales, it is necessary to obtain more objective measurements of its effectiveness as a stressor, specifically cortisol levels and peripheral measures of emotional activation, in order to establish the influence of the protocol on the activation of the HPA axis and the sympathetic nervous system.

Protocols based on the use of emerging technologies

Video games

Recent studies report the use of video games as an effective stress inducer, adding interactivity to generate greater immersion in the stressful situation. A preliminary protocol was designed by Geslin, Bouchard, and Richir (2011), using virtual reality. The procedure, which lasts eight to ten minutes, consists of a virtual journey in dark alleys with complete freedom of exploration, although there is only one route available, in which different stress inducing elements appear successively, such as a dog with attacking behavior, a narrow staircase, flashing

lights, an abandoned surgery room, etc. The protocol measures the fear response by self-reporting in an emotional response questionnaire, finding that the expected emotional activation is not achieved in general terms. Nevertheless, participants who do not consider themselves experienced players present activation superior to that experienced by experienced players (Geslin et al., 2011).

Subsequently, Madsen (2016) implemented a procedure based on the use of horror video games to induce fear. In this protocol, participants play the video game Konami's, P.T. ("Playable Teaser"), characterized by belonging to the genre of terror and having a first-person perspective, on the PlayStation 4 console, broadcast on a television screen. Members of the experimental group play on the console, while those of the control group observe the game on a monitor. The results of this study demonstrate statistically significant increases in recordings of electrodermal activity, respiratory rate and heart rate, as well as in self-report of fear of individuals belonging to the experimental group (Madsen, 2016). These procedures are innovative and bring useful proposals for the development of future studies. However, require a more exhaustive validation of the protocol, as well as the measurement of cortisol to establish the influence of the procedure on the activation of the HPA axis.

Virtual reality

Annerstedt et al. (2013) designed a procedure for stress induction by a virtual reality application that simulates the TSST, with the corresponding cognitive activities and social evaluation, supported by the use of previously recorded voices activated by the experimenters from an external room. The virtual environment is presented using the CAVE™ system with projections on three of the walls and floor and passive stereoscopy to generate 3D vision. The procedure generates significant increases in cortisol levels and heart rate (Annerstedt et al., 2013).

Subsequently, Breuninger, Sláma, Krämer, Schmitz, and Tuschen-Caffier (2017) designed a protocol for anxiety induction, based on the use of virtual reality technologies. The procedure comprises five sequential conditions, starting with a five-minute baseline in which a neutral video is displayed. Then, a five-minute training session is conducted teaching the participant how to use the virtual reality team's controls. Before, a contextualization phase of one to three minutes begins, participants are invited to imagine a situation that gives coherence and realism to the subsequent experimental situation. Next, a phase of stress induction begins, participants face an application of virtual reality with multiple stress factors, including an explosion and the impossibility of rescuing the victims, which is the task they must

perform. The last phase consists of a relaxation in a dark room. The results show an increase in sympathetic activity, reflected in significant differences in heart rate and skin conductance (Breuninger et al., 2017).

Sabo, Rajčáni and Ritomský (2018) designed recently a stress induction procedure to observe its effects on various speech characteristics. The procedure consists of exposing participants (seated) to a virtual reality application called "NoLimits Roller Coaster Simulation" in the HTC VIVE virtual reality system, which corresponds to a roller coaster ride lasting 2 minutes, 50 seconds. Results show a significant increase in physiological activation, measured by heart rate and skin resistance, although no effects on speech are found (Sabo et al., 2018). Considering the novelty of these procedures, related to the recent development of the technologies that make them possible, the results presented are still preliminary and require the validation of cortisol protocols and measurements (in which it has not been measured yet) to evaluate their effectiveness in activating the HPA axis. It is also necessary to collect a greater number of data to consolidate the validity of the results obtained and to consider the collateral effects that the use of virtual reality devices may have on people who suffer vertigo or who have higher levels of sensitivity to this kind of technology.

Augmented reality

Recent studies made it possible to design procedures for anxiety induction in the context of the study of the therapeutic effectiveness of augmented reality procedures for the treatment of phobias, which could eventually generate stress. Thus, Yeh, Li, Zhou, Chiu, and Chen (2018) designed an augmented reality procedure that consists of confronting the participants with a situation with multiple stressors such as a situation of terror in the first instance, supposed intermittent equipment failures, alarming noises, and a final fire scene. This procedure revealed to generate an increase in the skin conductance level and heart rate of participants (Yeh et al., 2018). Other studies, conducted by the same research group, present a variation of this protocol, making use of augmented reality applications with claustrophobic characteristics, generating increases in the heart rate of the participants (Tsai et al., 2018). Augmented reality protocols allow the inclusion of artificial elements designed for the experiment, in the real environment of the individual, which constitutes a significant advantage for these procedures, since they induce stress in more realistic contexts for the individual. Considering the recent development of these technologies, it is necessary to replicate the studies, validate the protocols and establish their relationship with the activity of the HPA axis.

