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Programa de Doctorado en Psicología

Mecanismos autonómicos de la modulación de la respuesta cardíaca de defensa

Doctoranda

Alba Garrido Muñoz

Directores

Dr. José Luis Mata Martín

Dr. Jaime Vila Castellar

UNIVERSIDAD DE GRANADA

Departamento de Personalidad, Evaluación y Tratamiento Psicológico

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Autor: Alba Garrido Muñoz
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ANOVA: analysis of variance

BP: blood pressure

CDR: cardiac defense response

ECG: electrocardiography

HP: heart period

HR: heart rate

IAPS: International Affective Picture System

ICG: impedance cardiography

IEE: intervalo entre estímulos

ITI: inter-trial Interval

PEP: pre-ejection period

RCD: respuesta cardíaca de defensa

SBP: systolic blood pressure

Capítulo 1:

Introducción

1.1. La respuesta de defensa

El concepto de defensa hace referencia al conjunto de respuestas o patrones de respuesta que aparece en los organismos ante la presencia de peligro o amenaza (D. C. Blanchard & R. J. Blanchard, 2008; Vila et al., 2009). En la mayoría de los casos, estos comportamientos defensivos no han aparecido como consecuencia de una experiencia previa específica con una amenaza, sino que guardan relación con las historias evolutivas de las especies que los manifiestan. Los organismos que han llevado a cabo respuestas defensivas más adaptativas son las que han logrado sobrevivir y reproducirse. Así, estas respuestas habrían ido evolucionando hasta llegar a constituir el repertorio de respuestas defensivas "preprogramado" del que disponen hoy en día las distintas especies, incluido el ser humano. Estas respuestas pueden asociarse rápidamente a través de condicionamiento con estímulos y situaciones amenazantes (D. C. Blanchard & R. J. Blanchard, 2008; R. J. Blanchard & D. C. Blanchard, 1989). Pero ¿en qué consiste ese repertorio de respuestas defensivas?

Ya en el año 1929, Walter Cannon acuñaba el término "*fight or flight*" ("lucha o huida") para describir la respuesta del ser humano frente a una amenaza. Desde entonces, se han hecho importantes avances en investigación que nos han llevado a ampliar esta definición de la respuesta defensiva. Una parte importante de la investigación se ha centrado en el estudio de la respuesta defensiva en animales (e.g., roedores, gallinas, primates, etc.) siguiendo la tradición etológica. La etología consiste en el estudio del comportamiento animal en sus medios naturales o en contexto de laboratorio, siempre y cuando el laboratorio esté diseñado de forma en que se incluyan las características naturales necesarias para permitir el desarrollo de un patrón de comportamiento animal completo (D. C. Blanchard & R. J. Blanchard, 1988).

Bracha et al. (2004) sugieren que, al detectarse una amenaza, los mamíferos presentan un patrón defensivo biológicamente determinado que consta de varias respuestas que tienen lugar de forma secuencial conforme se produce un incremento o una aproximación de la amenaza. Esta secuencia se enmarca dentro del ámbito de investigación etológico y fue propuesta originalmente por J. A. Gray (1988). Supone una reordenación y una ampliación de la concepción de la respuesta defensiva entendida como "*fight or flight*" (Cannon, 1929), e incluye estas cuatro respuestas: *freezing* (congelamiento), *flight* (huida), *fight* (lucha) y *fright* (inmovilidad tónica).

Según este planteamiento, lo primero que ocurre cuando un organismo detecta una amenaza es la aparición de la respuesta de congelamiento, que consiste en un incremento del estado de alerta o vigilancia. La respuesta de congelamiento puede entenderse como la tendencia a "parar, mirar y escuchar" que se encuentra asociada con el miedo. La investigación etológica ha demostrado que una presa que permanece "congelada" ante la presencia de otro organismo que supone una amenaza tiene más posibilidades de escapar. Por lo tanto, la respuesta de congelamiento supone una ventaja para la supervivencia. Esto es debido a que el córtex visual y la retina de los mamíferos carnívoros (y, en menor grado, del ser humano) ha evolucionado de forma que detectan antes el movimiento de los objetos que su color (Bracha et al., 2004).

Tras la respuesta de congelamiento, tiene lugar la respuesta de huida que consiste, como su propio nombre indica, en un intento de huida por parte del organismo. Si la huida no es posible, tiene lugar la siguiente respuesta defensiva dentro de la secuencia, la respuesta de lucha. Por último, si el organismo llega a entrar en contacto físico directo con la amenaza, se pone en marcha la última fase de la secuencia, que es conocida como respuesta de inmovilidad tónica y que también ha sido definida en la literatura como "hacerse el muerto". La inmovilidad tónica puede hacer que el depredador piense que la presa está muerta y que no se esfuerce en retenerla,

proporcionando a la presa una oportunidad para escapar e incrementando así sus posibilidades de supervivencia. Esta respuesta también puede explicar el comportamiento experimentado por algunas víctimas de violación durante el asalto sexual (Bracha et al., 2004).

Si bien la secuencia "*freeze, flight, fight, fright*" constituye una descripción de la respuesta defensiva que puede aplicarse tanto a los animales mamíferos como al ser humano, Bracha (2004) señala la existencia de una respuesta adicional específica para la secuencia del ser humano conocida como "*faint*" (desmayo). La respuesta de desmayo es inducida por el miedo experimentado tras, por ejemplo, la visualización de una jeringa, sangre o una herida. Se piensa que esta respuesta surgió como una forma de supervivencia ante episodios de violencia entre seres humanos durante el período Paleolítico, y esa es la razón por la que solo existe en el ser humano y no en otros animales mamíferos. Por lo tanto, según este autor, la secuencia "*freeze, flight, fight, fright, faint*" proporciona una descripción más completa de la respuesta defensiva humana.

La investigación realizada por otros/as autores con roedores en contexto de laboratorio (ver D. C. Blanchard, 1997), sugiere la existencia de al menos cinco comportamientos defensivos que son elicitados frente a una amenaza: *flight* (huida), *freezing* (congelamiento), *defensive threat* (amenaza defensiva), *defensive attack* (ataque defensivo), y *risk assessment* (evaluación de riesgo). Desde esta concepción de la defensa, se entiende que la respuesta defensiva que es emitida por el organismo depende principalmente de dos factores: las características del estímulo amenazante para el bienestar del organismo y la situación en la que se presenta la amenaza. Estos dos factores, así como los comportamientos que conforman la respuesta defensiva, muestran una gran consistencia a través de las especies, incluyendo al ser humano

(D. C. Blanchard et al., 2001; D. C. Blanchard & R. J. Blanchard, 2008; Edmunds, 1974; Hediger, 1968).

Por consiguiente, para poder llevar a cabo respuestas defensivas efectivas, los organismos deben ser capaces de reconocer los distintos tipos de estímulos amenazantes que existen (D. C. Blanchard & R. J. Blanchard, 2008). De manera general, pueden distinguirse tres tipos de estímulos amenazantes que pueden encontrarse en cualquier ambiente natural (Endler, 1986): depredadores, otros miembros de la misma especie y características peligrosas del ambiente.

La distancia defensiva, i.e., la distancia que existe entre la presa y el depredador, también determina el comportamiento defensivo. La respuesta de huida constituye la respuesta dominante y, cuando ésta no es posible, tiene lugar la respuesta de congelamiento. A mayor cercanía del depredador a la presa, se producirá una huida más rápida, cuando la huida es posible, y una respuesta de congelamiento más intensa, cuando la huida no es posible. Conforme la distancia defensiva disminuye y el contacto con el depredador se convierte en algo más inminente, aparece la amenaza defensiva, seguida por el ataque defensivo (D. C. Blanchard, 1997). Asimismo, es importante que la respuesta defensiva esté orientada con respecto a la fuente de amenaza, ya sea para acercarse a ella llevando a cabo comportamientos de amenaza defensiva y/o ataque defensivo, o para alejarse de ella, llevando a cabo comportamientos de huida hacia un lugar más seguro (D. C. Blanchard & R. J. Blanchard, 2008; R. J. Blanchard & D. C. Blanchard, 1989).

Sin embargo, hay muchas situaciones que suponen un peligro potencial pero no presentan una fuente de amenaza que se pueda identificar fácilmente. Las respuestas defensivas de huida, congelamiento, amenaza defensiva y ataque defensivo, no pueden ser utilizadas de manera efectiva hasta que se ha identificado la fuente de

amenaza, lo cual puede ocurrir cuando es demasiado tarde para la presa y ya no tiene opciones de supervivencia. En estos casos, es necesario iniciar la respuesta defensiva con otros comportamientos que han sido conceptualizados como evaluación de riesgo, que consiste en la inhibición de actividades que se llevan a cabo de manera habitual (e.g., alimentación, autocuidado o cuidado de los miembros más jóvenes del grupo), en favor de un patrón de observación y evaluación del ambiente en busca de señales de peligro (D. C. Blanchard & R. J. Blanchard, 1988, 2008; R. J. Blanchard, & D. C. Blanchard, 1989).

Fanselow (1991, 1994), por otra parte, basándose en los resultados de diversos estudios realizados con roedores en contexto de laboratorio (e.g., Fanselow & Lester, 1988), ha propuesto un modelo (*predator stage model*) según el cual el sistema defensivo consta de tres fases (o modos, según la terminología de Timberlake [e.g. Timberlake, 1993; Timberlake & Lucas, 1989]): *pre-encounter* (pre-encuentro), *post-encounter* (post-encuentro) y *circa-strike*. Las fases se encuentran distribuidas a lo largo de un *continuum* de inminencia predatoria, que ha sido descrita por Fanselow y Lester (1988) como la percepción que tiene la presa acerca de la probabilidad de ser consumida por un depredador. Una mayor inminencia predatoria implicaría un mayor nivel de miedo experimentado por la presa y viceversa. Por lo que, en último término, la activación de cada una de estas fases depende del nivel de miedo experimentado por el organismo. Además, cada una de estas fases se encuentra asociada con determinados comportamientos defensivos.

Las fases contenidas en este modelo han sido descritas por Fanselow (1994), utilizando como ejemplo la rata, del siguiente modo: Niveles bajos de miedo se asocian con el inicio de la primera fase, la fase de pre-encuentro. Ante la posibilidad de encontrarse con un estímulo amenazante, la rata modificará su patrón de actividad habitual de manera que se disminuyan los factores de riesgo a su propia de

supervivencia, al mismo tiempo que se siguen cubriendo los requerimientos energéticos de su organismo. Niveles moderados de miedo se encuentran asociados con la segunda fase, la fase de post-encuentro. La respuesta dominante durante esta fase en el caso de la rata será la respuesta de congelamiento. Por último, niveles muy elevados de miedo, tales como los inducidos por el contacto directo con un estímulo amenazante, se asocian con la fase *circa-strike* y conllevan la puesta en marcha de respuestas defensivas activas, como morder o saltar.

1.2. Organización motivacional de la emoción

Desde una perspectiva evolutiva, las emociones humanas, tales como el miedo, son consideradas disposiciones para la acción. Éstas habrían evolucionado a partir de estados preparatorios evocados por señales de peligro, en los que la supervivencia dependía de la capacidad para retrasar o incluso inhibir respuestas comportamentales más visibles. Las emociones, por tanto, derivan de una fase de la respuesta defensiva que está asociada con un estado de alerta del organismo, durante el cual éste se prepara para la posibilidad de llevar a cabo una respuesta defensiva más activa (Lang, 1995; Lang et al., 1997, 2000).

Lang et al. (1997) han planteado un modelo, conocido como modelo motivacional, según el cual las emociones se encuentran organizadas en torno a dos sistemas motivacionales, apetitivo y defensivo, que han evolucionado para mediar en interacciones con el ambiente que promueven o amenazan la supervivencia, respectivamente. Esta organización bifásica de la emoción ya había sido propuesta previamente por otros/as autores (e.g., Dickinson & Dearing, 1979; Konorsky, 1967; Mehrabian & Russell, 1974; Ortony et al., 1988; Osgood et al., 1957; Russell, 1980; Schlosberg, 1952; Shaver et al., 1987; Tellegen, 1985; Wundt, 1986). Según este modelo, el sistema apetitivo se activa en contextos que promueven la supervivencia e

incluye conductas como la alimentación, la reproducción y el cuidado de la progenie. Por el contrario, el sistema defensivo se activa en contextos que implican una amenaza, incluyendo un repertorio conductual básico basado en la lucha y en la huida. Estos sistemas son el reflejo de la activación de determinados circuitos neuronales en el cerebro que, presumiblemente, también serían los encargados de enviar señales a estructuras que median sistemas somáticos y autonómicos implicados en atención y acción (Bradley et al., 2001; ver también Davis, 2000; Davis & Lang, 2003; Fanselow, 1994; LeDoux, 1990).

El modelo motivacional considera que la emoción presenta dos parámetros básicos: valencia hedónica (o valencia, simplemente; i.e., motivación agradable-apetitiva o motivación desagradable defensiva) y *arousal* (i.e., grado de activación motivacional). Estos dos conceptos serían equivalentes a lo que ha sido denominado como dirección e intensidad en el ámbito de la investigación de la conducta motivada en animales (e.g., Hebb, 1949; Schneirla, 1959). La valencia y el *arousal* pueden ser considerados como un reflejo de la activación motivacional, de forma que la evaluación sobre agradabilidad o desagradabilidad indica qué sistema motivacional se encuentra activo, mientras que la evaluación sobre *arousal* indica la intensidad de la activación motivacional (Bradley et al., 2001; Bradley & Lang, 2007).

Las evaluaciones sobre el estado emocional realizadas a través de medidas de autoinforme no constituyen lecturas directas de la actividad de los circuitos motivacionales, estando afectadas por muchos otros factores, entre los que se encuentran factores de tipo personal, situacional y cultural. Aun así, cuando las personas evalúan la valencia y el *arousal* de un amplio rango de estímulos emocionales, entre los que se incluyen imágenes, palabras, sonidos y texto, aparece una distribución dentro del espacio afectivo bidimensional que es consistente con el modelo motivacional. Además, esta consistencia se mantiene a través de diferentes

idiomas y culturas, lo que sugiere la existencia de una determinación biológica subyacente (Bradley et al., 2001).

La Figura 1 representa el espacio afectivo bidimensional resultante de la evaluación de valencia y *arousal* de un conjunto de imágenes estandarizadas conocido como Sistema Internacional de Imágenes Afectivas (*International Affective Picture System*, IAPS), llevada a cabo por un amplio número de participantes. Más concretamente, esta figura hace referencia a las imágenes pertenecientes a la versión del IAPS del año 2005 (Lang et al., 2005). Tal y como puede observarse, el espacio afectivo adopta una forma similar a la de un *boomerang* en el que cada uno de los brazos representa cada uno de los sistemas motivacionales. Las líneas de regresión incluidas en la figura se basan en la correlación entre las evaluaciones de valencia y *arousal*, obtenidas de manera independiente para las imágenes con contenido emocional agradable y desagradable, e indican el grado con el que las imágenes activan los sistemas motivacionales apetitivo y defensivo. De este modo, el brazo superior del *boomerang* representa el sistema motivacional apetitivo. En un extremo del brazo los estímulos tienen valencia neutral y *arousal* bajo, mientras que en el otro extremo los estímulos tienen máxima agradabilidad y *arousal*. El brazo inferior representa el sistema motivacional defensivo. En un extremo de este brazo los estímulos tienen valencia neutral y *arousal* bajo, mientras que en el otro extremo los estímulos tienen máxima desagradabilidad y *arousal*. Además, las líneas de regresión correspondientes a las imágenes agradables y desagradables son similares a los gradientes de aproximación y evitación encontrados previamente por Miller (1959) durante el estudio de conducta motivada en ratas (Bradley et al., 2001; Bradley & Lang, 2007).

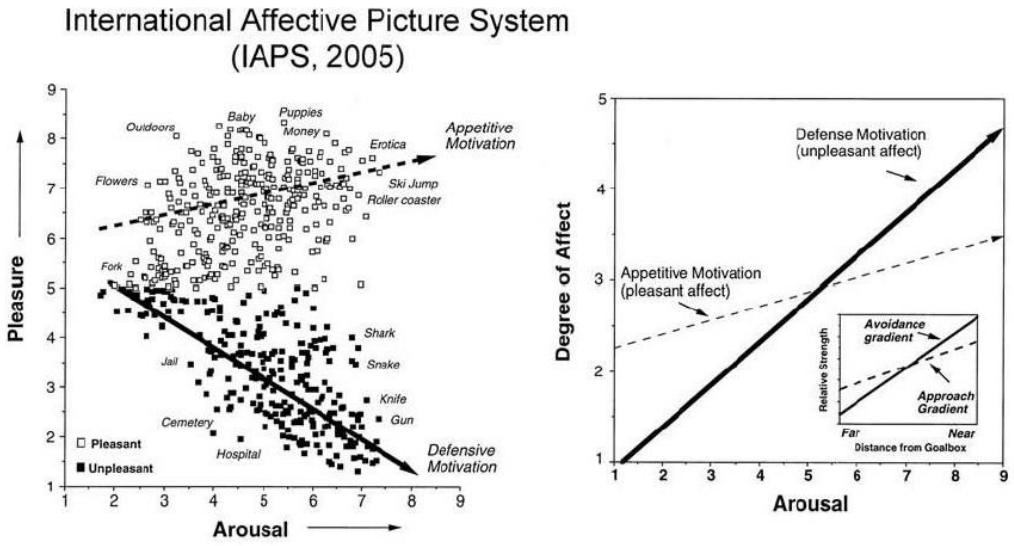


Figura 1. Imágenes con contenido emocional procedentes del IAPS (Lang et al., 2005), representadas en el espacio bidimensional definido por las puntuaciones medias en la evaluación de valencia (eje y) y *arousal* (eje x), procedentes de un número amplio de participantes. Extraído de Bradley & Lang (2007).

Asimismo, el modelo motivacional se ve respaldado por los resultados de varios estudios en los que se muestra cómo los factores que definen este espacio bidimensional, es decir, las evaluaciones de valencia y *arousal*, covarían sistemáticamente con reflejos biológicos que se encuentran asociados con la activación de los sistemas motivacionales apetitivos y defensivos (Bradley & Lang, 2007; Cuthbert et al., 1998; Greenwald et al., 1989; Lang et al., 1993).

1.3. El paradigma de visualización de imágenes y la hipótesis de *priming* motivacional

El paradigma de visualización de imágenes de Lang (1995) se ha utilizado con el objetivo de examinar el estado afectivo de los seres humanos en contexto de laboratorio. Además, este paradigma permite poner a prueba la hipótesis de *priming* motivacional (Lang, 1995), según la cual los reflejos defensivos, entre los que se

incluye la respuesta de sobresalto, incrementan su amplitud cuando la persona se encuentra motivada de forma aversiva (i.e., cuando su sistema motivacional defensivo se encuentra activado y su estado emocional es negativo); asimismo, los reflejos defensivos reducen su amplitud cuando la persona se encuentra motivada de forma apetitiva (i.e., cuando su sistema motivacional apetitivo se encuentra activado y su estado emocional es positivo).

Este paradigma consiste en la presentación de un estímulo acústico para evocar la respuesta de sobresalto durante la visualización de imágenes afectivas. La respuesta de sobresalto puede medirse de forma fiable a través del registro psicofisiológico del reflejo de parpadeo (cierre rápido del ojo), mediante electromiografía del músculo orbicular del ojo. En los primeros experimentos que utilizaron el paradigma de visualización de imágenes, se empleaban fotografías con contenido emocional como estímulos visuales. Actualmente, disponemos de un conjunto de imágenes estandarizadas conocido como IAPS, cuya última versión data del año 2008 (Lang et al., 2008) e incluye unas 1300 imágenes, así como las puntuaciones normativas de valencia, *arousal* y dominancia asociadas con cada imagen. (Lang, 1995; Lang et al., 2000).

Una serie de estudios realizados utilizando el paradigma de visualización de imágenes ha conseguido replicar en seres humanos el aumento de la amplitud del reflejo de sobresalto que había sido encontrado previamente en investigación animal (Lang, 1995). Además, los resultados obtenidos en dichos estudios, así como en otros más recientes (e.g., Bradley et al., 1990; Cobos et al., 2002; Sánchez et al., 2002; Vrana et al., 1988) apoyan la hipótesis de *priming* motivacional. La respuesta de sobresalto se inhibe cuando las personas visualizan imágenes con contenido emocional agradable y se potencia cuando visualizan imágenes con contenido emocional desagradable.

Tanto la inhibición como la potenciación de esta respuesta se intensifican conforme los estímulos visuales son evaluados como más activantes.

1.4. El circuito cerebral del miedo

Los sistemas motivacionales apetitivo y defensivo son el reflejo de la activación de circuitos neuronales, principalmente subcorticales, que a su vez son activados por reforzadores primarios, es decir, aquellos que poseen un valor reforzante biológicamente determinado. La información disponible en este sentido acerca del sistema motivacional defensivo proviene principalmente de investigación animal, particularmente de estudios de neurofisiología comportamental con ratas. Estos estudios constaban de procedimientos experimentales relativamente simples, en los que estímulos nocivos (e.g., *shock* eléctrico) eran emparejados con tonos y luces que previamente eran considerados como inocuos. De esta forma y mediante el uso de diversas herramientas neuroquirúrgicas, farmacológicas y electrofisiológicas, se ha conseguido trazar la cadena de activación que tiene lugar en el cerebro cuando un organismo se encuentra en una situación de peligro o amenaza (Lang et al., 1997, 1998, 2000).

Se ha demostrado de manera repetida que la amígdala desempeña un papel fundamental tanto para la expresión como para la adquisición del miedo condicionado (Davis, 1992; Gloor, 1960; T. S. Gray, 1989; Kapp & Pascoe, 1986; Kapp et al., 1984; LeDoux, 1987, 2000, 2003; Sarter & Markowitsch, 1985). Esta pequeña estructura con forma de almendra que se encuentra localizada en la profundidad de los lóbulos temporales, forma parte de los sistemas más antiguos del cerebro y puede actuar con relativa independencia de procesos cognitivos superiores, que aparecieron de forma más tardía en el cerebro (LeDoux, 1996; Rosen & Schulkin, 1998; Öhman & Mineka, 2001). La activación del sistema motivacional defensivo depende de las proyecciones

desde la amígdala hacia otras estructuras cerebrales. Lang et al. (1997,1998) han realizado una descripción paso a paso de la cadena de activación que se produce en el cerebro (ver Figura 2), basándose en los descubrimientos procedentes de investigación animal:

El *input* procedente del ambiente (estímulos aversivos condicionados e incondicionados) entra a través de los órganos sensoriales y, desde ahí, puede seguir dos vías. En la vía primaria éste pasa por el tálamo y se dirige hacia la amígdala; mientras que en la vía secundaria pasa por la corteza sensorial, por el tálamo y se dirige hacia la amígdala. Las eferencias procedentes de la amígdala constituyen las distintas ramas del circuito defensivo y cada una de ellas se encuentra asociada con distintos *outputs* o respuestas defensivas. La respuesta de sobresalto se encuentra modulada por proyecciones directas desde la amígdala hacia el núcleo reticular caudal del puente (e.g., Davis, 1989, 1997; Davis et al., 1988; Fendt et al., 1994); la respuesta autonómica (e.g., cambios en presión arterial), depende del buen funcionamiento de una ruta que parte de la amígdala hacia el hipotálamo (LeDoux, 1990); y los componentes somáticos se encuentran modulados por proyecciones desde la amígdala hacia la sustancia gris central: la sustancia gris central ventral está implicada en la respuesta de congelamiento, mientras que la sustancia gris dorsal es un componente crítico para la defensa activa, que incluye las respuestas de lucha y huida (ver Deapulis & Bandler, 1991; Fanselow et al., 1995).

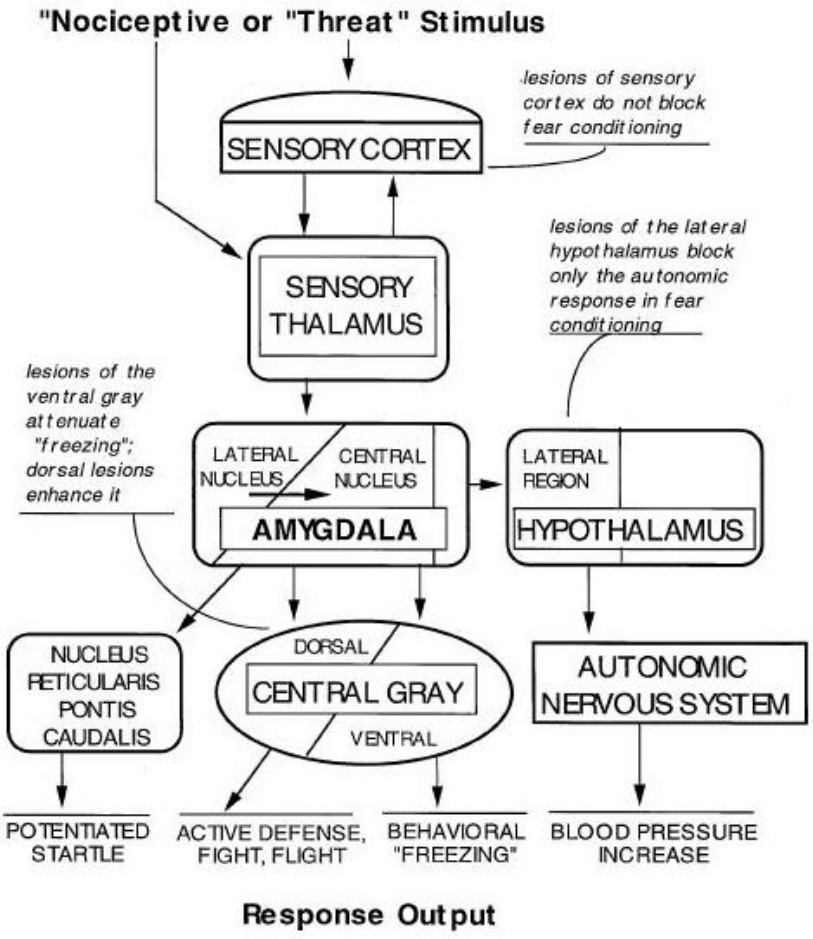


Figura 2. Neurofisiología del sistema motivacional defensivo en la rata. Extraído de Lang et al. (1998).

