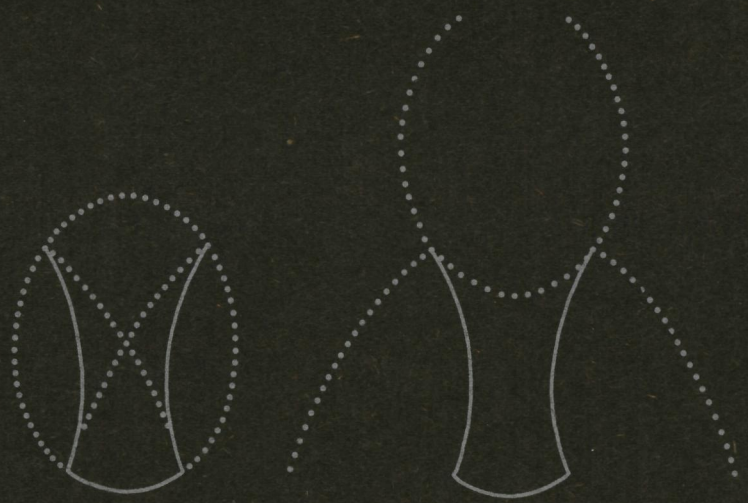

neural mechanisms of appetitive and aversive behavior

cees p.f. van der staak

NEURAL MECHANISMS OF APPETITIVE AND AVERSIVE BEHAVIOR

PROMOTOR:

Prof. J.M.H. Vossen

NEURAL MECHANISMS OF APPETITIVE AND AVERSIVE BEHAVIOR

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN DOCTOR
IN DE SOCIALE WETENSCHAPPEN
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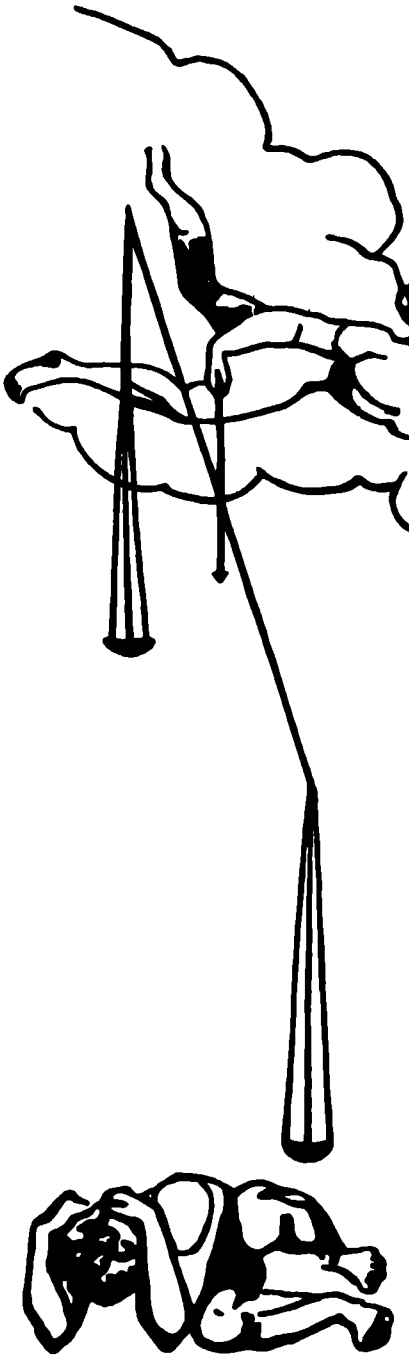
Aan mijn ouders

Aan Jomar

Aan Thomas en Lukas



"Let the Human Organs be kept in their perfect Integrity,
At will Contracting into Worms or Expanding into Gods,
And then, behold! what are these Ulro Visions .....?"

(William Blake Jerusalem
(1804-1820), plate 55, 36-38).

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- *Intra- and interhemispheric visual-motor coordination of human arm movements. Neuropsychol. 13, 4: 439-448, 1975.*
- *Habituation during cortical spreading depression in rats (abstract). Exp. Brain Res. 23: 205, 1975.*
- *Habituation to intense acoustic stimulation during cortical spreading depression in rats (co-author: W. Fischer). Physiol. Behav. (in press).*
- *Habituation and sensitization of the acoustic startle response during cortical spreading depression in rats. Physiol. Behav. 16,6,1976 (in press).*
- *Habituation of open field activity during cortical spreading depression in rats. (submitted for publication).*
- *Cortico-hypothalamic interactions in appetitive and aversive behaviour induced by hypothalamic stimulation in rats. In: A. Wauquier and E.T. Rolls (Eds.): Brain stimulation reward. Amsterdam: North Holland Publishing Company, 1976 (in press).*
- *Appetitive and aversive behavior induced by hypothalamic stimulation in rats during cortical spreading depression. (submitted for publication).*
- *Appetitive and aversive behavior induced by peripheral thermal stimulation during cortical spreading depression in rats (submitted for publication).*

CONTENTS

INLEIDING EN SAMENVATTING.

De neurale regulering van appetitief en aversief gedrag heeft de gedragswetenschappen van oudsher reeds beziggehouden. "..... la volonté qu'on a d'obtenir quelque bien ou de fuïr quelque mal, envoye promptement les esprits du cerveau vers toutes les parties du corps, qui peuvent servir aux actions requises pour cet effect; ..." (Descartes, 1644, blz. 141).

Het toestreven naar hetgeen als goed wordt ervaren en het zich afwenden van hetgeen als kwaad wordt ervaren behoorde volgens Descartes tot de fundamentele *passions*, die richting geven aan het gedrag. Hij schreef deze twee tegengestelde tendensen toe aan de werking van een en dezelfde *passion*, nl. *le desir*, het verlangen. Het gedrag van het redeloze dier wordt in dit verband volgens Descartes geheel en al bepaald door deze lichamelijke aandoeningen; de mens daarentegen beschikt over het vermogen bij het onderscheiden van goed en kwaad gebruik te maken van ervaring en rede; daardoor is hij tevens in staat zijn lichamelijke aandoeningen de baas te worden. Met andere woorden: het dierlijk gedrag wordt volledig beheerst door instinkten (aangeboren reaktiepatronen, die door bepaalde stimuli worden uitgelokt); het gedrag van de mens daarentegen is gesteld onder de kontrolerende werking van de rede. Deze strikte dichotomie tussen mens en dier, tussen rede en instinkt heeft eeuwenlang een bepalende invloed gehad op de bestudering van menselijk en dierlijk gedrag (Beach, 1955).

De strikte dichotomie tussen mens en dier werd ruim

honderd jaar geleden opgeheven in de evolutietheorie van Charles Darwin, die stelde dat het ontstaan der soorten, inklusief de mens, verlopen is langs lijnen van natuurlijke selektie. Deze theorie impliceert een verwantschap tussen de mens en andere diersoorten in de zin van een gemeenschappelijke oorsprong. De dichotomie tussen rede en instinkt werd hiermee overigens niet opgeheven. Om de theorie van Darwin ten aanzien van de kontinuïteit tussen mens en dier te ondersteunen werden aanvankelijk vooral twee soorten evidentie aangedragen: enerzijds werd getracht het bestaan van menselijke instinkten aan te tonen; anderzijds werd onderzoek verricht naar het bestaan van rede in het dier (met name intelligentie).

In de psychologie ontstond geleidelijk aan een anti-instinktbeweging, die de ontwikkeling van deze wetenschap in de eerste helft van de 20e eeuw sterk beïnvloed heeft. Het onderzoek werd overwegend gericht op de bestudering van leerprocessen. Men ontkwam overigens niet aan de noodzakelijkheid om binnen de verschillende leertheorieën instinkt-verwante begrippen te inkorporeren: o.a. onvoorwaardelijke reakties, uitgelokt door onvoorwaardelijke stimuli. Deze ontwikkeling binnen de psychologie werd voor het grootste gedeelte bepaald door onderzoekers in de Verenigde Staten en de Sovjet Unie en bleef lange tijd geïsoleerd van de ontwikkeling van de ethologie, die vooral in Europa werd ingezet. Zich baserend op Darwin als geestelijk vader, hield de ethologie zich vooral bezig met de bestudering van instinktief gedrag (Burghardt, 1973). De term instinkt is overigens in onbruik geraakt; tegenwoordig wordt meestal gesproken over *fixed action patterns* of

soort-specifiek gedrag.

Sinds enige jaren is er een ontwikkeling op gang gekomen, die zowel voor de psychologie als voor de ethologie van groot belang is. Deze ontwikkeling is erop gericht een integratie tussen psychologie en ethologie tot stand te brengen (Schneirla, 1966; Hinde, 1966; Burghardt, 1973). Behalve de wederzijdse methodologische verrijking, heeft de kommunikatie tussen beide disciplines ook op theoretisch niveau reeds belangrijke resultaten opgeleverd. Met name het onderzoek naar de biologische begrenzingen van leren (Seligman and Hager, 1972; Hinde and Stevenson-Hinde, 1973) is in dit verband van belang, omdat in dit onderzoek de centrale begrippen van beide disciplines, te weten leren en soort-specifiek gedrag, met elkaar gekonfronteerd worden. In het kader van dit onderzoek is duidelijk gebleken, dat bij het zoeken naar algemene wetmatigheden in leerprocessen rekening gehouden moet worden met de soort-specifieke en individu-specifieke reaktiewijzen, die aan deze leerprocessen ten grondslag liggen (Seligman and Hager, 1972; Nebylitsyn and Gray, 1972).

Beloning of bekrachtiging enerzijds en straf anderzijds zijn centrale begrippen in de leer-psychologie. Wanneer een of ander gedrag van het organisme systematisch gevolgd wordt door een bepaalde stimulus, dan kan de frekwentie van optreden van dit gedrag in de toekomst beïnvloed worden. Is er sprake van een verhoging van de frekwentie, dan spreekt men van een beloning-procedure; wordt de frekwentie daarentegen verlaagd, dan spreekt men van een straf-procedure. Of de aanbieding van een dergelijke stimulus bij een bepaald individu van een bepaalde diersoort

zal resulteren in een beloning- of in een straf-effekt, is afhankelijk van de onvoorwaardelijke reaktie, die dit individu ten aanzien van deze stimulus vertoont (Vossen, 1973). Met name de richting van de onvoorwaardelijke reaktie is hierbij van belang. De onvoorwaardelijke reaktie kan zich manifesteren als een toenaderingsreaktie naar de stimulus toe, of als een verwijderingsreaktie van de stimulus af. De aanbieding van een stimulus, die een toenaderingsreaktie induceert, heeft in het algemeen een beloning-effekt; de aanbieding van een stimulus, die een verwijderingsreaktie tot gevolg heeft, heeft daarentegen een straf-effekt (Glickman & Schiff, 1967). Toenadering wordt bij deze gedefinieerd als een verkleining van de afstand tussen organisme en stimulus, verwijdering als een vergroting van deze afstand. Toenadering kan in deze definitie bestaan uit een verplaatsing van het organisme naar de stimulus toe (bijv. dier nadert ander dier) of uit een verplaatsing van de stimulus naar het organisme toe (dier staat toe, dat ander dier nadert). Evenzo kan verwijdering bestaan uit een verplaatsing van het organisme van de stimulus weg (bijv. dier vlucht voor ander dier) of uit een verplaatsing van de stimulus van het organisme weg (dier vertoont *freezing* gedrag, waardoor ander dier wegloopt). Agressief gedrag lijkt op het eerste gezicht moeilijk te passen in deze indeling, maar ook agressief gedrag kan, al naar gelang het uiteindelijke resultaat van dit gedrag, worden omschreven als toenadering of verwijdering (Rasa, 1975).

"In the evolution of behavior, operations which approptiat-

ely increase or decrease distance, between organisms and stimulus sources must have been crucial for the survival of all animal types." (Schneirla, 1965, blz. 2). Theodore C. Schneirla, een van de weinige amerikaanse onderzoekers die zich reeds vanaf de dertiger jaren heeft beziggehouden met ethologisch onderzoek, heeft een belangrijke rol gespeeld bij het op gang komen van de kommunikatie tussen psychologie en ethologie (Piel, 1970). Centrale thema's in zijn werk waren de bifasische processen van toenadering (*approach*) en verwijdering (*withdrawal*).

Schneirla beperkte zijn begrippen *approach* en *withdrawal* tot de direkte toenadering tot en verwijdering van momentaan aanwezige stimuli; dit ter onderscheiding van zoek-gedrag (*seeking*) en vermijdings-gedrag (*avoidance*) ten aanzien van momentaan afwezige stimuli. Zoekgedrag is gericht op het toenaderen tot een stimulusbron, die zich buiten het momentane waarnemingsveld bevindt; vermijdingsgedrag is gericht op het verwijderd blijven van een stimulusbron, die zich buiten het momentane waarnemingsveld bevindt (Schneirla, 1959). Bij het tot stand komen van deze laatste gedragingen spelen leerprocessen een belangrijke rol. Zoek- en toenaderingsgedragingen worden in dit proefschrift aangeduid als appetitieve gedragingen; verwijderings- en vermijdingsgedragingen worden aangeduid als aversieve gedragingen. Deze terminologie is afkomstig van Craig (1918), die de tegengestelde affektieve toestanden van *appetite* en *aversion* als basiskondities beschouwde in de regulatie van het gedrag. De stimuli, die appetitief en aversief gedrag induceren, worden respektievelijk appetitieve en aversieve stimuli genoemd.

In dit verband moge opgemerkt worden dat een stimulus altijd in relatie staat tot andere stimuli. Daarom kan zich de situatie wel eens voordoen, dat het organisme zich verwijdert van een stimulus die normaliter appetitief is, om daardoor te ontsnappen aan een sterk aversieve stimulus, die met deze appetitieve stimulus gerelateerd is (bijv. *C.E.R.)*. Om dezelfde reden kan het ook wel voorkomen, dat het organisme een toenaderingsreaktie vertoont naar een stimulus, die normaliter aversief is, om daardoor een sterk appetitieve stimulus in het bereik te krijgen, die met deze aversieve stimulus gerelateerd is (bijv. *obstruction box* gedrag).

Schneirla (1959) stelde, dat de termen toenadering en verwijdering de enige empirische, objektieve termen zijn, die toepasbaar zijn op alle gemotiveerde gedragingen in alle diersoorten. Voor wat betreft het menselijke gedrag heeft hij deze stelling genuanceerd. In de loop van de menselijke ontogenie treedt een dusdanige differentiëring op in het gedrag, dat het bij de volwassene vaak moeilijk is uit te maken of een bepaald gedrag als een toenaderings- dan wel als een verwijderingsreaktie beschouwd moet worden. Bij het pasgeboren kind is het onderscheid tussen toenaderings- en verwijderingsreakties echter zeer duidelijk. Toenaderingsreakties manifesteren zich aanvankelijk als een ongedifferentieerde extensie en abduktie van alle ledematen en een draaien van het hoofd in de richting van de stimulus; geleidelijk aan krijgen deze reakties een meer gedifferentieerd karakter (o.a. reikbewegingen) en gaan ze een belangrijke funktie vervullen in het zich toewenden naar de wereld. Verwijderings-reakties daarentegen manifesteren zich als een flexie en

adduktie van alle ledematen; ook verwijderingsreakties worden geleidelijk aan gedifferentieerder (o.a. buigreflex); de funktie van deze reakties is gelegen in een zich afwenden van de wereld. Deze tegengestelde reakties van toenadering en verwijdering zijn door Buytendijk ook in het pasgeboren dier beschreven: "De grondkenmerken van het dierzijn, n.l. de geslotenheid en openheid komen daarbij (*bij de geboorte*) in een zeer bijzondere spanning, doordat de vreemde situatie het dier afscheidt, terugwerpt op zichzelf, d.w.z. een tendenz van terugtrekken, vlucht, bewegingsarmoede, gemis aan expansiviteit aanwezig is .... Tegelijkertijd is er echter bij het jonge dier een tegengestelde grondtendenz n.l. die, welke gericht is om zo spoedig mogelijk het evenwicht in de existentie te herstellen door het vormen van een kontakt met het nieuwe milieu." (Buytendijk, 1938, blz. 106)

In dit proefschrift wordt een aanzet gegeven tot een theorie omtrent de neurale regulering van appetitief en aversief gedrag. Aan deze theorie ligt een hypothese ten grondslag, die als volgt geformuleerd kan worden: als het organisme gekonfronteerd wordt met een stimulus, dan treden twee tegengestelde mechanismen in werking: een mechanisme, dat het organisme aanzet tot appetitief gedrag en een ander mechanisme, dat aanzet tot aversief gedrag. Diverse faktoren bepalen de relatieve sterkte van de aktiviteit van beide mechanismen (o.a.soort-specifieke kenmerken, individu-specifieke kenmerken, stimuluskenmerken en voorafgaande ervaring met dezelfde stimulus). Van de relatieve momentane aktivatie van beide mechanismen hangt af of het

overte gedrag zal bestaan uit appetitief of aversief gedrag. De hypothese van 2 tegengestelde mechanismen impliceert een verwantschap tussen de theorie, die in dit proefschrift geformuleerd zal worden, en andere *opponent-process* theorieën, die op het niveau van motivatie (Solomon & Corbit, 1974) en neurale organisatie (Hurvich & Jameson, 1974) ontwikkeld zijn. Ze is daarentegen in tegenspraak met de opvatting van theoretici als Descartes, die van mening zijn dat het onderscheid tussen appetitief gedrag en aversief gedrag zinloos is; in beide gevallen zou er namelijk sprake zijn van een zich begeven van een ongewenste situatie naar een gewenste situatie. Hoewel deze opvatting op het niveau van het overte gedrag verdedigbaar moge zijn, is het onwaarschijnlijk dat op het niveau van de neurale regulering van dit overte gedrag slechts één mechanisme werkzaam zou zijn; met name de effekten van intrakraniële stimulatie wijzen daarop (Glickman and Schiff, 1967).

Dit proefschrift is gebaseerd op een zestal onderzoekingen, op basis waarvan enige hypotheses met betrekking tot de neurale mechanismen van appetitief en aversief gedrag geformuleerd zullen worden. Enerzijds zijn de mechanismen van appetitief en aversief gedrag onderzocht met betrekking tot de horizontale overdracht van informatie tussen beide hersenhemisferen; anderzijds is de vertikale kommunikatie tussen neocortex en subkortikale strukturen onderwerp van onderzoek geweest.

In hoofdstuk 1 wordt een tweetal experimenten bij volwassen humane proefpersonen beschreven. In deze experimenten werd de visuo-motorische koördinatie onderzocht bij

het uitvoeren van abduktie-bewegingen (van het lichaam af) en adduktie-bewegingen (naar het lichaam toe) van linker- en rechterarm. Zowel de snelheid als de nauwkeurigheid van deze bewegingen werden onderzocht. Overeenkomstig de theorie van Schneirla zijn abduktie-bewegingen vroeg in de ontogenese te beschouwen als toenaderingsbewegingen, adduktiebewegingen als verwijderingsbewegingen. Het is aannemelijk, dat deze oorspronkelijke bipolariteit op latere leeftijd op een of andere wijze behouden blijft. Beide typen van bewegingen werden onderzocht in twee kondities. In de ene konditie moest interhemisferische informatie-overdracht plaatsvinden, terwijl in de andere konditie de visuomotorische koördinatie binnen één hemisfeer kon plaatsvinden. Op basis van de resultaten wordt een hypothese geformuleerd ten aanzien van de neurale banen via welke de sturing van abduktieve en adduktieve arm-bewegingen verloopt: abduktie-bewegingen worden gestuurd via een lateraal afdalend motorisch systeem, dat projekteert op de kontralaterale zijde van het ruggemerg; adduktie-bewegingen daarentegen worden gestuurd via een mediaal afdalend motorisch systeem, dat bilateraal op het ruggemerg projekteert. Op basis van de theorie van Schneirla kan dus gesteld worden, dat toenaderingsbewegingen van de arm worden gestuurd via een lateraal systeem met kontralaterale projektie, terwijl verwijderingsbewegingen worden gestuurd via een mediaal systeem met bilaterale projektie. De interpretatie van deze experimenten bij menselijke proefpersonen vertoont overeenkomsten met de resultaten van experimenten bij dieren, waarin de hypothalamus elektrisch gestimuleerd wordt: laterale stimulatie induceert in het algemeen toenaderings-

gedrag, mediale stimulatie verwijderingsgedrag (Stein, 1969).

In de experimenten die in de overige vijf hoofdstukken beschreven worden, werd gebruik gemaakt van ratten als proefdieren. Dit maakte het mogelijk in deze experimenten direkt in te grijpen in het funktioneren van de hersenen. De techniek die hierbij werd gebruikt is bekend onder de naam cortical spreading depression (CSD). CSD is als verschijnsel ontdekt door de braziliaanse fysioloog Leao in 1944 en is omstreeks 1960 in de fysiologische psychologie geïntroduceerd door de tsjechische onderzoeker, Dr. Jan Bures. Door middel van deze techniek kan een reversibele uitschakeling van de neocortex worden bewerkstelligd. Via een epidurale kanule wordt daartoe een oplossing van kaliumchloride op de dura gedruppeld. Deze ingreep veroorzaakt een plaatselijke depressie van de elektrische kortikale aktiviteit, die zich als een langzame golf over het gehele neocortex-oppervlak verspreidt. Meerdere depressiegolven in suksessie veroorzaken een relatief permanente onderdrukking van de neokortikale aktiviteit, die gedurende enige uren kan worden gehandhaafd. Na de beëindiging van de behandeling herstelt de aktiviteit zich weer. De CSD-techniek is zeer geschikt voor de bestudering van de relaties tussen beide hersenhemisferen en tussen de neocortex en subkortikale strukturen (Van der Staak, 1969; Bures e.a., 1974).

In de experimenten, die besproken worden in de hoofdstukken 2 tot en met 6, werden appetitieve en aversieve stimuli toegediend, door middel waarvan respektievelijk appetitief en aversief gedrag geïnduceerd werd. Hierbij

werden 2 test-procedures gebruikt. De eerste procedure was een habituatie-procedure; in deze procedure werden de stimuli toegediend, onafhankelijk van het gedrag van het proefdier. Herhaalde stimulusaanbieding resulteerde in een afname van de reaktie op deze stimuli. De tweede procedure was een konditioneringsprocedure; hierbij werd de aanbieding van de stimuli afhankelijk gemaakt van het optreden van een bepaalde response van het proefdier. Op deze wijze kon de frekwentie van de betreffende response verhoogd of verlaagd worden. Habituatie en konditionering zijn de belangrijkste processen, die plasticiteit in het gedrag brengen (Vossen, 1969).

In de hoofdstukken 2, 3 en 4 worden experimenten beschreven betreffende de effekten van CSD op habituatie. Hoewel er veel onderzoek is verricht naar de effekten van CSD op diverse leerprocessen, is er nauwelijks onderzoek geweest naar de effekten van CSD op habituatie, een van de meest elementaire vormen van plasticiteit in het gedrag. Habituatie kan gedefinieerd worden als een afname van een onvoorwaardelijke response als de uitvoering van deze response biologisch niet relevant blijkt te zijn (Vossen, 1973). In de experimenten, beschreven in de hoofdstukken 2 en 3, was habituatie aan aversieve geluidsstimuli objekt van onderzoek. In de experimenten van hoofdstuk 2 werd verstoring van drinkgedrag geregistreerd als reaktie op luid belgerinkel; het experiment van hoofdstuk 3 had betrekking op de registratie van schrikreakties op luide pieptonen. In het experiment, dat in hoofdstuk 4 beschreven wordt, werden de effekten van CSD op habituatie van lokomotie-gedrag in een nieuwe omgeving (*open field*) bestu-

deerd. Er werd aanvankelijk uitgegaan van een interpretatie van dit lokomotie-gedrag in termen van toenadering (i.c. exploratief gedrag, cfr. Vossen, 1966), maar later bleek deze assumptie niet houdbaar: in deze situatie kunnen zowel toenaderings- als verwijderingsreakties optreden: exploratie of ontsnapping (Archer, 1973).

De resultaten van deze habituatie-experimenten kunnen als volgt samengevat worden: CSD veroorzaakt een verhoogde sensitisatie; sensitisatie is gedefinieerd als een toename van arousal, die vooral bij herhaalde aanbieding van aversieve stimuli op de voorgrond treedt. Deze verhoogde sensitisatie wordt toegeschreven aan een disinhibitie van retikulaire arousal ten gevolge van de uitschakeling van de neocortex. Met andere woorden: de neocortex of een gedeelte ervan oefent onder normale omstandigheden een tonisch inhiberende invloed uit op de aktiviteit van die gedeelten van de retikulaire formatie, die verantwoordelijk zijn voor de sensitisatie-reaktie op aversieve stimuli. De retikulaire formatie is gelokaliseerd in de mediale regionen van de hersenstam. Er zijn overigens aanwijzingen dat de neurale bemiddeling van sensitisatie-reakties ook op het niveau van de thalamus plaatsvindt: bij herhaalde stimulatie van mediale regionen van de thalamus zijn sensitisatie-reakties gevonden, terwijl herhaalde stimulatie van laterale regionen vooral habituatie-reakties induceerde (Wester, 1971; Thompson e.a., 1973).