Conclusions

The study of stress, its nature, mechanisms and effects on cognition and behavior, constitutes an area of great research interest, for psychology and neurosciences, as it allows us to explain a series of frequent phenomena in the clinical, organizational, educational, legal, sportive, and other fields. The experimental study of these phenomena is productive in comparative research with non-human subjects. However, some cognitive and meta-cognitive elements require studies with humans. In this context, and knowing that experimental studies

require the manipulation of variables, it is necessary to design procedures for the induction of stress in human subjects that meet the ethical requirements necessary to avoid long-term effects on participants and produce a moderate level of stress by non-invasive protocols.

Thus, the protocols compiled in the present article show the results of the experimental search for ethical procedures, effective enough for the generation of stress in humans, as a state of the art that provides the theoretical, methodological and argumentative support necessary for the future construction of ethical and effective protocols, probably based on the use of technologies.

Ativação emocional em sujeitos humanos: procedimentos para a indução experimental de estresse

Resumo: O estudo dos efeitos do estresse sobre a cognição e o comportamento é uma área de especial interesse e desenvolvimento investigativo tanto para a psicologia como para as neurociências. A introdução de estresse constitui um elemento fundamental nestes estudos, pois permite a manipulação desta forma de ativação emocional como variável independente, para observar o seu efeito sobre o comportamento; porém, esta indução deve ser suficiente para gerar incrementos significativos nos níveis de cortisol e ao mesmo tempo cumprir os padrões éticos estabelecidos para a experimentação com humanos. O presente artigo tem como propósito aportar uma revisão geral sobre a ativação emocional, para aprofundar nos procedimentos utilizados na indução experimental de estresse em sujeitos humanos. Conclui-se que é requerido a elaboração de protocolos mais eficientes, e para tal, o uso de simulações e outras ferramentas tecnológicas pode ser de grande utilidade.

Palavras-chave: estresse, cortisol, ativação emocional, cognição, comportamento.

Activation émotionnelle chez l'homme: procédures pour l'induction expérimentale du stress

Résumé: L'étude des effets du stress sur la cognition et le comportement est un domaine d'intérêt particulier et de développement de la recherche tant pour la psychologie que pour les neurosciences. L'induction du stress constitue un élément fondamental de ces études, car elle permet la manipulation de ce mode d'activation émotionnelle en tant que variable indépendante, afin d'observer son effet sur le comportement; cependant, cette induction devrait être suffisante pour provoquer des augmentations significatives des taux de cortisol tout en respectant les normes éthiques établies pour l'expérimentation humaine. Le but de cet article est d'effectuer une revue générale de l'activation émotionnelle afin d'approfondir les procédures utilisées dans l'induction expérimentale du stress chez l'homme. Cet article conclut que la conception de protocoles plus efficaces est nécessaire; à cette fin, l'utilisation de simulations et d'autres outils technologiques peut être très utile.

Mots-clés: stress, cortisol, activation émotionnelle, cognition, comportement.

Activación emocional en sujetos humanos: procedimientos para la inducción experimental de estrés

Resumen: El estudio de los efectos del estrés sobre la cognición y el comportamiento es un área de especial interés y desarrollo científico, tanto para la Psicología como para las Neurociencias. La inducción de estrés constituye un elemento fundamental en estos estudios, pues permite la manipulación de esta forma de activación emocional, como variable independiente, para observar su efecto sobre la conducta; no obstante, esta inducción debe ser suficiente para generar incrementos significativos en los niveles de cortisol y cumplir los estándares éticos establecidos para la experimentación con humanos. El presente artículo tiene como propósito aportar una revisión general sobre la activación emocional, para profundizar en el conocimiento acerca de los procedimientos utilizados en la inducción experimental de estrés en sujetos humanos. Se concluye que se requiere el diseño de protocolos más eficaces, para lo cual, el uso de las simulaciones y otras herramientas tecnológicas puede ser de gran utilidad.

Palabras clave: estrés, cortisol, activación emocional, cognición, comportamiento.

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