1.5. El modelo de la cascada defensiva

Existe un número significativo de estudios en los que se ha utilizado el paradigma de visualización de imágenes y, aparte de la respuesta de sobresalto, se han registrado otras medidas de reactividad afectiva tales como la tasa cardíaca o la conductancia eléctrica de la piel. Los datos procedentes de estos estudios sugieren que más que una única respuesta que sirva como indicador de activación del sistema motivacional defensivo, lo que se observa es una cascada de respuestas psicofisiológicas que cambian de distinta forma y a distintos ritmos conforme la activación aumenta (Bradley, 2000; Bradley & Lang, 2007; Lang et al., 1997).

Por ejemplo, en algunos de los estudios se han comparado las respuestas psicofisiológicas de personas con y sin fobia específica. Ambos grupos presentan una potenciación de la respuesta de sobresalto durante la visualización de imágenes desagradables. En el caso de las personas con fobia específica, esta respuesta se potencia aún más durante la visualización de imágenes con contenido fóbico (Hamm et al., 1997; Sabatinelli et al., 1996). Con respecto a la respuesta en tasa cardíaca, las personas sin fobia específica presentan bradicardia (i.e., desaceleración de la tasa cardíaca) al visualizar imágenes desagradables; mientras que las personas con fobia específica presentan una aceleración de la tasa cardíaca al visualizar imágenes con contenido fóbico (Cook & Turpin, 1997; Hamm et al., 1997; Klorman & Ryan, 1980; Klorman et al., 1977). Además, en un contexto de visualización libre, las personas con fobia específica dejan de mirar las imágenes antes que las personas sin fobia específica. Todo esto sugiere que las imágenes desagradables producen una potenciación de los reflejos defensivos en personas tanto con y sin fobia específica. Sin embargo, otras medidas de tipo psicofisiológico y comportamental que son propias de la orientación atencional están ausentes cuando las personas con fobia específica procesan contenido que les produce miedo (Bradley, 2000; Lang, 1995).

Lang et al. (1997) han propuesto una adaptación del modelo animal de Fasenlow sobre la respuesta de defensa (*predator stage model*; Fanselow, 1991,1994) para explicar las reacciones psicofisiológicas humanas ante estímulos desagradables y amenazantes, lo que se conoce como modelo de la cascada defensiva (ver Figura 3). El modelo de Fasenlow sugiere que la respuesta defensiva consta de tres fases (pre-encuentro, post-encuentro y *circa-strike*) que aparecen de forma secuencial conforme aumenta la proximidad o inminencia de una amenaza. Según el modelo de la cascada defensiva, la intensidad emocional o *arousal* sería análoga a ese concepto de proximidad o inminencia de amenaza, y es lo que determinaría el cambio de una fase a otra.

Cuando el *arousal* aún se encuentra a niveles relativamente bajos, el patrón de reactividad psicofisiológica es consistente con la orientación y, por lo tanto, con una facilitación en el procesamiento de estímulos. Este patrón comprende la inhibición de la respuesta de sobresalto; bradicardia, que se vuelve más intensa y sostenida conforme la activación aumenta; e incrementos moderados en conductancia eléctrica de la piel, que aumentan tanto en frecuencia como en amplitud conforme la activación aumenta. Cuando el peligro se convierte en algo más inminente y nos acercamos a la fase *circa-strike*, el nivel de *arousal* se eleva, dando paso a una movilización metabólica y a la defensa activa. En ese momento el patrón psicofisiológico cambia de manera bastante significativa, observándose una potenciación de la respuesta de sobresalto, seguida por una aceleración de la tasa cardíaca que coincide temporalmente con el paso a la fase *circa-strike*; la conductancia eléctrica de la piel, por su parte, sigue aumentando conforme aumenta el nivel de activación (Bradley, 2000; Bradley et al., 2001; Bradley & Lang, 2007; Lang et al., 1997, 2000).

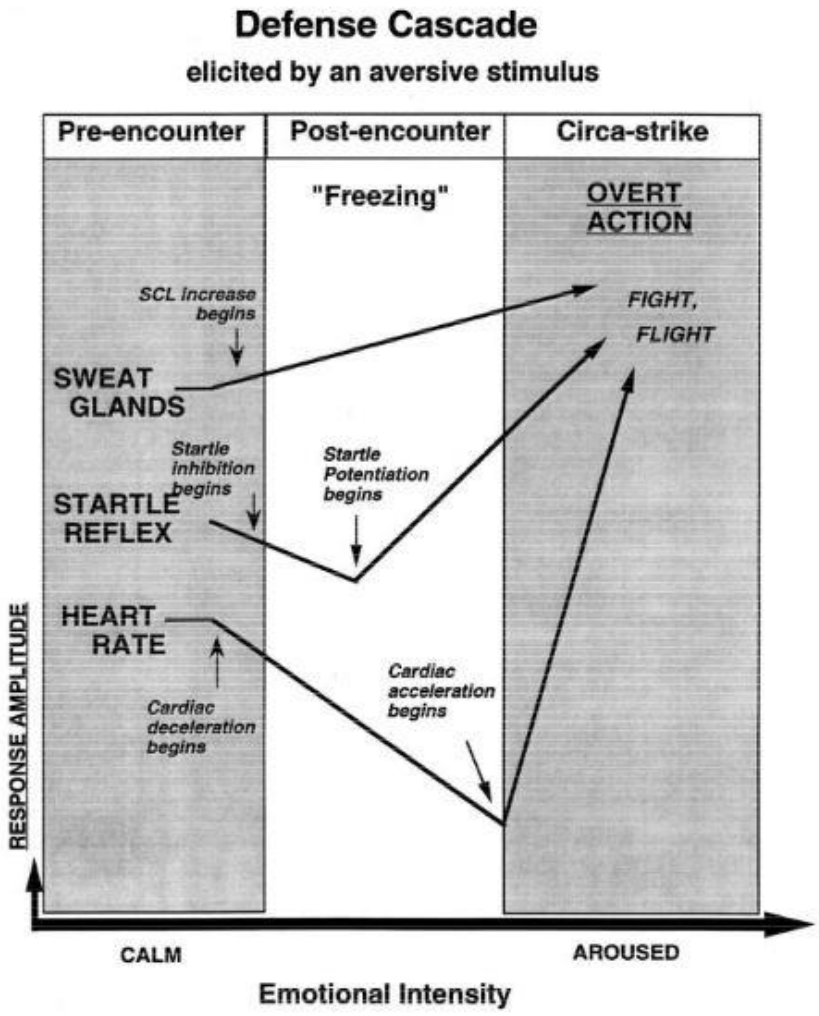


Figura 3. La respuesta defensiva humana según el modelo de la cascada defensiva (Lang et al., 1997): diferentes sistemas psicofisiológicos cambian a diferentes ritmos, en función de la intensidad de activación en el sistema motivacional defensivo. Extraído de Lang et al. (2000).

Los patrones autonómicos y somáticos que forman parte del sistema motivacional defensivo pueden agruparse en dos categorías: inmovilidad defensiva, que incluye respuestas como el congelamiento o el incremento del nivel de alerta o vigilancia, y en la que el organismo se comporta de manera pasiva pero está preparado para responder activamente si recibe mayor estimulación para ello; y acción defensiva, que incluye variaciones de las respuestas de lucha y huida ante una amenaza inminente. Por lo tanto, el modelo de la cascada defensiva considera la defensa como un

conjunto de respuestas que ocurren de manera secuencial a lo largo de dos fases: una primera fase en la que factores de tipo atencional facilitan la detección y procesamiento de una potencial amenaza; y una segunda fase en la que tienen lugar comportamientos de defensa activa (Lang et al., 1997, 2000).

Este modelo permite explicar por qué las personas con fobia específica presentan una aceleración cardíaca durante la visualización de imágenes con contenido fóbico. La activación de las personas con fobia específica al visualizar este tipo de imágenes es más alta en comparación con la activación de las personas sin fobia específica al visualizar imágenes desagradables. Este mayor nivel de activación hace que las personas con fobia específica se salten la primera fase de la cascada defensiva, durante la cual se produciría una bradicardia típica de la orientación, pasando a estar directamente en una fase más avanzada de postura defensiva (Bradley, 2000; Bradley y Lang, 2007).

1.6. Componentes cardíacos de la respuesta defensiva: la respuesta cardíaca de defensa

1.6.1. Aproximaciones tradicionales a la defensa cardíaca

Pueden identificarse dos grandes aproximaciones tradicionales a los componentes cardíacos de la defensa: la cognitiva y la motivacional. Por una parte, la aproximación cognitiva se basa en los trabajos de Ivan Pavlov sobre los reflejos de orientación y defensa (Pavlov, 1927), y enfatiza los procesos cognitivos asociados con la respuesta (Graham, 1992; Lacey & Lacey, 1974; Sokolov, 1963). Según esta aproximación, se considera que los estímulos ambientales producen cambios en la tasa cardíaca que representan mecanismos atencionales y perceptuales cuyo objetivo es facilitar o inhibir el procesamiento de dichos estímulos. El reflejo de orientación, que consiste en una

desaceleración de la tasa cardíaca ante estimulación novedosa y de nivel moderado, se encuentra asociado a aceptación sensorial y procesamiento del estímulo; mientras que el reflejo de defensa, que consiste en una aceleración de la tasa cardíaca ante estimulación aversiva e intensa, se encuentra asociado a rechazo sensorial (Vila et al., 2003, 2007).

Por otra parte, la aproximación motivacional se centra principalmente en emoción y motivación (Obrist, 1981; Steptoe & Vögele, 1991), basándose tanto en el término "*fight or flight*" ("lucha o huida") establecido por Walter Cannon para referirse la defensa como en la teoría del estrés propuesta por Hans Selye (Cannon, 1929; Selye, 1956). De acuerdo con esta aproximación, se considera que los estímulos ambientales producen cambios en la tasa cardíaca que reflejan ajustes metabólicos necesarios para proporcionar la energía requerida por el organismo para ajustar su comportamiento de forma adaptativa. Si el comportamiento más apropiado para una determinada situación es de tipo pasivo e implica no moverse, entonces se producirá una desaceleración de la tasa cardíaca; por el contrario, si el comportamiento más apropiado es de tipo activo, ya sea física o psicológicamente, entonces se producirá una aceleración de la tasa cardíaca (Vila et al., 2003, 2007).

1.6.2. El modelo atencional-motivacional de la respuesta cardíaca de defensa

Las aproximaciones cognitiva y motivacional fueron consideradas contrapuestas entre sí hasta la aparición del modelo de la cascada defensiva de Lang et al. (1997), que considera que la defensa presenta componentes tanto cognitivos como motivacionales y, por lo tanto, permite integrar las asunciones de ambas propuestas. Posteriormente, Vila et al. (2007) han propuesto el modelo atencional-motivacional con el objetivo de relacionar las fases propuestas en el modelo de la cascada defensiva con los distintos componentes de la respuesta cardíaca. Según este modelo, la respuesta cardíaca de

defensa (RCD) está caracterizada por un complejo patrón de cambios en la tasa cardíaca que se produce ante un sonido intenso e inesperado. Este patrón de respuesta presenta dos componentes acelerativos/desacelerativos en orden secuencial alterno y tiene una duración aproximada de 80 s.

El modelo atencional-motivacional sugiere que los componentes de la RCD tienen una significación tanto cognitiva como motivacional y se encuentran mediados por las dos ramas del sistema nervioso autónomo, la simpática y la parasimpática. La RCD representa la sucesión de dos fases: una fase atencional asociada al primer componente acelerativo/desacelerativo de la respuesta, durante la cual se produce una interrupción de la actividad y un incremento de la atención dirigida hacia señales externas con el objetivo de detectar una posible amenaza; y una fase motivacional asociada al segundo componente acelerativo/desacelerativo, durante la cual se llevan a cabo comportamientos de defensa activa (si se ha detectado una amenaza) o recuperación (si finalmente no se ha detectado ninguna amenaza) (Vila et al., 2007).

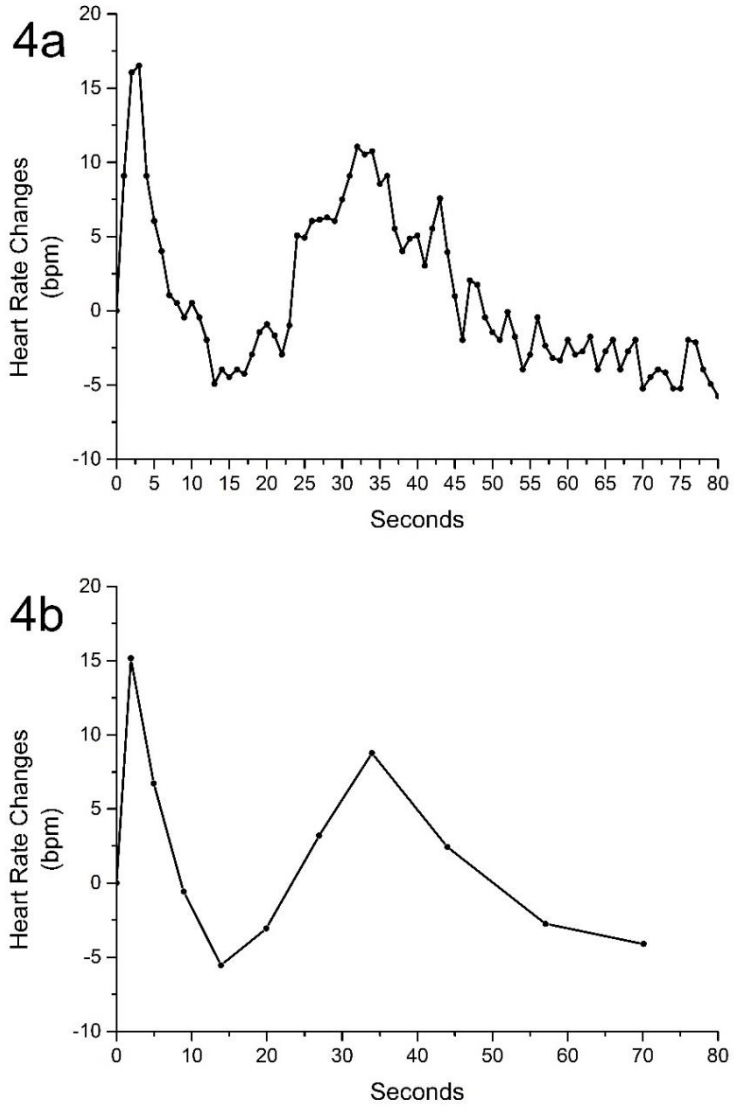
1.6.3. Investigación sobre la respuesta cardíaca de defensa

El modelo atencional-motivacional se basa en los resultados obtenidos en un número considerable de estudios en los que se ha utilizado como paradigma básico la presentación de un sonido intenso e inesperado para evocar la RCD durante la realización de diferentes tareas (Vila et al., 2007). A continuación, se realizará una descripción pormenorizada de la investigación realizada en torno a la RCD.

1.6.3.1. Forma de la respuesta

El patrón típico de la RCD presenta dos componentes acelerativos/desacelerativos en orden secuencial alterno (i.e., aceleración-desaceleración-aceleración-

desaceleración). En la Figura 4a se muestra la RCD de un grupo de 15 participantes, expresada como cambios en tasa cardíaca segundo-a-segundo con respecto a una línea de base de 15 s, durante los 80 s posteriores a la presentación de un estímulo evocador de la respuesta. En la Figura 4b se muestra la misma respuesta de manera simplificada, a través de la reducción de los 80 datos procedentes de los 80 s a 10, correspondientes a las medianas de 10 intervalos progresivamente más largos: dos intervalos de tres segundos, dos intervalos de cinco segundos, tres intervalos de siete segundos y tres intervalos de 13 segundos. Tal y como puede observarse en la figura, la primera aceleración tiene una latencia más corta que la segunda aceleración y los componentes acelerativos de la respuesta presentan una mayor amplitud que los componentes desacelerativos (Vila et al., 2007). Este patrón de respuesta fue descrito por primera vez por Fernández (1986b) y ha sido confirmado de manera repetida en estudios realizados posteriormente (e.g. Vila & Fernández, 1989; Vila et al., 1992, 1997).



Figuras 4a y 4b. Patrón típico de la respuesta cardíaca de defensa: respuesta de la tasa cardíaca promediada segundo-a-segundo (arriba) y los mismos datos expresados como las medianas de 10 intervalos (abajo) (todo expresado en puntuaciones diferenciales). Adaptación de Vila et al. (2007).

1.6.3.2. Características del estímulo evocador de la respuesta

Se han investigado varias características paramétricas del estímulo evocador de la RCD en seres humanos. La modalidad sensorial y la intensidad del estímulo fueron

examinadas en un estudio realizado por Vila et al. (1997), en el que se compararon estímulos de tipo auditivo, visual y electrocutáneo, manteniendo constante la intensidad subjetiva percibida por los/las participantes. Los resultados mostraron que las modalidades auditiva y electrocutánea son las únicas capaces de evocar el patrón de la RCD. Cuando el estímulo evocador es de intensidad alta (109 dB para la modalidad auditiva) los componentes acelerativos de la respuesta tienen una mayor amplitud; y ante un estímulo de intensidad moderada (79 dB para la modalidad auditiva) estos componentes tienen una menor amplitud, aunque el patrón típico con dos componentes acelerativos/desacerativos sigue presente. Ramírez et al. (2005) examinaron el tiempo de subida y la duración del estímulo evocador de la RCD mediante la manipulación de ambas características paramétricas, de forma que el estímulo se presentaba con un tiempo de subida de 0, 24, 48, 96 o 240 ms durante 50, 100, 250, 500 o 1000 ms. Los resultados obtenidos indicaron que el patrón de RCD no se ve afectado por el tiempo de subida pero sí por la duración, estando solo presente cuando el estímulo evocador tiene una duración de 500 y 1000 ms.

1.6.3.3. Diferencias individuales en la respuesta

Numerosos estudios han confirmado la existencia de diferencias individuales en el patrón de la RCD (Cloete, 1979; Eves & Gruzelier, 1984, 1987; Fernández, 1986a; Fernández & Vila, 1989b; Jung-Stalmann, 2003; Knott & Bulmer, 1984; Richards & Eves, 1991; Vila & Beech, 1978; Vila et al., 1992). Según Eves y Gruzelier (1984), las personas pueden clasificarse en dos grupos, “aceleradores” y “desaceleradores”, de acuerdo con la presencia o no de la segunda aceleración. Fernández y Vila (1989b) realizaron un análisis de *cluster* que confirmó la existencia de cuatro grupos según su patrón de respuesta, y que se encuentran representados en la Figura 5. Los grupos 1 y 2 presentan la segunda aceleración, mientras que los grupos 3 y 4 no la presentan. El grupo 1 presenta el patrón típico de la RCD con dos componentes

acelerativos/desacelerativos en orden secuencial alterno; el grupo 2 no presentan la primera desaceleración y su patrón consiste en una única aceleración prolongada; el grupo 3 muestra un retorno hacia la línea de base tras la primera aceleración; y el grupo 4 presenta una desaceleración prolongada tras la primera aceleración.

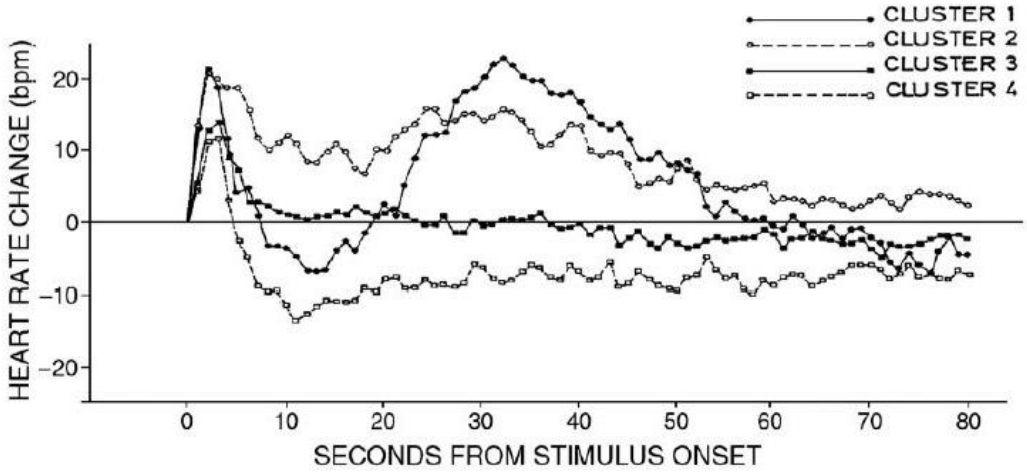


Figura 5. Diferencias individuales en la respuesta cardíaca de defensa: cuatro grupos descritos por Fernández & Vila (1989b) según su patrón de respuesta. Extraído de Vila et al. (2007).

Un estudio llevado a cabo por Fernández (1986a) ha corroborado la estabilidad a largo plazo de estas diferencias individuales en el patrón de la RCD, que se han asociado con varios factores biológicos y psicológicos como el ciclo menstrual (Vila & Beech, 1978), el género (Vila et al., 1992), los rasgos de personalidad (Cloete, 1979; Jung-Stalmann, 2003; Richards & Eves, 1991), la preocupación crónica (Delgado et al., 2009) y la ansiedad patológica (Kley, 2004; Viedma, 2008).

1.6.3.4. Habituación de la respuesta

Una de las características que presenta la RCD es su rápida habituación, lo que significa que su segundo componente acelerativo/desacelerativo disminuye hasta casi desaparecer tras la primera repetición de un estímulo evocador de dicha respuesta. Este efecto ha aparecido de manera consistente en diversos estudios que consistían en la presentación repetida de un estímulo acústico evocador de la RCD con un intervalo entre estímulos (IEE) de aproximadamente 2 min (e.g., Ramírez et al., 2005; Vila et al., 1997). En estos estudios, el primer componente acelerativo/desacelerativo también mostraba una tendencia a la habituación, aunque mucho menos pronunciada que para el segundo componente. También se ha observado que se produce una recuperación del patrón de respuesta al repetir el mismo procedimiento experimental tras varios meses (Fernández, 1986a), así como durante tareas de tipo dual en las que el sonido se presenta durante la realización de una tarea cognitiva (Turpin, 1986).

Guerra (2007) ha investigado los efectos de habituación y recuperación de la RCD durante una única sesión experimental mediante la manipulación del tipo de estímulo evocador de la RCD, que podía ser un ruido blanco o un grito humano (ambos equivalentes con respecto a intensidad, duración y tiempo de subida). El test psicofisiológico presentaba la siguiente secuencia: (a) primer período de adaptación de 10 min, (b) línea de base de 15 s, (c), tres presentaciones de un ruido blanco o un grito humano (orden contrabalanceado) con un IEE de 120 s, (d) segundo período de adaptación de 10 min, (e) línea de base de 15 s, (f) tres presentaciones de un ruido blanco o un grito humano (orden contrabalanceado) con un IEE de 120 s. Los resultados obtenidos en este estudio indicaron que se produce una recuperación completa del patrón de la RCD cuando el estímulo cambia de grito humano a ruido blanco.

Mata et al. (2009) también han examinado la habituación y recuperación de la RCD dentro de una misma sesión experimental mediante la manipulación del IEE. El test psicofisiológico consistió en tres presentaciones de un estímulo acústico evocador de la defensa cardíaca. El IEE entre la primera y la tercera presentación era de 30 min en todos los casos, mientras que el momento en el que se presentaba el segundo estímulo era manipulado de manera que el intervalo temporal entre el primer y el segundo estímulo aumentaba progresivamente, y el intervalo temporal entre el segundo y el tercer estímulo disminuía progresivamente (IEE: 2.5/27.5 min, 7.5/22.5 min, 12.5/17.5 min, 17.5/12.5 min, 22.5/7.5 min y 27.5/2.5 min). Los resultados de este estudio mostraron una habituación de la RCD tras un IEE corto (2.5 min), así como una recuperación tras IEE más largos, que es mayor conforme este intervalo temporal aumenta. Tanto la habituación como la recuperación se producen de manera más acentuada para el segundo componente acelerativo/desacelerativo que para el primer componente.

1.6.3.5. Significación cognitiva de la respuesta cardíaca de defensa

La significación cognitiva de la defensa cardíaca ha sido examinada en diversos estudios (Fernández & Vila, 1989a; Pérez et al., 2000; Vila et al., 1997) mediante el registro de la RCD y la manipulación de la orientación atencional (externa vs. interna). Estos estudios se basan en la hipótesis de aceptación-rechazo de Lacey y Lacey (1974), que forma parte de la aproximación tradicional cognitiva y asume que la dirección de los cambios en tasa cardíaca (aceleración o desaceleración) indica la intención del organismo para aceptar o rechazar estímulos ambientales. Más concretamente, esta hipótesis sugiere que la desaceleración de la tasa cardíaca se encuentra asociada con una facilitación de la aceptación sensorial y la aceleración cardíaca se encuentra asociada con el rechazo sensorial. De acuerdo con esto, la

RCD debería ir acompañada de una disminución en el procesamiento sensorial con el fin de rechazar el estímulo aversivo.

La investigación realizada por Fernández y Vila (1989a) consistió en la evocación de la RCD durante la realización de, entre otras, una tarea de tiempo de reacción simple (atención externa) y una tarea de aritmética mental (atención interna). Se encontró una relación positiva entre la presencia del segundo componente acelerativo en el patrón de la RCD y una mayor reactividad cardíaca durante la realización de la tarea de atención externa. Posteriormente, Vila et al. (1997) llevaron a cabo un estudio que consistió en la presentación de un estímulo acústico evocador de la RCD durante la realización de una tarea de atención externa, una tarea de atención interna o ninguna tarea (condición control). La tarea de atención externa era de seguimiento perceptivo y consistía en pulsar la tecla de un telégrafo cada vez que una luz se encendía. La tarea de atención interna también era de seguimiento perceptivo, pero en este caso consistía en pulsar la tecla de un telégrafo en consonancia con la percepción de cada latido del corazón. Los resultados de este estudio mostraron una potenciación del segundo componente acelerativo de la RCD, así como una reducción de su primer componente desacelerativo, durante la realización de la tarea de atención externa.

El estudio de Pérez et al. (2000) consistió en la evocación de la RCD durante la realización de una tarea de seguimiento perceptivo idéntica a la utilizada previamente por Vila et al. (1997) para inducir atención externa, la tarea de búsqueda en la memoria de Sternberg con dificultad fácil (atención interna), la tarea de búsqueda en la memoria de Sternberg con dificultad difícil (atención interna) o ninguna tarea (condición control). Se encontró una potenciación del segundo componente acelerativo de la RCD durante la tarea de atención externa, mientras que la manipulación de la dificultad de la tarea de atención interna no produjo ningún efecto. Más recientemente, Ramírez et al. (2010) han investigado la modulación atencional de la defensa cardíaca

mediante la presentación de un estímulo acústico evocador de la RCD y la utilización de las tareas de búsqueda visual y de búsqueda en la memoria de Sternberg para inducir atención externa e interna, respectivamente. En línea con los resultados de los estudios ya descritos, se encontró una potenciación del segundo componente acelerativo de la RCD durante la tarea de atención externa, en comparación con la tarea de atención interna, además de una reducción en el primer componente desacelerativo durante la tarea de atención externa, tal y como ocurría en el estudio de Vila et al. (1997).