In de laatste twee hoofdstukken worden experimenten beschreven, waarin een konditioneringsprocedure werd gebruikt: de aanbieding van appetitieve en aversieve stimuli

werd hierbij afhankelijk gemaakt van het optreden van een bepaalde response. In het verloop van het uitvoeren van de experimenten, die beschreven zijn in de hoofdstukken 2 t/m 4, werd het zwaartepunt van de vraagstelling geleidelijk verschoven. Aanvankelijk was de vraagstelling vooral gericht op de horizontale overdracht van informatie tussen beide hersenhemisferen; later kwam de nadruk echter steeds meer te liggen op de vertikale informatie-overdracht tussen de neocortex en subkortikale strukturen. Aan deze accentverschuiving lag ten grondslag het feit, dat gedurende CSD aanzienlijke veranderingen in de motivationele sfeer plaatsvinden: het dier eet niet meer, drinkt niet meer en vertoont veranderingen in andere gedragingen, die alle voor hun regulatie afhankelijk zijn van de integriteit van subkortikale strukturen, met name de hypothalamus. Uit elektrofysiologisch onderzoek is bovendien gebleken dat gedurende CSD de elektrische aktiviteit van o.a. de hypothalamus aanzienlijk gereduceerd is; dit wordt toegeschreven aan een stilvallen van tonische kortikofugale excitatie.

In het experiment, dat beschreven wordt in hoofdstuk 5, werd de hypothalamus met behulp van intrakraniële elektrodes geprikkeld; de invloed van CSD op de gedragseffekten van deze prikkeling werd bestudeerd. In 1954 is door Olds & Milner ontdekt, dat elektrische prikkeling van de hypothalamus een beloning-effekt kan hebben. Als een dier in staat wordt gesteld zichzelf door middel van het uitvoeren van een of andere handeling een elektrische prikkeling in de hypothalamus toe te dienen, dan zal het dit in hoge frekwentie gaan doen. Dit verschijnsel

staat sindsdien bekend onder de naam intrakraniële elektrische zelfstimulatie. In hetzelfde jaar 1954 rapporteerden Delgado e.a., dat elektrische prikkeling van de mesencefale retikulaire formatie een straf-effekt kan hebben; ook naar dit fenomeen is veel onderzoek verricht. Glickman en Schiff (1967) hebben de belonende, respektievelijk straffende waarde van intrakraniële stimulatie in verband gebracht met de gedragingen, die door deze stimulatie worden geïnduceerd. Belonende elektrische stimulatie induceert gewoonlijk appetitieve gedragingen; straffende stimulatie daarentegen induceert aversieve gedragingen.

In het omvangrijke onderzoek, dat betrekking heeft op de gedragseffekten van intrakraniële stimulatie, staat de gedachte centraal, dat het verschijnsel van intrakraniële zelfstimulatie de sleutel bevat voor het begrijpen van de neurale grondslagen van motivatie en bekrachtiging (Valenstein, 1973). Merkwaardig is, dat er slechts weinig onderzoekingen zijn, waarin de twee tegengestelde effekten van intrakraniële stimulatie (beloning vs. straf; appetitief effekt vs. aversief effekt) direkt met elkaar vergeleken worden. In het kader van bovengenoemde vraagstelling naar de neurale mechanismen van appetitief en aversief gedrag werd het experiment uitgevoerd, dat in hoofdstuk 5 wordt beschreven. In dit experiment werd de invloed van CSD op door hypothalamische stimulatie geïnduceerd appetitief en aversief gedrag onderzocht. Stimulatie in de laterale hypothalamus induceert in het algemeen appetitief gedrag, stimulatie in de mediale hypothalamus aversief gedrag (Stein, 1969). In het kader van dit experiment hebben we een testsituatie ontwikkeld, die vrij is van een aantal

verstorende faktoren, die in de gangbare testsituaties de interpretatie bemoeilijken. De ratten werden getest in een shuttle-box, waarin ze tegengestelde gedragingen moesten vertonen (over een barrière springen òf inhibitie van die response) om eenzelfde effekt te bereiken: òf zoveel mogelijk stimulatie verkrijgen (appetitief gedrag), òf zo weinig mogelijk (aversief gedrag). Sommige ratten werden gestimuleerd in de laterale hypothalamus, andere in de mediale hypothalamus.

De resultaten van dit experiment kunnen als volgt samengevat worden: CSD veroorzaakte een verstoring van appetitief gedrag, terwijl aversief gedrag niet werd aangetast. De resultaten van de zgn. ambivalente ratten rechtvaardigden de hypothese, dat door de elektrische hypothalamische stimulatie gelijktijdig appetitieve èn aversieve mechanismen werden geaktiveerd. Appetitieve mechanismen zijn op het niveau van de hypothalamus vooral gerangschikt in de laterale regionen, aversieve mechanismen in de mediale regionen (Stein, 1969). Het effekt van CSD op deze mechanismen kan beschreven worden als een blokkering van een tonische kortikofugale excitatie van de appetitieve mechanismen. De neocortex of een gedeelte ervan was in deze testsituatie betrokken bij de regulering van appetitief gedrag, terwijl de regulering van aversief gedrag niet afhankelijk was van deze kortikale betrokkenheid.

Nu is elektrische stimulatie van de hypothalamus een nogal onnatuurlijke vorm van stimulatie, en er zijn diverse onderzoekingen, waarin verschillen tussen de effekten van intrakraniële en "natuurlijke", perifere stimulatie

gevonden zijn. Dat vormde voor ons aanleiding een testsituatie te ontwikkelen, waarin appetitief en aversief gedrag door middel van perifere stimulatie in plaats van door middel van intrakraniële stimulatie geïnduceerd konden worden. In deze testsituatie, die vrijwel identiek is aan die, welke beschreven wordt in hoofdstuk 5, fungeerden veranderingen in bodemtemperatuur als stimuli, door middel waarvan binnen één stimulus-dimensie appetitief dan wel aversief gedrag geïnduceerd kon worden. Uit voor-experimenten was gebleken, dat binnen de range van 5-35 graden Celsius verhogingen van bodemtemperatuur appetitief gedrag induceerden, terwijl verlagingen van bodemtemperatuur binnen deze range aanleiding gaven tot aversief gedrag. In hoofdstuk 6 wordt een experiment beschreven, waarin de invloed van CSD op deze door perifere stimulatie geïnduceerde gedragingen werd nagegaan.

De resultaten van dit experiment vormen een bevestiging van de hypothese, die geformuleerd is op basis van het experiment met intrakraniële stimulatie: uitschakeling van de neocortex door middel van CSD veroorzaakte een verstoring van het appetitieve gedrag, terwijl het aversieve gedrag intakt bleef.

Een vergelijking van de habituatie-experimenten met de konditioneringsexperimenten levert een interessante tegenstelling op. De effekten van CSD in de habituatie-experimenten leiden tot de hypothese van een tonische kortikofugale inhibitie van die gedeelten van de retikulaire formatie, die betrokken zijn bij de sensitisatiereaktie op aversieve stimuli. De effekten van CSD in de

konditioneringsexperimenten leiden daarentegen tot de hypothese van een tonische kortikofugale excitatie van die gedeelten van de hypothalamus, die betrokken zijn bij appetitieve regulatie-mechanismen. De sensitisatie-mechanismen in de retikulaire formatie vertonen een mediale organisatie, terwijl de appetitieve mechanismen op het niveau van de hypothalamus lateraal zijn georganiseerd. Uitgaande van de hypothese, dat de tegengestelde appetitieve en aversieve mechanismen steeds gelijktijdig geaktiveerd worden, kan nu de volgende vraag worden gesteld. Kunnen de resultaten van zowel habituatie- als konditioneringsexperimenten misschien verklaard worden in termen van uitschakeling van één van beide kortikofugale projektie-systemen, of moet men aannemen dat beide kortikofugale projektie-systemen gedurende CSD komen stil te vallen? De resultaten van elektrofysiologische experimenten lijken een ondersteuning te vormen voor het tweede alternatief. In de retikulaire formatie zijn neuronen gevonden, die gedurende CSD hun aktiviteit verhoogden, terwijl de aktiviteit van vele neuronen in de laterale hypothalamus gedurende CSD vrijwel geheel tot stilstand kwam (Bures e.a., 1974). Deze blokkering van hypothalamische aktiviteit was niet een direkt gevolg van de toename van retikulaire aktiviteit, want in *cerveau isolé* preparaten bleek de toename van retikulaire aktiviteit gedurende CSD niet meer gevonden te worden; de blokkering van hypothalamische aktiviteit was daarentegen nog steeds aanwezig.

Gedurende CSD is de elektrische aktiviteit van de ge-

hele neocortex onderdrukt. Men kan zich de vraag stellen, of de effekten van CSD in de habituatie- en konditioneringsexperimenten veroorzaakt werden door de uitschakeling van de gehele neocortex, of dat uitschakeling van slechts een gedeelte van de neocortex verantwoordelijk was voor de gevonden effekten. Aan de hand van chirurgische lesie-experimenten is aangetoond, dat de prefrontale cortex betrokken is bij de neurale regulering van habituatie en sensitisatie; Thompson e.a. (1973) hebben in dit verband opgemerkt dat de effekten van pre-frontale lesies geïnterpreteerd kunnen worden als verhoogde sensitisatie ten gevolge van disinhibitie van retikulaire arousal. De prefrontale cortex is tevens nauw betrokken bij de regulering van appetitief gedrag (Routtenberg, 1971; Kolb and Nonneman, 1975); de effekten van CSD op door hypothalamische stimulatie geïnduceerd appetitief gedrag zijn vergelijkbaar met de effekten van prefrontale lesies op dit gedrag (Rolls and Cooper, 1974). De prefrontale cortex onderhoudt efferente verbindingen met limbische strukturen van diencephalon en mesencephalon, onder andere hypothalamus en retikulaire formatie. Op dezelfde wijze als andere schorsvelden de neokortikale uitbouw vormen van sensorische en motorische systemen, zo kan de prefrontale cortex beschouwd worden als de neokortikale uitbouw van het limbisch systeem (Nauta, 1964). Op grond van deze overwegingen lijkt de hypothese gewettigd dat zowel de CSD-effekten in de habituatie-experimenten als die in de konditioneringsexperimenten in feite toegeschreven kunnen worden aan de uitschakeling van de prefrontale cortex. Er zijn enige aanwijzingen, dat de inhibitie van sensitisatie-

mechanismen uitgaat van de mediale prefrontale cortex (Kolb, 1974), en dat de excitatie van appetitieve regulatiemechanismen bemiddeld wordt via de sulkale prefrontale cortex (Rolls and Cooper, 1974 ; Kolb and Nonneman, 1975). Ook op het niveau van de prefrontale cortex lijken de appetitieve mechanismen dus lateraal georganiseerd te zijn, de aversieve mechanismen mediaal. Bij de rat verlopen de efferente verbindingen van de sulkale prefrontale cortex onder andere naar de laterale hypothalamus; de mediale prefrontale cortex heeft efferente verbindingen naar de pretektale regionen in de middenhersenen (Leonard, 1969). De afferente verbindingen naar zowel sulkale als mediale prefrontale cortex zijn afkomstig van de mediodorsale kern van de thalamus (Leonard, 1969). De mediodorsale kern van de thalamus wordt ook een rol toegekend in de neurale regulering van appetitief en aversief gedrag (Keene and Casey, 1973).

Wanneer we de experimenten die beschreven worden in dit proefschrift in hun geheel overzien, dan is het mogelijk op basis van de resultaten het volgende theoretische model te formuleren met betrekking tot de neurale mechanismen van appetitief en aversief gedrag. Toenaderingsbewegingen van de ledematen, die zich vroeg in de ontogenese manifesteren als extensie- en abduktiebewegingen, worden gereguleerd door lateraal verlopende motorische systemen, die uitgaande van de linker en rechter hersenhemisfeer zich beperken tot het sturen van de kontralaterale ledematen. Verwijderingsbewegingen van de ledematen , die zich vroeg in de ontogenese manifesteren als flexie- en adduk-

tiebewegingen, worden gereguleerd door mediaal verlopende motorische systemen, die uitgaande van de linker en rechter hemisfeer een bilaterale sturing van de ledematen bewerkstelligen. De lateraal verlopende regulering van toenaderingsbewegingen der ledematen is gerelateerd aan eveneens lateraal verlopende mechanismen van appetitief gedrag, die zoek- en toenaderingsgedragingen van het organisme reguleren. De mediaal verlopende regulering van verwijderingsbewegingen der ledematen is gerelateerd aan eveneens mediaal verlopende mechanismen van aversief gedrag, die verwijderings- en vermijdingsgedragingen van het organisme reguleren. De tegengestelde mechanismen van appetitief en aversief gedrag zijn gelijktijdig aktief; bij de aanbieding van een stimulus hangt het van de relatieve momentane aktivatie van de appetitieve en aversieve mechanismen af of het overte gedrag zal bestaan uit appetitief of aversief gedrag ten opzichte van deze stimulus.

Voor de regulering van appetitief en aversief gedrag is de integriteit van de prefrontale cortex van essentieel belang. De sulkale prefrontale cortex oefent een tonisch exciterende invloed uit op appetitieve mechanismen in de laterale hypothalamus; de mediale prefrontale cortex oefent een tonisch inhiberende invloed uit op aversieve mechanismen in de mesencefale retikulaire formatie.

INTRODUCTION AND SUMMARY.

Since many years behavioral scientists have been interested in the neural regulation of appetitive and aversive behavior. "..... la volonté qu'on a d'obtenir quelque bien,ou de fuïr quelque mal, envoye promptement les esprits du cerveau vers toutes les parties du corps, qui peuvent servir aux actions requises pour cèt effect; ....." (Descartes, 1644, p. 141).

According to Descartes striving for something good and averting from something bad belong to the fundamental passions which direct behavior. He ascribed these two opponent tendencies to the operation of only one passion: desire. In Descartes' view the behavior of the brute animal is completely determined by his passions; on the contrary, the human being possesses the capacity to use experience and reason in distinguishing between good and bad; in this way he is also capable to control his passions. In other words: animal behavior is completely governed by instincts (innate reaction patterns elicited by particular stimuli); human behavior on the other hand is placed under the control of reason. For centuries this strict dichotomy between man and animal, between reason and instinct has had a determining influence on the study of human and animal behavior (Beach, 1955).

About hundred years ago Charles Darwin in his evolution theory rejected the strict dichotomy between man and animal. Darwin stated that the evolution of species, man included, has proceeded along lines of natural selection;

this theory implicates a congeniality of man and other animal species in the sense of a common origin. The dichotomy between reason and instinct was not removed in this theory. Early attempts to substantiate Darwin's theory of congeniality of man and other animal species implied a search for two kinds of evidence: on the one hand it was tried to prove the existence of human instincts; on the other hand the existence of reason in animals (particularly intelligence) was investigated.

Gradually in psychology an anti-instinct movement developed, which has had a strong influence on the development of this science in the first half of the 20th century. Research was mainly directed to the investigation of learning processes. This approach could not prevent the necessity to incorporate instinct-related concepts into the various theories of learning: for instance unconditioned reactions, elicited by unconditioned stimuli. This development in psychology was largely determined by researchers in the United States and the Soviet Union; for a long time it remained isolated from the development of ethology, which took largely place in Europe. With Darwin as spiritual founder, ethologists mainly studied instinctive behavior (Burghardt, 1973). Meanwhile the term instinctive behavior was replaced by the terms fixed action patterns or species-specific behavior.

Some years ago a new development started, which is of utmost importance for both psychology and ethology. This development is directed towards an integration of psychology and ethology (Schneirla, 1966, Hinde, 1966; Burghardt,

1973). Not only with respect to methodology, but also on a theoretical level the communication between both disciplines has produced important results already. Especially the research into the biological boundaries of learning (Seligman and Hager, 1972; Hinde and Stevenson-Hinde, 1973) is important, because the central concepts of both disciplines, viz. learning and species-specific behavior, are confronted with each other in this research. It has been clearly established that in the search for general laws of learning one has to take into account the species-specific and individual-specific reaction patterns, which form the basis of learning (Seligman and Hager, 1972; Nebylitsyn and Gray, 1972).

Reward or reinforcement on one side and punishment on the other side are central concepts in the psychology of learning. If a behavior of the organism is followed systematically by a specific stimulus, then the frequency of occurrence of that behavior can be changed. If frequency increases, the sequence is called a reward procedure; if frequency decreases, it is called a punishment procedure. Whether presentation of a stimulus to a certain individual of a certain species will result in a reward or punishment effect depends on the unconditioned reaction to this stimulus (Vossen, 1973). In particular, the direction of the unconditioned reaction is important. The unconditioned reaction may manifest itself either as an approach reaction towards the stimulus or as a withdrawal reaction away from the stimulus. Presentation of a stimulus that induces approach generally has a reward effect; on the contrary, pres-

entation of a stimulus that induces withdrawal has a punishment effect (Glickman and Schiff, 1967). Approach is defined here as a decrease of distance between organism and stimulus, withdrawal as an increase of this distance. In this definition approach can involve a movement of the organism towards the stimulus (e.g. an animal approaches another animal) or a movement of the stimulus towards the organism (the animal allows another animal to approach). In the same way withdrawal can involve a movement of the organism away from the stimulus (e.g. an animal flees from another animal) or a movement of the stimulus away from the organism (the animal freezes which causes another animal to go away). Aggressive behavior seems to fit in this classification rather difficultly at first sight, but, depending on the final result of this behavior, it may also be classified as approach or withdrawal (Rasa, 1975).

"In the evolution of behavior, operations which appropriately increase or decrease distance between organisms and stimulus sources must have been crucial for the survival of all animal types" (Schneirla, 1965, p. 2). Theodore C. Schneirla, an early American ethologist, has played an important role in initiating the communication between psychology and ethology (Piel, 1970). Main topics in his work were the biphasic processes of approach and withdrawal.

In his concepts of approach and withdrawal Schneirla referred to direct orientation with respect to present stimuli. These concepts were distinguished from the concepts of seeking and avoidance which referred to absent stimuli. Seeking involves approach towards a stimulus

outside the momentary perceptual field; avoidance involves withdrawal from a stimulus outside the momentary perceptual field (Schneirla, 1959). In seeking and avoidance, learning processes play an important role. In this dissertation seeking and approach behaviors are called appetitive behaviors; withdrawal and avoidance behaviors are called aversive behaviors. This terminology originates from Craig (1918), who considered the opponent affective states of appetite and aversion as constituents in the regulation of behavior. The stimuli which induce appetitive and aversive behavior, are respectively called appetitive and aversive stimuli. In this framework it should be noted, that a stimulus always has a relationship with other stimuli. Therefore, it may happen that an organism withdraws from or avoids a normally appetitive stimulus in order to withdraw from or avoid a stronger aversive stimulus, which this appetitive stimulus is related to (e.g. conditioned emotional response). In the same way an organism may seek or approach a normally aversive stimulus in order to seek or approach a stronger appetitive stimulus, which this aversive stimulus is related to (e.g. obstruction box behavior).

Schneirla (1959) stated that approach and withdrawal are the only empirical, objective terms applicable to all motivated behavior in all species. As far as human behavior is concerned, he has specified this statement. In the course of human ontogenesis behavior becomes so strongly differentiated, that in the adult it is often difficult to decide whether a particular behavior has to be considered an approach or a withdrawal behavior. In the human neonate, however, the distinction between approach and withdrawal

reactions is very clear. Originally, approach reactions manifest themselves as undifferentiated extension and abduction of all limbs and a turning of the head towards the stimulus; gradually these reactions become more and more differentiated (a.o. reaching movements) and start to play an important role in turning towards the world. On the other hand, withdrawal reactions manifest themselves as flexion and adduction of all limbs; withdrawal reactions also become differentiated (a.o. flexion reflex) and have a function in turning away from the world. Buytendijk had described these opponent reactions of approach and withdrawal also in animal neonates:
"De grondkenmerken van het dierzijn, n.l. de geslotenheid en openheid komen daarbij in een zeer bijzondere spanning, doordat de vreemde situatie het dier afscheidt, terugwerpt op zichzelf, d.w.z. een tendenz van terugtrekken, vlucht, bewegingsarmoede, gemis aan expansiviteit aanwezig is ..... Tegelijkertijd is er echter bij het jonge dier een tegengestelde grondtendenz n.l. die, welke gericht is om zo spoedig mogelijk het evenwicht in de existentie te herstellen door het vormen van een kontakt met het nieuwe milieu." (Buytendijk, 1938, p. 106)

In this dissertation an onset is given towards a theory concerning the neural regulation of appetitive and aversive behavior. Basic to this theory is a hypothesis which is formulated as follows: if the organism is confronted with a stimulus, two opponent mechanisms are set into operation: one mechanism prompting the organism to appetitive behavior and another mechanism prompting it to aversive

behavior. Various factors determine the relative strength of activity of both mechanisms, e.g. species-specific characteristics, individual-specific characteristics, stimulus characteristics and previous experience with the stimulus. Whether overt behavior will be appetitive or aversive depends on the relative momentary activation of both mechanisms. The hypothesis of two opponent processes implicates a relation between the theory formulated in this dissertation and other opponent-process theories, which have been formulated on the level of motivation (Solomon and Corbit, 1974) and neural organization (Hurvich and Jameson, 1974). On the contrary, the hypothesis is in contrast with the opinion of theorists like Descartes, who think the distinction between appetitive and aversive behavior to be senseless; according to them in both appetitive and aversive behavior one may speak of movement from a non-desirable to a desirable situation. Although this opinion may be defendable at the level of overt behavior, it is improbable that at the level of neural organization only one mechanism is operative; particularly the effects of intracranial stimulation substantiate an opponent process theory (a.o. Glickman and Schiff, 1967).

Six investigations are reported in this dissertation; the results of these investigations suggest some hypotheses concerning the neural mechanisms of appetitive and aversive behavior. On the one hand the mechanisms of appetitive and aversive behavior have been investigated with regard to the horizontal transfer of information between both hemispheres of the brain; on the other hand the vertical communication between neocortex and subcortical structures has

been investigated.

In chapter 1 two experiments in adult human subjects are described. In these experiments visual-motor coordination was investigated; subjects had to perform left and right arm abduction movements (away from the body) and adduction movements (towards the body). Both speed and accuracy of these movements were measured. According to Schneirla's theory early in ontogenesis abduction movements are to be regarded as approach movements, adduction movements as withdrawal movements. It is likely that this original bipolarity will be maintained in some way or another at a later age. Both movement types were investigated in two conditions. In one condition interhemispheric transfer of information had to take place; in the other condition visual-motor control could take place intrahemispherically. On the basis of the results a hypothesis is formulated concerning the neural pathways via which control of abductive and adductive arm movements is exerted: abduction movements are mediated by lateral descending motorsystems projecting to the contralateral side of the spinal cord; adduction movements are mediated by medial descending motor systems projecting to the spinal cord bilaterally. Therefore, on the basis of Schneirla's theory it may be stated, that approach movements of the arm are controlled by way of lateral systems with contralateral projection, while withdrawal movements of the arm are controlled by way of medial systems with bilateral projection. The interpretation of these experiments in human subjects shows resemblance to the results of animal experiments, in which the hypotha-

lamus is stimulated electrically: stimulation of the lateral hypothalamus generally induces approach movements, stimulation of the medial hypothalamus induces withdrawal movements (Stein, 1969).

In the experiments described in the remaining five chapters rats were used as subjects, thus enabling a direct intervention in the functioning of the brain. The technique which was used is known as cortical spreading depression (CSD). The phenomenon of CSD was discovered by the Brazilian physiologist Leao in 1944 and was introduced in physiological psychology by the Czech scientist Dr. Jan Bures about 1960. By way of this technique a reversible lesion of the neocortex can be induced. The CSD technique involves application of a solution of potassium chloride to the dura. This intervention causes a local depression of electrocortical activity, which spreads over the entire neocortical surface as a slowly propagated wave. Several depression waves in succession cause a relatively permanent depression of neocortical activity, which may be maintained for several hours. After termination of treatment activity slowly recovers. The CSD-technique is well suited for the investigation of interhemispheric and cortico-subcortical relations (Van der Staak, 1969; Bures et al, 1974).

In the experiments described in chapters 2-6 appetitive and aversive stimuli were presented in order to induce appetitive and aversive behavior, respectively. Two test procedures were used. The first one was a habituation procedure; in this procedure stimuli were presented independent of the behavior of the animal. Repeated stimulus presentat-

ion resulted in a decrease of the reaction to these stimuli. The second procedure was a conditioning procedure; in this procedure stimulus presentation was made contingent on the occurrence of a specified response of the animal. In this way the frequency of this response could be increased or decreased. Habituation and conditioning are the foremost processes, which enable behavioral plasticity (Vossen, 1969).