Los resultados de todos estos estudios sobre modulación atencional de la defensa cardíaca sugieren que existe una relación positiva entre la RCD y la orientación atencional externa, así como con procesos atencionales de aceptación sensorial. Por consiguiente, estos resultados no apoyan las asunciones de la aproximación tradicional cognitiva ni la hipótesis de aceptación-rechazo.

1.6.3.6. Significación motivacional de la respuesta cardíaca de defensa

La significación motivacional de la defensa cardíaca ha sido investigada en diferentes estudios (Ruiz-Padial et al., 2005; Sánchez et al., 2002, 2009) mediante el registro de la RCD y la manipulación del estado afectivo utilizando el paradigma de visualización de imágenes (Lang, 1995). Este paradigma consiste, como ya se ha explicado anteriormente, en la presentación de un estímulo acústico evocador de la respuesta de sobresalto durante la visualización de imágenes afectivas. Utilizando un estímulo acústico de larga duración (500 o 1000 ms) y tiempo de subida instantáneo, es posible evocar la respuesta de sobresalto y la RCD de forma simultánea para examinar el efecto que tiene la modulación emocional sobre ambos reflejos defensivos (Vila et al., 2007). Este paradigma también permite poner a prueba la hipótesis de *priming* motivacional (Lang, 1995), según la cual los reflejos defensivos incrementan su

amplitud cuando el organismo se encuentra motivado de forma aversiva y reducen su amplitud cuando se encuentra motivado de forma apetitiva.

La investigación realizada por Sánchez et al. (2002) consistió en la presentación de un estímulo acústico evocador de la RCD durante la visualización de imágenes con contenido emocional agradable, neutral y desagradable procedentes del IAPS. Se encontró una potenciación de la RCD ante imágenes desagradables, en comparación con imágenes agradables y neutras. Sin embargo, no se encontró una inhibición de la RCD ante imágenes agradables. La visualización de imágenes desagradables también provocó un cambio en el patrón bifásico típico de la RCD, de tal forma que la primera desaceleración desaparece y los dos componentes acelerativos se unen, conformando una única aceleración más larga y de mayor amplitud.

El estudio de Ruiz-Padial et al. (2005) consistió en la evocación de la RCD a participantes con fobia a las arañas y en la visualización de imágenes con contenido afectivo durante procedimientos de enmascaramiento efectivo y no efectivo. Las imágenes podían ser tanto de contenido fóbico (imagen de araña) como de contenido no fóbico (imagen de flor). Los resultados obtenidos mostraron una potenciación de la RCD ante imágenes de contenido fóbico, así como una modificación de su patrón de respuesta, que exhibía una única aceleración como la encontrada por Sánchez et al. (2002). Estos cambios en el patrón de la RCD fueron observables tanto en la condición de enmascaramiento efectivo como no efectivo, aunque presentaban una menor amplitud en la condición de enmascaramiento efectivo.

Sánchez et al. (2009) llevaron a cabo un estudio para examinar la modulación emocional de la RCD en participantes con fobia a la sangre (pero no a los animales) y participantes con fobia a los animales (pero no a la sangre). La presentación de un estímulo acústico evocador de la RCD era acompañada por la visualización de

imágenes que representaban sangre y animales temidos. Se encontró una potenciación de la RCD ante imágenes que representaban el objeto temido, en comparación con las imágenes del objeto no temido. Es decir, la RCD de los/las participantes con fobia a la sangre se potenciaba durante la visualización de imágenes que representaban sangre, mientras que la RCD de los/las participantes con fobia a los animales se potenciaba durante la visualización de imágenes que representaban animales temidos. También se observó una modificación en el patrón de la RCD durante la visualización de imágenes del objeto temido que era similar al encontrado previamente por otros/as autores (Ruiz-Padial et al., 2005; Sánchez et al., 2002).

Por otra parte, Ramírez et al. (2010) han examinado la significación cognitiva y motivacional de la RCD de manera conjunta en un estudio que no se basa en el paradigma de visualización pasiva de imágenes. En cambio, este estudio consistió en la presentación de un estímulo acústico evocador de la RCD durante la realización de una variante de la tarea de búsqueda visual de Sternberg (atención externa) en la que los estímulos visuales eran imágenes con contenido emocional agradable, neutral y desagradable procedentes del IAPS. La manipulación experimental tuvo como resultado una potenciación del segundo componente acelerativo de la RCD durante la visualización de imágenes desagradables, en comparación con imágenes agradables y neutras. Sin embargo, en este caso el patrón de la RCD no experimentó una modificación tan significativa como la observada en los estudios sobre modulación emocional basados en el paradigma de visualización de imágenes (Ruiz-Padial et al., 2005; Sánchez et al., 2002, 2009).

Los resultados de los estudios realizados en torno a la modulación emocional de la defensa cardíaca indican que existe una potenciación de la RCD durante la visualización de imágenes que activan el sistema motivacional defensivo. Por lo tanto, estos estudios apoyan la hipótesis de *priming* motivacional. Esta hipótesis también

sugiere que la visualización de imágenes que activan el sistema motivacional apetitivo inhibe los reflejos defensivos, lo cual ha sido corroborado en el caso de la respuesta de sobresalto (e.g., Bradley et al., 1990; Cobos et al., 2002; Vrana et al., 1988) pero en el de la RCD.

1.6.3.7. Influencia del sistema nervioso autónomo en la respuesta cardíaca de defensa

La influencia autonómica en la defensa cardíaca ha sido examinada en varios estudios (Fernández & Vila, 1989c; Reyes del Paso et al., 1993, 1994) mediante la evocación de la RCD junto con la utilización tanto de índices indirectos de la actividad autonómica como de procedimientos de tipo farmacológico. Esto es debido a que las dos ramas del sistema nervioso autónomo, simpática y parasimpática, no siempre actúan de manera recíproca sobre el corazón (Berntson et al., 1994; Gellhorn et al., 1941; Reyes del Paso et al., 2014), por lo que su implicación en esta respuesta no puede ser inferida exclusivamente a través de los cambios en casa cardíaca.

El estudio de Fernández y Vila (1989c) consistió en la evocación de la RCD durante el registro simultáneo del período cardíaco para la obtención de la RCD y del tiempo de tránsito de pulso como índice indirecto de la actividad del sistema nervioso simpático. Los resultados mostraron el patrón típico de la RCD con dos componentes aceleratívos/desaceleratívos en orden secuencial alterno. Durante el primer componente acelerativo/desacelerativo de la RCD, los cursos temporales del período cardíaco y del tiempo de tránsito de pulso presentaban direcciones opuestas; mientras que durante el segundo componente acelerativo/desacelerativo los cursos temporales de ambos índices presentaban una mayor similitud, existiendo coincidencias entre período cardíaco y tiempo de tránsito de pulso y, por lo tanto, sugiriendo la existencia de mediación simpática durante el segundo componente acelerativo/desacelerativo de

la RCD. Además, el curso temporal del tiempo de tránsito del pulso indicaba que la mediación simpática sobre la RCD comenzaba varios segundos previos al comienzo de la segunda aceleración, lo que ha llevado a estos/as autores a sugerir la existencia de mediación parasimpática de tipo inhibitorio durante el primer componente acelerativo/desacelerativo de la RCD, que desaparece posteriormente para permitir el comienzo de la segunda aceleración cardíaca.

El estudio de Reyes del Paso et al. (1993) incluye dos experimentos. En el primero de ellos se evaluó la validez del sinus arritmia respiratorio como índice indirecto de la actividad del sistema nervioso parasimpático durante procedimientos de respuesta fásica, tales como los que se utilizan para la evocación de la RCD. Una vez comprobada la validez de dicho índice, el segundo experimento consistió en la evocación de la RCD durante el registro simultáneo de la tasa cardíaca para la obtención de la RCD y del sinus arritmia respiratorio como índice indirecto de la actividad del sistema nervioso parasimpático. Se observó el típico patrón de respuesta de la RCD con dos componentes acelerativos/desacelerativos en orden secuencial alterno. El sinus arritmia respiratorio presentaba un patrón de respuesta que constaba de cuatro componentes (reducción, incremento, reducción e incremento) paralelos pero de dirección opuesta a los cambios en tasa cardíaca. Estos resultados han sido interpretados por los autores de este estudio como mediación parasimpática durante el primer componente acelerativo/desacelerativo de la RCD, con inhibición vagal durante la primera aceleración y activación vagal durante la primera deceleración; además de una interacción simpático-parasimpática durante el segundo componente acelerativo/desacelerativo de la RCD.

La investigación realizada por Reyes del Paso et al. (1994) consistió en la administración de metoprolol por vía intravenosa para bloquear la acción del sistema nervioso simpático, atropina por vía intravenosa para bloquear la acción del sistema

nervioso parasimpático o una solución salina (condición control). Seguidamente, se procedió a la presentación de un estímulo acústico para evocar la RCD y al registro simultáneo de la tasa cardíaca para la obtención de la RCD, del volumen sistólico como índice indirecto de la actividad del sistema nervioso simpático y de la presión sanguínea, que proporciona información indirecta tanto de la rama simpática como parasimpática del sistema nervioso autónomo. Se observó que tanto la primera aceleración como la primera y la segunda desaceleración de la tasa cardíaca se encontraban presentes en el patrón de respuesta de la RCD, sin embargo, había solo una tendencia a mostrar la segunda aceleración de la respuesta. El patrón de respuesta del volumen sistólico consistió en una reducción de corta latencia seguido por un incremento que se prolongaba hasta el final de la respuesta. El patrón de respuesta de la presión sanguínea consistió en un incremento durante la primera desaceleración de la RCD, una reducción durante la segunda aceleración y un ligero incremento durante la segunda desaceleración. Los resultados obtenidos en este estudio sugieren, según sus autores, una mediación parasimpática durante el primer componente acelerativo/desacelerativo y una interacción simpático-parasimpática durante el segundo componente acelerativo/desacelerativo de la RCD.

Recientemente, Árbol (2017) ha examinado la implicación simpática en la defensa cardíaca mediante la presentación de un estímulo acústico evocador de la RCD durante el registro simultáneo del período cardíaco para la obtención de la RCD y del período de pre-eyección, que constituye un índice de contractilidad miocárdica mediada por el sistema nervioso simpático (Berntson et al., 2016). En este estudio se observó el patrón típico de la RCD con dos componentes acelerativos/desacelerativos en orden secuencial alterno. El control cardíaco simpático, por su parte, presentaba un patrón de respuesta trifásico (reducción, incremento y reducción progresiva final). Los cursos temporales del período cardíaco y del período de pre-eyección presentaban direcciones opuestas durante el primer componente acelerativo/desacelerativo de la

RCD y direcciones bastante similares durante el segundo componente acelerativo/desacelerativo. Estos resultados indican una mediación de las dos ramas autonómicas en la RCD, con una mayor implicación parasimpática durante el primer componente acelerativo/desacelerativo y una mayor implicación simpática durante el segundo componente acelerativo/desacelerativo.

En general, los resultados obtenidos en estos estudios sugieren que la RCD se encuentra mediada por las dos ramas del sistema nervioso autónomo, con una mayor influencia parasimpática durante el primer componente acelerativo/desacelerativo y una mayor influencia simpática durante el segundo componente acelerativo/desacelerativo. Esto nos permite conocer con mayor profundidad cuales son los mecanismos fisiológicos subyacentes a la defensa cardíaca.

Capítulo 2:

Objetivos e hipótesis

2.1. Objetivo general

En el capítulo anterior se ha realizado una descripción pormenorizada de la RCD y de los estudios de investigación que se han llevado a cabo en torno a la misma. Varios de esos estudios (Fernández, 1986a; Guerra, 2007; Mata et al., 2009; Ramírez et al., 2005; Turpin, 1986; Vila et al., 1997) se han centrado en examinar una de las características más destacables de la RCD, su rápida habituación tras la repetición de un estímulo evocador de la RCD, que es especialmente pronunciada para el segundo componente acelerativo de la RCD. Se ha encontrado que IEE cortos producen habituación de la respuesta, mientras que IEE más largos producen recuperación. Algunos estudios (Fernández & Vila, 1989a; Pérez et al., 2000; Ramírez et al., 2010; Ruiz-Padial et al., 2005; Sánchez et al., 2002, 2009; Vila et al., 1997) han examinado la significación cognitiva y motivacional de la RCD. La significación cognitiva de la RCD se ha puesto a prueba mediante el registro de la RCD y la manipulación de la orientación atencional (externa vs. interna), encontrándose que existe una relación positiva entre la RCD y la orientación atencional externa, así como con procesos atencionales de aceptación sensorial. La significación motivacional de la RCD se ha puesto a prueba mediante el registro de la RCD y la manipulación del estado afectivo. Se ha encontrado que existe una potenciación de la RCD durante la visualización de imágenes con contenido emocional desagradable y que, por tanto, activan el sistema motivacional defensivo. Además, otros estudios (Árbol, 2017; Fernández & Vila, 1989c; Reyes del Paso et al., 1993, 1994) han examinado la influencia autonómica en la RCD mediante la evocación de la RCD junto con la utilización de distintos índices de la actividad autonómica y procedimientos de tipo farmacológico. Los resultados de estos estudios sugieren una mediación de las dos ramas del sistema nervioso autónomo en la RCD, con mayor influencia parasimpática durante el primer componente acelerativo/desacelerativo y mayor influencia simpática durante el segundo componente acelerativo/desacelerativo. De este modo, se ha ampliado nuestro

conocimiento acerca de los mecanismos fisiológicos subyacentes a la RCD. Sin embargo, aún se desconoce cómo podrían cambiar estos mecanismos si se utilizan procedimientos experimentales en los que, además de evocar la RCD, se manipule el IEE y factores de tipo atencional y emocional para producir habituación y recuperación de la respuesta, así como modulación atencional y emocional.

Por lo tanto, el objetivo general de la presente tesis doctoral consiste en avanzar en el conocimiento de las influencias autonómicas en la RCD en relación a procesos de habituación y recuperación de su patrón de respuesta, así como a su modulación tanto por factores atencionales como emocionales. Para la consecución de este objetivo se han realizado tres estudios experimentales.

2.2. Objetivos específicos e hipótesis

2.2.1. Sympathetic Contributions to Habituation and Recovery of the Cardiac Defense Response - Estudio 1

El objetivo del primer estudio consistió en examinar la implicación del sistema nervioso simpático en los procesos de habituación y recuperación de la defensa cardíaca. Para ello, se presentó en tres ocasiones un estímulo acústico con las características paramétricas apropiadas para evocar la RCD (i.e., un ruido intenso e inesperado) y se manipuló el IEE, de forma que había un IEE corto y un IEE largo que se presentaban en orden contrabalanceado. Se llevó a cabo el registro psicofisiológico de la tasa cardíaca y del período de pre-eyección. Este último constituye un índice del control cardíaco simpático. De acuerdo con los resultados obtenidos en el estudio de Mata et al. (2009), IEE cortos producen una habituación de la RCD mientras que IEE más largos producen una recuperación de la respuesta, que es mayor conforme este

intervalo temporal aumenta. Con este estudio se pretendía poner a prueba las siguientes hipótesis:

- La repetición del estímulo acústico evocador de la RCD tras un IEE corto está acompañada por una disminución en el control cardíaco simpático. Más concretamente, se produce una reducción de la actividad simpática durante el segundo componente acelerativo/desacelerativo de la RCD.

- La repetición del estímulo acústico evocador de la RCD tras un IEE largo está acompañada por un aumento en el control cardíaco simpático. Se produce un incremento de la actividad simpática durante el segundo componente acelerativo/desacelerativo de la RCD.

- Existe una correlación positiva entre la tasa cardíaca y el período de pre-eyección durante el segundo componente acelerativo/desacelerativo de la RCD.

2.2.2. Autonomic Contributions to Attentional Modulation of the Cardiac Defense Response - Estudio 2

El objetivo del segundo estudio consistió en examinar la implicación autonómica en relación con la modulación atencional de la defensa cardíaca. Esto se llevó a cabo mediante dos presentaciones de un estímulo acústico evocador de la RCD y la manipulación de la orientación atencional, a través de la realización de la tarea de búsqueda visual de Sternberg para inducir atención externa vs. ninguna tarea (condición control). Se registró la tasa cardíaca, el período de pre-eyección y la presión arterial sistólica. El período de pre-eyección constituye un índice del control cardíaco simpático y la presión arterial sistólica proporciona información indirecta tanto

sobre sobre el control cardíaco simpático como parasimpático. Con este estudio se pretendía poner a prueba las siguientes hipótesis:

- La manipulación de la orientación atencional para inducir atención externa produce una reducción de la primera desaceleración y una potenciación del segundo componente acelerativo/desacelerativo de la RCD.

- Las dos ramas del sistema nervioso autónomo, simpática y parasimpática, se encuentran implicadas en la modulación atencional de la RCD.

2.2.3. Autonomic Contributions to Attentional and Emotional Modulation of the Cardiac Defense Response: Estudio 3

El objetivo del tercer estudio consistió en examinar la implicación autonómica en relación con la modulación atencional y emocional simultáneas de la defensa cardíaca. Esto se llevó a cabo mediante dos presentaciones de un estímulo acústico evocador de la RCD; la manipulación de la orientación atencional a través de la realización de la tarea de búsqueda visual de Sternberg para inducir atención externa; y la manipulación del estado afectivo utilizando imágenes con distinta valencia (agradable, neutral o desagradable) como estímulos visuales que forman parte de la tarea. Se registró la tasa cardíaca, el período de pre-eyección y la presión arterial sistólica. El período de pre-eyección constituye un índice del control cardíaco simpático y la presión arterial sistólica proporciona información indirecta tanto sobre sobre el control cardíaco simpático como parasimpático. Con este estudio se pretendía poner a prueba las siguientes hipótesis:

- La modulación emocional de la RCD produce una potenciación del segundo componente acelerativo de la respuesta ante la visualización de imágenes desagradables, en comparación con imágenes agradables y neutras.

- Las dos ramas del sistema nervioso autónomo, simpática y parasimpática, se encuentran implicadas en la modulación atencional y emocional de la RCD.

Capítulo 3:

Sympathetic Contributions to Habituation and Recovery of the Cardiac Defense Response - Estudio 1

Publicado como:

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Abstract

The cardiac defense response (CDR) to intense auditory stimulation is characterized by two acceleration-deceleration heart rate (HR) components. This study investigated contributions of sympathetic cardiac control to habituation and recovery of the CDR. Fifty-six healthy subjects were presented with noise stimuli eliciting the CDR. Three stimuli were presented with short and long (2.5 min and 12.5 min) inter-trial intervals (ITIs). The pre-ejection period was recorded as an index of sympathetic cardiac control, in addition to HR. Repeated stimulation at short ITI was associated with marked habituation of the HR and sympathetic responses; both responses exhibited a degree of recovery with long ITI. Regarding the time course, the first acceleration-deceleration was accompanied by a decline and subsequent increase in sympathetic cardiac control. During the second acceleration-deceleration, the parameters exhibited parallel courses. These results suggest that the sympathetic contribution to the habituation and recovery is limited to the second HR component.

3.1. Introduction

The concept of defense refers to an organism's adaptive reactions when it is confronted with dangerous or threatening situations in order to ensure survival; it is closely related to the emotions of fear and anxiety, and alterations in defense are believed to be involved in the occurrence of anxiety disorders and post-traumatic stress (Lang et al., 2000; Lang & McTeague, 2009; Schalinski et al., 2013). Defense encompasses responses of various bodily systems, where research on cardiac defense has a long history in psychophysiology (Vila et al., 2007).

The defense cascade model (Lang et al., 1997) regards the defense response as a dynamic process that encompasses several successive response components, involving an initial attentional set for detection and analysis of potential threats, and a subsequent motivational set of active defensive behaviours. Thus, different stages of the defense response are considered, with their particular expressions differing according to the type and severity of the aversive event, its spatial and temporal proximity, and previous experience with the event type (D. C. Blanchard & R. J. Blanchard, 1988; Bracha, 2004; Facchinetti et al., 2006; Fanselow, 1994; Gallup, 1977; J. A. Gray, 1988; Lang et al., 1997; Marks, 1987).

Building on this theory, Vila et al. (2007) proposed the attentional-motivational model of the defense, relating different components of the cardiac response to specific stages of the defense cascade. This framework acknowledges that the cardiac response is characterized by a complex pattern of heart rate changes to an unexpected intense noise with two acceleration-deceleration components within a frame of 80 seconds after stimulus onset (see Figure 6). These components are believed to be mediated by sympathetic and parasympathetic cardiac control, and to be of cognitive and motivational significance. The response pattern typically involves two successive phases: an initial phase of heart rate acceleration, reaching its peak around seconds 2-

3, and deceleration linked to attentional and protective processes, including the interruption of ongoing activity and increased attention to external cues; and a second acceleration, that reaches its peak around seconds 30-35, and deceleration component representing motivational processes, particularly the preparation of active defensive behaviours and recovery if no real danger occurs.

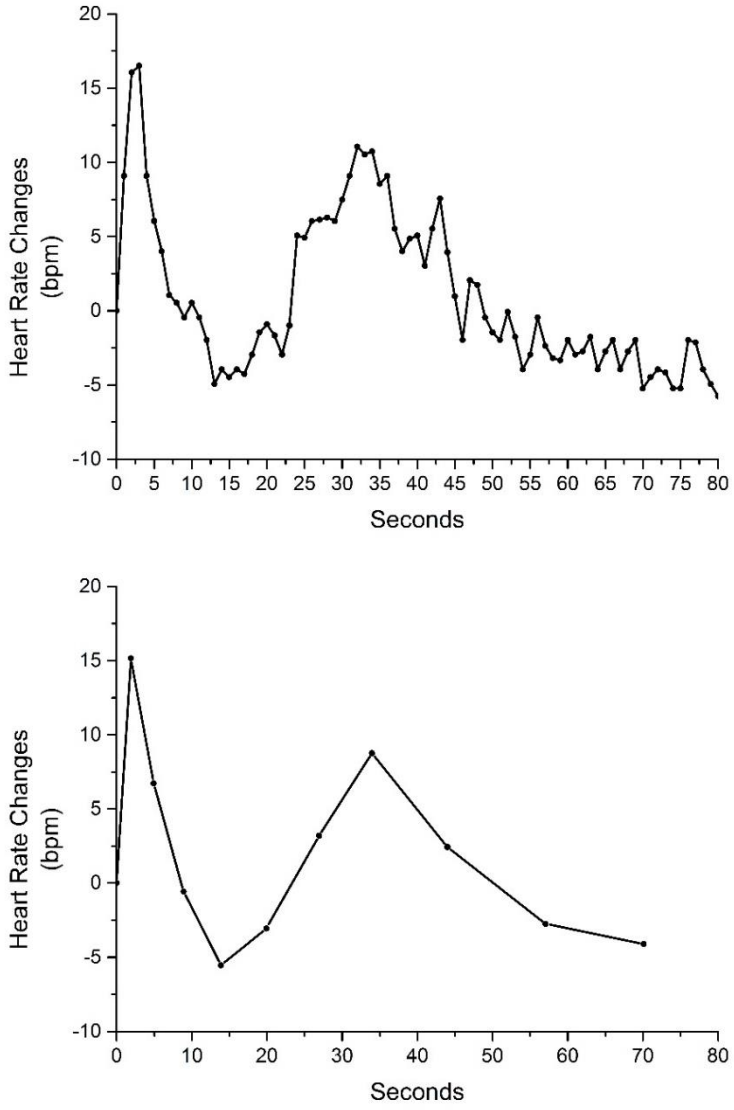


Figure 6. Typical pattern of the cardiac defense response: average second-by-second heart rate response (up) and the same data expressed in terms of the medians of 10 intervals (down) (Adapted from Vila et al., 2007).

A significant proportion of the research on the cardiac defense is based on a paradigm involving the presentation of an unexpected aversive noise under different task conditions, where beat-to-beat heart rate modulations constitute the main dependent variable (see Vila et al., 2007 for an overview of this research). An important finding pertains to habituation of the response following repetition of the acoustic stimulus, which is particularly pronounced in the second acceleration/deceleration component. This is a robust observation of studies during which the noise was repeatedly presented in a single session with inter-trial intervals (ITIs) of around 2 min (e.g., Ramírez et al., 2005; Vila et al., 1997). Moreover, recovery of the cardiac defense, defined in terms of reappearance of the response following a time interval, was seen when repeating the same experimental procedure after several months (Fernández, 1986).

Mata et al. (2009) investigated habituation and recovery of the cardiac defense response as a function of stimulus timing by systematically varying ITI. An acoustic stimulus eliciting the cardiac response was presented three times within a single session. While the interval between the first and the third presentation was fixed at 30 min, the timing of the second presentation was manipulated by successively increasing the interval between the first and second, and successively decreasing the interval between the second and the third presentation (ITIs: 2.5/27.5 min, 7.5/22.5 min, 12.5/17.5 min, 17.5/12.5 min, 22.5/7.5 min, and 27.5/2.5 min). As a result, the two response components showed pronounced habituation during presentation of the noise after a short ITI (2.5 min), in addition to recovery at long ITIs, which increased with increasing ITI duration. Both habituation and recovery were stronger for the second response component than for the first.

While the study of Mata et al. (2009) underlines that the strength of the cardiac defense response may substantially change according to repetitions and timing of stimulation,

not much is known about the physiological mechanisms underlying habituation and recovery as a function of the time interval between stimulus repetition. To address this gap in the literature, the present study aimed to investigate the autonomic nervous system mechanisms involved in these phenomena, where sympathetic contributions were of particular interest. The traditional view on cardiac defense, based on Cannon's fight/flight response (Cannon, 1929), assumes that the cardiac changes to an aversive stimulus are mediated by an increment in sympathetic activation accompanied by a reciprocal decrement in parasympathetic activation. However, it has been well established that in heart rate regulation, the two divisions of the autonomic nervous system do not necessarily act in a reciprocal manner (Berntson et al., 1993, 1994; Gellhorn et al., 1941; Reyes del Paso et al., 2014).

This is acknowledged by the theory of autonomic space (Berntson et al., 1991), which postulates three basic modes of action of the autonomic nervous system: (a) a coupled reciprocal mode, with a negative correlation between sympathetic and parasympathetic activity; (b) a coupled non-reciprocal mode, with a positive correlation between the activity of both branches (i.e., co-activation or co-inhibition); and (c) an uncoupled mode, in which both branches act independently of each other. In addition, non-linear interactions between sympathetic and parasympathetic influences in heart rate regulation have been established (Levy & Zieske, 1969). While effects of sympathetic activity on heart rate are rather weak during high background parasympathetic activity, vagal effects are even stronger during high background sympathetic activity (Uijtdehaage & Thayer, 2000). This mode of interaction is referred to as accentuated antagonism and reflects the vagal dominance in heart rate control (Morady et al., 1988; Uijtdehaage & Thayer, 2000).

To investigate sympathetic cardiac control during psychological processing, pharmacological methods as well as indirect indices (e.g., pulse transit time, indices of

cardiac contractility) are available (Berntson et al., 2016). Regarding sympathetic cardiac control during the cardiac defense, the indirect indices suggested a decrease in sympathetic cardiac control during the first acceleration component, followed by a gradual increase beginning during the first deceleration component, reaching its maximum at the second heart rate peak. Thereafter, sympathetic cardiac control decreases concurrently with heart rate (Fernández & Vila, 1989; Reyes del Paso et al., 1994). Recently, Árbol (2017) investigated sympathetic cardiac control during the cardiac defense response based on pre-ejection period (PEP) measurement in a single trial of the defense paradigm. This study confirmed the initial reduction of sympathetic cardiac control as well as the increase and decrease in the further course of the defense. PEP may be derived using impedance cardiography and constitutes a well-established index of sympathetic cardiac control (Berntson et al., 2016). However, it must be acknowledged that PEP represents an inotropic parameter, which provides information about beta-adrenergic influences on the myocardium (Berntson et al., 2016; Cacioppo et al., 1994; Hassan & Turner, 1983). Indirect measurement of sympathetic control of the sinus node, i.e. beta-adrenergic chronotropic control, is not available. Considering that sympathetic influences on cardiac contractility and heart rate may dissociate to certain degree, PEP should be regarded as a more general index of sympathetic cardiac control.

Altogether, these results suggest that the first acceleration/deceleration component is mainly mediated by the fast acting parasympathetic system. Apparently, here vagal withdrawal dominates over the simultaneous reduction of sympathetic cardiac control. In contrast, the sympathetic system may play a relevant role in the second component, where heart rate modulations seem to vary according to beta-adrenergic activation and inhibition (Fernández & Vila, 1989). The sympathetic origin of the second response component has also been confirmed by beta-adrenergic blockade using metoprolol (Reyes del Paso et al., 1994).

Building on this research, in the present study beat-to-beat changes in heart rate and PEP were recorded to explore sympathetic contributions to changes of the cardiac defense response over time, i.e., habituation and recovery. For this purpose, unexpected aversive noise was presented in repeated trials while systematically manipulating ITI (c.f. Mata et al., 2009). The following main hypotheses were tested: (1) Repetition of stimulus presentation at a short ITI will be accompanied by decreases in sympathetic cardiac control, with a reduction of sympathetic activation specifically during the second acceleration/deceleration component of the cardiac defense response; (2) Repetition of stimulus presentation at a long ITI will be accompanied by increases in sympathetic cardiac control, with increased sympathetic activation specifically during the second acceleration/deceleration component of the cardiac defense response; (3) Independently of ITI, heart rate and PEP will show positive correlations during the second acceleration/deceleration component of the cardiac defense response.

3.2. Method

3.2.1. Participants

A total of 56 university students (28 women and 28 men) aged between 18 and 45 years (*M* = 21.80, *SD* = 4.85) participated in the study. Individuals taking drugs affecting the central or autonomic nervous system, and those suffering from cardiovascular diseases or auditory or visual deficits, were excluded. All participants provided written informed consent to the study protocol and received course credits for their participation. The Ethics Committee of the University of Granada approved the study (approval number 423/CEIH/2017).

3.2.2. Study design

All participants were presented with an acoustic stimulus eliciting the cardiac defense response three times (Ramírez et al., 2005): white noise of 105 dB, 500 ms duration,

and instantaneous rise time. To manipulate habituation and recovery of the cardiac defense response, two different conditions of the ITI were used: ITI Condition 1 (ITI1 = 2.5 min, ITI2 = 12.5 min) and ITI Condition 2 (ITI1 = 12.5 min, ITI2 = 2.5 min). In a between-subjects design, participants were randomly assigned to these conditions (14 women and 14 men per condition).

The experimental paradigm comprised the following steps: (a) an initial 8 min rest period, (b) three trials of acoustic stimulation without prior warning under the corresponding ITI conditions, and (c) a second rest period of 90 s. Each trial included a pre-trial recording period of 10 s, stimulus presentation of 500 ms, and a post-trial recording period of 80 s. Participants were instructed to breathe normally during the test, to keep their eyes open, and to look at a fixation point located at a distance of 2 m from their eyes.

3.2.3. Instruments and recordings

3.2.3.1. Acoustic stimulation

The white noise was generated by a Coulbourn V15-17 audio system and an IMQ Stage Line PPA-1 amplifier. It was presented binaurally through AKG K-240 Monitor headphones (600 ohms). The intensity of the sound was calibrated using a sound level meter (model 2235; Brüel & Kjær Inc., Bremen, Germany) and an artificial ear (model 4153; Brüel & Kjær Inc.).

3.2.3.2. Psychophysiological recordings

3.2.3.2.1. Electrocardiography (ECG)

Beat-to-beat recordings of heart period (HP) during the 80 sec after stimulus onset, converted to weighted average every second, and transformed to differential score with respect to a HP baseline of 10 sec prior to stimulus onset, were used to describe the cardiac defense response. Then the 80 HP values were reduced to 10 values

corresponding to the mean of 10 progressively longer intervals: two intervals of three seconds, two intervals of five seconds, three intervals of seven seconds, and three intervals of 13 seconds. This definition follows the same criteria used in previous studies on the cardiac defense response, except that in the present study heart rate was substituted by HP (Mata et al., 2009; Vila et al. 2007). According to earlier suggestions, HP is preferred over heart rate in studies on autonomic mechanisms underlying cardiac changes (Berntson et al., 1995; Graham, 1978; Reyes del Paso & Vila, 1998). On the other hand, the 10 progressively longer intervals simplify the analysis allowing identification of the two-acceleration/deceleration components of the CDR: first acceleration/deceleration (intervals 1-3) and second acceleration/deceleration (intervals 4-10). HP was defined as the interval (in ms) between consecutive R-waves of the ECG. The ECG was recorded using a Biopac system (MP 150, Biopac Systems Inc., Goleta, CA, USA) with an ECG100C amplifier, at a sampling rate of 1000 Hz. Disposable Ag/AgCl electrodes filled with electrode paste were used in Einthoven's lead II configuration (right arm, left leg, ground electrode, right leg). AcqKnowledge 4.2. software (Biopac Systems Inc.) was applied for R-wave detection and manual artefact correction.

3.2.3.2.2. Impedance cardiography (ICG)

Sympathetic cardiac control was estimated based on beat-to-beat ICG recordings of the PEP during the 80 sec after each acoustic stimulus, using the same weighted average every second and the same baseline period as for HP. Similarly, the 80 PEP values were reduced to the means of the same 10 intervals. ICG was conducted using a Biopac system (MP 150, Biopac Systems Inc.) and a NICO100C amplifier (sampling rate, 1000 Hz). Disposable Ag/AgCl strip electrodes filled with electrode paste were attached in the tetrapolar configuration described by Kubicek et al. (1966). The upper voltage electrode was placed around the base of the neck and the lower voltage electrode around the thorax at the level of the xiphisternal junction; the two current

electrodes were fixed 3 cm distal from each of them. AcqKnowledge 4.2 software was used for ICG signal processing. PEP was defined as the period in ms between the onset of ventricular depolarization (Q-onset in ECG) and the beginning of left ventricular ejection (B-point in the first derivative of ICG signal) (Sherwood et al., 1990). The B-point was localized by using the algorithm known as *third derivative classification*, which has been suggested to be superior to other popular algorithms (Árbol et al., 2017). Automatic B-point detection was corrected manually when needed.

3.2.3.3. Self-report measures

Participants completed a post-experimental rating scale assessing the subjectively perceived intensity and unpleasantness of each of the three acoustic stimuli. The scale ranged from 0 (not at all intense/unpleasant) to 100 (extremely intense/unpleasant).

3.2.4. Procedure

Each participant attended a single laboratory session of approximately 60 min duration. Upon arrival, participants were invited to sit in an armchair and received information about the study. They were told that physiological data was going to be recorded in the resting state during the experiment. Moreover, they were instructed to ignore any noises that they heard during the session. According to the standard instructions of the cardiac defense procedure (see Vila et al., 2007), the aversive nature of the stimuli was not mentioned. Participants signed an informed consent form and completed a brief interview to evaluate the inclusion and exclusion criteria. Thereafter, the electrodes and the headphones were fitted. Participants were left alone in the dimly lit room during the test. After its completion, the post-experimental questionnaire was presented.

3.2.5. Statistical analysis

HP and PEP were converted into z scores to allow statistical comparison between HP and PEP data. In the first step of the statistical analysis, HP and PEP were analysed

separately by means of two 2(x3x10) analyses of variance (ANOVAs) with the between-group factor of ITI Condition (ITI Condition 1 vs. ITI Condition 2) and the two within-subject factors of Trial (Trials 1 to 3) and Time (the 10 intervals after the onset of the acoustic stimulus). In addition, both psychophysiological measures were analysed jointly. For this purpose, 2x10 ANOVAs were conducted with the between-group factors of Measure (HP vs. PEP) and Time. Six models were computed for the different conditions and trials. Regarding self-report measures, intensity and unpleasantness were analysed separately by means of two 2(x3) ANOVAs with the between-group factor of ITI Condition and the within-subject factor of Trial.

The Greenhouse-Geisser epsilon correction was applied to the within-subject factors. Results are provided with uncorrected df and corrected *p* values; partial eta squared (ηp^2) is indicated as a measure of effect size. Alpha was set at .05 for the ANOVAs; in follow-up analysis of significant interactions concerning Time, separate ANOVAs were conducted for each interval to identify the specific components of the cardiac defense response with significant effects, followed, when applicable, by multiple pair-wise comparisons using Bonferroni test. The correlation analysis was performed using Pearson bivariate correlation between the heart rate index (HP) and the sympathetic index (PEP) along the 10 intervals, joining both ITI conditions and the three trials.

3.3. Results

3.3.1. Cardiac defense response

Figure 7 denotes the time course of HP during the experimental task (to facilitate visual interpretation, inverted values are presented in the figure; as such, a reduction in HP represents an increase in heart rate, and vice versa). During all trials of the two ITI Conditions, an initial acceleration/deceleration component arose during the first 3 intervals (11 seconds) after the noise stimulus. In most of the trials, a less pronounced second acceleration/deceleration component was seen thereafter. During ITI Condition

1, heart rate was highest for Trial 1 followed by Trials 3 and 2. In contrast, during ITI Condition 2, heart rate was highest for Trial 2, followed by Trials 1 and 3.

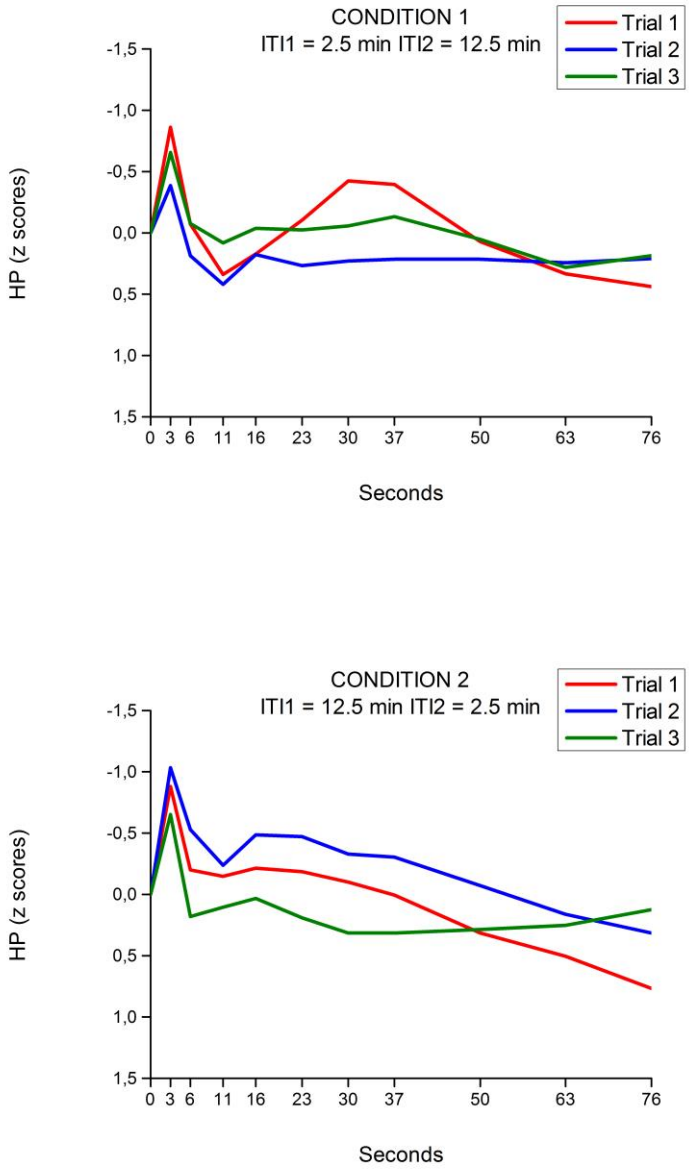


Figure 7. Course of the cardiac defense response: mean heart period across the 10 time-intervals (expressed in z scores) as a function of ITI conditions and trials.

The ANOVA for HP yielded a main effect of Time, $F(9,486) = 22.68$, $p < .001$, $\eta p^2 = .296$, and interaction effects of ITI Condition x Trial, $F(2,108) = 6.68$, $p = .002$, $\eta p^2 =$

.110, Trial x Time, $F(18,972) = 4.67$, $p = .001$, $\eta p^2 = .080$, and ITI Condition x Trial x Time, $F(18,972) = 3.63$, $p < .001$, $\eta p^2 = .063$. To further analyse the ITI Condition x Trial x Time interaction, three separate ANOVAs were computed for each trial, with the between-group factor of ITI Condition and the within-subject factor of Time. The models revealed effects of Time for all trials (Trial 1 $F[9,486] = 22.42$, $p < .001$, $\eta p^2 = .293$, Trial 2 $F[9,486] = 11.04$, $p < .001$, $\eta p^2 = .170$, Trial 3 $F[9,486] = 12.64$, $p < .001$, $\eta p^2 = .190$) and ITI Condition x Time interactions for Trial 1 ($F[9,486] = 3.24$, $p = .011$, $\eta p^2 = .057$) and Trial 2 ($F[9,486] = 3.17$, $p = .011$, $\eta p^2 = .055$). In addition, separate ANOVAs were conducted for each interval to identify for each condition the intervals with significant differences between the three trials. For ITI Condition 1 significant differences were found in intervals 1 ($F[2,54] = 3.93$, $p = .026$, $\eta p^2 = .127$), 5 ($F[2,54] = 5.62$, $p = .008$, $\eta p^2 = .172$), 6 ($F[2,54] = 8.93$, $p = .001$, $\eta p^2 = .249$), and 7 ($F[2,54] = 6.91$, $p = .004$, $\eta p^2 = .204$) . For ITI Condition 2 significant differences were found in intervals 2 ($F[2,54] = 6.15$, $p = .005$, $\eta p^2 = .185$), 5 ($F[2,54] = 6.79$, $p = .003$, $\eta p^2 = .201$), 6 ($F[2,54] = 4.42$, $p = .02$, $\eta p^2 = .141$), 7 ($F[2,54] = 5.68$, $p = .01$, $\eta p^2 = .174$), 8 ($F[2,54] = 3.11$, $p = .05$, $\eta p^2 = .103$), and 10 ($F[2,54] = 8.36$, $p = .001$, $\eta p^2 = .236$).

Pair-wise multiple comparisons between the three trials using Bonferroni test showed significant differences for ITI Condition 1 between the first and the second trial (short ITI) in intervals 1, 5, 6, and 7: lower heart rate for the second trial (all corrected ps $\leq$ .023); and between the second and the third trial (long ITI) in intervals 5 and 7: higher heart rate for the third trial (all corrected ps $\leq .037$). With regard to ITI Condition 2, pair-wise comparisons yielded significant differences between the first and the second trial (long ITI) in interval 8: higher heart rate for the second trial (corrected $p \leq .05$); and between the second and the third trial (short ITI) in intervals 2, 5, 6, and 7: lower heart rate for the third trial (all corrected ps $\leq .044$).

3.3.2. Sympathetic cardiac control

Figure 8 illustrates the time course of PEP (inverted values are presented; as such, a reduction in PEP represents an increase in sympathetic cardiac control, and vice versa). During most trials, an initial decrease of sympathetic cardiac control was seen, followed by a steep rise peaking between intervals 4 and 5, and a subsequent gradual decline. Substantial differences between trials arose after interval 2 up to the final interval. During ITI Condition 1, sympathetic cardiac control was highest for Trial 1, followed by Trials 3 and 2. In contrast, during ITI Condition 2, sympathetic cardiac control was lowest for Trial 3; only a minor difference arose between Trials 1 and 2.

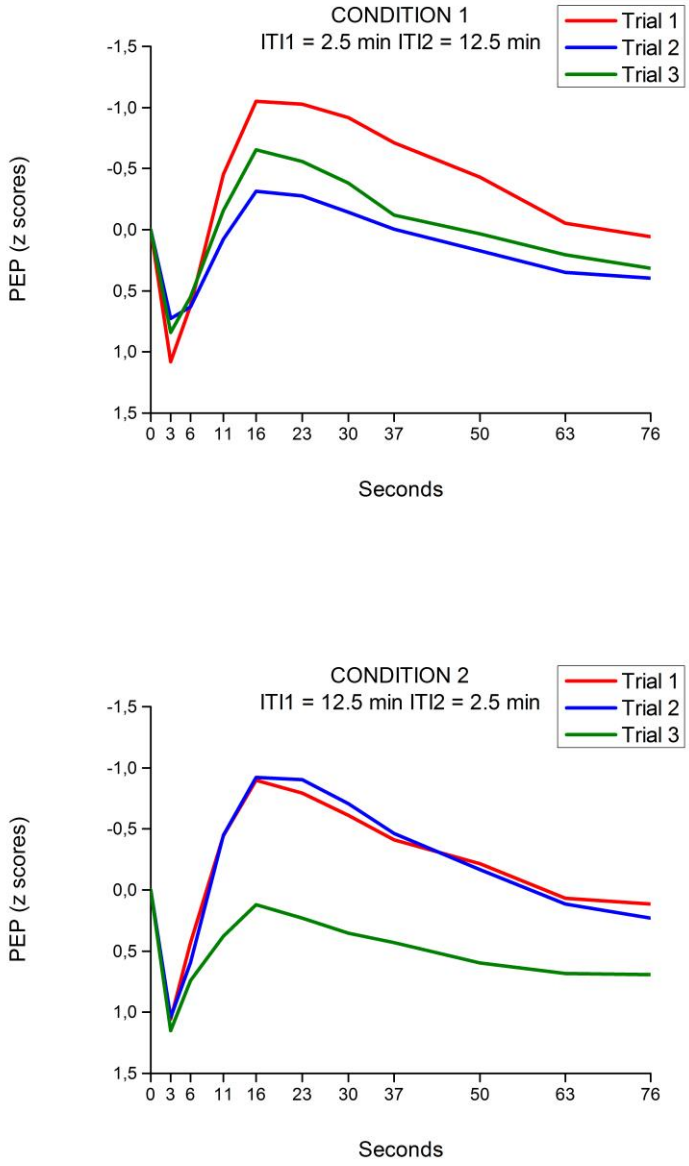


Figure 8. Course of sympathetic cardiac control: mean pre-ejection period across the 10 time-intervals (expressed in z scores) as a function of ITI conditions and trials.

The ANOVA for PEP revealed main effects of Trial, $F(2,108) = 10.47$, $p < .001$, $\eta p^2 = .162$, and Time, $F(9,486) = 128.76$, $p < .001$, $\eta p^2 = .705$, and interaction effects of ITI Condition x Trial, $F(2,108) = 7.59$, $p = .001$, $\eta p^2 = .123$, Trial x Time, $F(18,972) = 9.12$, $p < .001$, $\eta p^2 = .144$, and ITI Condition x Trial x Time, $F(18,972) = 7.81$, $p < .001$, $\eta p^2 = .126$. Follow-up analysis of the three-way interaction was accomplished through

ANOVAs for each trial with the between-group factor of ITI Condition and the within-subject factor of Time. A significant effect of Time arose for all trials (Trial 1 F[9,486] = 104.15, p < .001, ηp^2 = .659, Trial 2 F[9,486] = 68.79, p < .001, ηp^2 = .560, Trial 3 F[9,486] = 52.47, p < .001, ηp^2 = .493); moreover, ITI Condition x Time interactions were seen for the second and third trials (Trial 2 F[9,486] = 6.06, p = .002, ηp^2 = .101, Trial 3 F[9,486] = 3.46, p = .03, ηp^2 = .060). In addition, separate ANOVAs were conducted for each interval to identify for each condition the intervals with significant differences between the three trials. For ITI Condition 1 significant differences between trials were found in all intervals, except interval 2 (all ps ≤ .04 and all ηp^2s ≥ .116). For ITI Condition 2 significant differences between trials were found in all intervals, except intervals 1 and 2 (all ps ≤ .003 and all ηp^2s ≥ .121).

Bonferroni-corrected pair-wise comparisons between the three trials yielded significant differences for ITI Condition 1 between the first and the second trial (short ITI) in intervals 3 to 9: lower sympathetic control for the second trial (all corrected ps ≤ .03); the comparison between the second and the third trial (long ITI) did not yield significant results. For ITI Condition 2, this analysis revealed significant differences between the second and third trial (short ITI) in intervals 3 to 9: lower sympathetic cardiac control for the third trial (all corrected ps ≤ .045); the comparison between the first and the second trial (long ITI) did not yield significant results.

3.3.3. Cardiac defense response and sympathetic cardiac control

The comparison between the patterns of HP and PEP (Figure 7 and Figure 8) shows that, for all ITI conditions and trials, HP and PEP showed opposing time course patterns during the first 3 time-intervals (11 seconds) of the response: initial heart rate acceleration was followed by a heart rate decrease; sympathetic cardiac control initially decreased and thereafter increased. Relatively parallel time courses of both parameters arose during the remaining response.

The ANOVAs for the joint analysis of HP and PEP revealed Measure x Time interactions for all trials in both conditions (ITI Condition 1: Trial 1 F[9,243] = 38.42, p < .001, ηp^2 = .587, Trial 2 F[9,243] = 18.09, p < .001, ηp^2 = .401, Trial 3 F[9,243] = 24.45, p < .001, ηp^2 = .475; ITI Condition 2: Trial 1 F[9,243] = 30.43, p < .001, ηp^2 = .530, Trial 2 F[9,243] = 26.22, p < .001, ηp^2 = .490, Trial 3 F[9,243] = 20.37, p < .001, ηp^2 = .430), confirming the different time courses of both measures in all cases. In addition, a main effect of Time arose for all trials of both ITI conditions (ITI Condition 1: Trial 1 F[9,243] = 21.95, p < .001, ηp^2 = .448, Trial 2 F[9,243] = 6.44, p < .001, ηp^2 = .193, Trial 3 F[9,243] = 10.38, p < .001, ηp^2 = .278; ITI Condition 2: Trial 1 F[9,243] = 19.22, p < .001, ηp^2 = .416, Trial 2 F[9,243] = 16.44, p < .001, ηp^2 = .378, Trial 3 F[9,243] = 4.47, p < .003, ηp^2 = .142); a main effect of Measure was seen for the third trial of ITI Condition 2 (F[1,27] = 6.47, p = .017, ηp^2 = .193).

Follow-up analysis of the Measure x Time interactions was accomplished by separate ANOVAs for each interval to identify, for the three trials in both ITI conditions, the intervals with significant differences between the two measures. For all trials and conditions, significant differences were found in intervals 1 and 2 (all ps ≤ .02 and all ηp^2s ≥ .185): increased heart rate associated with decreased sympathetic activation. After interval 2, although both measures were moving in the same direction, some significant differences appeared with the sympathetic index being always higher than the heart rate index, except in ITI Condition 2 Trial 3. For ITI Condition 1, significant differences were found in Trial 1 (intervals 3 to 10, all ps ≤ .02 and all ηp^2s ≥ .173), Trial 2 (intervals 4 and 5, both ps ≤ .04 and both ηp^2s ≥ .146; and Trial 3 (intervals 4 and 5, both ps ≤ .02 and both ηp^2s ≥ .233). For ITI Condition 2, significant differences were found only in Trial 1 (intervals 4 to 6 and 8 to 10, all ps ≤ .05 and all ηp^2s ≥ .132).

3.3.4. Correlation between HP and PEP

Pearson correlation between the heart rate index (HP) and the sympathetic index (PEP) along the 10 intervals, joining both ITI conditions (n = 56) and the three trials, revealed one significant negative correlation in interval 1 (r = -.294, p = .028), corresponding to the first acceleration/deceleration, and two significant positive correlations in intervals 7 (r = .349, p = .008) and 8 (r = .301, p = .024), corresponding to the second acceleration/deceleration.

3.3.5. Self-report measures

Table 1 shows the mean and standard error for the subjective perception of intensity and unpleasantness of the noise as a function of ITI conditions and trials. Participants assigned to ITI Condition 1 rated the noise of Trial 1 as the most intense and unpleasant, followed by the Trials 3 and 2. Regarding ITI Condition 2, the noise of Trial 1 was rated as the most intense and unpleasant, followed by Trials 2 and 3.

Table 1. Mean (and standard deviation) of self-report noise intensity and unpleasantness as a function of ITI conditions and trials.