In the chapters 2, 3 and 4 experiments concerning the effects of CSD on habituation are described. Although much research has been directed to the effects of CSD on learning processes, few experiments have been directed to the effects of CSD on habituation, one of the most elementary forms of behavioral plasticity. Habituation may be defined as a decrement of an unconditioned response, if execution of this response has no biological relevance (Vossen, 1973). In the experiments described in chapters 2 and 3 habituation to aversive acoustic stimuli was investigated. In the experiments of chapter 2 arrest of ongoing drinking behavior in reaction to intense ringing of a door-bell was recorded; the experiment of chapter 3 concerned startle reactions to intense tone stimuli. In chapter 4 an experiment is described, in which the effects of CSD on habituation of locomotor activity in an open field were studied. Initially it was assumed that this locomotor activity has to be interpreted in terms of approach (i.c. exploration, cfr. Vossen, 1966); later it became clear that this assumption is not justified: in the open field approach as well as withdrawal reactions may occur: exploration or escape (Archer, 1973).

The results of these habituation experiments may be summarized as follows: CSD causes an increased sensitization; sensitization is defined as an increase in arousal following repeated presentation of aversive stimuli. This increased sensitization is ascribed to a disinhibition of reticular arousal, due to elimination of neocortical activity. In other words: normally the neocortex or part of it has a tonic inhibitory influence on the activity of those parts of the reticular formation, which are involved in the sensitization reaction to aversive stimuli. The reticular formation is located in the medial regions of the brain stem. It seems worthwhile to mention that there are indications that neural regulation of sensitization reactions also takes place at the level of the thalamus: repeated stimulation of medial thalamic regions induced sensitization reactions, while repeated stimulation of lateral regions induced mainly habituation reactions (Wester, 1971, Thompson et al, 1973).

In the experiments described in the last two chapters a conditioning procedure was used: presentation of appetitive or aversive stimuli was made contingent on the occurrence of a specified response. During the experiments described in chapters 2 - 4 emphasis had changed gradually. Initially we were mainly interested in horizontal transfer of information between both hemispheres; later on emphasis was laid more and more to the vertical transfer of information between neocortex and subcortical structures. This change of emphasis was instigated by the fact that substantial changes in motivation take place during CSD: the animal

does not eat or drink anymore and shows changes in other behaviors, the regulation of which depends on the integrity of subcortical structures, the hypothalamus in particular. Electrophysiological investigations have shown, that CSD causes a substantial reduction of hypothalamic activity; this is interpreted as an interruption of tonic corticofugal excitation.

In the experiment described in chapter 5 the hypothalamus was stimulated electrically by way of intracranial electrodes; the influence of CSD on the behavioral effects of this stimulation was studied. In 1954 Olds and Milner discovered, that electrical stimulation of the hypothalamus can have a reward effect in a learning situation. If a particular response of a rat is followed systematically by electrical stimulation in the hypothalamus, the animal will start to generate this response in a high frequency. Today intracranial electrical self stimulation is studied extensively. In the same year 1954 Delgado et al reported, that electrical stimulation of the midbrain reticular formation can have a punishment effect in a learning situation; this phenomenon has also been extensively investigated. Glickman and Schiff (1967) related the reward or punishment value of intracranial stimulation to the behaviors which are induced by such stimulation. Rewarding electrical stimulation usually induces appetitive behaviors; punishing stimulation induces aversive behaviors.

The extensive research on the behavioral effects of intracranial stimulation is based on the presupposition that these phenomena contain the key to understanding the neural bases of motivation and learning (Valenstein, 1973).

Curiously enough, only few investigators directly compared the two opposite effects of intracranial stimulation (reward vs. punishment; appetitive vs. aversive effect). In the experiment described in chapter 5 the influence of CSD was studied both on appetitive and aversive behavior induced by hypothalamic stimulation. Lateral hypothalamic stimulation generally induces appetitive behavior; medial hypothalamic stimulation induces aversive behavior (Stein, 1969). We developed a test situation devoid of a number of artefacts, which disturb interpretation in current test situations. The rats were tested in a shuttle box and had to generate opposite behaviors (barrier crossing response or inhibition of that response) in order to obtain the same result: either maximization of stimulation (appetitive behavior) or minimization of stimulation (aversive behavior). Some rats were stimulated in the lateral hypothalamus, others in the medial hypothalamus.

The results of this experiment may be summarized as follows: CSD disturbed appetitive behavior; it did rot disturb aversive behavior. The results of the ambivalent rats justified the hypothesis that electrical hypothalamic stimulation activates both appetitive and aversive mechanisms at the same time. At the hypothalamic level appetitive mechanisms are arranged laterally, while aversive mechanisms have a medial arrangement (Stein, 1969). The effect of CSD on these mechanisms may be described as an interruption of tonic corticofugal excitation of appetitive mechanisms. The neocortex or part of it was involved in the regulation of appetitive behavior in this test situation, while the regulation of aversive behavior was not

dependent on this cortical involvement.

Electrical hypothalamic stimulation is a rather unnatural type of stimulation and the effects of intracranial stimulation differ in some important respects from the effects of "natural", peripheral stimulation. Therefore, we developed another test situation using peripheral instead of intracranial stimulation. This test situation was almost identical to the test situation described in chapter 5, but now changes in floor temperature were used as stimuli in order to induce appetitive or aversive behavior within one stimulus dimension. Preliminar experiments had shown, that within the range of 5^{o}-35^{o} C a raise in floor temperature induced appetitive behavior, while a fall in floor temperature induced aversive behavior. In chapter 6 an experiment is described, in which the effects of CSD on these, by peripheral stimulation induced, behaviors were studied.

The results of this experiment confirm the hypothesis based on the intracranial stimulation experiment: depression of neocortical activity during CSD caused a disturbance of appetitive behavior, while aversive behavior remained intact.

A comparison between the habituation experiments and the conditioning experiments reveals an interesting contrast. The effects of CSD in the habituation experiments lead to the hypothesis of a tonic corticofugal inhibition of those parts of the reticular formation, which are involved in sensitization reactions to aversive stimuli. On the other hand, the effects of CSD in the conditioning ex-

periments lead to the hypothesis of a tonic corticofugal excitation of those parts of the hypothalamus, which are involved in appetitive regulation. The sensitization mechanisms in the reticular formation show a medial arrangement, while the appetitive mechanisms are arranged laterally at the level of the hypothalamus. Taking into consideration the hypothesis that the opponent mechanisms of appetitive and aversive behavior are always simultaneously activated, the following question may be put forward. It is possible to explain the results of both habituation and conditioning experiments in terms of interruption of one corticofugal projection system, or is it necessary to assume, that both corticofugal systems are interrupted during CSD? Results of electrophysiological experiments clearly substantiate the second alternative. In the reticular formation neurons have been found, which increased activity during CSD; the activity of many neurons in the lateral hypothalamus was completely blocked during CSD. This blocking of hypothalamic activity was not an indirect effect of the increase in reticular activity: in *cerveau-isolé* preparations the increase in reticular activity was not found anymore, while the blocking of hypothalamic activity was still present (Bures et al, 1974).

During CSD the electrical activity of the entire neocortex is depressed. Are the effects of CSD found in these experiments caused by depression of the entire neocortex, or was depression of only part of it responsible for these effects? In surgical lesion experiments it has been demonstrated, that the prefrontal cortex is involved in the

neural regulation of habituation and sensitization; Thompson et al (1973) suggested that the effects of prefrontal lesions may be interpreted as increased sensitization caused by disinhibition of reticular arousal. The prefrontal cortex is also intimately involved in the regulation of appetitive behavior (Routtenberg, 1971; Kolb and Nonneman, 1975); the effects of CSD on appetitive behavior induced by hypothalamic stimulation are comparable to the effects of prefrontal lesions on this behavior (Rolls and Cooper, 1974). The prefrontal cortex has efferent connections with limbic structures of diencephalon and mesencephalon, a.o. hypothalamus and reticular formation. In the same way as other cortical areas constitute a neocortical extension of sensory and motor systems, the prefrontal cortex may be considered as a neocortical extension of the limbic system (Nauta, 1964). These considerations justify the hypothesis that the CSD-effects found in both habituation and conditioning experiments are to be ascribed to depression of the prefrontal cortex. An experiment of Kolb (1974) suggests that the inhibition of sensitization mechanisms originates from the medial prefrontal cortex; the excitation of appetitive mechanisms seems to originate from the sulcal prefrontal cortex (Rolls and Cooper, 1974; Kolb and Nonneman, 1975). So, also at the level of the prefrontal cortex the appetitive mechanisms seem to have a lateral arrangement, the aversive mechanisms a medial one. In the rat the efferent connections of the sulcal prefrontal cortex course mainly to the lateral hypothalamus; the medial prefrontal cortex has efferent connections with the pretectal regions in the midbrain (Leonard, 1969).

The afferent connections of both sulcal and medial prefrontal cortex originate from the mediodorsal nucleus of the thalamus (Leonard, 1969). The mediodorsal nucleus of the thalamus is also supposed to play a role in the neural regulation of appetitive and aversive behavior (Keene and Casey, 1973).

On the basis of the experiments described in this dissertation a theoretical model is formulated concerning the neural mechanisms of appetitive and aversive behavior. Approach movements of the limbs, which manifest themselves early in ontogenesis as extension and abduction movements are controlled by way of laterally descending motor systems; these lateral systems originating from the left and right hemisphere are limited to the control of contralateral limbs. On the other hand, withdrawal movements of the limbs, which manifest themselves early in ontogenesis as flexion and adduction movements, are controlled by way of medially descending motor systems; these medial systems also originate from the left and right hemisphere but exercise a bilateral control of the limbs. The laterally descending systems of approach movements of the limbs are related to the lateral mechanisms of appetitive behavior, which regulate searching and approach behavior of the organism. The medially descending systems of withdrawal movements of the limbs are related to the medial mechanisms of aversive behavior, which regulate withdrawal and avoidance behavior of the organism. The opponent mechanisms of appetitive and aversive behavior are always simultaneously activated; given the presentation of a particular stimulus

it depends on the relative momentary activation of appetitive and aversive mechanisms whether overt behavior will manifest itself as appetitive or aversive behavior with regard to that stimulus. The prefrontal cortex plays an essential role in the regulation of appetitive and aversive behavior. The sulcal prefrontal cortex exerts a tonic excitatory influence on appetitive mechanisms in the lateral hypothalamus; the medial prefrontal cortex exerts a tonic inhibitory influence on aversive mechanisms in the midbrain reticular formation.

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CHAPTER 1. INTRA- AND INTERHEMISPHERIC VISUAL-MOTOR CONTROL OF HUMAN ARM MOVEMENTS.

1.1. *Abstract.*

In 2 experiments normal human subjects performed movements with the left or right arm. Visual-motor control was intrahemispheric or interhemispheric. Direction of movements was adductive or abductive. It was concluded that abductive movements are controlled by the contralateral hemisphere, while adductive movements can be controlled by either hemisphere.

It was also suggested via which pathways this control is exerted. Control of abductive movements was related to a lateral system, which projects to the contralateral side of the spinal cord; control of adductive movements was related to a medial system, which projects bilaterally to the spinal cord.

1.2. *Introduction.*

Regulation of movements on the basis of visual information is called visual-motor control. Two aspects can be distinguished here: Initiation of movement on appearance of a visual signal and guidance of movement on the basis of visual feedback. About the central neural pathways involved in the mediation of visual-motor control little is yet known (1).

One way to study the course of visual-motor control in the brain is to compare intrahemispheric with interhemispheric control. In the first case the relevant visual information is projected to the hemisphere from which the

motor control of a particular movement is supposed to originate; in the second case the visual information is projected to the contralateral hemisphere. In the first case the visual-motor control can take place within one hemisphere, while in the second case a communication has to be established between visual areas in one hemisphere and motor areas in the other. Therefore interhemispheric control would take more time and/or be less accurate (2).

In normal human subjects limitation of relevant visual information to one hemisphere can be accomplished by instructing the subject to keep his eyes fixed on a fixation point. Visual information about stimuli to the left of this fixation point will be projected to the visual areas of the right hemisphere of the brain; visual information about stimuli to the right of the fixation point will be projected to the visual areas of the left hemisphere. Information about stimuli very close to the left or right of the fixation point probably is projected not only to the visual areas of the contralateral hemisphere but also to those of the ipsilateral hemisphere. This may be accomplished either directly because of foveal overlap across the midline of the retina or indirectly by way of the corpus callosum (3).

About the motor control of unilateral movements much evidence has been gained from experiments with split-brain subjects: subjects in whom the forebrain commissures have been transsected. It appears that hand and finger movements are controlled exclusively by the contralateral hemisphere, while arm movements may be controlled by either hemisphere (4, 5). Another useful approach is the

study of motor performance in patients with unilateral brain lesions (6, 7). We have followed a third approach: experimentation on normal human subjects (8).

The investigations to be reported here originate from the question whether it can be shown in normal human subjects that different kinds of arm movements are under the visual-motor control of either of the two hemispheres. We have chosen adductive and abductive movements because of suggestions made by Schneirla (9) and Tarantino (10), that these two types of movements are controlled by different behavior regulating mechanisms. These are movements around the shoulder-joint: Adductive movements are defined as movements which generally bring the arm towards the body and abductive movements as movements which generally bring the arm away from the body. Speed and accuracy of adductive and abductive movements are investigated in the case of intrahemispheric control as well as in the case of interhemispheric control. Tasks were chosen so as to make visual guidance of the movements necessary, because Lehman (2) has suggested that this is an important factor in the determination of differences between intrahemispheric and interhemispheric control.

We speak of uncrossed (intrahemispheric) control, when the arm is moved towards a stimulus in the ipsilateral half of the visual field (right arm - right visual field, left arm - left visual field) and of crossed (interhemispheric) control when the arm is moved to a stimulus in the contralateral half (left arm - right visual field, right arm - left visual field). In the analyses the two uncrossed arm-field combinations are taken together and compared with the two crossed combinations taken together.

In this way uncrossed and crossed control can be compared without possible influence of systematic differences between the two arms or between the two halves of the retina (1).

If it is found that for a particular type of movement of one arm, speed and accuracy are higher during uncrossed control than during crossed control, it may be concluded that the visual-motor control of such movements is initiated from the contralateral hemisphere. In the case of crossed control, interhemispheric transfer of information should then have taken place; this takes time and causes loss of information (2). If no difference is found between uncrossed and crossed control, it may be concluded that this type of arm movement can be controlled by either hemisphere.

1.3. Experiment 1.

In this experiment speed of abductive and adductive movements was investigated during uncrossed and crossed visual-motor control.

Subjects.

Subjects were 10 right-handed undergraduate students in psychology. They were naive with respect to the task to be performed.

Apparatus and procedure.

The apparatus consisted of a 60 x 60 cm board, positioned at a table in front of the subjects, on which at

several places Widmaier switches could be mounted. These are buttons of 12 mm dia, which can be illuminated by a small bulb (6 V, 40 mA) located inside the button; when the button is pressed, illumination stops. There were always 3 buttons on the board: 1 start button and 2 target buttons at 6 cm to the left and right of the start button.

The subject had to perform 2-choice jump reactions: he kept the start button pressed by a finger of his right or left hand; he was instructed to release the start button and press the target button as quickly as possible at the onset of illumination of one of the target buttons. Time between onset of illumination of target button and release of start button (decision time) and time between release of start button and pression of target button (movement time) were recorded in milliseconds (ms). Sequence of illumination of left and right target button was random.

The subject was instructed to maintain fixation of a point in the middle of the board. Fixation was controlled by the experimenter who sat at the other side of the table. Head-movements were prevented by a chin rest. Distance from the eyes of the subject to the fixation point was about 40 cm.

Position of start and target buttons was changed after every 48 trials; there were 9 different positions (Fig. 1). Of these 48 trials, the first 24 were performed with one arm, the last 24 with the other arm. Sequence of positions of buttons and of hands performing the reactions was balanced over subjects. Inter-trial time was 8 sec.

In this way arm, visual field, distance of target

button from fixation point and direction of movement were ordered into a complete factorial design. Movements were obtained of left and right arm on the basis of uncrossed and crossed visual information; the target button was positioned at 6, 12, or 18 cm from fixation and the direction of movement was adductive or abductive.

Direction of movement could be specified as follows (see Fig. 1): when the hand was moved in the direction of the fixation point this movement was called adductive in the case of uncrossed control and abductive in the case of crossed control. When the hand was moved away from the fixation point, this movement was called abductive for uncrossed and adductive for crossed control. So it was *not* the movement of the *hand* with reference to the fixation point *but* the movement of the *arm* with reference to the body which specified the direction as adductive or abductive: towards the body and away from the body respectively. As hand and wrist of the subjects were observed to be kept in a relatively fixed position during movement, it seemed reasonable to specify the direction in terms of arm movements and not of hand movements.

Results.

Decision times (DT) or movement times (MT) which were longer than 1000 ms were discarded, because such long times only were obtained when the subject made an erroneous reaction. On the remaining data analyses of variance were done with Arm (left vs right), Control (uncrossed vs crossed) and Distance (6, 12, 18 cm from fixation) as fixed factors and Subjects (10 Ss) as a random factor (11).

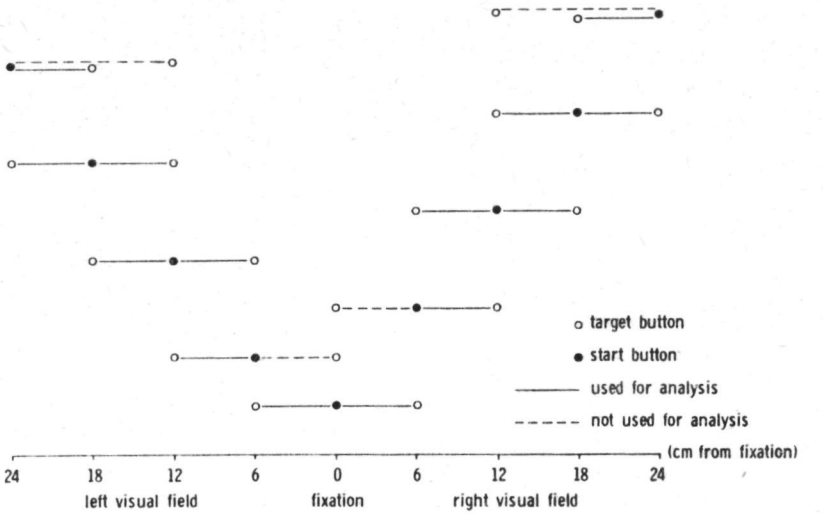

Fig. 1. Position of start and target buttons in Experiment 1.

This was done for adduction and abduction DT's separately and also for adduction and abduction MT's separately (Tables 1 and 2). The subjects effects were not tested for significance. The relevant means are depicted in Fig. 2.

As can be seen from Table 1 the factor Control was significant in the case of abductive DT's; uncrossed DT's were generally smaller than crossed DT's (Fig. 2). For adductive DT's this difference was not significant. The general tendency for DT's to increase with increasing distance from fixation was significant for abductive but not for adductive DT's. The only significant effect in adductive DT's was the Arm x Distance interaction.

For MT's the same general picture emerged; uncrossed MT's were significantly smaller than crossed MT's for abductive, but not for adductive movements. Abductive MT's

Adductive movements				Abductive movements			
Source	Error term	F	P	Source	Error term	F	P
A Arm	A X D	1 32		A Arm	A X D	0 26	
B Control	B X D	1 77		B Control	B X D	15 59	0 004
C Distance	C X D	1 16		C Distance	C X D	4 88	0 02
D Subjects				D Subjects			
A X B	A X B X D	2 93		A X B	A X B X D	0 12	
A X C	A X C X D	7 58	0 004	A X C	A X C X D	0 29	
B X C	B X C X D	0 47		B X C	B X C X D	3 24	0 06
A X B X C	A X B X C X D	0 63		A X B X C	A X B X C X D	0 38	

Table 1. Analyses of variance for decision times in Experiment 1.

Adductive movements				Abductive movemen s			
Source	Error term	F	P	Source	Frror term	F	P
A Arm	A X D	1 60		A Arm	A X D	0 10	
B Control	B X D	1 10		B Control	B X D	34 40	0 0004
C Distance	C X D	1 89		C Distance	C X D	9 72	0 002
D Subjects				D Subjects			
A X B	A X B X D	1 31		A X B	A X B X D	3 81	
A X C	A X C X D	2 73		A X C	A X C X D	1 18	
B X C	B X C X D	0 17		B X C	B X C X D	0 99	
A X B X C	A X B X C X D	0 42		A X B X C	A X B X C X D	1 06	

Table 2. Analyses of variance for movement times in Experiment 1.

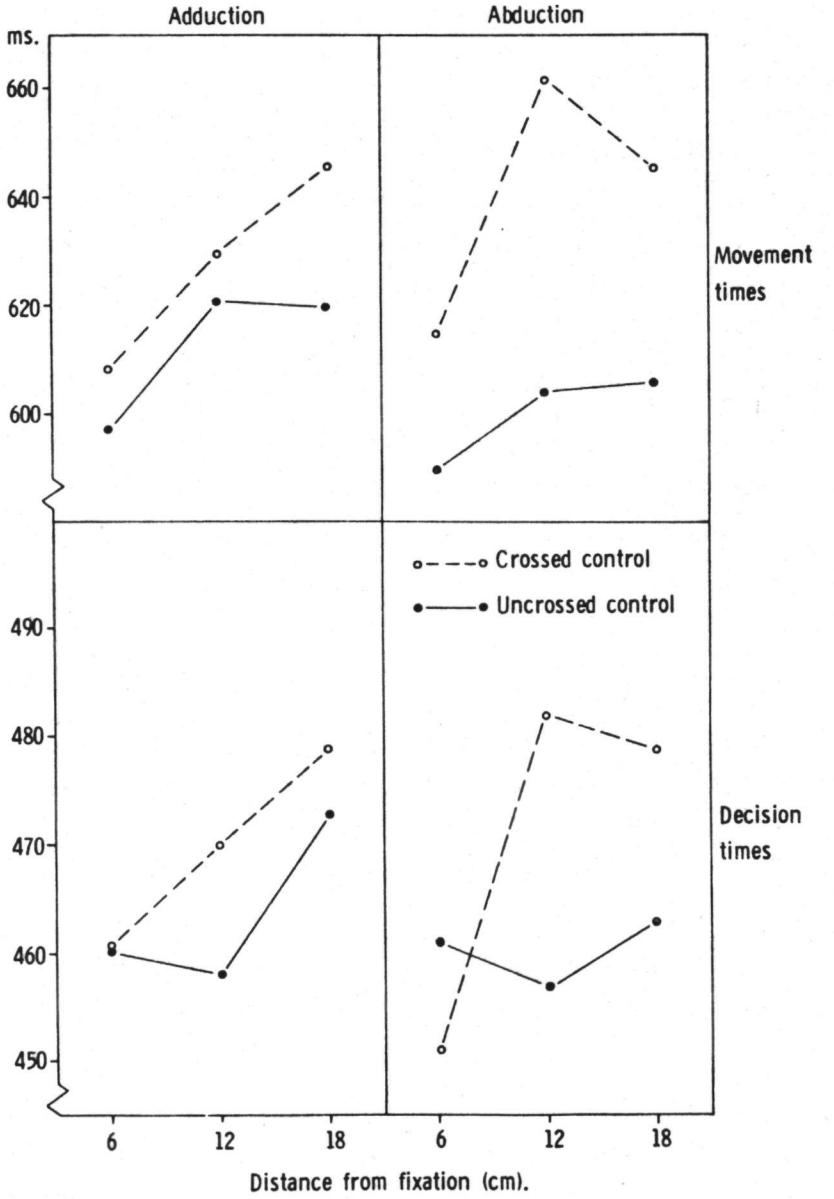

Fig. 2. Mean decision and movement times in Experiment 1.

increased significantly with increasing distance from fixation, while adductive MT's did not.

Discussion.

In this experiment it is clearly demonstrated that speed of initiation *and* speed of execution of abductive arm movements is higher in the case of uncrossed control than in the case of crossed control. This is interpreted as an indication that motor control of abductive arm movements is initiated normally from the contralateral hemisphere and that visual guidance of these movements also takes place from this hemisphere. (See Introduction.) For adductive arm movements these differences between uncrossed and crossed control did not reach significance, so it is concluded that these movements can be controlled by either hemisphere.

It seems reasonable to assume that for crossed abductive movements in this experiment time-consuming interhemispheric transfer of information had to take place. One may ask, however, whether this transfer took place via callosal connections between the visual cortices of both hemispheres or via another callosal or extra callosal pathway (2). The former pathway could only have played a role when the target button was close to the fixation point, because direct callosal connections are restricted to those parts of the visual cortex on which a small vertical strip in the middle of the visual field is projected (3).

Berlucchi et al (1) found in a reaction time experiment with normal human subjects that the difference between uncrossed and crossed reactions did not change

with changing distance of the visual stimulus from fixation (5, 20 and 35 degrees of visual angle). They concluded that on none of the distances interhemispheric transfer had taken place via direct callosal connections between the visual cortices. In our experiment the Control x Distance interaction just failed to reach significance for abductive DT's (Table 1), but on the basis of the graphical representation in Fig. 2 it could be tentatively hypothesized that on distance 6 (corresponding to about 9 degrees of visual angle) interhemispheric transfer took place via callosal connections between the visual cortices; this transfer took little time and no difference or only a small difference appeared between uncrossed and crossed DT's and MT's. On distances 12 and 18 (about 17 and 25 degrees of visual angle) then transfer had to take place via another more time consuming pathway, and therefore crossed DT's and MT's were substantially larger than uncrossed.