	ITI Condition 1			ITI Condition 2		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Noise intensity	78.33 (14.97)	65.19 (12.80)	74.44 (14.87)	78.42 (10.86)	73.13 (13.80)	66.88 (14.96)
Noise unpleasantness	84.62 (11.62)	65.96 (16.89)	72.31 (18.37)	79.17 (15.44)	74.79 (16.30)	65.83 (21.11)

The ANOVAs yielded a main effect of Trial (Intensity: F[2,108] = 10.98, p < .001, ηp^2 = .170; Unpleasantness: F[2,108] = 23.57, p < .001, ηp^2 =.300) and an interaction effect

of Trial x ITI Condition (Intensity: F[2,108] = 6.75, p=.002, ηp^2=.110; Unpleasantness: F[2,108] = 8.67, p = .001, ηp^2 = .140) for both intensity and unpleasantness ratings. Pair-wise comparisons showed significant differences between Trial 2 and the other two trials for intensity (all corrected ps ≤ .015) and between all trials for unpleasantness (all corrected ps ≤ .047) in ITI Condition 1, and between Trial 3 and the other two trials for intensity (all corrected ps ≤ .003) and between all trials for unpleasantness (all corrected ps ≤ .037) in ITI Condition 2.

3.4. Discussion

The aim of this study was to investigate sympathetic mechanisms related to habituation and recovery of the cardiac defense response. For this purpose, unexpected noise stimuli eliciting the response were presented at varying ITIs, while ECG and ICG recordings were obtained. Repeated noise stimulation at a short ITI of 2.5 min was associated with marked habituation of the heart rate and sympathetic cardiac control, indexed by PEP. Moreover, both types of responses exhibited a degree of recovery across a long ITI of 12.5 min. Subjective ratings of intensity and unpleasantness of the noise coincide with the psychophysiological data, with lower ratings in trials after a short ITI than after a long ITI for both ITI conditions and measures. Regarding the time course of the response, sympathetic cardiac control showed a short initial decline, followed by a steep rise and gradual decrease toward the end of the observation interval. While the typical biphasic pattern of heart rate modulations arose in most of the trials, heart rate and sympathetic cardiac control demonstrated opposing changes during the first phase, with one significant negative correlation in interval 1, and virtually parallel time course thereafter, with two significant positive correlations around the middle of the second acceleration/deceleration.

Habituation of the heart rate response at a short ITI, and recovery at a long ITI, were overall stronger for the second acceleration/deceleration component than the first one. This confirms previous studies suggesting virtual restriction of habituation of the cardiac defense to its second component at ITIs of similar durations, as in the present study (Mata et al., 2009; Ramírez et al., 2005; Vila et al., 1997). Likewise, in earlier studies, time-elapsing recovery within the same experimental session, and across repeated assessments after several months, was more pronounced for the second than the first component (Fernández, 1986; Mata et al., 2009). A somewhat unexpected result concerns the increase of the magnitude of the second response component between the first and second trial of the second condition (long ITI). As this increase was not preceded by habituation of the response, it cannot be interpreted in terms of recovery. Instead, processes of sensitization, presumably associated with enhancement of vigilance during expectation of the stimulus after the first trial, may be relevant.

The cardiac sympathetic response also showed pronounced habituation and reduced recovery at a short ITI in both task conditions; significantly lower sympathetic cardiac control during the repetition at short ITI arose in 7 consecutive intervals beginning with the 3rd interval. No distinct habituation and recovery of the cardiac sympathetic response was seen at a longer ITI. Therefore, it would appear that habituation of the sympathetic response across the short ITI was markedly stronger than its recuperation across the long ITI. Taking into account the substantial magnitude of the habituation of the sympathetic response, in addition to its time characteristics, one may conclude that it plays a key role in the habituation of the second component of the cardiac defense response. Increased responsiveness of the sympathetic system after its habituation may also be involved in the recovery of the heart rate response. However, as in our study, recovery of both types of responses was relatively small; the evidence of this effect is not as strong as that for habituation. In this regard, it may be considered that

the duration of the long ITI was not sufficient to enable substantial recovery of the responses. While Mata et al. (2009) showed recovery of the heart rate response using ITIs of up to 27.5 min, Fernández (1986) investigated recovery across repeated sessions with several months in between.

During the first 3 time-intervals of the response (11 seconds), heart rate and sympathetic cardiac control showed virtually mirror-inverted time course patterns: while heart rate exhibited a short-term rise and a subsequent decline during most trials, sympathetic cardiac control initially decreased and then gradually rose. The coincidence of sympathetic inhibition and heart rate acceleration supports the notion that the sympathetic nervous system plays a subordinate role in the first acceleration/deceleration component of the cardiac defense response (Fernández & Vila, 1989; Reyes del Paso et al., 1994). Instead, heart rate acceleration is likely to be caused by a reduction of vagal outflow to the sinus node. In the following phase of the response (i.e., after 11 seconds), the time courses of heart rate and sympathetic cardiac control were more similar to each other. Here, the increase in sympathetic cardiac control continued, whereas in most trials a second heart rate increase arose. The occurrence of the peak of sympathetic cardiac control did not exactly correspond to that of the second heart rate increase; however, both measures showed virtually parallel decreases after the second heart rate peak. The third trial of condition 2 should be noted as an exception, during which heart rate increased once again towards the end of the observation interval. Nevertheless, the similar courses of sympathetic cardiac control and heart rate confirm the view that the sympathetic system contributes to heart rate modulation during the second acceleration/deceleration component. This is confirmed by the significant positive correlations observed in our study between HP and PEP around the middle of the second acceleration/deceleration. It is also consistent with the finding of Árbol (2017) based on PEP measurement by ICG during elicitation of the cardiac defense response in a single trial, as well as earlier studies

using other indirect measures of sympathetic cardiac control, as well as pharmacological methods (Fernández & Vila, 1989; Reyes del Paso et al., 1994).

The present findings may be discussed within the framework of autonomic space (Berntson et al., 1991), according to which three basic modes of interactions between the sympathetic and parasympathetic systems are distinguished: a coupled reciprocal mode, a coupled non-reciprocal mode, and an uncoupled mode. During the first 3 intervals (11 seconds) of the response, sympathetic cardiac control and heart rate showed nearly mirror-inverted time courses. Assuming that at this stage heart modulation mainly underlies vagal control, both systems may have interacted in a coupled non-reciprocal mode. While during the initial heart rate increase (i.e., the first interval of 3 seconds) co-inhibition prevailed, co-activation arose after the first heart rate peak. Apparently, vagal cardiac control counteracted activity of the sympathetic system, reducing its influence on heart rate to a minimum. The mode of interaction changed after the third interval (11th second), where both assessed parameters showed a parallel rather than opposing time course. While the increase in sympathetic cardiac control continued until approximately the fourth interval (16th second), our data do not allow conclusions regarding the role of the parasympathetic system during the second response component. Vagal withdrawal may have supported the second heart rate acceleration, with both systems acting in a coupled reciprocal mode. However, an uncoupled mode with dominance of sympathetic over parasympathetic influences is also feasible.

As initially stated, sympathetic and parasympathetic influences on heart rate may also interact in a non-linear fashion. In terms of accentuated antagonism, sympathetic chronotropic effects are substantially attenuated by concurrent parasympathetic activity and parasympathetic effects are even stronger during sympathetic activity (Uijtdehaage & Thayer, 2000). Taking this into account, during the first 3 intervals (11 seconds) of

the response, concurrent parasympathetic activation may have impeded heart rate acceleration due to sympathetic activity. On the other hand, during this period, heart rate deceleration resulting from parasympathetic activity may even have been boosted by concurrent sympathetic activation. The parallel time course of sympathetic cardiac control and heart rate after the 3rd interval may suggest stronger sympathetic chronotropic influences. However, in light of accentuated antagonism this may only apply when assuming rather small parasympathetic influences during this period.

In this study, PEP was applied for the first time as an index of sympathetic cardiac control in research on habituation and recovery of the cardiac defense response. Our findings are in line with those using PEP in the investigation of the defense in a single trial (Árbol, 2017), as well as earlier studies based on other methods (Fernández & Vila, 1989; Reyes del Paso et al., 1994), and thus support the suitability of PEP in this context. However, as sympathetic influences on heart rate cannot be quantified using indirect measures, in future research our results may be complemented by pharmacological methods. Another limitation of this study pertains to the restriction of the analysis to sympathetic cardiac control; as such, conclusions regarding the contributions of other regulatory mechanisms must remain speculative. In addition to vagal withdrawal, inhibition of function of the cardiac baroreflex may be involved in heart rate acceleration during the two components of the defense. While vagal influences on heart rate can be determined using pharmacological blockade, baroreflex function can be non-invasively quantified using sequence analysis of beat-to-beat blood pressure and HP (Berntson et al., 2016; Duschek et al., 2013). The application of these methods in upcoming studies may provide further insight into the autonomic mechanisms involved in the cardiac defense and its modulation through habituation and recovery. A final methodological limitation to take into account is that, although alcohol and caffeine intake, cigarette smoking and physical activity were assessed and

no statistical differences were found between groups, participants were not instructed to abstain from these activities prior to experimental session.

The present findings support the attentional-motivational model of the defense (Vila et al., 2007), according to which the cardiac response is mediated by both branches of the autonomic nervous system. However, this model also emphasizes the behavioural significance of the response: while the first acceleration/deceleration component is believed to represent attentional allocation towards a potentially threatening cue, the second component has been linked to motivation and the initiation of defensive behaviours. One may thus consider connections between these psychological processes and specific autonomic mechanisms. It might be speculated, for example, that vagal withdrawal during the first component is related to attentional processing, whereas sympathetic activation during the second one may be linked to motivation and behavioural preparation. It would certainly be worthwhile to address such interactions between autonomic function and behavioural features in future research. The clinical significance of the cardiac defense is underlined by its alterations in mental disorders, particularly anxiety disorders and post-traumatic stress. While exaggerated heart rate responses have repeatedly been reported in affected individuals (Lang et al., 2000; Lang & McTeague, 2009; Viedma, 2008), research on possible peculiarities in habituation and recovery remains scant (Schalinski et al., 2013). However, deficient adjustment of the defense response during repeated exposure to potentially threatening stimuli, in addition to generally increased sympathetic responsiveness, may characterize these conditions and may certainly be of interest in upcoming studies.

In summary, the present study confirmed the role of the sympathetic nervous system in the cardiac defense. In addition to mediating cardiac activation during the response, sympathetic mechanisms may also contribute to modulations of the strength of the response across repeated elicitations: while a reduction in sympathetic responsiveness

may underlie habituation of cardiac reactivity, increased sympathetic activation after longer resting intervals may lead to its recovery. Concerning the time course of the response, heart rate and sympathetic cardiac control exhibited nearly mirror-inverted patterns during the initial phase, indicating co-activation of both branches of the autonomic nervous system. The sympathetic system is likely to dominate heart rate regulation in the further course of the response. Contributions of vagal and baroreflex mechanisms to the defense response, and its modulations through habituation and recovery, could be addressed in future research, in addition to the role of cardiac autonomic control in behaviours associated with the particular components of the response.

Capítulo 4:

Autonomic Contributions to Attentional Modulation of the Cardiac Defense Response - Estudio 2

Abstract

The cardiac defense response (CDR) to aversive auditory stimulation is characterized by two acceleration/deceleration heart rate (HR) components. This study investigated attentional modulation of the CDR and the role of autonomic cardiac control therein. In 60 healthy subjects, the CDR was elicited, while HR, pre-ejection period (PEP), and systolic blood pressure (SBP) were recorded. Half of the subjects performed a visual search task following the noise stimulus; the other half was exposed to the stimulus without any subsequent task. The task led to potentiation of the second acceleration/deceleration and a trend towards a reduction of the first deceleration. Moreover, there was a greater SBP decline during the second component. Autonomic recordings suggested mediation of the first component by parasympathetic cardiac control; sympathetic and parasympathetic mechanisms contribute to the second component. The potentiation of the CDR due to attentional modulation may relate to increased parasympathetic withdrawal.

4.1. Introduction

The cardiac defense response (CDR) is characterized by a complex pattern of heart rate (HR) modulations that typically occurs after intense and unexpected auditory stimulation (Vila et al., 2007). It involves two acceleration/deceleration HR components arising within approximately 80 s. According to the psychophysiological literature, there are two classical approaches to understanding the CDR: the first is based on Ivan Pavlov's work about the orienting and defense reflexes (Pavlov, 1927), and emphasizes cognitive processes associated with the response (Graham, 1992; Lacey & Lacey, 1974; Sokolov, 1963). It posits that environmental stimuli trigger HR changes, which represent specific attentional and perceptual mechanisms aiming to facilitate or inhibit stimulus processing. The CDR is understood as an attentional mechanism contrary to orienting that entails sensory rejection. The second approach mainly refers to emotion and motivation (Obrist, 1981; Steptoe & Vögele, 1991); it builds on Walter Cannon's concept of the fight-flight response and Hans Selye's stress theory (Cannon, 1929; Selye, 1956). Here, HR responses to environmental stimuli are postulated to reflect metabolic adjustment that optimizes energy supply to the body, which is required for adaptive behaviours.

These traditional concepts were considered controversial until the development of an integrative model, referred to as the defense cascade (Lang et al., 1997). This approach considers defense as a set of responses that occur sequentially during two phases: a first phase in which attentional factors facilitate detection and processing of a potential threat; and a second phase in which protective actions occur. The reactions occurring during these two phases vary according to the type and intensity of the aversive event, previous experience thereof, and its spatial and temporal proximity (D. C. Blanchard & R. J. Blanchard, 1988; Bracha, 2004; Facchinetti et al., 2006; Fanselow, 1994; Gallup, 1977; J. A. Gray, 1988; Lang et al., 1997; Marks, 1987).

Vila et al. (2007) proposed an attentional-motivational model of the CDR in an attempt to relate the assumptions of the defense cascade model - namely the sequence of protective responses from freeze to flight, fight and faint - to specific cardiac response components. The model suggests that the components of the CDR are mediated by both branches of the autonomic nervous system, sympathetic and parasympathetic, and are associated with specific cognitive and motivational processes. According to this model, the first acceleration/deceleration component represents an attentional phase, including interruption of ongoing activity, increased attentional arousal and processing of the aversive stimulus; the second acceleration/deceleration component reflects a motivational phase, including active protective behaviours or recovery if no substantial danger is detected.

The attentional-motivational model partly builds on a series of studies (Fernández & Vila, 1989a; Pérez et al., 2000; Vila et al., 1997) in which the CDR was recorded during manipulations of attentional orientation, based on the intake-rejection hypothesis (Lacey & Lacey, 1974). According to this hypothesis, HR deceleration is associated with facilitation of sensory intake, while HR acceleration relates to a state of internal cognitive elaboration and the intention to reject environmental input. A positive relationship between the presence of the second accelerative component in the CDR pattern and a greater cardiac activity was found when participants performed an external attention task (Fernández & Vila, 1989a). Furthermore, potentiation of the second accelerative component of the CDR was observed when participants performed a task requiring external attention, but not during internal attentional orientation (Pérez et al., 2000; Vila et al., 1997). Ramírez et al. (2010) investigated attentional modulation of the CDR using Sternberg's visual search and memory search tasks to induce external and internal attention, respectively. In line with the described studies, the second CDR component was greater during the external attention task than during the internal attention task. Some of these studies also revealed a reduction of the first

deceleration component due to external attention (Ramírez et al., 2010; Vila et al., 1997).

Ramírez et al. (2010) claimed that the findings pertaining to attentional modulation of the CDR can also be explained by Posner's attentional model (Posner, 1994). According to this theory, three attentional networks can be distinguished: the alertness network, involved in maintaining an appropriate state of vigilance; the anterior attentional network, mediating executive control; and the posterior attentional network, relevant to the selection of information from sensory input. Activity of the alertness network was suggested to have an inhibitory relationship with the anterior attentional network and an excitatory relationship with the posterior attentional network. Therefore, the CDR, during which an unexpected noise increases alertness, may be potentiated by an external attention task (posterior attentional network) but not by an internal attention task (anterior attentional network).

Regarding autonomic mediation of the CDR, it must be recognized that the sympathetic and parasympathetic systems do not always exert reciprocal cardiac influences (Berntson et al., 1994; Gellhorn et al., 1941; Reyes del Paso et al., 2014); therefore, autonomic contributions to the CDR cannot be exclusively inferred from HR modulations. The theory of autonomic space of Berntson et al. (1991) proposed three different modes of action of the autonomic nervous system: (a) a coupled reciprocal mode (negative correlation between sympathetic and parasympathetic activity); (b) a coupled non-reciprocal mode (positive correlation between the activity of both branches; i.e., co-activation or co-inhibition); and (c) an uncoupled mode (independent activity of the two systems).

In a number of studies, sympathetic and parasympathetic mediation of the CDR was studied using indirect indices (e.g., stroke volume, pulse transit time or respiratory

sinus arrhythmia); the pharmacological effects of atropine and metoprolol have also been investigated (Fernández & Vila, 1989c; Reyes del Paso et al., 1993, 1994). Their findings suggested parasympathetic dominance during the first acceleration/deceleration component of the CDR, and sympathetic-parasympathetic interaction (with sympathetic dominance) during the second component. Recently, Árbol (2017) and Garrido et al. (2020) investigated sympathetic control during the elicitation of the CDR in a single trial, as well as across repeated elicitations; in the latter case, habituation and recovery of the response were quantified. In both studies, the pre-ejection period (PEP) was recorded as an index of sympathetically mediated myocardial contractility (Berntson et al., 2016). Both studies showed nearly opposite patterns of HR and sympathetic cardiac control during the first acceleration/deceleration component and largely parallel patterns during the second acceleration/deceleration component. This support the notion of co-activation of both branches, with parasympathetic dominance, during the first component of the CDR, and sympathetic dominance during the second component.

In the present study, we investigated autonomic control in connection with attentional modulation of the CDR. For this purpose, HR, PEP, and systolic blood pressure (SBP) were recorded during a test in which an experimental group completed Sternberg's visual search task immediately after the presentation of unexpected aversive noise; a control group was exposed to aversive noise without any subsequent task. Whereas PEP was expected to provide specific information on sympathetic cardiac control, SBP was expected to provide indirect information on both sympathetic and parasympathetic cardiac control. The following hypotheses were tested: (1) The typical time course pattern of the CDR will be modified by external attention, with a reduction of its first deceleration and a potentiation of its second acceleration/deceleration component; (2) The underlying mechanisms of this modulation will involve both sympathetic and parasympathetic systems.

4.2. Method

4.2.1. Participants

Participants were 60 university students (46 women and 14 men) aged between 17 and 26 years (*M* = 20.80, *SD* = 2.10). Individuals taking drugs affecting the central or autonomic nervous system, as well as those suffering from cardiovascular diseases or auditory or visual deficits, were excluded from the study. All participants provided written informed consent to take part in the study and received course credits for their participation. The Ethics Committee of the University of Granada approved the study (approval number 423/CEIH/2017).

4.2.2. Study design

All participants were presented with an acoustic stimulus twice, with an inter-trial interval (ITI) of 12.5 min; the stimulus had characteristics appropriate to elicit the CDR (Ramírez et al., 2005): white noise of 105 dB, 500 ms duration, and instantaneous rise time. A between-subjects design was used and the participants were randomly assigned to one of the two experimental conditions: visual search task (Condition 1) or no task (Condition 2) (23 women and 7 men per condition).

The experimental paradigm proceeded as follows: (a) initial 10 min rest period, (b) two trials of acoustic stimulation without prior warning, followed by the visual search task (or by no task depending on the condition) during an 80 s period, with the 12.5 s ITI, and (c) a final rest period of 55 s. Each trial included a 15 s pre-trial recording period, 500 ms acoustic stimulus presentation period, and 80 s post-trial recording period. Participants were instructed to keep their eyes open, look at a computer screen located approximately 50 cm from their eyes, and breathe normally during the test.

4.2.3. Visual search task

A variant of Sternberg's visual search task (Sternberg, 1969) was employed in Condition 1. The task was programmed using E-Prime 2.0 software (Psychology Software Tools, Inc., Sharpsburg, PA, USA) and presented on a Dell computer using a 48 cm (19 inch) Dell monitor. Visual stimuli consisted of 22 neutral pictures (Self-Assessment Manikin scales of *valence* [*M* = 5.41, *SD* = 0.59] and *arousal* [*M* = 3.73, *SD* = 0.40]) selected from the International Affective Picture System (IAPS; Lang et al., 2008) using the Spanish norms (Moltó et al., 1999, 2013; Vila et al., 2001). IAPS codes of each picture are provided in Footnote 1[1].

In this task, participants had to search for a variable target and determine whether or not it was present in an array of stimuli scattered randomly over the computer screen. Each trial comprised the following steps: (a) a fixation point was presented in the centre of the screen for 500 ms (simultaneous with the acoustic stimulus); (b) the target stimulus was shown at the same position for 2500 ms; (c) a mask (#) was presented at the same position for 500 ms; and finally, (d) an array of four stimuli scattered randomly across the screen appeared and remained until the participant executed a response (or until the maximum presentation duration of 1500 ms had elapsed). Participants were instructed to respond as quickly and accurately as possible by pressing (with their index finger of the right hand) a yellow-coloured key ('B') if the target was present in the array or a blue-coloured key ('N') if the target was not present in the array.

[1] Codes of the 22 IAPS neutral pictures used in the study: 2102, 2104, 2191, 2372, 2377, 2383, 2393, 2396, 2400, 2411, 2435, 2488, 2513, 2515, 2521, 2575, 2595, 2749, 2840, 2870, 5455, 7550.

4.2.4. Instruments and recordings

4.2.4.1. Acoustic stimulation

The white noise was generated by a Coulbourn V15-17 audio system and an IMQ Stage Line PPA-1 amplifier, and presented binaurally through AKG K-240 Monitor headphones (600 ohms). The intensity of the sound was calibrated using a sound level meter (model 2235; Brüel & Kjær Inc., Bremen, Germany) and an artificial ear (model 4153; Brüel & Kjær Inc.).

4.2.4.2. Psychophysiological recordings

4.2.4.2.1. Electrocardiography (ECG)

Beat-to-beat HR recordings were used to characterize the CDR, which was defined by the 80 s after the acoustic stimulus onset, converted to a weighted average every second, and transformed to a differential score with respect to a 15 s baseline prior to acoustic stimulus onset. The 80 HR values were reduced to 10 values corresponding to the median of 10 progressively longer intervals, following the criteria used in previous studies of the CDR (Mata et al., 2009; Vila et al., 2007): two intervals of three seconds, two intervals of five seconds, three intervals of seven seconds, and three intervals of 13 seconds. The use of these intervals simplifies the analysis and allows identification of the first (intervals 1-4) and second (intervals 5-10) acceleration/deceleration components of the CDR. The ECG recordings were accomplished by means of a Grass polygraph (PRS3, model 07E0229G; West Warwick, RI, USA) with a P511 AC amplifier, and wired to a Biopac system (MP 150; Biopac Systems Inc., Goleta, CA, USA) (sampling rate, 1000 Hz). Disposable Ag/AgCl electrodes filled with electrode paste were used in Einthoven's lead I configuration (right clavicle, left clavicle, ground electrode, left leg). AcqKnowledge 4.2. software (Biopac Systems Inc.) was used for ECG signal processing, R-wave peak detection and manual artefact correction. HR was defined as the average number of R-waves per minute.

4.2.4.2.2. Impedance cardiography (ICG)

Beat-to-beat ICG recordings of the PEP during the 80 s period after acoustic stimulus onset were used to estimate sympathetic cardiac control. In the same manner as for the HR, the 80 PEP values were converted to a weighted average every second, transformed to a differential score, and reduced to 10 values. ICG was recorded using a Biopac system (MP 150; Biopac Systems Inc.) and a NICO100C amplifier (sampling rate, 1000 Hz). Disposable Ag/AgCl strip electrodes filled with electrode paste were attached in the tetrapolar configuration described by Kubicek et al. (1966). The upper voltage electrode was placed around the base of the neck, and the lower voltage electrode around the thorax at the level of the xiphisternal junction. The two current electrodes were fixed at a distance 3 cm from each other. PEP was defined as the period (in ms) between the onset of ventricular depolarization (Q-wave onset in ECG) and the beginning of left ventricular ejection (B-point in the first derivative of the ICG signal) (Sherwood et al., 1990). Due to the difficulty of identifying Q-wave onset in the ECG record of some individuals, R-wave onset was chosen instead as the fiducial point to calculate PEP, as suggested by Bernston et al. (2004). AcqKnowledge 4.2 software was used for ICG signal processing, Q-wave peak detection, and B-point detection. The B-point was localized using the algorithm known as *third derivative classification*, which has been suggested to be superior to other popular algorithms (Árbol et al., 2017). Automatic B-point detection was corrected manually as necessary.

4.2.4.2.3. Blood pressure (BP)

SBP was calculated through beat-to-beat recordings of the BP during the 80 s period after acoustic stimulus onset. The 80 SBP values were converted to a weighted average every second, transformed to a differential score and reduced to 10 values, as was done for the HR. BP recordings were conducted using a CNAP Monitor 500 (model NIBP100D-1; CNSystems, Graz, Austria) wired to a Biopac system (MP 150; Biopac Systems Inc.) and a DA100C amplifier (sampling rate, 1000 Hz). Continuous

BP measurements were taken at the proximal joints of the index and middle fingers of the left hand, which was positioned at the level of the heart. SBP was defined as the maximum peak in mmHg of every systolic wave of the BP. AcqKnowledge 4.2. software (Biopac Systems Inc.) was employed for systolic wave detection and manual artefact correction.

4.2.4.3. Behavioural measures

Reaction time in ms, as well as the number of correct, incorrect, and missed responses, were recorded for the visual search task (Condition 1).

4.2.4.4. Subjective measures

All participants completed a post-experimental rating scale evaluating the subjectively perceived intensity and unpleasantness of the two acoustic stimuli. The scale ranged from 0 (not at all intense/unpleasant) to 100 (extremely intense/unpleasant). Participants from Condition 1 also completed a post-experimental rating scale assessing the subjectively perceived attentional demands required for the visual search task. The scale ranged from 0 (attention is not required at all) to 100 (a high level of attention is required). In addition, neutral pictures used for the visual search task in Condition 1 were rated using the Self-Assessment Manikin *valence* and *arousal* scales (Lang et al., 2008).

4.2.5. Procedure

Each participant attended a single laboratory session that lasted approximately 60 min. Upon arrival, participants were invited to sit in an armchair and received information about the study. They signed an informed consent form and completed a brief interview to assess their suitability for the study according to the inclusion and exclusion criteria. Participants assigned to Condition 1 were told that they would have to perform a task immediately after the presentation of the acoustic stimulus. Participants assigned to

Condition 2 were asked to look at a fixation point located on the computer screen, and to ignore the acoustic stimulus that they were going to hear during the test. The aversive nature of the acoustic stimuli was not mentioned, in accordance with the standard instructions for the cardiac defense procedure (see Vila et al., 2007). The electrodes and headphones were fitted and participants were left alone in a dimly lit room during the test. The post-experimental questionnaires were then completed.

4.2.6. Statistical analysis

HR, PEP, and SBP were analysed separately by means of three 2(x2x10) analyses of variance (ANOVAs) with the between-group factor of Condition (Condition 1 vs. Condition 2), and the two within-subject factors of Trial (Trial 1 vs. 2) and Time (the 10 time-intervals after the onset of the acoustic stimulus). Regarding subjective measures, intensity and unpleasantness of the acoustic stimuli were analysed separately by means of two 2(x2) ANOVAs with the between-group factor of Condition and the within-subject factor of Trial. The Greenhouse-Geisser epsilon correction was applied to the within-subject factors. Results are provided as uncorrected df and corrected *p* values; partial eta squared (ηp^2) is provided as a measure of effect size. Alpha was set at .05 for the ANOVAs.

4.3. Results

4.3.1. Cardiac defense response

Figure 9 depicts the time course of HR during the experimental task. In all trials and conditions, a first acceleration/deceleration component arose during intervals 1-4 after acoustic stimulus presentation. It was followed by a second acceleration/deceleration component during intervals 5-10, which was more pronounced for Trial 1 than Trial 2 in both conditions. The HR increase during the second acceleration/acceleration component was greater in Condition 1 (visual search task) than Condition 2 (no task)

for both trials. Furthermore, for Trial 1 the first deceleration was greater in Condition 2 than in Condition 1.

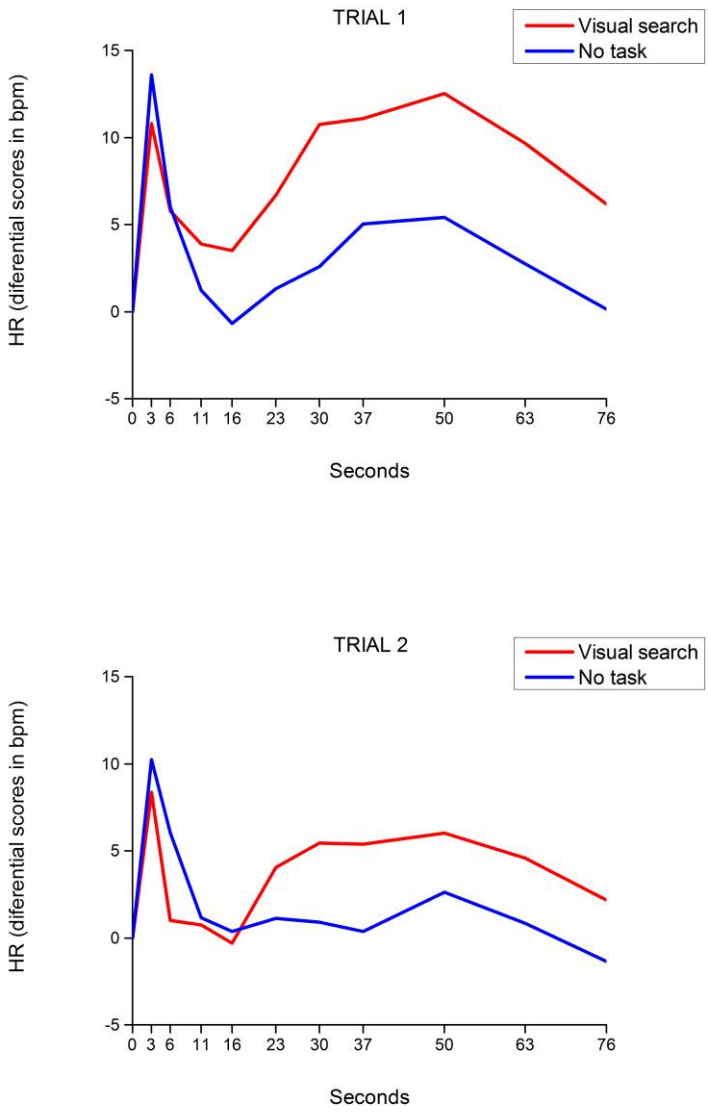


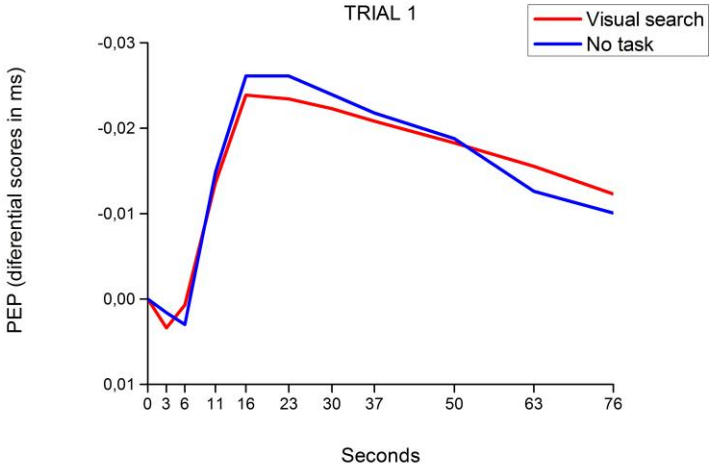
Figure 9. Course of the cardiac defense response: heart rate across the 10 time-intervals (expressed as differential scores) as a function of trial and condition.

The ANOVA for HR yielded main effects of Trial (F[1,58] = 8.35, p = .005, ηp^2 = .126) and Time (F[9,522] = 12.42, p < .001, ηp^2 = .176), in addition to a Condition x Time interaction (F[9,522] = 4.02, p = .007, ηp^2 = .065). Follow-up analysis of this interaction

was accomplished using separate ANOVAs for the first (intervals 1-4) and second (intervals 5-10) acceleration/deceleration component, to identify components showing differences between the two conditions. A significant Condition effect arose for the second component (F[1,58] = 5.71, p = .020, ηp^2 = .090), in which participants from Condition 1 displayed a greater HR response. No significant Condition effect was seen for the first component (F[1,58] = 0.06, p = .813, ηp^2 = .001). However, there was a trend towards a smaller response during the first component in Condition 1 than in Condition 2, but only for Trial 1 (Condition x Trial x Time interaction: F[3,174] = 2.51, p = .076, ηp^2 = .042).

4.3.2. Sympathetic cardiac control

Figure 10 shows the time course of PEP (inverted values are presented; as such, a reduction in PEP reflects an increase in sympathetic cardiac control, and vice versa). In all trials and conditions, sympathetic cardiac control decreased during the first two intervals. A steep increase subsequently occurred, reaching its peak around interval 4, followed by a progressive decline until the last interval. Although the response pattern was similar in both trials and conditions, the response was greater during Trial 1.



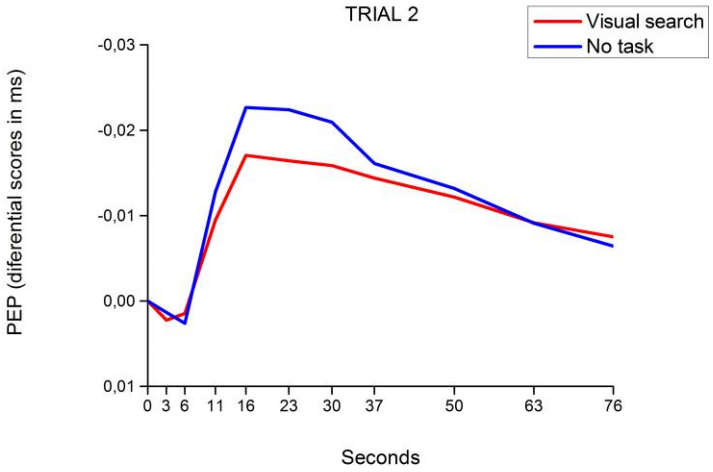


Figure 10. Course of sympathetic cardiac control: pre-ejection period across the 10 time-intervals (expressed in differential scores) as a function of trial and condition.

The ANOVA for PEP revealed main effects of Trial (F[1,58] = 15.52, p < .001, ηp^2 = .211) and Time (F[9,522] = 86.14, p < .001, ηp^2 = .598), and a Trial x Time interaction (F[9,522] = 5.80, p < .001, ηp^2 = .091). To further analyse the interaction, two separate ANOVAs were computed for each trial, with the between-group factor of Condition and the within-subject factor of Time. Only the Time effect reached significance for both

trials (Trial 1 F[9,522] = 80,74, $p < .001$, $\eta p^2 = .582$, Trial 2 F[9,522] = 58.06, $p < .001$, $\eta p^2 = .500$).

4.3.3. Systolic blood pressure

Figure 11 illustrates the time course of SBP. In all trials and conditions, SBP increased and peaked around interval 2. The subsequent time course varied between the two conditions. In Condition 2, SBP decreased until interval 3 and remained virtually stable during the remaining response in both trials. In Condition 1, the SBP decrease was far greater than in Condition 2; this difference was larger for Trial 1 than for Trial 2.

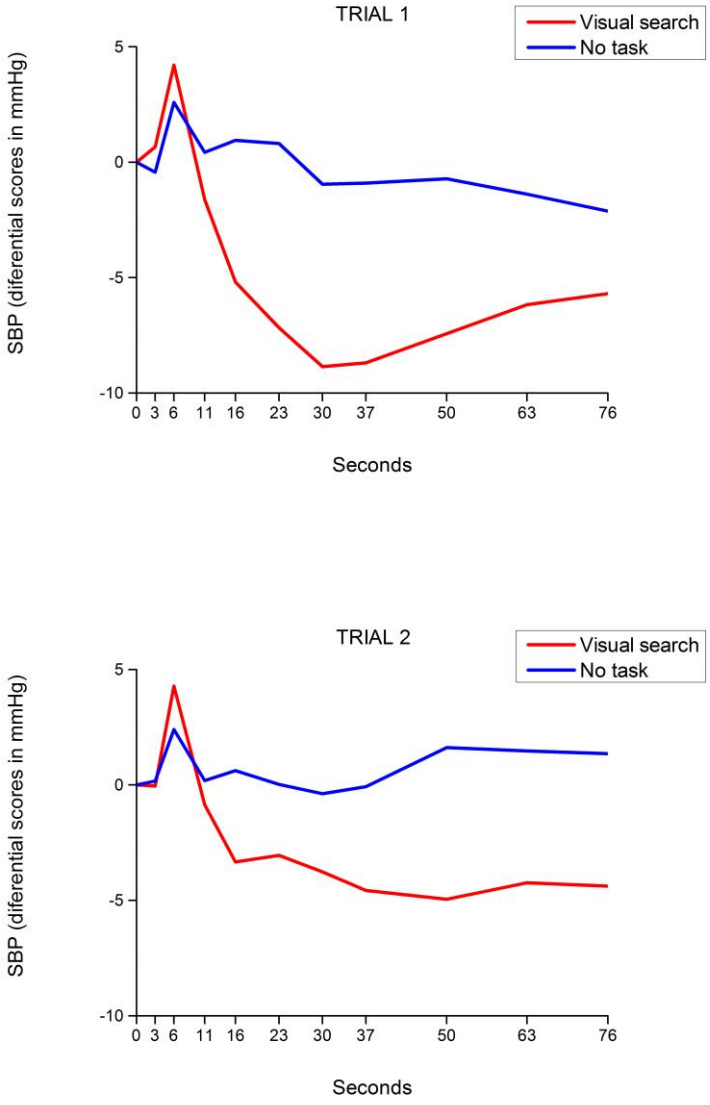


Figure 11. Course of systolic blood pressure: systolic blood pressure across the 10 time-intervals (expressed in differential scores) as a function of trial and condition.

The ANOVA for SBP yielded a main effect of Time (F[9,522] = 13.42, p < .001, ηp^2 = .188), and Condition x Time (F[9,522] = 6.44, p < .001, ηp^2 = .100) and Condition x Trial x Time interaction effects (F[9,522] = 2.61, p = .048, ηp^2 = .043). Follow-up analysis of the three-way interaction was accomplished using ANOVAs for each trial with the between-group factor of Condition and within-subject factor of Time. For both trials, the models revealed effects of Time (Trial 1 F[9,522] = 13.15, p < .001, ηp^2 =

.185, Trial 2 F[9,522] = 6.29, p < .001, ηp^2 = .098) and Condition x Time interactions (Trial 1 F[9,522] = 6.13, p < .001, ηp^2 = .096, Trial 2 F[9,522] = 4.25, p = .005, ηp^2 = .068). In addition, separate ANOVAs were conducted for each interval to identify the intervals for each trial with differences between the two conditions. Condition effects were seen in intervals 4-8 (all ps ≤ .001) for Trial 1; and in intervals 7-10 (all ps ≤ .003) for Trial 2.

4.3.4. Task performance

In the visual search task (Condition 1), the mean reaction time was 756.09 ms (*SD* = 206.34 ms), with 89.27 % correct responses, 1.77 % incorrect responses, and 8.96 % missed responses.

4.3.5. Subjective measures

Mean ratings on the visual search task were 71.83 (*SD* = 21.56) for attentional demand, 5.68 (*SD* = 1.73) for picture valence, and 3.82 (*SD* = 1.94) for picture arousal. Table 2 shows, for all participants, the mean intensity and unpleasantness ratings for the acoustic stimuli as a function of Condition and Trial. Participants in both conditions rated the noise of Trial 1 as more intense and unpleasant than that of Trial 2. The ANOVAs revealed a main effect of Trial for intensity (F[1,58] = 8.33, p = .005, ηp^2 = .126) and unpleasantness (F[1,58] = 19.02, p < .001, ηp^2 = 247).

Table 2. Mean (*SD* in parentheses) noise intensity and unpleasantness ratings as a function of condition and trial.

	Condition 1 (*N* = 30)		Condition 2 (*N* = 30)	
	Trial 1	Trial 2	Trial 1	Trial 2
Noise intensity	70.17 (12.21)	66.67 (17.09)	74.67 (17.61)	67.17 (19.81)
Noise unpleasantness	70.17 (15.17)	62.67 (19.06)	78.83 (18.46)	67.83 (20.50)

4.4. Discussion

The aim of the study was to examine attentional modulation of the CDR and the role of autonomic cardiac control therein. In half of the participants, the presentation of unexpected aversive noise was followed by an external attentional task, while ECG, ICG, and BP recordings were obtained. The typical pattern of the CDR, consisting of two acceleration/deceleration components, was observed in all trials and conditions. The participants presented with the task (Condition 1), as compared to the control group (Condition 2), exhibited potentiation of the second acceleration/deceleration component of the CDR, which was larger in Trial 1 than in Trial 2. A trend towards a reduction of the first deceleration component in Condition 1 arose in the first trial. No differences in sympathetic cardiac control, indexed by PEP, were seen between groups (Condition 1 vs. Condition 2). However, for both trials, a smaller SBP response during the second acceleration/deceleration component, arose in participants performing the task (Condition 1).

In both conditions, HR modulations were smaller during the second trial than during the first trial. This reflects rapid habituation of the CDR and replicates the findings of a

number of previous studies (e.g., Garrido et al., 2020; Mata et al., 2009; Ramírez et al., 2005; Vila et al., 1997). In those studies, as in the current one, habituation was largely restricted to the second acceleration/deceleration component of the CDR. Also, we observed a markedly smaller sympathetic cardiac response in the second trial. This is in accordance with a recent study (Garrido et al., 2020) and supports the notion of sympathetic contributions to habituation of the CDR. The SBP decrease observed during the period corresponding to the second acceleration/deceleration component of the CDR was smaller in the second than in the first trial, suggesting habituation of the BP response. Moreover, on a behavioural level, subjective ratings of the intensity and unpleasantness of the noise were lower overall for the second trial than for the first trial.

The observed modulations of HR and sympathetic cardiac control may be explained within the framework of autonomic space (Berntson et al., 1991). As mentioned above, this theory posits three modes of interaction between the two branches of the autonomic nervous system: a coupled reciprocal mode, a coupled non-reciprocal mode, and an uncoupled mode. During the first acceleration/deceleration component of the CDR, in both conditions, HR and sympathetic cardiac control showed almost opposite courses. Therefore, the initial HR increase cannot be explained by sympathetic activation; instead, it may be due to stronger parasympathetic inhibition (co-inhibition). In the same way, the subsequent HR deceleration may be explained by parasympathetic activation, which dominates over simultaneously occurring sympathetic activation (co-activation). This indicates that both autonomic branches act in a coupled non-reciprocal mode during the first acceleration/deceleration component, where the cardiac influence of the parasympathetic system exceeds that of the sympathetic system.

During the second acceleration/deceleration component of the CDR, in both conditions, HR and sympathetic cardiac control showed relatively similar courses. However, when the second HR acceleration started, sympathetic cardiac control was already maximal and progressively decreased thereafter. Therefore, it is likely that the second acceleration was also due to parasympathetic inhibition, which was more pronounced in Condition 1. This points toward a coupled reciprocal mode with parasympathetic dominance during the second acceleration component of the CDR. During the second HR deceleration, sympathetic cardiac control also decreased; therefore, this component may be explained by sympathetic inhibition and/or parasympathetic activation (coupled reciprocal mode or uncoupled mode). Our findings corroborate previous research suggesting mediation of the first acceleration/deceleration component of the CDR by the parasympathetic system (Árbol, 2017; Fernández & Vila, 1989c; Garrido et al., 2020; Reyes del Paso et al., 1993, 1994). However, they indicate that parasympathetic influences also contribute to the second CDR component, at least to HR acceleration. This may also be relevant to attentional modulation of the CDR. The present findings support the notion that parasympathetic cardiac control is involved in the reduction of the first HR deceleration and potentiation of the second CDR component during external attention.

Changes in BP are mediated by the sympathetic and parasympathetic nervous systems (Berntson et al., 2016). As SBP and sympathetic cardiac control showed almost opposite courses during the first acceleration/deceleration component of the CDR, the initial BP response cannot be explained by sympathetic influences. Instead, early SBP modulations may result from parasympathetically mediated HR acceleration and deceleration. The subsequent course of the SBP response differed between the two experimental conditions in both trials. When participants performed the visual search task (Condition 1), SBP was lower than in the control condition (Condition 2) during the period corresponding to the second acceleration/deceleration component of

the CDR. For the first trial, the difference between conditions was even greater than for the second trial.

It is important to note that execution of the visual search task was associated with a greater HR increase during the second component, but with greater SBP decline. This divergence is difficult to explain. The role of the parasympathetic system in cardiovascular regulation is restricted to chronotropic cardiac influences; therefore, it may modulate BP only through changes in HR. Considering this, and taking the opposite directions of the condition effects for HR and SBP into account, a parasympathetic origin of the greater SBP decreases in the first condition can be ruled out. Sympathetic cardiac control was not altered by the experimental manipulation; as such, there is also no evidence of sympathetic mediation of the condition effect on SBP. However, it should be noted that sympathetic control was only quantified using PEP. While PEP is an index of beta-adrenergic inotropic myocardial influences, BP also plays a role in alpha-adrenergic control of vasomotor tone (Berntson et al., 2016). As such, it may be that the stronger SBP decline associated with the visual search task resulted from stronger sympathetically mediated vasodilation. This hypothesis could be tested in future studies through assessment of peripheral resistance, for example.

Our study constitutes the first attempt to determine the autonomic mechanisms involved in attentional modulation of the CDR. Nevertheless, it must be acknowledged that parasympathetic activity was only inferred from HR. Moreover, PEP constitutes an ionotropic parameter that provides information about beta-adrenergic influences on the myocardium (Berntson et al., 2016; Cacioppo et al., 1994; Hassan & Turner, 1983). No chronotropic parameters are available to estimate sympathetic control of the sinus node, and a degree of dissociation between HR and sympathetically mediated myocardial contractility is plausible. Another limitation pertains to the lack of an internal

attentional orientation task condition, which would provide greater insight into attentional modulation of the CDR.

Taken together, the present findings support the attentional-motivational model of the CDR, which posits that the CDR, and its behavioural effects, are mediated by both branches of the autonomic nervous system (Vila et al., 2007). The relevance of the CDR to cognition is reflected in its modulation through manipulation of attentional requirements. External attention was associated with a reduction of the first deceleration, and potentiation of the second acceleration/deceleration component of the CDR. In earlier research on modulation of the CDR by external attention, these effects were explained by the congruence between the attentional demands required by the visual search task and the attentional demands required for detecting and processing the aversive stimulus (both external) (Vila et al., 2007). That is, shared attentional demands would reduce the time needed for detecting and processing the aversive stimulus, thereby shortening the first acceleration/deceleration component of the CDR; in turn, this would bring forward and increase the second acceleration/deceleration component. Furthermore, potentiation of the CDR provoked by the visual search task suggests involvement of attentional mechanisms of sensory intake in the response, which would be contrary to the assumptions of the intake-rejection hypothesis and the traditional cognitive approach to the CDR (Graham, 1992; Lacey & Lacey, 1974; Sokolov, 1963). Nevertheless, these results are in line with Posner's attentional model (Posner, 1994), where increased alertness due to the presentation of an unexpected noise (alertness network) would be potentiated by an external attention task (posterior attentional network).

In conclusion, this study confirmed modulation of the CDR through external attention and furthered our understanding of the autonomic mechanisms involved therein. Attentional effects were seen for both components of the CDR. Modulation of the first

component, which relates to attentional resource allocation and the detection and processing of possible threats, seems to be mediated by parasympathetic cardiac influences. Moreover, the parasympathetic system may also contribute to the effects of attention on the second component, which is associated with actual defensive behaviours. Sympathetic cardiac control may play a subordinal role in attentional modulation of the CDR. Attentional modulation of the BP response to aversive stimulation was observed for the first time in this study. While parasympathetic mechanisms may account for the initial SBP increase, the autonomic mediation of the subsequent SBP decrease, which was sensitive to attentional orientation, remains to be clarified.

Capítulo 5:

Autonomic Contributions to Attentional and Emotional Modulation of the Cardiac Defense Response - Estudio 3

Abstract

This study investigated autonomic mediation during attentional and emotional modulation of the cardiac defense response (CDR), a complex pattern of heart rate (HR) modulations comprising two accelerative/decelerative components. Forty-eight healthy women were presented twice with noise stimuli for eliciting the CDR in the context of a visual search task using pleasant, neutral or unpleasant pictures as visual stimuli. HR, pre-ejection period (PEP) and systolic blood pressure (SBP) were recorded. The pictures with emotional content provoked a potentiation of the second acceleration component of the CDR, which was stronger for the pleasant pictures than for the unpleasant ones. SBP seemed to be not affected by the emotional modulation. Autonomic recordings suggested parasympathetic mediation during the first component as well as sympathetic and parasympathetic mediation during the second component of the CDR. The potentiation of the CDR due to emotional modulation of the CDR seems to be mediated parasympathetically.

5.1. Introduction

Defense reaction refers to an organism's psychophysiological mechanisms that are activated when encountering a dangerous or threatening situation. Rather than constituting a single response, defense comprises a cascade of psychophysiological responses which occur sequentially in two phases, as postulated by the cascade defense model (Lang et al., 1997): an initial phase with predominance of attentional factors aimed at detecting and processing a possible threat; and a subsequent phase with motivational factors aimed at active defense. These psychophysiological responses change at different rates depending on the type and severity of the aversive event, previous experience with the event type, and its spatial and temporal proximity (D.C. Blanchard & R. J. Blanchard, 1988; Bracha, 2004; Facchinetti et al., 2006; Fanselow, 1994; Gallup, 1977; J. A. Gray, 1988; Lang et al., 1997; Marks, 1987).

In addition to offering a description of the defense responding process from a naturalistic perspective, this model facilitates the integration of the opposing ideas from the two classical approaches to the cardiac components of the defense. From the cognitive perspective, based on Ivan Pavlov's work (Pavlov, 1927) about the orienting and defense reflexes, defense response is understood as an attentional mechanism that entails sensory rejection, contrary to orientation (Graham, 1992; Lacey & Lacey, 1974; Sokolov, 1963). From the motivational perspective, based on Walter Cannon's concept of the fight-flight response and Hans Selye's stress theory (Cannon, 1929; Selye, 1956), this response is understood as a response mobilization mechanism which is contrary to relaxation (Obrist, 1981; Steptoe & Vögele, 1991).

The attentional-motivational model was proposed by Vila et al. (2007) meaning to connect the assumptions from the defense cascade model with the cardiac defense response (CDR), which is characterized by a complex pattern of heart rate (HR) modulations to an intense and unexpected auditory stimulation. The response pattern

shows two acceleration/deceleration components in alternating order for approximately 80 s. This model suggests these components have a cognitive and motivational significance and are mediated by both sympathetic and parasympathetic branches of the autonomic nervous system. The CDR represents the succession of two phases: the first acceleration/deceleration component is linked to an attentional phase, including interruption of ongoing activity and heightened attention to external cues; the second acceleration/deceleration component is linked to a motivational phase, encompassing active defensive behaviours or recovery if no substantial danger occurs.

The attentional-motivational model is built on the results from a considerable number of studies which used a paradigm involving the presentation of intense and unexpected auditory stimulation for eliciting the CDR under different task conditions. Cognitive significance of the CDR was investigated in several studies (Fernández & Vila, 1989a; Pérez et al., 2000; Vila et al., 1997) through recording of the CDR during manipulations of attentional orientation. These studies are based on the intake-rejection hypothesis (Lacey & Lacey, 1974), which posits HR deceleration is related with facilitation of sensory intake and HR acceleration is associated to a state of internal cognitive elaboration and the intention to reject environmental input. According to this hypothesis, the CDR should be accompanied by a decrease in sensory processing in order to reject the aversive acoustic stimulus. Fernández & Vila (1989a) found a positive relationship between the presence of the second accelerative component in the CDR pattern and a greater cardiac activity during external attentional orientation. Moreover, potentiation of the second acceleration component of the CDR was observed when participants performed a task requiring external attention, but not when performing one requiring internal attention (Pérez et al., 2000; Vila et al., 1997). Later, Ramírez et al. (2010) accomplished another study for examining attentional modulation on the CDR using Sternberg's visual search task and Sternberg's memory search task as external and internal attention tasks, respectively, and found that the second

component was greater during the external attention task. Some of these studies also revealed a reduction of the first deceleration component on account of external attention (Ramírez et al., 2010; Vila et al., 1997).

Findings concerning attentional modulation of the CDR can also be explained by Posner´s attentional model (Posner, 1994), as suggested by Ramírez et al. (2010). This theory suggests the existence of three attentional networks: the alertness network, involved in maintaining an appropriate vigilance state; the attentional network, relevant to executive control; and the posterior attentional network, involved in selection of information from sensory input. The alertness network has an inhibitory relationship with the anterior attentional network and an excitatory relationship with the posterior attentional network. Hence, the CDR, elicited by an unexpected noise and represented by the alertness network, may be potentiated by an external attention task (posterior attentional network) but not by an internal attention task (anterior attentional network).