The difference between the results of the experiment of Berlucchi et al (1) and of our experiment may be ascribed to differences in the task. Our subjects had to make a decision about the direction of movement; moreover their movements had to be guided visually. May be in our experiment performance of the response demanded a higher degree of cortical control than in the experiment of Berlucchi et al (1).

1.4. Experiment 2.

In this experiment we tried to replicate the findings of Experiment 1 with another task. Moreover, this time not

speed but accuracy of arm movements was measured. We expected to find the same differences between abductive and adductive movements as in experiment 1.

Subjects.

The subjects were 12 right-handed undergraduate students. They were other students than the subjects in Experiment 1 and were also naive with respect to the task to be performed.

Apparatus and procedure.

The task in this experiment was the so-called reciprocal tapping task (12). The subject held a metal stylus in his hand. He was instructed to hit two rectangular target strips alternately with this stylus; these strips were mounted side by side on a wooden board. Both target strips (width 0.5 cm) were flanked to the left and the right with error strips (width 1.5 cm). Target strip and error strips had a different color: copper vs nickel. The number of hits, undershoots and overhoots were recorded on 6 counters. After 1 of the counters of a group of 3 strips had recorded, none of the counters in that group would score again until one of the opposite set of strips had been touched.

The subject was again instructed to maintain fixation of a point in the middle of the board and this fixation was controlled in the same way as in Experiment 1. Head movements were prevented by a chin rest. The distance from the eyes of the subject to the fixation point was about 40 cm.

Both sets of strips were always positioned on the same side of the fixation point, either to the left or to the right. The subject worked either with the left or right arm. The centres of the target strips could be on one of the following distances from the fixation point: 2 and 6 cm, 2 and 8 cm, 2 and 10 cm, 10 and 6 cm, 14 and 8 cm, 18 and 10 cm. Only the performances at distances 6, 8 and 10 cm were used for analysis, because at these distances both abductive and adductive movements were obtained. The target strips on these distances will be called relevant target strips in the following. The length of the movements was 4, 6 and 8 cm on distances 6, 8 and 10 respectively. By complete factorial combination 24 conditions were obtained. In the same way as in Experiment 1 movements were obtained of left and right arm on the basis of uncrossed and crossed visual information; the relevant target strip was positioned at 6, 8 or 10 cm from fixation and the direction of movement was abductive or adductive. Each condition was given twice to every subject. The sequence in which these conditions were presented was balanced over subjects and two sessions in such a way that on each session every subject were given all conditions once.

Speed of tapping movements was kept constant by instructing the subject to let his taps coincide with the sound of a metronome which was given every 800 ms. This was done to prevent a speed-accuracy trade-off. In every presentation of a condition 40 taps were made by the subject, 20 on the irrelevant target strip and 20 on the relevant target strip. As every condition was given twice,

40 relevant taps were obtained per condition. Accuracy of movements in a certain condition was expressed as the percentage of hits in these 40 taps.

Results.

In Table 3 the results of the analyses of variance for the movement accuracy scores are given. Again these analyses were done for adductive and abductive movements separately. Arm (left vs right), Control (uncrossed vs crossed) and Distance (6, 8, 10 cm from fixation) were treated again as fixed factors and Subjects (12) as random factor. The relevant means are depicted in Fig. 3.

For abductive movements accuracy was higher during uncrossed control than during crossed control. For adductive movements there was no significant difference between uncrossed and crossed control.

Both for adductive and abductive movements there was a highly significant decrease in accuracy with increasing distance from fixation. For adductive movements the factor Arm was significant (mean accuracy 38.5 and 45.8 for left and right arm respectively). For abductive movements, finally, there was a significant Arm x Control x Distance interaction (Fig. 4).

Discussion.

The results of this experiment substantiate the conclusion drawn from Experiment 1. In Experiment 1 abductive movements were initiated and executed with higher speed under uncrossed than under crossed control; in this

Adductive movements				Abductive movements			
Source	Error term	F	P	Source	Error term	F	P
A: Arm	A X D	4.73	0.05	A: Arm	A X D	1.59	
B: Control	B X D	0.73		B: Control	B X D	7.19	0.02
C: Distance	C X D	17.03	0.0001	C: Distance	C X D	16.03	0.0001
D: Subjects				D: Subjects			
A X B	A X B X D	0.46		A X B	A X B X D	0.39	
A X C	A X C X D	0.08		A X C	A X C X D	0.05	
B X C	B X C X D	1.00		B X C	B X C X D	1.09	
A X B X C	A X B X C X D	0.30		A X B X C	A X B X C X D	4.50	0.02

Table 3. Analyses of variance for movement accuracy scores in Experiment 2.

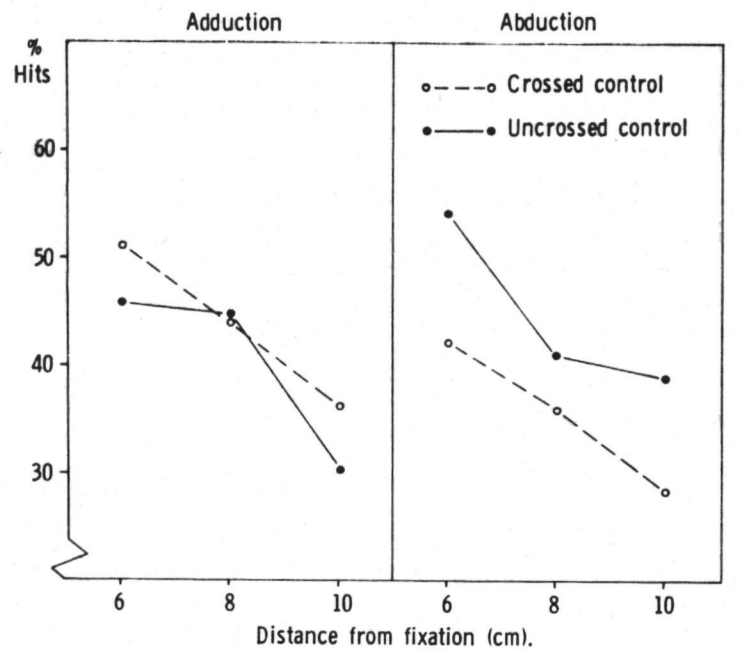

Fig. 3. Mean accuracy scores in Experiment 2.

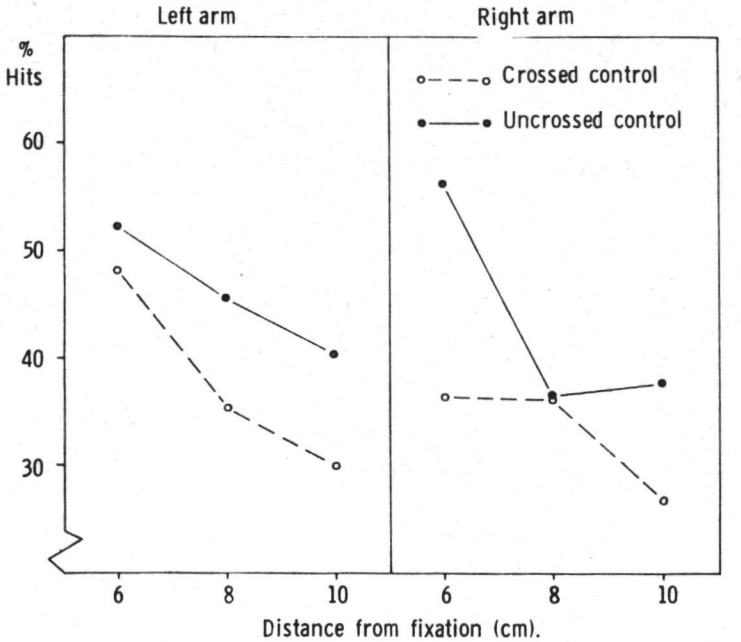

Fig. 4. Mean accuracy scores for abductive movements with left and right arm in Experiment 2.

experiment these same movements appeared to be more accurate under uncrossed control. For adductive movements no such difference was found in Experiment 1 as was also the case in this experiment.

Accuracy decreased with increasing distance from fixation. It must be mentioned, however, that as distance of target strip from fixation point increased the length of movement also increased (4, 6 and 8 cm respectively, see Procedure). This may also have contributed to the decreasing accuracy.

In the discussion of Experiment 1 it was tentatively hypothesized that in the crossed visual-motor control of abductive movements interhemispheric transfer took place

via callosal connections between the visual cortices on distance 6, while on the other 2 distances transfer had to take place via another more time consuming pathway. In this experiment no such tendency was found (Fig. 3). There was, however, a significant Arm x Control x Distance interaction (Table 3). In Fig. 4 this interaction is graphically represented. It can be seen that for the left arm the results show some resemblance to the movement times in Experiment1: the difference in movement accuracy between uncrossed and crossed control was twice as large on distances 8 and 10 as on distance 6. Of course the eccentricity of the target strips in this experiment was not the same as in Experiment 1; in Experiment 1 the distances corresponded to 9, 17 and 25 degrees of visual angle, while in this experiment, they corresponded to about 9, 11.5 and 14 degrees. Nevertheless the picture looks the same. For the right arm the picture looks quite different: movements under uncrossed control were more accurate than under crossed control on distances 6 and 10, but not on distance 8. The reason for this difference between left and right arm is not clear. It could be an indication that the callosal connections between the visual cortices of both hemispheres are not symmetrical in man.

1.5. General Discussion.

In the Introduction we stated that if speed or accuracy of movements of one arm is higher during uncrossed than during crossed control, this may be taken as evidence that these movements are normally controlled by

the contralateral hemisphere. If no differences are found between uncrossed and crossed control, then it may be concluded that these movements can be controlled by either hemisphere. The two experiments which are described here appear to have demonstrated that abductive movements of the arm are controlled by the contralateral hemisphere, while adductive movements can be controlled by either hemisphere. This holds for the initiation as well as for the execution of these movements.

Such a different control of abductive and adductive movements is not without sense. Abductive movements of one arm will mostly be directed towards targets which are in the ipsilateral half of the visual field. Adductive movements may also be directed towards targets in the ipsilateral half of the visual field, but besides may extend towards targets in the contralateral half. Thus, for the visual-motor control of abductive arm movements the relevant visual information will generally be projected to the contralateral hemisphere; for adductive arm movements this information is sometimes projected to the contralateral, sometimes to the ipsilateral hemisphere. Therefore, it would be efficient if adductive movements could be controlled by either hemisphere; for abductive movements control by only the contralateral hemisphere would suffice.

When it is said that abductive arm movements are normally controlled by the contralateral hemisphere, this does not necessarily imply that control by the ipsilateral hemisphere is impossible under all circumstances. In this respect there may be a difference with splitbrain research, where it is generally held that arm movements *can* be

controlled by either hemisphere (4, 5).

The results of these experiments could be explained in another way: maybe abductive arm movements were slower and less accurate under crossed than under uncrossed control because of physical constraints and not because these movements had to be performed in the crossed visual field. Under crossed visual control movements always had to be performed on the crossed side of the body. It is difficult to envisage, however, why this explanation would hold for abduction movements and not for adduction movements. The results of a previous investigation (13) make this explanation also improbable. In that experiment subjects had to perform reciprocal tapping movements between targets on the ipsilateral and contralateral side of the body with either hand and under two visual conditions. In one condition eye movements were allowed, in the other condition fixation was required. The results of that experiment clearly demonstrated that the accuracy of visually guided arm movements depends on the side of the visual field in which these movements take place.

On the basis of the results of these experiments it is possible to make some suggestions about the course of the descending pathways via which the control of abductive and adductive movements is exerted. In neuroanatomical research in the rhesus monkey a distinction is made between direct and indirect corticospinal connections (5). The direct corticospinal connections mainly terminate in the contralateral side of the spinal cord; these connections partly consist of direct cortico-motoneuronal fibers which are important in the control of individual finger movements (14). The indirect connections can be subdivided

into two pathways: in the first place there is a contralateral pathway which consists of ipsilateral cortical projections to the cells of origin of a lateral brainstem pathway, which projects in turn to the contralateral side of the spinal cord; secondly there is a bilateral pathway which consists of bilateral cortical projections to the cells of origin of a ventromedial brainstem pathway, which projects in turn bilaterally to the spinal cord. Thus, there is an indirect contralateral pathway which takes a more lateral course and an indirect bilateral pathway which takes a more medial course.

If it is plausible that control of abductive movements takes place via the lateral pathway and of adductive movements via the ventromedial pathway, this would explain the differences found in our experiments. Lawrence and Kuypers (15) came to the conclusion that the ventromedial pathway is especially concerned with maintenance of erect posture and integrated movements of body and limbs; superimposed on this, independent control of the extremities, mainly the hand, should take place via the lateral pathway. However, individual movements of the hand and arm are mostly directed away from the body, i.e. in an abductive direction. The monkeys of Lawrence and Kuypers (15) in which the lateral pathway was interrupted failed in this type of movement: the arm hung loosely from the shoulder and there was a definite impairment in reaching out for food. This seems to indicate that abductive movements are controlled via the lateral pathway. Adductive movements very often have the function of bringing objects with one hand into the reach of the other hand; i.e. in bimanual

coordination. For the control of these movements the bilateral ventromedial pathway would be more suited.

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CHAPTER 2. HABITUATION TO INTENSE ACOUSTIC STIMULATION DURING CORTICAL SPREADING DEPRESSION IN RATS

(Co-author: Wolfgang Fischer).

2.1. *Abstract.*

Few investigations have focused on the effects of cortical spreading depression (CSD) on habituation. In the 2 experiments to be reported here long term habituation to intense acoustic stimulation was investigated. Arrest of ongoing drinking behavior was used as an index of habituation. In the first experiment habituation training during bilateral CSD was compared to habituation training under nondepressed conditions; testing for long term habituation took place under nondepressed conditions in both groups. In contrast to the control group the bilateral CSD group did not show long term habituation. In the second experiment habituation training during CSD in one hemisphere did not result in long term habituation when testing took place during CSD in the contralateral hemisphere. Possible explanations of these results are discussed.

2.2. *Introduction.*

For about 20 years cortical spreading depression (CSD) has been used as a reversible decortication technique in behavioral research and learning processes in particular have been extensively investigated (3, 12, 13). Thus it is puzzling that habituation, which may be considered as an elementary form of behavioral plasticity, has received such little attention: only two investigations have

focused on the effects of CSD on behavioral habituation (11, 14).

Behavioral habituation refers, within certain limits, to decrement of an unconditioned response as a result of repeated or continuous exposure to the response eliciting stimulus (9). Usually a distinction can be made between short term habituation, i.e. decrement of response magnitude within a single session, and long term habituation, i.e. the decremental effect of habituation in one session on response magnitude in a later session.

In surgical decortication experiments in rats the frontal cortex has been found to be involved in short term habituation (10) and long term habituation (7), but these findings are debatable (8, 16).

During CSD the electrical activity of the total cortex, including the frontal cortex, in one or both hemispheres is depressed. It was decided to start a systematic investigation into habituation of several behavioral reactions during reversible decortication caused by CSD.

Two types of behavioral reaction which are frequently used in habituation experiments are startle response and open field activity; it has been suggested that habituation of these reactions is mediated by different processes (15). A third behavioral reaction sometimes used is arrest of ongoing behavior (mostly drinking) by introduction of an unfamiliar stimulus (4, 5). If this stimulus has been exposed before, arrest of ongoing behavior will be less than if it is presented for the first time. It was decided to investigate habituation of all three types of behavioral reaction during CSD. In the experiments to

be reported here arrest of ongoing drinking behavior was used as an index of prior habituation to intense acoustic stimulation.

2.3. *Experiment 1.*

In this experiment habituation training was given during bilateral CSD or under nondepressed conditions. Testing for long term habituation took place under nondepressed conditions only.

Subjects.

48 Male albino rats (WAG strain) were used. They weighed about 350 g at the time of surgery and were housed in individual cages in a stock-room; food and water were available ad lib. until the first postoperative day when they were deprived of water. They were kept on a 12 hr. light/12 hr. dark cycle and were tested during the light period.

Surgery.

The rats were anesthetized with HYPNORM (10 mg fluanisone, 0.2 mg phentanylcitrate pro ml; 0.08 cc/100 g i.m.), followed by NEMBUTAL (60 mg/ml; 0.03 cc/100 g i.v.). Trephine openings measuring 3 mm in diameter were drilled in the skull about 4 mm behind bregma and about 3 mm to the left and the right of the midline; care was taken not to damage the dura.

Stainless steel epidural cannulae (length 6.5 mm with an internal diameter of 1.5 mm) were placed in these

openings; 0.5 mm from the bottom of the cannulae was a flange of 0.5 mm with which they rested on the skull to prevent pressure on the dura. The cannulae were fixed to the skull with dental cement and fixation screws. Then the dura was moistened with saline and the cannulae were closed with a screwmounted plunger to prevent dehydration. Saline was refreshed on the first postoperative day.

Apparatus.

The testing box was made of plywood, had a 30 x 30 cm metal floor and was 20 cm high; a nipple from which the rat could drink extended from the ceiling of the box. By drinking the rat closed an electrical circuit (2 uA) which triggered a running time meter.

Five training boxes which were identical in shape to the testing box contained no drinkometer circuit. A door-bell was placed in front of the testing box (sound pressure level about 95 dB measured inside the box). Testing box and door-bell were located in a sound-proof cabin. Animals could be observed through observation windows in testing box and sound-proof cabin. Training boxes were located in the stock room.

Procedure.

Animals were randomly assigned to one of four groups: two groups that received bilateral CSD-treatments (Bi+ and Bi-) and two control groups (Co+ and CO-); each group consisted of 12 subjects. From the first post-operative day (day 1) onwards all animals were set on a 24-hours

water deprivation schedule. On day 2 they were trained to drink in one of the training boxes for 45 minutes; on day 3 this time was reduced to 15 minutes.

After drinking training on day 3 bilateral CSD was induced in the animals of groups Bi+ and Bi-. This was achieved by removing the plungers and filling the cannulae with a solution of 25% KCl; cannulae were closed with small screws. Effectiveness of CSD-treatment was tested by examining placing reflexes which are impaired during CSD (3). Animals of control groups Co+ and Co- received the same treatment except that saline was used instead of the KCl solution.

After this treatment animals were returned to their home cages. Cages of Bi+ and Co+ animals were then placed inside the sound-proof cabin next to the door-bell where they were exposed to the sound of the bell which rang intermittently for 13 minutes with a pattern of 2 seconds on - 8 seconds off.

After this exposure KCl was removed and the cannulae were rinsed with saline; animals were then returned to the stock-room.

Bi- and Co- animals received the same treatment except that their cages were not placed inside but outside the sound-proof cabin, so that they would not be exposed to the sound of the bell.

On day 4 the whole procedure of day 3 was repeated with the only difference that drinking training was now given in the testing box instead of the training box and drinking activity was recorded for 15 minutes after the first contact with the water nipple.

On day 5 habituation testing took place. All animals received the same treatment; no CSD was induced.

They were placed in the testing box. After the first contact with the water nipple recording of drinking activity started and continued for 15 minutes. At the beginning of the third minute the bell started to ring in the same pattern as on the preceding days: 2 seconds on - 8 seconds off. This ringing continued for 13 minutes until the end of the session. Bi- and Co- animals heard this sound for the first time, while Bi+ and Co+ animals had been exposed to it on the preceding two days. Bi+ animals had been under bilateral CSD during these prior exposures, while the cortices of Co+ animals had not been depressed.

Results.

In Figure 1 results of the 4 groups are depicted. Mean drinking times are expressed as seconds of drinking in the first 5 two-minute periods; also the standard errors of the means are given. The results of the last 5 minutes are discarded because long term habituation effects appeared only during the first period that the sound was introduced.

Group Co+ was compared with group Co-, group Bi+ with group Bi-, so the only difference between compared groups is that one had received prior habituation training and the other had not. In this way CSD-effects on habituation may be separated from other subsequent CSD-effects, which could otherwise confound interpretation. As it was found that the assumption of equal covariances was not met on

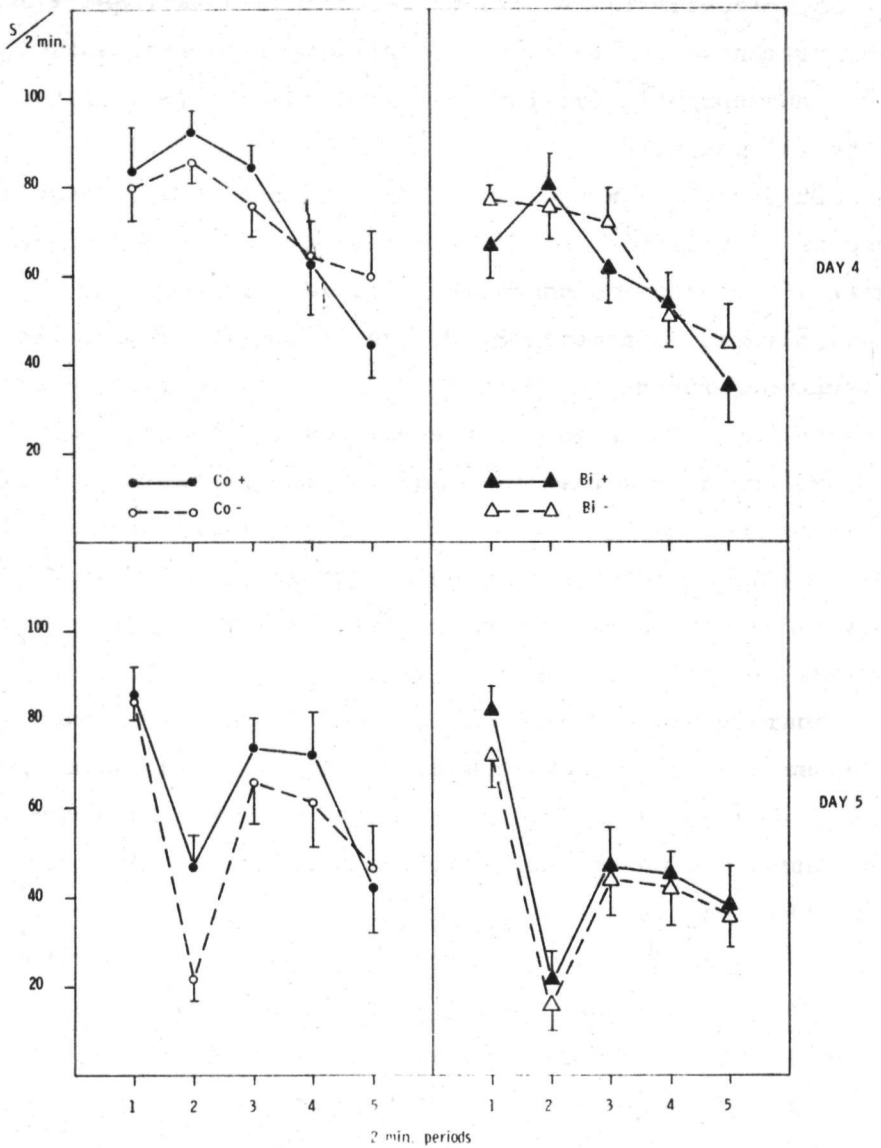

Fig. 1. Mean drinking times and standard errors in seconds per 2 minutes in experiment 1. Co denotes the control group, Bi the bilateral CSD group; the signs + and - refer to the presence or absence of habituation training.

day 5, a multivariate analysis of variance of repeated measurements, in which the 5 periods were variables, was most appropriate (2); for this analysis the program of Finn (6) was used.

On day 4 drinking was not interrupted by the sound of the bell. Comparisons between Co+ and Co- groups did not reveal any significant difference, neither multivariate, nor univariate in any period. The same was found for groups Bi+ and Bi- on day 4.

On day 5 the bell started to ring at the beginning of the second two-minute period. Although the over-all difference between groups Co+ and Co- was not significant (multivariate $F_{5,18} = 1.83$; $p = .16$), both groups differed significantly in the second period (step down $F_{1,22} = 7.86$; $p = .01$); in the other periods the differences were not significant. As the only difference between groups Co+ and Co- was prior exposure of the sound of the bell to group Co+, it may safely be concluded that the smaller disruption of group Co+ at the introduction of the bell was due to prior habituation.

For group Bi+, which also had experienced 2 prior exposures to the sound of the bell, a different picture emerged. The over-all difference between groups Bi+ and Bi- was not significant (multivariate $F_{5,18} = .27$; $p = .92$). Also the univariate differences between both groups were not significant in any period; for the second period the step-down F-test revealed: step down $F_{1,22} = .07$; $p = .79$. So group Bi+ reacted as if it had heard the bell for the first time.

It may be concluded therefore that repeated exposure

to intense acoustic stimuli during bilateral CSD does not lead to long term habituation effects during later reexposure in a normal state.

2.4. *Experiment 2.*

In the second experiment to be reported here the so-called reversible split brain design (3) was used. Rats were exposed to the sound of the bell during unilateral CSD. Later disruption of drinking by this sound was tested for half of the rats during CSD in the contralateral hemisphere; as a control group the other half was tested during CSD in the same (ipsilateral) hemisphere. No comparisons were made between habituation training and testing within groups, but only, as in experiment 1, of habituation testing between groups.

Subjects.

24 Male albino rats (WAG strain) were used. They weighed about 350 g at the time of surgery and were kept under the same circumstances as the rats in experiment 1.

Surgery and apparatus.

In all animals cannulae were implanted over the cortex of each hemisphere in the same way as in experiment 1. Also the same apparatus was used.

Procedure.

Animals were randomly assigned to one of two groups: UI or UC. Each group consisted of 12 subjects. Both

groups were exposed to the sound during unilateral CSD. Group UI was tested for habituation during ipsilateral CSD, group UC during contralateral CSD.
Procedure was almost the same as for the Bi+ group in experiment 1; it differed only in 3 respects.

In the first place water deprivation was not started on the first but on the second post-operative day (day 2); this was done to allow better post-operative recovery. In the second place drinking training was reduced by one day: on day 3 animals spent 45 minutes in the training box and on day 4, 15 minutes, after the first contact with the nipple, in the testing box. Exposure to the sound of the bell was given after drinking training on days 3 and 4. Habituation testing took place on day 5 again. The third difference concerned the elicitation of CSD of course. Before the exposure to the bell on days3 and 4 plungers were removed from both cannulae; one cannula was filled with saline, the other with 25% KCl solution. For half of the subjects in each group the left hemisphere was depressed, for the other half the right one was. Effectiveness of CSD was tested by examining contralateral placing reflexes. After exposure to the bell KCl was removed again and the cannulae were rinsed with saline. Before the drinking test on day 5 another CSD treatment was given; in the UI group the same hemisphere was depressed as on the preceding days; in the UC group the contralateral hemisphere was depressed.

Results

Results of experiment 2 are depicted in Figure 2 in the

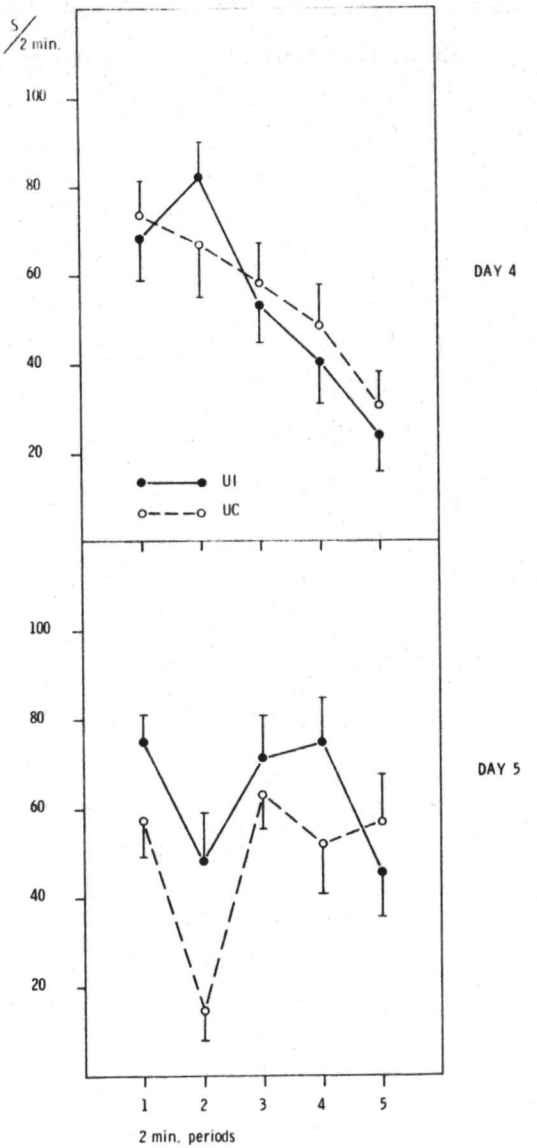

Fig. 2. Mean drinking times and standard errors in seconds per 2 minutes in experiment 2. UI denotes the ipsilateral CSD-group, UC the contralateral CSD-group.

same way as in experiment 1. Also the same MANOVA's were done. On day 4, when drinking was not interrupted by the ringing of the bell, no significant differences were found between groups UI and UC, neither multivariate nor univariate in any period.

On day 5 the over-all difference between groups UI and UC was again not significant (multivariate $F_{5,18} = 1.50$, $p = .24$). In the second period the univariate F-test revealed a significant difference (univariate $F_{1,22} = 6.35$; $p = .02$); using the first period as a covariate, however, the step down F-test just failed to reach significance (step down $F_{1,22} = 3.72$; $p = .06$). In the other four periods the difference between groups UI and UC was not significant. Therefore, it may be tentatively concluded that at the introduction of the sound of the bell group UC showed less long term habituation effects than group UI.

The difference in the first period failed to reach significance (univariate $F_{1,22} = 2.91$; $p = .10$), but results could be taken as a small indication of lower drinking time in group UC (Fig. 2); as unilateral CSD produces a slight transient adipsia at first application (2), this adipsia may have been manifested during contralateral CSD, while it did not show up during ipsilateral CSD, which was applicated for the third time. In that case the reduced step down F-value in the second period could also be explained.

2.5. *Discussion.*

In Experiment 1 original habituation for group Bi+

took place in a bilateral decorticate state; testing for long term habituation was done in a normal state (both hemispheres undepressed). Results for group Bi+ did not differ from those of group Bi- which was naive at the time of testing. So in contrast to group Co+, group Bi+ did not show any long term habituation.

This outcome does not necessarily imply that short term habituation was also disrupted in group Bi+. Nadel (11) who investigated decline of activity in a novel environment found that short term habituation was undisturbed during bilateral CSD while long term habituation was absent. Short term habituation during bilateral CSD could not be tested in the present experiments because bilateral CSD induces adipsia (3) and interruption of drinking was used as an index of habituation. For testing of short term habituation to intense acoustic stimuli during bilateral CSD direct measurement of startle response seems to be more appropriate.

Squire (14) investigated habituation in a reversible split brain experiment; he measured decline of activity in a novel environment. Testing for long term habituation effects revealed no differences between ipsilateral and contralateral CSD treatments. These results are contrary to those of experiment 2: testing for habituation to intense acoustic stimuli during contralateral CSD revealed less habituation, as compared to testing during ipsilateral CSD.

This difference between habituation to a novel environment and habituation to intense acoustic stimuli supports the suggestion that habituation to these different

stimulus situations is mediated by different processes (15).

The absence of long term habituation in group Bi+ may be explained in different ways. It could be that information about the repeated stimulus exposure was not permanently stored during bilateral CSD; this could have been due either to direct inactivation of the cortex or to remote effects of CSD on some subcortical structure (3). Another possibility is that information was permanently stored in non-depressed neural structures, but that this information was not retrievable when the cerebral cortices were no longer depressed. These two explanations may be considered as "central" hypotheses: they state that long term habituation deficits were directly caused by a change in the state of the central mechanisms in which storage or retrieval of information had to take place. Such a "central" hypothesis may also explain the reversible split brain results in experiment 2. The design of both experiments does not permit a choice, however, between the storage failure and retrieval failure hypothesis.

A third hypothesis by which the present results could be explained is a stimulus control hypothesis (13) which takes into consideration that CSD changes the internal and external stimulus situation; long term habituation deficits should then have been caused by a change in the total stimulus situation, which resulted in generalization decrement, rather than by a change in central mechanisms.

This discussion about "central" and stimulus control hypotheses resembles the discussion with respect to state

dependent learning which may also be explained by "central" or stimulus hypotheses (1).

Further experiments are needed to determine which of these hypotheses is most suited to explain CSD-effects on habituation.

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CHAPTER 3. HABITUATION AND SENSITIZATION OF THE ACOUSTIC STARTLE RESPONSE DURING CORTICAL SPREADING DEPRESSION IN RATS.

3.1. *Abstract.*

Habituation of the acoustic startle response was tested in eight groups of rats which received different CSD-treatments on three successive days. Both short term habituation and long term habituation were investigated. Bilateral CSD did have a significant effect on short term habituation, but not unilateral CSD. This effect was interpreted as a temporary increment of sensitization in the beginning of the session. Neither unilateral CSD nor bilateral CSD did have an effect on long term habituation as long as no treatment change took place. Treatment change resulted in an increased startle amplitude if it was a change from no CSD to unilateral or bilateral CSD. The reverse change had no such effect. These results were interpreted as evidence for a gradual compensation over sessions for the initial CSD-induced sensitization increment. The results were discussed in connection with the results of earlier experiments on CSD and habituation.

3.2. *Introduction.*

Cortical spreading depression (CSD) is a reversible decortication technique which has been widely used in neuro-behavioral research in rats (1). In previous experiments (19) it was shown that long term habituation to intense acoustic stimulation was seriously disturbed

when stimulation was presented during CSD. Long term habituation is defined as a decrement of response magnitude over sessions as contrasted with short term habituation which is defined as a decrement of response magnitude within a single session. In those experiments disruption of ongoing drinking behavior was used as an index of habituation. This procedure, however, had an important limitation. CSD induces adipsia (1) and therefore habituation could not be measured during CSD but only later on when normal cortical function had recovered. For this reason only CSD-induced effects on long term habituation could be investigated in this previous study.

Moreover, because of the imcompleteness of the experimental design, it could not be decided how the results could most suitably be explained.

Therefore it was decided in the experiment to be reported here to investigate habituation of the acoustic startle response during CSD. In a preliminary investigation it was found that these startle responses were not eliminated during CSD. By measuring startle responses not only long term habituation but also short term habituation could be investigated. Moreover, in this way long term habituation could be measured not only in an undepressed state but also during CSD. This made it possible to use 2x2 designs which are also frequently used in state dependent learning studies and which enable a comparison of the suitability of the possible hypotheses by which the results can be explained (15).

The acoustic stimulus parameters were chosen so as to resemble as much as possible those of Davis (4). Davis

had found that the differential processes of habituation and sensitization, which have been postulated to operate on different levels of neural functioning (10, 18) may also be demonstrated in startle response habituation. Startle response habituation may be considered therefore as the net result of a decremental process (habituation) and an incremental process (sensitization); it must be noticed that a distinction is made here between response habituation as an operationally defined phenomenon and habituation as an inferred construct (18). For example Groves & Lynch (9) had suggested that the frequently found effects of frontal cortex lesions on response habituation (8) may be ascribed to an increased state of sensitization caused by disinhibition of reticular arousal. For these reasons it seemed worthwhile to investigate whether CSD had effects on habituation and/or sensitization.

3.3. Methods.

Subjects.

80 Male albino rats (WAG strain) were used. They weighed about 300 g at the time of surgery and were housed in individual cages in a stock-room. Food and water were available ad lib. They were kept on a 12 hr. light/12 hr. dark cycle and were tested during the light period.

Surgery.

The rats were anesthetized with HYPNORM (10 mg fluani-

zone, .2 mg phentanyl citrate pro ml; .08 cc/100 g i.m.) followed by NEMBUTAL (60 mg/ml; .03 cc/100 g i.v.). Over the cortex of each hemisphere trephine openings were drilled in the skull, measuring 3 mm in diameter, in which stainless steel epidural cannulae were placed. These cannulae were 6.5 mm long and had an internal diameter of 1.5 mm; at a distance of .5 mm from the bottom of the cannulae was a flange of .5 mm with which they rested on the skull to prevent pressure on the dura. Cannulae were fixed to the skull with dental cement and fixation screws. The dura was moistened with saline and the cannulae were closed with a screwmounted plunger to prevent dehydration. Saline was refreshed daily.

Apparatus.

Amplitude of startle response was recorded in a small cage (15 x 8 x 8 cm) made of aluminium and perspex and mounted on a 5 mm thick rubber base. It was located in a sound-proof cabin. The top and the four walls of the box were heavily perforated to allow free passage of air. Startle stimuli resembled those of Davis (4). With an interstimulus interval of 30 s tone stimuli (4000 Hz, 100 ms) were given; intensity was 102 dB measured inside the cage. The tone was generated by a 4000 Hz oscillator, amplified by a 25 w stereo amplifier and provided by 2 speaker boxes each containing 4 speakers of 6 w. The speaker boxes were located to the left and to the right of the startle cage.

A phonograph cartridge rested on the top of the cage. The voltage output of the cartridge crystal was amplified

and fed into an U.V. recorder (S.E. 3006), located outside the sound-proof cabin. Cage movement resulted in vertical deflections of the trace on the recording paper. The relation between cage movement amplitude and trace deflection on the recording paper was tested by dropping different weights on the floor of the startle cage; amplitude of trace deflection was proportionate to weight. The cartridge signal resulting from a startle response of a rat inside the cage had a frequency of about 150 Hz. The signal was sent through a low-pass filter to prevent appearance of the 4000 Hz signal on the U.V. record. Two seconds before presentation of a startle stimulus the U.V. recorder was triggered automatically and ran for 4 seconds with a paper speed of 50 cm/min. Startle amplitude was determined by measuring trace amplitude to the nearest mm and converting this value to millivolts.

Procedure.

Subjects were randomly assigned to one of 8 groups (Fig. 1); each group consisted of 10 subjects. The experiment lasted 10 weeks; in each week 8 subjects were used, one of each group. Six subjects which had to be discarded because of ineffective CSD-treatment were replaced by other rats which were tested in the 11th week.

On the fourth day after surgery (day 1) the first habituation session took place. Before this session one of 3 treatments was given: no CSD (N), unilateral CSD (U) or bilateral CSD (B). Bilateral CSD was induced as follows. The plungers were removed and the cannulae were fill-

ed with a 25% KCl solution; cannulae were closed with small screws. Effectiveness of CSD treatment was tested before and after the habituation session by examining placing reflexes which are impaired during CSD (1). The unilateral CSD and no CSD treatments were exactly the same as the bilateral CSD-treatment except that in the unilateral CSD treatment only one cannula was filled with KCl, the other with saline and in the no CSD treatment both cannulae were filled with saline.

After treatment the rat was placed in the startle cage. Five minutes later the first startle stimulus was presented. The whole session consisted of presentation of 50 tone stimuli with an interstimulus interval of 30 s. After the session the animal was removed from the cage; cannulae were cleaned and rinsed with saline; then the animal was returned to its home cage. The order of testing the animals of the different groups was varied from week to week to balance diurnal rhythm effects (6).

24 Hours later (day 2) all rats were subjected to the same CSD treatment and habituation session. Another 24 hours later (day 3) the last habituation session took place, but now for several groups the CSD treatment had been changed (Fig. 1).

From Fig. 1 it may be seen that on day 1 and day 2 treatment did not change; so for day 1 and day 2 the 8 groups of Fig. 1 could be combined to form 3 larger groups: group N (no CSD, n=30), group U (unilateral CSD, n=30) and group B (bilateral CSD, n=20). On day 3 for some groups the treatment was the same as on the preceding days (groups NNN, UUU and BBB), while it was changed

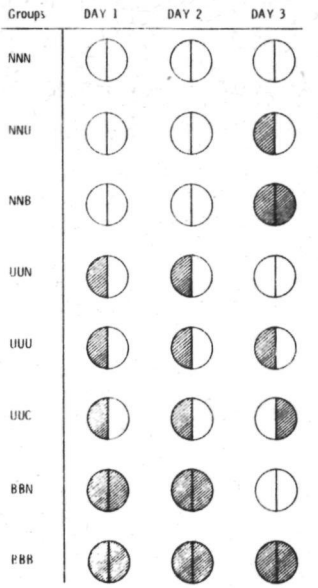

Fig. 1. Successive CSD treatments of the 8 groups. The hemicircles indicate brain hemispheres; hatching indicates cortical spreading depression. N: no CSD; U: unilateral CSD; B: bilateral CSD.

in the other groups. In a typical 2x2 design in state dependent learning experiments two treatments are used: a drug treatment and a no drug treatment; each of the 4 possible successions of treatments is given to a different group of subjects: no drug - no drug, no drug - drug, drug - no drug and drug - drug. It may be seen that groups NNN, NNB, BBN and BBB form such a 2x2 design, in which bilateral CSD is analogous to a drug treatment. In the same way groups NNN, NNU, UUN and UUU form a 2x2 design, in which unilateral CSD is analogous to drug treatment.

Finally groups UUU and UUC form a so called reversible split-brain design (1): both groups are tested during unilateral CSD on day 1 and day 2; on day 3 group UUU is

tested during CSD in the same hemisphere, while group UUC is tested during CSD in the contralateral hemisphere.

3.4. Results and conclusions.

For individual animals the mean startle amplitudes of successive days were calculated; then the means of these day scores were calculated for different groups. For day 1 and day 2 this was done for the large groups N, U and B; for day 3 this was done for the 8 groups of Fig. 1. In Fig. 2 these mean day scores and their standard errors are depicted; it may be seen that the large groups of animals which received the same treatment on day 1 and day 2 are split into 2 or 3 smaller groups with different treatments on day 3.

First the results of day 1 and day 2 will be presented. The differences between the day scores of the component small groups (NNN - NNU - NNB, UUN - UUU - UUC and BBN - BBB) on day 1 and day 2 were tested by way of univariate analyses of variance. No significant differences were found, so there were no objections to combine them to form the large groups N, U and B. Long term habituation which manifested itself as a decrement of startle amplitude between day 1 and day 2 was tested for significance in groups N, U and B. The differences between the mean startle amplitudes of day 1 and day 2 were tested by one-tailed t-tests for related samples which gave significance for all 3 groups (group N: $t=2.44$, $df=29$, $p=.01$; group U: $t=4.03$, $df=29$, $p=.0002$; group B: $t=1.89$, $df=19$, $p=.04$). So it may be concluded that the non-depressed group N did show long term habituation between

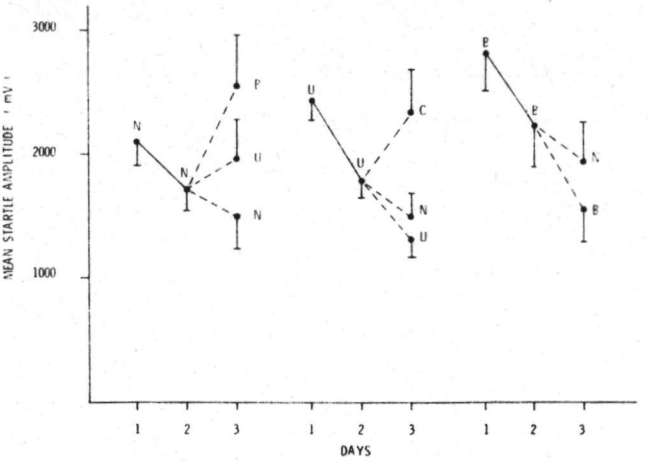

Fig. 2. Mean day scores of startle amplitude and S.E.M.'s of groups N (n=30), U (n=30), and B (n=20) on days 1 and 2 and of the 8 groups of Fig. 1 (n=10) on day 3. N: no CSD; U: unilateral CSD; B: bilateral CSD. See text for further explanation.

day 1 and day 2 and also that neither unilateral nor bilateral CSD prevented this long term habituation from occurring.

Short term habituation on day 1 and day 2 was analysed as follows. For individual animals the mean startle amplitudes of successive blocks of 5 trials were calculated (4). In this way 10 block scores were obtained for day 1 and 10 for day 2. In Fig. 3 these mean block scores and standard errors of groups N, U and B are presented. The significant long term habituation in all groups which was found in comparing day scores is also visible in Fig. 3: block scores are generally lower on day 2 than on day 1.

It is clear that all 3 groups did also show short term habituation: initially there was a sharp decrement

in startle amplitude, but in the course of the session amplitude changes became much smaller. It seemed, however, that the shape of the curves and mainly the steepness of the initial decrement differed between groups. To test these differences for significance a multivariate analysis of variance (7) was done; the 10 block scores were taken as variables, groups (N, U and B) and days (1 and 2) were treated as factors, error term was the pooled within-group variance-covariance matrix. The difference between day 1 and day 2, indicating long term habituation, was significant as was to be expected (multivariate $F=2.53$, $df=10/145$, $p=.008$). There were no significant interaction effects between groups and days (multivariate $F= 1.05$, $df=20/290$, $p=.40$). So it may be concluded that long term habituation did not differ between groups. To test group effects two comparisons were made, one between groups U and N, the other between groups B and N. Group U did not differ significantly from group N (multivariate $F=1.12$, $df=10/145$, $p= .35$). The comparison between groups B and N did reveal a significant difference, however (multivariate $F=3.81$, $df=10/145$, $p=.0002$).
So it may be concluded that bilateral CSD had a clear effect on short term habituation as compared to a control treatment (group N), while unilateral CSD had not.

The difference between group B and group N was further analyzed. From Fig. 3 it may be seen that eventually groups B and N reached the same asymptotic level of startle amplitude. The largest differences seem to have occurred in the first blocks. In the frame of the multi-

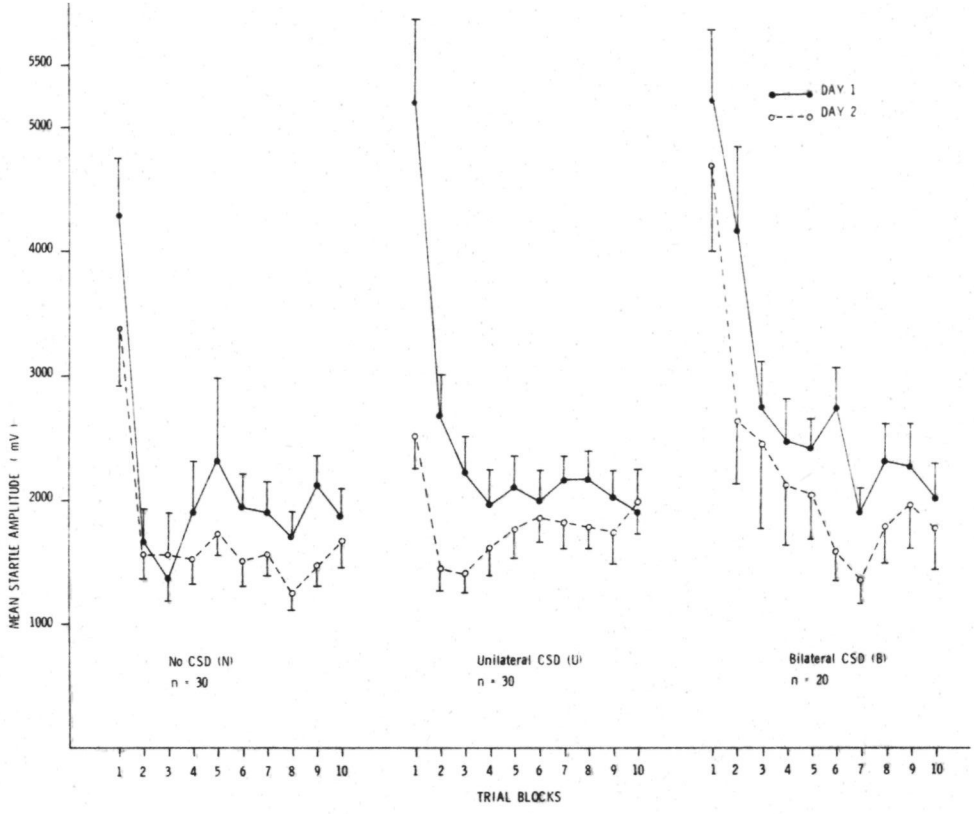

Fig. 3. Mean block scores of startle amplitude and S.E.M.'s of groups N, U and B on days 1 and 2.

variate analysis of variance also univariate F-tests were performed for successive blocks. Group B differed significantly from group N in block 1 (univariate F=4.26, df=1/154, p=.04), in block 2 (univariate F=23.67, df=1/154, $p < .0001$) and in block 3 (univariate F=10.31, df=1/154, p=.002). In block 4 the difference was no longer significant (univariate F=2.92, df=1/154, p=.09). It was decided, therefore, to take a closer look at the individual trial scores of the first three blocks.

In Fig. 4 the mean scores of trials 1-15 and 46-50 of groups B and N on day 1 and day 2 are presented. From Fig. 3 one might get the impression that group B had a higher initial startle amplitude than group N, but Fig. 4 shows that this is not the case. The differences between groups B and N on trials 1-15 were tested by way of a multivariate analysis of variance with trials as variables; this was done for day 1 and day 2 separately.

On day 1 group B differed significantly from group N (multivariate F=1.99, df=15/34, p=.05). The univariate F-tests revealed that groups B and N did not differ in the first 3 trials; in trials 4- 12 and in trial 15 startle amplitude of group B was significantly higher than the amplitude of group N ($p<.05$); in trials 13 and 14 the difference was not significant.

On day 2 the picture was about the same, but now the over-all difference between groups B and N was not significant anymore (multivariate F=1.45, df=15/34, p= .18). The univariate F-tests revealed significant differences only in trials 9, 10, 12 and 13.