Motivational significance of the CDR has been studied in a series of studies (Ruiz-Padial et al., 2005; Sánchez et al., 2002, 2009) in which the CDR was recorded during emotional modulation using Lang's startle probe paradigm (Lang, 1995). The results of these studies showed a potentiation of the CDR when participants were viewing phobic or unpleasant pictures, in comparison with pleasant and neutral ones. Moreover, the typical biphasic CDR pattern underwent a profound modification, in which the first deceleration disappeared and the two acceleration components merged into a single larger and more prolonged acceleration. More recently, Ramírez et al. (2010) investigated concurrent cognitive and motivational significance of the CDR using Sternberg's visual search task to induce external attention, as well as adding pictures with different emotional valence to the task. The experimental manipulation resulted in a greater second acceleration component when participants were viewing unpleasant pictures than when viewing pleasant and neutral ones. However, the CDR pattern did

not change so drastically as in previous studies, which was thought to be due to methodological differences between the startle probe paradigm and the visual search task. Altogether, these results suggest a potentiation of the CDR by viewing unpleasant pictures, providing partial support to the motivational *priming* hypothesis (Lang, 1995), which posits a potentiation and inhibition of the defensive reflexes when the defensive and appetitive motivational systems are activated, respectively.

Another significant part of the studies contributing to the development of the attentional-motivational model have examined autonomic mediation of the CDR. However, it has to be taken into consideration that the two branches of the autonomic nervous system, sympathetic and parasympathetic, do not always act over the heart reciprocally (Berntson et al., 1994; Gellhorn et al., 1941; Reyes del Paso et al., 2014). Consequently, the theory of the autonomic space postulated by Berntson et al. (1991) suggests three different modes of action of the autonomic nervous system: (a) a coupled reciprocal mode (negative correlation between the activity of both branches); (b) a coupled non-reciprocal mode (positive correlation between the activity of both branches, i.e., co-activation or co-inhibition); and (c) an uncoupled mode (both branches act independently from each other). Therefore, a sufficient understanding of autonomic contributions to the CDR requires the use of additional indices together with HR.

In this way, research on autonomic mediation of the CDR (Fernández & Vila, 1989c; Reyes del Paso et al., 1993, 1994) has included indirect indices (e.g., stroke volume, pulse transit time or respiratory sinus arrhythmia) as well as pharmacological methods (atropine and metoprolol). Overall, results suggested parasympathetic dominance during the first acceleration/deceleration component, and sympathetic-parasympathetic interaction (with sympathetic dominance) during the second acceleration/deceleration component of the CDR. Later studies (Árbol, 2017; Garrido et al., 2020) used pre-

ejection period (PEP) as an index of sympathetically mediated myocardial contractility (Berntson et al., 2016). Results from both of them point towards a co-activation of both branches, which parasympathetic dominance, during the first acceleration/deceleration component, and sympathetic dominance during the second acceleration/deceleration component of the CDR.

The aim of the present study was to investigate autonomic contributions to attentional and emotional modulation of the CDR. HR, PEP, and systolic blood pressure (SBP) were recorded during a test in which the presentation of unexpected aversive noise was followed by the immediate realization of Sternberg's visual search task, using pictures with different emotional valence as visual stimuli for each one of the three experimental groups (pleasant, neutral or unpleasant). PEP was expected to provide specific information on sympathetic cardiac control; and SBP was expected to provide indirect information on both sympathetic and parasympathetic cardiac control. The following hypotheses were tested: (1) The time course pattern of the CDR will be modified by emotional modulation, with a larger potentiation of the second acceleration component of the CDR in the group viewing unpleasant pictures; (2) The underlying mechanisms of attentional and emotional modulation will involve both sympathetic and parasympathetic systems.

5.2. Method

5.2.1. Participants

Participants were 48 university students (all women) aged between 17 and 26 years (*M* = 20.64; *SD* = 1.94). Exclusion criteria comprised taking drugs affecting the central or autonomic nervous system as well as suffering from cardiovascular diseases or auditory or visual deficits. All participants provided written informed consent to the study protocol and received course credits for their participation. The Ethics Committee of the University of Granada approved the study (approval number 423/CEIH/2017).

5.2.2. Study design

All participants were presented with an acoustic stimulus twice, with an inter-trial interval (ITI) of 12.5 min; the stimulus had characteristics appropriate to elicit the CDR (Ramírez et al., 2005): white noise of 105 dB, 500 ms duration, and instantaneous rise time. A between-subjects design was used and the participants were randomly assigned to one of the three experimental conditions: visual search task with pleasant pictures (Condition 1), visual search task with neutral pictures (Condition 2), and visual search task with unpleasant pictures (Condition 3).

The experimental paradigm had the following sequence: (a) initial 10 min rest period, (b) two trials of acoustic stimulation without prior warning, followed by the visual search task (with the corresponding set of pictures depending on the condition) during an 80 s period, with the 12.5 s ITI, and (c) a final rest period of 55 s. Each trial included a 15 s pre-trial recording period, 500 ms acoustic stimulus presentation period, and 80 s post-trial recording period. Participants were instructed to keep their eyes open, look at a computer screen located approximately 50 cm from their eyes, and breathe normally during the test.

5.2.3. Visual search task

A variant of Sternberg's visual search task (Sternberg, 1969) was employed. The task was programmed using E-Prime 2.0 software (Psychology Software Tools, Inc., Sharpsburg, PA, USA) and presented on a Dell computer using a 48 cm (19 inch) Dell monitor. Visual stimuli consisted of 22 pleasant pictures (Self-Assessment Manikin scales of *valence*: M = 6.98, SD = 1.57; and *arousal*: M = 6.38, SD = 2.10), 22 neutral pictures (*valence*: M = 5.43, SD = 1.43; *arousal*: M = 3.80, SD = 1.91), and 22 unpleasant pictures (*valence*: M = 1.64, SD = 1.02; *arousal*: M = 6.93, SD = 2.11) selected from the International Affective Picture System (IAPS; Lang et al., 2008) using the Spanish norms (Moltó et al., 1999, 2013; Vila et al., 2001). The three categories of

pictures differed significantly in *valence*, whereas significant differences in *arousal* just occurred for neutral pictures. IAPS codes of each picture are provided in Footnote 2[2].

The visual search task consisted of searching for a variable target and detecting if it was present of not in an array of stimuli randomly located over the computer screen. Each trial comprised the following steps: (a) a fixation point was presented in the centre of the screen for 500 ms (simultaneous with the acoustic stimulus), (b) the target stimulus was shown at the same position for 2500 ms, and (c) a mask (#) was presented at the same position for 500 ms; and finally, (d) an array of four stimuli scattered randomly across the screen appeared and remained until the participant executed a response (or until the maximum presentation duration of 1500 ms had elapsed). Participants were instructed to respond as quickly and accurately as possible by pressing (with their index finger of the right hand) a yellow-coloured key ('B') if the target was present in the array or a blue-coloured key ('N') if the target was not present in the array.

5.2.4. Instruments and recordings

5.2.4.1. Acoustic stimulation

The white noise was generated by a Coulbourn V15-17 audio system and an IMQ Stage Line PPA-1 amplifier, and presented binaurally through AKG K-240 Monitor headphones (600 ohms). The intensity of the sound was calibrated using a sound level

[2] Codes of the 66 IAPS pictures used in the study. Pleasant pictures: 4311, 4604, 4611, 4631, 4643, 4650, 4653, 4658, 4659, 4660, 4668, 4669, 4680, 4687, 4690, 4692, 4693, 4694, 4695, 4697, 4698, 4800. Neutral pictures: 2102, 2104, 2191, 2372, 2377, 2383, 2393, 2396, 2400, 2411, 2435, 2488, 2513, 2515, 2521, 2575, 2595, 2749, 2840, 2870, 5455, 7550. Unpleasant pictures: 2691, 3051, 3053, 3059, 3064, 3100, 3181, 3185, 3225, 6520, 6825, 9163, 9250, 9254, 9265, 9412, 9415, 9420, 9427, 9433, 9435, 9491.

meter (model 2235; Brüel & Kjær Inc., Bremen, Germany) and an artificial ear (model 4153; Brüel & Kjær Inc.).

5.2.4.2. Psychophysiological recordings

5.2.4.2.1. Electrocardiography (ECG)

Beat-to-beat HR recordings were used to describe the CDR, which was defined by the 80 s after the acoustic stimulus onset, converted to a weighted average every second, and transformed to a differential score with respect to a 15 s baseline prior to acoustic stimulus onset. The 80 HR values were reduced to 10 values corresponding to the median of 10 progressively longer intervals, following the criteria used in previous studies of the CDR (Mata et al., 2009; Vila et al., 2007): two intervals of three seconds, two intervals of five seconds, three intervals of seven seconds, and three intervals of 13 seconds. The use of these intervals simplifies the analysis and allows identification of the first (intervals 1-4) and second (intervals 5-10) acceleration/deceleration components of the CDR. The ECG recordings were accomplished by means of a Grass polygraph (PRS3, model 07E0229G; West Warwick, RI, USA) with a P511 AC amplifier, and wired to a Biopac system (MP 150, Biopac Systems Inc., Goleta, CA, USA) (sampling rate, 1000 Hz). Disposable Ag/AgCl electrodes filled with electrode paste were used in Einthoven's lead I configuration (right clavicle, left clavicle, ground electrode, left leg). AcqKnowledge 4.2. software (Biopac Systems Inc.) was used for ECG signal processing, R-wave peak detection and manual artefact correction. HR was defined as the average number of R-waves per minute.

5.2.4.2.2. Impedance cardiography (ICG)

Beat-to-beat ICG recordings of the PEP during the 80 s after acoustic stimulus onset were used to estimate sympathetic cardiac control. In the same manner as for the HR, the 80 PEP values were converted to a weighted average every second, transformed to a differential score, and reduced to 10 values. ICG was recorded using a Biopac

system (MP 150, Biopac Systems Inc.) and a NICO100C amplifier (sampling rate, 1000 Hz). Disposable Ag/AgCl strip electrodes filled with electrode paste were attached in the tetrapolar configuration described by Kubicek et al. (1966). The upper voltage electrode was placed around the base of the neck, and the lower voltage electrode around the thorax at the level of the xiphisternal junction. The two current electrodes were fixed at a distance 3 cm from each other. PEP was defined as the period (in ms) between the onset of ventricular depolarization (Q-wave onset in ECG) and the beginning of left ventricular ejection (B-point in the first derivative of the ICG signal) (Sherwood et al., 1990). Due to the difficulty of identifying Q-wave onset in the ECG record of some individuals, R-wave onset was chosen instead as the fiducial point to calculate PEP, as suggested by Bernston et al. (2004). AcqKnowledge 4.2 software was used for ICG signal processing, Q-wave peak detection, and B-point detection. The B-point was localized using the algorithm known as *third derivative classification*, which has been suggested to be superior to other popular algorithms (Árbol et al., 2017). Automatic B-point detection was corrected manually as necessary.

5.2.4.2.3. Blood pressure (BP)

SBP was calculated through beat-to-beat recordings of the BP during the 80 s period after acoustic stimulus onset. The 80 SBP values were converted to a weighted average every second, transformed to a differential score and reduced to 10 values, as was done for the HR. BP recordings were conducted using a CNAP Monitor 500 (model NIBP100D-1; CNSystems, Graz, Austria) wired to a Biopac system (MP 150, Biopac Systems Inc.) and a DA100C amplifier (sampling rate, 1000 Hz). Continuous BP measurements were taken at the proximal joints of the index and middle fingers of the left hand, which was positioned at the level of the heart. SBP was defined as the maximum peak in mmHg of every systolic wave of the BP. AcqKnowledge 4.2. software (Biopac Systems Inc.) was employed for systolic wave detection and manual artefact correction.

5.2.4.3. Behavioural measures

Reaction time in ms, as well as the number of correct, incorrect, and missed responses, were recorded.

5.2.4.4. Subjective measures

The subjectively perceived attentional demands required for the visual search task were evaluated using a post-experimental rating scale which ranged from 0 (attention is not required at all) to 100 (a high level of attention is required). Emotional pictures used for the visual search task were rated using the Self-Assessment Manikin *valence* and *arousal* scales (Lang et al., 2008). Moreover, the subjectively perceived intensity and unpleasantness of the two acoustic stimuli was assessed using a post-experimental rating scale which ranged from 0 (not at all intense/unpleasant) to 100 (extremely intense/unpleasant).

5.2.5. Procedure

Each participant attended a single laboratory session that lasted approximately 60 min. Upon arrival, participants were invited to sit in an armchair and received information about the study. They signed an informed consent form and completed a brief interview to assess their suitability for the study according to the inclusion and exclusion criteria. Participants were told that they would have to perform a task immediately after the presentation of the acoustic stimulus. The aversive nature of the acoustic stimuli was not mentioned, in accordance with the standard instructions for the cardiac defense procedure (see Vila et al., 2007). The electrodes and the headphones were fitted and participants were left alone in a dimly lit room during the test. The post-experimental questionnaires were then completed.

5.2.6. Statistical analysis

HR, PEP, and SBP were analysed separately by means of three 3(x2x10) analyses of variance (ANOVAs) with the between-group factor of Condition (Conditions 1-3), and the two within-subject factors of Trial (Trial 1 vs. 2) and Time (the 10 time-intervals after the onset of the acoustic stimulus). Regarding behavioural measures, reaction time and accuracy were analysed separately by means of two 3(x2) ANOVAs with the between-group factor of Condition and the within-subject factor of Trial. Regarding subjective measures, attentional demand was analysed using a one-way ANOVA with the between-group factor of Condition. *Valence* and *arousal* ratings for the emotional pictures were analysed separately by means of two 3(x22) ANOVAs with the between-group factor of Condition and the within-subject factor of Picture (the 22 pictures used during the task). Intensity and unpleasantness of the acoustic stimuli were analysed separately by means of two 3(x2) ANOVAs with the between-group factor of Condition and the within-subject factor of Trial. The Greenhouse-Geisser epsilon correction was applied to the within-subject factors. Results are provided as uncorrected df and corrected *p* values; partial eta squared (ηp^2) is provided as a measure of effect size. Alpha was set at .05 for the ANOVAs. In follow-up testing Bonferroni corrections were applied to account for multiple comparisons.

5.3. Results

5.3.1. Cardiac defense response

Figures 12 and 13 show the time course of HR during the experimental task. In all trials and conditions, a first acceleration/deceleration component arose during intervals 1-4 after acoustic stimulus presentation. It was followed by a second acceleration/deceleration component during intervals 5-10, which was more pronounced for Trial 1 than Trial 2 in all conditions. The HR increase during the second acceleration/deceleration component was greater in Condition 1 (pleasant), followed by Conditions 3 (unpleasant) and 2 (neutral) for Trial 1. The HR increase during the

second component was greater for Condition 1 than for the other two conditions for Trial 2.

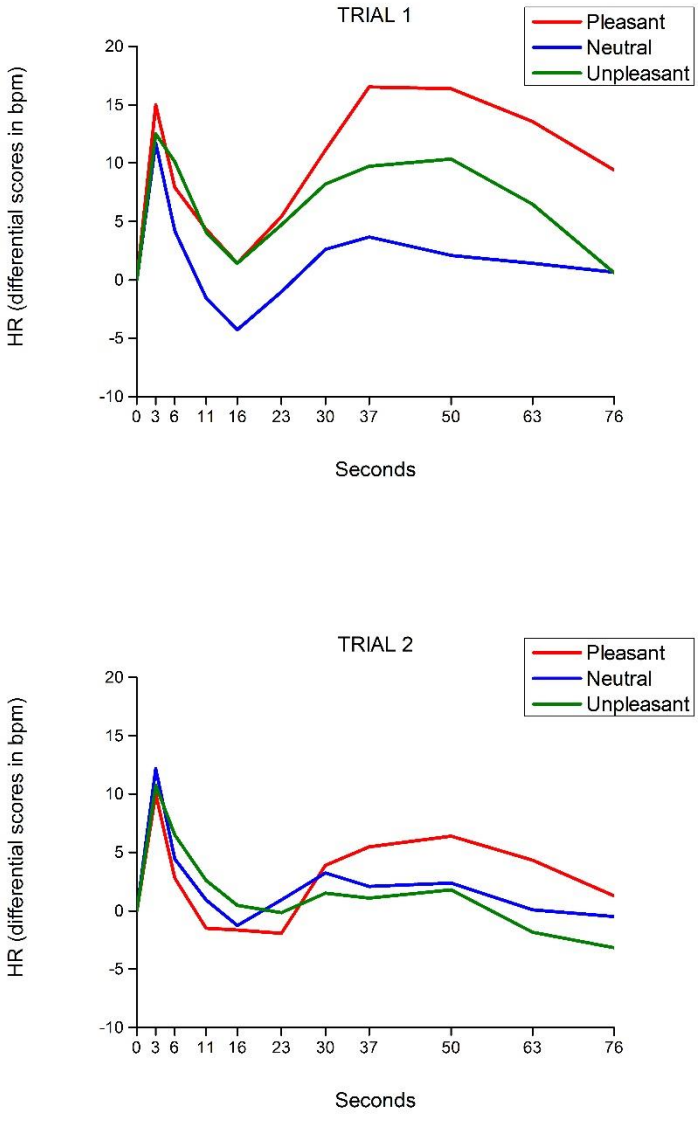


Figure 12. Course of the cardiac defense response: heart rate across the 10 time-intervals (expressed as differential scores) as a function of trial and condition.

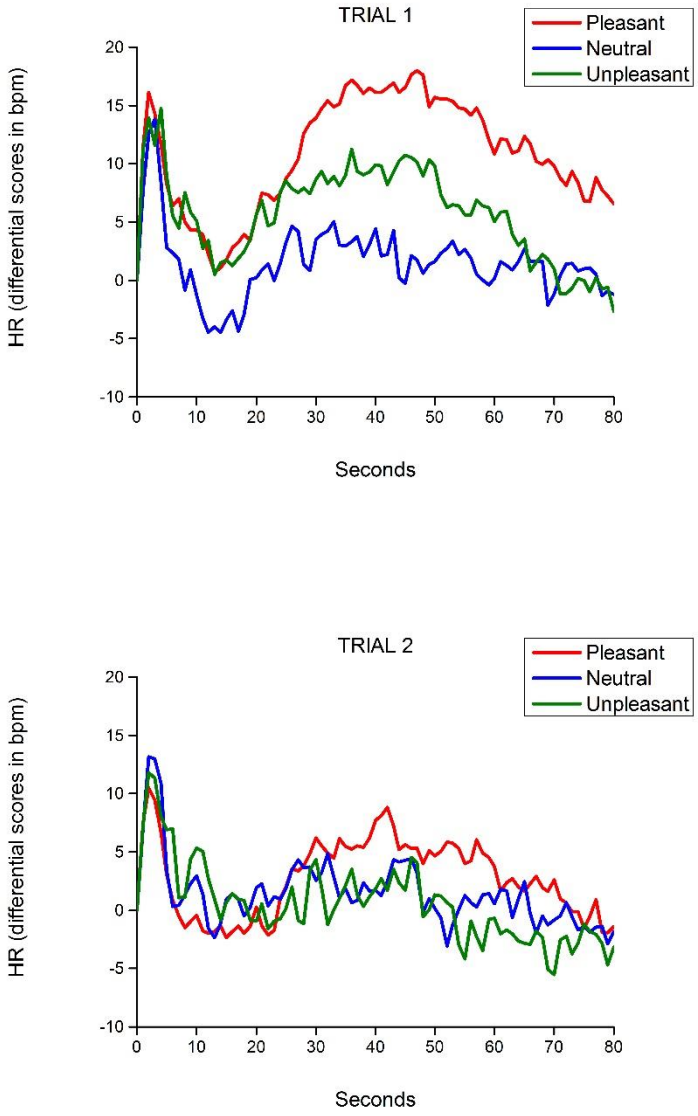


Figure 13. Course of the cardiac defense response: heart rate across the 80 seconds (expressed as differential scores) as a function of trial and condition.

The ANOVA for HR yielded main effects of Trial (F[1,45] = 13.78, p = .001, ηp^2 = .234) and Time (F[9,405] = 21.02, p < .001, ηp^2 = .318), and interaction effects of Condition x Trial (F[2,45] = 4.82, p = .013, ηp^2 = .176), Condition x Time (F[18,405] = 2.36, p = .034, ηp^2 = .095), and Trial x Time (F[9,405] = 6.80, p < .001, ηp^2 = .131). Follow-up analysis was accomplished through ANOVAs for each trial with the between-group factor of Condition and the within-subject factor of Time. The models revealed effects

of Time for both trials (Trial 1 F[9,405] = 21.85, p < .001, ηp^2 = .327, Trial 2 F[9,405] = 14,47, p < .001, ηp^2 = .243) and a Condition x Time interaction for Trial 1 (F[18,405] = 2.47, p = .018, ηp^2 = .099). In addition, separate ANOVAs were conducted for each interval to identify the intervals with differences between the two conditions. Condition effects were seen in intervals 7-9 (all ps ≤ .048). Pair-wise multiple comparisons between the three conditions using Bonferroni test showed significant differences between the first and the second condition in intervals 7 and 8: lower HR for the second condition (all corrected ps ≤ .041). No other comparisons were significant.

5.3.2. Sympathetic cardiac control

Figures 14 and 15 illustrate the time course of PEP (inverted values are presented; as such, a reduction in PEP reflects an increase in sympathetic cardiac control, and vice versa). In all trials and conditions, sympathetic cardiac control decreased during the first two intervals. It was followed by a steep rise peaking around interval 4. Thereafter, a gradual decline was seen until the last interval. The pattern was virtually the same for all trials and conditions. Nevertheless, the overall magnitude of the response was greater during Trial 1.

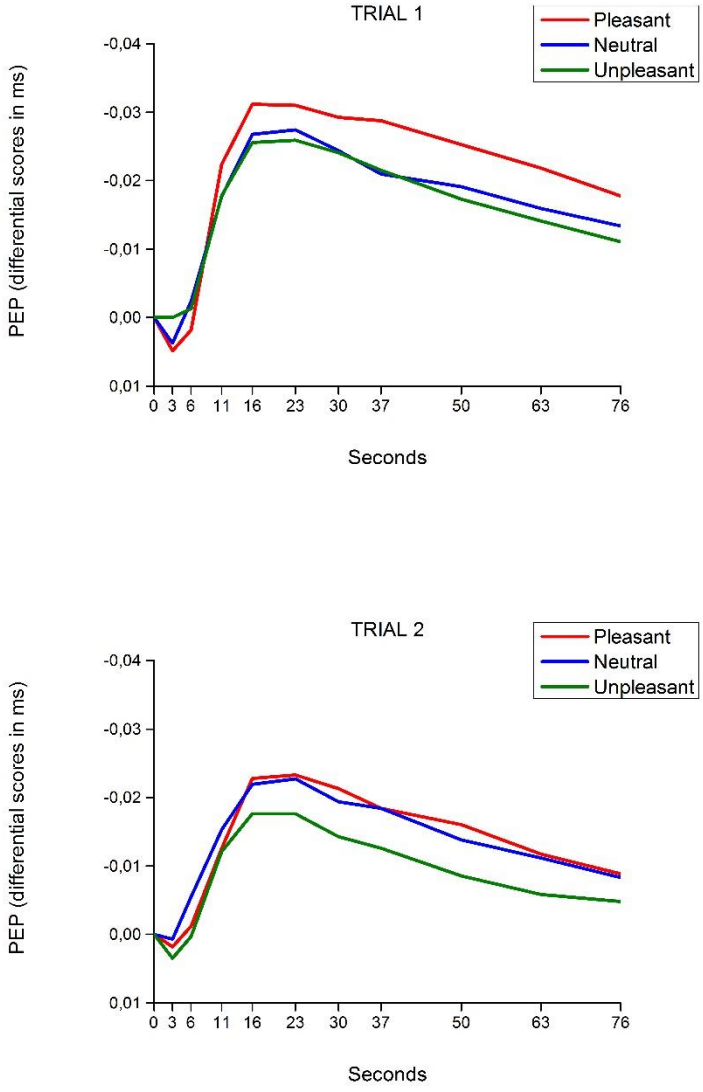


Figure 14. Course of sympathetic cardiac control: pre-ejection period across the 10 time-intervals (expressed in differential scores) as a function of trial and condition.

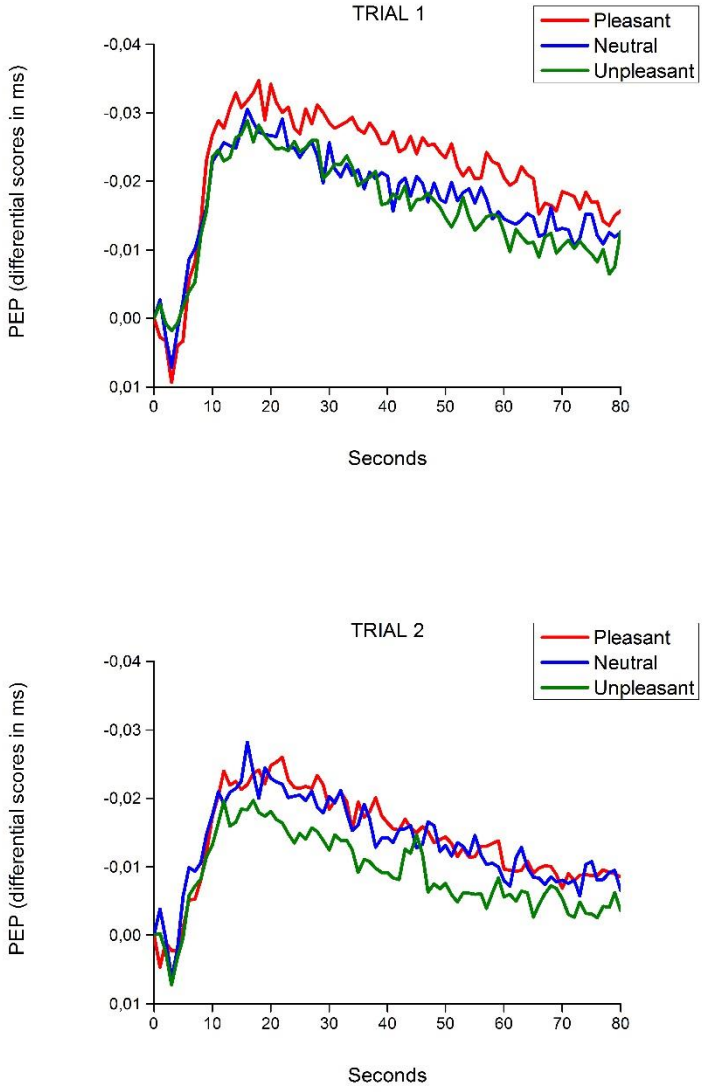


Figure 15. Course of sympathetic cardiac control: pre-ejection period across the 80 seconds (expressed in differential scores) as a function of trial and condition.