Now the short term habituation results of Fig. 4 may

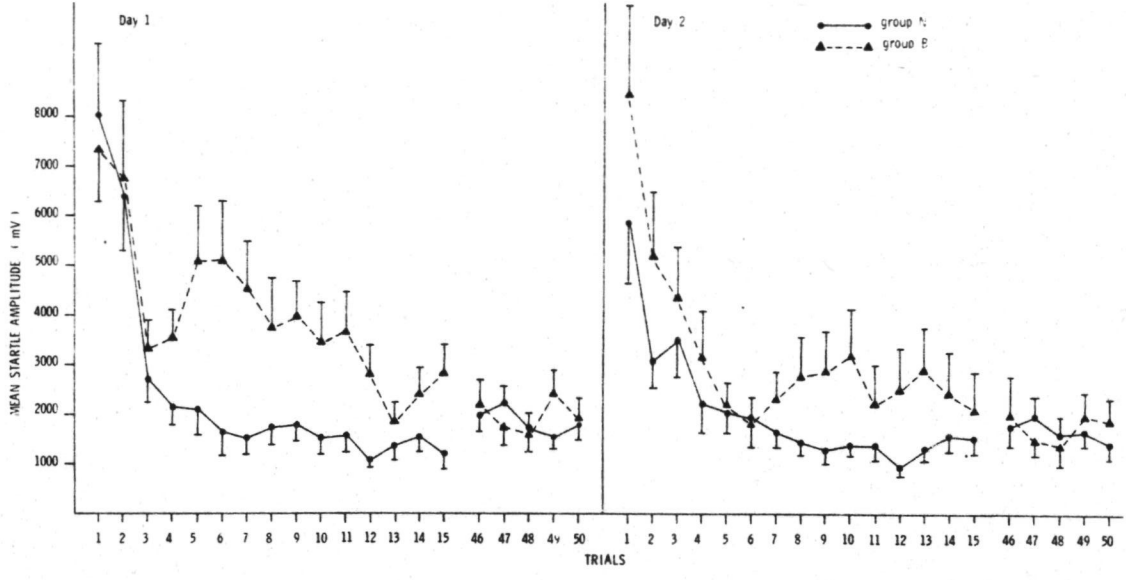

Fig. 4. Mean trial scores of startle amplitude and S.E.M.'s of groups N (n=30) and B (n=20) on days 1 and 2. N: no CSD; B: bilateral CSD.

be interpreted as follows. Group B and group N started at the same startle amplitude on day 1. In the first few trials there was a sharp decrement to a lower amplitude in both groups. Group N remained on this low amplitude level during the following trials (with possibly only later on in the session a temporary increment; see Fig. 3). From trial 4 onwards, however, startle amplitude of group B became higher than that of group N and remained higher during a number of following trials. At the end of the session group B had returned again to the same low amplitude level as group N. On day 2 the same general picture emerged, but now the higher amplitude of group B was less impressive and did appear only later on in the session. If the short term habituation curves are considered to be the net result of a decremental habituation process and an incremental sensitization process, then the difference between groups B and N is most suitably interpreted as an increased sensitization in group B which develops after the first few trials. This increased sensitization, however, was only temporarily influential and had disappeared again at the end of the session. On day 2 the increased sensitization of group B had become somewhat less influential than on day 1.

Now the results of day 3 will be considered. On this day a treatment change had taken place for several groups (see Fig. 1).

It was decided to make no comparisons on day 3 between groups that had been subjected to different treatments on that day. Such comparisons would not differentiate between short term effects due to treatment

and long term effects due to treatment change. Therefore the mean startle amplitudes on day 3 of each group, in which a change of treatment had taken place, were compared to the corresponding scores of the group with the same treatment on day 3, but in which no change of treatment had taken place (Fig. 2).

Two pairs of comparison were of interest, as they concerned groups which together formed a 2x2 design in an analogous way to state dependent learning studies (see procedure). It was hypothesized that change of treatment would result in an increase of startle amplitude; comparisons were done by one-tailed t-tests for independent samples. The first pair of comparisons concerned the following 4 groups: NNN, NNB, BBN and BBB. Group NNB did have a significantly higher startle amplitude on day 3 than group BBB ($t=2.04$, $df=18$, $p=.03$), while group BBN and group NNN did not differ significantly ($t=1.04$, $df=18$, $p=.16$). The second pair of comparisons concerned the groups NNN, NNU, UUN and UUU. Here startle amplitude of group NNU was significantly higher than that of group UUU ($t=1.80$, $df=18$, $p=.04$), while groups UUN and NNN did not differ significantly ($t=.01$, $df=18$, $p=.50$).

So the change of a no CSD treatment on days 1 and 2 to a bilateral or unilateral CSD treatment on day 3 resulted in a higher startle amplitude on day 3 as compared to the corresponding groups in which the CSD treatment was already given for the third day. The change of a bilateral or unilateral CSD treatment to a no CSD treatment had no such effect.

Recently Overton (15) has presented a number of pos-

sible patterns of results in 2x2 designs and their interpretation. The asymmetrical pattern which was obtained in this experiment corresponds with one of the patterns presented by Overton; that pattern was interpreted as to be the result of development of tolerance to an initial drug effect; the initial drug effect is compensated for. If the CSD effects in this experiment are considered to be analogous to drug effects, it may be concluded that CSD had an initial effect on startle response amplitude but that this effect was compensated for in the course of successive habituation sessions. As was concluded earlier, this initial effect consisted of a temporary sensitization increment in the beginning of a session.

Group UUU and group UUC formed a reversible split-brain design. The difference on day 3 between these groups was significant ($t=2.71$, $df=18$, $p=.01$). So a change of treatment from CSD in one hemisphere to contralateral CSD resulted in an increment of startle amplitude as compaired to an ipsilateral CSD-treatment.

3.5. *Discussion.*

The conclusions of this experiment about habituation of the acoustic startle response during CSD may be summarized as follows.

1. Bilateral CSD did have a significant effect on short term habituation as compared to a non-depressed condition. This effect was further analyzed and interpreted as a temporary sensitization increment in the beginning of the session. Unilateral CSD did not show this

effect.

2. Unilateral and bilateral CSD did not prevent long term habituation from occurring if no change of treatment took place between sessions. This long term habituation could not be differentiated from long term habituation in an undepressed condition.

3. Change of treatment resulted in an increased startle amplitude if it was a change from no CSD to unilateral or bilateral CSD. The reverse change had no such effect. These results were interpreted as evidence for a gradual compensation over sessions for the initial CSD-induced sensitization increment.

4. Change of treatment from CSD in one hemisphere to contralateral CSD also resulted in an increased startle amplitude.

Groves & Lynch (9) have suggested that the frequently found impairment of habituation after cortical lesions is to be ascribed to increased sensitization caused by disinhibition of reticular arousal. This suggestion is substantiated in this experiment at least as far as decortication by CSD is concerned. The findings of electrophysiological experiments that CSD is accompanied by reticular excitation may also bear on this suggestion, although it is not quite clear whether this excitation is due to an elimination of inhibitory cortico-reticular activity or to a temporary discharge of excitatory cor-

tico-reticular activity accompanying CSD onset (1). Perhaps the CSD-induced disinhibition concerns those reticular neurons which have been shown to act as sensitization neurons (11).

CSD-induced sensitization increment was manifested only temporarily in this experiment. These results are different from those of Davis & Sheard (5) who investigated startle response habituation after lesions of the raphe nuclei. They also found an increased sensitization, but in that case it was reinstated on subsequent days.

At first sight it may seem surprising that the change of treatment from CSD in one hemisphere to contralateral CSD did result in an increased startle amplitude, while the change from unilateral CSD to no CSD and also from bilateral CSD to no CSD did not result in such an increment. This difference may be explained, however, if one assumes that there was sensitization increment both during unilateral and bilateral CSD, but that this increment was opposed by compensatory processes taking place in non-depressed neural structures. Such compensatory processes have also been suggested in explaining CSD-effects on learning (2). After habituation during CSD in one hemisphere a change to CSD in the other hemisphere would block part of these compensatory processes and thereby cause a new increment of sensitization. After a change from unilateral or bilateral CSD to no CSD no such blocking would take place. That the initial sensitization increment was only manifested during bilateral and not during unilateral CSD may then be interpreted as follows. During unilateral CSD stronger compensatory pro-

cesses did take place because there were more non-depressed neural structures than during bilateral CSD; so during unilateral CSD the increased sensitization effect was compensated for before it could become manifest in the startle response habituation curve.

In a previous investigation (19) it was found that a change of treatment from bilateral CSD to no CSD and from unilateral CSD in one hemisphere to contralateral CSD did result in a clear disturbance of long term habituation. It could not be decided there whether the results were best explained by a storage failure, a retrieval failure or a stimulus control hypothesis. In the present experiment storage of long term habituation did take place during CSD. As far as the stimulus control hypothesis (16) is concerned it appeared that the effects of treatment change were not symmetrical for changes in opposite directions. Therefore, in view of the present results retrieval failure caused by treatment change seems to be the most adequate explanation for the results of this previous investigation; this is also most in accord with recently proposed memory theories (12, 13).

It is puzzling, however, that a change of treatment from bilateral CSD to no CSD did result in disturbed long term habituation in the previous investigation, while in the present experiment it did not. Maybe the explanation of this difference has to be sought in the way in which habituation to intense acoustic stimulation was measured in both experiments. In the present experiment startle amplitude was measured, while in the previous experiment reduction of ongoing activity was recorded. It has been

demonstrated (3) that these two reactions to intense acoustic stimuli may be dissociated: Prior aversive stimulation appeared to result in a reduction of interstimulus activity as compared to a control group, while startle amplitude was not affected. These results were interpreted as an indication that activity reduction as reaction to intense acoustic stimuli is a more reliable measure of arousal or emotionality than startle amplitude (3).

In this experiment CSD appeared to result in increased sensitization; sensitization is defined as a general arousal level (18). This increased arousal level, however, was manifested only temporarily in startle amplitude. It is not unlikely that a more reliable measure of arousal like activity reduction, which was used in the previous experiment (19), would show a longer lasting CSD-effect on arousal. This could explain why the treatment change from bilateral CSD to no CSD did result in disturbed long term habituation in the previous experiment but not in this one. It must be hypothesized, then, that CSD did cause an increased arousal or emotionality not only during exposure to intense acoustic stimulation in the CSD-state, but also during later re-exposure in an undepressed state. This suggestion, however, will have to be investigated more specifically in further experiments.

The results of this experiment are also different from those found in two other experiments on CSD and habituation. Nadel (14) investigated decline of activity in a novel environment during unilateral and bilateral

CSD. In contrast to this experiment he found that during bilateral CSD short term habituation was unaffected, while long term habituation was absent. Squire (17) investigated habituation in a reversible split-brain experiment; he also measured decline of activity in a novel environment. In contrast to this experiment he found no difference in long term habituation between testing during ipsilateral and contralateral CSD. In a previous investigation (19) also a difference was noted between habituation to a novel environment and habituation to intense acoustic stimulation. These differences substantiate the hypothesis that habituation to these different stimulus situations is mediated by different processes (20).

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CHAPTER 4. HABITUATION OF OPEN FIELD ACTIVITY DURING CORTICAL SPREADING DEPRESSION IN RATS.

4.1. *Abstract.*

Habituation of locomotor activity in an open field was investigated in 6 groups of rats which received different CSD-treatments on 2 successive days. Both short term habituation and long term habituation were investigated.

During bilateral CSD short term habituation was slower and less negatively accelerated, whereas during unilateral CSD it was not different from control. Long term habituation within treatments was significant for the control treatment and approached significance for the bilateral CSD treatment; for the unilateral CSD treatment no significant long term habituation was found. Long term habituation after treatment change revealed state dependent learning effects which were ascribed to retrieval failure, caused by the change in the functioning of the brain.

4.2. *Introduction.*

Behavioral habituation may be defined, within certain limits, as a decrement of a species-characteristic response occurring as a result of repeated or continuous stimulation (8). Usually a distinction is made between short term habituation, i.e. decrement of response magnitude within a single session, and long term habituation, i.e. decrement of response magnitude over sessions. Habituation

as a decremental process is thought to be opposed by an incremental process of sensitization (15).

In a previous investigation (16) it was shown that cortical spreading depression (CSD), which is a reversible decortication technique (3), did have an effect on the acoustic startle response in rats; this effect was interpreted as a temporary increment of sensitization in the beginning of the session; the habituation process was not disturbed during CSD.

Some discrepances exist between the results of startle response habituation (16) and those of earlier investigations concerning the relation between habituation and CSD (11, 14). It has been suggested (16) that these discrepances were due to a difference in experimental procedure. In the startle response habituation experiments discrete startle reactions to discrete stimuli were measured, while in the earlier investigations activity reduction was measured in function of continuous exposure to a novel stimulus situation. It has been found that these two types of habituation may be dissociated (19). Therefore, in the present experiment habituation of open field locomotor activity was determined during different CSD-treatments.

4.3. Methods.

Subjects.

60 Male albino WU (SPF63 Cpb) rats (10) were used. They weighed about 300 g at the time of surgery and were housed in individual cages. Food and water were available

ad lib. The rats were kept on a 12 hr. light/12 hr. dark cycle and were tested during the light period.

Surgery.

The rats were anesthetized with HYPNORM (10 mg fluanizone,.2 mg phentanyl citrate pro ml; 0.1 cc/100 g i.m.). Over the posterior cortex of each hemisphere trephine openings were drilled into the skull, measuring 3 mm in diameter, in which stainless steel epidural cannulae (length 6.5 mm with an internal diameter of 1.5 mm) were placed; care was taken not to damage the dura. At a distance of 0.5 mm from the bottom of the cannulae was a flange, which rested on the skull to prevent pressure on the dura. The cannulae were fixed to the skull with dental cement and fixation screws. The dura was moistened with saline and the cannulae were closed with a screw-mounted plunger to prevent dehydration. Saline was refreshed daily.

Apparatus.

Locomotor activity was observed in a square open field measuring 100 x 100 cm (17); the wooden floor was painted white; black lines divided it in 36 equal squares. The walls were 35 cm high; 3 walls were made of wood and painted white, the fourth was made of plexiglass to allow observation of the animal. The perforated top also consisted of plexiglass.

Procedure.

Subjects were randomly assigned to one of 6 groups and were tested on 2 successive days; each group consisted of 10 subjects initially, but 7 animals were discarded because of dura damage during surgery or ineffective CSD treatment.

The groups were: N-N (no CSD - no CSD, n=9), N-B (no CSD - bilateral CSD, n=9), B-B (bilateral CSD - bilateral CSD, n=8), B-N (bilateral CSD - no CSD, n=8), U-U (unilateral CSD - ipsilateral CSD, n=10) and U-C (unilateral CSD - contralateral CSD, n=9); the first letter refers to the treatment of the first testing day, the second letter to the treatment of the second testing day.

On the second day after surgery (day 1) the first observation session took place. Before the session one of 3 treatments was given: no CSD, unilateral CSD or bilateral CSD. Bilateral CSD was induced as follows. The plungers were removed and the cannulae were filled with a 25% KCl solution; cannulae were closed with small screws. Effectiveness of CSD treatment was tested before and after the habituation session by examining placing reflexes. The unilateral CSD and no CSD treatments were exactly the same as the bilateral CSD treatment except that in the unilateral CSD treatment only one cannula was filled with KCl, the other with saline, and in the no CSD treatment both cannulae were filled with saline. 10 Minutes after treatment the rat was placed in the middle of the open field. Time-sampling observation started after 30 seconds; every 10 seconds an observer recorded the square in which the animal was located (17). Duration of the session was 25 minutes which resulted in a total of 150 observations per

session. After the session the animal was removed from the open field; cannulae were cleaned and rinsed with saline and the animal was returned to its home cage. 24 Hours later (day 2) the second habituation session took place. CSD treatment could be the same as on the first day or different depending on the group the animal belonged to. Observation of open field activity was the same as on day 1.

4.4. Results.

Locomotor activity was analyzed as follows. For 2 successive observations it was scored whether or not the animal had moved within the 10 seconds interval to a different square of the open field. This was done for all 149 pairs of successive observations of a session. The mean number of displacements was calculated for 10 2.5 minutes periods of day 1 and for the whole 25 minutes sessions of day 1 and day 2. On day 2 reduction of locomotor activity within the session was not analyzed to prevent confounding of short term and long term habituation effects. Long term habituation was analyzed by comparing the session scores of day 1 and day 2. In Fig. 1 session means and standard errors of the 6 groups are depicted. Long term habituation within treatments was tested in groups N-N, U-U and B-B (one-tailed t-tests for related samples); the reduction in the number of displacements between day 1 and day 2 was significant for group N-N ($t=4.89$, $df=8$, $p=.001$), not significant for group U-U ($t=.59$, $df=9$, $p=.29$) and just failed to reach

significance in group B-B ($t=1.73$, $df=7$, $p=.06$). Therefore, it is concluded that long term habituation of locomotor activity did occur in group N-N and probably also in group B-B, whereas it was absent in group U-U.

For the analysis of the effects of treatment change on long term habituation 2 designs were used: the 2x2 design which is frequently used in state dependent learning studies (12) and the reversible split-brain design (3). Groups N-N, N-B, B-N and B-B formed the 2x2 design; groups U-U and U-C formed the reversible split-brain design. The data of the 2x2 design were analyzed by way of a multivariate analysis of variance (6) with 2 factors: treatment on day 1 (N or B) and treatment on day 2 (same or different); number of displacements on day 1 and day 2 were taken as 2 variables; multivariate error term was the pooled within-group variance-covariance matrix. The first factor (treatment N or B on day 1) was not significant, neither multivariate, nor univariate on day 1 or day 2. So bilateral CSD caused no difference from control in the total locomotor activity in the first session. The second factor (same or different treatment on day 2) was significant (multivariate $F_{2,29}=4.27$; $p=.02$); the univariate tests showed that the difference on day 1 was not significant; however, the step down F-test on day 2 (taking the results of day 1 as covariate) revealed, that the groups with the same treatment on day 2 as on day 1 (N-N and B-B) differed significantly from groups N-B and B-N, which had a different treatment on day 2 (step-down $F_{1,30}=8.52$; $p=.007$).

There was no significant interaction between factors

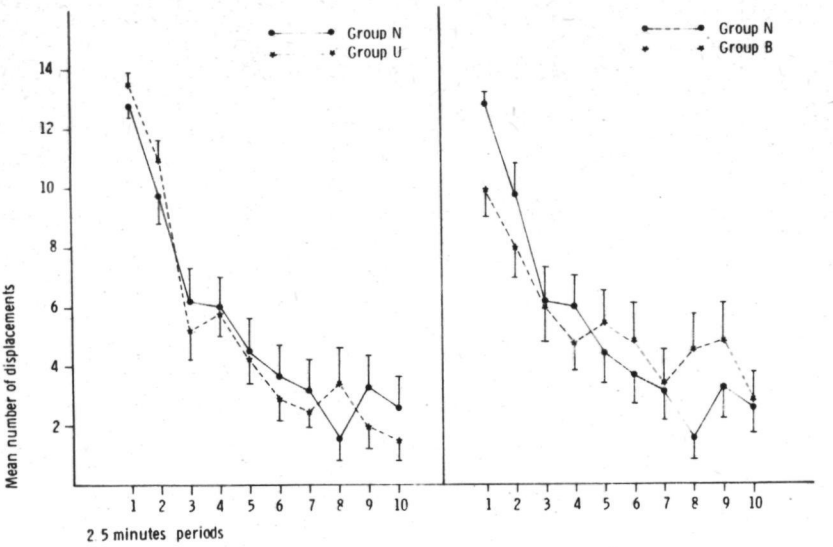

Fig. 1. Mean number of displacements and S.E.M.'s of the 6 groups on day 1 and day 2. N: no CSD; B: bilateral CSD; U: unilateral CSD; C: contralateral CSD.

1 and 2, neither multi-variate, nor univariate on either day. Therefore, it is concluded that, whereas the 4 groups showed no difference on day 1, on day 2 the groups with a different treatment as on day 1 had a significantly higher locomotor activity than the groups with no treatment change. This symmetrical pattern of results corresponds to the pattern which is obtained in case of a state dependent learning effect (12).

The reversible split-brain design was also analyzed by way of a multivariate analysis of variance with days as variables. Groups U-U and U-C did not differ significantly, neither multivariate, nor univariate on day 1 or day 2.

For the analysis of short term habituation the numbers

of displacements in the 10 2.5 minutes periods of day 1 were used. The differences on day 1 between groups N-N and N-B, between groups U-U and U-C and between groups B-B and B-N were analyzed by way of univariate analyses of variance (unweighted means solution) with the 10 periods as repeated measurements (20). As these groups did not differ significantly, they were combined to form the larger groups N (n=18), U (n=19) and B (n=16) respectively. Relevant comparisons within these 3 groups are between groups U and N and between groups B and N. These comparisons were made by way of 2 univariate analyses of variance with the 10 periods as repeated measurements; means and standard errors are depicted in Fig. 2.

In the comparison between groups U and N it was found that these groups did not differ significantly; the factor periods was highly significant ($F_{9,315}=43.55$; $p < .0001$), but the groups x periods interaction was not. The comparison between groups B and N revealed no significant difference between groups; again the factor periods was highly significant ($F_{9,288}=20.66$; $p<.0001$), but so was the interaction between groups and periods ($F_{9,288}=1.90$; $p=.05$). The interaction was submitted to further analysis by tests on trends (20). The difference in linear and quadratic trends were found to be significant ($F_{1,32}=11.29$; $p=.002$ and $F_{1,32}=4.95$; $p=.04$ respectively), whereas the difference in cubic, quartic and quintic trends were not. Therefore, it may be concluded that short term habituation, manifesting itself as a decrement of locomotor activity over periods, did take place in all 3 groups. Group U did not differ from group N in this res-

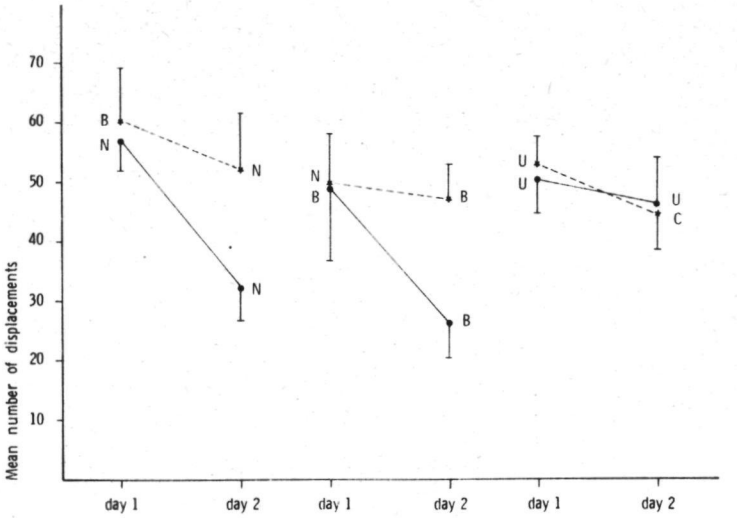

Fig. 2. Mean number of displacements and S.E.M.'s in the 10 periods of day 1. Comparison between groups U and N and between groups B and N.

pect, but group B did; the difference in linear and quadric trends may be interpreted as a slower and less negatively accelerated short term habituation of group B.

Qualitative observation of the behavior in the open field revealed some interesting differences between groups. Most striking was a large reduction of rearing and grooming activity during bilateral CSD, probably due to the impairment of cortical postural reactions (3). During bilateral CSD the animals occasionally bumped their nose against the wall as if they had not noticed it. During unilateral CSD the behavior was not markedly different from control, except that the animals occasionally displayed contralateral circling behavior (18).

4.5. Discussion.

In a previous investigation (16) it was found that CSD had an effect on the acoustic startle response, which was interpreted as a temporary increment of sensitization; sensitization has been defined as a general arousal level (15). In the present experiment, in which CSD effects on open field activity were investigated, it seems not justified to interpret the findings in terms of arousal, as an increment of arousal might have caused either an increment or a decrement of locomotor activity (1). Therefore, the present findings are only interpreted in terms of locomotor activity and not in terms of underlying processes.

Bilateral CSD caused no difference from control in the total locomotor activity in the first session. This finding is in contrast with another investigation relating CSD and open field activity (4). But maybe this discrepance might be explained by the difference in session durations, being 90 minutes in the latter study and 25 minutes in the present investigation.

Short term habituation manifesting itself as a reduction of locomotor activity within the first session was found to take place in the control group, as well as during unilateral and bilateral CSD. During unilateral CSD short term habituation did not differ from control, whereas during bilateral CSD it did. During bilateral CSD short term habituation was slower and less negatively accelerated as compared to control.

This finding is in contrast with Nadel (11), who

claimed to have found normal short term habituation of exploratory activity in a novel situation during bilateral CSD; in that study, however, activity was measured by an ultrasonic activity device, which did not differentiate between locomotion and other kinds of activity. The present finding is more in accord with the experiment of Kolb (9) who found an impaired short term habituation of open field activity after bilateral medial frontal lesions. Therefore, it is suggested that the bilateral CSD-effect on short term habituation of open field activity was in fact caused by the depression of medial frontal cortex activity.

Long term habituation within treatments was analyzed by comparing total locomotor activity of day 1 and day 2. In the control condition locomotor activity was significantly reduced, and also during bilateral CSD the reduction approached significance. Therefore, this finding seems to be in contrast with Nadel's experiment (11), in which an absence of long term habituation during bilateral CSD was reported. As stated above, in the latter study activity was measured by an ultrasonic device; possibly this device measured also other activities except locomotor. Remarkably, during unilateral CSD no long term habituation was found; in a preliminary replication the same absence of long term habituation was found in an U-U group of 12 animals. The reason for this absence is not clear.

Long term habituation after treatment change was analyzed in a 2x2 design (groups N-N, N-B, B-N and B-B) and in a reversible split-brain design (groups U-U and U-C).

The 2x2 design revealed a symmetrical state dependent learning effect (12). In a recent study (7) heart rate conditioning during CSD was investigated in a 2x2 design with the same symmetrical pattern of result. Such result could be interpreted in terms of Schneider's stimulus control hypothesis (13). This theory takes into consideration that CSD changes the internal and external stimulus situation. Long term habituation deficits after treatment change should then have been caused by a change in stimulus situation, which resulted in generalization decrement, rather than by a change in central mechanisms. Although both stimulus and central hypothesis have been proposed to explain state dependent learning effects, considerable doubts have been raised against the stimulus hypothesis (2, 5). Therefore, it is hypothetized that retrieval failure, caused by a change in the functioning of the brain and not stimulus control, was responsible for the state dependent learning effect.

The reversible split-brain design revealed no differences between groups U-U and U-C. In a previous study relating CSD and habituation of locomotor activity (14) ipsilateral and contralateral groups did not differ either. In that study, however, both groups showed long term habituation, while in the present study both groups showed an absence of it.

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CHAPTER 5. APPETITIVE AND AVERSIVE BEHAVIOR INDUCED BY HYPOTHALAMIC STIMULATION IN RATS DURING CORTICAL SPREADING DEPRESSION.

5.1. *Abstract.*

Electrodes were implanted in the lateral or medial hypothalamus in rats. Electric stimulation via these electrodes could induce appetitive or aversive behavior, thus providing respectively positive or negative reinforcement. The rats were tested in a shuttle-box in 2 contingencies and had to generate opposite behaviors (barrier crossing response or inhibition of that response) in order to obtain the same result (either maximization or minimization of stimulation). The effects of contralateral, ipsilateral and bilateral cortical spreading depression on these behaviors were established. The results were interpreted as an increased aversiveness of hypothalamic stimulation during cortical spreading depression due to reversible elimination of the telencephalic involvement in the regulation of appetitive behavior.

5.2. *Introduction.*

Reversible decortication experiments in rats using the technique of cortical spreading depression (CSD) have clearly demonstrated the existence of cortico-hypothalamic interactions. CSD consists of a temporary depression of the electrical activity of the total neocortex of one or both hemispheres, which is achieved by application of a KCl-solution onto the dura. Besides this direct effect

CSD also has remote electrophysiological effects, which are ascribed to a silencing of tonic corticofugal connections (BURES, BURESOVA & KRIVANEK, 1974). One of the remote effects of CSD is a temporary depression of EEG and single unit activity in the lateral hypothalamic area (WEISS & FIFKOVA, 1961, BURES, BURESOVA, FIFKOVA, OLDS, OLDS & TRAVIS, 1961). This electrophysiological depression is parallelled by a depression of consummatory behaviors, which depend on the integrity of the lateral hypothalamic area: during bilateral CSD rats are aphagic (TEITELBAUM 1971) and adipsic (LEVITT & KRIKSTONE 1968).

Consummatory behaviors like eating and drinking may be induced by electrical stimulation in the lateral hypothalamic area; during CSD these stimulation induced consummatory behaviors are blocked. (HUSTON & BURES 1973).

Another effect of hypothalamic stimulation is its positive or negative reinforcement effect (VALENSTEIN 1973): the frequency of a certain response may be increased if this response is followed by onset of offset of hypothalamic stimulation. Positive reinforcement effects are mostly obtained by stimulation in lateral hypothalamic regions; negative reinforcement effects by stimulation in medial regions (OLDS 1962, STEIN 1969, VALENSTEIN 1973). Frequently ambivalent effects are found: short lasting stimulation is positively reinforcing, but if continued it becomes negatively reinforcing (BOWER & MILLER, 1958). If a suitable test is used, it is found that stimulation effects from most medially or laterally placed electrodes are more or less ambivalent (ATRENS & VON VIETINGHOFF-RIESCH, 1972, MENDELSON & FREED, 1973,

SCHMITT, VERGNES & KARLI, 1973).

GLICKMAN & SCHIFF (1967) explicitly linked the positive and negative reinforcement effects of brain stimulation to the direction of the behaviors which can be induced by stimulation via the same electrode. They stated "that serious confusions have arisen in the brain stimulation literature from investigators failing to properly discriminate whether these sequences of behavior constitute approach or withdrawal". In the following the terms appetitive and aversive behavior are used. Appetitive behavior is defined as a response directed towards obtaining brain stimulation, aversive behavior as a response directed towards stopping it. The induction of appetitive behavior is considered to have a positively reinforcing effect, while the induction of aversive behavior is supposed to be negatively reinforcing.

The effects of CSD on stimulation induced appetitive and aversive behavior have also been investigated. It was found that appetitive behavior induced by lateral hypothalamic stimulation was completely blocked by bilateral CSD, less completely by ipsilateral CSD and still less by contralateral CSD (BURES, BURESOVA, FIFKOVA, OLDS, OLDS & TRAVIS, 1961, RüDIGER & FIFKOVA, 1963). With respect to aversive behavior induced by dorsomedial tegmental stimulation it was reported that during bilateral CSD only the operant component was inhibited, while the reflex component remained intact (BURES, BURESOVA, FIFKOVA, OLDS, OLDS & TRAVIS, 1961). Results were interpreted by these authors as a depression of operant control mechanisms and/or as a reduction of hypothalamic excitability.

These interpretations are debatable because of the kind of behavior which was reinforced. The response in these experiments, to which onset of offset of stimulation was made contingent, was lever pressing. It is possible that the animals could not meet the motor requirements of this response during CSD, as CSD causes a loss of cortical postural reflexes (BURES, BURESOVA & KRIVANEK, 1974). Moreover, lever pressing for hypothalamic stimulation is more equivalent to a consummatory response than to an operant response in the usual sense (MILNER 1970, p. 389) and it has been established that the elicitation of consummatory behavior by hypothalamic stimulation is blocked during CSD (HUSTON & BURES, 1973). So possibly lever pressing was blocked in the same way as other stimulation induced consummatory behaviors are blocked during CSD. VALENSTEIN (1964) has given some other arguments, which question the general validity of lever pressing as a measure of reinforcement strength. Therefore, we developed a test in which the motor requirements of the operant response are much lower and in which the operant response is not likely to be contaminated by elicited consummatory responses. It tried to combine the advantages of the approach and escape test, developed by Olds (1960), and the preference test, developed by Valenstein and Meyers (1964). It was expected that in this test the positive or negative reinforcement strength of brain stimulation could be established more unequivocally. With this test the effects of CSD on appetitive and aversive behavior induced by hypothalamic stimulation were investigated.

5.3. Method.

Subjects.

A total of 22 male albino WU (SPF63 Cpb) rats (LOOSLI 1975) completed the experiment; several rats were discarded because of ineffective electrode placement or ineffective CSD treatment. The animals weighed about 300 g at the time of surgery; they were kept on a 12 hr. light/12 hr. dark cycle and were tested during the dark period in a dark room.

Surgery.

The rats were operated upon twice. Both times anesthetization was induced with Hypnorm and a supplementary dose of Nembutal. In the first operation bipolar electrodes (Plastic products company, MS 303, SS, 008") were implanted in the lateral or medial hypothalamus; the electrodes were insulated except for the cross section of the tips. Coordinates were respectively: 5.4 mm anterior, 1.5 mm lateral, 9.0 mm below dura and 5.4 mm anterior, 0.75 mm lateral, 9.0 mm below dura (PELLEGRINO & CUSHMAN 1967); so the only difference was in the lateral coordinate. In the second operation stainless steel epidural cannulae (length 6.5 mm with an internal diameter of 1.5 mm) were implanted; they were placed in trephine openings measuring 3 mm in diameter, which were drilled in the skull over the posterior cortex of each hemisphere; care was taken not to damage the dura. At a distance of 0.5 mm from the bottom of the cannulae was a flange of 0.5 mm, which rested on the skull to prevent pressure on the dura. The cannulae

were fixed to the skull with dental cement and fixation screws. The dura was moistened with saline and the cannulae were closed with a screwmounted plunger to prevent dehydration. Saline was refreshed daily.

Apparatus and Stimulation Parameters.

The rats were tested in a Plexiglass shuttle box, measuring 40x25x40 cm. The two 20x25 cm Pertinax platforms were separated by a 2.5 cm Pertinax barrier and rested on microswitches. The rat's weight on either platform depressed microswitches; by the rat moving from one platform to the other the brain stimulation circuit could be activated or interrupted. Barrier crossing responses were counted by an observer and recorded on a Campden 654 cumulative recorder. The electrical brain stimulation was generated by a Grass S48 stimulator followed by a Grass SIU5 stimulus isolation unit and a Grass CCU1A constant current unit. It consisted of quasi-biphasic pulse trains: one 300 msec train per second, 100 pulses per second, 0.2 msec pulse width; effective current strength was established for each individual rat and varied between 60-125 uA.

The stimulation was provided to the animal via a phone jack and a spring-loaded cord.

Procedure.

After electrode implantation the rats were first tested in the shuttle box according to the rate - independent method of VALENSTEIN and MEYERS (1964) in order to establish effective current strength. Current strength was

considered to be effective if it induced clear appetitive and/or aversive barrier crossing responses. Thereafter the rats were trained in the shuttle box in two contingencies; these contingencies were presented successively and lasted 10 minutes each. In one contingency a barrier crossing response resulted in a 5-seconds presentation of intermittent brain stimulation having the above mentioned parameters. (ON-response); in the other contingency the same response resulted in a 5-seconds interruption of otherwise continuous intermittent stimulation (OFF-response). So a rat could maximize stimulation by generating many ON-responses and few OFF-responses (appetitive behavior) or it could minimize stimulation by generating few ON-responses and many OFF-responses (aversive behavior). Training was continued in several daily sessions until stable performance was obtained. Then the rats were operated upon for the second time in order to implant the epidural cannulae. On the third day after the second operation 5 days of testing started (Fig. 1). On day 2, 3 and 4 contralateral, ipsilateral or bilateral CSD was induced in the following way. The plungers were removed and the cannulae were filled with a 25% KCl solution or with saline; cannulae were closes with small screws. Effectiveness of CSD treatment was tested by examining contralateral placing reflexes which are impaired during CSD (BURES, BURESOVA & KRIVANEK, 1974). Before and after this CSD-treatment a 10 minutes ON-response test and a 10 minutes OFF-response test were given (pretreatment and posttreatment tests). Order of ON- and OFF-testing and order of contralateral, ipsilateral and bilateral CSD

were balanced over subjects. After the posttreatment tests the cannulae were cleaned and rinsed with saline. The three experimental days were preceded and followed by control days, on which the procedure was the same except that now saline was applicated to both hemispheres.

Histology.

At the completion of the experiment the brains were removed and fixed in formalin. Then they were frozen and sliced in 25 μ sections; the sections were stained with cresyl-violet to provide histological confirmation of the electrode placements.

5.4. *Results.*

Effective stimulation could induce one of three different types of behavior (Fig. 2). Stimulation was termed appetitive, if the animal tended to maximize stimulation: many ON-responses, few OFF-responses. Stimulation was termed aversive, if the animal tended to minimize stimulation: few ON -responses, many OFF-responses. Finally, stimulation was termed ambivalent, if the animal generated a high frequency of ON-responses and also a high frequency of OFF-responses. As a criterion to label rats as appetitive, ambivalent or aversive a so-called appetition score was determined: appetition score = (ON-responses x 100)/(ON-responses + OFF-responses); this score has a maximum of 100 and a minimum of 0. An animal with a score higher than 66 was labeled as appetitive; if the score was between 33 and 66, the animal was called ambivalent; final-

	pre-treatment test	treatment	post treatment test
day 1	on - off	saline	on - off
day 2	on - off	contralateral csd	on - off
day 3	on - off	ipsilateral csd	on - off
day 4	on - off	bilateral csd	on - off
day 5	on - off	saline	on - off

order of on- and off-testing was balanced over subjects
* order of contralateral, ipsilateral and bilateral csd was balanced over subjects

Fig. 1. Design of treatments and tests on the 5 testing days.

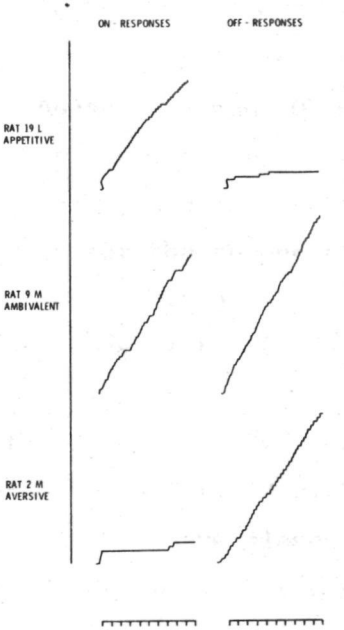

Fig. 2. Representative examples of ON- and OFF-responding of appetitive, ambivalent and aversive rats. Duration of testing was 2 x 10 minutes. Each response resulted in 5 steps (one per sec) on the cumulative recorder.

ly, an animal with a score lower than 33 was labeled as aversive. Of the 22 animals which completed the experiment, 9 were found to be appetitive, 7 ambivalent and 6 aversive.

The mean pretreatment appetition scores over the 5 test days were 76.4, 51.2 and 22.0 for the appetitive, ambivalent and aversive groups respectively.

In Fig. 3 the mean number of pretreatment and posttreatment ON- and OFF-responses are presented for the 3 groups over the 5 test days. This figure shows that in appetitive and ambivalent rats during CSD there was a clear tendency of a decrease in ON-responses and an increase in OFF-responses. This tendency seems to have been larger during ipsilateral CSD than during contralateral CSD and was still larger during bilateral CSD. In aversive rats this tendency was not so clear.

In Fig. 4 the results are presented in another way. Here the appetition scores are given. It can be seen that the pretreatment scores remained relatively constant over the 5 test days. Differences between pretreatment and posttreatment scores of the 3 groups were tested for significance by two-tailed t-tests for related samples. No significant differences appeared on the first control day ($t = .16$, .06 and .92 for appetitive, ambivalent and aversive rats respectively) nor on the last control day ($t = 1.30$, .17 and .89 respectively). Also during contralateral CSD the differences were not significant ($t = 1.86$, .93 and 1.42 respectively). During ipsilateral CSD posttreatment scores were significantly lower than pretreatment scores for appetitive and ambivalent rats, but this difference

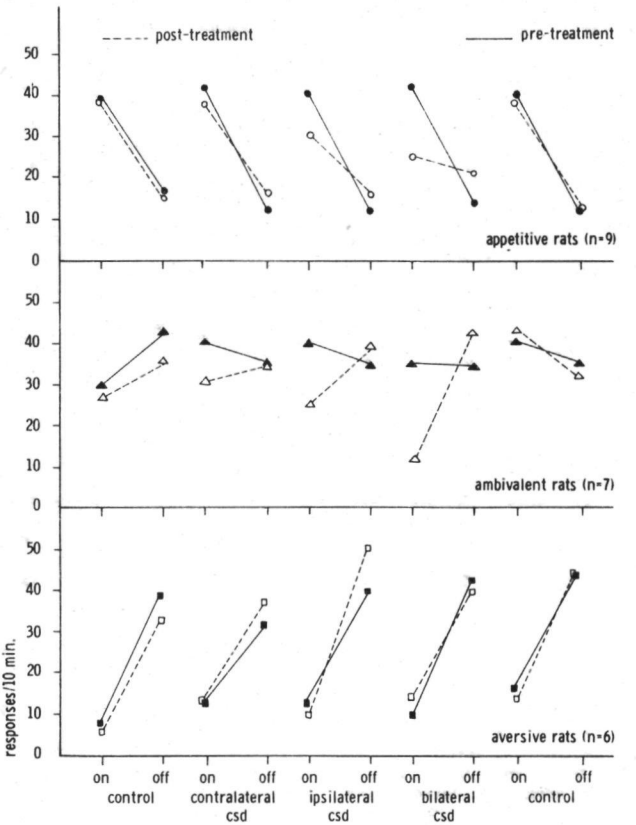

Fig. 3. Pretreatment and posttreatment results on the 5 testing days: mean number of ON- and OFF-responses per 10 minutes of appetitive, ambivalent and aversive rats.

failed to reach significance for aversive rats (t=3.39/p =.01, t=2.94/p=.03 and t=2.03/p=.10 respectively). During bilateral CSD posttreatments scores were again lower than pretreatment scores for appetitive and ambivalent rats, while the difference for aversive rats was not significant (t=3.16/p=.01, t=5.18/p=.002, t=.91/p=.40 respectively). The mean posttreatment score of appetitive rats during bilateral CSD was lowered to the level of the pretreatment scores of the ambivalent rats. In the same way the posttreatment score of ambivalent rats was lowered during bilateral CSD to the pretreatment level of the aversive rats.

The results of the histological determination of electrode placements are depicted in Fig. 5. One ambivalent and one aversive placement could not be determined since the removal of the electrode had produced substantial tissue damage. All but one appetitive placements were found to be more or less lateral to the fornix; on the other hand all ambivalent and all aversive placements were medial to the fornix.

5.5. Discussion.

From the results of this experiment the following conclusions can be drawn.

1. Operant behavior, reinforced by hypothalamic stimulation, was not blocked by CSD in this experiment. Although their performance changed, even during bilateral CSD appetitive, ambivalent and aversive rats still

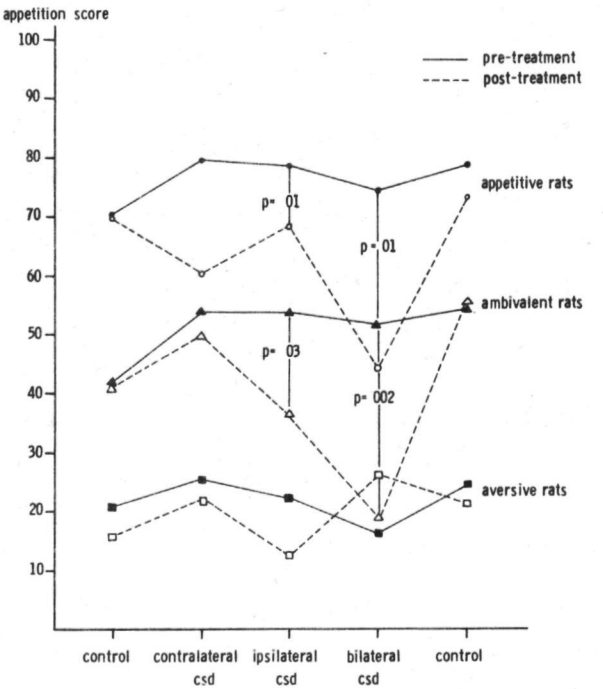

Fig. 4. Pretreatment and posttreatment results on the 5 testing days: mean appetition scores of appetitive, ambivalent and aversive rats. P-values of significant two-tailed t-tests for related samples are represented.

showed a substantial number of ON- and/or OFF-responses. Therefore, the complete blocking of operant hypothalamic approach and tegmental escape behavior, found by BURES, BURESOVA, FIFKOVA, OLDS, OLDS & TRAVIS (1961), was likely due to the fact that the response, that was used, was lever pressing. Apparently this response was blocked in the same way as other consummatory behaviors, to which it is equivalent (HUSTON & BURES, 1973, MILNER, 1970); another possibility is that the motor requirements could not be met due to a loss of cortical postural reflexes (BURES, BURESOVA & KRIVANEK, 1974).

2. During CSD an increased aversiveness of hypothalamic stimulation appeared to exist. This increased aversiveness manifested itself as a decrease of ON-responses, an increase of OFF-responses and a resulting decrease of appetition scores. It was found for appetitive lateral placements as well as for ambivalent medial placements. The absence of a comparable finding for aversive placements is probably due to a bottom effect: apparently stimulation via these electrodes was so aversive already, that the appetition score could not be reduced any further by CSD.

3. In terms of operant conditioning the increased aversiveness may be interpreted as a change of the reinforcement value in negative direction. It is suggested that the test, used in this experiment, provides a valid test of the reinforcement value of brain stimulation: The animal has to show opposite behaviors (barrier

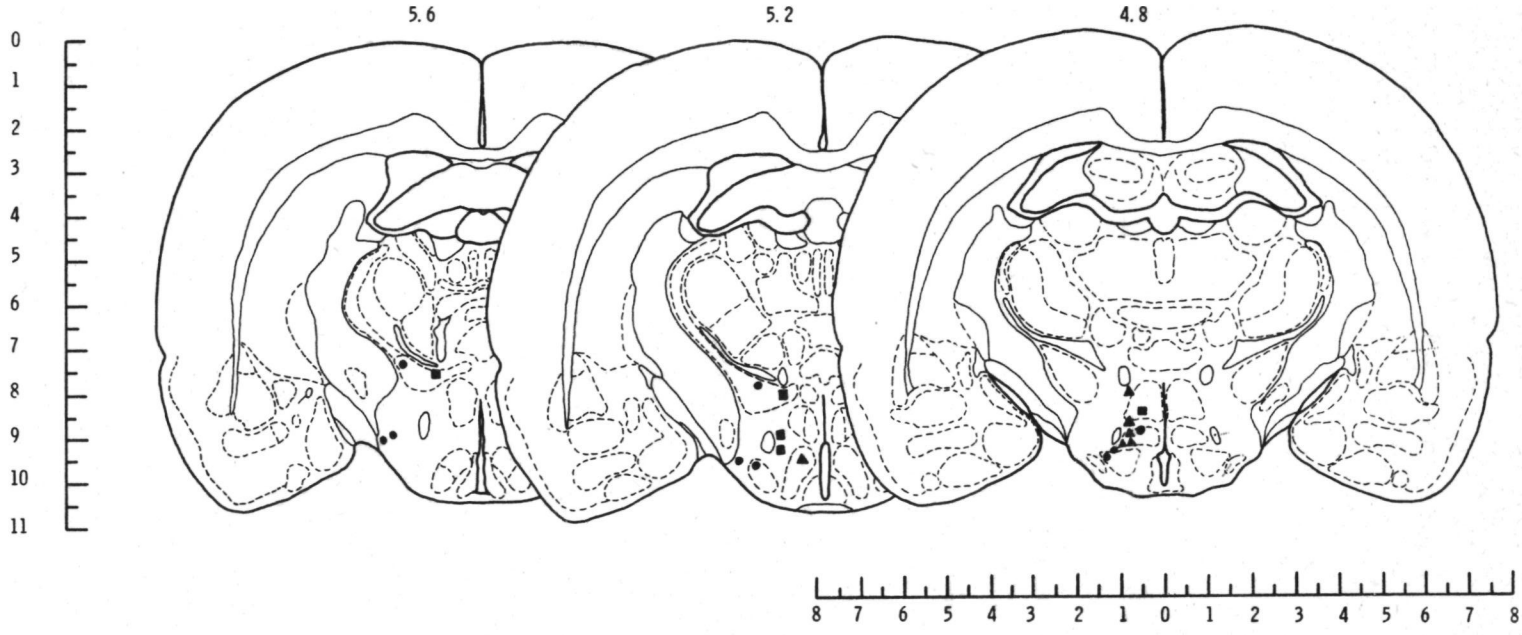

Fig. 5. Electrode placements of appetitive (●), ambivalent (▲) and aversive (■) rats. Section drawings are from Pellegrino and Cushman (1967).

crossing response or inhibition of this response) in order to obtain the same result (either maximization or minimization of stimulation). In this respect this test resembles the test of OLDS (1960). It differs from OLDS' test, however, in that the motor requirements are low (only locomotion) and that contamination by stimulation induced consummatory behaviors is not likely to occur. It was found that individual pretreatment appetition scores remained relatively constant over days; therefore, it is suggested that this test is also a reliable one.

4. The effect of CSD on the behavior of the animals was mainly ipsilateral. This may be taken as evidence that it was really due to a disruption of cortico-hypothalamic interactions and that it was not some general effect. In this respect the results confirm the findings of RÜDIGER & FIFKOVA (1963).