The ANOVA for PEP revealed main effects of Trial (F[1,45] = 18.26, p < .001, ηp^2 = .289) and Time (F[9,405] = 90.63, p < .001, ηp^2 = .668), and a Trial x Time interaction (F[9,405] = 10.70, p < .001, ηp^2 = .192). To further analyse the Trial x Time interaction, two separate ANOVAs were computed for each trial, with the between-group factor of Condition and the within-subject factor of Time. The Time effect reached significance

for both trials (Trial 1 F[9,405] = 90.15, p < .001, ηp^2 = .667, Trial 2 F[9,405] = 51.15, p < .001, ηp^2 = .532).

5.3.3. Systolic blood pressure

Figures 16 and 17 depict the time course of SBP. In all trials and conditions, an initial increase of SBP was seen, which reached its peak around interval 2. This increase was larger for Trial 1 than for Trial 2. Subsequently, SBP decreased until interval 4, and remained virtually stable during the remaining response. While the pattern was virtually the same for all trials and conditions, the response was greater during Trial 1.

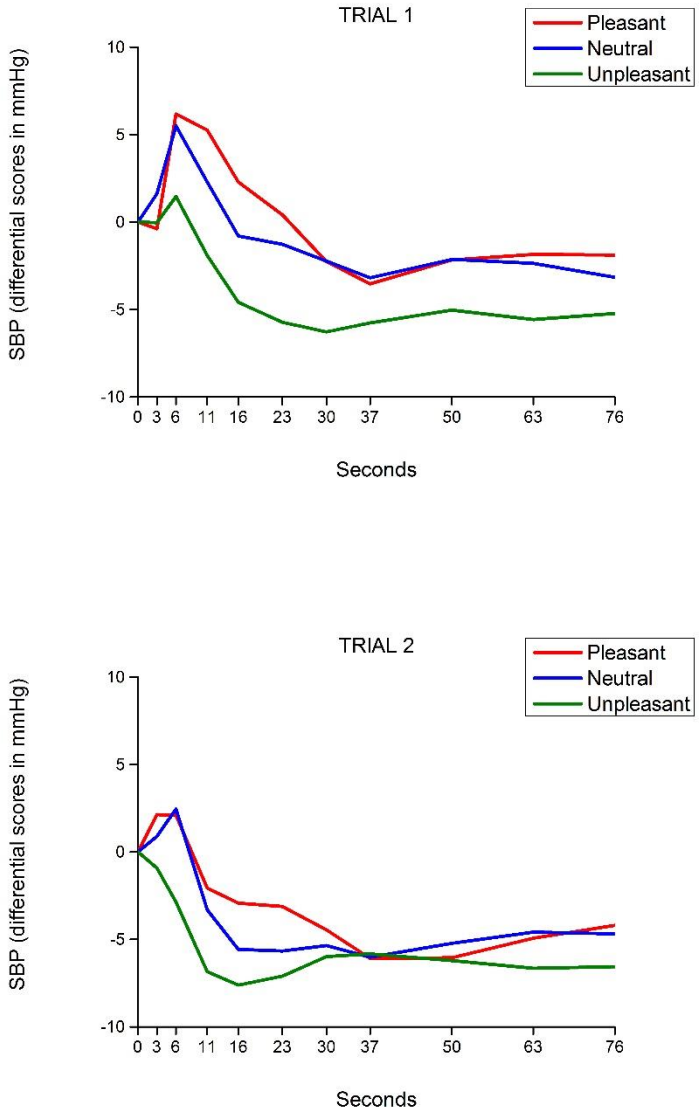


Figure 16. Course of systolic blood pressure: systolic blood pressure across the 10 time-intervals (expressed in differential scores) as a function of trial and condition.

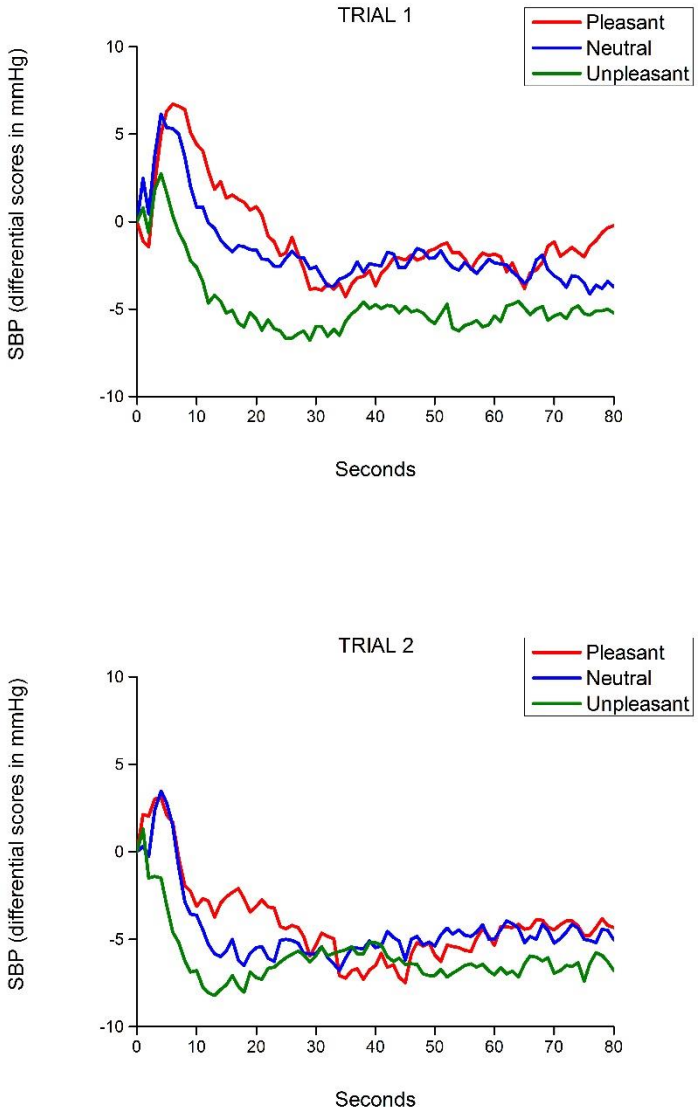


Figure 17. Course of systolic blood pressure: systolic blood pressure across the 80 seconds (expressed in differential scores) as a function of trial and condition.

The ANOVA for SBP yielded a main effect of Time (F[9,405] = 16.47, p < .001, ηp^2 = .268) and a Trial x Time interaction (F[9,405] = 6.29, p < .001, ηp^2 = .123). To further analyse the Trial x Time interaction, two separate ANOVAs were computed for each trial, with the between-group factor of Condition and the within-subject factor of Time. A significant effect of Time arose for both trials (Trial 1 F[9,405] = 14.84, p < .001, ηp^2 = .248, Trial 2 F[9,405] = 13.33, p < .001, ηp^2 = .229).

5.3.4. Task performance

Table 3 shows, for all participants, the mean reaction time and the percentage of correct, incorrect, and missed responses as a function of Condition. As can be observed, reaction time was shorter for participants from Condition 2, followed by participants from conditions 3 and 1; and accuracy was higher for participants from Condition 3, followed by participants from conditions 2 and 1. The ANOVAs yielded main effects of Condition (F[2,45] = 17.76, p < .001, ηp^2 = .441) and Trial for reaction time (F[1,45] = 20.26, p < .001, ηp^2 = .310), and a main effect of Condition for accuracy (F[2,45] = 3.48, p = .039, ηp^2 = .134). Bonferroni-corrected pair-wise comparisons between the three conditions showed significant differences between Condition 1 and the other two conditions for reaction time (all corrected ps ≤ .001); and significant differences between the first and the third condition for accuracy (corrected p = .049). No other comparisons were significant.

5.3.5. Subjective measures

Table 3 also displays the mean of subjective attentional demand requirements for task performance, as well as *valence* and *arousal* ratings for the emotional pictures, as a function of Condition. Attentional demand ratings were higher for Condition 1, followed by Conditions 3 and 2. Picture *valence* ratings were higher for Condition 1, followed by Conditions 2 and 3; and picture *arousal* ratings were higher for Condition 3, followed by Conditions 1 and 2. Whereas the ratings of the pictures were consistent with the Spanish norms, the participants rated the pleasant pictures as slightly less pleasant and less arousing as well as the unpleasant pictures as slightly less unpleasant and less arousing. The ANOVA for attentional demand did not yield significant results. The ANOVAs for picture *valence* and *arousal* revealed a main effect of Condition for both *valence* (F[2,45] = 171.62, p < .001, ηp^2 = .884) and *arousal* (F[2,45] = 13.82, p < .001, ηp^2 = .381). Bonferroni-corrected pair-wise comparisons between the three conditions showed significant differences between Condition 3 and the other two conditions for

valence (all corrected $p < .001$), and between Condition 2 and the other two conditions for *arousal* (all corrected $p \leq .001$). As expected, unpleasant pictures differed from pleasant and neutral conditions in valence; and pleasant and unpleasant pictures did not differ in *arousal*. However, pleasant and neutral pictures did not differ significantly in valence.

Furthermore, Table 4 shows, for all participants, the mean intensity and unpleasantness ratings for the acoustic stimuli as a function of Condition and Trial. Participants from all conditions rated the noise of Trial 1 as more intense and unpleasant than that of Trial 2. The ANOVAs for noise intensity and unpleasantness yielded main effects of Condition (Intensity: $F[2,45] = 14.40$, $p < .001$, $\eta p^2 = .390$; Unpleasantness: $F[2,45] = 7.02$, $p = .002$, $\eta p^2 = .238$) and Trial (Intensity: $F[1,45] = 14.42$, $p < .001$, $\eta p^2 = .243$; Unpleasantness: $F[1,45] = 14.61$, $p < .001$, $\eta p^2 = .245$). Bonferroni-corrected pair-wise comparisons between the three conditions showed significant differences between Condition 1 and the other two conditions for both intensity (all corrected ps $< .001$) and unpleasantness ratings (all corrected ps $\leq .015$).

Table 3. Mean (*SD* in parentheses) of reaction time and subjective data, along with the percentage of correct, incorrect, and missed responses, as a function of condition.

	Condition 1 (*N* = 16)	Condition 2 (*N* = 16)	Condition 3 (*N* = 16)
Behavioural data			
Reaction time	1020.83 (78.06)	763.35 (144.62)	857.52 (136.22)
Correct responses	83.59	91.99	94.14
Incorrect responses	6.45	6.84	3.52
Missed responses	9.96	1.17	2.34
Subjective data			
Attentional demand	76.25 (9.04)	67.38 (23.98)	75.63 (15.48)
Picture *valence*	6.20 (1.26)	5.71 (1.78)	2.03(1.59)
Picture *arousal*	5.52 (1.72)	3.81 (1.68)	6.10 (2.14)

Table 4. Mean (*SD* in parentheses) noise intensity and unpleasantness ratings as a function of condition and trial.

	Condition 1 (*N* = 16)		Condition 2 (*N* = 16)		Condition 3 (*N* = 16)	
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Subjective data						
Noise intensity	75.31 (11.32)	65.00 (13.54)	88.40 (8.13)	82.68 (12.65)	89.38 (10.73)	83.13 (13.15)
Noise unpleasantness	77.69 (14.10)	66.13 (18.28)	87.83 (9.99)	81.70 (14.29)	90.19 (13.13)	83.81 (14.61)

5.4. Discussion

The purpose of this study was to investigate the role of autonomic cardiac control in attentional and emotional modulation of the CDR. During the experiment, the presentation of unexpected aversive noise was followed by the realization of an external attention task which included pleasant, neutral or unpleasant pictures as visual stimuli; while ECG, ICG, and BP recordings were obtained. The typical biphasic pattern of the CDR, consisting of two acceleration/deceleration components in alternating order, arose for all trials and conditions. The amplitude of the second accelerative component was larger when participants viewed pleasant pictures (Condition 1) in Trial 1, followed by unpleasant pictures (Condition 3) and neutral pictures (Condition 2). Significant differences in intervals 7 and 8 were found between Condition 1 and Condition 2. These differences between conditions in the HR response coincide with the results obtained on a behavioural level. Participants from Condition 1 responded slower and with less accuracy to the visual search task. However, the subjective ratings of intensity and unpleasantness of the noise were lower for Condition 1 than for the other two conditions. No differences in sympathetic cardiac control, indexed by PEP, and SBP were seen between the three conditions.

The HR response pattern exhibited habituation following repetition of the acoustic stimulus, which was more pronounced for the second acceleration/deceleration component of the CDR. Rapid habituation is a characteristic of the CDR that has been observed in numerous studies (e.g. Garrido et al., 2020; Mata et al., 2009; Ramírez et al., 2005; Vila et al., 1997). The sympathetic cardiac control and SBP responses also showed a smaller response in the second trial than during the first trial. Habituation of the sympathetic cardiac control after the first trial, which was also found in a previous study of Garrido et al. (2020), suggests a sympathetic involvement in habituation of the CDR. According to the habituation effect observed through trials, subjective ratings of

intensity and unpleasantness of the noise were lower for the second trial than the first trial in all conditions.

The findings relating to HR and sympathetic cardiac control responses can be better understood within the theory of the autonomic space (Berntson et al., 1991). As previously mentioned, this framework posits three different modes of interaction between the two branches of the autonomic nervous system: a coupled reciprocal mode, a coupled non-reciprocal mode, and an uncoupled mode. During the first acceleration/deceleration component of the CDR, in all conditions, HR and sympathetic cardiac control showed almost mirror-inverse time courses. Sympathetic inhibition during HR increase may be explained by an even stronger parasympathetic inhibition (co-inhibition). In the same manner, sympathetic activation during HR decrease may be explained by a stronger parasympathetic activation (co-activation). Therefore, the functioning of a coupled non-reciprocal mode between the two autonomic branches, with parasympathetic dominance, would be feasible during the first acceleration/deceleration component of the CDR.

During the second acceleration/deceleration component of the CDR, in all conditions, HR and sympathetic cardiac control displayed rather similar courses. However, sympathetic activation reached its maximum peak when HR activation started, and decreased gradually from then on. This suggests a coupled reciprocal mode of interaction between the two branches, with parasympathetic dominance, during the second acceleration component. Regarding the second deceleration component, sympathetic inhibition during HR decrease can be explained by sympathetic inhibition and/or parasympathetic activation, thus, both systems may act in a coupled reciprocal mode or an uncoupled mode.

These findings are in line with previous research that indicates a parasympathetic mediation during the first acceleration/deceleration component of the CDR (Árbol, 2017; Fernández & Vila, 1989c; Garrido et al., 2020; Reyes del Paso et al., 1993, 1994). Nevertheless, those studies also suggested an overall sympathetic dominance during the second acceleration/deceleration component of the CDR, whereas our study points toward a parasympathetic dominance during its second acceleration. In addition, HR response during the second acceleration component is more pronounced when viewing emotional pictures, especially pleasant ones, than neutral pictures. No differences in sympathetic cardiac control were seen between the three conditions, so this effect of emotional modulation may be attributed to vagal withdrawal.

SBP time course was similar to HR and opposite to sympathetic cardiac control during the first acceleration/deceleration component of the CDR in all conditions. As SBP is mediated by both sympathetic and parasympathetic branches of the autonomic nervous system, changes in SBP cannot be explained by sympathetic cardiac control and may be due to parasympathetic influences. During the second acceleration/deceleration component of the CDR, in all conditions, HR as well as sympathetic cardiac control displayed opposite time courses in relation with SBP. As is the case with the first component, SBP during the second component cannot be explained by sympathetic cardiac control. The parasympathetic system exerts exclusively chronotropic influences over the heart, which means it only modulates BP through changes in HR. As HR and SBP take opposite time courses, SBP cannot be explained either by parasympathetic cardiac control. It should be taken into account that PEP constitutes and index of beta-adrenergic ionotropic myocardial influences but BP also plays a role in alpha-adrenergic control of vasomotor tone (Berntson et al., 2016). Therefore, the divergence between HR and SBP patterns during the second component of the CDR may be explained instead by sympathetic mediated

vasodilatation. This could be tested in future studies through assessment of peripheral resistance.

Autonomic mechanisms involved in attentional and emotional modulation of the CDR were investigated for the first time in this study. The addition of emotional content to the visual search task provoked differences in the HR response between conditions during the second acceleration component which may be explained by a reduction of vagal outflow to the sinus node. One limitation of this study pertains to the use of PEP as index of sympathetic cardiac control. PEP constitutes an ionotropic parameter that provides information about beta-adrenergic influences on the myocardium (Berntson et al., 2016; Cacioppo et al., 1994; Hassan & Turner, 1983). No chronotropic parameters are available to estimate sympathetic cardiac control influences over the heart and a certain degree of dissociation between HR and sympathetic mediated myocardial contractility is plausible. In addition, no parameter was used for estimating parasympathetic cardiac control, which was just inferred from HR. Sympathetic and parasympathetic contributions to the CDR could also be determined in future research by the application of other methods like pharmacological blockade or by quantification of the baroreflex function.

Altogether, these results provide additional evidence to the attentional-motivational model of the CDR, which posits that the CDR is mediated by both branches of the autonomic nervous system and has both cognitive and motivational significance (Vila et al., 2007). In this study, the addition of emotional content to the visual search task resulted in a potentiation of the second acceleration component of the CDR by viewing emotional pictures in comparison with neutral pictures, which may be understood as an effect of emotional arousal. Pleasant pictures presented the largest amplitude of the second acceleration component. Hence, these results do not support the motivational priming hypothesis (Lang, 1995), according to which it was expected a stronger

potentiation of the CDR by viewing unpleasant pictures that would activate the defensive motivational system.

In conclusion, this study confirmed emotional modulation of the CDR and broadened our understanding of the autonomic mechanisms involved therein. Emotional effects were seen for the second component of the CDR, which is associated with active defensive behaviours in the presence of threat. More specifically, these effects were seen during the second acceleration component, which is mainly mediated by the parasympathetic cardiac system. Therefore, parasympathetic cardiac control may play a key role in emotional modulation of the CDR. On the contrary, sympathetic cardiac control does not seem to intercede in this kind of modulation. Moreover, the BP response seems to be mediated parasympathetically during the first component while its autonomic mediation during the second component of the CDR remains to be clarified.

Capítulo 6:

Conclusiones

Las principales conclusiones de la presente tesis doctoral son las siguientes:

- La presentación repetida de estimulación auditiva intensa e inesperada para evocar la RCD con un IEE corto (2.5 min) se encuentra asociada con la habituación de la respuesta de tasa cardíaca y el control cardíaco simpático, mientras que ambas respuestas muestran un cierto grado de recuperación con un IEE largo (12.5 min). Tanto la habituación como la recuperación de la RCD son más pronunciadas para el segundo componente acelerativo/desacelerativo que para el primer componente acelerativo/desacelerativo.

- En relación a la implicación simpática en los procesos de habituación y recuperación de la RCD, durante el primer componente acelerativo/desacelerativo, los patrones de respuesta de la tasa cardíaca y el control cardíaco simpático sugieren que las dos ramas del sistema nervioso autónomo, simpática y parasimpática, tienen un modo de funcionamiento acoplado no-recíproco con dominancia parasimpática; durante el segundo componente acelerativo/desacelerativo, serían posibles tanto un modo de funcionamiento acoplado recíproco como uno desacoplado, con dominancia simpática.

- El sistema nervioso simpático parece tener un papel fundamental en los procesos de habituación y recuperación de la RCD y su mediación se encuentra limitada al segundo componente acelerativo/desacelerativo.

- Se confirma la modulación atencional de la RCD mediante inducción de atención externa. Los efectos atencionales son observables para los dos componentes de la RCD, con una potenciación del segundo componente

acelerativo/desacelerativo y una tendencia a la reducción del primer componente desacelerativo.

- Se confirma la modulación emocional de la RCD. Se observa un efecto de *arousal* emocional para el segundo componente de la RCD, con una potenciación del segundo componente acelerativo durante la visualización de imágenes afectivas en comparación con imágenes neutras. Las imágenes agradables son las que presentan una mayor amplitud del segundo componente acelerativo.

- En relación a la implicación autonómica tanto en la modulación atencional como en la modulación atencional y emocional simultáneas de la RCD, durante el primer componente acelerativo/desacelerativo, los patrones de respuesta de la tasa cardíaca y el control cardíaco simpático indican que las dos ramas tienen un modo de funcionamiento acoplado no-recíproco con dominancia parasimpática; durante el segundo componente acelerativo, ambas ramas tienen un modo de funcionamiento acoplado recíproco con dominancia parasimpática; mientras que durante el segundo componente desacelerativo, los sistemas simpático y parasimpático podrían estar funcionando tanto con un modo acoplado recíproco como con un modo desacoplado. Además, la respuesta de presión arterial parece estar mediada por el sistema nervioso parasimpático durante el primer componente acelerativo/desacelerativo de la RCD, mientras que la mediación autonómica en dicha respuesta durante el segundo componente acelerativo/desacelerativo aún se desconoce.

- El sistema nervioso parasimpático parece estar implicado en la modulación atencional de la RCD mediante inducción de atención externa.

- El sistema nervioso parasimpático también parece estar implicado en la modulación emocional de la RCD.

- Estos hallazgos apoyan el modelo atencional-motivacional de la RCD, según el cual la RCD se encuentra mediada por las dos ramas del sistema nervioso autónomo, simpática y parasimpática, y tiene una significación tanto cognitiva como motivacional.

Capítulo 7:

Conclusions

Based on the results obtained in this thesis, the following main conclusions are drawn:

- Repeated intense and unexpected auditory stimulation for eliciting the CDR at a short ITI (2.5 min) is associated with marked habituation of the heart rate response and sympathetic cardiac control, whereas both responses exhibit a degree of recovery at a long ITI (12.5 min). Habituation and recovery of the CDR are overall stronger for the second acceleration/deceleration component than the first acceleration/deceleration component.

- Regarding sympathetic contributions to habituation and recovery of the CDR, during the first acceleration/deceleration component, the HR and sympathetic cardiac control time courses suggest both branches of the autonomic nervous system, sympathetic and parasympathetic, act in a coupled non-reciprocal mode with parasympathetic dominance; during the second acceleration/deceleration component, a coupled reciprocal mode as well as an uncoupled mode, with sympathetic dominance, is feasible.

- The sympathetic nervous system may play a key role in habituation and recovery of the CDR and its mediation is limited to the second acceleration/deceleration component.

- Attentional modulation of the CDR through external attention is confirmed. Attentional effects were seen for both components of the CDR, with a potentiation of the second acceleration/deceleration component and a trend towards a reduction of the first deceleration component.

- Emotional modulation of the CDR is confirmed. An emotional arousal effect is seen for the second component of the CDR, with a potentiation of the second

acceleration component by viewing emotional pictures in comparison with neutral pictures. Pleasant pictures presented the largest amplitude of the second acceleration component.

- Regarding autonomic contributions to attentional modulation as well as concurrent attentional and emotional modulation of the CDR, during the first acceleration/deceleration component, the HR and sympathetic cardiac control time courses indicate both branches act in a coupled non-reciprocal mode with parasympathetic dominance; during the second acceleration component, both branches act in a coupled reciprocal mode with parasympathetic dominance; whereas during the second deceleration component, the sympathetic and parasympathetic systems may act in a coupled reciprocal mode as well as in an uncoupled mode. Moreover, the BP response seems to be mediated parasympathetically during the first component while its autonomic mediation during the second component of the CDR remains to be clarified.

- The parasympathetic nervous system may be involved in attentional modulation of the CDR through external attention.

- The parasympathetic nervous system may also be involved in emotional modulation of the CDR.

- These findings support the attentional-motivational model of the CDR, which posits that the CDR is mediated by both branches of the autonomic system, sympathetic and parasympathetic, and has both cognitive and motivational significance.

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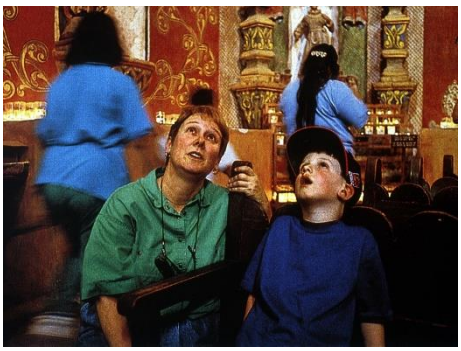
Anexos

Anexo 1

Imágenes del IAPS utilizadas en el Estudio 2


OXYGEN

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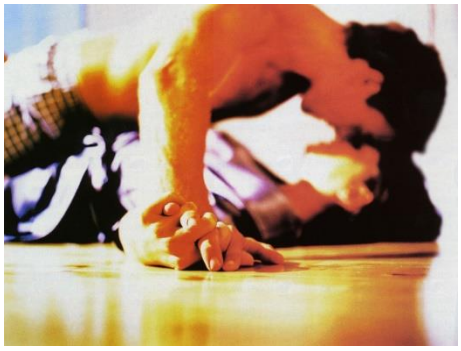
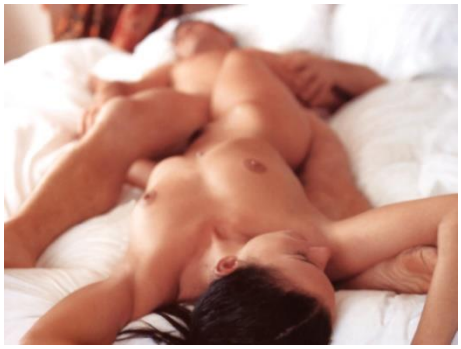
Anexo 2

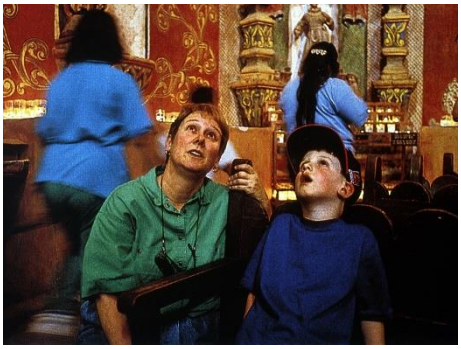
Imágenes del IAPS utilizadas en el Estudio 3 - Imágenes agradables





Imágenes del IAPS utilizadas en el Estudio 3 - Imágenes neutras


OXYGEN

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Imágenes del IAPS utilizadas en el Estudio 3 - Imágenes desagradables