The effect of CSD in this experiment resembles the effect of lesions in the ventro-medial tegmentum, which have also been found to increase the aversiveness of hypothalamic stimulation (SCHMITT, VERGNES & KARLI, 1973). For the increased aversiveness of hypothalamic stimulation during CSD the following explanation is suggested. a) By stimulation in lateral or medial hypothalamus both an appetitive and an aversive system are activated. b) Placement of electrode and intensity of stimulation determine the degree of activation of both systems and thereby the net effect of stimulation on the behavior of the

animal. c) The excitability of the appetitive system is reduced by ipsilateral CSD, while the excitability of the aversive system remains unchanged; the net effect is an increased aversiveness of stimulation. The reduced excitability of the appetitive system during ipsilateral CSD was likely due to a depression of frontal cortex activity, as the frontal cortex has been found to be involved in appetitive or reward pathways (ROUTENBERG, 1971). Recently it has been suggested that a region in or near the sulcal prefrontal cortex is involved in brain-stimulation reward (ROLLS & COOPER, 1974). The same region has also been implicated in the regulation of food and water intake (KOLB & NONNEMAN, 1975). Therefore, it is hypothetized that the frontal cortex forms the highest level of a system, involved in the regulation of appetitive (i.e. approach) behavior. It cannot be excluded, however, that also with respect to the frontal involvement in hypothalamic self-stimulation a lever-pressing test may produce results dissimilar to a test, in which stimulation is made contingent to a locomotor response (BERDASHKEVICH & SHIK, 1971). One may wonder, whether such telencephalic involvement may also be found for the regulation of aversive (i.e. withdrawal) behavior. CARLTON & MARKIEWICZ (1973) have reported experiments in which hippocampal spreading depression was found to disturb aversive behavior (retrieval of conditioned fear), while appetitive behavior remained intact. So maybe the hippocampus or part of it (JARRARD, 1973) is involved in the regulation of aversive behavior.

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CHAPTER 6. APPETITIVE AND AVERSIVE BEHAVIOR INDUCED BY PERIPHERAL THERMAL STIMULATION DURING CORTICAL SPREADING DEPRESSION IN RATS.

6.1. *Abstract.*

Eight groups of eight rats were tested in a shuttle box. Floor temperature of each of the two compartments of the box could be set on one of two possible values by way of thermostatically controlled water which was circulating under the floor. In this way locomotor responses from one compartment to the other were made contingent to change in floor temperature. Temperature changes were within the range of 5^0 - 35^0 C. A change from a higher floor temperature to a lower floor temperature was aversive and produced a relatively low level of responding; on the other hand a change from a lower floor temperature to a higher one was appetitive and produced a relatively high level of responding. During bilateral CSD appetitive responding was significantly reduced, while aversive responding was not changed, as compared to a nondepressed control condition. These results were interpreted as follows: CSD causes a reduction of the excitability of an appetitive mechanism, whereas the excitability of the aversive mechanism remains unchanged.

6.2. *Introduction.*

In the search for the neural basis of complex learning the involvement of neocortical structures is usually implicated (Russell, 1971). For about two decennia cortical

spreading depression (CSD) is used as a reversible decortication technique in neuro-behavioral research in rats; learning processes in particular have been investigated (Bures et al, 1974). The investigation of learning in a bilateral decorticate state has been limited almost exclusively to aversively motivated tasks. The reason for this limitation is evidently that the unconditioned responses to aversive foot-shocks, which are conventionally used as unconditioned stimuli in such tasks, although reduced are not completely lost during bilateral CSD (Thompson & Enter, 1967). On the other hand, the unconditioned responses to stimuli like food and water (eating and drinking), which are conventionally used in appetitively motivated tasks, are completely depressed during CSD (Bures et al, 1974).

A discontinuity exists between shock-motivated aversive learning tasks and food - or water - motivated appetitive learning tasks. Results of shock-motivated and food - or water - motivated tasks are often difficult to compare because of differences in procedure and especially because of differences in the type of unconditioned reaction to these different types of stimuli. Therefore, if one is interested in the differential effects of CSD on appetitive and aversive motivation, preferably a test situation should be developed, in which appetitive and aversive behavior can be studied under the same procedure and under stimulation on one and the same stimulus dimension. In this respect, appetitive behavior is defined as a response directed towards the source of stimulation, i.e. an approach response; aversive behavior, on the other hand, is defined as a response directed away from the stimulation

source, i.e. a withdrawal response (cfr. Schneirla, 1959).

To meet the above mentioned requirements we developed a test situation in which appetitive or aversive intracranial electrical stimulation was used (Van der Staak, 1975[a]): in a shuttle box locomotor responses were made contingent to onset or offset of electrical stimulation of lateral or medial hypothalamus. The effects of CSD on appetitive and aversive responses were investigated. The results were interpreted in terms of a theory of two opponent mechanisms (cfr. Van der Staak, 1975[b]). According to this theory the response of an organism to a stimulus is determined by two opponent mechanisms: an appetitive mechanism, driving the organism towards approach, and an aversive mechanism, driving it towards withdrawal. On the basis of the results of this experiment the suggestion was made that CSD reduced the excitability of the appetitive mechanism, while the excitability of the aversive mechanism remained unchanged.

Now, intracranial electrical stimulation is a rather unnatural type of stimulation and the effects of intracranial stimulation on behavior differ in some important respects from the effects of peripheral stimulation (Milner, 1970). Therefore, for the experiment to be reported here we developed another test situation, in which peripheral instead of intracranial stimulation was used in order to induce appetitive and aversive behavior; in all other respects the procedure in this test was chosen so as to be as much comparable as possible to the procedure in the intracranial stimulation test. Only a few modifications had to be made, which were due to the peculiarity

of the peripheral stimulation. Change in floor-temperature was chosen as peripheral stimulation. This choice was instigated by the finding that thermotropic locomotion in rats is dependent on the integrity of the lateral hypothalamus and that hypothalamic control of this behavior is disturbed during CSD (Rüdiger & Seyer, 1965). Preliminar experiments (Receveur, 1974) had shown that within the range of 5^0 C - 35^0 C a rise in floor-temperature induced appetitive behavior: when given the choice between two floors with different temperatures, the rat approached and stayed on the floor with the higher temperature. This finding implicated, of course, that a fall in floor-temperature induced aversive behavior: the rat withdrew from and stayed away from the floor with the lower temperature. In the same experiments it was also found that a change from 5^0 C to 20^0 C or vice versa produced a high level of motivation (high approach to 20^0 C, high withdrawal from 5^0 C), whereas a change from 20^0 C to 25^0 C or vice versa produced a lower level of motivation.

In the experiment to be reported here the effect of bilateral CSD on appetitive and aversive behavior, induced by changes in floor temperature, was investigated. Two levels of appetitive and aversive motivation were used. Because the change in floor temperature was a time-consuming process, after each temperature-change contingent response a time-out period had to be inserted to re-establish the starting-situation for the next trial. During this time-out period the animal was free to respond. This allowed a comparison of the effects of CSD on contingent and non-contingent responses.

6.3. Methods.

Subjects.

64 Male albino WU (SPF63 Cpb) rats were used. They weighed about 300 g at the time of surgery and were housed in individual cages. Food and water were available ad lib.; room temperature was kept at 20^0 C. The rats were kept on a 12 hr. light/12 hr. dark cycle and were tested during the light period.

Surgery.

The rats were anesthetized with hypnorm (10 mg fluanizone, 0.2 mg phentanyl citrate pro ml, 0.08 cc/100 g i.m.) followed by nembutal (60 mg/ml; 0.03 cc/100 g i.v.). Over the posterior cortex of each hemisphere trephine openings were drilled into the skull, measuring 3 mm in diameter, in which stainless steel epidural cannulae (length 6.5 mm with an internal diameter of 1.5 mm) were placed. Care was taken not to damage the dura. At a distance of 0.5 mm from the bottom of the cannulae was a flange, which rested on the skull to prevent pressure on the dura. The cannulae were fixed to the skull with dental cement and fixation screws. The dura was moistened with saline and the cannulae were closed with screw-mounted plungers to prevent dehydration. Saline was refreshed daily.

Apparatus.

The rats were tested in a shuttle box, measuring 20x60x20 cm. On the bottom of each of the two compartments

was a 20x30x0.7 cm reservoir, through which water was pumped. The top of the reservoir, which formed the floor of the box, consisted of a 0.1 mm thick copper foil to permit quick temperature exchange between the water of the reservoir and the feet of the rat; the other sides of the reservoir were made of thermally isolated brass. The box had no barrier, so the animal could move freely from one compartment to the other. The plexiglass walls of the box were coated with black adhesive foil except for the front wall in which two noncoated hinge doors allowed observation of the animal. All four walls were heavily perforated to permit free passage of air. The top of the box consisted of a plywood enclosure in which two 5W light bulbs were mounted; through the ground-glass bottom of the enclosure a dim illumination of the box was provided.

The temperature of the water, which was pumped through the reservoirs on the bottom of the box, could be changed. Two circulations were possible for each compartment reservoir. Two circulating water baths with thermostatic control provided the water and by opening or closing a system of solenoid valves it could be chosen which of the two circulations was pumped through the reservoir (capacity: 12 l/min).

Temperature combinations, which were used in the different experimental conditions, were either 5^0 C and 20^0 C or 20^0 C and 35^0 C. It took about 15 sec to raise or lower the box-floor temperature from one temperature to the other; temperature changes were measured on the floor of the box by way of iron-constantan thermocouples.

Procedure.

Subjects were randomly assigned to one of eight groups; each group consisted of eight subjects. On the second day after surgery (day 1) the first session took place. Before the session one of two treatments was given: no CSD (N) or bilateral CSD (B). There were four N-groups and four B-groups. Bilateral CSD was induced as follows. The plungers were removed and the cannulae were filled with a 25% KCl solution; cannulae were closed with small screws. Effectiveness of CSD treatment was tested before and after the session by examining placing reflexes. The no CSD treatment was exactly the same as the bilateral CSD treatment except that saline was used instead of the KCl solution.

Ten minutes after treatment the rat was placed in the shuttle box; floor temperature was 20^0 C in both compartments. Five minutes later testing was begun by changing the floor temperature of one compartment. This could be a temperature change in the compartment the animal was located in at the moment or in the opposite one; the change could be either a rise to 35^0 C or a fall to 5^0 C. In this way a locomotor response of the rat from one compartment into the other resulted in a change from one floor-temperature to another: from 20^0 C to 5^0 C, from 35^0 C to 20^0 C, from 20^0 C to 35^0 C or from 5^0 C to 20^0 C. On the basis of previous experiments (Receveur, 1974) these contingencies were called high aversive (i.e. high withdrawal from the opposite compartment), low aversive (i.e. low withdrawal from the opposite compartment), low appetitive

(i.e. low approach to the opposite compartment) and high appetitive (i.e. high approach to the opposite compartment). It will be clear that the contingencies were defined as appetitive or aversive depending of whether they resulted in approach to or withdrawal from the opposite compartment, respectively. Each of the four N-groups and each of the four B-groups was tested in one of the four contingencies.

If the animal made such a contingent response, a time-out period of 60 sec started: the changed floor temperature was brought back to 20^0 C again, so after about 15 sec no temperature difference between the compartments existed anymore. After the 60 sec time-out period, in which the rat was free to move from one compartment to another (non-contingent responses), the next trial was started: floor temperature of one compartment was raised or lowered again, depending of the experimental condition, until the animal made a contingent response. Such contingent response started the next time-out period, etc. This procedure was continued for a duration of 25 minutes after which the session was ended. The rat was removed from the box; cannulae were cleaned and rinsed with saline and the animal was returned to its home cage. 24 Hours later (day 2) and 48 hours later (day 3) all animals received the same testing session, preceded by the same treatment, depending of the experimental condition.

6.4. Results.

For each of the three sessions the number of contingent responses and the mean number of non-contingent res-

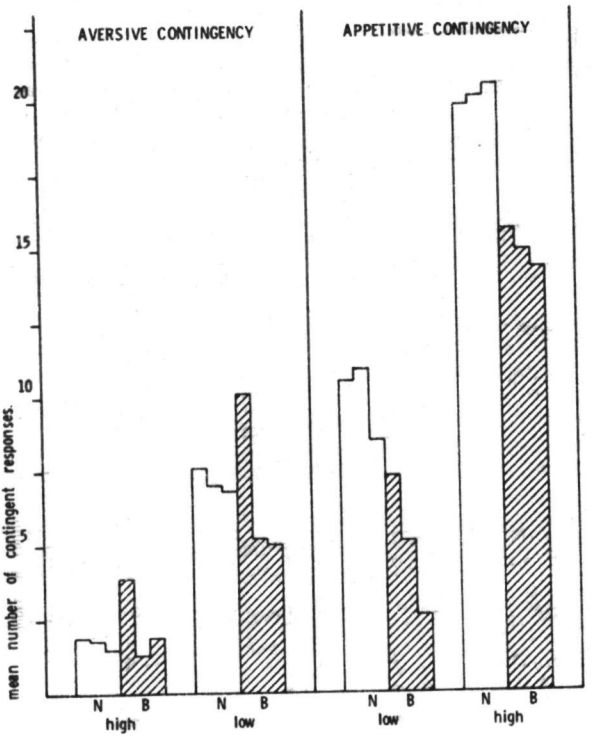

Fig. 1. Mean number of contingent responses per day for the aversive and appetitive contingencies. Results of low and high levels of motivation are differentiated. N: no CSD treatment; B: bilateral CSD treatment. The 3 different levels within each column denote the mean values of the 3 consecutive testing days.

ponses per time-out were recorded. In Fig. 1 the mean numbers of contingent responses of the eight groups are depicted. The results of the groups with aversive contingency conditions in which responses produced a lower temperature, and those of the groups with appetitive contingency conditions, in which responses produced a higher temperature, were analyzed separately. This was done by way on univariate analyses of variance with Treatments (N vs B) and Level of motivation (high vs low) as fixed factors and repeated measurements on Days 1, 2 and 3 (Winer, 1970). The results of the groups with aversive contingency conditions were as follows. There were no significant differences between the N-groups and the B-groups. The difference between the low aversive contingency and the high aversive contingency groups, however, was significant ($F_{1,28} = 42.71$, $p < .001$) and so was the difference between days ($F_{2,56} = 8.72$, $p < .001$). Of the interactions ontly the Treatments x Days interaction was significant ($F_{2,56} = 5.10$, $p = .009$). These results may be interpreted (see Fig. 1) as a higher number of contingent responses during the low aversive contingency condition than during the high aversive one. Furthermore it may be concluded that the number of contingent responses decreased over days. The significant Treatment x Days interaction, finally, seems to be caused by the high response level of the B-groups on day 1.

For the groups, in which an appetitive contingency was induced between response and temperature, the results were different. The difference between N-groups and B-

groups was significant now ($F\ 1,28 = 14.78$, $p < .001$). Also the low appetitive and high appetitive contingency groups differed significantly ($F\ 1,28 = 57.68$, $p < .001$). Difference between days was not significant, nor any of the interactions. So, it may safely be concluded, that the high appetitive contingency condition produced a higher level of responding than the low appetitive condition. Moreover, bilateral CSD caused a reduction of appetitive responding, both in the low appetitive and in the high appetitive contingency condition.

The difference between the N-groups and B-groups in the appetitive contingency condition could have been due to a reduced locomotor facility during bilateral CSD. If this was so, the reduced level of responding of the B-groups should have been manifested not only in the contingent responses but also in the non-contingent responses during the time-out periods. To investigate this possibility for the non-contingent responses the same analyses of variance were done as for the contingent responses. As the number of time-out periods per session differed between subjects, the mean number of non-contingent responses per time-out per session was taken as variable; in Fig. 2 the groups means are presented. For the groups with aversive contingency conditions the analysis revealed no significant differences at all, neither in the main effects (Treatment, Level of motivation, Days), nor in any of the interactions. For the groups with appetitive contingency conditions only one effect was found to be significant: the B-groups differed significantly from the

N-groups ($F\ 1,28 = 4.24$, $p = .04$) and this difference may be interpreted as a higher level of responding of the B-groups. So, the results of the analyses of the non-contingent responses were different from those of the contingent responses. Whereas the B-groups showed a lower level of contingent responding in the appetitive conditions as compared to the N-groups, at the same time their level of non-contingent responding in the appetitive conditions was found to be higher.

6.5. *Discussion.*

In this experiment shuttle box responses could result in one of two possible effects. In some groups contingent responses resulted in a fall of floor temperature. As far as these aversive contingency conditions were concerned, bilateral CSD had no effect on the number of contingent responses as compared to control, neither for the high aversive condition, nor for the low aversive condition. In other groups contingent responses resulted in an appetitive rise of floor temperature. Bilateral CSD caused a significant reduction in the number of appetitive contingent responses as compared to control, as well for the low appetitive condition as for the high appetitive condition. This reduction of appetitive contingent responding was not due to an impaired motor facility during bilateral CSD, as in the same appetitive conditions the number of non-contingent responses was raised as compared to control. Therefore, these results substantiate the hypothesis that CSD reduces the excitability of appetitive mechanisms

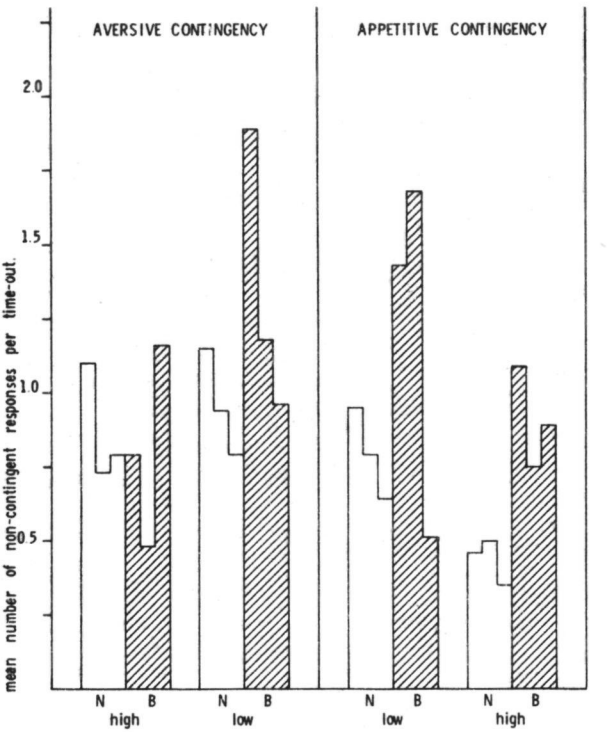

Fig. 2. Mean number of non-contingent responses per time-out per day for the aversive and appetitive contingencies. Results of low and high levels of motivation are differentiated. N: no CSD treatment; B: bilateral CSD treatment. The 3 different levels within each column denote the mean values of the 3 consecutive testing days.

while the excitability of aversive mechanisms remains unchanged (Van der Staak, 1975[a]): this hypothesis appears to hold not only with regard to intracranial electrical stimulation, but also with regard to peripheral thermal stimulation.

The results of this experiment also substantiate the finding by Rüdiger and Seyer (1965) of an absence of thermotropic locomotion during bilateral CSD. Rüdiger & Seyer (1965) made no distinction between appetitive and aversive thermotropic behavior. On the basis of the present experiment their conclusion may be specified: only the appetitive component of thermotropic behavior is disturbed during CSD. Rüdiger and Seyer (1965) further demonstrated that the CSD-effect on thermotropic behavior was due to a reduced hypothalamic excitability. The hypothalamic involvement in the regulation of thermoregulatory behavior has been clearly established (Corbit, 1970; Hardy, 1973). This may explain, therefore, why the CSD-effect on behavior induced by peripheral thermal stimulation was equal to the CSD-effect on behavior induced by intracranial electrical stimulation (Van der Staak, 1975[a]).

The significant differences in contingent responding between high and low aversive conditions and between high and low appetitive conditions, substantiate the finding of Receveur (1974) that a change in floor temperature from 5^0 C to 20^0 C or vice versa produces a high level of motivation (appetitive or aversive motivation, respectively), whereas a change from 20^0 C to 35^0 C or vice versa produces a lower level of motivation.

The decrease of aversive contingent responding over days was only small and was not parallelled by a significant increase of appetitive contingent responding over days. Moreover, the differences between aversive and appetitive contingent responding and between high and low levels of motivation manifested themselves from the beginning of training onwards and did hardly change anymore.

Therefore, although the induced contingencies were of the operant type, it seems that the results have been due to elicitation rather than operant conditioning. In this respect, the contingency between locomotion and floor temperature resembles the contingency between freezing and foot-shock, for which Bolles and Riley (1973) obtained comparable results. According to Bolles and Riley (1973) these results suggest another look at the operant-respondent distinction.

In this experiment changes in floor temperature were made contingent to locomotor responses. This test situation is different from other test situations which are used in experiments on thermoregulatory behavior. Usually, the animal is required to make a manipulative response (bar-pressing) in order to obtain changes in ambient temperature (Corbit, 1970). Rüdiger & Seyer (1968) investigated the effects of CSD and unilateral hypothalamic lesions on bar-pressing for radiant heat in a cold environment. CSD, induced in the hemisphere contralateral to the hypothalamic lesion, cuased an increased bar-pressing rate for radiant heat. These results seem to be in contradiction to the results of Rüdiger and Seyer (1965) and of the present experiment, in which a locomotor response was used as operant. This

contradiction suggests a closer investigation of CSD-effects on thermoregulatory behavior, in which bar-pressing and locomotion are explicitely compared in the frame of the above mentioned distinction between respondents and operants.

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CURRICULUM VITAE

Cees P.F. van der Staak is geboren op 29 juli 1945 te St. Michielsgestel. Na de lagere school bezocht hij het St. Janslyceum te 's Hertogenbosch, alwaar hij in 1963 het diploma gymnasium β behaalde. Van 1963 tot en met 1969 studeerde hij psychologie aan de Katholieke Universiteit te Nijmegen en legde in december 1969 cum laude het doktoraalexamen psychologie af. In zijn stage-periodes heeft hij zich bezig gehouden met onderzoek naar lateralisatie-verschijnselen (vakgroep Psychologische Funktieleer) en met persoonlijkheidsonderzoek bij dove kinderen (Instituut voor Doven, St. Michielsgestel). Sinds januari 1970 is hij verbonden als wetenschappelijk medewerker aan de vakgroep Vergelijkende en Fysiologische Psychologie van het Psychologisch Laboratorium der Katholieke Universiteit te Nijmegen. In het najaar van 1970 is hij gedurende 3 maanden werkzaam geweest op het Nederlands Centraal Instituut voor Hersenonderzoek te Amsterdam, daartoe in staat gesteld door de "European Training Program in Brain and Behaviour Research".

STELLINGEN

1. In de evolutie van het gedrag zijn appetitieve en aversieve mechanismen van doorslaggevend belang geweest voor de overleving.

2. Herhaalde of kontinue stimulatie induceert vaak een aanvankelijk toenemende en later afnemende reaktie, welke door Groves & Thompson beschreven is in termen van habituatie en sensitisatie; het is zinvoller deze veranderingen in reaktie te beschrijven in termen van differentiele habituatie in appetitieve en aversieve mechanismen.

3. Het is onmogelijk de belonende of straffende waarde van intrakraniele stimulatie ondubbelzinnig vast te stellen aan de hand van de response-frekwentie in een skinner-box, de in dit proefschrift gepresenteerde shuttle-box procedure biedt deze mogelijkheid wel.

4 Bij het onderzoek naar het antagonisme van appetitieve en aversieve mechanismen kunnen verhoging vs. verlaging van bodemtemperatuur beschouwd worden als geschikte onvoorwaardelijke stimuli, omdat ze aangrijpen op een en hetzelfde homeostatisch regelmechanisme; de gangbare stimuli (voedsel of water vs. elektrische schokken) zijn in dit verband minder geschikt

5. In de neuropsychologische diagnostiek kan een vruchtbaar gebruik gemaakt worden van de stelling, dat de relatieve kracht en souplesse van abduktie- en adduktie-bewegingen der ledematen een uitdrukking vormen van de relatieve werkzaamheid van de antagonistische neurale mechanismen van toenadering en verwijdering.

6. De bipolaire mechanismen van appetitief en aversief gedrag houden elkaar normaliter in balans; vele gedragsstoornissen zijn te interpreteren als een doorslaan van deze balans ten gevolge van een te langdurig overwicht van een van beide mechanismen.

7. Bij het onderzoek naar algemene wetmatigheden in leerprocessen moet rekening worden gehouden met soort-specifieke en individu-specifieke reaktiewijzen, die aan deze leerprocessen ten grondslag liggen.

8. Psychologie is een biologische wetenschap; in de huidige psychologie-opleiding komt dit te weinig tot uitdrukking.

9. Doofheid veroorzaakt niet alleen bijzondere problemen in de kognitieve maar ook in de emotionele ontwikkeling; het doven-onderwijs zou gebaat zijn met een fundamenteel onderzoek naar de emotionele ontwikkeling van het dove kind.

10. Peuterspeelzalen kunnen een belangrijke bijdrage leveren aan de kognitieve, emotionele en sociale ontwikkeling van het jonge kind.

11. Het boek "Een soort vuur" van P.J. Stolk verdient evenveel aandacht als het boek "Wie is van hout" van J. Foudraine.

12. De resultaten van split-brain onderzoek leveren interessante vragen op voor iedere mensbeschouwing.

13. Door wetenschappelijk onderzoek kan de mens wetmatigheden ontdekken in zijn gedrag; het geheim van zijn bestaan laat zich daardoor niet ontsluieren.

14. Het symbool van de kelk als levengevende bron is te herleiden tot een zich openend ei.

Nijmegen, 28 november 1975 C.P.F. van der Staak

Cover: B.A. Fekkes